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Abstract

Oxaliplatin is an anticancer drug widely used in cancer chemotherapy. This thesis evaluates whether a specific dose-banding scheme for oxaliplatin could replace the individual dosing method that is currently used in the oxaliplatin administration. Dose-banding was introduced into UK clinical practice in 2001, as it reduces delays in patients receiving their treatment and, through quality control and end-product testing, safeguards the infusion quality and patient safety.

A range of studies were included in this thesis: an extended stability study on oxaliplatin infusions using a sequential temperature cycling design; studies on oxalate, a potential degradation product and metabolite of oxaliplatin which has been linked to oxaliplatin neurotoxicity and the development of an *ex vivo* pharmacokinetic (PK) simulation model to compare the effect of different oxaliplatin dosing methods on its therapeutic outcomes.

The shelf-life of oxaliplatin infusions over a concentration range of 0.2 mg/mL – 0.7 mg/mL is extended to 84 days when stored at 2 – 8℃ plus a further 7 days after being left at room temperature (25℃) for 24 hours. This ensures the unused oxaliplatin infusions are safe to be re-issued to patients, which could reduce drug wastage. The oxalate study suggests that the dose-limiting neurotoxicity of oxaliplatin is unlikely to be directly related to the oxalate produced from oxaliplatin degradation in infusions or from the non-enzymic transformation of oxaliplatin *in vivo* because the oxalate levels from these routes are minor compared to the endogenous level. The safety and efficacy of

dose-banding schemes was demonstrated by comparing the simulated PK characteristics gained from the *ex vivo* model. Dose-banding with the ±10% maximum deviation was selected as the most promising dosing scheme for oxaliplatin.

Finally, recommendations are made concerning the introduction of oxaliplatin dose-banding scheme into clinical practice, and on the benefits of harmonised dose-banding schemes.

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Chapter 1: Literature review and general introduction to this thesis

1.1 General introduction

1.1.1 Background

Oxaliplatin is a relatively new cytotoxic drug from the platinum group, which was approved in 1999 for clinical use in European countries and in 2002 for use in the United States. (1) Compared with other drugs in platinum group, such as cisplatin and carboplatin, oxaliplatin is more active against a wider range of tumours, especially the cisplatin-sensitive and cisplatin-/carboplatin-resistant cancers, such as colon cancer. (2) (3) Furthermore, oxaliplatin has a favourable toxicity profile with less side-effects to patients during the treatment. (4) The dose-limiting side effect of oxaliplatin is neurotoxicity, which occurs in around 48% of patients studied receiving oxaliplatin treatment with 5-FU/LV (5-fluorouracil and leucovorin). (5) Oxaliplatin is currently used in chemotherapies for metastatic colorectal cancers as the first-line treatment with the combination of 5-fluorouracil (5-FU) and leucovorin (LV). (6) Oxaliplatin is also used in a variety of adjuvant treatments for other cancers without licencing approval, such as advanced breast cancer, non-small cell lung cancer, ovarian cancer, hepatocellular carcinoma and non-Hodgkin's lymphoma. (7) Due to its broad panel of anti-tumour activity, oxaliplatin certainly has significant potential in treatments for different diseases in clinical practice. (4)

At present, oxaliplatin is normally administered by the individualized dosing method, with which the drug dose is calculated individually for each patient based on their BSA (Body Surface Area) and the recommended oxaliplatin dose (usually 85 mg/m^2) from the SPC (Summary of Product Characteristics). (5) However, the individual preparation of oxaliplatin infusions with timely provision has increased the demand on pharmacy cytotoxic compounding services, which

brings a challenge for the hospital pharmacy aseptic unit. (8) (9) Problems associated with the individual dosing method include: increasing the workload and stress for pharmacy and nursing staff involved; greater risk of errors in the calculation of doses and volumes during the preparation of drug infusions; delays for patients awaiting treatment and disruption of outpatient scheduling. (8) (9) To improve the chemotherapy service for patients, the implementation of a dose-banding scheme could be considered as an alternative dosing method for oxaliplatin administration.

Dose-banding is a new approach for chemotherapy administration which was introduced by *R J Plumridge* and *G J Sewell* in 2001. (8) With dose-banding schemes, doses of the cytotoxic drug are divided into a series of dose bands and a standard dose is selected for each band (normally the middle point of the band). The prescribed dose for an individual patient is calculated from his/her BSA as usual, which is then fitted into one of these dose bands in the dose-banding scheme. Subsequently, the standard dose from that band will be administered to the patient. Without the need for preparing individualized dose for each patient before every treatment course, the application of standard doses in the dose-banding schemes allows the advanced preparation of drug infusions within the hospital or by outsourced manufacturers, which can also permit streamlined preparation and rigorous quality control. (8) (10) To ensure the pharmacological effectiveness and safety of the drug during the advanced preparation and storage time before administration to patients, long-term stability must be demonstrated for the cytotoxic drug infusion. (9) Furthermore, the pharmacokinetic (PK) behaviour of the drug in terms of the amount of drug available to the tissues, identified as the area under the drug plasma-

concentration versus time curve (AUC) should also be confirmed to ensure the therapeutic efficacy and an acceptable safety profile. (11)

Therefore, to develop a specific dose-banding scheme for oxaliplatin administration, more data is required in two aspects: 1) the extended physical and chemical stability period of oxaliplatin infusions; 2) confirmation of an acceptable therapeutic efficacy and toxicity using the pharmacokinetic profile of oxaliplatin as a surrogate for the clinical effect of implementing the dose-banding scheme. Thus, a stability study of oxaliplatin infusions and a PK simulation study with different dosing methods were both conducted and presented in this thesis. In addition, to improve the understanding of oxaliplatin side-effects, especially neurotoxicity, more studies on oxalate in human plasma were included since the oxalate has been anecdotally implicated in oxaliplatin-associated neurotoxicity. (12)

1.1.2 Studies and experiments

The organisation of this thesis is set out below:

Chapter 1 (Literature review): The relevant literature on cancer, chemotherapeutic drugs and oxaliplatin was reviewed in this chapter. In order to fully understand the development of oxaliplatin, different cancers and anticancer drugs were discussed and drugs from the three generations of the platinum group were compared. Recent research on oxaliplatin was reviewed in this chapter including studies on the mechanism of anti-tumour activity; pharmacokinetics/pharmacodynamics in preclinical and clinical studies; cancers which can be treated with oxaliplatin chemotherapies and the related therapeutic issues; comparisons of oxaliplatin to cisplatin and/or carboplatin; toxicities with oxaliplatin treatment.

Chapter 2: The extended stability study on oxaliplatin infusions in 5% glucose is reported in this chapter. The long-term stability data of oxaliplatin infusions is required to support the development of a specific dose-banding scheme for oxaliplatin administration in clinical practice. However, the SPC (Summary of Product Characteristics) from the manufacturer and previous stability studies on oxaliplatin infusions were limited by various conditions and issues. Therefore this study, which employed a novel sequential temperature cycling design, explored the long-tern stability of standard oxaliplatin infusions under both storage and in-use conditions. The development and validation of the required analytical methodology is also described in this chapter.

Chapter 3: A series of oxalate studies are described in this chapter. The peripheral neuropathy side-effects associated with oxaliplatin chemotherapy have been attributed to oxalate, which is both a potential degradation product from oxaliplatin breakdown in infusions, and also a “metabolite” which occurs non-enzymically in the blood following oxaliplatin administration. The purpose of these studies was to determine the concentrations of oxalate that can arise from long-term storage of oxaliplatin infusions (studied in Chapter 2), and the oxalate produced non-enzymically in blood after oxaliplatin administration to patients. Thus, oxaliplatin in human plasma was prepared and stored at 37℃ to simulate the body temperature. The concentration of oxalate was then measured using a validated assay. The amount of oxalate produced from oxaliplatin by both of these routes was then compared to the endogenous level of oxalate from dietary intake to establish if the oxaliplatin administration could produce a significant increase in oxalate levels.

Chapter 4: This chapter describes the development and application of an *ex vivo* simulation model on oxaliplatin pharmacokinetic (PK) studies. Previous

studies (13) on comparisons between individualised treatments and dose-banding have used key PK parameters (e.g. AUC, C_{max}) as a surrogate for clinical outcomes such as tumour response and toxicity, where small differences would be difficult to measure and very large numbers of patients would be required. In addition, the clinical PK studies are invasive (blood sampling) and inconvenience for patients, and so an *ex vivo* PK model was developed to simulate drug clearance and test the different dosing options. Five different dosing methods were compared with simulations on 10 assumed patients using this PK model. These studied dosing methods included: individual dosing, dose-banding scheme with 5% maximum deviation from prescribed dose, dose-banding scheme with 10% maximum deviation from prescribed dose, logarithmic dose-banding scheme and flat-fixed dosing. Although it was necessary to scale-down the *ex vivo* model for practical reasons, the model was able to replicate the technical challenges of infusion administration posed by different dosing schemes. Key pharmacokinetic parameters (AUC, C_{max} and T_{max}) were compared to establish if the dosing methods influenced the availability of oxaliplatin to the tissues, which would in turn influence both toxicity and therapeutic outcomes.

Chapter 5: This chapter presents concluding remarks and recommendations for further work.

1.1.3 Contributions to knowledge

The results from studies described in this thesis broadened the current knowledge of oxaliplatin and indicated the feasibility of dose-banding implementation for oxaliplatin administration in clinical practice.

The new extended shelf-life of oxaliplatin infusions (in the concentration of 0.2 mg/mL – 0.7 mg/mL) was demonstrated, which also enabled the unused oxaliplatin infusions to be returned to stock and re-issued later with safety. This extended stability study with the sequential temperature cycling design has also set a benchmark for stability studies required to support dose-banding application.

The oxalate study in Chapter 3 proved that the amount of oxalate in both oxaliplatin infusions and human plasma are relatively small and unlikely to cause any toxic effects on patients. This result enhanced the knowledge on potential contribution of oxalate to oxaliplatin-related neurotoxicity implied in previous literature, and also brought alternative hypotheses surrounding the role of oxalate in neurotoxicity for further research.

From simulation results with the *ex vivo* PK model described in Chapter 4, dose-banding schemes with 5% and 10% maximum deviation from the prescribed dose both demonstrated an acceptable inter-individual variability of PK characteristics. In addition, the mean values of AUC and C_{max} and their standard deviation (SD) for the dose-banding schemes were comparable to the individualized dosing. As a result, dose-banding schemes simulated in this study should be able to provide the acceptable and comparable therapeutic efficacy and toxicity for oxaliplatin administration in clinical practice. Among different dose-banding schemes, the 10% deviation scheme required less standard infusions was highly recommended for chemotherapy administration.

1.2 Literature review

1.2.1 Introduction to cancer

1.2.1.1 Cancer definition

There are approximately 50 trillion (50×10^{13}) cells in a human body which can make up bones, tissues and organs. (14) Cells can be differentiated and specialized to perform individual tasks in different groups, and a cell life-cycle includes growth, division, differentiation, senescence and apoptosis. Normally, cell proliferation can be controlled with cell process and only occur when required, which shows the fine balance between growth-promoting and growth-restraining systems. However, sometimes this balance can be disrupted and the regulation control of cell growth can be lost. These abnormal cells then have the potential to develop into cancer. (14)

Cancer is defined as a disease which is caused by the failure of the process controlling cell growth. There are over one hundred different kinds of cancer and almost all of these cancers have different tumour locations, types and treatments. (14) Even in the same tumour type, significant differences in tumour growth and response to chemotherapy may be observed. (15)

Compared with normal cells, tumour cells exhibit continued cell proliferation, loss of differentiation and failure to undergo apoptosis. (14) Generally, cancer starts with a single cell that has undergone mutations; it can not only increase the cell's growth but also result in the loss of normal controls of cell proliferation. The initial mutation would lead cells to form a genetically homogeneous clone. Additional mutations occur to encourage cell growth further and raise sub-clones in the tumour with different properties, which result in heterogeneous cells. (14) Tumours are classified into two main groups, benign and malignant

tumours. Benign tumours are relatively mild because the cells are growing within a well-defined capsule and usually maintain the original characteristics of cells with well differentiated functions. On the other hand, malignant tumours are life-threatening and always invade surrounding tissues and metastasize to different areas of the body. (14)

Tumour cells exhibit a number of features which distinguish them from normal cells: (14)

1) The tumour cells are not dependent on growth factors which are necessary in normal cells.
2) Normal cells need to be in contact with the cell surface in the extracellular environment which is not required in tumour cells.
3) The tumour cell growth is not restricted by the presence of other cells.
4) Tumour cells are less adhesive than normal cells.
5) Finally, tumour cell proliferation is not responsive to tissue density.

1.2.1.2 Cancer causes

Carcinogenesis is a multistep process which involves a series of mutations resulting in the increased cellular proliferative capacity, invasiveness and metastatic potential of the cell. (15) Normally it is inaccurate to consider single agent as the cause of cancer. (15) There are a number of different factors, called risk factors which affect the likelihood of cancer developing including host factors and exogenous agents. (16) Host factors include hormonal patterns and immunological capacities, which are more important in the development stage of cancer than in genetic influence. (16) Even if cancer is caused by mutations, this does not mean the disease is heritable. (16) It has been found that in most patients who have a so-called heritable cancer the carcinogenesis requires

particular environmental factors. (16) Although the role of genetics should not be overstated, genetics can still play an important role in the control of cancer, such as 1) identification of individuals who are at risk of a specific cancer to help build preventive or screening strategies for the individual or family; 2) identification of cancer subtype to tailor the treatment for targeting specific disease. (16)

Current knowledge of cancer and carcinogenesis indicates that environmental factors play a significant role in the development of most of the common cancers associated with fatality. (17) These external risk factors can be categorized into 3 main groups: physical agents (e.g. asbestos, ultraviolet light), chemical agents (e.g. tobacco) and biological agents (e.g. Aflatoxins). (18)

Generally, physical factors include ionizing and non-ionizing radiation as well as certain materials with significant physical properties such as asbestos. (16) Most common causes, for example tobacco and alcohol, are normally considered as chemical factors. Biological factors include various viruses, such as hepatitis B virus leading to liver cancer, human papilloma virus leading to cervical cancer. (16) In addition, there is increasing recognition of cancers potentially caused by lifestyle factors like diet, physical activity and alcohol consumption. (16)

Tobacco is as known the main cause of cancer-related death and the most common cause of lung cancer. (19) The risk of lung cancer is related to the dose of carcinogen (tobacco daily consumption), the duration of smoking and the intensity of exposure (smoking device used, such as cigarette, cigar, pipe, hookah, etc.). (18)

Heavy alcohol drinking is another main cause of cancers in different sites including the oral cavity, pharynx, larynx, oesophagus and liver, and probably breast and colorectal. (20) These cancers are linearly related to the mean daily alcohol consumption. (18)

Many carcinogenic factors are also associated with occupation, including exposure to asbestos. (18) Occupational exposure to asbestos under domestic circumstances leads to the risk of mesothelioma while the non-occupational exposure to asbestos may cause lung cancer. (18) (21) In addition to asbestos exposure, many other occupational factors often result in various cancers of lung, skin, urinary tract, nasal cavity and pleura. (21) The industries with evidence of carcinogenic risk include agriculture, construction, demolition, shipbuilding, shipbreaking, petroleum, metal and rubber. (16) (22)

Diet has an important role in the cause of cancer, and more than 30% of human cancers are considered to be related to diet and nutrition. (18) Obesity increases the risk of several common cancers such as colorectal and breast cancer. (23) Some studies show that certain substances contained in vegetables and fruits can protect against cancers; while it has also been indicated that the excess amount of some animal products could increase the risk of colorectal and probably breast cancer as well as other diseases. (23)

Furthermore cancers could also be caused by other factors, such as environmental pollution, medical interventions including drugs used and infections. (15)

1.2.1.3 Cancer classification

Tumours from one organ always show apparently different behaviour from those originating from another organ. (17) As a result, classifying cancers into

various types would help with diagnoses and treatment. Generally, cancers can be divided into two groups, benign cancer and malignant cancer on the basis of behavioural characteristic. (17) There are over 100 different types of cancers arising from different cells, among which the most common types are carcinomas, sarcomas, lymphomas and leukaemia. (15) For histological classification, those cancers are usually sub-classified by the types of organs or tissues that they originate from but not the ones they metastasize to. (24)

Carcinomas are the most common type of cancer which constitutes approximate 90% of cancers occurring in a year. (15) Carcinomas can originate from several different organs including skin; lining of the internal organs such as lungs, stomach and intestine; form glands such as breast and prostate. (15) Carcinomas can be further classified by the appearance of the cells they originate from, for example squamous carcinomas (arise from skin, lung, mouth, throat and oesophagus), adenocarcinomas (arise from breast, bowel, lower oesophagus, stomach and ovary), transitional cell carcinomas (arise from bladder) and small cell carcinomas (arise from lung). (24)

Sarcomas derive from the connective tissues and are rarely found in humans. (15) The cells of these supportive tissues are called “fibroblasts” and these tissues include bone, fat and muscle, which are also named as fibrous tissues, ligaments and tendons. (25) Carcinomas and sarcomas are both solid tumours that affect discrete organs. (26) Three main available treatment options for solid tumours are surgery, radiotherapy and chemotherapy. These modalities can be used either alone or as combinations depending on the disease. (26)

Lymphomas are malignant tumours of lymphoid tissues which spread via the lymph nodes. (27) Lymphomas originate from lymphocyte cells, a particular type of white blood cells, which metastasize throughout the body via the lymph

glands and blood. (24) According to the affected cell types, the lymphomas can be divided into two major groups called Hodgkin's disease (HD) and non-Hodgkin's lymphomas (NHLs). (24) HD always presents as a painless enlargement of lymphnodes which is often observed in the neck. (27) NHLs are a different malignancy group which change from indolent, slow growing tumours to an aggressive, rapidly fatal disease. The prognostic important "B symptoms" include fever, night sweats and weight loss and also malaise, itching or pain in enlarged nodes after drinking alcohol. With fatigue and weakness of the patient's body, "B symptoms" may appear with the NHLs. (24) The treatments of patients with HD and NHLs are different. In general, HD is sensitive to radiotherapy and chemotherapy. However, 50 – 60% NHLs has to be treated with combination chemotherapy. (27)

Leukaemia is not a common type of cancers, which arises from bone marrow cells that are responsible for making the white blood cells to build up the immune system. (24) With leukaemia, the number of abnormal white cells increases which would damage the patient body in two main ways: reduction of the normal function of white blood cells and restriction of the space for new normal white cells. (24) Unlike other cancers, Leukaemia can be originally defined as acute or chronic based on the condition of the patient. (28) For acute leukaemia, infection, anaemia and bleeding are common symptoms but life threatening. For chronic leukaemia, the main symptoms are not always specific, such as malaise, "B symptoms", abdominal discomfort and sometimes hepatomegaly. The treatment of leukaemia, which is generally based on combination chemotherapy, is still improving with more focused therapy and supportive care. Bone marrow transplantation is also increasingly used in the treatment for all kinds of leukaemia. (28)

1.2.1.4 Cancer diagnosis

Diagnosis of cancer requires both careful clinical assessment and detailed diagnostic investigations including endoscopy, histopathology, imaging, cytology and laboratory studies. (16) An early diagnosis can increase the possibility of cure in many kinds of cancers (but not all of them) and reduce the morbidity that results from both disease and treatment. (16)

Many symptoms may alert the patient or their physician to the possibility of cancer, but normally no specific symptoms could be found. (29) The cancer symptoms are various and depend on the site of tumour, the size of tumour, which tissue has been invaded and whether the tumour has metastasized to other parts of the body. (29) In addition to more severe symptoms (e.g. coughing up blood, chest pain and persistent profound diarrhoea), there may be other lighter symptoms such as slight shortness of breath, general feelings of fatigue and loss of appetite. (29) All these symptoms may have different causes unrelated to cancer, and therefore an expert diagnosis is necessary. (29)

The definitive way to diagnose cancer is to use the histological examination of a biopsy sample from the tissue suspected to contain cancer cells. (29) Different techniques are also required for correcting the cancer diagnosis, including aspiration biopsy, needle biopsy, incisional biopsy and excisional biopsy. (16) Other tests could also be undertaken to assist with the diagnosis, such as blood and urine tests, X-rays, CT (Computerized Tomography) scans, MRI (Magnetic Resonance Imager) scans and scanning of different tissues. (30)

Blood and urine tests can give the information about anaemia, bone marrow, liver and kidneys function, and can also estimate the cancer-specific biomarkers

(29) such as prostate specific antigen (PSA) levels, which can be elevated in both malignant and benign prostatic disease. (31)

Tumour markers are the molecules produced by tumours, which can characterize various cancers and be properly employed to identify them. (31) As a result, tumour biomarkers sometimes can be used to support the diagnosis of the specific cancers. However, some biomarkers are not sufficiently specific or sensitive enough to contribute to a definitive diagnosis. (25) For example, carcinoembryonic antigen (CEA) is a protein secreted by colon and rectal carcinomas as well as cancers of breast, lung and pancreas, which can be used as a biomarker for cancer diagnosis. However, the CEA is not only expressed on the surface of cancer cells, but also some embryonic cells types. (25) Therefore, the determination of CEA could not directly lead to the definite diagnosis of cancer. Furthermore, since the CEA can be only detected with advanced tumours, it is thereby not used frequently in early diagnostic testing of cancers. (25) There are some other examples of tumour biomarkers, such as human chorionic gonadotropin (HCG) produced by choriocarcinoma, adrenocorticotrophic hormone (ACTH) coming from pituitary gland by squamous cancer of lungs and alpha-fetoprotein (AFP). (31) All these biomarkers can be used to assist diagnosis and also to monitor the programme of treatment. (31) However, caution must be exercised as the elevated bio-marker levels may arise from non-neoplastic conditions. (31)

X-ray and computerised – tomography (CT) are imaging techniques which could give the visualization of any suspected tumour. (15) There are two different kinds of X- rays: plain X-rays and contrast X-rays. Compared with the plain X-ray, the contrast X-ray is more useful for cancer diagnosis since the tumour outline can be shown on the X-ray with an injection or a drink of the certain

substance. (29) Moreover, CT and MRI can provide more details about the structure of vital organs. (29) The only way to give a conclusive diagnosis of cancer and confirmation of malignancy is the histological examination of a biopsy sample obtained from the abnormal cells. (29) This requires the expertise of an experienced pathologist to confirm the diagnosis. (30) For leukaemia, blood samples and bone marrow biopsies are taken for the examination in place of the tissue samples biopsy used for solid tumour diagnosis. (15)

Some cancers can be diagnosed without biopsy. For example, abnormal cells in the sputum can be seen and tested for lung cancer; for the cancers related to intestines, a few drops of fluid gathered in the intestine cavity can be removed by needles and tested in the laboratory. (29) The diagnosis for breast cancer is based on laboratory assessment of Fine-Needle Aspiration (FNA) biopsy, which has been used since 1930's. (32) These tests can not only facilitate the diagnosis of cancers but also assist with the choice of various treatments. (30)

1.2.1.5 Cancer staging

Before selecting the treatment strategy, some key pieces of information on the extent of disease are required from the reported results in cancer patients, such as presenting sites, histological type and the anatomic extent of the disease. (33) This identification of cancer extension has been termed "cancer staging". (33) Staging is the fundamental assessment of tumour burden and its anatomical dissemination, and can be sub-divided by clinical, radiological and pathological staging. (34)

Staging at presentation is significant for both patients and clinicians who could use cancer staging systems to make an accurate prediction of prognosis and

plan the next treatment modalities. (34) Recently, the TNM classification system became the global benchmark for reporting the extent of malignant disease, and a main factor of prognosis for predicting the result of cancer treatment. (33) The TNM system was invented in 1940s by *Pierre Denoix* (35) and has evolved into a comprehensive and broadly applicable system, which is now generally accepted by most cancer research groups. (35)

In TNM system, malignancy can be described with three scales: T, the extent of the primary tumour; N, absence or presence and extent of regional lymph nodes metastases; M, absence or presence of distant metastases. (34) Following that, numerical values are added to indicate the extent of local and distant spread of tumour. (35)

The different meanings for symbols in this system are shown below: (34)

For T

Tx: primary tumour cannot be assessed;

T0: no evidence of primary tumour;

Tis: carcinoma in situ;

T1, T2, T3, and T4: increasing size and/or local extent of primary tumour.

For N

Nx: regional lymph node metastases;

N0: no regional lymph node metastases;

N1, N2, N3: increasing involvement of regional lymph nodes

For M

Mx: presence of distant metastases;

M0: no distant metastases;

M1: distant metastases.

The distant metastases mentioned above are different from the lymph node metastases. (34) Lymph node involvement is a direct extension of primary tumour into lymph nodes and the involvement of distant nodes is referred to as the “distant metastases which are other than regional”. (34)

There are also some other systems used for assessing the likelihood of recurrence of disease around the tissue part where the surgery was applied to. In the meantime, the high-risk tumour groups can be identified and treated more appropriately with the assistant of using these staging systems. (24)

For example, according to the TNM classification system, lung cancer can be staged based on an assessment of the presence of distant metastases and the condition of chest and mediastinum. (18) For colon cancer, ‘Dukes’ classification’ has been used successfully as an indicator of prognosis. (17) Cancers of major organ systems including bowel, however, mostly use more than one staging classification. (17) The ‘Jass classification’ is used as one of the supportive systems of staging rectal carcinoma. (17) For different cancers, the same feature in clinical outcomes could be inspected with a broadly looking by using combined systems. (17)

1.2.1.6 Treatment options of cancer

The basic principles of cancer treatment are similar all over the world, while the management depends on local patterns such as the commonest cancer types and the relative proportions presenting at early or late stages of cancer. (16) These proportions come from not only the prevailing circumstances but also the results of early detection and screening. (16) The successful treatment for

neoplastic diseases is to inflict the maximum damage on the tumour stem cells and the minimum toxicity on normal tissue cells. (17) In different countries, however, the specific treatments are adopted based on the availability of human, physical and financial resources, as well as political considerations. (16) Even though there are some differences in treatment principles between individual countries, the primary goals of cancer treatment are the same, which are aimed at cure, life prolongation and improvement of quality of life. (16) Generally, the cure rate for many types of cancers is relatively low, and if the cancer cannot be cured the paramount goal is to maintain the highest possible quality of life. (18) It means curative and palliative treatment is not mutually exclusive but supportive to each other. (16)

The principle methods of cancer treatment are surgery, radiotherapy and chemotherapy, which also include hormonal manipulation and psychosocial support. (16) These therapies can be used alone or in combinations (18), and the main management of cancer treatment is usually the surgical resection assisted with systemic chemotherapy or localized irradiation. (17) The effectiveness of all cancer treatments varies greatly and depends on the site of disease and a number of social factors. (16)

Surgery is based on physical removal of the tumour mass while radiotherapy and chemotherapy expose tumour to toxic ionizing radiation and cytotoxic chemicals, respectively, to destroy cancer cells without isolating and removing them. (31) The practical experience suggests that the multidisciplinary management model is more effective than the sequential independent modalities to patients with various cancers. (16) For example, the combination therapy of surgery and radiotherapy is suitable for both local and regional tumours with curative effect in early stages. (16)

1.2.1.6.1 Surgery

Surgery is the first line attack for most cancers (15) and also the most effective modality for local diseases. (31) It contributes essentially to diagnosis, staging and treatment of local tumours through removal of tumour masses, which could also provide palliation and reduction of some complications. (16)

The role of surgery in the cancer management depends on whether the tumour is benign or malignant. Benign tumours could normally be cured completely by the surgical therapy. (15) However, there are some life-threatening benign tumours, which are either inoperable (mainly because of the difficult anatomical location) or untreatable (because of infection or compression of vital structures when they increase in size). (15) For malignant tumours, the success of surgery mainly depends on the complete removal of tumour cells, because any remaining tumour cells will remain viable and are capable for metastasis. (15)

Surgery can cure the early-stage tumours which have not invaded normal tissues. (15) Once the invasion starts, it becomes difficult to guarantee that the entire tumour cell population can be removed completely. (15) Over 50% of cancers have metastasized already by the time they are diagnosed. (15) As a result, it requires not only surgery but also radiotherapy and/or chemotherapy to build up a combined therapeutic modality. (15) Even in a combined therapy model, the removal of specimens by surgery is important to: 1) determine the extent of tumour progress and designing the further treatment; 2) sometimes eliminate the isolated metastatic tumours; 3) reduce pain and disease symptoms in advanced cases. (15)

Some cancers can be cured by the surgery alone, such as cancers of oesophagus, lung, liver and stomach. On the other hand, for some cancers

surgery could only influence the course of treatment, for examples, the early stage solid tumours (e.g. Dukes A or T1 colon tumours), early prostate, breast cancer and oral cavity cancers, and early skin tumours. (16)

However, the ‘un-resectionable tumour’ cannot be separated by surgery, which are either from the inaccessible anatomic sites or the extensive intermingling of tumour and critical normal tissue. (31) The need for anaesthesia, loss of haemostasis and infection are the general risks in the surgery. (31) Another serious issue of surgery found in the clinical experience is that any remaining cancer cells are provoked to spread to other tissues faster than before. (31)

1.2.1.6.2 Radiation therapy

As one of the main treatments for localized tumours, radiotherapy can increase mutations by damaging DNA and sometimes kill cells completely to create more extensive damage. (15) The damage caused by radiation is based on disruption of genetic material and is lethal to most cells which are rapidly dividing including both cancer cells and normal cells. (15) The proliferating cells are very sensitive to radiation and the cells that are capable for proliferation but not actively dividing at the exposure time can still be destroyed by radiation with higher doses. (31) There are several different types of radiation, such as X-rays, radiation from the decay of radioactive elements (cobalt) and particles or electrons beams produced by linear accelerators. (15)

Generally, radical radiotherapy can influence the cure of cancers of head, neck, cervix and prostate and early stage Hodgkin’s disease (HD) as well as various un-resectable brain tumours in young people. (16) Sometimes the radiotherapy is a preferred alternate to surgery for curative treatment in localized tumours, but in other cases radiotherapy is more suitable in the treatment for tumours

with special locations where the operation is not possible. (15) For example, in laryngeal cancer, radiation is usually used for the treatment instead of surgery since it does not affect the ability of speech while the surgery may damage the vocal cords and cause the loss of speech. (15) In addition, skin cancers at sites like the eyelid or nose tip are often treated by radiation. (15) In clinical practice, cancer treatment is based on the combinations of surgery and radiation, surgery and chemotherapy and sometimes chemotherapy and radiation. Radiation is used to kill remaining cells around the surgical sites when the combination treatment is chosen for certain cancers. (15) For instance, the combination treatment of surgery and radiation is usually used in the early stage of breast cancer. During the treatment, surgery is used to remove only primary tumour and affected lymph nodes while radiation is used to destroy the remaining tumour cells at the surgical margin. (15) Furthermore, for some cancers occurred in certain tissues that are sensitive to radiation, such as testicular cancers, the treatment is limited to surgery combined with radiotherapy in regional lymph nodes. (15)

Meanwhile, radiotherapy in the palliative treatment is of great value in life-threatening situations such as profuse bleeding from tumours or superior vena cava syndrome. (16) It is also valuable in cases of secondary pain under bone metastasis and bleeding, and of compressive syndromes caused by tumours including spinal cord compression and cerebral metastatic disease. (16)

Two main methods used for radiotherapy are tele-therapy and brachytherapy, which provide the quality assurance to meet the requirements of imaging and medical physics services. (16) For tele-therapy, cobalt machines and accelerators are used. For brachytherapy, the choice of equipment depends on the dose rate, and normally caesium is used under low dose rate (LDR) and

iridium or cobalt is used in high dose rate (HDR). (16) With all these equipment, the radiotherapy staffs are required to work in accordance with a registerable qualification and clearly defined protocols. (16)

There are, however, still a number of side effects and limitations with radiotherapy resulting from the high sensitivity of rapidly dividing normal cells. (31) The blood-forming cells in the bone marrow, the cells that line the intestine, skin cells, the hair forming cells and the cells of reproductive organs are included. (15) Damage of these normal cells causes various side effects such as anaemia, nausea, vomiting, mucositis, diarrhoea, skin damage, hair loss, infection and haemorrhage as well as sterility. (31) The extent of the side effects from radiotherapy relates to the amount of radiation delivered and the area of body that is irradiated. (15) Therefore, the dose of radiotherapy must be limited, which can also facilitate the control of the chronic side effects in non-proliferative tissues and/or in slowly renewing tissues like liver and kidney. (31) Dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) can be referred to limit the radiation exposure in the dose-limiting tissue to ensure that patients receive the safe amount of radiation without life-threatening toxicity. (31)

1.2.1.6.3 Chemotherapy

The surgery and/or radiotherapy could be used effectively in treatments for localized cancers, but are usually limited by the metastasis of cancer cells to other distant sites of the body. (15) These metastatic growths are too small to be detected at first diagnosis, which means a systematic treatment is necessary to kill cancer cells throughout the body. (15) As a result, chemotherapy is required in the effective cancer treatments. Chemotherapy can lead to cure of some disseminated diseases, such as Hodgkin’s disease (HD) and high grade non-Hodgkin lymphomas including Burkitt lymphomas, germ cell tumours,

leukaemia and small cell lung cancer in the limited stage. (16) In addition, chemotherapy is an important part of palliative treatment of cancer and can also assist in the control of symptoms in some cancers such as metastatic breast cancer, prostate cancer and low-grade non-Hodgkin disease. (16) For instance, anti-androgens and anti-oestrogens do not kill cancer cells but occupy the respective cytosolic receptors and block the action of trophic hormones. (17) The primary goal of chemotherapy is to kill cancer cells while conserving normal tissues and consequently achieve a balance between cancer cell death and normal cell survival in the cancer treatment. (15)

The target of chemotherapy, like radiotherapy, is rapidly proliferating cells in tissues, which means chemotherapy is another type of cytoreductive therapy. (31) Most anticancer drugs increase apoptosis rather than cell necrosis, and on scale this is the most significant cell death mode in the antineoplastic therapy. (17) Chemotherapeutic drugs are generally classified according to the different mechanisms of their anticancer activity. (30) Three of the main anticancer mechanisms are inhibition DNA synthesis, induction DNA damage and blocking cell division. (15) The principle of antimetabolites is interfering with one or more steps during the synthesis of DNA by incorporating a false substrate into the DNA as the biological base materials in an active tumour cell. (30) In this way, DNA cannot replicate in tumour cells which leads to the block of cell division and/or the death of dividing cells. (15) The antimetabolites include methotrexate, 5-fluorouracil, cytosine arabinoside, mercaptopurine, thioguanine and hydroxyurea. (15) By chemically reacting with DNA molecules directly these agents can cause several types of damage in the tumour cell and block further DNA replication. (15) However, some chemotherapeutic agents which can directly damage the DNA may sometimes encourage the development of

secondary cancers, particularly leukaemia. (15) Alkylating agents are a major group of DNA-damaging drugs, including cyclophosphamide, ifosphamide, melphalan, bischloroethylnitrosourea (BCNU), cyclohexyl chloroethyl nitrosourea (CCNU), thiotepa, chlorambucil and procarbazine. (15) Other drugs that damage the DNA molecules in different ways include bleomycin, cisplatin, mitomycin C, daunomycin, etoposide (VP-16) and teniposide. (15) In addition to having direct effects on DNA, there are also some other ways to inhibit cellular processes in tumour cells. For example, actinomycin D can interfere with cell division by blocking gene expression rather than replication. (15) Paclitaxel, vincristine and vinblastine usually inhibit the movement of chromosomes. (15) Furthermore, in acute leukaemia, leukemic cells cannot synthesize their own asparagine and have to depend on endogenous supply; the enzyme asparaginase can block the growth of leukaemia cells by degrading the amino acid asparagine in the blood. (15)

Chemotherapeutic agents can also be classified according to whether they are cell cycle-specific or cell cycle phase-specific. (17) Cell cycle phase-specific drugs include antimetabolites and antimitotic drugs, and cell cycle-specific drugs include anti-tumour antibiotics, alkylating agents and platinum-related compounds. (17)

However, chemotherapeutic drugs are not specific for cancer cells, which eradicate dividing cells in renewing tissues including both tumour and normal tissues. (31) The toxicity and side effects of chemotherapy depends on the relative sensitivities of cancer and normal cells. (15) Several common side effects (e.g. nausea and vomiting) could occur during the chemotherapy, but only a small proportion of chemotherapeutic drugs can cause really severe adverse effects. (30) Some widely used chemotherapeutic agents can cause

hair loss while the hair can grow out again after stopping the treatment. (30) The bone marrow suppression (myelosuppression) may cause various adverse effects, such as anaemia, various infections resulted from the lower white cell count and bleeding tendency caused by the loss of the platelet cells. (30) The vincristine and vinblastine drug group can cause peripheral neuropathy giving pins-and-needles or numbness symptoms by affecting the nerves in hands and feet. (30) Cisplatin can damage the kidneys and cause deafness. (30) Although chemotherapy is associated with significant adverse effects, it is the only modality which can potentially treat all kind of malignant metastatic cancer including micro-metastatic disease with unknown locations of tumour. (31)

A major obstacle to the ultimate success of cancer chemotherapy is the ability of malignant cells developing drug resistance. (17) After the chemotherapy, some tumour cells may remain and develop new tumour tissues which would be resistant to the drug that the tumour is exposed to. (15) A number of different kinds of mutation can confer resistance of tumour cells to chemotherapeutic drugs. (15) These cells sometimes are resistant to a single agent, like the DHFR gene to methotrexate, but in other cases the tumour cell can become resistant to multiple drugs. (17) Single-drug resistance is often attributed to the development of alternate bio-synthetic pathway in the cell, and to alternation or over-expression of the drug targets. (17) Multi-drug resistance is related to the over-expression of a membrane-bound energy-dependent drug efflux pump. (17)

However there are some solutions for the drug resistance problem and the use of combinations of chemotherapy agents in the treatment is one of them. The administration that combines several different chemotherapeutic agents can reduce the possibility of tumour resistance, as it is generally unlikely that the

tumour cells can be resistant to all of the used drugs. (15) To avoid multi-drug resistance, a spectrum of drugs should be selected, ideally without overlapping side-effects. (15) For practical administration, the combination chemotherapy is superior to a single agent, and its schedule usually aims at using drugs from different categories to gain the maximum tumour cell killing and the minimum side effects. (30)

The clinical use of chemotherapy also requires highly trained physicians, expensive drugs, close monitoring of laboratory tests and skilful nursing support. (16)

1.2.2 Anticancer drugs

1.2.2.1 Alkylating agents

Alkylating agents are one of the earliest classes of chemotherapeutic drugs, which were introduced into clinical practice since 1940s. (36) The first attention for alkylating agents came from mustard gas, a chemical weapon used in the First World War. (36) Nitrogen mustard was then introduced into clinical use as the first alkylating agent after several studies of the mustard gas, which started the modern evolution of cancer chemotherapy. (37)

Alkylating compounds contain alkyl groups (C_nH_{2n+1}) and are capable of forming covalent linkages with other molecules. (36) The general formula of an alkylating reaction (alkylation) can be expressed as $R\text{-}CH_2\text{-}X + Y = R\text{-}CH_2\text{-}Y + X$. (36) Moreover, the alkylating reaction can be divided into two types due to different agents, which are those that can directly react with biological molecules and other agents forming into the reactive intermediate first. These two types are named S_N1 and S_N2 (S_N denotes 'Substitution Nucleophilic'), and their reaction formulas are illustrated in *Figure 1-1* below. (38)

$$RX \rightarrow R^+ + X^- \overset{Y^-}{\rightarrow} RY + X^-$$
$$S_N\,1\;Reaction$$

$$RX + \; Y^- \rightarrow [Y \cdots R \cdots X]^{\delta^- \quad \delta^-} \rightarrow RY + X^-$$
$$S_N\,2\;Reaction$$

Figure 1-1, S_N1 and S_N2 reactions of alkylating agents through different routes.

For the therapeutic alkylating agents, five major structural classes are considered as common agents: nitrogen mustards, aziridines, alkyl sulphonates, nitrosoureas and the mechanistically distinct platinum-containing drugs. (39)

There are two main anticancer mechanisms for the alkylating agents reacting with biological molecules *in vivo* at physiological temperature and pH. (36) One of them is combining with biologically active molecules by covalent bonds, such as nucleic acid, proteins, amino acid and nucleotides. These alkylating agents also have the potential of damaging cell membranes, depleting amino acid stores and inactivating enzymes which are necessary for supporting the protein synthesis and the construction of new DNA strands. (36) However, the most important action of alkylating agents to damage the DNA of tumour cells is to form a cross-linkage between DNA chains. (36) Alkylating agents are divided into two different groups according to the number of alkyl groups in the drug, which are mono-functional and bi-functional. (40) The most effective drugs used in cancer treatment are the bi-functional alkylating agents, which have two alkyl groups to form covalent bonds at two nucleophilic sites, usually the N-7 and sometimes O-6 position on the purine base guanine. (36) It means that these alkyl groups can bind to a guanine base on the two opposite strands (inter-strand) and/or on the same strand (intra-strand). (41) For mono-functional alkylating agents with only one alkyl group, one site of guanine bases can be alkylated. Guanine bases with the alkyl bridge are prevented from acting as

templates for the formation of new DNAs. (39) *Figure 1-2* below shows different alkylating situations resulted from the various reactions between alkylating agents and the DNA. (39) Adducts generated from these reactions inhibit DNA replication and cell division.

A number of drugs from different alkylating agent groups are currently used in cancer chemotherapy. From the nitrogen mustard group there are mechlorethamine, chlorambucil, melphalan, cyclophosphamide and ifosfamide. From aziridines group, there are triethylenemelamine, thio-tepa, mitomycin C and altretamine. For alkyl sulphonates which have reactive methyl sulfonate groups, busulphan is the most common agent. From nitrosoureas group, 2-chloroethylnitrosoures (CENUs), carmustine (BCNU), lomustine (CCNU), methyl-CCNU (semustine) and chlorozotocin are used in the clinic. (39) Platinum-based agents will be discussed in following chapters.

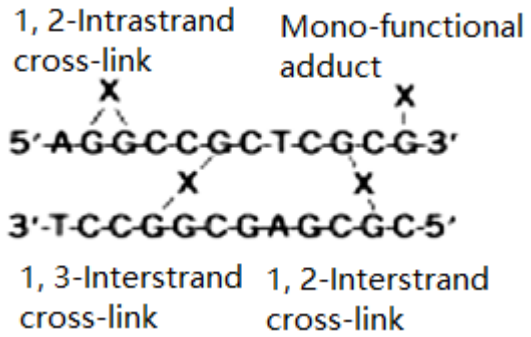

Figure 1-2, Mono-functional adduct, 1, 2- and 1, 3- inter-strand, and 1, 2- intra-strand cross-links induced by the DNA interactive agents. X = alkylating agent. (41)
[Permission to reproduce this figure has been granted by John Wiley and Sons.]

Cyclophosphamide (CP), for instance, is one of the most valuable nitrogen mustards, which is widely used in treatments of various kinds of cancer. (36) The molecular formula of cyclophosphamide is $C_7H_{15}C_{12}N_2O_2P{\cdot}H_2O$ with the molecule weight of 279.1. The chemical name of cyclophosphamide (CP) is known as 2-[bis(2-chloroethyl) amino] tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate and the structure formula is shown below (*Figure 1-3*).

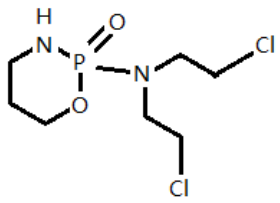

Figure 1-3, The chemical structure formula of cyclophosphamide (CP).

Cyclophosphamide is an inactive pro-drug with high stability, which needs to be activated into 4-hydroxycyclophosphamide by the hepatic mixed function oxidase system. (39) (42) This metabolite is most significant for the function of cyclophosphamide since its degradation process forms into effective nor-nitrogen mustard after distributing throughout the body. (39) Following the activation of cyclophosphamide, DNA alkylation is effected by reactive nitrogen mustard molecules. This mechanism is also representative of most drugs with chloroethyl groups as shown in *Figure 1-4*. (39)

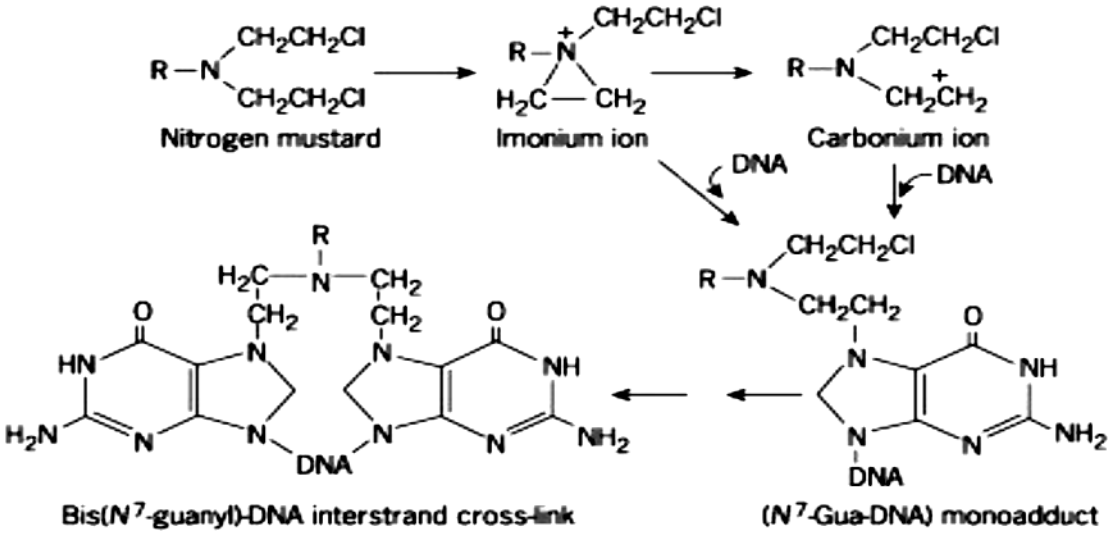

Figure 1-4, Mechanism of DNA alkylation by a reactive nor-nitrogen mustard molecule and formations of inter-strand cross-link and mono-functional adduct. (39) *[Permission to reproduce this figure has been granted by John Wiley and Sons.]*

Cyclophosphamide has been incorporated into the treatment of most paediatric and adult malignancies, such as sarcoma, leukaemia, lymphomas and breast cancer. (43) Cyclophosphamide has also played a major role in the improvement of treatment to bone and soft tissue sarcoma both in children and adults. (44) In addition, cyclophosphamide is used in patients with responsive

chronic lymphocytic leukaemia (CLL) in the combination with fludarabine and rituximab (known as FCR). (45) (46) For lymphoma, the combination chemotherapy regimen of cyclophosphamide, doxorubin, vincristine and prednisone (CHOP) has been used as a standard treatment for a long time. (47) The role of cyclophosphamide in the treatment of breast cancer is complex. On one hand, cyclophosphamide is commonly used with concurrent docetaxel and doxorubicin as the neoadjuvant chemotherapy for patients who have locally advanced breast cancer. (48) On the other hand, metronomic low-dose cyclophosphamide can improve the clinical outcome in advanced-stage breast cancer patients, as a result of the induction of stable tumour-specific T-cell responses. (49)

Cyclophosphamide has a number of adverse effects including cardio-toxicity, nephrotoxicity, neurotoxicity, infertility, bladder toxicity, myelosuppression and leukaemogenesis. (43) To prevent serious bladder toxicities, the use of mesna (2-mercaptoethanesulfonic acid) has been considered and frequently recommended from textbooks and clinical guidelines. Mesna works by forming a complex with acrolein which is the toxic metabolite of cyclophosphamide. (50) But there is no direct evidence for its effectiveness in preventing bladder cancer in humans. Compared with other drugs in the nitrogen mustard group, cyclophosphamide has less risk of gastrointestinal toxicity, thrombocytopenia and alopecia which mostly depends on the dosage of cyclophosphamide. (36)

Not all alkylating agents are used frequently in clinical practice in the UK, but bendamustine, as an alkylating drug, is unique with both alkylating and antimetabolite properties. (51) Bendamustine, 4-{5-[bis (2-chloroethyl) amino]-1-methyl-2-benzimidazolyl} butyric acid hydrochloride, is a bifunctional alkylating agent and the chemical structure is shown in *Figure 1-5*. (52) As one of the

licensed approval nitrogen mustard, bendamustine is also known as Treanda® and Ribomustin®. (53) Its empirical molecular formula is C16H21Cl2N3O2· HCl and its molecular weight is 394.7. (52)

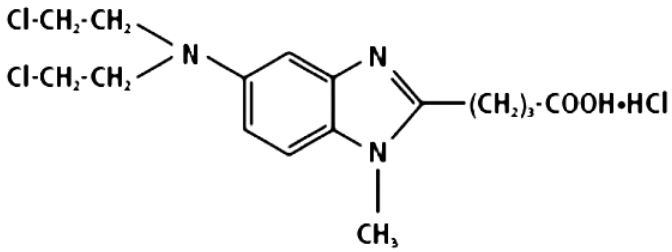

Figure 1-5, Chemical structure formula of bendamustine with HCl.

The anticancer activity of bendamustine is structurally related to three elements: 2-chloroethylamine alkylating group, benzimidazole ring and butyric acid side chain. (54) The action mechanism of bendamustine is similar to the other nitrogen mustard agents which is using the 2-chloroethylamine alkylating group to react with N7-Gua or O6-Gua in the DNA in order to crosslink DNA strands and subsequently break them. (39) However, bandamustine appears to provide more extensive and durable DNA strand breaks compared with other 2-chlorothylamine alkylates. (51) Bendamustine has been proven with the distinctive preclinical activity in cell lines, and also shows the clinical activity in patients with cancers that are resistant to alkylating agents. (55) The clinical indications of bandamustine include a range of diseases, such as non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, multiple myeloma, breast cancer and small-cell lung cancer. (54)

1.2.2.2 Antimetabolites

Antimetabolite chemotherapeutic agents have a long history in cancer treatment for almost 50 years and are continued using today. (56) (57) As the second family of cytotoxic drugs to be discovered, antimetabolites could interfere some

essential metabolic processes by using their structurally similarity to the biochemical materials within tumour cells. (36) (58)

The general mechanism of antimetabolite drug activity is related to the metabolites which contribute to the formation of nucleic acid and protein needed for cell division. (36) There are two important ways that antimetabolites can prevent cell division by replacing the normal metabolites: incorporation into new nuclear material (inhibit purine and pyrimidine metabolism); combination with vital enzymes in an irreversible reaction. (36) (59) The major classes of antimetabolites are antifolates, cytidine analogues, pyrimidine analogues, adenosine analogues and purine analogues. (57) According to the Food and Drug Administration (FDA, US), there are 14 purine and pyrimidine antimetabolites in total which account for nearly 20% of all the drugs used for cancer treatment. (60) Methotrexate (MTX), N-(phosphonacetyl)-L aspartate (PALA), 5-fluorouracil (5-FU) and the new thymidylate synthesis (TS) inhibitions all work on key steps in the principal pathways of pyrimidine and also purine biosynthesis. (61) Another significant antimetabolite target is to bind with the dihydrofolate reductase (DHFR) which is a key enzyme in DNA synthesis. (56) Folate bio-synthetises pathways are shown in *Figure 1-6* below, according to which the inhibitors are aiming at the vital enzymes such as dihydrofolate reductase (DHFR), thymidylate synthase (TS) and glycinamide ribonucleotide formyltransferase (GARFT). (62) Dihydrofolate reductase (DHFR) is normally inactivated by methotrexate (MTX), which leads to the inhibition of tetrahydrofolates (FH_4) synthesis and arresting the formation of purine and pyrimidine. (36) On the other hand, thymidylate synthase (TS) can be blocked by 5-fluorouracil (5-FU) to prevent the formation of thymine and cytosine, and subsequently arrest the production of new DNAs. (36) Similar to 5-FU, another

more efficient drug, capecitabine (Xeloda), is developed as a pro-drug of 5-FU, which improves the drug tolerability and intra-tumour drug concentrations due to its conversion into 5-FU. (63)

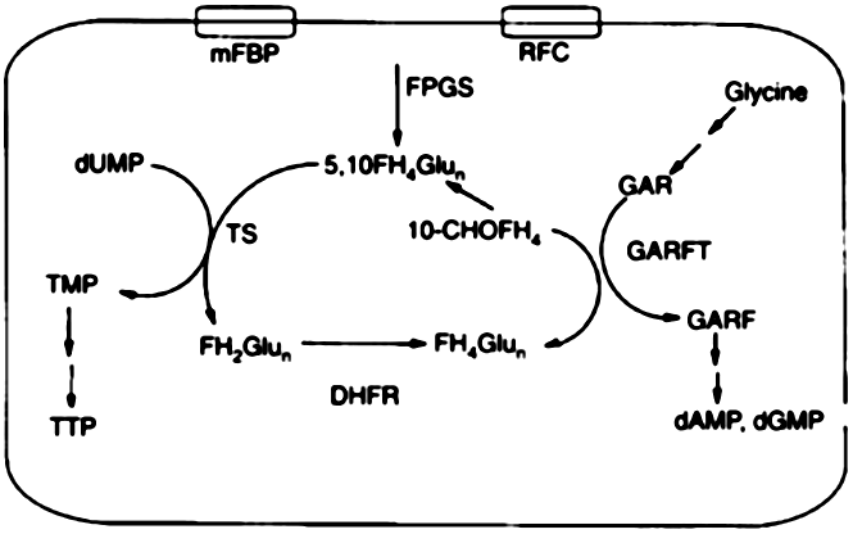

Figure 1-6, Folate bio-synthetises pathways and inhibitors. mFBP, membrane-associated folate binding protein; RFC, reduced folate carrier; FPGS, folylpolyglutamate synthase; dUMP, deoxyuridine monophosphate; FH_4, tetrahydrofolate; GAR, glycinamide ribonucleotide; TMP, thymidine monophosphate; FH_2, dihydrofolate; TS, thymidylate synthase; DHFR, dihydrofolate reductase; Glu_n, polyglutamate; dAMP, deoxyadenosine monophosphate; dGMP, deoxyguanosine monophosphate; TTP, thymidine triphosphate. (62) *[Permission to reproduce this figure has been granted by Nature Publishing Group.]*

Methotrexate (MTX) is a principal example for antimetabolites as a folic acid antagonist. (35) (57) The systematic name of MTX is 4-Amino-4-deoxy-10-met hylpteroyl-L-glutamic acid and the molecule formula is $C_{20}H_{22}N_8O_5$ with a molecule weight of 454.44. (64) MTX is a structural analogue of dihydrofolic acid (*Figure 1-7*) which can replace the dihydrofolate and react with the enzyme dihydrofolate reductase (DHFR) since that MTX has 100,000 times greater affinity for dihydrofolate reductase (DHFR) than dihydrofolate. (35) (61)

In simple terms, the mechanism of MTX starts from the inhibition of the conversion of dihydrofolate to tetrahydrofolate, and subsequently restricts the supply of deoxythymidine triphosphate and purine nucleotide. (57) (64) As a result, pyrimidine nucleotide synthesis and purine biosynthesis are inhibited,

and the formation of DNA, RNA and protein are interfered. (59) (61) In addition, MTX could also inhibits folate-dependent enzymes such as thymidylate synthesis (TS) and other enzymes involved in purine synthesis leading to the depletion of three nucleotide pools: guanine nucleotide triphosphate (GTP), adenine nucleotide triphosphate (ATP) and thymidine triphosphate (TTP). (57) (61)

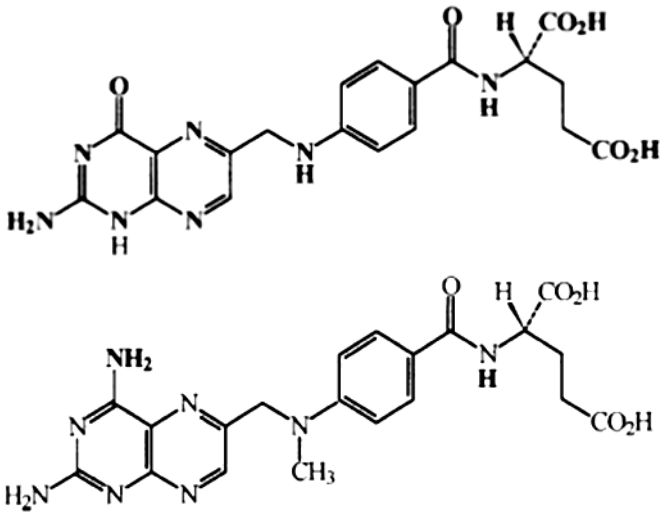

Figure 1-7, Chemical structure formulas of dihydrofolic acid (above) and methotrexate (bottom). (65) *[Permission to reproduce this figure has been granted by Elsevier.]*

Several purine-based and pyrimidine-based drugs exhibit anticancer activity against various solid tumours, leukaemia and lymphomas. (66) As one of them, MTX is used in the chemotherapy of different human malignancies, such as lymphoblastic leukaemia, lymphoma, breast cancer, head and neck cancer, and osteosarcoma. (59) For children with acute lymphoblastic leukaemia (ALL), in particular, MTX acts as a key agent in the treatment. The high-dose regimen contributes to the increased cure rate and improves patients' prognosis. (67) Even though the determinants of MTX in the treatment of ALL are not clear, MTX effects on prophylaxis of the central nervous system (CNS) damage involve the MTX in the intensification regimen and the maintenance therapy. (67) (68) High-dose methotrexate has been reported as the most active drug for

newly diagnosed primary CNS lymphoma. (69) MTX, as a single or combined modelling, expresses considerable activity in different kinds of lymphoma, and those drug combinations include MTX and irradiation (IR) for NK/T-cell lymphoma, high-dose MTX and temozdomide (HD-MTX-TMZ) for the primary central nervous system lymphoma (PCNSL). (70) (71) In addition, MTX is also used in the treatment of inflammatory diseases. (64) Recently, MTX has been used as an immunosuppressive agent in the organ transplantation and additionally in the treatment of some autoimmune diseases, such as rheumatoid arthritis (RA), and severe asthma. (64) Furthermore low MTX doses can be administered through oral or parental routes for the treatment of rheumatoid arthritis and psoriasis. (72) (73)

As usual, the balance between the clinical efficacy and side effects of MTX is important and has been the subject of continued research. (74) It can be discussed in two aspects: the low dose and the high dose of MTX. The low MTX dose used for benign disorders or continuation therapy of ALL and NHL rarely cause any significant and uncontrollable toxicity other than bone-marrow suppression and mild hepatotoxicity, which never leads to severe liver fibrosis. (74) For high-dose MTX (HD-MTX), more significant side-effects are observed, including reversible myelosuppression, mucositis, hepatotoxicity, and reduced kidney function, as well as both transient and persistent neurotoxicity. (74) With the long period use of MTX, several side effects can also occur during the treatment including mucosal, ulceration, stomatitis, bone marrow suppression, loss of appetite with drug induced hepatic fibrosis and cirrhosis. (73) Among all these toxicities, the intestinal damage or enterocolitis is one of the major toxic effects, which results in malabsorption syndrome and could disrupt the chemotherapy regimen in the meantime. (64) To reduce the adverse effects of

MTX, new approaches directing to the target cells (e.g. Leukocytes) have been evaluated. (64) (73) As an activated form of folic acid, leucovorin can terminate the metabolic pathway blocked by MTX to reverse most of its side-effects, apart from the nephrotoxicity. (74)

Another issue limiting the use of MTX in cancer chemotherapies is the drug resistance of tumour cells. (61) The mechanism of the MTX resistance is still not clearly known nowadays, but there are some hypothesises including cellular deletion of the folate transporter, loss of the ability of polyglutamate folates, and amplification of genes encoding for dihydrofolate reductase. (57) As a result, MTX is often used in combination treatments with other drugs to prevent the development of its resistance, which could modulate the expression of genes involved in MTX resistance. (75)

Another typical antimetabolite drug, 5-Fluorouracil (5-FU), is one of the first examples of the series of fluoropyrimidine. (36) (60) In addition, 5-FU is also the most important and simplest among these fluoropyrimidine compounds, which consists of a uracil molecule with a fluorine atom substituted for the number five hydrogen atom as shown in *Figure 1-8*. (36) (76)

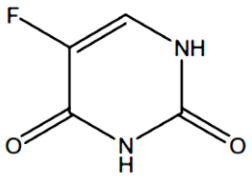

Figure 1-8, Chemical structure formula of 5-fluoroucil (5-FU).

The mechanism of 5-FU reaction is not fully acknowledged but it is believed to be related with the interference of DNA synthesis and mRNA translation. (36) (77) Division of tumour cells can be inhibited by 5-FU in two different ways: 1) blocking the enzyme thymidylate synthesis to arrest the production of new DNA;

2) incorporation into RNA in place of uracil or thymine to inhibit RNA synthesis. (36)

5-Fluorouracil has been used in daily clinical oncology practice for almost 50 years and continues to be widely used in the management of several common malignancies, such as cancers of the colon, breast and skin. (78) (79) However, treatments containing 5-FU are observed with several side effects as a result of its nonspecific cytotoxicity. (77) These include hematologic toxicity (leukopenia including neutropenic fever and anaemia, and thrombocytopenia), gastrointestinal toxicity (oral and intestinal mucositis, stomatitis, diarrhoea, nausea and vomiting), and less frequent dermatologic toxicity (hand-foot syndrome and hair loss). (80)

1.2.2.3 Antitumor antibiotics

Antitumor antibiotics are a group of important anti-cancer drugs which were originally derived from microorganisms by fermentation. (34) (81) Unlike other antibiotics used for infections, antitumor antibiotics have cytotoxicity which limits the value of antimicrobial agents but ensures its efficacy in malignant conditions. (82) These antibiotic agents can interfere with the proliferation of malignant cells through several different mechanisms. Most of antitumor antibiotics are cell-cycle non-specific while a few of them are cell-cycle specific, and some could be considered with both properties. (81) (83)

There is controversy concerning the mechanisms of the anticancer activity of antitumor antibiotics. (83) However, some evidences were found for the following modes:

Ⅰ. Intercalation: antitumor antibiotics can bind to the double helical structure of DNA molecule and cause strand breakage. (82) (83)

Ⅱ. Disrupting RNA synthesis: RNA synthesis can be inhibited by several different ways such as inhibiting the DNA-dependent RNA synthesis, directly inhibiting RNA synthesis, altering DNA to block transcription by forming tight adducts with it. (17) (34) (82) (83)

Ⅲ. Antimitotic effects: the free radical formation provided by antitumor antibiotics can disrupt the DNA chain and prevent mitosis. (81)

Ⅳ. Membrane binding: antibiotics agents alter the cellular membranes by increasing their permeability to various ions.

Ⅴ. Alkylation: some antibiotics are even demonstrated with their activity that resembles alkylating agents or antimetabolites to block DNA replication. (83)

Ⅵ. Others: some other models are considered to contribute to the antitumor effect, for instance metal ion chelating with antibiotics can produce cytotoxic compounds and then inhibit topoisomerase enzymes. (81)

Antitumor antibiotics can be classed into two groups: anthracyclines and non-anthracyclines, among which anthracyclines are more important while the other group are also of value in cancer treatments. (36) Several anthracycline agents are used widely in the treatment of haematological and solid tumours, such as daunorubicin, doxorubicin and epirubicin. (36) (84) The first antitumor anthracycline was daunorubicin isolated in 1963, and doxorubicin was introduced around the 1970s and then became into the best known and the most widely used anthraquinone anticancer agent for treatments of malignant cancers. (85) (86)

Doxorubicin is commonly prescribed as a small molecular chemotherapeutic drug for the treatment of an extensive range of solid tumours due to its broad

antitumor activity. (87) (88) (89) The molecular formula of doxorubicin is $C_{27}H_{29}NO_{11}$ and the chemical structure is shown in *Figure 1-9*, with the comparison to the structure of daunorubicin. (86)

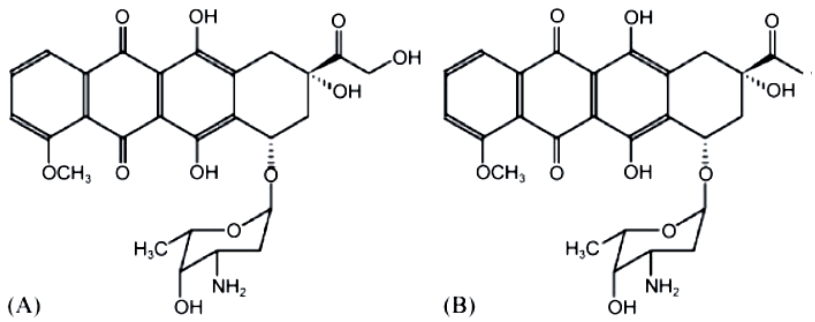

Figure 1-9, Chemical structure formulas of doxorubicin (A) and daunorubicin (B). *[Permission to reproduce this figure has been granted by John Wiley and Sons.]*

The mechanism of the anticancer action of doxorubicin is partly attributed to its complexation with DNA. (89) The data from the UV-resonance Raman spectra shows that doxorubicin can interact with the elements on positions of adenine and guanine. N7 positions of purines are the proton-acceptor sites of adenine and guanine rings, which could be connected with the portion of doxorubicin hydroxyl group via a hydrogen bond formation. (89) This interaction causes a partial deformation of the hydrogen bonds between the adenine and thymine as well as the cytosine and guanine bases as shown in *Figure 1-10*. (89)

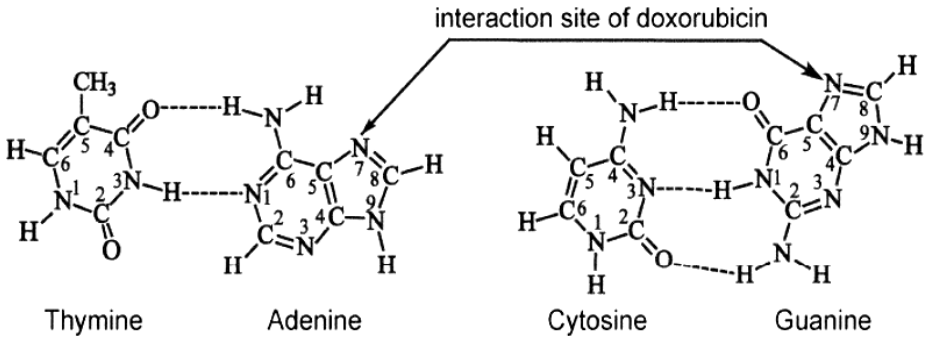

Figure 1-10, Diagram of the interaction site of doxorubicin on adenine and guanine rings of DNA. *[Permission to reproduce this figure has been granted by Korean Chemical Society.]*

With the most effective activity against cancers, doxorubicin can be prescribed for a broad range of solid tumours such as breast carcinoma, bronchogenic

carcinoma, gastric cancers and soft tissue sarcomas. (36) However, for both daunorubicin and doxorubicin, various side effects can occur with their treatments including severe myelosuppression, development of drug resistance and considerable nausea and vomiting. (36) (86)

Doxorubicin is associated with a dose-related cardio-toxicity which limits the use of doxorubicin in clinical chemotherapies. (84) (85) Cardiotoxicity, as a further adverse effect, is proved to be a fatal factor depending on if it is acute or, more commonly, chronic. (88) (90) The acute or late-stage cardiotoxicity may manifest itself as congestive heart failure (CHF), arrhythmias or conductivity dysfunction, associated with electrocardiographic (ECG) abnormalities, a pericarditis-myocarditis syndrome, and occasionally myocardial infarction and sudden death. (84) (85) (90) Because that the development of cardiomyopathy is related to the cumulative drug dose, the cardiotoxicity can be minimized by limiting the dosage of doxorubicin. (36) (88) In the doxorubicin treatments, the incidence of cardiotoxicity is 3% at 400 mg/m^2, 7% at 500 mg/m^2 and 18% at 700 mg/m^2. As a result, the total drug dose has been recommended to be better below 550 mg/m^2. (36) (84) As a small-molecule chemotherapeutic agent, doxorubicin is commonly limited by a lack of specificity in targeting, which could potentially cause those dose-limiting toxicities via the drug exposure to non-target tissues in patient's body. (91) Therefore the adverse effect of cardiotoxicity from doxorubicin could be caused by its release to the cardiovascular system when the drug is trying to accurately target the cancer sites *in vivo*. (91)

On the other hand, the major drawback of doxorubicin is the onset of resistance. (92) As an anthracycline, doxorubicin is a substrate for several ATP-binding cassette (ABC) membrane transporters including P-glycoprotein (Pgp),

multidrug-resistance related protein-1 and -2, and breast cancer resistance protein. (92) (93) As a result, doxorubicin is actively extruded from cells by over-expression these pumps, which reduces the intracellular retention of the drug. (92) To reduce the drug resistance, several inhibitors of Pgp (P-glycoprotein) have been designed and tested which turned out to have poor specificity and high toxicity in clinical trials. (92) In recent years, to improve the antitumor efficacy and ameliorate dose-related toxicity, various drug delivery technologies have been developed to increase the specificity of drug targeting including lipid-based (liposome), micelles and conjugated to a polylysine dendrimer. (85) (86) (91) Meanwhile, the nanoparticle-mediated drug delivery has been proven with effectiveness to overcome the MDR (multidrug resistance). (93) Liposomes can passively target the drug and reduce the drug toxicity to normal tissues by utilising the increased permeability of the tumour vascular system, which leads to the drug accumulation in tumour tissues. (86) Micelle-encapsulated doxorubicin (M-DOX) can improve the drug penetration and accumulation in tumour sites leading to a higher antitumor activity. (86) According to preclinical and clinical studies, the liposome-encapsulated doxorubicin formulation has also improved the pharmacokinetics distribution of doxorubicin especially the accumulation in tumour tissues and provided a greater antitumor efficacy than free doxorubicin. (85) (86)

Additionally, another way to improve the anticancer activity and reduce the toxicity of chemotherapeutic agents is to develop new analogues for cancer treatments. (36) For example, epirubicin, a stereoisomer of doxorubicin, is now commonly used in the treatment of breast cancer. (84) Epirubicin has less cardio-toxicity on an mg/m^2 unit basis than doxorubicin. However, epirubicin is

tended to be prescribed at 25-50% higher dosage than doxorubicin to achieve the similar antitumor benefit. (84)

1.2.2.4 Vinca alkaloids

Some plants have been found to have anticancer activity, which encouraged a great deal of effort to screen the suspect plants for potential cytotoxic drugs. Even though only few relative agents have clinical impact, a number of plant extracts have been screened for their cytotoxicity against mammalian cells. (17) Plant alkaloids are nitrogenous bases, and a majority of them have medicinal properties, for example, cocaine, morphine, quinine and atropine. (36) The group of plant alkaloids called vinca alkaloids have been isolated from the common periwinkle plant Vinca rosea for more than 60 years. (82) As one of the most useful cytotoxic agents, this vinca alkaloid group supports the traditional belief of the strong healing powers from the periwinkle. (82) In clinical practice, three vinca alkaloids have been demonstrated with good therapeutic effect in cancer treatments, named vincristine, vinblastin and vindesine. (24) (36)

Tubulin is the key point in the anticancer mechanism of vinca alkaloids which are lethal to mitotic cells. (24) In the mitotic process, tubulin is an intracellular protein that polymerises into microtubules. Microtubule has many functions such as the mitosis transport of solutes, cell movement and maintaining the structural integrity of the cell. (36) The most important function of the micro-tubular proteins is to form the cell mitotic spindle and to arrange the chromatid pairs during metaphase. (24) (82) The mechanisms of the anticancer activity of these three drugs are similar and all related to the binding of tubulin. The major antineoplastic effects of vinca alkaloids are exerted during the metaphase of the mitotic process starting with binding tightly to tubulin. (17) (82) By preventing the polymerisation of tubulin subunits into microtubules, the vinca alkaloids can

block the cell spindle formation. (24) (36) The mitosis is arrested in metaphase, and the death of cells ensues since they are unable to divide. (17) (82) For this reason, vinca alkaloids are known as ‘spindle poisons’. Other relevant properties of the antitumor effect of vinca alkaloids include inhibition of thymidine incorporated into DNA; inhibition of uridine incorporated into RNA; disrupting synthesis of both RNA and intracellular proteins at high doses. (17) (82)

Vincristine (VCR) is one of the most widely used chemotherapeutic agents in clinical practice, which has been known for its medicinal properties since the 17th century. (94) (95) It was first used in adults and children successfully as a cytotoxic agent in 1962 and then remains a key component of chemotherapy regimens for many paediatric cancers. (94) (95) The molecular formula of vincristine is $C_{46}H_{58}N_4O_{10}$, and the chemical structure is shown in *Figure 1-11* along with another analogue vinblastine (VLB). (96) (97)

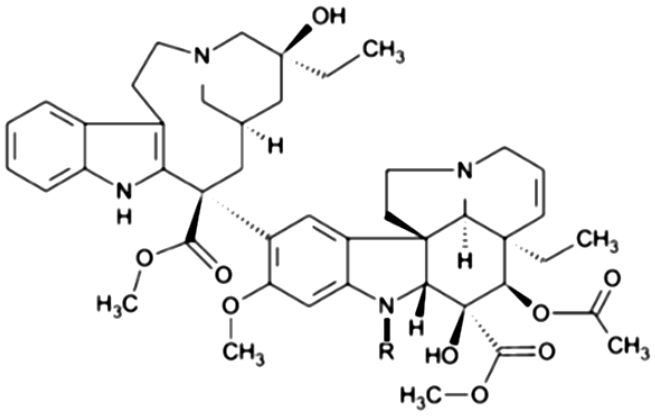

Figure 1-11, Chemical structure formulas of vincristine (R=CHO) and vinblastine (R=CH_3). *[Permission to reproduce this figure has been granted by Elsevier.]*

The mechanism of the action of vincristine is as described above about the interaction with tubulin which causes interference with microtubules and mitotic spindle dynamics, the disruption of intracellular transport, and thereby arresting cell division in metaphase. (94) (98) The tumour blood flow would reduce and

cell apoptosis would arise as the ultimate result from the vincristine-mediated cellular disruption. (94)

In medicinal folklore, vincristine has been described as being effective for the treatment of haemorrhage, scurvy, toothache, wound healing, diabetic ulcers and hyperglycaemia. (94) Vincristine is also extensively used in combinations with the other agents for treatments of a number of human carcinomas, such as acute lymphoblastic leukaemia (ALL), malignant lymphoma and solid tumours like breast cancer. (98) (99) (100)

The main dose-limiting side effect of vinca alkaloids is the dose-dependent cumulative peripheral neuropathy. (98) For different vinca alkaloids, the related neurotoxicity is qualitatively similar but quantitatively different following the order: vincristine > vindesine > vinblastine > vinorelbine. (99) In terms of neuropathy, the limitations of the therapeutic potential of vincristine are the lack of proven neuroprotective measures and the incomplete understanding of pharmacokinetics and pharmacogenetics mechanisms of the drug. (94) The neurotoxicity associated with vincristine is related to the drug dosage, frequency of drug administration and patients' age, but can be generally reversible with slow recovery. (94) (99) (101) Vincristine-induced neuropathy is normally mild but sometimes with severe consequences including an autonomic and peripheral sensory-motor polyneuropathy and, reported only rarely, the severe partial or total paralysis. (94) (99) The most common clinical symptoms of neurotoxicity, appearing in 2 to 19 weeks after the commencement of vincristine administration, include loss of deep tendon reflexes (may be associated with foot drop and gait disturbance), jaw pain, constipation and ileus. (94) (99) Some circumstances can enhance the vincristine neurotoxicity, such as: 1) if the recommended dose of 12 mg is exceeded; 2) if the patient is hypersensitive to

the drug; 3) if there is a pre-existing liver dysfunction or a hereditary neuropathy; 4) if other drugs are concomitantly used, such as allopurinol, erythromycin, isoniazid, mitomycin C, phenytoin and itraconazole. (99) (100) These drugs can prolong the exposure to VCR through inhibiting the enzymes of cytochrome P-450 in the liver or blocking P-glycoprotein pumps and interfere with the metabolism of vincristine. (102)

Thereby, the neurotoxicity of vincristine can be increased by drug interactions when the drug is applied in a combination treatment course. (102) A careful neurological examination and detailed family history record should be accomplished before initiating the vincristine treatment. It is necessary to keep vincristine neuropathy as a differential diagnosis in patients with ALL (Acute Lymphoblastic Leukaemia) who may develop neurological symptoms. (99)

Other non-neural side effects of vinca alkaloids are markedly less common, including alopecia and myelosuppression. (94)

1.2.3 Platinum drugs

1.2.3.1 Development

Platinum-based compounds have been used worldwide in treatments of different types of cancer since cisplatin was introduced into clinical use in the 1970s. (103) (104) As the first platinum drug used for cancer patients, cisplatin (*Figure 1-12*) is one of the most active agents in chemotherapies for treatments of a variety of malignancies, such as head and neck, bladder, cervical and lymphomas cancers, especially testicular and ovarian carcinoma. (103) (104) (105) However, the clinical utility of cisplatin has been restricted by two major limitations: severe toxicity and tumour resistance. (104) (106) (107) (108)

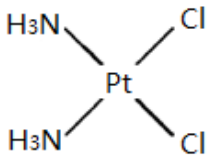

Figure 1-12, Chemical structure formulas of cisplatin, cis-diamminedichloro platinum (Ⅱ).

The dose-limiting toxic effects of cisplatin include nephrotoxicity, high emetogenic potential (e.g. nausea and vomiting), ototoxicity and neurotoxicity (e.g. peripheral neuropathy). (107) (109) (110) The renal toxicity can be circumvented by adequate intravenous hydration, slow cisplatin infusion rates and simultaneous administration of mannitol, and the emesis can be effectively controlled by the combination treatment with dopamine agonists, corticosteroids and/or serotonin antagonists. (109) However, these support measures are not effective for all patients and besides the ototoxicity and neurotoxicity remain difficult to control. (109) The sensory neuropathy becomes evident after having received a cumulative dose of cisplatin higher than 300 mg/m^2, and has been reported occurring in 90% of patients receiving the cisplatin-based chemotherapy. (111) (112) Another severe toxicity of cisplatin, ototoxicity, occurs in up to 93% of patients after the administration of cisplatin. (113) This progressive hearing loss is often irreversible, which leads to lower quality lives for survivors from cancer. (113) (114) In attempt to overcome the renal and gastrointestinal side effects of cisplatin, the second-generation platinum Ⅱ compound, carboplatin (*Figure 1-13*) was developed as a cisplatin analogue to conserve the therapeutic effectiveness and meanwhile provide a more acceptable side-effect profile. (106) (109) Carboplatin substantially diminishes the major toxicities caused by cisplatin (e.g. emesis) and also avoids ototoxicity and nephrotoxicity. (107) (110) As a result, carboplatin has replaced cisplatin in many chemotherapeutic regimens and emerged as the first-line treatment of

choice for patients with advanced ovarian cancer. (104) (109) However, the clinical use of carboplatin is also limited by its primary toxic effect of myelosuppression (bone marrow suppression) which could possibly cause the thrombocytopenia. (103) (104) (107) (110) Since the renal excretion is the main pathway of carboplatin elimination (65% of the administered dose excreted into urine within the first 24 hours (115)), the clearance of carboplatin in patient's body mostly depends on the individual renal function. (116) In addition, the major adverse effect of carboplatin, thrombocytopenia, is highly correlated with its pharmacokinetic characteristic of AUC (Area Under the concentration-time Curve). (117) Thereby, unlike other cytotoxic drugs whose doses are calculated with the patient's body surface area (BSA), carboplatin dosing is generally calculated using the Calvert formula: (116) (117) (118)

$$\text{Dose (mg)} = \text{Target AUC (mg} \cdot \text{min/mL)} \times [\text{GFR (mL/min)} + 25]$$

(AUC = area under the concentration-time curve; GFR = glomerular filtration rate)

This Calvert formula related with AUC and GFR is the most widely used formula for dose calculation and prescription of carboplatin in clinical practice. (115) (118) (119)

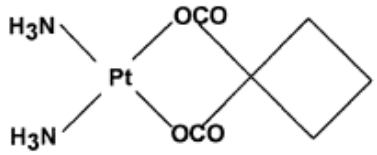

Figure 1-13, Chemical structure formula of carboplatin, cis-diammine (1,1- cyclobutane dicarboxylate) platinum (II).

Tumours that are resistant to both cisplatin and carboplatin cover some of the most frequent human tumour types, and their resistance can be classed into intrinsic resistance and acquired resistance. (105) (107) Cisplatin is inactive against some cancers related to the intrinsic resistance, such as colon cancer

and non-small cell lung cancers. (107) Some tumours show well responding to cisplatin at previous stage of the therapy, while cisplatin cannot produce long-lasting remissions afterwards. The resistances that normally emerge during the course of therapy are known as acquired resistance. (107) The mechanisms of tumour resistance are with multifactor, but can be discussed in two broad categories: before and after the drug binds to DNA. (104) (106)

First, the tumour resistance occurs before the binding of platinum to DNA, which is mediated by inadequate levels of platinum to reach target DNA. (106) The relevant mechanisms of this type resistance are shown below: (*Figure 1-14*)

1) Reduce the influx of the drug by losing the main copper transporters (CTR1) which can facilitate the platinum transport across the cell membrane. (106)
2) Increase the cellular detoxification due to raising the levels of glutathione and metallothionein. (104) In the cytoplasm, the chemically reactive aqua species from cisplatin and water preferentially react with tripeptide glutathione or metallothioneins. Therefore, the increase of glutathione and metallothionein can effectively bind to the activated platinum in the cytoplasm before it can reach the nucleus. (104) (106)
3) Enhance the efflux of the drug by activating the exporters of platinum. Generally, platinum exports from cells through the copper exporters ATP7A and ATP7B as well as the glutathione S-conjugate export GS-X pump (MRP2 or ABCC2). (106)

Second, the tumour resistance is mediated after DNA binding. (111) After entering the nucleus, the activated aqua platinum species would bind with the target DNA on position N7 of guanine (intra-strand and/or inter-strand

crosslink) and produce the platinum-DNA adducts. (111) To contribute to the platinum drug resistance, nucleotide-excision repair (NER), as the main removal pathway of these DNA adducts, could be increased to remove the platinum-DNA adducts before the apoptotic signalling pathways are triggered. (109) (111) Additionally, the increased tolerance to platinum-DNA adducts can lead to tumour resistance either through the loss of DNA mismatch repair (NMR), bypassing of DNA adducts by polymerase β and η, or through down-regulation of apoptotic pathways. (109) (111)

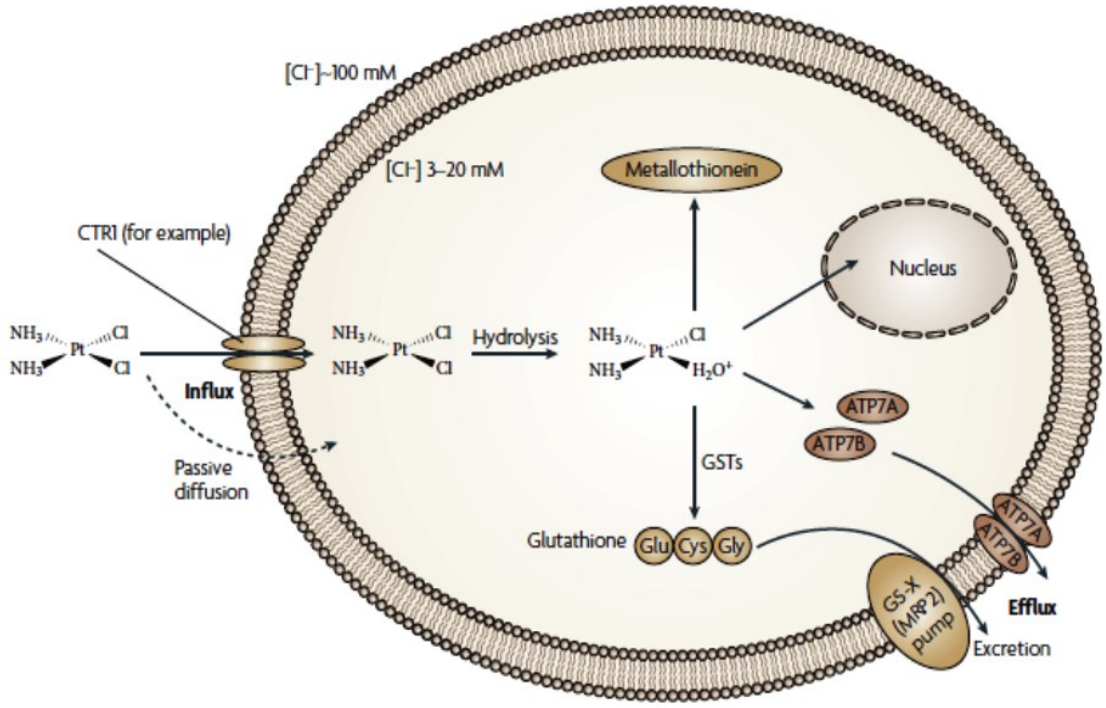

Figure 1-14, Tumour resistance to cisplatin and carboplatin mediated by inadequate levels of platinum reaching the target DNA. GSTs: glutathione S-transferases; CTR1: copper transporter; ATP7A, ATP7B: copper exporter. (106) *[Permission to reproduce this figure has been granted by Nature Publishing Group.]*

To circumvent the tumour resistance and broaden the clinical utility of platinum-based agents, new platinum analogues, such as oxaliplatin, have been developed. (106) Oxaliplatin shows more benefits than cisplatin and carboplatin with less toxic effects, and it is the only third generation of platinum agent currently used in clinical practice of cancer chemotherapy. (105) (109) It has been proposed that oxaliplatin has no inheritance cross resistance with both

cisplatin and carboplatin, and thereby oxaliplatin can be used in the treatment of colorectal cancer which is extremely insensitive to the earlier platinum analogues. (109) Furthermore, the toxicity profile of oxaliplatin is favourable, with the incidences of just 1% and 3% respectively for oto- and renal toxicity. (109)

Cisplatin, carboplatin and oxaliplatin have been approved with varied licenced indications by different regulatory authorities in countries and areas, such as MHRA (Medicines and Healthcare products Regulatory Agency, UK) and FDA (Food and Drug Administration, US). (106) (120) (121) (122)

From MHRA, cisplatin has the broadest therapeutic indications including the advanced or metastasised testicular cancer, ovarian cancer, bladder carcinoma, squamous cell carcinoma of the head and neck, non-small cell and small cell lung carcinoma, as well as cervical carcinoma (in the combination with other chemotherapeutic drugs or with radiotherapy). (120) Carboplatin is only indicated for the treatment of small cell lung carcinoma and advanced ovarian carcinoma of epithelial origin either as the first line therapy or the second line therapy after other treatments have failed. (121) Oxaliplatin, in combination with 5-fluorouracil (5-FU) and folinic acid (FA), can be used for treatment of metastatic colorectal cancer and adjuvant treatment of stage Ⅲ (Duke' C) colon cancer after complete resection of the primary tumour. (122)

From FDA, licenced indications of cisplatin are limited with metastatic testicular, metastatic ovarian and transitional bladder cancer. (106) Carboplatin can only be used for the treatment of advanced ovarian cancer as the first line treatment or the second line with palliative treatment. (106) Furthermore, oxaliplatin has been approved for the second line and previously untreated or adjuvant

treatment of metastatic colorectal cancer with 5-FU with leucovorin (LV) by FDA. (106)

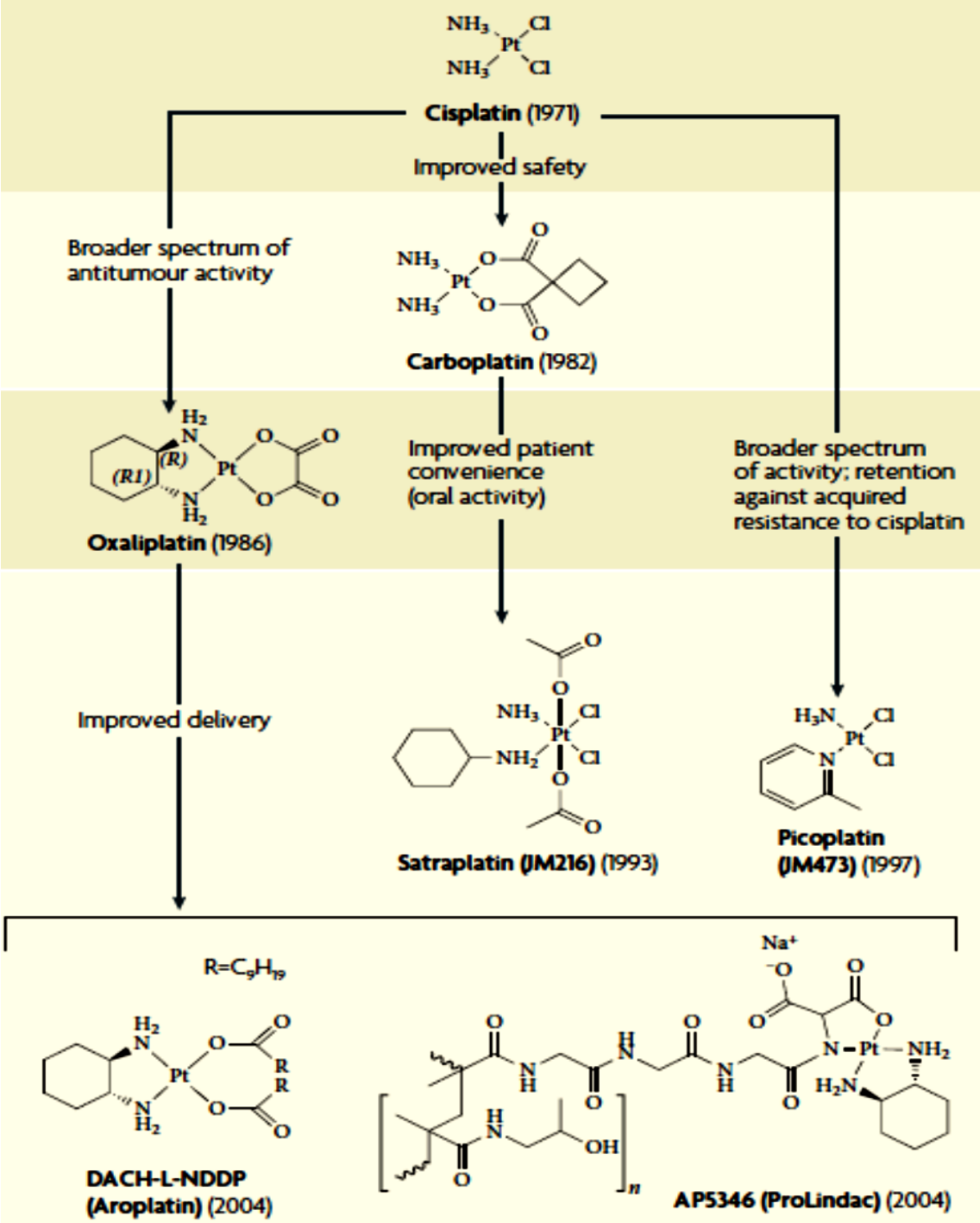

Figure 1-15, The current 'family tree' of platinum drugs and the generalized rationales underlying their development. *Dates after drug names indicate when each drug was first given to patients. DACH: diamino-cyclohexane, contained in oxaliplatin and aroplatin.* (106) *[Permission to reproduce this figure has been granted by Nature Publishing Group.]*

In recent years, studies of platinum have been increased, which focus on different aspects of the possible developments on platinum. For examples, the

elucidation of mechanisms of tumour resistance to platinum drugs, the introduction of new platinum-based agents (oxaliplatin, satraplatin and picoplatin) and clinical combination studies using platinum drugs with resistance modulators or new molecularly targeting drugs. (106) The current developments of platinum agents are briefly shown in *Figure 1-15*. (106)

1.2.3.2 Oxaliplatin

1.2.3.2.1 Biotransformation and mechanism of action

The chemical structure of oxaliplatin (trans-[(1R,2R)-1,2-cyclohexanedi- amine-N,N0]oxalato(2-)-O,O0 platinum) is shown in *Figure 1-16*, which contains a 1,2-diaminocyclohexane group (DACH) and a bidentate oxalate ligand. Compared with other stereochemical isomers of the oxalate-Pt-DACH, the trans-1-(R,R)-DACH-Pt isomer has been shown with the most cytotoxicity and subsequently been selected for developing oxaliplatin. The development of platinum drugs and oxaliplatin was discussed in the above section, which has indicated that the primary advantage of oxaliplatin is the anticancer efficacy against some tumours that are resistant to cisplatin and carboplatin. However, the specific mechanism of oxaliplatin anticancer activity in tumour cells is difficult to ascertain.

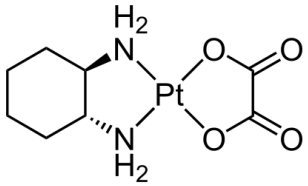

Figure 1-16, Chemical structure of oxaliplatin.

Generally, the principle anticancer mechanism of oxaliplatin is believed to be similar to cisplatin, in that the formation of platinum-DNA adducts results in the cell death. (123) (124) The most preferable site on DNA strand for both cisplatin and oxaliplatin to bind to is the same, which is the N-7 positions of guanine and

adenine. (109) Oxaliplatin could react with both nuclear and cellular DNA and form in three kind of adducts: intra-strand, inter-strand and DNA protein cross-links adducts. (4) (123) The intra-strand adduct is formed by the cross-link between oxaliplatin and NDA through adjacent guanines and occasionally adenines, which is the most abundant among all three adducts. (123) Among those intra-strand DNA-platinum adducts, 60% are bound between two guanine bases and between an adenine and a guanine is 30% of the intra-strand adducts. (109) The other kind of platinum-DNA binds are only about 1% compared to all platinum adducts, including inter-strand and DNA-protein cross-links. (109) Moreover, oxaliplatin can break more DNA strand compared to cisplatin. (109) With these DNA lesions and damages, oxaliplatin could inhibit both DNA replication and transcription and sequentially cause the apoptosis of tumour cells. (4) However, with equal or frequently more cytotoxicity compared to cisplatin, oxaliplatin has surprisingly lower reactivity to crosslink with the DNA. (4) (125) Furthermore, oxaliplatin degrades in a high rate *in vivo* with the half-life about 15 minutes, while the reaction between oxaliplatin and DNA is proved very slow. Therefore, except for the intact oxaliplatin, one or more biotransformation products from oxaliplatin might also be able to cause the fatal DNA adducts. (124) (125)

After administration to patients, about one third of oxaliplatin irreversibly binds to erythrocytes and another one third reacts with various albumins and plasma proteins. (124) The remaining free oxaliplatin degrades with an extensive non-enzymatically biotransformation, and subsequently produces different complexes under different conditions. (124) The biotransformation of oxaliplatin *in vivo* is shown in *Figure 1-17*, which could be divided into two phases with different half-life time for the degradation of oxaliplatin. According to some

previous studies, before reacting into Pt(DACH) complexes, oxaliplatin rapidly dehydrates into the monodentate intermediate with the oxalate ring open which could then form into other platinum complexes. (125) It has been indicated that the half-life for the Phase I was only 16 minutes, while the half-life for oxalate ligand lost was up to 92 minutes which could represent Phase II. (126)

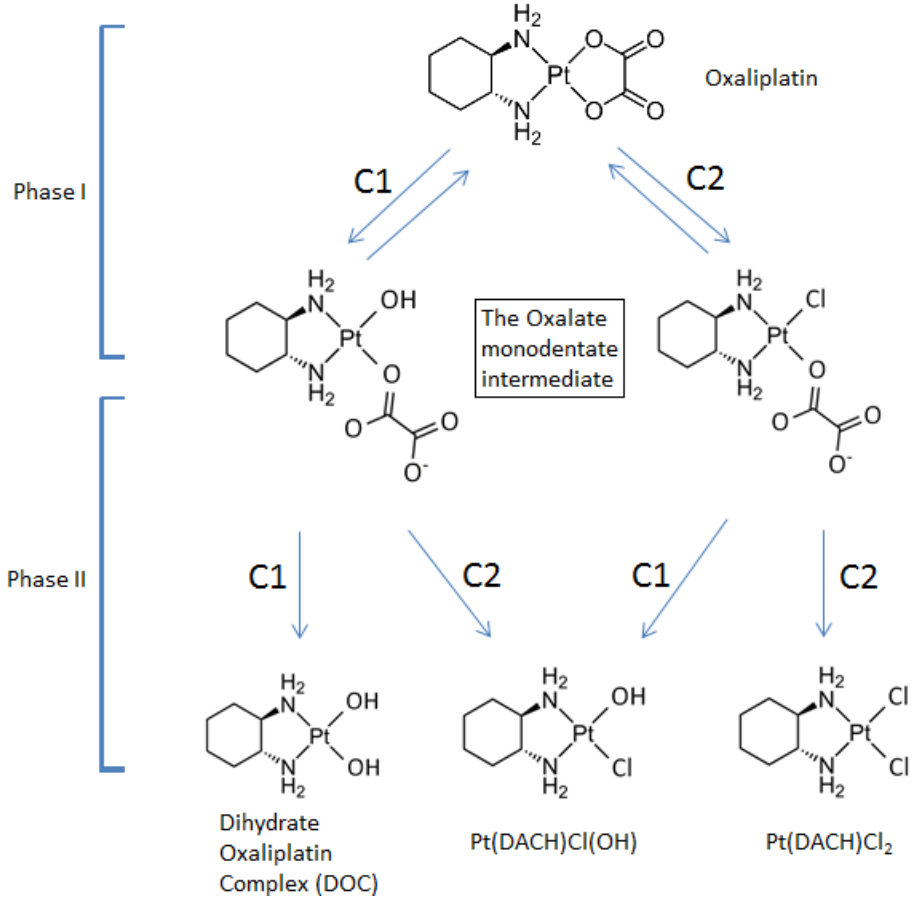

Figure 1-17, Non-enzymatic biotransformation of oxaliplatin *in vivo*, with the oxalate monodentate intermediate which is produced rapidly in Phase I of oxaliplatin degradation. The reactions are slightly slower in Phase II compared to Phase I. The oxaliplatin complexes included in this figure are produced under different conditions: ***C1 = Alkaline hydrolysis by nucleophiles; C2 = Acid hydrolysis by chloride***. The intermediates are oxaliplatin with the oxalate ring open, which are respectively protonated and deprotonated.

However, nucleophiles causing the alkaline hydrolysis in human bodies, such as endogenous bicarbonate and dihydrogen phosphate, present very low reactivity to oxaliplatin under the physiological conditions. (125) (126) Therefore, the dihydrated oxaliplatin could not be produced much in plasma, even though the DOC (dihydrate oxaliplatin complex) is more cytotoxic than

oxaliplatin. (125) $Pt(DACH)Cl_2$ could be produced by both the intact oxaliplatin and the intermediate with oxalate which can be formed due to the high concentration of extracellular chloride. (127) Additionally, $Pt(DACH)Cl_2$ is also more cytotoxic to tumour cells than oxaliplatin, but its contribution to the total cytotoxicity of oxaliplatin *in vivo* is unclearly acknowledged. (125) Consequently, considering those studies on oxaliplatin, *Elin Jerremalm* (125) has suggested in his review that the intact oxaliplatin is acting as the main cytotoxic component in blood. (125)

If the cytotoxicity is only related to the intact oxaliplatin, with increased lipophilicity oxaliplatin is supposed to be more readily taken up by cells and reacted with the nuclear DNA. (124) On the other hand, the sulphur-containing compounds in blood are very reactive to oxaliplatin, including glutathione, methionine and cysteine. (124) But the sulphur-platinum species are most likely inactive, which could influence the cellular effects of the drug. (124) (125) From all these studies, it was shown that the intact oxaliplatin degrades rapidly and forms with a wide range of blood components in. As a result, oxaliplatin could not produce as many DNA adducts as cisplatin. However, the measurable cytotoxicity of oxaliplatin is frequently higher to tumour cells than cisplatin. (4) Therefore, the mechanism of anticancer activity of oxaliplatin might also involve other cellular effects through other components in the blood rather than the DNA itself. (124) (125) It has been indicated that RNA and proteins can also interfere with the anticancer activity mechanism of oxaliplatin. (109) In a study with mouse leukaemia cell cultures, oxaliplatin inhibited RNA synthesis while no positive result was observed with cisplatin. (109) In addition, with conjugating to different cellular proteins, oxaliplatin can inactivate those proteins and subsequently interfere with the cell functions to cause more cell deaths. (109)

Other potential mechanism of oxaliplatin cytotoxicity is also suggested from different studies. For example, the inhibition of the thioredoxin system, an important cellular redox system, causes the cell apoptosis. It has been proved that the cisplatin–glutathione complex could inhibit the thioredoxin reductase by binding to a selenocysteine-containing active site on thioredoxin reductase and subsequently induce cell death. (128) Therefore it might also be triggered by oxaliplatin-sulphur compound, which is similar in chemical structure to cisplatin. (124) (125) Furthermore, the possibility has also been suggested that platinum-sulphur complex could act as a potential drug reservoir and be activated for the subsequent DNA bindings. (124)

1.2.3.2.2 Pharmacokinetics (PK)

Pharmacokinetics (PK) studies are very important for the clinical development of drugs. To evaluate the therapeutic efficacy and safety of oxaliplatin, various clinical and preclinical studies have been conducted under different conditions, and a wide range of PK characteristics were achieved. Oxaliplatin is normally administrated by intravenous infusions within 5% glucose, in which case the absorption of oxaliplatin would be 100% in a patient's blood circulation. Therefore, PK studies of oxaliplatin mostly aimed at the representative PK parameters involved in the processes of drug distribution and elimination, which are reviewed in the following paragraphs.

After administration to patients, oxaliplatin immediately transfers through the blood circulation and distributes widely among various tissues/organs in the body within a short distribution time. (3) From previous studies, the estimated distribution volume for oxaliplatin was different for total (protein-bound and intact fractions) and intact (free) oxaliplatin, but with high values for both. (11)

(109) For the total amount of oxaliplatin (including protein-bound and un-bound oxaliplatin) in plasma, the distribution volume was around 35 L, while for the un-bound oxaliplatin in PUF (plasma ultra-filtrate) the value ranged from 349 – 812 L. (11) (109) This large distribution volume for oxaliplatin could be explained by its nature of lipophillicity from the DACH ligand. (11) In addition, the irreversible bindings to proteins, DNA and other cellular macromolecules could potentially overestimate the distribution volume of oxaliplatin. (11) With a 2-hour infusion of oxaliplatin, the C_{max} (the maximum drug concentration in plasma after administration) normally appears at the very end of the infusion, of which value depends on the dosage of administered oxaliplatin. (11) (129) With the standard dose of oxaliplatin suggested in SPC (Summary of product characteristics), the C_{max} value after 2-hour infusion of 85 mg/m^2 oxaliplatin was observed as 3.6 μM in PUF. (125) The previous study conducted with oxaliplatin in different infusion duration presented that prolonging the infusion time (from 2 – 6 hours) reduced the mean plasma C_{max} by approximately 32%. (11) In order to obtain the full profile of oxaliplatin PK process, various simulation models for oxaliplatin PK analysis have been applied in previous studies. (130) Information about the pharmacokinetics characteristics of oxaliplatin and their modelling systems are described in detail in Chapter 4 (refer to Section 4.1.1).

Among those previous studies, the most suitable PK models for oxaliplatin were selected with 2-compartment, 3-compartment and non-compartment models. (3) (131) AUC (area under the drug concentration-time curve) is an important PK parameter representing the drug exposure in the human body, which can be calculated from the simulation models. (3) The mean $AUC_{0\text{-}inf}$ values for 85 mg/m^2 oxaliplatin were presented differently in different studies after the 2-hour infusion, which were 4.25, 118 and 252 μg-hr/mL respectively for PUF, plasma

and blood cells. (11) Within 48 hours after oxaliplatin administration, more than 50% of the platinum complexes were eliminated through the kidney by urine. (129) Consequently, the glomerular filtration is proven to be the principal mechanism of oxaliplatin elimination by the kidney. Therefore renal clearance could be roughly considered as the total clearance for oxaliplatin elimination from plasma/PUF. (3) The value of oxaliplatin clearance from PUF was indicated as 18.5 L/h at 85 mg/m^2 dosage, which was higher than the average human glomerular filtration (≈7.5 L/h). (11) To investigate the PK variations for patients with renal impairment, a PK study of oxaliplatin on cancer patients with normal renal function and renal impairment was conducted by *Chris H.Takimoto*. (129) Results from different groups of patients with different renal functions has shown that patients with renal impairment had lower clearance for PUF platinum and subsequently the platinum exposure was higher in those patients. (129) However, the corresponding increase of the clinical toxicity related to oxaliplatin was not observed. (129) According to those results, a hypothesis about oxaliplatin elimination was proposed, which suggested that the clearance mechanism for cytotoxic platinum complexes eliminated within the initial phase was not related to renal function. (129) However, the particular mechanism of the effect of renal impairment on platinum clearance still has not been proven. (11) (129)

Time course of the PK process for oxaliplatin could be characterized by three phases, including the short initial α-phase, the longer β-phase and the prolonged terminal γ-phase. (11) (129) The distributions phase of oxaliplatin comprises the α-phase and β-phase, of which the average half-lives were indicated respectively as 0.28 hour and 16.3 hour from previous studies. (11) During this time, the intact oxaliplatin and anti-tumour reactive platinum

complexes were distributed in two ways: 1) rapid removal and binding to tissues; 2) clearance from the systematic circulation through glomerular filtration. (11) The prolonged γ-phase is actually the terminal elimination phase of oxaliplatin and the value of its half-life largely varies in different studies with the rough range from 240 hour to more than 600 hour. (129) The big $T_{1/2}$ variation for γ-phase was generally caused by variation in the experiment conditions in different studies, such as the sampling time design and the detection limits from different determination methods. (11) For example, oxaliplatin results detected with FAAS and ICO-MS were significantly different with the respective accuracy of each assay. Even, after comparison between results from studies using the same detection technic, the terminal elimination phase for oxaliplatin was proven to be long and the average $T_{1/2}$ could be considered as about 250 hours. (131) This terminal phase could represent the slow release of the platinum bound reversibly to some cellular macromolecules, such as amino acid and plasma proteins. (11) Meanwhile, platinum change occurred during the terminal γ-phase only comprise the inactive platinum conjugates. (11) Therefore, the shorter distribution phase is more clinically relevant as its $T_{1/2}$ could represent the change of pharmacologically reactive platinum complexes.

Detailed PK parameters of oxaliplatin collected from previous literature are presented in *Table 4-1* (refer to Chapter 4), with different experimental conditions and data analysis. To understand the big variability among those PK data from different studies, more effective factors to experiment results should be concerned, including the experiment design, detection sample types, method selection, patient number and conditions.

In different studies, different matrices were applied on oxaliplatin determination, including blood, plasma and plasma ultra-filtrate (PUF). (11) As discussed above, after administration, most oxaliplatin binds irreversibly to erythrocytes and various albumins in blood and plasma. These products of oxaliplatin are pharmacologically inactive and without cytotoxicity, while the free intact oxaliplatin is capable of being ultra-filtered from plasma. (125) Therefore, the determination of oxaliplatin in plasma ultra-filtrate is considered as the most acceptable approach to investigate the platinum PK processes. (11) (109) In early PK studies, oxaliplatin was only tested with blood and plasma samples. (11) After more was discovered about the oxaliplatin cytotoxicity mechanisms, the important role of free intact oxaliplatin in its anti-tumour activity has been confirmed. As a result, most of the recent PK studies on oxaliplatin started to include the platinum determination in PUF samples together with plasma and occasionally full blood samples. (11) (125) However, it has been indicated that the free platinum concentration is probably overestimated by the amount of platinum in PUF which could also include the inactive oxaliplatin bound to amino acids and other small biomolecules from blood. (109) Although, the overestimated result from PUF samples can still be analysed with the total oxaliplatin concentration in plasma/blood samples, in order to observe the relationship between active and inactive platinum and subsequently learn about the pharmacological behaviour of active platinum complexes. (109)

As discussed above, determination assays for oxaliplatin used in its PK studies are required with good precision and accuracy on samples of blood, plasma and PUF. According to previous PK studies, different techniques were applied to detect oxaliplatin in those samples, among which FAAS (Flameless Atomic Absorption Spectrometry) and ICP-MS (Inductively Coupled Plasma - Mass

Spectrometry) were the most commonly selected. (11) FAAS and ICP-MS could be both applied to several different types of samples, such as full blood, plasma, PUF and urine samples. (129) (132) (133) Assays validated from these two methods were all with good accuracy and precisions, and with FAAS the assay for oxaliplatin samples (including blood, plasma and PUF) could test within the concentration range of 4 ng/mL – 400 ng/mL. (132) Various assays using ICP-MS were validated and applied in previous studies, while one of those studies on cancer patients with different renal functions has indicated different detection range of oxaliplatin in different samples. (129) The test concentration ranges were 1 – 250 ng/mL for PUF samples and 0.1 – 10 μg/mL for plasma, and the lowest platinum concentration that can be tested was 0.1 μg/mL in urine samples. (129) The HPLC system with an UV detector was also used for oxaliplatin determinations in some PK studies. (11) With further development, a HPLC-MS (High Performance Liquid Chromatography tandem with mass spectrometry) method was validated for the quantification of intact oxaliplatin in human plasma, which could test the oxaliplatin concentration as small as 20 ng/mL within the range of 20 ng/mL – 1000 ng/mL. (125) (134)

Compared with cisplatin and carboplatin, oxaliplatin has a similar drug exposure in patient bodies after administration, which indicates that the therapeutic effect from these three drugs should be without significant difference. (11) The main difference between oxaliplatin and cisplatin/carboplatin is caused by the carrier ligands. The DACH-platinum complexes produced from oxaliplatin are more lipophilic, which is easier to distribute into tissues and subsequently increases the volume of oxaliplatin distribution in PUF. (11) While no platinum accumulation was observed in PUF after the oxaliplatin administration of 85 mg/m^2 every 2 weeks. (135) (136) Furthermore, in studies with chemotherapy

combination of oxaliplatin and 5-Fluorouracil (5-FU), the significant PK interactions were not found between these two drugs in patients. (11) This result could indicate that the additive anti-tumour activity obtained from the combination chemotherapy is not caused by the major alterations in drug exposure, and the potential mechanism for the enhanced efficacy of oxaliplatin is still unknown. (11)

1.2.3.2.3 Clinical use and toxicities

The development of platinum drugs was presented above in Section 1.3.1, according to which oxaliplatin is probably the most successful compound from the newest generation and widely applied in chemotherapies for various cancers at present. In fact, after cisplatin and carboplatin, a range of platinum compounds with the DACH ligand were studied, some of which were even tested and evaluated in preclinical and/or early clinical trials. (4) Because those DACH-platinum compounds have shown good anti-tumour activity in cell lines that are cisplatin-sensitive and/or carboplatin-resistant. (4) As one of the DACH-platinum compounds that passed the initial tests and selected for preclinical studies, oxaliplatin has competed the others with its auspicious characteristics, such as stability, formulation, solubility and safety issues. (4) Furthermore, it has been indicated from previous studies that the activity of oxaliplatin could be remained without the cross-resistance in cisplatin-resistant cell lines, as well as a 5-FU-resistant and a doxorubicin-resistant cell line. (4) The DACH-platinum complex as oxaliplatin could exist in three isomeric conformations which could express their anti-tumour activities by interacting with DNA in different ways. (4) However, according to a previous study published in 1978, the trans-1-(R,R)-DACH-Pt isomer was indicated as the most effective agent against the tumour cells that are resistant to other platinum drugs. (137) Therefore, the

stereochemical isomeric conformation of oxaliplatin was confirmed and after approved with valid preclinical and clinical results oxaliplatin is currently applied in clinical practice in many countries around the world, including Europe, Asia and America. (11)

In clinical chemotherapies, oxaliplatin was approved for the treatment of metastatic colorectal cancer generally with 5-FU (5-fluorouracil) and leucovorin. (5) (6) Colon cancers have shown with the primary resistance to both cisplatin and carboplatin treatments either as single agents or in combinations. (123) However, the cytotoxic activity of oxaliplatin was observed against colorectal cancer, which is the first platinum-based drug that shows convincing clinical activity on colorectal cancers. (1) (4) Oxaliplatin was first launched in France in 1996 and subsequently accepted in the rest of Europe since 1999, while in the United States oxaliplatin was approved by the FDA (Food and Drug Administration, US) a few years later in 2002. (1) Moreover, the clinical application of oxaliplatin is indicated differently in Europe and US as presented below:

- In the US, oxaliplatin was approved for the initial treatment of advanced colorectal cancer in combination with infusions of 5-FU and LV (leucovorin) in 2004. (138) But initially in 2002, it was recommended to be used as the second-line treatment for patients with metastatic carcinoma of the colon or rectum when the disease had recurred and progressed after the first-line therapy (bolus 5-FU/LV and irinotecan) failed. (1)
- In Europe, oxaliplatin in combination with 5-FU/LV was initially indicated as a first-line treatment for patients with metastatic colorectal cancer. (1)

> In 2004, an extended indication of oxaliplatin was approved through the Mutual Recognition Procedure, which included the 'Adjuvant treatment of stage III (Dukes' C) colon cancer after complete resection of the primary tumour'. (139)

Colorectal cancer was initially treated by the antimetabolite 5-FU (5-fluorouracil) with its biomodulator (LV, leucovorin) before new active medicines were discovered. (1) After approval in 1995, irinotecan started to be used as the main agent for the first-line treatment of colorectal cancers associated with 5-FU. (1) However, after oxaliplatin was introduced into clinical chemotherapies, the treatment combinations with oxaliplatin have competed with irinotecan, because the oxaliplatin treatment has a favourable toxicity profile and therapeutic efficacy variability. (1) (6)

Even though, oxaliplatin are only approved for the treatment of colon cancers so far, the cytotoxic activity of oxaliplatin in various tumour cell lines and cancer types was investigated and presented in a range of preclinical and clinical studies. (4) (123) Oxaliplatin is cytotoxic to a broad range of cell lines some of which are cisplatin-resistant, including colon, ovarian, cervix, lung cancer cells and leukemia cell lines. (4) In previous *in vivo* and *in vitro* studies, oxaliplatin has shown activity against a number of tumours, including breast cancer, gastric cancer, renal cell carcinoma and sarcoma. (4) As a platinum drug, the possible use of oxaliplatin in treatments for those cancers that response to cisplatin and/or carboplatin are also considered. Recently, more clinical studies were conducted on some cancers that are occasionally treated with chemotherapeutic combinations containing oxaliplatin as the second-line treatment, such as advanced breast cancer, ovarian cancer, hepatocellular carcinoma and non-Hodgkin's lymphoma. (7) Studied on 23 patients with

metastatic breast cancer, oxaliplatin in off-label use was indicated with little value to the combination chemotherapy. (140) While for the platinum-resistant ovarian cancer, the combination of gemcitabine and oxaliplatin (GEMOX) showed encouraging activity and manageable toxicity, which indicated the potential of this combination treatment for patients with ovarian cancer, especially for those who have been pre-treated with other chemotherapeutic agents. (141) As a result, the GEMOX has been tested in a large number of patients (n=204) with advanced hepatocellular carcinoma (HCC), and the result of acceptable tumour responses has demonstrated the therapeutic efficacy and safety of GEMOX for the treatment of advanced HCC. (142) In addition, GEMOX (gemcitabine and oxaliplatin) could also be applied to the treatment of advanced non-small cell lung cancer, which was indicated with good activity and toleration in studied patients. (143) For other chemotherapy combinations, the significant activity of OFAR2 (including oxaliplatin, fludarabine, cytarabine and rituximab) regimens was found in the treatment for non-Hodgkin's lymphoma. (144) Furthermore, the potential of oxaliplatin in chemotherapies against pancreatic cancer and gastrointestinal cancer was also in development in clinical studies. (1) (139)

Compared with cisplatin and carboplatin, oxaliplatin has a preferable toxicity profile with mild myeloid-suppression and moderate gastrointestinal side effects to patients. (1) (129) The gastrointestinal toxicity presented by nausea and vomiting are common with platinum drugs, which could be systematically prevented by the antiemetic of 5-HT_3 antagonists which could significantly reduce the severity of this toxicity. (123) (129) Severe toxicity effects associated with cisplatin, otoxicity and nephrotoxicity were not observed in clinical studies of oxaliplatin. (123) (129) However, the dosing administration of oxaliplatin is

limited by the major toxicity of the transient peripheral neuropathy, which is characterized by paresthesia, dysesthesia and hypoesthesia in hands, feet, perioral area or throat in patients. (5) (123) (129) The neurotoxicity of oxaliplatin expresses differently during the chemotherapy treatment, which was classified in two types as following:

I. An acute sensory neuropathy occurred rapidly during the infusion and lasted for hours to a few days. (5) (123) These symptoms can be fully reversed within 14 days after the completion of oxaliplatin treatment. (5) However, the acute neuropathy frequently recurs with the accumulative oxaliplatin from further dosing schedules. (5) In addition, acute neurological symptoms are proven to be triggered and exacerbated by contact with cold temperature, in which case ice from food or drink should be avoided during the infusion administration of oxaliplatin. (5) (145)

II. A persistent (> 14 days) chronic sensory neuropathy normally occurs with oxaliplatin treatment following the acute neuropathy events but it could also appear without prior symptoms. (5) The chronic neuropathy is also one of the severe side effects for cisplatin, which mechanism is believed to be related to the platinum-DNA bindings in neuron cells. (12) (146) Compared to the acute neuropathy, deficits in proprioception might be developed in persistent neurotoxicity, which can interfere with daily activities for a number of patients. (5) Furthermore, these symptoms of persistent neuropathy may be reduced in some patients upon the discontinuation of oxaliplatin treatment. (5)

Mechanisms of these two types of neurotoxicity associated with oxaliplatin are not clearly acknowledged at the moment. Several hypotheses were proposed

and investigated in previous studies, which lead to a few options after the exclusion of irrelative factors: 1) interference with voltage-gated ion channels either by the oxalate produced from oxaliplatin degradation which can chelate with the extracellular calcium ions, or directly by oxaliplatin or oxaliplatin intermediates (such as $[Pt(DACH)oxCl]^-$); 2) the apoptosis of sensory neurons could be caused by the DNA damage from oxaliplatin/cisplatin bindings. (145) (146) In a previous study on dorsal root ganglion (DRG) neurons, which has been proven to be the major damage site in chronic neurotoxicity, the results of oxalate treatment on those neurons indicated that oxalate did not cause significant cell damage and is consequently not involved in chronic neurotoxicity of oxaliplatin. (146) However, the potential of oxalate related to the acute neurotoxicity occurred with oxaliplatin treatment was not excluded, which was believed with great values in the relative researches for oxaliplatin. (12) (146) (147) To prevent the neurotoxicity from oxaliplatin, different clinical approaches were tested, and the calcium-magnesium infusions have shown good efficacy as an adjuvant agent in clinical practice. (147) Detailed information reviewed from previous studies on the relationship between oxalate and neurotoxicity of oxaliplatin was discussed in Chapter 3, please refer to Section 3.1.1.

In addition to neurotoxicity, thrombocytopenia is also a frequent side effect observed with oxaliplatin treatment, which normally appears in up to 70% patients as a mild or moderate toxicity but occasionally becomes the major issue that could delay or discontinue the chemotherapy treatment. (7) (123) The mechanism of thrombocytopenia has been indicated with mild to moderate bone marrow suppression in previous studies. New mechanisms were also demonstrated from recent studies, including splenic sequestration of platelets,

the relevant liver damage induced from oxaliplatin and immune chrombocytopenia. (7)

In summary, oxaliplatin is currently used as the first-line treatment of metastatic colon cancers and adjuvant treatments for various cancers. Although oxaliplatin has a favourable toxicity profile compared with other platinum drugs, mechanisms of those major side effects (such as neurotoxicity and thrombocytopenia) are still in need of further studies in order to discover effective preventions and for the improvement of clinical use with oxaliplatin. From previous *in vivo* and *ex vivo* studies, the value of oxaliplatin in clinical chemotherapies was indicated, and oxaliplatin could be applied to a wider range of clinical use with appropriate development.

1.2.4 Dose-banding

Drug dosing is always a key issue in chemotherapy with the 'dose-effect' relationship of cytotoxic drugs. (13) Doses of most anticancer drugs have currently been calculated on the basis of BSA (Body Surface Area) in clinical practice since 1958. (8) (13) The only exception is carboplatin, for which the dose is calculated on the basis of patient renal function and pre-defined pharmacokinetic targeted AUC (area under the plasma concentration-time curve). (8) (9) The use of BSA can provide individualized dose of cytotoxic drugs for each patient in accordance with the evaluation of haematology test results and renal and hepatic function as well as other indicators of the patient's clinical status. (8) (9) Chemotherapy, in now, is mostly administered in the outpatient setting, which means that drug infusions, with bespoke dose calculated according to BSA, are required immediately after the patients' assessment and the authorization of their prescription by the oncologist during

their visit to the outpatient department. (8) (9) As a result, this individual preparation of chemotherapeutic drug infusions, with timely provision, increases the demand on pharmacy intravenous admixture and anticancer drug compounding service bringing a challenge for hospital pharmacy aseptic units. (8) (9) This situation could cause several problems, such as delays for patients awaiting treatment and disrupting scheduling; limiting the number of patients that hospital clinics can treat in the same time; increasing the stress for oncology outpatients, pharmacy and nursing staff involved; the lack of specialist nursing resources in or beyond the normal working hours. (8) (9) In this case, new approaches for chemotherapy administration are required among which the pre-preparation of batch-scale aseptic infusions is attractive since it can permit streamlined preparation and rigorous quality control. (10) However, this would introduce a limitation of using standard doses, when in practice it is necessary to calculate bespoke, individual patient doses using BSA. To overcome this difficulty, the dose-banding approach has been developed for chemotherapy preparation, since it allows standard batch-prepared infusion bags or syringes to be used for individual patients where doses are based on BSA. (10) (13)

Dose-banding has been defined as “a system whereby through agreement between prescribers and pharmacists, doses of intravenous cytotoxic drugs calculated on an individualized basis that are within defined ranges or bands are rounded up or down to predetermined standard doses.” (8) The maximum variation of the adjustment between the standard dose and the doses constituting each band is not more than 5%. (8) (10) Therefore, a range of prefilled syringes or infusions, manufactured by pharmacy staff or purchased from commercial sources, can then be used to administer the prescribed drug dose for chemotherapy. (8) (10)

These ranges (or bands) of doses based on BSA are predefined and a pre-determined standard dose is applied to each band usually as the midpoint of the band which means the standard dose should be used for the patients whose prescribed doses are included in the same dose band. (13) (148) Dose-banding schemes are constructed individually for each drug to make sure the variation should not be more than 5% from the prescribed dose. (8) (148) Moreover, the standard dose applied for patients are prepared from the combinations of pre-filled syringes or pre-prepared infusion bags, which are the only drug solutions stored and used directly in the clinical administration of chemotherapy with dose-banding scheme. (8) (148) An example of a part of a dose-banding scheme is shown in *Figure 1-18*, which presents some dose bands of methotrexate in the chemotherapy of CMF (Cyclophosphamide; Methotrexate; 5-Flourouracil).

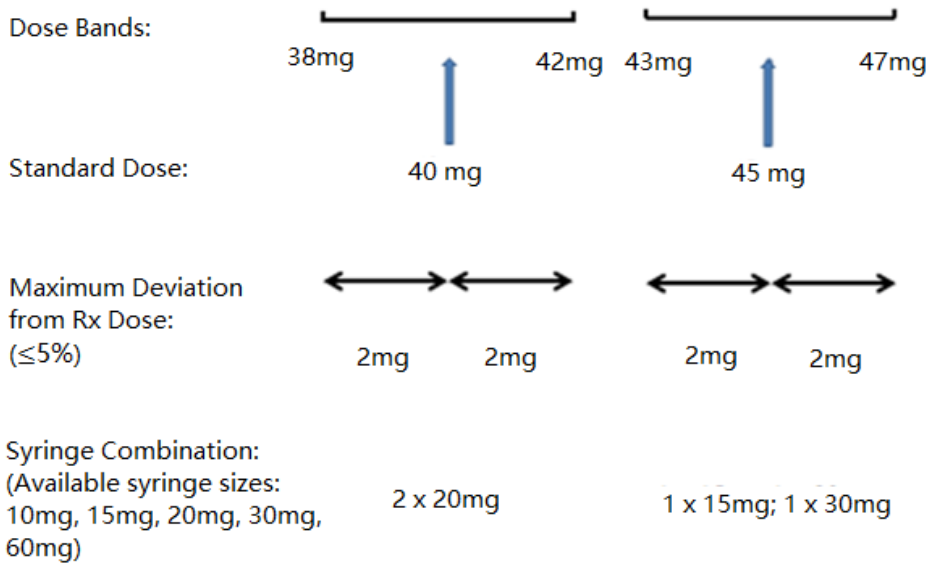

Figure 1-18, Examples of a part of the dose-banding scheme of methotrexate in CMF (Cyclophosphamide; Methotrexate; 5-Flourouracil) chemotherapy, inside which the dose of methotrexate is 40 mg/m^2 based on BSA. Two lower dose ranges are shown above: 38 mg – 42 mg and 43 mg – 47 mg. The standard doses of 40 mg and 45 mg can be provided within these two bands and the maximum variance is 2 mg which is not more than 5% from the prescribed dose. The pre-produced syringes of methotrexate are made in 5 different sizes to be combined into several different standard doses of each band, which are 10 mg, 15 mg, 20 mg, 30 mg and 60 mg. Rx dose: prescription dose.

With more than 10 years use in the UK, dose-banding has shown various benefits in clinical practice. (9) The main advantages of dose-banding are described in three aspects below:

1, Safety and quality: By improving workload planning of the pharmacy production, dose-banding can reduce errors related to the cytotoxic drugs preparation and the stress from pharmacists and nurses. (148) In addition, dose-banding simplifies the process of dose calculation and provides the possibility of the end-product testing of batches, such as drug assay, drug identification and infusion sterility. (8) (148) Therefore, dose-banding enables the prospective quality control for validated batches and pre-filled syringes, and thus increases the accuracy and safety of chemotherapy administrations.

2, Good for pharmacy staff: dose-banding reduces the stress on pharmacists and nursing staff to decrease the risk of errors in the preparation and administration of chemotherapy. (8) (148) Using the ‘ready-to-use’ batches or pre-filled syringes, the occupational exposure risk to the staff that is responsible for handling cytotoxic chemotherapy is also reduced. Furthermore, providing good quality data on the drug infusion stability is available, and the shelf-life of the drug infusions could be prolonged to minimise/eliminate the drug wastage. (8) (9) (148)

3, Good for patients: dose-banding has been shown to decrease patients’ waiting time since their infusions are pre-prepared and readily available, avoiding the delay of up to several hours while bespoke chemotherapy infusions are prepared. In addition, dose-banding can also provide the possibility to give treatments to patients nearer their homes, in GP clinics and on any weekday. (10) (148)

However, there are still some potential disadvantages, such as the dose variance from dose-banding might result in an unacceptable total variance from the intended dose when combined with errors incurred in the estimation of BSA ; the need to combine different syringes/infusions (two or three) giving to a standard dose may add some errors in practice. (8) (148) Furthermore, not all patients are suitable for dose-banding approach. Patients with extreme conditions, such as children, cachexic patients and obese patients, might require more accurate drug dosage for the treatment. (10)

Several anticancer drugs have been successfully administered with dose banding schemes in clinical practice in the UK, including cyclophosphamide, methotrexate, doxorubicin, epirubicin, vincristine, 5-FU (Fluorouracil) and folinic acid. (148) Two main conditions are required for the cytotoxic drugs to be dose banded, which are clinical acceptability of 5% variation from the prescribed dose and longer stability duration (ideally at least 3 months) to provide a reasonable shelf-life for pre-preparation of standard infusions. (8) (148)

In summary, pre-filled syringes and infusion bags for dose-banding can be manufactured from a licensed pharmacy facility or the pharmaceutical industry, and then used in clinical practice associated with trained pharmacy staff, especially pharmacists, nurses and oncologists. (8) (148) (149) As a result, the implementation of dose-banding in clinical chemotherapy practice requires well-designed infusion stability studies and clinical evidence that dose banding is acceptable in the oncology practice with no adverse effect on patient outcomes. (149) Determination of stability of the drug infusions is necessary to ensure that the drug potency is not reduced and that harmful degradation products or drug precipitates are not produced during prolonged storage. (8) Thereby the safe use of drug infusions for dose-banding scheme can be supported by long-term

stability data of pre-prepared syringes and infusions. (148) There are few clinical studies using key pharmacokinetic measures as a surrogate to research on the implementation of dose-banding. (13) (149) These studies indicate that there is no significant difference between the pharmacokinetic profiles obtained with dose-banding and individual dosing based on BSA. (10) (13) Thus, dose-banding is currently considered to be acceptable in clinical practice. (8) Furthermore, the administration of dose-banding not only facilitates the quality control of cytotoxic drug infusions but also improves the services to patients compared with the traditional (individual) dose preparation. (8) (10) (13)

1.2.5 Summary of the literature review

Cancer is a type of disease caused by abnormal cell growth with the potential to expand and/or metastasize to normal tissues/organs of the body. The causes of cancers are generally considered as the host factors and the exogenous/environmental factors, among which the environmental factors are the key issue to prevent the development of cancer. There are more than 100 types of cancer, which can be classified into four main kinds, which are carcinoma, sarcoma, lymphoma and leukaemia. For different types of cancer, different treatment will be selected and applied to patients, including surgery, radiation therapy and chemotherapy, which can be used independently or within combinations. Compared with other treatments, chemotherapy can provide systematic effects against metastatic and disseminated cancers with various anticancer drugs, such as alkylating agents, antimetabolites, anti-tumour antibiotics and vinca alkaloids. The anticancer activity of those drugs used for chemotherapies aims at different cancers with different mechanisms. Alkylating agents are based on the mechanism of damaging the DNA by cross-link DNA strands and subsequently causing cell apoptosis. Platinum group is one of the

major alkylating agents that are currently used for various cancers in clinical chemotherapies. In platinum group, cisplatin, carboplatin and oxaliplatin are three main drugs with outstanding cytotoxicity to a number of cancer cell lines.

Oxaliplatin is the third generation of platinum drugs, which can be used for cisplatin- and/or carboplatin-resistant cancers with different activities and mechanisms. Since approached in France in 1996, oxaliplatin has been used for metastatic colorectal cancers as the first-line treatment. Meanwhile, increasing preclinical and clinical studies have shown the wider range of tumours that oxaliplatin is reactive to, which has improved the clinical use of oxaliplatin. However, the dose-limiting side effect of oxaliplatin is neurotoxicity, which mechanism has not been clearly defined. As a result, the search for suitable adjuvant agents for the effective prevention of neurotoxicity associated with oxaliplatin treatment requires further study.

Furthermore, dose-banding has been introduced as an alternative drug administration method to improve the clinical administration of anticancer drugs, which are normally prepared by the individual dosing scheme based on patient's BSA (Body Surface Area). With more advantages for patients, pharmacists and manufacturers, dose-banding is currently applied to many chemotherapeutic drugs in the UK. However, the application of dose-banding in clinical practice requires further investigations of the specific drug to ensure the therapeutic efficacy and safety for its chemotherapies.

As a regular chemotherapeutic drug used frequently in clinical practice, the administration of oxaliplatin can be improved with dose-banding schemes.

Therefore, a well-designed infusion stability study is required to indicate that oxaliplatin infusions are suitable for preparation in advance and safe to use after

a long-term storage (at least 3 months). In addition, the dose-banding schedule for oxaliplatin must be proven with appropriate pharmacological efficacy and acceptable toxicity to patients, which could be demonstrated from a pharmacokinetic study with clinical or laboratory-based experiments. Furthermore, studies on side effects produced from oxaliplatin would be useful and supportive to improve the clinical chemotherapies with oxaliplatin.

Chapter 2: Sequential-temperature stability study on oxaliplatin infusions

2.1 Introduction

2.1.1 Oxaliplatin background

Oxaliplatin is a third-generation platinum Ⅱ compound which has been developed by replacing the diamino ligand attached to Pt with 1, 2 – diamino-cyclohexane (DACH) and adding an oxalate ligand as a 'leaving group'. (134) As a novel platinum drug, the use of oxaliplatin could increase the number of cancer types which can be treated by platinum, and overcome some of the present limitations of platinum-based chemotherapy such as the resistance tumour cells have developed against cisplatin and carboplatin. (150) In clinical practice, oxaliplatin is currently used in the treatment of many types of tumours such as colorectal cancer, refractory breast cancers, non–small-cell lung cancer, and non-Hodgkin's lymphoma. (151) The mechanism of anti-tumour activity of oxaliplatin is based on activation of oxaliplatin derivatives following: displacement of the labile oxalate ligand leaving groups, and the active platinum species form both inter- and intra- standard platinum-DNA crosslinks. (127) As a result, replication and transcription of DNA are inhibited, and moreover the drug has cell - cycle nonspecific cytotoxicity. (109)

In clinical practice, oxaliplatin is normally administered to patients by individual dosing method. Oxaliplatin doses for each patient are calculated according to patient BSA values. Like other frequently-prepared chemotherapy infusions, the preparation and administration of oxaliplatin individual doses has added stress to pharmacy aseptic compounding units. Furthermore, the waiting time for patients is prolonged because of the labour-intensive preparation of individual infusions and the associated large workloads. (8) (9) In order to reduce the preparation workload for pharmacy compounding units and improve the

timeliness and safety of drug administration to patients, dose-banding schemes offer many advantages for oxaliplatin infusions, including: 1) improved quality control on oxaliplatin infusions because dose-banding facilitates end-product testing; 2) ease of preparation for pharmacy compounding units by use of batch-scale preparation; 3) reduce waiting time for patients because their banded-dose of chemotherapy is available immediately after the prescription has been authorised. (152) The benefits of dose-banding are well documented for a range of chemotherapy infusions used in clinical practice in the UK. (9) To develop a specific dose-banding scheme for oxaliplatin administration, the stability of oxaliplatin infusions must be sufficient to permit the preparation of infusions in advance. The validation of extended shelf-life requires appropriate stability studies to be conducted on oxaliplatin infusions at relevant concentrations and storage conditions.

Oxaliplatin is normally prepared with 5% glucose to gain the individual dose for each patient in clinical administration. According to previous studies on oxaliplatin degradation, it has been proven that oxidative degradation is the major route of oxaliplatin degradation within aqueous solutions, such as glucose solution. (153) In 5% glucose, oxaliplatin is considered to be fairly stable and slowly degrades into oxalate and diaquated DACH-platinum complexes, such as [Pt(DACH)$(OH)_2$]. (154) (See *Figure 2-1*) As discussed in Chapter 1, the degradation process of oxaliplatin could be significantly influenced by the presence of chloride ion and the pH of the solution. (127) For example, in human blood, where the chloride concentration is relatively high (97 – 107 mmol/L), oxaliplatin can rapidly degrade into [Pt(DACH)Cl_2] and/or [Pt(DACH)Cl(OH)] by non-enzymatic biotransformation. (154) (127) For this reason, oxaliplatin infusions are normally prepared in 5% glucose infusions as

opposed to sodium chloride diluent. In recent years, polyolefin infusion bags have replaced those made of PVC to avoid the requirement for plasticisers (typically phthalates) that can leach into the drug infusion. (155) Therefore, polyolefin bags of 5% glucose were used in this stability study on oxaliplatin infusions. (127) (156)

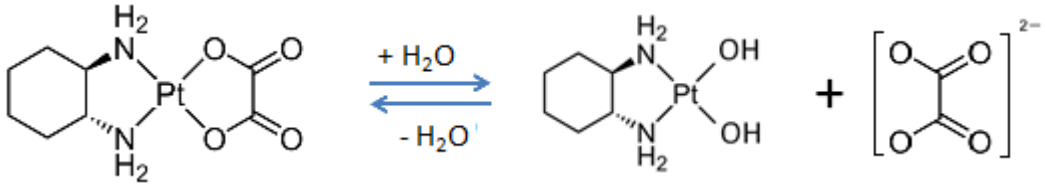

Figure 2-1, The hydrolytic degradation of oxaliplatin in aqueous solutions, resulting in the production of [Pt(DACH)(OH)] and oxalate ions.

The stability information of oxaliplatin is generally indicated within the SPC (Summary of Product Characteristics) produced from the manufacturer. However, the shelf-life stated in SPC is very short for oxaliplatin infusions after dilution from the stock vial (5 mg/mL), and is given as 6 hours at room temperature (25℃) and only 24 hours at 2 - 8℃. (122) To extend the shelf-life of oxaliplatin infusions, several stability studies have been conducted under different conditions. In a previous study in 2007, oxaliplatin infusions of 0.7 mg/mL concentration were prepared with 5% glucose in PVC-free bags, and stored separately at 3 - 7℃ and 20 - 24℃ for 30 days. (154) The chemical stability of oxaliplatin in those infusions was determined by an HPLC assay, while the physical stability was only examined visually with the evaluation of colour change and visible particle formations in the infusion. (154) With over 90% remaining concentrations of oxaliplatin in all infusions, the study results supported the extended 30 day stability of oxaliplatin infusions (0.7 mg/mL) under the storage at both temperatures. (154) Furthermore, a more recent study in 2009 demonstrated longer stability period for oxaliplatin infusions. (151) In this study, oxaliplatin infusions at a concentration of 0.25 mg/mL were prepared

with 5% glucose. After storage for 90 days separately at 4℃ and 25℃, 97.8% and 96.4% of the oxaliplatin was retained, respectively, compared with the initial concentrations. (151) Although the recommended shelf-life of oxaliplatin was increased to 90 days as a result of this study, the data obtained were limited in several ways:

- The concentration range of oxaliplatin infusions in previous stability studies could not fully cover the range required in clinical practice. The average BSA (body surface area) range of cancer patients in UK can be considered as 0.59 m^2 to 2.06 m^2. (157) With the licenced oxaliplatin dosage of 85 mg/m^2 (122) this give a required dose range of approximately 50 mg – 175 mg. Assuming that 5% glucose bags in 250 mL are used for oxaliplatin dilutions, the concentration of oxaliplatin in those infusions should then be within the range of 0.2 mg/mL – 0.7 mg/mL. The results from the two previous stability studies described above were limited with a single test concentration, which could not represent the concentration range of oxaliplatin infusions used in clinical practice.
- The physical stability of oxaliplatin infusions was only assessed by visual examination in both studies, including colour change and visible particle precipitation. In this case, the formation of sub-visual particles was not tested, which could significantly influence the physical stability of infusions. (158)
- The acceptance criteria used for appraising the oxaliplatin concentration change in both studies was the same: ± 10%. For cytotoxic drugs with a narrow therapeutic window, the criteria for the concentration variation

should be more restricted to improve patient safety and a limit of ± 5% has been proposed in stability studies on cytotoxic drugs. (149) This is particularly important when the identity, pharmacology and toxicology of any breakdown products are not known. However, it is notable that in the second study the actual variation of oxaliplatin concentration was within 5% of the original concentration. This suggests that the extended 90 day shelf-life for 0.25 mg/mL oxaliplatin infusions from this study would have been achieved even if stricter acceptance criteria had been applied.

- The parallel temperature design was applied in all previous stability studies on oxaliplatin infusions. This limits the interpretation of extended shelf-lives to single temperature conditions and does not account for the variable temperatures encountered by infusions during storage and administration to the patient. (159)

Given the limitations of pre-existing stability data for oxaliplatin infusions, and the need for rigorous data to support extended shelf-lives of batch-manufactured infusions at a range of standard concentrations required for dose-banding, an appropriately designed stability study reflecting these needs was required.

2.1.2 Experiment design

In order to improve the stability data for oxaliplatin infusions, a sequential temperature design was applied in this study. (160) Compared to the studies conducted with a parallel temperature design, the sequential temperature approach provides the stability data with different and/or mixed temperature conditions, including temperature cycling, which simulates expected situations in clinical practice. With valid stability data, drug wastage could be significantly

reduced in situations where staff forgets to return an infusion bag to the fridge immediately after receipt, or where a patient's treatment has been delayed for clinical reasons after the infusion was prepared or taken out of refrigerated storage. (159) The sequential temperature schedule was designed by the addition of two regular storage temperatures: the refrigerated storage temperature of 2 – 8℃ and the room temperature of 25℃ at which the infusions are administered to the patient. For instance, the infusion bags can be stored in the fridge at 2 – 8℃ for a defined period and then incubated at 25℃ for another 24 hours afterwards to simulate long-term refrigerated storage followed by a period of time at room temperature consistent with transport of the infusion around the hospital and administration to the patient in the day-case clinic. The specific schedule of the sequential temperature cycling designed for this stability study is described in detail in Section 2.3.1.1.

As discussed above, the concentration of oxaliplatin infusions is normally prepared within the range of 0.2 mg/mL – 0.7 mg/mL in clinical practice. To ensure the stability data from this study is practically applicable, oxaliplatin infusions were prepared in two different concentrations of 0.2 mg/mL and 0.7 mg/mL. Thus, results from this study should be able to cover all oxaliplatin infusions administered to patients within the expected BSA range.

It was important to evaluate both chemical and physical stability of oxaliplatin infusions in this study. Chemical stability was determined by monitoring the drug-degradation using High Performance Liquid Chromatography (HPLC) with a fully validated stability-indicating method, which was also validated for precision, linearity of response and stability indication. (161) (162) Oxaliplatin concentrations were determined at different time-points for each infusion group,

and the concentrations at each time were compared to oxaliplatin concentrations obtained at time zero.

Previous stability studies on oxaliplatin infusions were limited in their assessment of physical stability, but in this study a systematic multi-parameter analysis was adopted including visual appearance, sub-visual particulate counting and pH measurement as well as weight changes monitoring. (158) Sub-visual particulate counts were used to monitor the aggregation of micro-precipitates which can affect the physical stability of infusions and remain undetected by the visual examination. (149) (163) Meanwhile, infusion weight changes were monitored to examine the loss of water through the infusion container membrane, which can change the oxaliplatin concentration of infusion and may even mask drug loss through degradation. (149)

2.1.3 Aims and objectives

This stability study aims to support a dose-banding scheme, which is based on batch-preparation of a range of standard infusions, for oxaliplatin administration by investigating and validating the extended stability of oxaliplatin infusions in 5% glucose.

The objectives of this stability study also include:

- To develop and validate the methodology to rigorously evaluate the physical and chemical stability of oxaliplatin infusions;
- To design a robust sequential-temperature cycling schedule for this study in order to determine the stability of oxaliplatin infusions under ‘real-life’ conditions encountered in clinical practice;

- To identify the optimum shelf-life of oxaliplatin infusions within the constraints of laboratory-based study, which could be referred and applied to clinical and pharmaceutical practice.

2.2 Materials and methods

2.2.1 Materials

2.2.1.1 Chemical and reagents

Oxaliplatin concentrate solution 5 mg/ mL, batch 871SD008/2, expiry 11/2013 *(Fresenius Kabi Oncology Plc., UK)*

Glucose 5% w/v Freeflex 250 mL polyolefin bags, batch 14DL7107, expiry 08/11/2013 *(Fresenius Kabi, UK)*

Sodium phosphate monobasic monohydrate (ACS reagent, 98.0 - 102.0%) *(Sigma-Aldrich, Japan)*

Sodium phosphate dibasic (HPLC grade) *(Sigma, USA)*

Sodium hydroxide 1M (HPLC grade) *(Fisher Scientific, UK)*

Acid ortho-phosphoric (HPLC grade) *(Fisher Scientific, UK)*

Methanol (HPLC grade) *(Fisher Scientific, UK)*

Hydrochloric acid 1M *(Fisher Scientific, UK)*

Multifunction peroxide solution 3% *(Specsavers, UK)*

Screen washing liquid *(Halfords, UK)*

pH electrode storage solution; pH 4.01 Buffer solution; pH 7.00 Buffer solution *(Thermo Fisher Scientific, USA)*

Sterile water for irrigation and dilution, batch 12E22B28, expiry 04/2015 *(distilled and de-ionised) (Baxter, UK)*

2.2.1.2 Consumables

10 mL Luer-lock syringes *(Codan Medical ApS, UK)*

50 mL Luer-lock syringes *(BD Plastipak, UK)*

0.8mm × 40mm needles, 21G × $1^1/_2$'', LOT 120507, expiry 04-2017; 1.1mm × 40mm needles, 19G × $1^1/_2$'', LOT 120411, expiry 03-2017 *(BD Microlance, UK)*

Screw-cap polypropylene tubes 7 mL *(Fisher Scientific, UK)*

Screw-cap polypropylene tubes 20 mL *(Gosselin SAS, UK)*

HPLC autosampler crew-cap vials 2 mL and caps *(Chromacol Ltd., UK)*

Cellulose acetate filters (pore size 0.45 µm) *(Sartorius Stedium Biotech, Germany)*

Disposable polypropylene pipettes 3 mL *(Fisher Scientific, UK)*

Micropipette tips 1000 µl *(Gilson SAS, France)*

Prep Pad, 70% Isopropyl Alcohol BP *(A CliniMed Group Company, UK)*

2.2.1.3 Equipment and instrumentation

Analytical balance: Sartorius Basic 100g - 0.0001g, 4-figure balance *(Sartorious Ltd., UK)*

Balance: SG-601(600g ×0.1g) 2-figure *(Fisher Scientific, UK)*

Refrigerator at 2 – 8℃ *(Fisher Scientific, UK)*

Incubator at 25℃ *(Gallenkamp, Japan)*

Digital thermometers *(Fisher Scientific, UK)*

Micropipette: Proline Plus 100 – 1000 µL *(Biohit Limited, UK)*

pH meter : Orion 5 Star Benchtop *(Thermo Scientific, UK)*

Magnetic stirrer : HI 190M *(Hanna Instruments, UK)*

Glass filter holder and flask *(Emil company, UK)*

Filter vacuum pump: FB 70155 *(Fisher Scientific, UK)*

Static water bath: Clifton Range *(Nickel-Electro Ltd., UK)*

Class Ⅱ safety cabinet: Biological Class Ⅱ containment *(Bigneat Ltd., UK)*

Particle counter: LiQuilaz LS200, attached with software PSS-view *(Particle Measuring Technique GB Ltd., UK)*

Glassware: Grade A, flasks, measuring cylinders and beakers *(Fisher Scientific, UK)*

HPLC Column: Water Spherisorb® (CNRP) 4.6 mm×250 mm stainless steel, packed with 5.0 µm bonded phase cyano CNRP Column *(Waters Limited, UK)*

HPLC System: *(Jasco UK, Essex UK)*

Intelligent Column Thermostat, CO-2060 plus

Intelligent Auto-sampler, AS 2057 plus

Quaternary Gradient Pump, PU-2089 plus

Multiwavelength Detector, FP 2020

LE-Net Ⅱ/ADC

Chrompass Software version 1.8.6.1

2.2.2 HPLC assay

2.2.2.1 Conditions

A bonded-phase cyano column of Water Spherisorb® (CNRP), 5.0 µm particle size and 4.6 mm×250 mm, was used to detect and separate oxaliplatin in a validated HPLC method. The HPLC assay for the quantification of oxaliplatin in infusions was adapted and developed from the European Pharmacopoeia (EP) by optimizing the detection conditions within the Jasco system of HPLC, including mobile phase composition, column temperature, flow rate and UV wavelength for detection. (164)

Compared with the phosphate mobile phase with 1% acetonitrile described in EP, larger fraction of organic phase has shown better peak shape and effective separation of oxaliplatin from interference peaks in chromatograms under isocratic conditions. A range of mobile phase containing 5% - 10% methanol was tested with the standard solution of oxaliplatin under isocratic conditions. According to those tests, the effective chromatogram with good separation of oxaliplatin was obtained using the mobile phase with 5% methanol. As a result, the mobile phase prepared with 5 mM phosphate buffer at pH 6.5 and 5% methanol was selected and used in this assay.

To optimize the peak shape and separation of oxaliplatin in chromatogram, temperature of the column oven was adjusted into 35℃, which provided sharper peaks of oxaliplatin with appropriate peak heights and good separation between different peaks. In order to short the retention time and modify the peak shape, the flow rate in this assay was adjusted into 1.0 mL/min from 1.2 mL/min that stated in the method from EP. In addition, oxaliplatin showed maximum absorbance at 210 nm after testing the standard solution of oxaliplatin in the UV

wave length ranging from 200 nm to 250 nm. Therefore, the wave length 210 nm was applied in the UV diode-array detector in this HPLC assay. The software of Jasco ChromPass version 1.8.6.1 was used to collect and analyse the chromatographic data. HPLC conditions are shown below:

Flow rate = 1.0 mL/min; Injection volume = 20 µL; Run time = 12 mins; Detection wave length = 210 nm; Temperature of column oven = 35℃.

Column maintenance: The HPLC column was conditioned at the flowrate of 1.0 mL/min every 14 days with: HPLC grade water for 20 minutes; methanol and water with 50:50 (v/v) for 20 minutes; 100% methanol for 20 minutes; methanol and water 50:50 (v/v) for 20 minutes; HPLC grade water for 20 minutes. In addition, daily storage of the column required flushing with HPLC grade water for 20 minutes followed by 50:50 methanol : water (v/v) for 20 minutes prior to shut down.

The column and system performance data were calculated with the equations referring to EP (European Pharmacopoeia). (162)

$$N = 5.54\left(\frac{t_R}{W_h}\right)^2$$

(N = the number of theoretical plates; t_R = retention time; W_h = width of the peak at half-height.)

$$A_s = \frac{W_{0.05}}{2d}$$

(A_s = symmetry factor; $W_{0.05}$ = width of the peak at one twentieth of the peak height; d = distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.)

For this HPLC assay, the initial number of theoretical plates was N = 2628, and the peak is symmetrical with the A_s = 1.48. Variation from those performance

parameters of ＞10% triggered column re-conditioning in advance of the normal 2 week schedule. (165)

2.2.2.2 HPLC method validation

The HPLC method described in Section 2.2.2.1 was validated monthly over the duration of the stability study. Such validations comprised intra- and inter-day precision, linearity of response and specificity (stability-indication). All the acceptance criteria, except for stability-indication, in this section are referred from British Pharmacopoeia (BP) and European Pharmacopoeia (EP). (162) (166)

2.2.2.2.1 Intra-day precision

Vials of oxaliplatin solution (5 mg/mL) were used to prepare all the samples for intra-day precision including one standard solution and six test solutions which were diluted volumetrically to the concentration of 20μg/mL. Six test dilutions were transferred to sample vials and analysed in triplicate using a HPLC method sequence created especially for intra-day precision. The process was completed within the same day. Mean values of peak heights and standard deviation (RSD) were calculated:

$$\mathrm{RSD} = \left(\frac{\mathrm{SD}}{\mathrm{x}}\right) \times 100\%$$

(SD = standard deviation; x = mean concentration)

Acceptance criteria: RSD ＜ 2.0%

2.2.2.2.2 Inter-day precision

A standard solution of 20 μg/mL was prepared in the first day of the inter-day precision from the initial 5 mg/mL oxaliplatin vial. This standard solution was stored at 4℃ and used for the whole term of this precision test in 6 consecutive

days when 6 test solutions (20 μg/mL) were prepared on each day. Replicated test solutions were injected in triplicate each day for 6 days, and from the mean peak heights the RSD value was calculated using the equation referred in section 2.2.2.2.1.

Acceptance criteria: RSD ＜ 2.0%

2.2.2.2.3 Linearity of response

1 mL of 5 mg/mL oxaliplatin solution was diluted volumetrically with HPLC grade water in a grade A volumetric flask (50 mL) to give a 0.1 mg/mL solution. Then a series of calibration solutions were prepared by diluting the 0.1 mg/mL solution from 0.25, 1.25, 2.5, 5 mL into 25 mL and 8, 12, 18 mL, measured with an automatic pipette, into 20 mL separately with HPLC grade water in grade A volumetric flasks to give a calibration plot with the oxaliplatin concentrations of 1.0, 5.0, 10.0, 20.0, 40.0, 60.0 and 90.0 μg/mL.

This concentration range included the concentrations of samples expected to be analysed in this stability study.

Data of 7 mean peak heights from 3 replicate injections for each solution were plotted against concentration and were fitted to a linear relationship using the least-squares regression analysis.

Acceptance criteria: R^2 ≥ 0.995; and even distribution of data on regression line by visual inspection.

2.2.2.2.4 Stability-indicating capability validation

The degradation of oxaliplatin is mainly affected by acid or base catalysed nucleophilic substitution or aquation reactions. (126) (127) These mechanisms were used in forced degradation studies to demonstrate the stability-indicating

attributes of the HPLC assay, in which 5 individual oxaliplatin solutions were subjected to different treatments to accelerate oxaliplatin degradation prior to HPLC assay. Considering the concentration of the control solution is 20 µg/ mL, 10 times more concentrated solutions of 200 µg/mL were prepared to be diluted 10 times later. Five of 10 mL grade A volumetric flasks were used and labelled A, B, C, D, E. 1 mL of oxaliplatin solution (200 µg/mL) was pipetted into each flask followed with 1 mL 0.1 HCl added in flask B, 1 mL 0.1 NaOH added in flask C, 1 mL 3% H_2O_2 added in flask D and 1 mL HPLC grade water added in flask E. Flask A (control) was kept in the fridge at the temperature of 2 - 8℃ and all the other flasks were stoppered and incubated in water bath at 50℃ for 30 minutes. After all the flasks equilibrated to room temperature, 1 mL 0.1 NaOH was added into flask B and 1 mL 0.1 HCl into flask C to neutralise the acid and alkaline treatments, respectively, and then all 5 flasks were adjusted to volume with HPLC grade water. The solutions were then diluted to give a nominal drug concentration of 20 µg/mL and analysed in duplicate by the HPLC assay.

The peak heights of solutions from flasks B – E were then compared against those of the control solution (flask A).

2.2.3 Physical stability tests

2.2.3.1 Visual appearance

To examine the physical change in infusion bags, for instance the infusion colour and particulate contamination, the infusion bags were viewed against both dark and white backgrounds under the normal laboratory lighting conditions. In addition, a 5% glucose bag with no added drug was used as a comparison against test infusion bags.

Acceptance criteria: clear, colourless and free from visible particles.

2.2.3.2 Sub-visual particle counting

A LiQuilaz LS200 Particle counter, working with the light-blockage principle, was used to quantify 10μm and 25μm diameter particles in samples to provide an indication on whether particulate aggregation was occurring during the storage of oxaliplatin infusions. (158) Before sampling, the fluid pathway was flushed with screen washing liquid for 3 times and another 3 times with distilled water. Samples were filled into 7 mL screw-cap polypropylene tubes which had been pre-rinsed with water for irrigation (BP) for 5 times in advance. Each sample in 5 mL was measured twice and the mean value was recorded. Between sampling, the system was flushed 3 times with distilled water for irrigation (BP).

Pharmacopoeia Acceptance criteria: the count of 10 μm particle ≤ 25 units per millilitre; the count of 25 μm particle ≤ 3 units per millilitre. However, this test was used semi-quantitatively to identify any trends in particulate aggregation at each sample point.

2.2.3.3 pH

pH variation may indicate significant chemical and physical changes in oxaliplatin infusions. An Orion 5 Star GLP pH meter with combination glass electrode was used to measure the pH of samples at each time point. For calibrating the pH meter, the reference buffer solutions at pH 4.01 and pH 7.00 were used each time before sampling. Between samples, distilled water was used to rinse the glass electrode, and automatic temperature compensation was deployed.

Acceptance criteria: ± 1.0 pH unit.

2.2.3.4 Weight change

The infusion bags were weighed to assess moisture loss or gain across the container wall at each test point using a 4-figure analytical balance. Calculations were based on the weight of after sampling from the previous test point and the weight recorded immediately prior to the next sampling, and the weight differences were recorded.

Acceptance criteria: ＜0.2% variation from the weight of last sample time.

2.3 Experimental

2.3.1 Study design

2.3.1.1 Sequential temperature cycling

The sequential temperature study design was selected to closely simulate the temperatures and conditions experienced by oxaliplatin infusions in pharmaceutical storage and clinical practice.

The infusion bags were divided into 7 groups each containing 3 infusions following the time schedule of 1, 3, 7, 14, 28, 56, 84 days which are durations for infusion bag storage under refrigerated condition (2 – 8℃). The scheme is shown below, where x is the number of days of refrigerated storage.

Step A: Storage at 2 – 8℃ (refrigerated temperature) for x days and sample analysis.

Step B: Incubation at 25℃ (room temperature) for 24 hours and sample analysis (a).

Step C: Storage at 2 – 8℃ (refrigerated temperature) for 7 days and sample analysis (b).

If x = 1, 3, 7, 14 days, the process includes Step A and Step B.

If x = 28, 56, 84 days, the process includes Step A, Step B and Step C.

This design allows information to be obtained about temperature cycling effects with this infusion in the first 2 weeks of the study. If the infusion remains within specification during this study phase, it is then possible to proceed to the more rigorous cycling which replicates an infusion bag being removed from the refrigerator, taken to the clinical treatment area (room temperature) and then returned to refrigerated storage to simulate situations such as treatment delays.

2.3.1.2 Selection of infusion concentrations

The two concentrations used in this study were 0.2 mg/mL (the lowest) and 0.7 mg/mL (the highest). As recommended in the SPC, the dosage of oxaliplatin should be 85 mg/m^2 based on BSA (Body Surface Area). (122) The concentration range used in this study was commonly prepared in clinical practice to meet typical patients' BSA ranging from 0.59 m^2 to 2.06 m^2. (157) In addition, higher dosage of oxaliplatin for patients with larger BSA could also be covered in this concentration range by preparing oxaliplatin infusions in different volume of glucose.

2.3.2 Preparation of oxaliplatin infusion bags

All of the oxaliplatin infusions were prepared, sampled and diluted in a Biomat AC Class Ⅱ safety cabinet to insure maintenance of asepsis and safe handling of cytotoxic drugs. The principles of 'Good Pharmaceutical Manufacturing Practice' were followed throughout the whole study.

There were 8 groups of bags in total which comprise the 7 sample groups of group A, B, C, D, E, F, G and a spare group H to cover any leaking or damaged

infusion bags. For each group, 6 bags were prepared, comprising 3 bags at the lowest concentration of 0.2 mg/mL and 3 bags at the higher concentration of 0.7 mg/mL.

For 0.2 mg/mL bags, 10 mL oxaliplatin solution concentrate (5 mg/mL) was diluted into 250 mL, 5% glucose in a polyolefin bag after 10 mL of the 5% glucose solution had been withdrawn from the bag. The bag was then gently mixed. For 0.7 mg/mL bags, 35 mL oxaliplatin was diluted into 250 mL, 5% glucose in a polyolefin bag following the withdrawal of 35 mL of 5% glucose solution.

After preparation of each group of infusion bags, the infusions were left to stand for 30 minutes before sampling (time zero) in order to provide time for complete mixing.

All bags were labelled clearly with the drug name, concentration, group number, infusion volume and date of preparation.

The ‘spare’ group could be used to replace infusions from any group if required.

Because oxaliplatin is light sensitive (122), each infusion bag was placed in light-protecting plastic bags to ensure protection from light throughout the entire study.

2.3.3 Stability determination

2.3.3.1 Chemical stability

The HPLC method used for oxaliplatin assay to assess chemical stability was validated monthly, which equated to 4 times in total during the whole study. At each sample point, 1 mL infusion sample was withdrawn from each infusion bag (triplicate bags for each group and concentration) and diluted to the test

concentration. Using a previously calibrated automatic pipette, samples were transferred into grade A volumetric flasks and adjusted to volume with HPLC grade water:

For 0.2 mg/mL infusions, 1 mL sample was diluted in a 10 mL volumetric flask to provide a nominal concentration of 0.02 mg/mL.

For 0.7 mg/mL infusions, 1 mL sample was diluted in a 20 mL volumetric flask to provide a nominal concentration of 0.035 mg/mL.

The external standard method of HPLC assay was used and the concentration of the external standard solution was 0.02 mg/mL. The standard solution of oxaliplatin at 0.02 mg/mL was prepared by diluting 0.04 mL, 5 mg/mL oxaliplatin concentrate with deionized water into a 10 mL grade A volumetric flask. Oxaliplatin concentrate vial used for standard solution preparation was from the same batch that was used for preparing oxaliplatin infusion bags for each group. Duplicate injections of each test dilution were bracketed with duplicate injections of the standard solution.

Quantification of oxaliplatin in samples:

The mean peak heights of duplicate sample solutions and the two 'bracketing' standard chromatograms were recorded for each sample point. The calculation equation for the concentration of oxaliplatin in samples is:

$$C_t = \left(\frac{H_t}{H_s}\right) \times D \times C_s$$

(H_t = mean peak height of test sample solution; H_s = mean peak height of standard solution; D = dilution factor of test sample solution; C_s = concentration of oxaliplatin in standard solution.)

2.3.3.2 Physical stability

For physical stability tests, two samples of 3 mL and 5 mL volume were transferred to sterile polypropylene vials. All of the physical tests were processed at each sample point, e.g. t = 0, t = x, t = a and/or t = b (refer to Study Design section 2.3.1.1).

Visual examination was conducted using the method described in Section 2.2.3.1, and the calculations of weight differences followed the method described in Section 2.2.3.4. The pH variation was measured using the method in Section 2.2.3.3 and calculated with respect to the initial pH-value from time point zero. Sub-visual particle counts were measured with the method discussed in Section 2.2.3.2 and the differences were recorded to establish any trends in the variation of 10 µm and 25 µm particle counts.

2.4 Results

2.4.1 Development and validation of HPLC method for oxaliplatin

A simple, effective HPLC assay was developed and validated for the analysis of oxaliplatin in 5% glucose infusions. A low concentration of phosphate buffer salts was used in the mobile phase of this method to minimise nucleophilic substitution reactions of oxaliplatin when samples were diluted for assay in the mobile phase. (109) In addition, the mobile phase was adjusted for a pH of 6.5 to reduce the possibility of oxaliplatin degradation. (126) (156)

The method was re-validated monthly in terms of specificity (stability-indication), intra-day precision, inter-day precision and linearity of analytical response, referred to by the International Conference of Harmonisation (ICH) Harmonised

Tripartite Guideline, 'Validation of analytical Procedures: TEXT AND METHODOLOGY Q2(R1). (161)

During these validations, precision of the method was analysed with the calculation of the RSD (relative standard deviation) using the equation stated in Section 2.2.2.2.1. As shown in *Table 2-1*, the RSD of Inter-day precision and Intra-day precision were respectively within the limits of $< 2.0\%$, which means the method is repeatable according to British Pharmacopoeia (BP). (161) (166) In addition, the calibration plot was shown to be linear over the concentration range 1.0 – 90.0 µg/mL (n=7), with the equation of y = 1.0076x + 0.0969 (R^2 = 0.9999) derived from the least-squares regression analysis, and an even distribution of the data on the regression line can be inspected visually (*Figure 2-2*). Results of the stability-indicating capability validation of the developed method are presented in *Table 2-2*. These results demonstrate that oxaliplatin can degrade in the presence of HCl, NaOH or H_2O_2, and additional peaks arising from degradation products were separated from the oxaliplatin analytic peak. This HPLC assay for oxaliplatin was therefore considered to be stability indicating.

In summary, all validations of the HPLC assay based on linearity of analytical response, intra- and inter- day precision and stability-indicating ability confirmed the assay as being suitable for oxaliplatin drug stability studies in pharmaceutical infusions.

Table 2-1, Inter-day precision data for the HPLC assay of oxaliplatin during 6 continuous days and intra-day precision data of oxaliplatin assay with 6 triplicate assays within one day.

Precision Type	Injection Identifier	Oxaliplatin Concentration (µg/mL)	RSD (%, n=6)
Inter-day Precision	Day1	20.0396	0.3228
	Day2	20.1009	
	Day3	20.1026	
	Day4	20.1593	
	Day5	19.9728	
	Day6	20.0450	
Intra-day Precision	Test1	19.8609	0.3246
	Test2	19.9851	
	Test3	19.9004	
	Test4	19.9683	
	Test5	20.0396	
	Test6	19.9854	

RSD = Relative Standard Deviation; concentrations are calculated from mean peak heights of triplicate injections. Inter-day study: 06/12/12-11/12/12 Intra-day study: 06/12/12

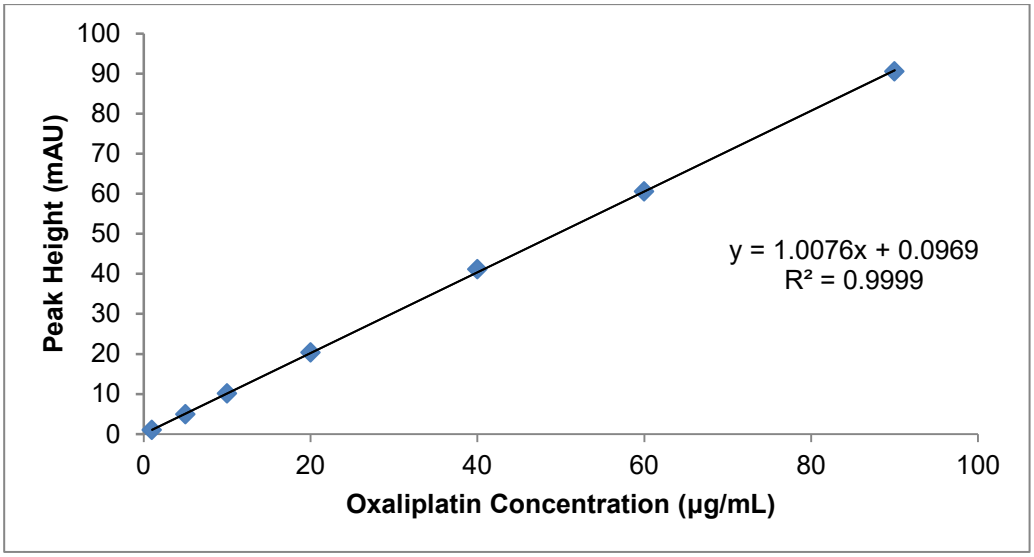

Figure 2-2, Oxaliplatin linearity of response plot with analytic concentrations and peak heights obtained with the HPLC assay.

Table 2-2, Oxaliplatin degradation and the presence/absence of additional peaks in stability-indicating validation of the HPLC assay.

Flask	Oxaliplatin Conc. (μg/mL)	Treatment 1	Treatment 2	Treatment 3	Oxaliplatin Remaining Conc. (as % of the control)	Number of Additional Peak	Oxaliplatin Peak Resolution
A	20	None	2-8℃, control	None	100	0	N/A
B	20	0.1 mL, 0.1M HCl	50℃, 30 min	1 mL,0.1M NaOH	N/A	1	resolved
C	20	1 mL, 0.1M NaOH	50℃, 30 min	1 mL, 0.1M HCl	28.72	1	resolved
D	20	1 mL, 3%H_2O_2	50℃, 30 min	None	N/A	2	resolved
E	20	1 mL, Water	50℃, 30 min	None	96.61	0	N/A

Flask A is the control solution in this validation and the results from other flasks were compared with flask A. N/A = not applicable.

2.4.2 Results from stability studies of oxaliplatin infusions

Chemical Stability data for oxaliplatin infusions (0.2 mg/mL and 0.7 mg/mL) in 250 mL 5% glucose bags under the sequential-temperature cycling storage condition have been calculated based on peak heights, and are presented in *Table 2-3*.

Results of sub-visual particle counts measured at 10μm and 25μm diameter by per millilitre are shown in *Table 2-4*.

The changes of pH-value and bag weight are both recorded in *Table 2-5*, where the data are compared to the initial values from T0.

Each table contains data from 7 groups of Group A – G, and all infusions remained clear and colourless and free from visible particles throughout the whole course of this study.

Table 2-3, Chemical stability of oxaliplatin infusions (0.2 mg/mL and 0.7 mg/mL) under the sequential-temperature storage conditions (Group A – G), showing the percentages of the remaining oxaliplatin with respect to the initial concentrations at T_0.

Infusion Group	Nominal oxaliplatin Conc. (mg/mL)	Conc. T_0 (mg/mL)	Conc. T_x Relative to T_0 [(SD)%]	Conc. T_{x+a} Relative to T_0 [(SD)%]	Conc. T_{x+a+b} Relative to T_0 [(SD)%]
Group A (x=1)	0.2	0.1840	98.57 (1.0)	98.38 (0.3)	101.80 (0.5)
	0.7	0.6490	98.92 (0.2)	97.97 (0.5)	102.04 (0.04)
Group B (x=3)	0.2	0.1888	100.06 (1.3)	99.17 (0.6)	99.64 (0.9)
	0.7	0.6666	100.02 (0.7)	99.61 (0.7)	100.08 (1.1)
Group C (x=7)	0.2	0.1902	98.58 (0.3)	98.09 (0.9)	99.48 (0.5)
	0.7	0.6642	98.63 (0.2)	98.80 (0.4)	100.15 (0.4)
Group D (x=14)	0.2	0.1892	100.55 (0.4)	99.30 (0.2)	97.61 (0.5)
	0.7	0.6642	100.04 (0.3)	99.65 (1.0)	98.32 (0.5)
Group E (x=28)	0.2	0.1914	102.12 (1.7)	97.89 (0.7)	100.21 (0.4)
	0.7	0.6745	101.46 (0.07)	97.35 (0.05)	101.38 (0.3)
Group F (x=56)	0.2	0.1922	96.58 (0.2)	98.98 (0.6)	98.39 (0.8)
	0.7	0.6637	97.23 (0.9)	99.31 (0.4)	100.13 (1.2)
Group G (x=84)	0.2	0.1868	103.03 (0.9)	99.73 (0.9)	99.93 (0.8)
	0.7	0.6566	99.80 (1.6)	99.65 (1.7)	98.64 (0.9)

Conc. = concentration; SD = Standard deviation; T_0 = Time zero; all the values are average value. Sequential-temperature cycling conditions for each group: x = days for storage at 2 – 8 ℃; a = 24 hours at room temperature; b = 7 days storage at 2 – 8 ℃.

Table 2-4, Sub-visual particle counts (per mL) of oxaliplatin infusions (0.2 mg/mL and 0.7 mg/mL) at 10 µm and 25 µm under the sequential-temperature storage conditions (Group A – G).

Infusion Group	Nominal oxaliplatin Conc. (mg/mL)	Particle Counts T_0 (per mL)		Particle Counts T_x (per mL)		Particle Counts T_{x+a} (per mL)		Particle Counts T_{x+a+b} (per mL)	
		10 µm	25 µm	10 µm	25 µm	10 µm	25 µm	10 µm	25 µm
Group A (x=1)	0.2	3.7	0.0	2.2	0.0	15.5	0.0	1.8	0.0
	0.7	9.3	0.0	3.0	0.0	1.2	0.0	1.2	0.0
Group B (x=3)	0.2	4.5	0.0	3.2	0.2	2.2	0.0	2.3	0.0
	0.7	5.7	0.0	1.0	0.0	3.5	0.0	1.2	0.0
Group C (x=7)	0.2	2.2	0.0	2.3	0.0	1.7	0.0	2.7	0.0
	0.7	6.2	0.0	2.5	0.2	1.7	0.0	3.2	0.0
Group D (x=14)	0.2	2.5	0.2	2.2	0.0	2.5	0.2	3.8	0.0
	0.7	15.5	0.0	1.0	0.0	2.7	0.0	3.3	0.0
Group E (x=28)	0.2	4.8	0.0	4.2	0.2	5.2	0.2	6.7	0.0
	0.7	5.2	0.0	6.2	0.5	5.2	0.3	10.0	0.2
Group F (x=56)	0.2	3.2	0.0	6.5	0.2	4.8	0.0	5.3	0.2
	0.7	3.0	0.2	3.2	0.0	4.0	0.2	3.5	0.0
Group G (x=84)	0.2	3.5	0.0	3.8	0.0	4.3	0.0	8.0	0.5
	0.7	7.5	0.0	3.3	0.0	4.8	0.0	5.0	0.0

Conc. = concentration; SD = Standard deviation; T0 = Time zero; all the values are mean values, n=3. Sequential-temperature cycling conditions for each group: x = days for storage at 2 – 8 ℃; a = 24 hours at room temperature; b = 7 days storage at 2 – 8 ℃.

Table 2-5, The pH-value and weight of oxaliplatin infusions (0.2 mg/mL and 0.7 mg/mL) and the percentage variations in pH compared to the initial value (T_0) under the sequential-temperature storage conditions (Group A – G).

Infusion Group	Nominal oxaliplatin Conc. (mg/mL)	T_0		T_x		T_{x+a}		T_{x+a+b}		Weight Change in T_x (g)	Weight Change in T_{x+a} (g)	Weight Change in T_{x+a+b} (g)
		pH	pH Difference Relative to Initial T_0 (%)	pH	pH Difference Relative to Initial T_0 (%)	pH	pH Difference Relative to Initial T_0 (%)	pH	pH Difference Relative to Initial T_0 (%)			
Group A (x=1)	0.2	4.38	0.0000	4.42	0.9130	4.39	0.2280	4.41	0.6850	0.0000	0.0000	0.0667
	0.7	4.41	0.0000	4.47	1.3610	4.43	0.4540	4.47	1.3610	0.0333	0.0333	0.0000
Group B (x=3)	0.2	4.28	0.0000	4.36	1.8690	4.39	2.5700	4.38	2.3360	0.0333	0.1000	0.0670
	0.7	4.36	0.0000	4.45	2.0640	4.46	2.2940	4.46	2.2940	0.1000	0.0667	0.0000
Group C (x=7)	0.2	4.27	0.0000	4.41	3.2790	4.43	3.7470	4.42	3.5130	0.0667	0.0000	0.0667
	0.7	4.33	0.0000	4.47	3.2333	4.48	3.4642	4.46	3.0020	0.0667	0.0000	0.0667
Group D (x=14)	0.2	4.38	0.0000	4.36	0.4566	4.36	0.4566	4.39	0.2283	0.0000	0.0333	0.1000
	0.7	4.44	0.0000	4.43	0.2252	4.43	0.3352	4.46	0.4505	0.0333	0.0000	0.0333
Group E (x=28)	0.2	4.40	0.0000	4.43	0.6818	4.45	1.1364	4.42	0.4545	0.1000	0.0667	0.1000
	0.7	4.45	0.0000	4.48	0.6742	4.50	1.1236	4.47	0.4494	0.1000	0.1000	0.1000
Group F (x=56)	0.2	4.40	0.0000	4.39	0.2273	4.42	0.4545	4.39	0.2273	0.1667	0.0667	0.1000
	0.7	4.44	0.0000	4.42	0.4505	4.45	0.2252	4.43	0.2252	0.1333	0.0000	0.0333
Group G (x=84)	0.2	4.40	0.0000	4.38	0.4550	4.40	0.0000	4.40	0.0000	0.2000	0.0000	0.0333
	0.7	4.44	0.0000	4.39	1.1260	4.43	0.2250	4.43	0.2250	0.1667	0.0333	0.1000

Conc. = concentration; SD = Standard deviation; T0 = Time zero; all the values are average value. Sequential-temperature cycling conditions for each group: x = days for storage at 2 – 8 ℃; a = 24 hours at room temperature; b = 7 days storage at 2 – 8 ℃.

2.5 Discussion

2.5.1 Analysis of results on chemical stability

Specific monographs for oxaliplatin infusions are not included in official documents, such as European Pharmacopoeia (EP) or British Pharmacopoeia (BP). However, in the Revision Bulletin of The United States Pharmacopoeia the oxaliplatin injection is referred to and it is required that these injections should contain NLT (not less than) 90.0% and NMT (not more than) 110.0% of the labelled dosage of oxaliplatin. (167) The ± 10% acceptance criterion has also been widely used in a majority of stability studies of cytotoxic drugs in Europe. (154) (168) Recently, however, there has been a growing consensus that the practical stability limits should be defined by taking into account certain factors such as the therapeutic index of the drug, its clinical use, safety and potency and its pharmacodynamics/pharmacokinetic variability. (149) By this criteria it has been recommended that the stability limit for anticancer drugs with low therapeutic indices should be restricted to ± 5%, (149) and for this reason the acceptance criteria of 95% - 105% was adopted for oxaliplatin concentration in this study.

Table 2-3, which shows the experimentally derived chemical stability data of oxaliplatin infusions from this study, demonstrates that the drug concentrations did not vary by more than ± 4% of the initial concentrations at any of the sample points, which meets the ± 5% acceptance limit. On the other hand, these accepted concentration changes suggest no significant absorption of oxaliplatin onto the container walls, which is another main potential cause of drug loss occurring independently and/or in addition to the drug degradation.

In addition to the stability of oxaliplatin itself, the degradation products and metabolites of oxaliplatin must be considered in order to ensure the safe use of drug infusions. For instance, oxalate is one of the main degradation products of oxaliplatin, and is also a calcium chelator which may partly contribute to the neurotoxicity of oxaliplatin. (147) From *Figure 2-1* showing the degradation of oxaliplatin, it could be deduced that 1 mol oxaliplatin can liberate 1 mol oxalate if fully degraded. Oxalate generally exists in several different forms such as oxalic acid (*Figure 2-3*), and as oxalate salts such as sodium and calcium oxalate. Because the oxalic acid is the most basic form among this group, it can be used as an example in this study. (126) (127) (147) Hypothesis of the worst case permitted by the ± 5% degradation limits imposed above means 5% of oxaliplatin totally degrade into oxalic acid, and the equation below can be used to calculate the content mass of oxalic acid. (162) (166)

$$M = \frac{m}{n}$$

(M = the molar mass; m = the mass; n = the number of moles)

For the infusion bags in high concentration of 0.7 mg/mL and low concentration of 0.2 mg/mL, a 5% loss of oxaliplatin by degradation would produce oxalic acid of 1.9825 mg and 0.5663 mg respectively in each dose. Acute toxicity studies indicate that oxalic acid is of moderate to high toxicity by the oral route in mammalian species, and the LD50 (lethal dose to kill 50% of test population) orally in rats is reported as 375 mg/kg of body weight. (169) (170) The data for oxalic acid toxicity to human beings are insufficient, but the LDL_0 (minimal dose found to be lethal) orally in human has been documented as 71 mg/kg. (170) The overall average BSA of patients in UK is 1.79 m^2 which requires an oxaliplatin dose of 152.15 mg based on 85 mg/m^2 standard dose, and the

degradation of 5% of this dosage would give at most 2.0 mg oxalic acid. This is well within the limit range of LDL_0 mentioned above, although the limits refer to the oral route whereas oxaliplatin is administered by the IV route. (122) (157) With the reservation that the bioavailability of oral oxalate will be lower than the IV route, the acceptance criteria of oxaliplatin stability studies of ± 5% can be justified. Moreover no additional HPLC peaks contributed by other degradation species were apparent in this study. However, the possibility of other oxaliplatin degradation products cannot be ruled out as some species may not be detected by the UV detection system in this HPLC assay. (162) (166)

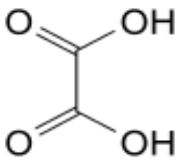

Figure 2-3, Chemical structure formula of oxalic acid (ethanedioic acid).

The current lack of data provided by pharmaceutical companies and difficulties in retrieving the experimental data from industry are a critical issue for stability forecasting of cytotoxic drug preparations in current practice. (171) For oxaliplatin, there are only few published studies available in the public domain concern with chemical and physical stability, and SPCs (Summary of Product Characteristics) provided by different companies show a high variation in shelf life statements. (172) The most recent SPC available gives a shelf-life for oxaliplatin infusions in 5% glucose of 48 hours at 2 – 8℃ and 24 hours at 25℃. (122) It is likely however, that the short shelf-lives supported by the pharmaceutical industry are associated more with concerns about sterility of aseptically manipulated infusions than physical and chemical stability. Of the reports currently available (see Section 2.1.1 of Introduction), the longest period of oxaliplatin infusion stability reported was 90 days at temperature of 4℃ or

25℃ with a drug concentration of 0.25 mg/mL. (151) All these studies explored exposure to light and it was demonstrated that exposure to light did not significantly affect the stability of oxaliplatin infusions. (151) (154) (168) (173)

Several limitations have been found with these previous stability studies on oxaliplatin infusions. For example, only one study considered the oxaliplatin dosage based on the recommended BSA calculation of 85 mg/m^2, while the concentrations or concentration ranges selected by other studies were not evidence-based. (154) (173) The acceptance criteria for oxaliplatin concentrations applied in previous studies were all 90% - 110%, which is not appropriate for a cytotoxic drug with narrow therapeutic index, as discussed previously.

Additionally, the various temperatures experienced by oxaliplatin infusions in clinical practice and pharmaceutical storage, and the transfer of infusions between these different conditions were not considered in these studies. (151) (154) (168) (173) Unlike this study (Refer to *Table 2-3*) previous stability studies of oxaliplatin infusions fail to meet the current requirements of clinical practice and dose-banding. (8) (149) This study, on the other hand, included a rigorously derived concentration range of 0.2 mg/mL to 0.7 mg/mL based on the licenced BSA dose (122), an acceptance criteria of 95 – 105% for oxaliplatin concentration concordant with current thinking on cytotoxic drug stability, and finally the use of a sequential temperature design which is distinct from the other studies on the stability of oxaliplatin infusions.

This study is also distinct from others in that it provides evidence that infusion bags can remain stable when exposed to room temperature (25℃) for 24 hours and returned to a refrigerator of 2 – 8℃ for further 7 days. (159) Because the

sequential-temperature design provides definitive physical and chemical stability data under the additive storage conditions at 2 – 8℃ and 25℃, and also accounts for the effects of temperature cycling, it is possible to assign shelf-lives that have the potential to reduce drug wastage. The study design, which incorporates triplicate infusion evaluation at each temperature/concentration combination, improves rigour over previous studies.

2.5.2 Analysis of results on physical stability

2.5.2.1 pH change

pH is one of the most important characteristics that could influence the stability of oxaliplatin infusions in several ways as shown below.

Firstly, the ionisation, and hence solubility, of most substances is affected by the pH of the solution, which means not only could the physical stability of oxaliplatin be affected, but the pH change could also affect other components of the infusion. (162) (174) A pH change may therefore affect the efficacy and safety of infusions as well as its suitability for intravenous injection. (122) (175)

Extremes of pH are associated with vascular pain and phlebitis, although other factors are also implicated including osmotic pressure of the infusion, size of the vein used, size and material of the catheter and infusion periods. (176) According to a previous study, the optimal infusion pH for the peripheral vein is about 6.5 units. (177) The pH of oxaliplatin infusions in the range of 4.27 – 4.50 are shown in the results section (*Table 2-5*), it is considered that this low pH partly causes the negative side effect on patients reported in clinical practice. (176) (177) Therefore any further decrease in pH is not desirable.

In addition, pH is one of the factors contributing to the degradation rate of oxaliplatin in aqueous media. (127) It has been established that oxaliplatin is

normally transformed by water and nucleophiles such as Cl^- and HCO_3^-, while the main source for the pH variation is CO_2 diffusion through the wall of the plastic bag and subsequent acidification producing HCO_3^-. (127) (149) Therefore, the pH variation caused by CO_2 contamination would increase the rate of oxaliplatin degradation and potentially influence the stability of the infusions.

Therefore the pH of oxaliplatin infusions should be monitored carefully and reported throughout the study period, and its variations must be properly interpreted. (149)

As shown in *Table 2-3*, pH-values in this study varied slightly at each time point, while the maximum pH deviations of the infusions of 0.2 mg/mL and 0.7 mg/mL were 0.16 and 0.15 units, respectively, from the initial values. Because these differences are much lower than the pre-defined acceptance criteria of ± 1.0 unit, the pH variations observed in this study were considered to be of no pharmaceutical or clinical significance. (162) (166)

Furthermore, there could be several reasons for such minor changes in pH, such as the initial pH of oxaliplatin concentrate (normal pH range is 4.0 – 7.0) (122) and 5% glucose infusions (pH range is 3.5 – 6.5, BP) (166); and finally dissolved CO_2 from air in the infusion bag could also have a small effect on pH.

In previous stability studies on oxaliplatin infusions physical stability, physical stability tests were limited to only visual appearance and pH measurement. (154) (168) However, sub-visual particulate counts and weight change monitoring should also be applied in stability studies to ensure physical stability and water-transfer across the container walls are monitored effectively. (149) (161) Without this, it would be possible to miss particulate aggregation at the

sub-visual level and to overlook water loss from the infusion that could mask drug loss due to chemical degradation.

2.5.2.2 Weight change

Variations in the concentration of oxaliplatin could also occur as a result of weight changes due to the diffusion of water through the plastic bag wall. (149) Weight changes observed in this study, shown in *Table 2-5*, did not exceeded 0.0750%, which implies that the oxaliplatin concentration was not significantly influenced by the loss or gain of water. (149) It is important to test for the moisture loss from infusion bags, since significant permeation (e.g. ≥ 2%) and water loss from the infusion could mask the loss of oxaliplatin occurring as a result of drug degradation.

2.5.2.3 Visible appearance and particulate counts

Both visual and sub-visual particle characterisation always has significance in stability studies, which should be well defined and standardized. (149) (163) Such particulate examinations are crucial to prevent potential adverse effects from particle contamination. (149) (163) For example, particles can cause toxicity to specific tissues or organs; or distribute via blood and block the blood flow causing an embolism if they reach a certain size. (163) Another severe effect is that particles could be identified by the immune system as foreign materials, which could result in an immune reaction causing secondary effects. (163) Furthermore, the particulate nature of the drug can affect its bioavailability, solubility, filterability, all of which may result in an unfavourable clinical outcome. (163)

In this study, the particle examination was divided into two sections, visual particle inspection and sub-visual particle evaluation, each of which was determined by a different technique. (158) (178)

The visual test can detect formation of visual particles and also any change to the initial colour of solutions. (149) (178) In this study, the infusion bags were examined against both black and white backgrounds under normal laboratory lighting, enabling a visual qualitative assessment to be achieved. (162) (178) Any colour changes would be easily observed because of the colourless and transparent characteristics of oxaliplatin infusions. (162) As shown in the result section (Section 2.4.2), all the infusion solutions remained clear and colourless with no visible particles throughout this study period.

With no visible changes apparent, micro-precipitates can still appear, and by increasing in size with time could eventually induce the formation of visible precipitates. (149) Therefore, a sub-visual particulate evaluation is required for stability studies. Using the method from pharmacopoeia, a sub-visual particle counter based on light obscuration was used to count sub-visual particles of 10 μm and 25 μm diameter in this study. (162) (178) In accordance with the International Pharmacopoeia requirements, the sub-visual particulate count (*Table 4*) was expressed as counts per millilitre for oxaliplatin infusion bags with nominal volume of 250 mL, and remained well below the criteria of ≤ 25 units per millilitre for 10 μm particles and ≤ 3 units per millilitre for 25 μm particles. (162) (178) These results indicated that there is no significant sub-visual particle contamination in oxaliplatin infusions with time. Because there was no marked increase in the counts for the larger 25 μm particles over time, there was no pharmaceutically significant particle aggregation. (149) This was important to

establish as the temperature cycling used in this sequential-temperature design could have induced particulate growth. (179)

2.6 Limitations, further work and clinical application

2.6.1 Limitations

The range of oxaliplatin concentrations used in this study was 0.2 mg/mL to 0.7 mg/mL which has been calculated from the average patients' BSA in the range of 0.59 – 2.06 m^2 and the oxaliplatin dose of 85 mg/m^2 provided on SPC. (122) (157) This range of oxaliplatin concentrations will cover the majority of patients but the requirements of patients with extreme BSA values were not considered in this study. (157) In clinical practice, such extreme cases are normally excluded from dose-banding schemes and doses are provided on a patient-specific basis.

The stability duration of oxaliplatin infusions was limited to 92 days through all the temperature cycles in this study with the design of sequential-temperature cycling. There is the possibility that longer stability duration of oxaliplatin infusions might be required, while the result of this study is limited to satisfy the demand. Nevertheless, this 92 days shelf-life of oxaliplatin infusion is sufficient for clinical application, and it also could facilitate a 'dose-banding' scheme which ideally requires 3 months of infusion stability in storage. (8) Therefore, the design and duration of this study could be considered reasonable. In the UK, the MHRA regulatory inspectorate are reluctant to approve infusion shelf-lives for aseptic products beyond 3 months because of microbiological and container integrity concerns. (180)

According to EP (European Pharmacopeia), plastic containers for parenteral infusions are manufactured from polymers, and the most commonly used

polymers are polyethylene, polypropylene and polyvinyl chloride (PVC). (162) Considering the limited experimental conditions (e.g. limited storage space), there was only one type of infusion container used in this study, which was polyolefin, a newer plastic from Fresenius Kabi, UK, which does not leach plasticisers into the infusion. (181) However, this modern material is now widely adopted into clinical practice and polyolefin is seen as the 'gold-standard' for infusion containers. The data reported in this study could not be extrapolated to containers constructed of other materials without additional experimental work to certify that there is no effect on either chemical or physical stability of the infusion.

Additionally, oxaliplatin concentrate of 5 mg/mL from Fresenius Kabi Oncology Plc. was the only formulation and brand used in this study. There are two different formulations of oxaliplatin manufactured from different suppliers, which are lyophilised powder and concentrate injection. (122) (182) Considering oxaliplatin concentrate is widely used to be diluted with glucose in clinical practice, this formulation was selected in this study while the other brands and formulations of oxaliplatin were not included. The use of cytotoxic drug formulations presented as lyophilised powders is declining on the basis of the additional reconstitutions required and perceived additional risks of cytotoxic exposure to pharmacy staff.

2.6.2 Potential avenues for further investigation

This study has not involved the validation of long-term storage of oxaliplatin infusions at ambient temperature of 25 ℃. Considering pharmaceutically practical conditions with limited refrigerated storage space, drug infusions could be prepared and stored at room temperature, therefore the duration of infusion

stability under this condition might be useful. (182) (173) However, for most aseptically-prepared infusions refrigerated storage is preferred to limit the proliferation of any inadvertent microbiological contamination. To realistically replicate the various practical situations, additional experimental stressed-conditions studies could be included, such as exposure to light for a short time period, accidental freezing, or exceeding temperature limits for a few hours. (149) Moreover, it has been shown in this study that 'in-use' infusion bags could be returned to the refrigerated temperature (2 – 8℃) and stored for a further 7 days while maintaining stability. This period could potentially be extended within the experiment design to offer more information for hospitals or clinical departments on stability in cases where treatment delays are longer than 7 days, and further increase the flexibility of oxaliplatin infusions use. (36)

2.6.3 Clinical application

In addition to extending the shelf-life of oxaliplatin infusions in 5% w/v glucose to 84 days, this study also demonstrated that it is safe to use these infusions which have been placed at 25℃ and returned in refrigerator for another 7 days. The conclusion from the sequential-temperature cycling included in this study could avoid unnecessary wastage of re-issued drugs in some situations, such as the unexpected failure of a refrigerator, bags stored at room temperature or the return of unused bags from the clinic because of treatment delays. (149) (159) The range of concentrations in this study (Section 3.1.2) was selected carefully based on the BSA dose of 85 mg/m^2, which would include virtually all requirements for cancer patients. (122) In this case, the data from this study could facilitate a 'dose-banding' scheme. This approach is predicated on long term chemical and physical infusion stability to allow anticancer drug infusions to be prepared in advance. (13)

However, as the microbiological quality depends on the individual working and handling practice, the application of the extended shelf-lives requires rigorously controlled and validated pharmacy aseptic units accredited for the preparation of oxaliplatin infusions. (162) (173) Thus, with the secure knowledge of the production environment and appropriate storage conditions, all the results from this study could be applied for clinical practice by adopting rigorous preparation procedures and using appropriately trained staff in the preparation process. (149)

2.7 Conclusion

Oxaliplatin infusions at 0.2 mg/mL and 0.7 mg/mL concentrations in 250 mL 5% glucose contained in polyolefin bags were chemically and physically stable for at least 84 days at 2 – 8℃. For the temperature sequential section, oxaliplatin infusions were also stable under the further storage at 25℃ for 24 hours and the following 7 days at 2 – 8℃ in a refrigerator. All the bags were stored with protection from light. This result indicated a long-term stability period for oxaliplatin infusions of concentrations between 0.2 and 0.7 mg/ mL, which allows the pre-preparation of oxaliplatin infusions for dose-banding schemes.

Chapter 3: Studies of oxalate formation in oxaliplatin solutions

3.1 Introduction

3.1.1 Background

3.1.1.1 Oxalate and neurotoxicity from oxaliplatin

As a third generation of platinum compound, oxaliplatin can be used for treatment against some tumours which exhibit primary resistance to cisplatin and carboplatin, which are from the same cytotoxic drug group. (123) Even though oxaliplatin has a more tolerable side-effect profile compared with cisplatin and carboplatin, sensory neurotoxicity is the main dose-limiting toxicity in oxaliplatin chemotherapies. (3) (146)

About 85 – 95% patients with oxaliplatin treatment suffer from the sensory neuropathy during and/or shortly after the infusion, which might be triggered and aggravated by exposure to low temperatures. (12) (109) The particular neurosensory toxicity caused by oxaliplatin is acute and rapidly reversible, which can be characterized by paraesthesia and dysesthesia of hands, feet and perioral area, and jaw tightness. (12) (109) The chronic neuropathy from oxaliplatin (which also occurs with cisplatin) is believed to be a cumulative effect, which may increase in both duration and severity with repeated administration of oxaliplatin infusions. (12) (109) (183) These symptoms can be reversed within 4 – 6 months following completion or discontinuation of treatment, without any permanent impairment of sensory functions in patients. (109) (146) Thereby the circadian-guided dosing of oxaliplatin is potentially effective in limiting the neurological toxicities. (109) (146) Compared to the continuous infusion treatment, the dosing schedule based on circadian rhythm could also increase the anti-tumour activity of oxaliplatin. (109)

Mechanisms for both types of neurotoxicity of oxaliplatin are still not clear, but previous studies have been conducted on the basis of various hypotheses in the literature. (109) (147) (183) Those hypotheses were all based on the degradation process of oxaliplatin in blood circulation of patient bodies.

After administration to patients, one third of the oxaliplatin dose binds irreversibly to erythrocytes and one third can form complexes with albumin and other plasma proteins. (124) The remaining free fraction of oxaliplatin normally undergoes non-enzymatic biotransformation and the oxalate ligand of oxaliplatin can be displaced by non-enzymatic hydrolysis. (109) (Shown in *Figure 3-1*) Degradation products of oxaliplatin, Pt-DACH (R-diamino-cyclohexane platinum) and oxalate, subsequently react with endogenous chloride and water to form monochloro, dichloro and diaquo complexes with chloride glutathione, methionine and cysteine. (109) (124) As a result, oxalate complexes are the main degradation products from the initial non-enzymic transformation of oxaliplatin *in vivo*.

Two hypotheses aimed at chronic neuropathy and transient acute neurotoxicity have been widely considered for oxaliplatin neurotoxicity, which focus on platinum-DNA interactions and ion-channel involvement. (147) (183) In both cases, the major damage sites of the neurotoxicity from oxaliplatin are peripheral sensory neurons and dorsal root ganglia (DRG) cells. (146)

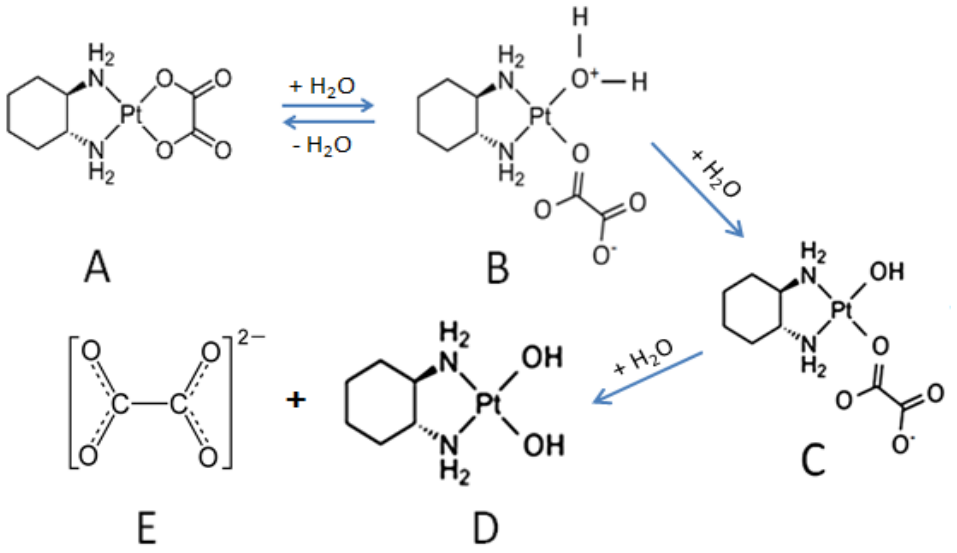

Figure 3-1, The initial non-enzymatic hydrolysis biotransformation of oxaliplatin *in vivo*. [A: oxaliplatin; B: the protonated oxalate monodentate intermediate; C: the de-protonated oxalate monodentate intermediate; D: dehydrated oxaliplatin complex; E: oxalate.]

The cumulative chronic neurotoxicity of oxaliplatin also appears with other platinum drugs, such as cisplatin, and might be correlated with the Pt-DNA binding. (146) A previous study showed that compared with cisplatin oxaliplatin produced fewer Pt–DNA adducts and was less neurotoxic to DRG neurons *in vitro*. (146) The inhibition to rRNA synthesis by oxaliplatin was indicated as a mechanism of damage of sensory nerve nucleoli, which was recognised by measuring the nucleolar shrinkage and disintegration. (183) Another hypothesis arising from previous cisplatin studies indicated that the accumulation of heavy metal (Pt) in neurons could influence on their torpidity. (147)

The acute, but rapidly reversible neuropathy is specifically associated with oxaliplatin therapies rather than other cytotoxic drugs in the platinum group, and it is believed to be related to ion-channels located in cellular membrane. (12) (147) The acute neurosensory symptoms caused by oxaliplatin are similar to the side-effect of some drugs/toxins that react with neuronal or muscular ion-channels. (12) (147) In this case, it is suspected that the neurotoxicity is caused by the interaction of oxaliplatin and one or more channel subtypes. (12) The

acute neurological symptoms might also reflect the hyper-excitability of peripheral nerves resulted from the transient channelopathy induced by oxaliplatin. (12) According to the degradation process of oxaliplatin (refer to *Figure 3-1*), the voltage-gated ion channels could be interfered with by its degradation products, such as oxalate and the charged chloride complex. (145) It has been proven that the monochloro- complex of oxaliplatin has no general surface-charge effect on those ion channels, which means oxalate, as the main degradation product of oxaliplatin, could be the most significant cause of the neurotoxicity. (145)

Oxalate produced from oxaliplatin might accumulate and interfere with intracellular divalent cation, such as calcium (Ca^{2+}) and/or magnesium (Mg^{2+}), and break the intracellular homeostasis within the neuronal systems. (147) Oxalate is a well-known calcium chelator, which could disrupt the voltage-gated sodium ion (Na^{+}) channels, especially Ca^{2+}-dependant Na^{+} channels, via Ca^{2+} chelation in sensory neurons. (12) (109) Then the disordered Na^{+} channel might induce the acute toxicity to neuron cells and subsequently result in neuropathy. (109)

If it could be verified that the intracellular Ca^{2+}/Mg^{2+} reduces in neuro systems of the patient who suffers neurotoxicity from oxaliplatin chemotherapy, then the possibility of oxalate being the main cause of neurotoxicity should be considered. A previous study demonstrated that during the oxaliplatin chemotherapy for colorectal cancer, the use of additional infusions containing calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions could reduce the incidence of sensory neurotoxicity without any adverse influence on tumour response. (109) Therefore, oxalate might be involved in the neurotoxicity of oxaliplatin through interference with the voltage-gated sodium channel.

The hypotheses mentioned above are all subject to a lack of evidence to indicate the clear mechanism of neurotoxicity associated with oxaliplatin treatment. However, the association between oxalate and neurotoxicity is one of the more plausible theories, and so this study examined the prevalence of oxalate derived from oxaliplatin in both, oxaliplatin infusions and human plasma.

3.1.1.2 Oxalate toxicity to human body

Oxalate, as shown in *Figure 3-2*, is a dianion with formula of $C_2O_4^{2-}$, which has been found in a wide range of plants and high consumption foods. (184) Oxalate is also an important metabolite in the human body. (185) For humans, the intake of oxalate in regular daily diet is within the average range of 130 mg – 152 mg per day. However it is thought that only about 50 mg oxalate can be absorbed by the human body. (186) The amount of oxalate in animals and humans originates from two sources: i) metabolic end-product produced largely in the liver; ii) absorbed directly from food in the gastrointestinal tract (GIT). (186) As shown in *Figure 3-3*, oxalate in blood circulation is mainly produced metabolically in the liver (85% - 90%), whereas food provides only 2% - 10% of total oxalate in blood. Most oxalate from food turns into an energy source by the intestinal bacterial or be eliminated in faeces. (186) The main pathway for oxalate elimination is through the kidney, where 90% - 95% of blood oxalate is removed from organism and excrete in urine. (186) The remaining 5% - 10% oxalate is secreted in the intestine. (186)

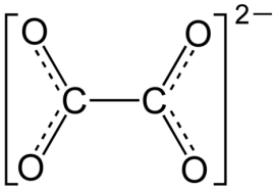

Figure 3-2, Chemical structure of oxalate anion.

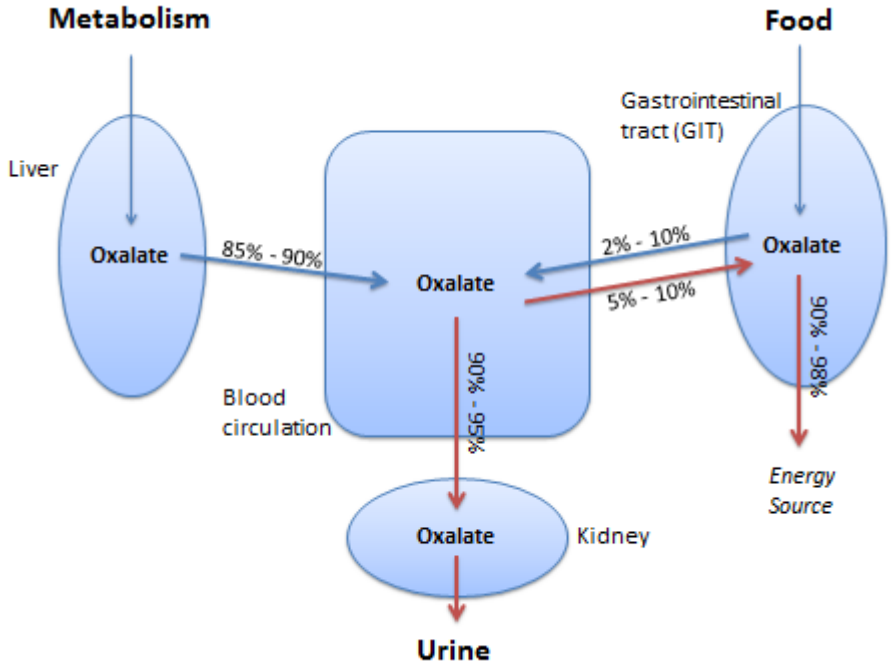

Figure 3-3, Pathways of oxalate in animal/human body. Oxalate in blood circulation is generated from two sources: internal metabolism and food absorption. The excretion of blood oxalate is mainly through kidney, while the un-absorbed oxalate in food is mostly used to provide energy for the body.

As a regular compound in general diet, overdosed oxalate could cause kidney stones by chelation with calcium ions (Ca^{2+}). (187) In human/animal body, oxalate can rapidly form crystals with calcium ions and produce calcium oxalate (CaOx), which is the most abundant compound in the formation of kidney stones. (186) (188) Kidney stones are the combination of protein complexes and crystal formations, including calcium oxalate (CaOx), calcium phosphate (CaP), purines and other uncommon stones. (186) (187) In more than 80% cases of urolithiasis, calcium oxalate is the predominant compound in kidney stones. (186) (188) In kidney, crystallisation occurs in several stages including solid phase precipitations, growth, aggregation and stone formation. (186) However, these kidney stones could only happen under the abnormal conditions in human body, such as damage to the integrity of tubular epithelium or higher tubular oxalate concentration. (186) (188) Furthermore, oxalate also has proven toxicity to renal tubular cells at high concentrations of ≥ 5 mmol/L.

(188) Therefore, the physiological concentration of oxalate in the body (plasma and urine samples) is a key factor to indicate and prevent renal toxicity and kidney stones.

For a healthy human person, the physiological concentration of oxalate from urine samples is within the range of ≤ 1mmol/L (188) (189) and the plasma oxalate is normally lower at concentrations of 1 – 3 µmol/L. (190) (191) However, after oxaliplatin administration, the plasma oxalate in patients could be increased by the oxalate derived from oxaliplatin degradation.

In this study, the amount of oxalate produced in oxaliplatin infusions was determined, together with a simulation of the non-enzymic transformation of oxaliplatin *in vivo* in which oxaliplatin was incubated with blood plasma at physiological temperature (37℃).

3.1.2 Method selection for oxalate assay

3.1.2.1 Oxalate assays with HPLC methods

As a normal constituent of food, oxalate is commonly monitored in water, agriculture, plants and food quality control since its potential toxicity to human bodies. (184) In previous studies on oxalate, various assay methods were applied with the most common detection methods of: HPLC, Gas Chromatography (GC) and Spectrophotometric/Colorimetric (590 nm) in conjunction with soluble oxalate oxidase and peroxidase.

In this study, oxalate concentration was required to be detected in samples that contain various components including oxaliplatin. Compared with spectrophotometric method, HPLC offers a separation process to gain more accurate result of oxalate concentrations in different samples. Furthermore, unlike GC, no solvent extraction process was required with aqueous samples in

HPLC methods. *Table 3-1* shows various HPLC assays for oxalate determination, from which a specific assay was developed and validated for oxalate determination in this study.

A HPLC assay with C_{18} column modified from the literature was tested with samples of oxaliplatin infusions to separate oxalate in chromatography. But oxalate could not be completely separated from oxaliplatin and the shape of oxalate peak was undesirable. In general, ODS columns (Silica-based C_{18} column) were often used for analysis of oxalate. However, oxalate is highly hydrophilic and poorly retained on ODS columns, which raised the difficulty to achieve the adequate oxalate separation in the chromatogram. (192) To overcome this problem, an amide column was used in this study, the Inertsil Amide column (4.6 mm × 250 mm and with 5.0 µm particle size from GL Science). The porous silica gel, packed in this amide column, has been chemically bonded with carbamoyl group and the polar interaction increases retention of oxalate on the stationary phase. (192) As a polar solute, oxalate would be better resolved by this assay in the HILIC mode (hydrophilic interaction chromatography) with a more polar mobile phase containing 65% acetonitrile. (193)

Table 3-1, Summary of published Liquid Chromatography methods for oxalate determination in biological samples.

Literature	**EP 2012** (164)	**Rajendra B. Kakde 2012** (194)	**Vinay B Patel 2012** (195)	**Tarkatti Kaleemullah 2011** (196)	**T. Machold 2009** (197)
Substance	Oxalic acid	Escitalopram oxalate & Clonazepam	Escitalopram oxalate (ESC) & Etizolam (ETI)	DPTTA in Escitalopram oxalate	Low-molecular-weight carboxylates
Column	C_{18}; 5 µm; 4.6×250 mm	C_{18}; 5 µm; 4.6×250 mm	C_{18}; 5 µm; 4.6×150 mm	C_8; 5 µm; 4.6×150mm	C_{18}; 5 µm; 4.6×150 mm
Flow Rate	2.0 mL/min	1.5 mL/min	1.0 mL/min	1.0 mL/min	1.0 mL/min
Temperature	40 ℃	40 ℃	30 ℃	25 ℃	25 ℃
Injection Volume	20 µL	20 µL	10 µL	20 µL	20 µL
Wavelength	205 nm	268 nm	254 nm	240 nm	215 nm
Run Time	10 min	10 min	10 min	15 min	10 min
Mobile Phase	acetonitrile & phosphate	acetonitrile & 50 mM phosphate	acetonitrile & 0.005 M Hexane Sulfonic Acid	acetonitrile & 0.02% (v/v) of Orthophosphoric acid	25 mM KH_2PO_4 & H_3PO_4
Retention Time	2.7 min	6.6 min & 9.4 min	3.66 min & 8.07 min	4.0 min	1.7 min (Oxalic acid)

Continued

Literature	**C. Murphy 2009** (198)	**Joseph E. Zerwekh 1983** (199)	**Evelyn A. Havir 1982** (200)	**Hua-Wei Xu 2006** (185)	**Warren D. Holloway 1989** (201)	**Alastair B. Ross 1999** (202)
Substance	Oxalic acid	Urinary oxalate	Separation of organic acids	oxalate, citrate, and malate	Organic Acids	Oxalate
Column	C_{18}; 4 µm; 4.6×250 mm	High Pressure Liquid Chromatograph (Water Corasil II Column)	Aminex HP-87H column (9µm; 7.8×300mm)	C_{18}; 5µm; 4.6×250mm	ion-exclusion column(HPX-87H); 300 mm X 7.8 mm	ion exclusion column (HPX-87H); 300×7.8 mm
Flow Rate	4.0 mL/min	2.0 mL/min	0.6 mL/min	1.0 mL/min	0.5 mL/min	0.6 mL/min
Temperature	37 ℃	N/A	25℃	N/A	N/A	N/A
Injection Volume	20 µL	5.0 µL	N/A	N/A	N/A	N/A
Wavelength	210nm	211 nm	210 nm	220 nm	214 nm	210 nm
Run Time	35min					
Mobile Phase	0.25% KH_2PO_4 & 2.5 mM $C_{16}H_{36}N{\bullet}HSO_4$	120 mmol/L phosphate buffer	0.03 M H_2SO_4	0.5% KH_2PO_4 & 0.5 mM TBA	0.125 M H_2SO_4	0.0125 M H_2SO_4
Retention Time	N/A	35 s	N/A	N/A	9.8min	7.0min

3.1.2.2 Oxalate assay kit

The validated HPLC assay with an amide column mentioned above was sufficient for oxalate determination in oxaliplatin infusions (in 5% glucose). However, for plasma/PUF (plasma ultra-filtrate) samples, it was envisaged that this HPLC assay might not be capable of resolving the oxalate peak from the interference of various constituents in plasma samples. Furthermore, this HPLC assay was not expected to be sensitive enough to examine the low concentrations of oxalate in plasma/PUF samples. In plasma/PUF samples, the concentration of oxalate would be expected to be around 5 µmol/L. (191) Therefore, an alternate oxalate assay with better specificity and sensitivity was considered for oxalate determinations in plasma/PUF samples.

Enzymatic methods with spectrophotometer/colorimeter were used in previous studies in literature, which were generally applied with oxalate oxidase and peroxidase. (191) (190) Compared to HPLC assays, spectrophotometric methods for oxalate determination were capable of higher sensitivity with a working concentration range of 0.6 µmol/L – 20 µmol/L. (191)

As a result, an oxalate kit, from Trinity Biotech, specific for oxalate determination was selected to test plasma/PUF samples, since this method met both specificity and selectivity criteria required in this study. The enzymatic method with this oxalate kit was based on the oxidation of oxalate (by oxalate oxidase), which can produce hydrogen peroxide (H_2O_2). The quantity of H_2O_2, measured by a peroxidase-catalysed reaction, could reflect the concentration of oxalate in samples.

The assay with this oxalate kit was originally designed for quantifying oxalate in urine samples. However, a study carried out by *Ladwig* (191) has suggested

that this assay was applicable for plasma oxalate with a good linearity over a concentration range of 1 – 50 µmol/L. (191) In addition, more validation tests were conducted before the experiment to confirm the accuracy and precision for oxalate determination in this study. The mechanism and validation of this assay were described in details in Section 3.2.4 (page 160) below.

3.1.3 Experiment design

As mentioned above, oxalate was postulated to be the main cause of the neurotoxicity associated with oxaliplatin treatment. However, the mechanism of this neuropathy side effect was not clearly understood. According to the published literature oxalate was only proved to be associated with neuropathy. Because oxalate was a common metabolite in the human body, it was reasonable to expect that the oxalate in the human body would need to reach a critical concentration to cause any toxic effect in organs. After the administration of oxaliplatin, oxalate produced from oxaliplatin degradation would accumulate with the endogenous oxalate in human plasma. The total amount of oxalate in plasma might then reach the toxic concentration and consequently trigger the neurotoxicity in patients. Studies on oxalate production and accumulation at each stage of oxaliplatin degradation (before and after administration to patients) were conducted to investigate the relationship between oxalate and the neurotoxicity from oxaliplatin.

3.1.3.1 Preliminary experiment with oxaliplatin infusions

The ‘Stability study of oxaliplatin infusions’ was included in Chapter 2 (page 97) in this thesis. That stability study indicated that the stability period for oxaliplatin could be extended to at least 84 days when infusions were stored at 2 - 8℃ following a sequential temperature conditioning. During the experiment, those

oxaliplatin infusion bags were stored at 2 - 8℃ for most times, and also incubated at room temperature of 25℃ for 24 hours. Oxaliplatin infusions remained stable and drug concentration remained within the acceptance criteria of ± 5% of original concentration.

After the stability study, samples (10 mL) of those oxaliplatin infusions were retained and stored at 2 - 8℃ within well labelled containers for the potential use in the oxalate study. In order to investigate the amount of oxalate produced in oxaliplatin infusions, these samples of oxaliplatin infusions (in 5% glucose) were then tested in a preliminary experiment before the main oxalate study. Results of this preliminary study would inform the design of the following study by indicating the concentration of oxalate that might be expected in oxaliplatin infusions, and also give an estimate of the time course of oxalate production in oxaliplatin infusions.

In this preliminary study, concentrations of oxaliplatin and oxalate were detected from those infusions which have been stored at 2 - 8℃ for different time periods. The experiment procedure of this preliminary study was described in Section 3.3.1 (page 168), and the result was also discussed in Section 3.4.2 (page 181) below.

3.1.3.2 Experiment with plasma samples

To simulate the degradation process of oxaliplatin in blood circulation of human bodies, 0.2 mg/mL samples of oxaliplatin were diluted with human plasma and incubated at 37℃ throughout the experiment. Oxaliplatin and oxalate concentrations were periodically tested in this experiment to determine the oxaliplatin degradation dynamics.

Since plasma proteins could potentially bond with oxaliplatin/oxalate, the total amount of oxaliplatin/oxalate in human plasma included the quantity of bound (with proteins) and un-bound (free) fractions of oxaliplatin/oxalate. To investigate the concentration dynamics of both bound (with proteins) and un-bound (free) oxaliplatin/oxalate within plasma samples, 4 groups of oxaliplatin solutions were prepared and sampled in different ways as described below:

Group A (The control group): Oxaliplatin infusions in this group were prepared with 5% glucose and stored under the same conditions with all other groups. Within this group, changes of oxaliplatin/oxalate concentration in glucose were monitored throughout the experiment. This result can be used as the benchmark comparison reference for other plasma/PUF groups.

Group B: The total amount of oxaliplatin/oxalate (including bound and un-bound fractions) in plasma was determined in this group. For this group, oxaliplatin were prepared with human plasma, and samples were treated with acetonitrile to precipitate proteins and break the bond between proteins and oxaliplatin/oxalate in plasma. (203)

Group C: Concentrations of un-bound (free) oxaliplatin/oxalate in plasma were determined in Group C. The un-bound (free) oxaliplatin is normally considered as the therapeutic effective substance in bodies after the oxaliplatin administration to patients. Oxaliplatin solutions in this group were also prepared in human plasma and samples were prepared without any special treatment, from which only concentrations of un-bound oxaliplatin/oxalate were detected.

Group D: In order to detect the concentration change of oxaliplatin/oxalate without interference from plasma proteins, Group D was prepared by diluting oxaliplatin with plasma ultra-filtrate (PUF). (204)

The concentration dynamics of oxalate in plasma and PUF samples was observed and compared with the referred average level in plasma of human bodies presented in published literature. The potential relationship between the neurotoxicity associated with oxaliplatin treatment and oxalate can be investigated in this study.

Before the main experiment with those 4 groups mentioned above, a trial test was conducted in order to optimize test parameters for the main study. The experimental design described above was fully applied in this trial study using the HPLC assay validated for oxalate determination. However, oxalate results from this trial test showed significant variation and randomness, which indicated that the HPLC assay was not suitable and applicable for oxalate determination in plasma/PUF samples. Based on this trial test, some operational and experimental details were modified for the main experiment, such as sample preparations, the acetonitrile treatment process and the ultra-filter centrifuge procedure.

3.1.4 Purpose and objectives

The main purpose of this oxalate study can be divided into two parts: 1) to simulate the oxaliplatin degradation process in blood circulation in human bodies; 2) to investigate the potential relationship between oxalate and neurotoxicity associated with oxaliplatin chemotherapy.

Additional objectives for those experiments also include:

- To detect the concentration of oxalate in oxaliplatin infusions that are prepared for chemotherapy administration;
- To investigate the possibility of potential toxicity to human bodies caused by oxalate increasing from oxaliplatin degradation in plasma;

- To investigate the degradation process of oxaliplatin in PUF (plasma ultra-filtrate) and indicate the difference between oxaliplatin degrading in plasma and PUF;
- To estimate the concentration dynamics of un-bound (free) oxaliplatin in human plasma, and indicate the influence of oxaliplatin degradation to the drug therapeutic efficacy.

3.2 Materials and methodology

3.2.1 Materials

3.2.1.1 Chemicals

Oxaliplatin concentrate solution, 5 mg/mL; batch no. PP02066; expiry 10/2015 (Accord Healthcare Limited, UK)

Oxalic acid powder, 99.999%; batch no. #MKBC3170V (Sigma-aldrich, USA)

Human plasma, Grade A, 2 × 250 mL plasma bag (Derriford Hospital, UK)

Glucose 5% w/v Macoflex, Lot. 899914122H, expiry 09/2016 (Macopharma, UK)

Sodium phosphate monobasic monohydrate (ACS reagent, 98.0 - 102.0%) (Sigma-Aldrich, Japan)

Sodium phosphate dibasic (HPLC grade) (Sigma, USA)

Sodium hydroxide 1 M (HPLC grade) (Fisher Scientific, UK)

Acid ortho-phosphoric (HPLC grade) (Fisher Scientific, UK)

Methanol (HPLC grade) (Fisher Scientific, UK)

Hydrochloric acid 1 M (Fisher Scientific, UK)

pH electrode storage solution, 4.01 Buffer solution and 7.00 Buffer solution (Thermo Fisher Scientific, USA)

Sterile water for irrigation and dilution, batch 12E22B28, expiry 04/2015 (distilled and de-ionised) (Baxter, UK)

Reagents in oxalate assay Kit: (Lot: D035002, expiry 05/06/2016, Trinity Biotech, Ireland)

Oxalate reagent A: DMAB 3.2 mmol/L; MBTH 0.22 mmol/L; Buffer pH 3.1 ± 0.1.

Oxalate reagent B: oxalate oxidase (Barley) 3000 u/L; Peroxidase (Horseradish) 100.000 u/L.

Sample diluent: EDTA 10 mmol/L; Buffer pH 7.6 ± 0.1.

Sample purifier tubes: Activated charcoal.

3.2.1.2 Consumables

20 mL Luer-lock syringes (Codan Medical ApS, UK)

0.8 mm × 40 mm needles, 21G × 11/2'', LOT 120507, expiry 04-2017 (BD Microlence, UK)

1.1 mm × 40 mm needles, 19G × 11/2'', LOT 120411, expiry 03-2017 (BD Microlance, UK)

Plus blood collection tubes, 10 Ml, LOT 380202, expiry 03/2015 (BD Vacutainer, UK)

Centrifugal tubes with filter, 4 mL and 15 mL (Amicon, UK)

Screw-cap sterile polypropylene tubes in 5 mL and 20 mL (Gosselin SAS, UK)

HPLC auto-sampler crew-cap vials 2 mL and caps (Chromacol Ltd., UK)

Cellulose acetate filters, pore size 0.45 µm (Sartorius Stedium Biotech, Germany)

Disposable polypropylene pipettes 3 mL (Fisher Scientific, UK)

Micropipette tip 200 µL and 1000 µL (Gilson SAS, France)

Prep Pad, 70% Isopropyl Alcohol BP (A CliniMed Group Company, UK)

Cellulose acetate filter paper: pore size: 0.45; Lot: 0114111061305763 (Sartorius Stedim Biotech GmbH, Germany)

RC-Vliesver starkt filter paper: pore size: 0.45; Lot: 0314184061305223 (Sartorius Stedim Biotech GmbH, Germany)

3.2.1.3 Equipment

Spectrophotometer with cuvettes, CE 1010; Serial No. 90506 (Cecil Instruments, Cambridge England)

Centrifuge, Serial No. 30002493; Catolog No. 11175603 (Jouan, UK)

Analytical balance: Sartorius Basic 100 g - 0.0001 g 4-figure balance (Sartorious Ltd., UK)

Refrigerator at 2 – 8℃ (Fisher Scientific, UK)

Incubator at 37℃ (Gallenkamp, Japan)

Digital thermometers (Fisher Scientific, UK)

Micropipette: Proline Plus 100 – 1000 µL (Biohit Limited, UK)

Micropipette: Proline Plus 10 – 100 µL (Biohit Limited, UK)

Orion 5 Star Benchtop with glass electrode (Thermo Scientific, UK)

Magnetic stirrer : HI 190M (Hanna Instruments, UK)

Glass filter holder and flask (Emil company, UK)

Filter vacuum pump: FB 70155 (Fisher Scientific, UK)

Class Ⅱ safety cabinet: Biological Class Ⅱ containment (Bigneat Ltd., UK)

Grade A, flasks, measuring cylinders and beakers (Fisher Scientific, UK)

HPLC Column: Water Spherisorb® (CNRP) 4.6 mm×250 mm stainless steel, packed with 5.0 μm bonded phase cyano CNRP Column (Waters Limited, UK)

HPLC Column: Inertsil Amide column, 5.0 μm particle size and 4.6 mm × 250 mm, serial no. 4BI41544. (GL Science, Japan)

HPLC System 1: (Jasco UK, Essex UK)

Intelligent Column Thermostat, CO-2060

Intelligent Auto-sampler, AS 2057

Quaternary Gradient Pump, PU-2089

Multiwavelength Detector, MD 2010

LE-Net Ⅱ/ADC

Chrompass Software version 1.8.6.1

HPLC System 2: (Jasco UK, Essex UK)

Intelligent Column Thermostat, CO-2067

Intelligent Auto-sampler, AS 2057 plus

Quaternary Gradient Pump, PU-2080

Multiwavelength Detector, MD 2010

LE-Net Ⅱ/ADC

Chrompass Software version 1.8.6.1

3.2.2 HPLC assay for oxaliplatin

The HPLC assay for oxaliplatin concentration detection was the same assay used in the stability study of oxaliplatin infusions described in Chapter 2 (page 108). Detailed validation of this assay was shown in Section 2.2.2.2 in Chapter 2 (page 110), although the setting conditions were slightly different as listed below:

Column: bonded-phase cyano column of Water Spherisorb (CNRP), 5.0 μm particle size and 4.6 mm × 250 mm, serial no. 0159311311.

Mobile phase: 5 mM phosphate buffer in pH 6.5 and 5% methanol.

System: Jasco 1 system with Jasco ChromPass version 1.8.6.1.

Method: Flow rate = 1.0 mL/min;

Injection volume = 20 μL;

Run time = 10 minutes;

Detection wave length = 210 nm;

Temperature of column oven = 35℃.

The standard solution of oxaliplatin used in this study was in the concentration of 20 μg/mL in order to match up with the expected oxaliplatin concentration in samples.

3.2.3 HPLC assay for oxalate determination in oxaliplatin infusions

3.2.3.1 Method conditions

Various oxalate assay techniques were discussed in the introduction section above (Section 3.1.2), the HPLC assay with an amide column was selected in

this study for oxalate determination. This assay was used to detect concentrations of oxalate in oxaliplatin infusion samples (diluted in 5% glucose), with method conditions shown below:

Column: Inertsil Amide column from GL Science, 5.0 µm particle size and 4.6 mm × 250 mm, serial no. 4BI41544.

Mobile phase: 30 mM Na_2HPO_4 buffer in pH 6.5 with 65% acetonitrile.

System: Jasco 2 system with Jasco ChromPass version 1.8.6.1.

Method: Flow rate = 1.0 mL/min;

Injection volume = 5 µL;

Run time = 10 minutes;

Detection wave length = UV 210 nm;

Temperature of column oven = 50℃.

Standard oxalate solution (10 µg/mL) was used in this external standard method. The amide column used in this study was chemically bonded with carbamoyl groups, on which oxalate was well retained unlike the regular ODS columns. The column was not suitable for the regular conditioning with 100% water; therefore 100% acetonitrile and 50% acetonitrile (in water) were used to condition this column every 2 days.

3.2.3.2 Method validation

The HPLC assay for oxalate determination was validated with intra- and inter-day precision and linearity of response. The acceptance criterion in this section was taken from the British Pharmacopoeia (BP) and European Pharmacopoeia (EP), which requires relative standard deviation (RSD) ≤ 2% for precisions. (166) (205) Oxalate stock solution of 1 mg/mL was prepared in advance from

the Oxalic acid powder (99.999%), in order to prepare all oxalate solutions in different concentrations required by those validation tests.

Intra-day precision: On the same day, one standard solution and one test solution were prepared in the same concentration of 10 µg/mL by diluting the oxalate stock solution (1 mg/mL) with water. The test solution was replicated from 5 individual vials in a HPLC method sequence created especially for intra-day precision. Mean values of oxalate concentrations calculated from chromatogram peak heights were compared and the relative standard deviation (RSD) were calculated with the equation,

$$\mathrm{RSD} = \left(\frac{\mathrm{SD}}{\mathrm{x}}\right) \times 100\%$$

[SD = Standard deviation; x = Mean concentration of oxalate.]

Inter-day precision: A standard oxalate solution of 10 µg/mL was prepared in the first day of the inter-day precision from the 1 mg/mL oxalate stock. This standard solution was used for the whole term of this precision test in 6 continuous days when 5 test solutions (10 µg/mL) were prepared on each sample day. Replicated test solutions were injected each day for 6 days, and from the mean oxalate concentrations calculated from peak heights the RSD value was calculated using the equation referred above.

Linearity of response: Oxalate stock solution (1 mg/mL) was diluted volumetrically with HPLC grade water in a grade A volumetric flask, to give 10 mL oxalate solution in 0.1 mg/mL. Then a series of calibration solutions was prepared by diluting the 0.1 mg/mL solution from 0.01, 0.05 mL into 10 mL and 0.05, 0.15, 0.25, 0.5, 1.0 mL into 5 mL separately with HPLC grade water in grade A volumetric flasks, to give a calibration plot with the oxalate concentrations of 0.1, 0.5, 1.0, 3.0, 5.0, 10.0, 20.0 and 100.0 µg/mL.

Concentrations of these 8 test solutions ranged from 0.1 – 100.0 µg/mL, which covers all oxalate concentrations expected to be tested in this study.

Eight mean peak heights from 3 replicate injections for each solution were plotted against concentration and were fitted to a linear relationship using the least-squares regression analysis.

The acceptance criterion for linearity validation was $R^2 \geq 0.995$ and even distribution of data on regression line by visual inspection.

During validation tests, it was found that the oxalate peak could be better separated from other interfering peaks when diluting oxalate samples with the mobile phase. Therefore, to obtain better chromatogram data, all samples tested with this HPLC assay for oxalate were prepared in the mobile phase instead of deionized water.

Result tables of validation tests for the HPLC assay of oxalate are shown below in result section, Section 3.4.1.1 (page 178).

3.2.3.3 Quantification of oxalate concentrations

The values of peak heights of oxalate in samples were recorded with the two 'bracketing' standard chromatograms at each sample point. Oxalate concentrations were calculated by using the equation below:

$$C_{test} = \frac{H_{test}}{H_{STD}} \times C_{STD} \times D$$

[C_{test} = Concentration of oxalate in samples; H_{test} = Peak height of oxalate in test sample solution; H_{STD} = Mean peak height of standard solution; C_{STD} = Concentration of standard solution; D = Dilution factor].

The standard solution (STD) involved in the above equation was the oxalate solution in concentration of 10 µg/mL. Additionally, the dilution factor of oxalate samples was the same in this study, as D = 10.

3.2.4 Oxalate assay kit for oxalate determination in human plasma

3.2.4.1 Assay mechanism

The selected assay for oxalate was an enzymatic method based on the oxidation of oxalate with oxalate oxidase. Hydrogen peroxide (H_2O_2) was produced in oxalate oxidation, which can be measured by a peroxidase-catalyzed reaction. Two enzymatic reactions involved in this assay were shown below:

$$\text{Oxalate} + O_2 \xrightarrow{\text{Oxalate Oxidase}} 2CO_2 + H_2O_2$$

$$H_2O_2 + \text{MBTH} + \text{DMAB} \xrightarrow{\text{Peroxidase}} \text{Indamine Dye} + H_2O$$

[MBTH: 3-methyl-2-benzothiazolinone hydrazine; DMAB: 3-(dimethylamino) benzoic acid]

Indamine dye yielded by the peroxidation of H_2O_2 had an absorbance maximum at 590 nm wavelength in spectrophotometer. Due to these reactions, the intensity of Indamine dye colour directly reflected the amount of H_2O_2, and consequently correlates with the concentration of oxalate in samples.

The enzymes and reactants required for these reactions were provided within the oxalate kit, as well as sample diluent and purifier tubes. Separate vials were used to seal different reagents, which are described in details below.

- **Oxalate Reagent A**: DMAB 3.2 mmol/L; MBTH 0.22 mmol/L; Buffer pH 3.1 ± 0.1

- **Oxalate Reagent B**: oxalate Oxidase (Barley) 3000 µ/L; Peroxidase (Horseradish) 100.000 µ/L
- **Sample diluent**: EDTA 10 mmol/L; Buffer pH 7.6 ± 0.1

The reagents in the oxalate kit were for '*ex vivo* diagnostic use', which only required normal precautions exercised in handling regular laboratory reagents. These reagents were provided in light proof vials as dry powder, which can be stored for long period at refrigerated temperature of 2 – 8℃. However, after dilution in deionized water for oxalate determination, the reconstituted reagents had various stability periods in different environments. Reconstituted oxalate Reagent A and Reagent B were only stable for 1 day at ambient temperature of 18 – 26℃, but at 2 – 8℃ they can be stored for 1 month (30 days). Reconstituted oxalate Reagent B also can be frozen at -20 ℃ for up to 30 days and unfrozen at room temperature before use. For sample diluent, the reconstituted solution can be stored for 1 week at ambient temperature 18 – 26℃ and 3 months at refrigerated temperature 2 – 8℃. All dissolved reagents needed to be warm up to approximately 37 ℃ before each assay. As the crystalline material might be formed in reagent vials during the storage in the refrigerator, warming the reagents up to room temperature can dissolve those precipitates and prevent associated errors in oxalate concentration results. Sample purifier tubes provided in the kit contained activated charcoal, which could be stored at room temperature (18 – 26℃) without any specific storage requirements.

The reagents should not be used if there was any moisture penetration in the dry reagent powder. The reconstituted reagents should be clear and free of

particulate matter. If the reagents developed haziness due to bacterial contamination, they should be discarded.

3.2.4.2 Sample preparation

The assay with the oxalate kit was originally designed for oxalate determination in urine samples, but it was also capable of testing oxalate in human plasma samples because: 1) the EDTA contained in sample diluent could prevent plasma clotting in samples; 2) sample purifier tubes can be used to purify human plasma; 3) plasma samples can be ultra-filtered in centrifuge to avoid the effect of various compounds in plasma to the oxalate detection.

In this oxalate assay, samples have to be prepared following the operation steps below:

1. Prepare sample diluent from the initial vial of dry reagent powder, which was described in details in the following section of oxalate determination (Section 3.2.4.3);
2. Pipette 2 mL or any suitable volume of samples and plasma control into a series of appropriately labelled sterile tubes;
3. Add equal volume (sample volume mentioned in Step 2) of sample diluent into each tube and mix them properly;
4. Adjust pH of these mixture in each tube into the pH range of 5.0 – 7.0 using 1 M hydrochloric acid or 1 M sodium hydroxide;
5. Pipette a proper volume of diluted samples and plasma control into labelled sample purifier tubes and intermittently mix for 5 minutes (A rotator mixer is recommended for mixing.);
6. Transfer these mixture into labelled ultra-filter tubes and centrifuged for 20 minutes at 4100 rpm;

7. The supernatants of ultra-filtrate samples and plasma control are ready to be detected.

Before sample preparation, all sterile vials, sample purifier tubes and ultra-filter tubes were prepared and labelled with proper information including group number, sample type and experiment date. For pH adjustment of diluted samples and plasma control, the pH meter was validated every day and the glass electrode was washed with deionized water before and between each sample. Oxalate Reagent A and Reagent B didn't need to be prepared and used during sample preparation, while Sample diluent was prepared in advance to dilute samples and plasma control.

3.2.4.3 Oxalate determination schedule

In this assay, some equipment was required but not provided within the kit, including spectrophotometer (with cuvettes), pipettes and centrifuge with fitted ultra-filter tubes. The spectrophotometer used in this assay was able to accurately measure various samples that have the absorbance at 590 nm. The selected pipette devices were within the correct working range to accurately transfer the liquid volumes required. Ultra-filter tubes used for purifying samples and plasma control were in the size of 4 mL in order to handle the required sample volume. Additionally small ultra-filter tubes could ensure more samples can be loaded in the centrifuge at any one time, which can shorten the experiment time and consequently reduce the detection errors in oxalate results caused by temporal difference.

Before the determination of oxalate in samples, reagents in the oxalate kit were prepared with deionized water and underwent the detailed preparation shown below.

Reagent A was reconstituted with the volume of deionized water as indicated on the vial label (10 mL). Then stoppered the vial and inversely mixed the reagent until it is completely dissolved.

Reagent B required different volume of water to dissolve in, which should be in accordance with the instruction on the vial label (2 mL). The reconstituted Reagent B cannot be mixed by shaking the vial; instead it was mixed immediately by gentle inversion.

Sample Diluent was prepared at the very beginning prior to sample preparation as mentioned in the above section (Section 3.2.4.2). According to the vial label of sample diluent, it was dissolved in 100 mL deionized water using a separated container in appropriate size. The entire powder from the vial was transferred into a labelled container (100 mL flask) and mixed with 100 mL deionized water. Since the dissolution procedure took round 0.5 – 1.0 hour, the preparation of sample diluent was conducted in advance of the whole experiment.

In addition, **standard oxalate** of 45 µg/mL was required in this assay as the reference value for the calculation of oxalate concentration in samples and plasma control. Standard oxalate was prepared by diluting 0.225 mL, 1 mg/mL oxalate stock in 5 mL flask to obtain the concentration of 45 µg/mL.

Oxalate concentrations of the treated samples and plasma control were determined as described below:

I. Warm oxalate reagents (Reagent A and Reagent B) to assay temperature (18 – 37℃);
II. Label sterile tubes for various solutions tested in this assay, including Reagent Blank, Standard oxalate, Plasma Control and Samples;
III. Pipette 1 mL oxalate Reagent A in each sterile tube;

IV. Pipette 50 µL Water / Standard oxalate (45 µg/mL) / Plasma Control / Samples in the tubes with matching labels respectively; (water is for the tube of Reagent Blank; Plasma control and Samples have all been treated as mentioned above)

V. Pipette 0.1 mL oxalate reagent B into each tube and mix immediately by gentle inversion;

VI. Incubate the tubes at room temperature for 5 minutes; (to accurate the time, samples are better tested in a small number each time)

VII. Calibrate the spectrophotometer at 590 nm wavelength and record the absorbance of pure water as a correction reference for samples;

VIII. Record the readings of absorbance (A) for Water, Reagent Blank, Standard oxalate, Plasma Control and Samples at 590 nm in the spectrophotometer as the mean value of triplicate tests;

Calculation of oxalate concentrations in plasma control and samples is referred to the calculation section below (Section 3.2.4.4).

3.2.4.4 Calculation of oxalate Concentration

In this assay, the absorbance (A) of Water and Reagent Blank were used to obtain the corrected absorbance (∆A) of Standard oxalate, Plasma control and oxalate samples. The corrected absorbance (∆A) can be calculated following Equation 1 – 4 as shown below.

$$\Delta\text{A Blank} = \text{A Blank} - \text{A Water} \quad \text{Equation 1}$$

$$\Delta\text{A Standard} = (\text{A Standard} - \text{A Water}) - \Delta\text{A Blank} \quad \text{Equation 2}$$

$$\Delta\text{A Plasma control} = (\text{A Plasma control} - \text{A Water}) - \Delta\text{A Blank} \quad \text{Equation 3}$$

$$\Delta A\ \text{Sample} = (A\ \text{Sample} - A\ \text{Water}) - \Delta A\ \text{Blank} \quad \text{Equation 4}$$

Calibrated with ∆A value of Standard oxalate, oxalate concentrations in Samples were calculated following Equation 5 and Equation 6 in different unit. ∆*A Sample* in these two equations can be replaced by ∆*A Plasma control* to calculate the oxalate concentration in Plasma control. The dilution factor of 2 in the equation represented the 1 : 1 dilution of samples/plasma control in Sample diluent as described in sample preparation section above.

$$\text{Oxalate concentration}\ (\mu g/mL) = \frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Standard}} \times 45 \times 2 \quad \text{Equation 5}$$

$$\text{Oxalate concentration}\ (mmol/L) = \frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Standard}} \times 0.5 \times 2 \quad \text{Equation 6}$$

[45=Concentration (µg/mL) of oxalate in standard; 0.5=Concentration (mmol/L) of oxalate in standard; 2=Dilution factor]

3.2.4.5 Method Validation

The performance characteristics of this assay were provided in the method instruction with the oxalate assay kit with the validation results including linearity, sensitivity, precision and recovery studies. (189)

A total of 30 urine specimens with oxalate concentrations ranging from 0.1 - 1.2 mmol/L were assayed. The linearity of oxalate within this range turned out with a correlation coefficient of 0.99 and the regression equation was y = 0.94x + 0.008. The sensitivity validation of this assay indicated an absorbance change of 0.150 corresponding to oxalate concentration of 0.1 mmol/L, when a spectrophotometer is used for the measurement under the stated conditions. Within-run and run-to-run precision studies showed that coefficient variations with 3 different urine samples were both lower than 2.18%. Known amounts of

oxalate were added to 59 urine specimens and the oxalate concentration was determined using this assay to obtain percentage of oxalate recovery. The recovery of added oxalate ranged from 93 - 107%, with the mean value of 100.5%.

All these validation tests were operated with urine samples, which was the sample subject that this assay originally focused on. To ensure that the plasma samples could be tested with this oxalate assay kit, additional validation tests were conducted with oxalate in the plasma samples.

A series of calibration oxalate samples were prepared within human plasma by diluting the oxalate stock solution (1 mg/mL) into concentrations of 1 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL. This range was sufficient to cover the oxalate concentration of all samples expected in this study. Each sample was tested in triplicate with the spectrophotometer and the mean value of absorbance (A) was used for the calculation. The absorbance values of tested oxalate were plotted against the nominal concentrations and fitted to a linear relationship using the least-squares regression analysis. Furthermore, oxaliplatin to a concentration of 20 µg/mL was added to each plasma sample in this test, in order to investigate the effect of oxaliplatin on oxalate determination within this assay. Results of this oxalate assay validation was discussed in the result section below (Section 3.4.1.2).

3.2.5 pH measurement

pH variation may indicate significant chemical and physical changes in plasma samples with oxaliplatin. An Orion 5 Star pH meter with combination glass electrode was used to measure the pH of samples at each time point. For calibrating the pH meter, the reference buffer solutions at pH 4.01 and pH 7.00

were used every time before sampling. Between samples, distilled water was used to rinse the glass electrode, and the automatic temperature compensation was deployed.

3.3 Experimental

Two experiments were conducted to investigate the oxalate production from the oxaliplatin degradation process. The preliminary study was conducted with oxaliplatin infusions prepared with 5% glucose, in order to determine the amount of oxalate produced in oxaliplatin infusions before administration to patients for chemotherapy. The main experiment was designed with human plasma samples to simulate the oxaliplatin degradation in blood circulation in patients. In this experiment oxalate's growth associated with oxaliplatin degradation was also examined in plasma samples.

3.3.1 Preliminary study with oxaliplatin infusions (with 5% glucose)

3.3.1.1 Infusion groups

Oxaliplatin infusions used in this preliminary study were those infusions (in 5% glucose) stored from the stability study presented in Chapter 2 (page 97). As mentioned in Chapter 2 (Section 2.3.1), 7 groups of oxaliplatin infusions were prepared in two different concentrations, 0.2 mg/mL and 0.7 mg/mL, in order to test the stability period of oxaliplatin infusions within the working concentration range. Each group was stored following the sequential temperature design for different time period at refrigerated and room temperature. (Refer to Chapter 2, page 114) According to results of the stability study, oxaliplatin infusion bags in all groups were found to be stable at the end of the experiment period and

oxaliplatin concentration variations were within the criteria of ± 5%. Those infusion bags were stored at 2 – 8℃ in light proof bags since the finish of the stability study, which were capable of being used for the investigation of oxalate produced in oxaliplatin infusions. Those infusion bags were prepared on different dates and stored for various time periods during and after the stability study. As a result, a series of oxalate concentrations produced in oxaliplatin infusions can be obtained for 7 different time periods in a range of 24 days, which were 553, 560, 567, 570, 571, 572 and 577 days. Details of storage time for each oxaliplatin group are shown in *Table 3-2* below.

Table 3-2, Storage time period of oxaliplatin infusion bags with 5% glucose in each group during and after the previous stability study described in Chapter 2.

Group	Storage time during the stability study of oxaliplatin infusions			Storage time after Stability Study	Storage time in total (days)
	Stored at 2-8℃ (days)	Stored at 25℃ (days)	Stored at 2-8℃ (days)	Stored at 2-8℃ (days)	
Group A	1	1	7	568	577
Group B	3	1	7	561	572
Group C	7	1	7	556	571
Group D	14	1	7	548	570
Group E	28	1	7	531	567
Group F	56	1	7	496	560
Group G	84	1	7	461	553

During the stability study of oxaliplatin infusions, the storage conditions of each group were following the sequential temperature design at different temperatures of 2-8℃ or 25℃.

Samples from each group tested in this preliminary study were compared with the data from T0 and the last sampling point of the stability study. These oxaliplatin variations would represent the change of oxaliplatin during the additional storage time period after the stability study.

Oxalate might exist in oxaliplatin infusions as a related substance from the original oxaliplatin vials. Reference to EP (European Pharmacopeia), within oxaliplatin infusions the concentration of oxalate impurity should not be more than 0.1% of oxaliplatin concentration. (164) Therefore, oxalate concentrations

detected from the oxaliplatin infusions in this study would indicate the total amount of oxalate in oxaliplatin infusions, which contained 1) the oxalate impurity originally existing in oxaliplatin vials; 2) the oxalate produced from oxaliplatin degradation through the entire storage period for each group.

3.3.1.2 Oxaliplatin and oxalate determination

Standard solutions of oxaliplatin and oxalate were both prepared on the same day before testing the samples from selected groups. Oxaliplatin standard solution (STD) was in 20 μg/mL, which was prepared in HPLC grade water following $0.04\ \mathrm{mL}, 5\ \mathrm{mg/mL} \rightarrow 5\ \mathrm{mL}, 20\ \mu\mathrm{g/mL}$ to obtain 5 mL STD solution. Oxalate standard solution (STD) was at concentration of 10 μg/mL, and was prepared in mobile phase used in oxalate assay following $0.05\ \mathrm{mL}, 1\ \mathrm{mg/mL} \rightarrow 5\ \mathrm{mL}, 10\ \mu\mathrm{g/mL}$ to obtain 5 mL STD solution.

From each group, infusion samples were selected in each concentration (0.2 mg/mL and 0.7 mg/mL) and tested in this preliminary study. Infusion samples were labelled as: 0.2A3, 0.7A2, 0.2B1, 0.7B3, 0.2C2, 0.7C1, 0.2D2, 0.7D3, 0.2E3, 0.7E2, 0.2F3, 0.7F1, 0.2G3, 0.7G2.

10 mL of samples were withdrawn from oxaliplatin infusions in each group by 20 mL syringes with luer-lock. For each sample, 1 mL was used for oxaliplatin assay, 1 mL was for the determination of oxalate with the HPLC method and the remaining sample solution was for pH measurement. For pH measurement, samples were tested immediately without any preparation. But for determinations of oxaliplatin and oxalate, samples were prepared and diluted in the following ways:

With the **oxaliplatin assay**, oxaliplatin infusion samples in concentrations of 0.2 mg/mL and 0.7 mg/mL were diluted with deionized water respectively by 10 and

20 times. For the **oxalate assay**, samples were prepared in the mobile phase used in the HPLC method, and diluted in the same factors as for the oxaliplatin assay.

Due to the amount of samples from those 7 groups, Group A, B, C and D were tested in the same day while other groups were tested together in the next day. During the sample time, two HPLC systems were used respectively for oxaliplatin and oxalate assays at the same time to gain the concentration result of both oxaliplatin and oxalate with less timing errors.

3.3.2 Main study of oxalate formation in human plasma after oxaliplatin administration

This experiment was to investigate the oxalate change in human plasma after administered oxaliplatin to give a concentration of 0.2 mg/mL. The degradation process of oxaliplatin in human plasma was simulated by incubating plasma sample groups at body temperature of 37℃. For oxalate determination, the HPLC assay used for the preliminary study described above was also tested with plasma samples in this experiment. Results showed that this HPLC assay was not suitable for testing human plasma samples. Therefore, an enzymatic method for oxalate determination in plasma samples was selected instead, since this assay could meet both specificity and selectivity criteria required in this study.

3.3.2.1 Group design

As mentioned in Section 3.1.3.2, 4 groups of oxaliplatin (0.2 mg/mL) solutions were prepared respectively in 5% glucose (Group A), human plasma (Group B, C) and PUF (Group D) in this study. In order to simulate the oxaliplatin degradation in blood circulation after administration to patients, oxaliplatin

concentration (0.2 mg/mL) prepared in those sample groups was carefully selected as approximately 100 times the oxaliplatin C_{max} (the peak platinum concentration) in plasma and PUF (plasma ultra-filtrate) referring to the literature of PK studies on oxaliplatin. (129) In addition, all sample groups were incubated at 37℃ within light-proof bags to simulate the human body temperature. The Control group, Group A, of oxaliplatin in 5% glucose solutions was prepared and stored under the same condition as other groups to ensure its comparability.

Details of each oxaliplatin group were described in *Table 3-3* below.

Table 3-3, Preparation and sampling information for Group A, Group B, Group C and Group D examined in this study.

Groups	Group A	Group B	Group C	Group D
Diluent	5% Glucose	Human Plasma	Human Plasma	PUF
Prepared oxaliplatin concentration	0.2 mg/mL	0.2 mg/mL	0.2 mg/mL	0.2 mg/mL
Number & Size of oxaliplatin solutions*	3 × 50 mL	3 × 50 mL	3 × 50 mL	3 × 50 mL
Sample treatment before assays	None	Acetonitrile	None	None
Detected substances in samples	All oxaliplatin/oxalate	Protein-Bound and un-bound (free) oxaliplatin/oxalate	Un-bound (free) oxaliplatin/oxalate	All oxaliplatin/oxalate

** Oxaliplatin solutions for each group were prepared in triplicate and stored in 10 mL blood tubes with EDTA.*

3.3.2.2 Study procedure

These 4 groups of 0.2 mg/mL oxaliplatin solutions in different diluents were stored at 37℃ in the incubator for 5 days to simulate the body temperature. To investigate the oxaliplatin degradation process, samples were withdrawn and tested at each day including 6 tests at sample points of Day 0 (T_0), Day 1, Day 2, Day 3, Day 4 and Day 5. The initial (T_0) oxaliplatin and oxalate

concentrations were the reference values for subsequent time-points. Samples taken from each solution in the group were divided into three portions respectively for oxaliplatin HPLC assay, oxalate assay with enzymatic method and pH measurement. The HPLC assay for oxaliplatin determination was described in details in the methodology section (Section 3.2.2). While for oxalate determination, an enzymatic method with oxalate assay kit was used to detect oxalate concentration in each sample. As a result, samples were prepared in two different ways respectively for oxaliplatin (by HPLC method) and oxalate (by enzymatic method) determinations, which are described in detail below (Section 3.3.2.3.2).

Furthermore, a group of blank plasma was also tested under the same condition to investigate the endogenous oxalate in human plasma and its concentration change without the interference from oxaliplatin. Within this group, 3 samples (of 15 mL volume each) of blank plasma were incubated at 37 ℃ while 15 mL blank plasma was stored at 2 – 8 ℃ as control. The blank plasma samples were kept in blood container tubes with EDTA for 5 days and tested for oxalate concentration each day using the same oxalate assay kit. Additionally, the pH of blank plasma samples was also measured every day. Results from this group were used as references for the analysis of those results from oxaliplatin sample groups.

3.3.2.3 Study preparation

3.3.2.3.1 Group preparation

Three sample solutions were included in each group and prepared following the operating process as described below.

Group A: 2 mL of the oxaliplatin concentrate stock solution (5 mg/mL) was diluted with 48 mL 5% glucose to prepare 0.2 mg/mL oxaliplatin solutions in 50 mL flask. These solutions were mixed in inversion and left to stand for 30 minutes before being transferred into the storage container in order to give them enough time for complete mixing. Then sample solutions of oxaliplatin in 5% glucose were stored separately in 5 × 10 mL labelled blood tubes with EDTA anti-coagulant.

Group B & C: 50 mL human plasma was withdrawn from 250 mL plasma bag by luer-lock syringes and kept in a clean container before the preparation of sample solutions. Diluted 2 mL of oxaliplatin concentrate stock (5 mg/mL) with 48 mL human plasma to gain the oxaliplatin concentration of 0.2 mg/mL. This 50 mL solution was prepared and mixed completely in 50 mL flask and immediately transferred into 5 × 10 mL blood tubes with EDTA for storage to prevent the potential plasma clotting.

Group D: To prepare 50 mL PUF, 60 – 70 mL human plasma was ultra-filtrated centrifuged with Amicon Ultra-15 centrifugal filter devices (up to 15 mL) spinning at 4100 rpm × g for approximately 3 hours. Then 48 mL of PUF was mixed with 2 mL, 5 mg/mL oxaliplatin stock solution to obtain 0.2 mg/mL oxaliplatin solution in PUF. The solution was then completely mixed in 50 mL flask and stored in 5 × 10 mL blood tubes with EDTA.

All solutions with oxaliplatin were prepared, sampled and diluted in a Biomat AC Class II safety cabinet and labelled clearly with the drug name, concentration, group number, solution name and date of preparation. After first samples were withdrawn from each group, all oxaliplatin solutions were then covered by light-proof bags and immediately put into the incubator at 37℃.

The blank plasma group only required human plasma without any additional substances, which included 3 × 15 mL test plasma stored in blood tubes with EDTA at 37℃ in the incubator.

3.3.2.3.2 Sample preparation

Samples from different group were prepared following the schedule as shown below:

Group A (Control group):

- 8 mL sample was withdrawn from each sample solution;
- 4.5 mL sample was used for pH measurement;
- 0.5 mL sample was diluted into 5 mL with deionized water for oxaliplatin assay in the HPLC system;
- 3 mL sample was prepared for oxalate assay following the procedure of oxalate assay kit:
  1) 3 mL sample was pipetted and mixed with 3 mL of sample diluent in the labelled tube;
  2) Adjusted the pH to range of 5.0 to 7.0 with 1 N hydrochloric acid (HCl);
  3) The diluted sample was transferred to a sample purifier tube and mixed for 5 minutes;
  4) Then the sample was transferred from purifier tube into ultra-filter tubes (10 kDa MWCO) and centrifuged at 4000 rpm for 20 minutes.
- 1 mL of the centrifuged sample was diluted into 5 mL with deionized water, from which 50 µL of the dilution was used to test the oxalate concentration with reagents in the oxalate assay kit following the determination procedure described in methodology above (refer to Section 3.2.4.3).

Group B:

- 8 mL sample was withdrawn from each sample solution;
- 5 mL sample was used for pH measurement;
- 3 mL sample was mixed with 3 mL Acetonitrile in an ultrasonic machine for 15 minutes to gain a 6 mL mixture;
- 3 mL of this mixture was ultra-filter centrifuged and 1 mL filtrate was then diluted into 5 mL with deionized water for oxaliplatin assay in HPLC system;
- The remaining 3 mL mixture was prepared for the oxalate determination following the procedure of oxalate assay kit, which was same with Group A as described above.
- 2 mL of the centrifuged sample was diluted into 5 mL with deionized water, and 50 µL of the dilution was used for determination of oxalate with oxalate assay kit.

Group C:

- 8 mL sample was withdrawn from each sample solution;
- 4 mL sample was used for pH measurement;
- 1 mL sample was centrifuged to gain 0.5 mL filtrate to prepare 5 mL testing sample with deionized water for oxaliplatin determination with the HPLC method;
- 3 mL sample was prepared for oxalate assay in the same way with Group A described above following the procedure of oxalate assay kit;
- 1 mL of the centrifuged sample was diluted into 5 mL with deionized water, from which 50 µL of the dilution will be used for determination of oxalate with oxalate assay kit.

Group D:

- 8 mL sample was withdrawn from each sample solution;
- 4.5 mL sample was used for pH measurement;
- 0.5 mL sample was diluted into 5 mL with deionized water for oxaliplatin assay in HPLC system;
- 3 mL sample was prepared for oxalate assay following the procedure of oxalate assay kit, which was same to Group A;
- 1 mL of the centrifuged sample was diluted into 5 mL with water, from which 50 µL of the dilution was used for oxalate determination by oxalate assay kit.

The plasma control required in the oxalate assay was prepared as it was described in the methodology section in Section 3.2.4.2. Oxalate concentrations were detected and quantified in a spectrophotometer following the determination procedure of the oxalate assay kit described in Section 3.2.4.3 above.

In the blank plasma group, all samples were only tested for oxalate concentrations by an enzymatic method with the oxalate assay kit. At each time point, 2 mL samples were taken and detected following the preparation and determination schedule described in the oxalate assay in Section 3.2.4.

3.3.2.3.3 Preparation of other solutions

Standard solutions of oxaliplatin and oxalate used in their assays were both prepared in deionized water from stock solutions following the schedule below:

Oxaliplatin Standard solution (STD):

$$0.02\ \text{mL}, 5\ \text{mg/mL} \rightarrow 10\ \text{mL}, 10\ \mu\text{g/mL}$$

Oxalate Standard solution (STD):

$$0.45\ \text{mL}, 1\ \text{mg/mL} \rightarrow 10\ \text{mL}, 45\ \mu\text{g/mL}$$

The oxalate stock solution of 1 mg/mL used above was freshly prepared by dissolving the Oxalic acid powder (99.999%) in deionized water. The preparation of other solutions and reagents required for oxalate assay was presented in Section 3.2.4.3 above.

3.4 Results

3.4.1 Validation results for oxalate assays

3.4.1.1 Validations of the HPLC assay for oxalate determination

The HPLC method for oxalate determination in oxaliplatin infusion samples was validated in terms of intra- and inter-day precisions and linearity of analytical response, with reference to the International Conference of Harmonisation (ICH) Harmonised Tripartite Guideline, 'Validation of analytical Procedures: Text and Methodology Q2 (R1)'. (161) Precision of the method was analysed with the calculation of RSD (relative standard deviation) using equation stated in above section (Section 3.2.3.2).

As shown in *Table 3-4*, the RSD of Inter-day precision and Intra-day precision were both within the limits of 2.0%, which demonstrated that this method was repeatable by standards set in British Pharmacopoeia (BP). (166) (161) In addition, the calibration plot was shown to be linear over the concentration range of 0.1 – 20.0 µg/mL (n=7), with the equation y = 0.006x + 7E-05 (R^2 = 0.9999) from the least-squares regression analysis, and an even distribution of the data on the regression line was observed by visual inspection (as shown in *Figure 3-4*).

Table 3-4, Inter-day precision data for the oxalate HPLC assay during 5 continuous days and intra-day precision data of 5 injections within one day.

Precision Type	Injection Identifier	Oxalate Concentration (µg/mL)	RSD (%, n = 5)
Inter-day Precision	Oxalate Day1	10.6792	1.61
	Oxalate Day2	10.3206	
	Oxalate Day3	10.7386	
	Oxalate Day4	10.6380	
	Oxalate Day5	10.4776	
Intra-day Precision	Oxalate Test1	11.9984	0.89
	Oxalate Test2	12.0462	
	Oxalate Test3	11.9639	
	Oxalate Test4	12.2110	
	Oxalate Test5	12.1658	

RSD = Relative Standard Deviation; concentrations were calculated from mean peak heights of two continuous injections in HPLC system.
Inter-day study: 04/06/14-10/06/14 Intra-day study: 29/05/14

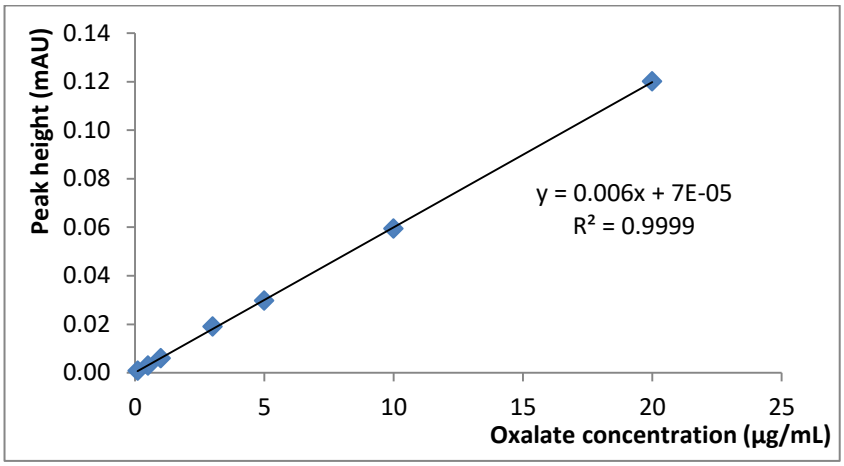

Figure 3-4, Oxalate linearity plot with analytic concentrations and peak heights obtained with the HPLC assay in oxaliplatin infusion samples.

In summary, all validation results of this HPLC assay based on intra- and inter-day precisions and linearity of analytical response confirmed the assay as being suitable for oxalate determination in oxaliplatin infusions in 5% glucose solution.

3.4.1.2 Oxalate assay kit validations

To ensure the oxalate assay kit was applicable for oxalate determination in human plasma samples, the linearity validation of assay response for oxalate in plasma was conducted with the resulting plot shown in *Figure 3-5* below. A series of oxalate samples were prepared from the dilution of oxalate stock

solution (1 mg/mL) with human plasma, including 7 oxalate samples in concentrations of 1 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL and 100 µg/mL. Furthermore, 20 µg/mL oxaliplatin was added to each plasma sample in this test in order to investigate the interference of oxaliplatin on oxalate determination in plasma samples with this assay.

According to *Figure 3-5*, the regression equation from the least-squares regression analysis was y = 0.008 + 0.0691 (R^2 = 0.9998), which indicated the good linearity of this oxalate assay in plasma samples. Moreover, the added oxaliplatin (20 µg/mL) might have slightly increased the concentration of oxalate but did not influence the linear plot in the result.

Therefore, the oxalate assay kit could be applied in this study to quantify oxalate concentrations in plasma samples without the interference of oxaliplatin.

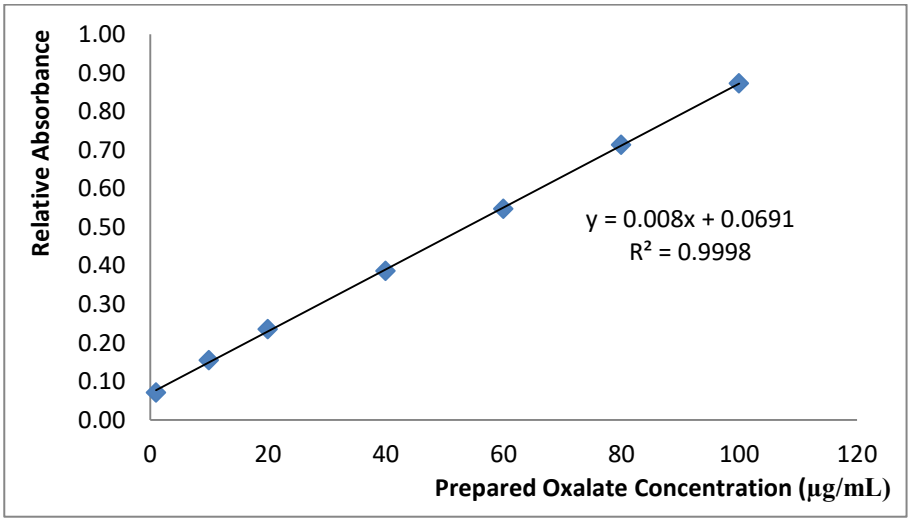

Figure 3-5, Linearity result of oxalate determination in human plasma by using the oxalate assay kit. All samples were prepared with added oxaliplatin at 20 µg/mL.

3.4.2 Preliminary study results on oxaliplatin infusions

3.4.2.1 Determination of oxaliplatin and oxalate concentrations

Oxaliplatin and oxalate concentrations tested in oxaliplatin infusion samples from each group were shown in *Table 3-5* below, in which the oxaliplatin data

given at time zero was from the previous stability study presented in Chapter 2 (page 121). Compared with the initial value at T_0, oxaliplatin concentrations slightly increased after storage for long periods of time and the concentration differences were within the range of 0.05% - 8.49%. The oxaliplatin concentration difference compared to T_0 was calculated from the equation,

$$\text{Concentration Difference (\%)} = \frac{(C_s - C_{T0})}{C_{T0}} \times 100\%$$

[C_s: oxaliplatin concentration at sample time; C_{T0}: oxaliplatin concentrations at time zero.].

Even though samples tested in this preliminary study were from oxaliplatin infusions in different concentrations. The variation percentage of oxaliplatin did not appear to be concentration dependent over the oxaliplatin concentration range of 0.2 mg/mL to 0.7 mg/mL. There was no obvious relationship between oxaliplatin concentration and storage time period for each group.

Table 3-5, Oxaliplatin and oxalate concentrations in oxaliplatin infusions from Group A – G, and the oxaliplatin concentration difference between sample time and time zero.

Group	Total Storage Duration	Nominal Concentration of Oxaliplatin	Oxaliplatin Concentration at T_0 (mg/mL)	Oxaliplatin Concentration at Sample Time (mg/mL)	Oxaliplatin Concentration Difference Between Sample Time and T_0 (%)	Oxalate Concentration at Sample Time (µg/mL)
A	577 days	0.2 mg/mL	0.1817	0.1914	+ 5.34	1.8660
		0.7 mg/mL	0.6499	0.7051	+ 8.49	3.1653
B	572 days	0.2 mg/mL	0.1893	0.1926	+ 1.74	2.1580
		0.7 mg/mL	0.6609	0.6923	+ 1.59	3.6257
C	571 days	0.2 mg/mL	0.1893	0.1923	+ 1.59	2.1841
		0.7 mg/mL	0.6609	0.6806	+ 2.98	4.3933
D	570 days	0.2 mg/mL	0.1880	0.1980	+ 5.32	1.5800
		0.7 mg/mL	0.6624	0.6927	+ 4.57	3.9401
E	567 days	0.2 mg/mL	0.1907	0.1958	+ 2.67	2.0932
		0.7 mg/mL	0.6731	0.6872	+ 2.10	4.0847
F	560 days	0.2 mg/mL	0.1911	0.1912	+ 0.05	2.6425
		0.7 mg/mL	0.6634	0.6713	+ 1.29	4.9521
G	553 days	0.2 mg/mL	0.1868	0.1906	+ 2.03	2.2496
		0.7 mg/mL	0.6561	0.6855	+ 4.48	4.6857

T_0: Time zero in the stability study; Sample time: The date that samples from different group were tested in this study; Storage Duration included storage time during and after the stability study.

As shown in *Table 3-5* above, more oxalate was produced in those infusions with higher oxaliplatin concentration (0.7 mg/mL). In 0.7 mg/mL oxaliplatin infusions, the concentration of oxalate was within the range of 3.1653 – 4.9521 µg/mL; while in 0.2 mg/mL oxaliplatin infusions, oxalate concentrations was shown in the range of 1.5800 – 2.6425 µg/mL. Relationships between the concentration of oxalate produced in oxaliplatin infusions and the storage time period were illustrated in *Figure 3-6*. As shown in this figure, oxalate concentrations slightly reduced along with the extension of storage time for oxaliplatin infusions in both concentrations of 0.2 mg/mL and 0.7 mg/mL. While for 0.7 mg/mL oxaliplatin infusions, concentrations of oxalate were higher and with larger temporal variation.

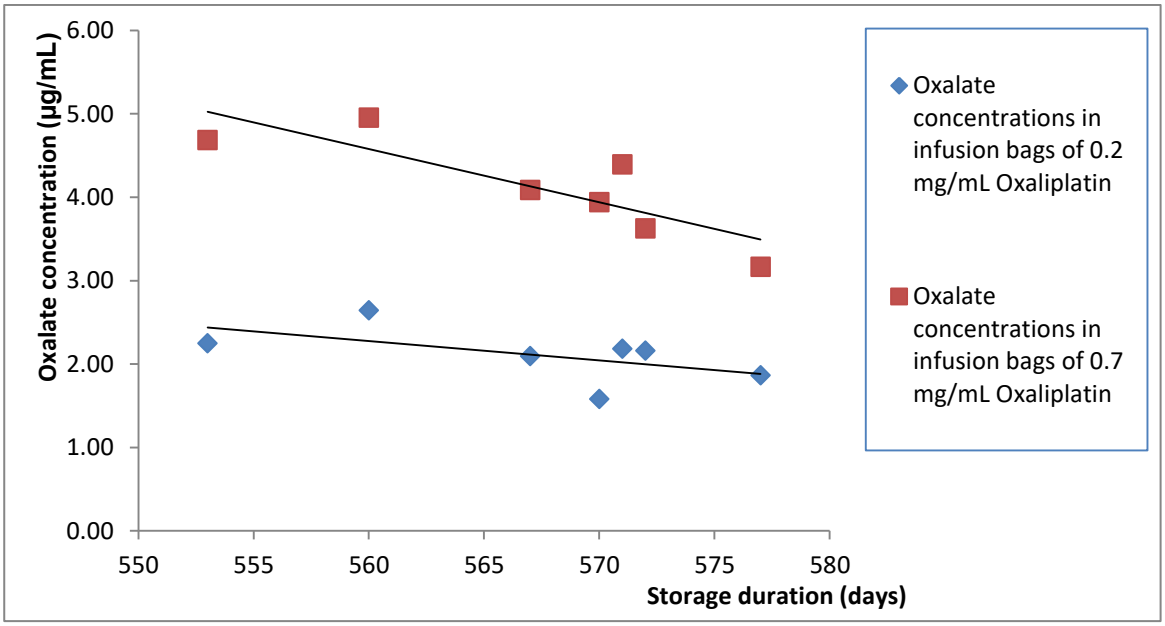

Figure 3-6, Oxalate concentration change in oxaliplatin infusions at different concentrations of 0.2 mg/mL and 0.7mg/mL during the long-term storage period.

3.4.2.2 pH variations

pH measurements for each oxaliplatin infusion at sample times were shown in *Table 3-6*, which also included pH variations compared to the initial values from Time zero (T_0). pH values for all oxaliplatin infusions were within the range of

4.32 – 4.56. The largest pH change over the entire storage time among different oxaliplatin groups was only + 0.19.

Table 3-6, pH measurements for oxaliplatin infusions in Group A – G and the pH difference between sample time and time zero.

Group	Total Storage Duration	pH at T_0	pH at sample time	pH difference
A	577 days	4.36	4.48	+0.12
		4.41	4.52	+0.11
B	572 days	4.28	4.44	+0.16
		4.36	4.53	+0.17
C	571 days	4.28	4.47	+0.19
		4.33	4.52	+0.19
D	570 days	4.37	4.32	-0.05
		4.43	4.48	+0.05
E	567 days	4.38	4.45	+0.07
		4.48	4.56	+0.08
F	560 days	4.36	4.42	+0.06
		4.45	4.52	+0.07
G	553 days	4.36	4.43	+0.07
		4.42	4.50	+0.08

T_0: Time zero in the stability study; Sample time: the date that samples from different group were tested in this study; Storage Duration included storage time during and after the stability study.

3.4.3 Results of the oxalate study with human plasma

As mentioned above in Section 3.3.2, four groups of oxaliplatin sample solutions were prepared respectively in 5% glucose, human plasma and PUF, and then incubated at 37 ℃ for 5 days throughout the entire study of oxalate formation in human plasma with added oxaliplatin.

3.4.3.1 Oxaliplatin concentration change in human plasma

Table 3-7 shows oxaliplatin concentration results for Group A – D. Differences between oxaliplatin concentrations tested at sample time and at time zero (T_0) are also presented in *Table 3-7*, which could show the oxaliplatin degradation process in different solvents (5% glucose, plasma and PUF) in this study.

For Group A, oxaliplatin degraded slowly at a constant rate in 5% glucose when incubated at 37℃. However, the degradation of oxaliplatin in human plasma at 37℃ was different as shown in results from Group B and Group C. In human plasma, oxaliplatin concentrations were reduced significantly within the first day, and then maintained this concentration for the duration of the experiment. Group D showed the change of oxaliplatin in PUF (plasma ultra-filtrate), in which the concentration of oxaliplatin reduced to about 30% of the initial value (at T_0) after the first day stored at 37℃ and then it remained constant.

For all four sample groups (Group A – D), *Figure 3-7* showed the change of oxaliplatin concentrations along with the study time period when stored at 37℃. Oxaliplatin concentrations plotted were the mean values from triple sample solutions in each group, and the standard deviations are shown as error bars in the figure. For Group A, the regression equation of this plot was $y = - 16.789x + 155.2$ ($R^2 = 0.9811$), according to which it could be indicated that the degradation of oxaliplatin in glucose was approximately linear following the time. (Refer to *Figure 3-7*) For Group B, C and D, changes of oxaliplatin concentrations in these groups were similar over the incubation time at 37℃ as shown in *Figure 3-7*.

Table 3-7, Oxaliplatin concentrations at each sample time for Group A – D during incubation at 37℃, and the variations of oxaliplatin concentrations with respect to the initial values at T_0.

Group Identifier	Day 0 (T_0)	Day 1		Day 2		Day 3		Day 4		Day5	
	Conc. (μg/mL)	Conc. (μg/mL)	Conc. Relative to T_0 (%)	Conc. (μg/mL)	Conc. Relative to T_0 (%)	Conc. (μg/mL)	Conc. Relative to T_0 (%)	Conc. (μg/mL)	Conc. Relative to T_0 (%)	Conc. (μg/mL)	Conc. Relative to T_0 (%)
A (Control)	157.8290	140.6313	89.11	114.0717	72.29	106.5970	67.54	85.1450	53.95	75.0930	47.58
B	66.8428	15.8087	23.77	18.3373	27.57	16.3587	24.61	14.3910	21.64	18.1713	27.31
C	61.8797	4.3623	7.05	4.5027	7.07	6.0293	9.75	6.2580	10.10	6.2480	10.11
D	116.2983	34.0350	29.25	9.5550	8.22	5.9377	5.10	5.8173	5.01	6.1470	5.30

Conc.: mean values of oxaliplatin concentration from triplicate samples in each group; Day 0: the start day of the 5 day storage period as Time zero for sample tests; Conc. Relative to T_0: the proportion of oxaliplatin concentration at sample time compared to the concentration at T0.

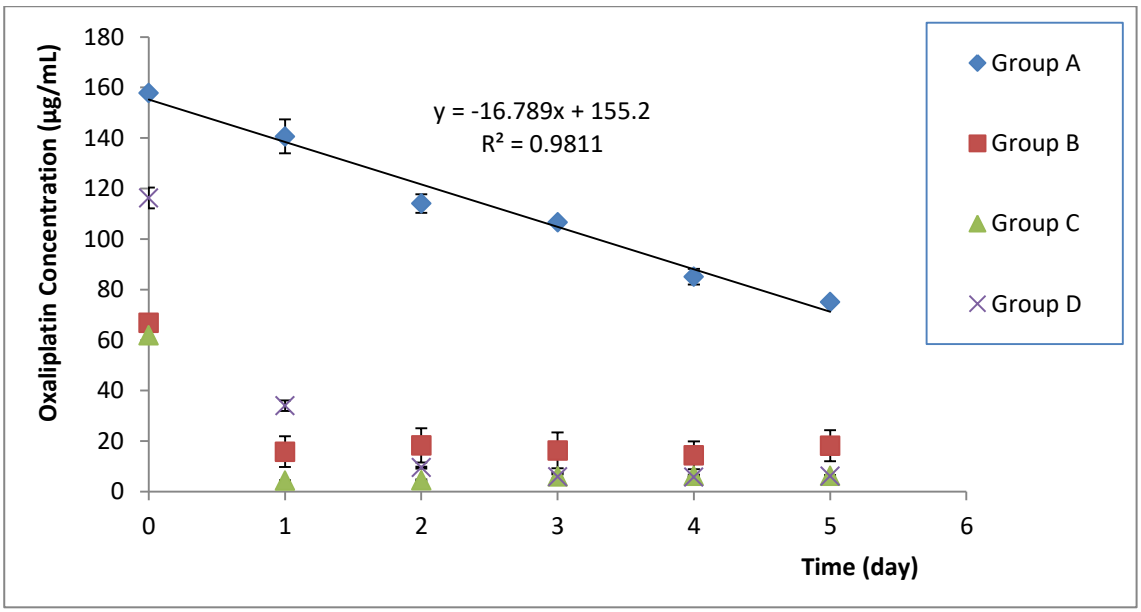

Figure 3-7, Changes of oxaliplatin concentrations in different samples from Group A - D during incubation at 37℃.

3.4.3.2 Oxalate variations in human plasma with/without added oxaliplatin

The oxalate result for Group A – D is shown in *Table 3-8* with the concentration change between each sample time and the initial values tested at T_0. In contrast to the reduction of oxaliplatin, oxalate concentrations have increased in different rates for different groups during the experiment time when incubating at 37℃. Results from the blank plasma group were also presented in *Table 3-8*. The initial concentration of oxalate in blank plasma samples was detected as lower than 1 µg/mL at T_0. The control plasma was also tested every day along with other tests but stored at 2 - 8℃ during the experiment. Oxalate concentration changes of the control plasma and test samples from the blank plasma group are shown in *Figure 3-8* below.

Table 3-8, Oxalate concentrations at each sample time for Group A – D and the blank plasma group during incubation at 37℃, and the variations of oxalate concentrations with respect to the initial values at T_0.

Group Identifier	Day 0 (T_0)	Day 1		Day 2		Day 3		Day 4		Day 5	
	Conc. (μg/mL)	Conc. (μg/mL)	Increased Conc. Relative to T_0 (μg/mL)	Conc. (μg/mL)	Increased Conc. Relative to T_0 (μg/mL)	Conc. (μg/mL)	Increased Conc. Relative to T_0 (μg/mL)	Conc. (μg/mL)	Increased Conc. Relative to T_0 (μg/mL)	Conc. (μg/mL)	Increased Conc. Relative to T_0 (μg/mL)
A (Control)	-1.8417	6.6532	8.4950	12.6188	14.4605	13.5263	15.3680	16.1943	18.0361	24.5566	26.3983
B	9.8226	42.1371	32.3145	41.1126	31.2900	37.5505	27.7279	39.2712	29.4486	46.8622	37.0396
C	9.0041	41.7339	32.7298	42.9444	33.9403	45.8277	36.8236	49.5952	40.5911	51.9782	42.9741
D	4.5020	36.4919	31.9899	40.0950	35.5929	50.6729	46.1709	43.7247	39.2227	45.8390	41.3370
Blank Plasma Group	0.4487	1.5000	-0.0205	1.7100	0.1895	2.3100	1.8613	3.1177	2.6690	2.4466	1.9979

Conc.: mean values of oxalate concentration from triplicate samples in each group; Day 0: the start day of groups storage as Time zero for sample tests.

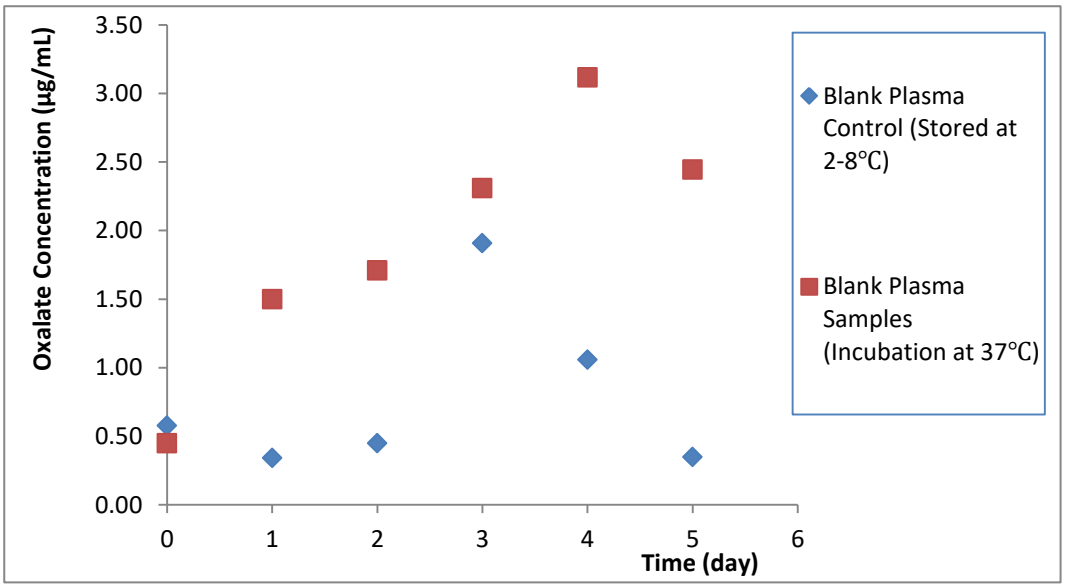

Figure 3-8, Oxalate concentration changes in the control plasma and samples from the blank plasma group, which were stored separately at 2 – 8 ℃ and at 37℃. *[The concentration used in the blank plasma sample plot was the average value calculated from triple tests.]*

Oxalate changes of Group A – D are shown in *Figure 3-9*, and for each group oxalate concentrations on these plots were mean values from triplicate samples with the standard deviations as error bars in the figure. As shown in *Figure 3-9*, the increasing of oxalate concentrations in Group A was not quite linear but the trend was obvious during 5 days storage at 37℃. Concentration changes of oxalate in Group B, C and D throughout the incubation (37℃) time period were also presented in *Figure 3-9*. For these three groups, oxalate was produced largely within the first storage day and the concentrations of oxalate increased at a low rate for remaining days of the experiment.

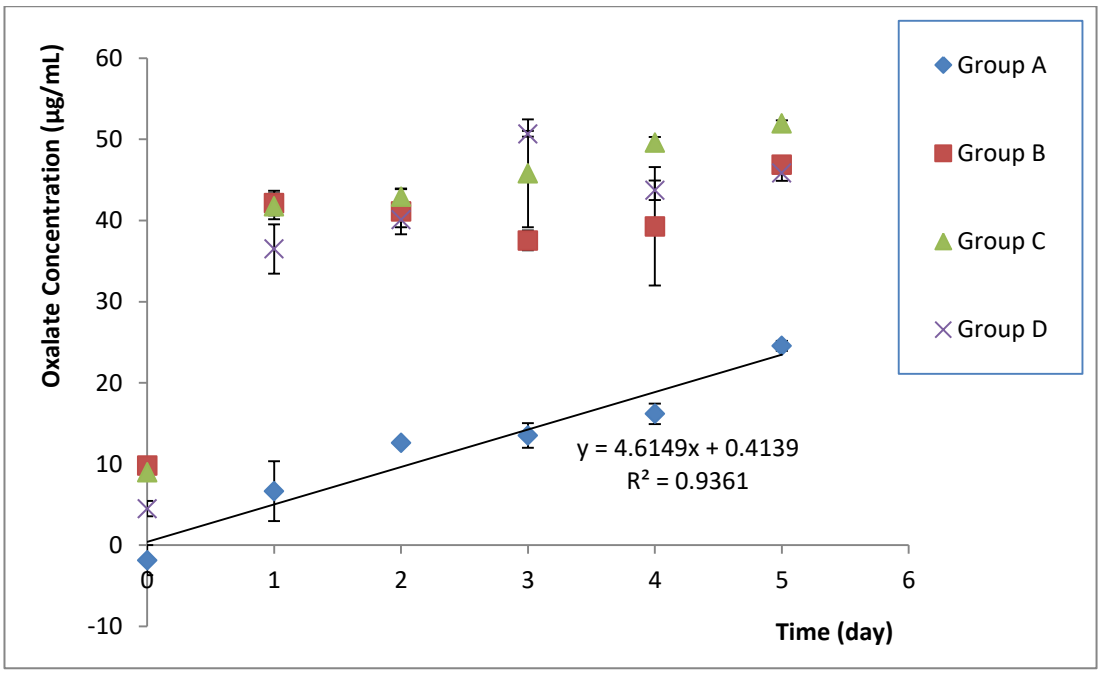

Figure 3-9, Changes of oxalate concentrations in different samples from Group A, B, C and D during incubation at 37℃.

3.4.3.3 pH variation trend

pH measurements for Group A – D at each sample time point was shown in *Table 3-9* with the pH variation compared to the initial value at T_0.

In Group B, C and D, the largest pH variation was only 0.29 among all samples which was too small to indicate any significant change in those solutions. Although the pH variations in Group A were also quite small (within the range of 0.28 – 0.71), there was a reducing trend of pH values in this group as shown in *Figure 3-10*. There were few potential reasons for this pH change which are explored later in the discussion section (Section 3.5.3.4) of this chapter.

Table 3-9, pH measurements at each sample time for Group A – D with the variation compared to the initial value at T_0.

Group Identifier	Day 0 (T_0)	Day 1		Day 2		Day 3		Day 4		Day 5	
	pH	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)
A (Control)	5.25	4.92	-0.33	4.84	-0.42	4.74	-0.51	4.63	-0.62	4.60	-0.65
B	7.11	6.94	-0.17	7.23	0.11	7.1	-0.02	7.2	0.09	7.18	0.07
C	7.06	7.02	-0.03	7.19	0.14	7.23	0.17	7.33	0.27	7.06	0.00
D	7.09	7.11	0.01	7.14	0.05	7.28	0.18	7.25	0.15	7.34	0.25

pH measurements presented in this table were mean values from triplicate samples in each group; Day 0: the start day of groups storage as Time zero for sample tests; pH difference to T_0: difference between the pH of each sample and the initial value at T_0.

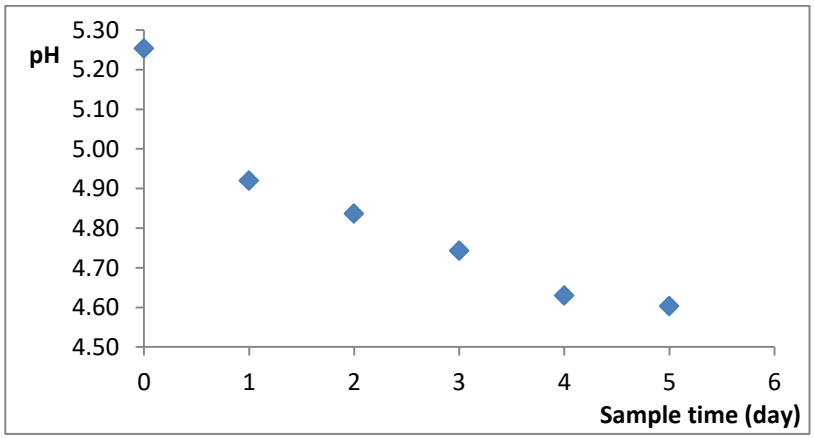

Figure 3-10, pH variation of oxaliplatin infusion samples in Group A during the incubation at 37℃.

3.5 Discussion

3.5.1 Preliminary study on oxaliplatin infusions

The preliminary experiment utilised oxaliplatin infusions from the previous stability study (refer to Chapter 2, page 97) were stored at 2 - 8℃ for more than 550 days, with different storage periods for each group. The determination of oxaliplatin and oxalate concentrations from those samples, provide useful benchmark information of the production and growth of oxalate in oxaliplatin infusions (including 0.2 mg/mL and 0.7 mg/mL oxaliplatin infusions) after prolonged storage.

Results of oxaliplatin and oxalate concentrations from those infusions were presented in *Table 3-5* above. Variations of oxaliplatin concentrations over the storage time were shown in *Figure 3-11* below, in which oxaliplatin concentrations were presented as % variation with respect to the initial values at T0 (referring to the results of the previous stability study in Chapter 2, page 121).

Two separate data series were included in *Figure 3-11*, which presented the concentration variation of oxaliplatin in the infusion bags prepared respectively in 0.2 mg/mL and 0.7 mg/mL. Oxaliplatin concentrations from all infusions have increased compared with the initial value from time zero. However variations detected in 0.7 mg/mL oxaliplatin infusions were slightly higher than the other ones in 0.2 mg/mL infusions at most sample times. Before 575 days, variations of oxaliplatin concentrations in both infusions were within the strict criteria range of ± 5%, while after stored for 577 days the variation of oxaliplatin in 0.7 mg/ml infusions was up to 8.49%.

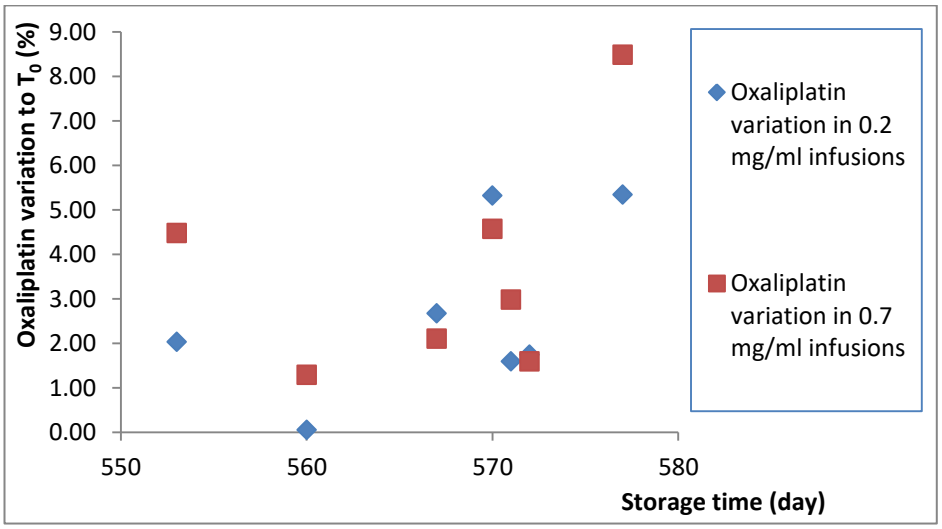

Figure 3-11, Oxaliplatin variations over storage time in infusions that prepared in different oxaliplatin concentrations, 0.2 mg/mL and 0.7 mg/mL. *[T_0 = Time zero; oxaliplatin variation = the proportion of oxaliplatin concentration difference between sample and time zero compared to the initial value from time zero; the oxaliplatin variations shown in this figure are all positive values.]*

Concentrations of oxalate were also determined in those oxaliplatin infusions with a validated HPLC assay. Oxalate results in this preliminary study showed the existence of oxalate in oxaliplatin infusions after storage for a prolonged period. In addition, the amount of oxalate produced from oxaliplatin degradation was detected in those infusions, which could be used to estimate the quantity of oxalate given to patients through oxaliplatin infusions in clinical practice. Consequently, the potential for oxaliplatin infusions to cause oxalate-mediated toxicity was demonstrated.

As shown in *Table 3-10*, measured oxalate concentrations in all samples from those infusions stored for a long period were very low. When compared with oxaliplatin infusions of 0.2 mg/mL, slightly more oxalate was produced from the oxaliplatin degradation in 0.7 mg/mL infusions during the storage time. This may suggest that, oxaliplatin infusions prepared in higher concentrations might produce more oxalate before drug administration, which could increase the potential risk of oxalate-mediated toxicity for patients.

Table 3-10, Oxalate concentrations (mmol/L) in samples from different infusions (prepared with 0.2 mg/mL and 0.7 mg/mL oxaliplatin) after stored at 2 - 8℃ for a period of time.

Group Identifier	Storage period (day)	Oxalate concentrations in samples (mmol/L)	
		0.2 mg/mL oxaliplatin Infusions	0.7 mg/mL oxaliplatin Infusions
A	577	0.02119	0.03596
B	572	0.02452	0.04119
C	571	0.02481	0.04991
D	570	0.01795	0.04476
E	567	0.02378	0.04641
F	560	0.03002	0.05626
G	553	0.02556	0.05324

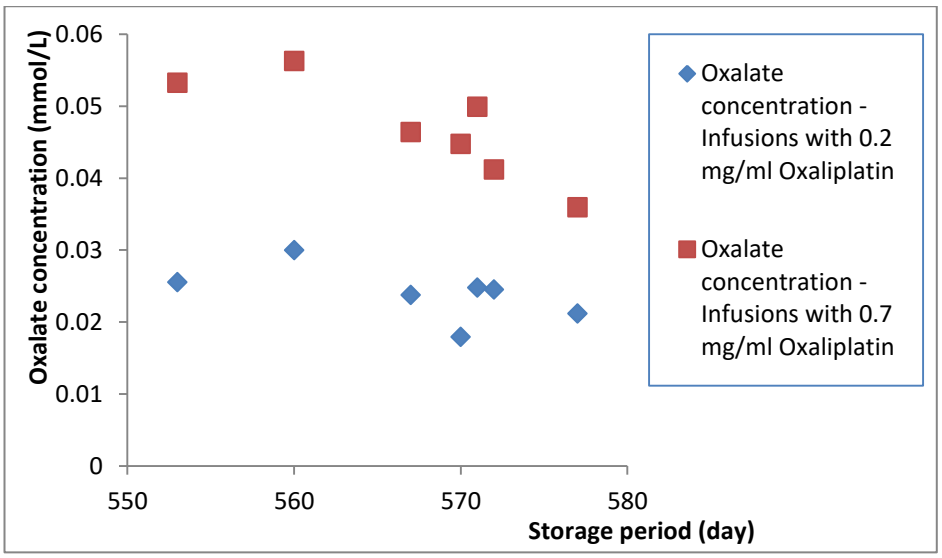

Figure 3-12, Oxalate concentrations (mmol/L) in infusions prepared with 0.2 mg/mL and 0.7 mg/mL oxaliplatin during long-term storage at 2 - 8℃.

As shown in *Figure 3-12*, oxalate concentrations in 0.2 mg/mL oxaliplatin infusions slightly decreased over the storage period. While for 0.7 mg/mL infusions, the reducing trend of oxalate concentrations was more obvious with a larger slope. It can be assumed that before 560 days oxalate concentrations were higher than 0.03002 mmol/mL and 0.05626 mmol/mL respectively for oxaliplatin infusions of 0.2 mg/mL and 0.7 mg/mL.

From previous literature, concentrations of plasma oxalate in humans were determined by various methods. (206) From methods with different accuracy and precision, different results were presented for oxalate concentrations in human plasma and blood samples. (191) (206) In healthy human body, oxalate concentration in blood could range from less than 1 µmol/L to more than 400 µmol/L according to the previous published studies. (206) However, the general consensus of the concentration range for plasma oxalate in healthy humans is 1 – 3 µmol/L, (191) (206) (207) and this range was adopted as a reference for this study.

As shown in *Table 3-10*, oxalate in 0.2 mg/mL oxaliplatin infusions were within the range of 0.01795 mmol/L – 0.03002 mmol/L, while for 0.7 mg/mL infusions

the concentrations were higher from 0.03596 mmol/L to 0.05626 mmol/L. Obviously, all infusions tested in this study were with higher concentration of oxalate than likely to be found in human plasma (1 – 3 μmol/L). (191) However, the maximum amount of oxalate in those infusions of 250 mL was only 0.66 mg and 1.24 mg respectively for 0.2 mg/mL and 0.7 mg/mL oxaliplatin infusions. Compared with the amount of oxalate (at most 50 mg) taken by people through regular diet, the amount of oxalate detected in oxaliplatin infusions was much lower. (186) According to the maximum amount of oxalate (1.24 mg) in 0.7 mg/mL oxaliplatin infusions, the expected plasma concentration of oxalate was 2.8062 μmol/L (calculated with the average blood volume of 5 L (208)), which was within the reference range of oxalate concentration in healthy human bodies. Furthermore, it has been indicated that oxalate was only toxic to renal tubular cells when its concentration reached as high as 5 mmol/L. (188)

3.5.2 Oxalate assay selection for plasma samples

In order to select a sufficient assay for oxalate determination in plasma samples, the validated HPLC assay used in the preliminary study (refer to Section 3.3.1.2) was also tested to determine oxalate in plasma samples. Two groups of plasma samples with added oxaliplatin and one group of oxaliplatin in PUF were included in this trial test. In contrast to the preliminary study with oxaliplatin infusions (prepared in 5% glucose), results from the plasma sample groups displayed huge variations of oxalate concentrations in each sample. Additionally, the blank PUF tested by the same HPLC assay has shown much higher concentrations of oxalate compared to other sample groups with added oxaliplatin. These results potentially indicated the endogenous oxalate existing in plasma and PUF, and also brought the negation of the precision and

accuracy of the oxalate HPLC assay for plasma/PUF samples applied in this trial.

The HPLC assay for oxalate determination selected for the preliminary study (Section 3.3.1.2) was in Hydrophilic Interaction Chromatography (HILIC) mode with an improved amide column from GL science. As discussed above, the HILIC was a preferable technique for polar and/or basic solute, which could provide much higher solute diffusivity and highly symmetrical peak shapes. (193) For polar solute such as oxalate, the HILIC should be better than the regular reversed-phase chromatography. One of the major disadvantages for HILIC was the heavy reliance on the aprotic solvent (such as acetonitrile), and consequently the mobile phase applied in this assay was prepared with 65% acetonitrile. (193) This assay has been improved and validated for oxalate determination in samples of oxaliplatin infusions with 5% glucose in the preliminary study (Section 3.2.3.2). During those validations, it was found that sample dilution in the mobile phase with higher aprotic solvent could improve the chromatogram result for oxalate separation in the HPLC system. Thus, samples tested in this HPLC assay were all diluted within the mobile phase (containing 65% acetonitrile) before being injected into the HPLC system.

However, for plasma/PUF samples, dilution in the solvent with high percentage of acetonitrile might not be suitable considering the deproteinisation effect of acetonitrile on plasma samples. Although plasma samples were ultra-filtered in advance following the preparation schedule, the oxalate determination results in HPLC system could still be influenced by various components in human plasma/PUF. With the diluted samples, the HPLC system can be potentially affected in several ways, such as damage or blockage in the HPLC column;

flowrate change in the system; interference on target substance separation in determination.

Therefore, the HPLC assay with amide column was excluded for oxalate determination in plasma samples, and an alternative enzymatic method with an oxalate assay kit was used in the main study.

3.5.3 Oxaliplatin and oxalate change in plasma during the incubation at 37℃

The main experiment of 'oxalate study in human plasma with oxaliplatin' was designed with 4 groups of oxaliplatin in different diluents which were incubated at 37℃ in order to simulate the degradation process of oxaliplatin in patient bodies. A group of blank plasma was also tested in this study to provide the oxalate data in blank plasma after treatment and storage under the same conditions as the other sample groups.

3.5.3.1 Oxaliplatin degradation in plasma samples

According to the result of this experiment (*Table 3-7*), oxaliplatin prepared in plasma – Groups B and C – degraded at a high reaction rate within the first day of the experiment and then entered a period of steady-state concentration. The degradation process of oxaliplatin in PUF, obtained from Group D, was similar to those results from plasma groups, which has also been reported in a previous study carried by *Jerremalm, Elin.* (124) The degradation process of oxaliplatin within the first day could not be indicated in detail by the results achieved from this study due to the low temporal resolution achievable. But this experiment could at least confirm the very short half-life ($t_{1/2}$) of oxaliplatin in the initial degradation after administration. With the extensive non-enzymatic chemical transformation in human blood, the half-life of oxaliplatin *in vivo* has

been estimated at around 15 minutes in published literature. (124) (131) The half-life of oxaliplatin in human plasma could be approximately calculated as 12 minutes with results from this experiment, which was close to the reference value of 15 minutes. (131)

In this study, Group B was prepared with 0.2 mg/mL oxaliplatin in human plasma and intended to investigate the total amount of oxaliplatin concentrations (including free and protein-bound oxaliplatin) in plasma samples. Group C was assayed for the concentration of un-bound (free) oxaliplatin in plasma. Thus, concentrations of oxaliplatin bound with proteins in plasma could be calculated following the equation,

$Conc.(bound) = Conc.(total) - Conc.(unbound)$ *[Conc.: concentration]*.

Samples from Group B were treated with acetonitrile to break down bonds between oxaliplatin and plasma proteins. This acetonitrile deproteinisation process was validated with the recovery rate of 108.32% and the oxaliplatin concentration found in plasma without acetonitrile treatment was about 54.04% of the total amount of oxaliplatin. As for oxaliplatin results from Group B and Group C, shown in *Figure 3-13* below, the total oxaliplatin was approximately twice the amount of the un-bound oxaliplatin on average.

As shown in *Figure 3-13*, the total amount of oxaliplatin was degraded significantly in the first day, and so was the un-bound (free) oxaliplatin. However, throughout the experiment the observed concentration of protein-bound oxaliplatin remained unchanged with few small fluctuations. It demonstrated that only un-bound or “free” oxaliplatin could undergo degradation. This significant result suggested that it is likely that only the free drug would contribute to therapeutic and adverse effects. In terms of drug

degradation and possible liberation of oxalate, the focus should be on the un-bound fraction (free) of oxaliplatin.

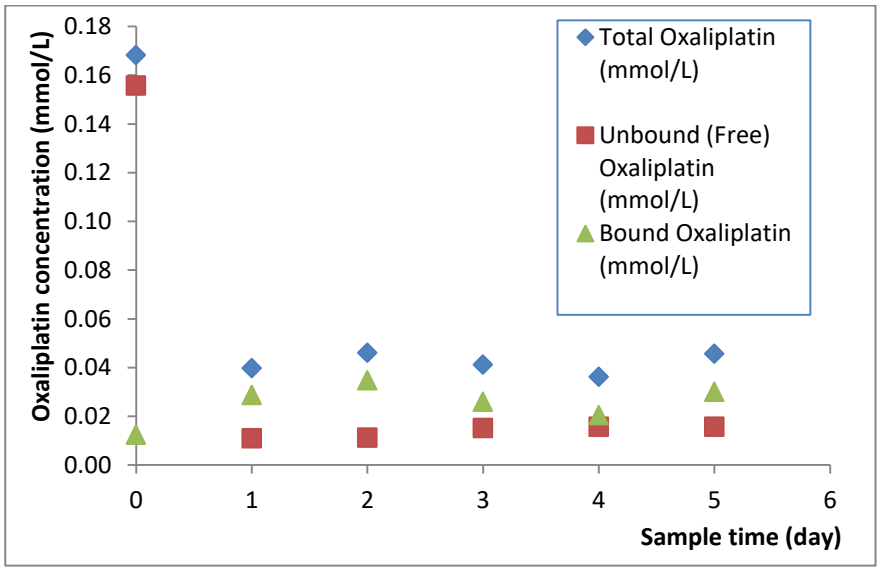

Figure 3-13, Concentrations of total, bound and un-bound (free) oxaliplatin calculated from result of Group B and Group C at each sample day.

Figure 3-14 showed concentrations of oxaliplatin in samples from Group C and Group D, which represented the different degradation process of oxaliplatin in plasma and PUF. As shown in the figure, oxaliplatin in PUF started with a higher concentration but degraded down to the same value as the un-bound oxaliplatin in plasma. The PUF used in this study was prepared by subjecting a certain amount of human plasma to ultra-filter centrifuge. From previous literature, it was believed that within the PUF chloride ion (Cl^-) was more concentrated than the plasma that PUF was prepared from. (209) Moreover, it has been proved that oxaliplatin can be 'bio-transformed' essentially by water and nucleophiles, such as Cl^-. (127) However, Cl^- concentrations in both plasma and PUF were not measured in this study. It cannot be indicated that the different Cl^- concentrations contained in plasma and PUF were the reason for different oxaliplatin concentrations examined in plasma and PUF samples.

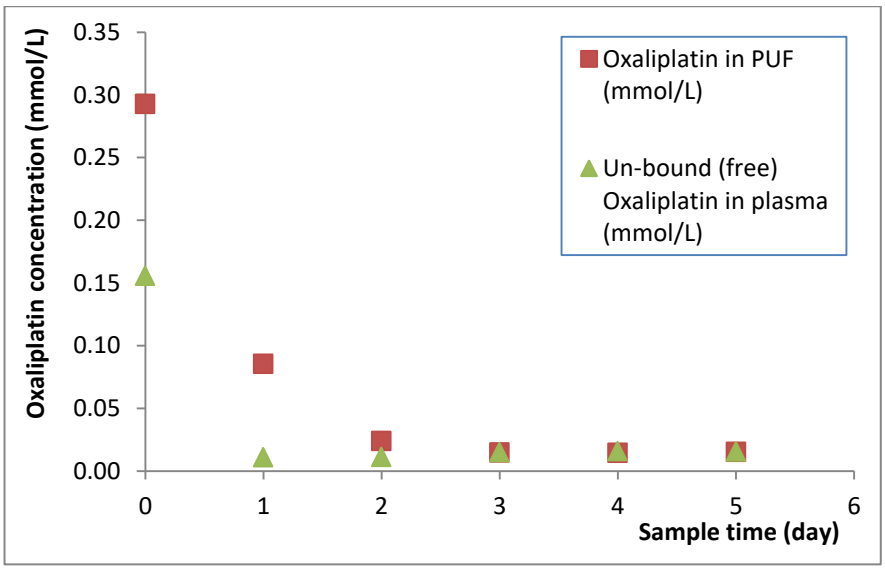

Figure 3-14, Concentrations of un-bound (free) oxaliplatin in plasma from Group C and oxaliplatin in PUF (plasma ultra-filtrate) from Group D at each sample day.

3.5.3.2 Oxalate growth in plasma samples

Since only un-bound (free) oxaliplatin could degrade in plasma samples (as discussed above), there should be the same amount of oxaliplatin degraded in these two plasma groups of Group B and Group C. Because the majority of oxalate in plasma was produced from oxaliplatin degradation, oxalate concentration values and variation tendency were highly similar for Group B and C as shown in *Figure 3-15*. These results also suggested it was less possible that protein bonds occurred between oxalate and plasma proteins, since the acetonitrile treatment operated on Group B samples did not affect the similarity of oxalate result from these two groups. After storage at 37℃ for 5 days, the oxalate result from Group B and C could both represent the growth of oxalate concentration in human plasma after administration with oxaliplatin.

The variation tendency of oxalate concentrations in PUF samples of Group D was similar to the other two plasma groups (Group B and C) with slightly lower values in each sample. This concentration difference between oxalate in plasma

and in PUF samples could possibly be explained by the endogenous oxalate existing in human plasma.

In *Figure 3-15*, oxalate concentrations in plasma samples of Group B and C were corrected with the endogenous oxalate detected in blank plasma control. The average oxalate concentration in blank plasma control was 0.0475 mmol/L, which was close to the average value (0.0317 mmol/L) of the concentration difference between plasma and PUF samples. According to *Figure 3-15*, oxalate concentrations detected in plasma samples (Group B and C) were close to those results from PUF samples (Group D) after correction with the endogenous value of oxalate in blank plasma.

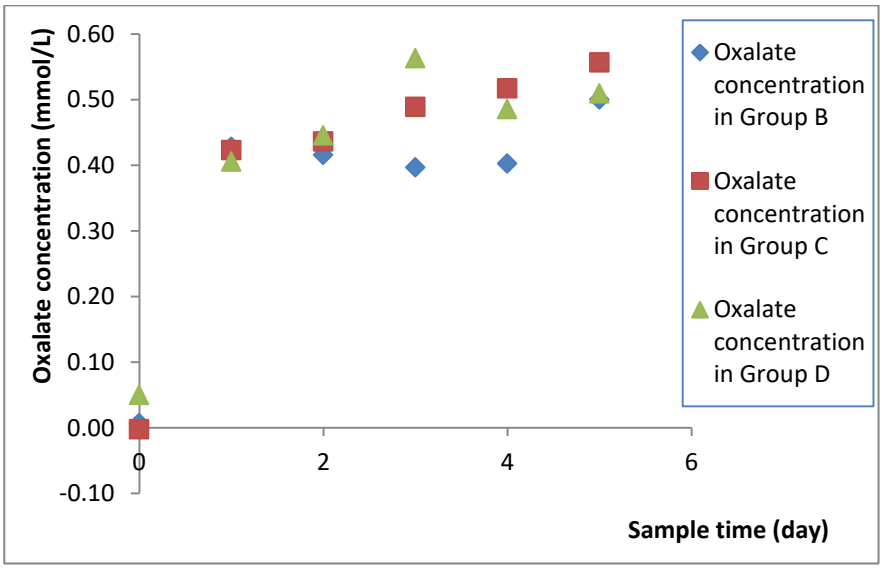

Figure 3-15, Oxalate concentrations for Group B and Group C after correction with the endogenous oxalate value from blank plasma control; and oxalate concentrations for Group D. *[Group B: oxaliplatin samples prepared in plasma and treated with acetonitrile to detect the total amount of oxaliplatin including protein-bound and un-bound (free) oxaliplatin; Group C: oxaliplatin samples prepared in plasma to detect the un-bound (free) oxaliplatin; Group D: oxaliplatin samples prepared in PUF (plasma ultra-filtrate)]*

3.5.3.3 Reconciliation of oxalate production and oxaliplatin degradation

3.5.3.3.1 In oxaliplatin infusions (with 5% glucose) when stored at 37℃

In this study, the control group (Group A) was prepared with 0.2 mg/mL oxaliplatin in 5% glucose, in order to show the change of oxaliplatin and oxalate in glucose infusions after incubation at 37℃ for 5 days. As shown in *Figure 3-7* and *Figure 3-9* above (in Section 3.4.3), concentration changes of both oxaliplatin and oxalate from Group A were both almost linear. In samples of Group A, oxaliplatin concentrations increased with the regression equation of $y = -16.789x + 155.2$ $(R^2 = 0.9811)$, while the concentration of oxalate was with the reduction plot of $y = 4.6149x + 0.4139$ $(R^2 = 0.9361)$. Considering the stability of oxaliplatin in infusions that stored in refrigerated conditions at 2 - 8℃, this result indicated that oxaliplatin degradation in 5% glucose infusions was influenced by temperature (37℃). Therefore, in clinical practice the storage conditions (especially temperature) for oxaliplatin infusions should be under strict control to prevent the potential risk of increased oxaliplatin degradation at higher temperature.

In oxaliplatin infusions, oxalate was produced from oxaliplatin degradation, and theoretically its concentration increase should be in proportion to the concentration loss of oxaliplatin. As shown in *Figure 3-16* below, the concentration increasing trends were at similar rates for oxaliplatin loss and oxalate gain. However, these concentration changes for oxaliplatin and oxalate were not equal in Group A.

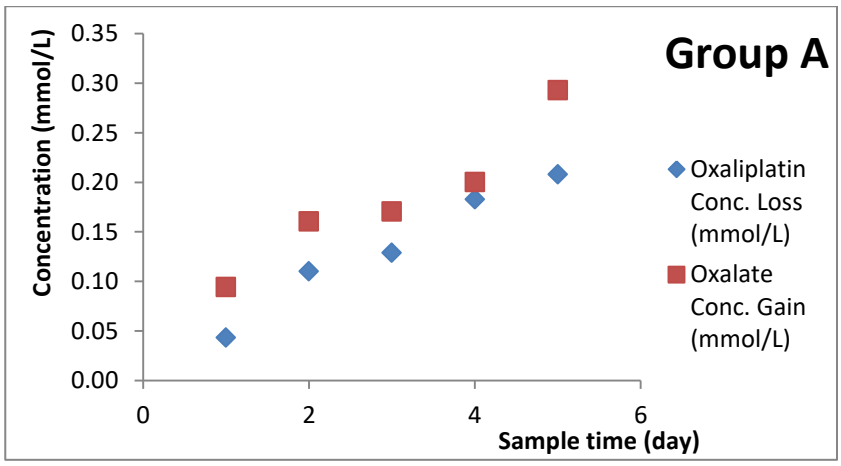

Figure 3-16, Concentrations of oxaliplatin loss and oxalate gain in oxaliplatin infusions with 5% glucose from Group A tested at Day 1 – Day 5. *[Conc. = Concentration in oxaliplatin infusions prepared with 5% glucose.]*

Infusion samples from Group A were prepared with only oxaliplatin and 5% glucose, thereby the potential interference to oxalate production could be from glucose, oxaliplatin and oxaliplatin degradation products, such as $[Pt(DACH)]^{2+}$ (1,2-diaminocyclohexyl-platinum) complexes. Because the oxalate assay used in this study was operated with a spectrophotometer at 590 nm, neither glucose nor $[Pt(DACH)]^{2+}$ complexes can be absorbed and determined at this wavelength according to European Pharmacopoeia. (164) In this case, concentration differences between oxaliplatin loss and oxalate gain at each sample time were not caused by interference from other components in oxaliplatin infusions.

One potential reason for the concentration gap between oxaliplatin loss and oxalate gain could be the pH adjustment to the samples used for oxalate determination during the experiment. As mentioned in Section 3.3.2.3, diluted samples were adjusted to pH 5.0 – 7.0 with 1 N hydrochloric acid (HCl) for the oxalate assay, while samples for oxaliplatin determination were injected into the HPLC system without pH adjustment. It has been demonstrated in previous studies that oxaliplatin decay in aqueous media can be affected by the chloride concentration and pH of the solution. (127) As a result, during the sample

preparation procedure oxaliplatin might be further degraded and consequently produced more oxalate in those samples for oxalate determination. This hypothesis was not verified in this study, in which case the concentration difference between oxaliplatin and oxalate change in infusions cannot be fully explained. However, these concentration differences obtained in Group A could be used as reference to other plasma/PUF groups, in which samples were prepared following the same schedule. The average value of the concentration difference calculated at each sample time was approximate 0.0571 mmol/L, and results of other plasma/PUF groups were corrected with this value and discussed in the following section.

3.5.3.3.2 In plasma/PUF samples when stored at 37℃

As discussed above, oxalate has been implicated in neurotoxicity associated with oxaliplatin chemotherapy and it could also be nephro-toxic when the plasma oxalate concentration increased to a certain level. (12) (188) Therefore, the relationship between oxalate concentration change and oxaliplatin degradation in plasma and PUF (plasma ultra-filtrate) was the focus of this study.

Figure 3-17 and *Figure 3-18* showed the relationship between concentrations of oxaliplatin loss and oxalate gain in plasma samples from Group B and Group C. Oxalate concentration gains shown in these two figures were corrected by three factors, which were 1) the endogenous oxalate tested in plasma control at each sample time; 2) the concentration gap between oxaliplatin loss and oxalate gain observed from the control group, Group A (refer to the above Section 3.5.3.3.1); 3) the increase of endogenous oxalate in blank plasma samples when stored at 37℃ for 5 days, which will be discussed in the following paragraph.

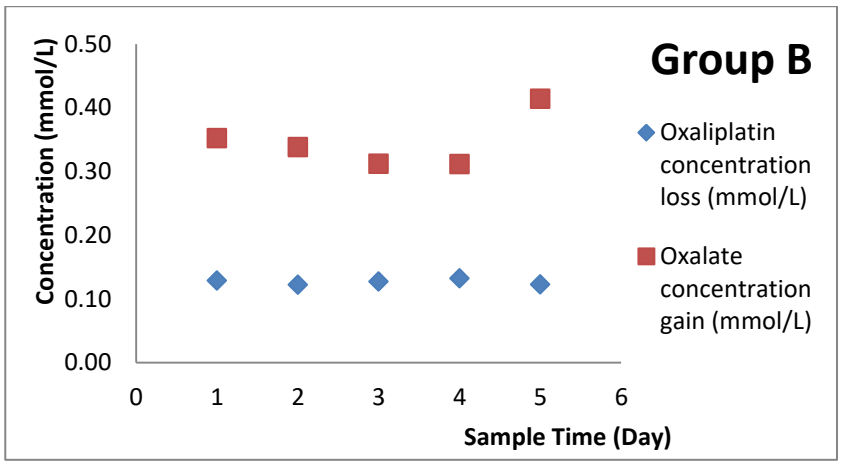

Figure 3-17, The relationship between concentrations of oxaliplatin loss and oxalate gain in Group B. *[Oxalate concentrations have been corrected by three factors.]*

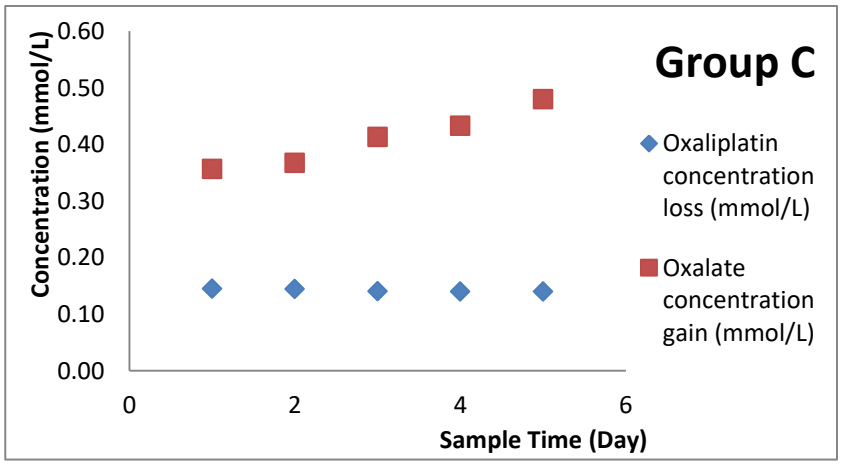

Figure 3-18, The relationship between concentrations of oxaliplatin loss and oxalate gain in Group C. *[Oxalate concentrations have been corrected by three factors.]*

According to those figures above, the oxalate concentration gained in plasma samples was higher than the oxalate that can be explained by oxaliplatin degradation. It could indicated that after oxaliplatin administration the change of oxalate in human plasma was mainly related to non-enzymic transformation of oxaliplatin but partially affected by other factors, such as the endogenous oxalate that originally existed in human plasma.

A group of blank plasma was tested to investigate concentration variations of the endogenous oxalate when incubated at 37℃ for the period of this experiment. All samples in this control group were blank plasma without any additional substances. Table 3-11 presented results of this plasma control

group, and mean values of oxalate concentrations from three tests were shown in *Figure 3-19*.

Table 3-11, Mean values of oxalate concentrations from the blank plasma group after incubated at 37℃ for 5 days.

Test Identifier	Oxalate Concentration (mmol/L)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Plasma Control	0.0064	0.0038	0.005	0.0212	0.0118	0.004
Plasma Test 1	0.0085	0.011	0.016	0.028	0.0353	0.0272
Plasma Test 2	0.0043	0.019	0.021	0.024	0.0332	0.0252
Plasma Test 3	0.0021	0.02	0.02	0.025	0.0353	0.0291
The mean oxalate Conc. among three samples	-0.0014	0.0129	0.014	0.0045	0.0228	0.0232

Conc. = Concentration; the mean oxalate Conc. was the average value calculated from three plasma tests at each sample day, which has been corrected by the oxalate concentrations in plasma control.

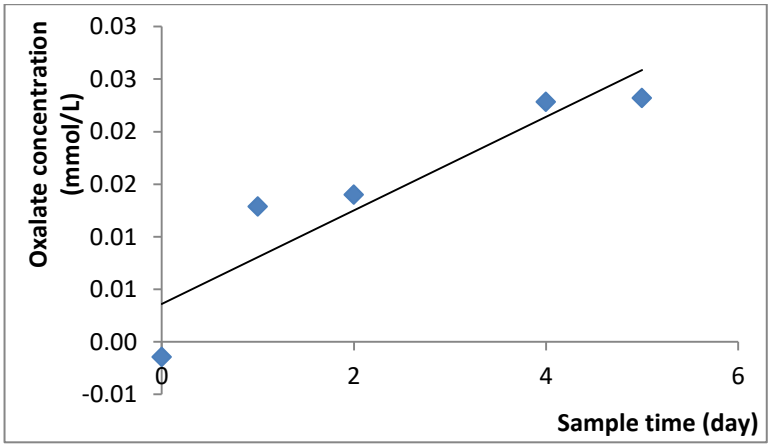

Figure 3-19, Oxalate concentrations in blank plasma tests after incubation at 37℃ for 5 days. *[All concentrations were corrected by plasma control at each day.]*

To simplify the analysis, data from Group C will be referenced in the discussion below, since it could represent the oxaliplatin and oxalate status in plasma as discussed in above sections (Section 3.5.3.1 and Section 3.5.3.2).

Assuming that the concentration of oxalate produced from oxaliplatin degradation was equal to the loss of oxaliplatin which was 0.1448 mmol/L at the first day in Group C. The total increased oxalate in plasma samples observed was 0.3568 mmol/L which suggested the extra oxalate generated from somewhere unknown was 0.2237 mmol/L. As shown in *Table 3-11*, after

storage under the same conditions at 37℃, oxalate concentrations in blank plasma were increased, and within the first day 0.0143 mmol/L oxalate was gained. Subtracting the endogenous oxalate increased by plasma itself, there was still 0.2094 mmol/L more oxalate that could not be traced back to the source.

However, after excluding the effect of temperature on plasma, it could at least be confirmed that the unexplained concentration change of oxalate was related to oxaliplatin that was administered to plasma samples. Except for the degradation reaction, oxaliplatin might have other influences on human plasma, which could promote the oxalate production in plasma. Within the first day after administration 0.2 mg/mL oxaliplatin could cause an increase of oxalate in human plasma of approximate 0.3542 mmol/L. As described above, oxaliplatin concentrations were stabilized around 0.0138 mmol/L in plasma after the first day, while oxalate was still increasing in a gentle slope. (Shown in *Figure 3-18*) Therefore, those hypothetical effects from oxaliplatin might also interfere in the second phase of this experiment (Day 2 – Day 5). Although it was not investigated in this study, the potential relationship between oxaliplatin and endogenous oxalate in human plasma could be of interest for further studies on related subjects in the future.

Those hypothetical effects of oxaliplatin on plasma could be related to a number of issues, such as 1) the sudden increase of oxalate after oxaliplatin administration might influence the endogenous oxalate in plasma; 2) oxaliplatin might have triggered some reactions between substances contained in plasma; 3) other degradation products from oxaliplatin might be involved.

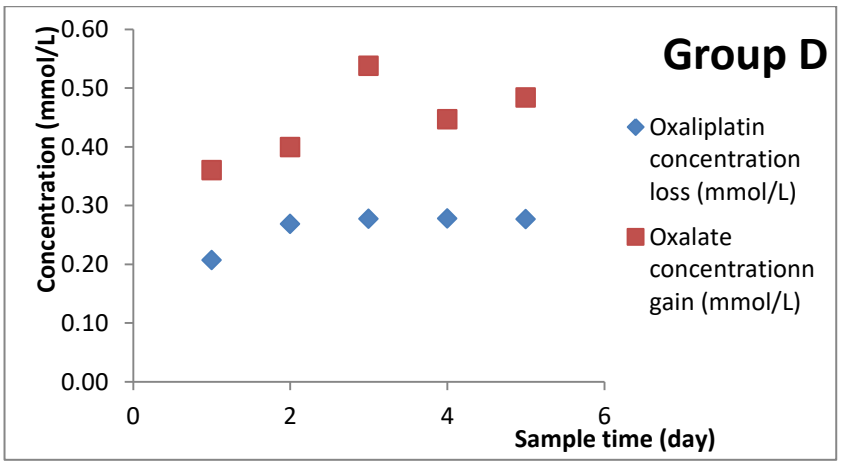

Figure 3-20, The relationship between concentrations of oxaliplatin loss and oxalate gain in Group D.

The relationship between concentrations of oxaliplatin loss and oxalate gain in PUF (Group D) was shown in *Figure 3-20*. As discussed in the above sections, changes of oxaliplatin and oxalate in PUF were similar to those results in plasma, with the exception that the decay of oxaliplatin in PUF occurring at a higher degradation rate. More oxaliplatin was degraded and consequently more oxalate should be produced compared to plasma samples. However, when compared to the results from plasma groups, the difference between oxaliplatin loss and oxalate gain was slightly smaller in PUF. This meant the extra oxalate produced from plasma by the hypothetical effects from oxaliplatin was less in PUF than in plasma. In this case, the potential mechanism for the oxaliplatin influence on plasma might be more related with the substances which exist in human plasma but are not included in PUF.

3.5.3.4 pH variation

pH measurements of samples from each group were subject to no interference from other potential factors since those samples were all measured directly from the blood container. During 5 days incubation at 37℃, pH variations of samples from Group A, B, C and D were all relatively small.

Figure 3-10 presented in Section 3.4.3.3 (page 189) showed the reducing trend of pH values from oxaliplatin infusion samples in the control group of Group A. Two potential causes for pH reduction were considered: oxaliplatin degradation and change of glucose solvent. This pH reduction trend did not appear in other oxaliplatin groups that were prepared with plasma and PUF, in which case pH reduction should not be attributed by oxaliplatin degradation. Thus the result suggested that after incubation at 37℃, the glucose related reaction might be triggered which influenced the pH value of the solution.

A previous study on glucose degradation was conducted by *Per Kjellstrand* (210), which indicated that temperature was the most important factor affecting the degradation of glucose among all storage conditions. Furthermore, pH reduced concurrently with glucose degradation under the storage temperature of 40℃ and the decrease rate for pH variation was higher with higher storage temperature. (210)

Therefore, the pH reduction trend occurred with samples from Group A in this experiment could be explained by the degradation of glucose after storage at 37℃ for 5 days.

3.5.4 Clinical significance

In the preliminary study, as discussed above, oxaliplatin infusions prepared with 5% glucose were tested for both oxaliplatin and oxalate determination. After being stored at 2 – 8 ℃ for a long period of 577 days, the maximum amount of oxalate detected from 0.7 mg/mL oxaliplatin infusions (250 mL) was 1.24 mg, which was relatively small compared with the level of endogenous plasma oxalate. Therefore, the amount of oxalate produced in oxaliplatin infusions was acceptable and safe to human bodies. Consequently, in clinical practice

oxaliplatin infusions freshly prepared or with short-term storage at 2 - 8℃ before the drug administration should be free from any potential toxicity related to/caused by oxalate. It could even be speculated that after being stored for 3 months (the proven stability period for oxaliplatin infusions from the previous stability study described in Chapter 2, page 136), the amount of oxalate generated in oxaliplatin infusions should be too small to cause any potential toxic effects to patients.

In the main experiment, oxalate was detected within plasma/PUF samples after administration with oxaliplatin. However, the concentration of oxaliplatin (0.2 mg/mL) applied in this study was 100 times higher than the C_{max} value quoted in studies in the published literature. (11) Therefore, oxaliplatin and oxalate concentrations obtained from this study were reduced by 100 times to simulate the *in vivo* situation of plasma in patients. The highest concentration of oxalate obtained in plasma samples was 4.997 µmol/L after scaling down by 100 times and corrected by the plasma control test. This oxalate concentration value could be used to represent the highest oxalate concentration in the plasma of a patient's body within 5 days after the administration of oxaliplatin. According to the previous literature, the concentration of plasma oxalate in healthy bodies was generally considered in 1 – 3 µmol/L, while in some cases it could be as high as 10 µmol/L but without any toxicity or potential damage to human bodies. (191) (207) Therefore, after oxaliplatin administration the amount of oxalate increased in plasma samples in this study was highly unlikely to cause significant toxic effects on patients.

As mentioned in above sections, oxalate was the most likely suspect for causing the neurotoxicity associated with oxaliplatin therapies. Some previous published studies have excluded several potential factors that may cause the

neurotoxicity, and suggested that oxalate could be involved since it is the main degradation product of oxaliplatin. (12) (183)

Regardless of the unknown mechanism of the acute neurotoxicity associated with oxaliplatin treatments, in order to cause any toxicity or damage, the amount of oxalate in blood circulation of human bodies had to be in the abnormal conditions or reach the toxic concentration level. (145) (146) However, results from this study proved that the amount of oxalate did not increase to abnormal level neither in oxaliplatin infusions nor in human plasma after administration of oxaliplatin infusions.

The experiment on plasma/PUF samples was only conducted for 5 days after drug administration. As shown in result tables (Section 3.4.3.2, page 187), oxalate concentrations were possessed a slight increasing trend at the end of the experiment, and were likely to increase further after these 5 days. Nevertheless, the acute neurotoxicity reported in the literature normally appeared very quickly after the oxaliplatin administration. In this case, results of this study should be sufficient to analyse this type of neurotoxicity associated with oxaliplatin therapies.

In summary, according to this study the amount of oxalate in both oxaliplatin infusions and in plasma (after oxaliplatin administration) was within the safe level for patients. Oxalate might not be the relative factor to the acute neurotoxicity which has been observed with oxaliplatin treatment.

This study also showed that storage temperature conditions could have a significant influence on oxaliplatin infusions. In the preliminary study, more oxalate was detected in infusions with higher prepared oxaliplatin concentration (0.7 mg/mL). Therefore, with the concern of individual dosage for patients

oxaliplatin infusions prepared in clinical practice should be better calculated and prepared in lower concentration to reduce the risk for producing more oxalate.

In addition, it was proven that oxaliplatin degraded faster in 5% glucose when stored at high temperature (37℃) (Section 3.4.3.1). Thus, the storage condition for oxaliplatin infusions in 5% glucose should be strictly controlled without exposure to high temperature even for a short time period. In clinical practice, the storage of oxaliplatin infusions should take extra concern in order to prevent any potential risk for patients.

3.5.5 Limitations

In the preliminary study (refer to Section 3.5.1), oxaliplatin infusions in 5% glucose from the previous stability study (described in Chapter 2, page 97) were used for oxalate and oxaliplatin determinations after a long-term storage at 2 – 8℃. Results from the preliminary study suggested that the amount of oxalate produced in oxaliplatin infusions was speculated to be safe for patients. However, results could be more convincing if the oxalate determination would have been carried out concurrently with the oxaliplatin assay for all groups at each sample time during the stability study presented in Chapter 2.

In clinical practice and dose-banding schemes, oxaliplatin infusions would not be used after a long-term storage, such as 500 days. The amount of oxalate detected in this preliminary study could not directly indicate the safety level of oxalate existing in oxaliplatin infusions that normally administered to patients. In addition, the increase tendency of oxalate in oxaliplatin infusions was not fully analysed in this preliminary study because of the lack of data from the great time gap during the long storage period.

3.6 Conclusion

The amount of oxalate produced in oxaliplatin infusions (in 5% glucose) after long-term storage at 2 – 8℃ was relatively small and should not be considered harmful or toxic to human bodies.

The concentration of oxalate increased in plasma after oxaliplatin administration when stored at 37℃ for 5 days. However, the total amount of increased plasma oxalate was relatively low, which suggested that oxalate might not be the cause of the acute neurotoxicity associated with oxaliplatin therapies.

Chapter 4: An *ex vivo* pharmacokinetics (PK) simulation study of oxaliplatin administered with different dosing schemes

4.1 Introduction

4.1.1 Pharmacokinetics (PK)

4.1.1.1 Introduction to PK

Pharmacokinetics, as a branch of pharmacology, is the attempt to discover what happens to a drug from its administration to the complete elimination from the body. (211) (212) Generally, after oral administration a drug will be subjected to four processes in the body, which are absorption (A), distribution (D), metabolism (M) and excretion (E). (212) In the case of intravenous administration, the absorption phase is effectively instantaneous.

Pharmacokinetics is the study of the time course of these four processes for drugs and the relationships between drug dose, plasma concentration and pharmacological (therapeutic and toxic) effect within the ADME processes. (211) (213) Key pharmacokinetic parameters related to the ADME processes include bioavailability (bioequivalence), apparent volume of distribution, biotransformation and clearance. (211) (213)

Bioavailability is normally used to describe the rate and extent of drug absorption (A), which could be defined as the fraction of the administered drug dose that reaches systemic circulation. (211) According to this definition, different administration routes of drugs result in different bioavailability (the bioavailability for a drug administered intravenously can be defined as 100%). (211) (214) Thus, bioavailability is an important tool to determine the differences in drug absorption rates between non-intravenous forms of drug administration such as oral and percutaneous drug-delivery. (211) (213)

For a given drug distribution (D) process, the apparent volume of distribution (V_d) represents the relationship between the drug concentration in blood/plasma and the amount of drug in the whole body. (211) (215) However, there is no obvious relationship between the apparent and real volumes of drug distribution in patients' bodies. (211) (215) The real volume of drug distribution is related to the different parts of the body fluid, including vascular fluid or blood, extracellular fluid and intracellular fluid. (211) (213) In general, for drugs bound to plasma proteins, the apparent volume of distribution is smaller than the real distribution volume; for drugs bound to extravascular tissues, the apparent volume of distribution is larger than the real volume of distribution. (211) (213) The apparent volume of drug distribution can be used to estimate the theoretical distribution volume and facilitate the production of the desired drug concentration in blood circulation. (211)

Total clearance is another important pharmacokinetic parameter, which is used to characterize drug elimination (containing elements of both metabolism and excretion). (211) (216) Clearance indicates the volume of plasma (or blood) from which the drug is completely removed or cleared in a certain time period. (216) Drugs can be cleared from the body through various organs with different mechanisms, including renal filtration and secretion, hepatic biotransformation, biliary excretion, gastrointestinal tract clearance and pulmonary clearance. (211) (216) Total/systemic clearance is the sum of all individual clearance that contribute to the overall elimination of a drug, as shown in the equation: $CL_t = CL_r + CL_h + CL_b + CL_{other}$. *[CL_t: total body clearance; CL_r: renal clearance; CL_h: hepatic clearance; CL_b: biliary clearance; CL_{other}: clearance by all other routes.]* (211) (216)

To conceptualize the interactions between the drug and organisms, a number of models have been developed. (216) The most commonly applied approach in pharmacokinetic studies is the compartmental model, which represents the body as a system of compartments to simplify complicated physiological systems. (211) (217) These compartments obviously are not physiologic or anatomically related, but this modelling system gives the best approximation to reality. (216) In compartmental models, the rates of transfer/elimination between/from compartments are assumed to follow first-order or linear kinetics. (211) (217) There are some drugs with nonlinear absorption, distribution or elimination characteristics, for such cases the 'population' model is preferred with additional consideration of random effects. (211) (217) Among the compartmental models, the one-compartment model is the simplest and most-frequently used model for drugs that distribute throughout the body relatively rapidly or instantaneously in some cases. (211) (217) Multi-compartment models can be applied to those drugs that have different distribution rates to different tissues. (211) In multi-compartment models, the highly perfused organs/tissues and blood may be treated as the central compartment, while the poorly perfused organs/tissues may be considered as peripheral compartments. (211)

Another modelling method used commonly is the non-compartment model, which can be applied to any compartmental model with fewer restrictive assumptions than other approaches. (130) (211) (217) In addition, based on the estimation of total drug exposure (refer to AUC), a non-compartment model can be used to estimate the drug's associated pharmacokinetic parameters, such as bioavailability, clearance, apparent volume of distribution, half-life ($t_{1/2}$), T_{max}, C_{max} and the fraction of a drug dose converted to a specific metabolite. (211)

(130) A non-compartment model can also be used to predict the average steady-state concentration (C_{ss}) and the time required to reach a certain fraction of the steady-state concentration of a drug. (211) Therefore, a non-compartment model was selected for this study to obtain required pharmacokinetic parameters which would indicate the pharmacologic effects of oxaliplatin with different dosing and administration schemes.

4.1.1.2 The importance of PK

Pharmacokinetics is one of the fundamental scientific disciplines for the design and development of new drugs and for the reassessment of old drugs. (211) (218)

The general drug design comprises several steps, including: 1) setting up a valid hypothesis of the target enzyme/receptor for the disease; 2) testing the biological activities with suitable models (or surrogate markers); 3) screening the *ex vivo* and *in vivo* biological activities of the new drug molecules. (218)

Several compounds will be tested for their *ex vivo* activities as drug candidates during the screening stage. (216) (218) However, the result from *ex vivo* and animal experiment could be misleading without considering their pharmacokinetic characteristics, for example a promising compound could be excluded from the experiment with an inappropriate animal species or poor experimental design. (218) Early and rapid pharmacokinetic information from estimations are essential to select the optimal drug candidates with good bioavailability, desired half-life ($t_{1/2}$) and suitable first-pass metabolism. (211) (218) In the development stage, minimal toxicity and maximum efficacy of selected drug candidates are the focus, which requires the most available pharmacokinetic data for the drug on absorption, distribution, metabolism and

excretion processes. (211) (218) In addition, pharmacokinetics could explain the underlying mechanisms of other important issues in drug development, such as drug-drug interaction, inter-individual variability of the drug efficacy and the relevant safety/risk assessment for inter-relationships between animal and human experiments or between *ex vivo* and *in vivo* studies. (211) (218) (219)

In order to achieve the efficacy and safety of drug treatments, clinical pharmacokinetics, based on the knowledge of drug pharmacokinetics, is directly applied to therapeutic situations during the clinical practice of pharmaceutical care. (218) (219) Clinical pharmacokinetics can be applied to optimise the drug regimen, with relation to the dosage adjustment, drug formulation selection and optimal drug administration route. (213) (219) Besides this, the therapeutic drug monitoring (TDM) for drugs with narrow therapeutic window or high toxicity also requires the application of clinical pharmacokinetics, where plasma drug concentrations are determined during treatments and compared with defined, clinically-acceptable limits. (211) (213) (219)

4.1.1.3 PK parameters

A graphic profile of drug pharmacokinetics is the curve of plasma drug concentration versus time, represented as a typical time course in *Figure 4-1* below. Within this typical curve, there are four primary parameters used to characterise the PK of a drug substance: AUC, C_{max}, T_{max} and $T_{1/2}$. After the drug is administered, the highest concentration in blood/plasma (C_{max}) will be achieved at a certain time point (T_{max}), which should be compared to the effective level of drug C_{max} required to provide good therapeutic efficacy for patients. (212) (219) C_{max} and T_{max} exhibit larger variability due to the administration route than drug dosage. (212) (220) The hierarchical order of

different levels of C_{max} and T_{max} achieved with different drug administration routes is shown below: (212) (220)

C_{max}: Intravenous bolus>Intravenous infusion>Oral medicine>Intramuscular injection

T_{max}: Intravenous bolus<Intravenous infusion<Oral medicine<Intramuscular injection.

$T_{1/2}$ is one of the main pharmacokinetic parameters of a drug, which represents the time for the drug concentration to be reduced by half. (211) $T_{1/2}$ is normally used to estimate the drug elimination and the time period of body exposure to a drug. (212) (216) In multiple-dosing methods, $T_{1/2}$ has value in determining of the frequency of drug dosing in order to obtain the desired blood/plasma drug concentration in a patient's body. (211) (220)

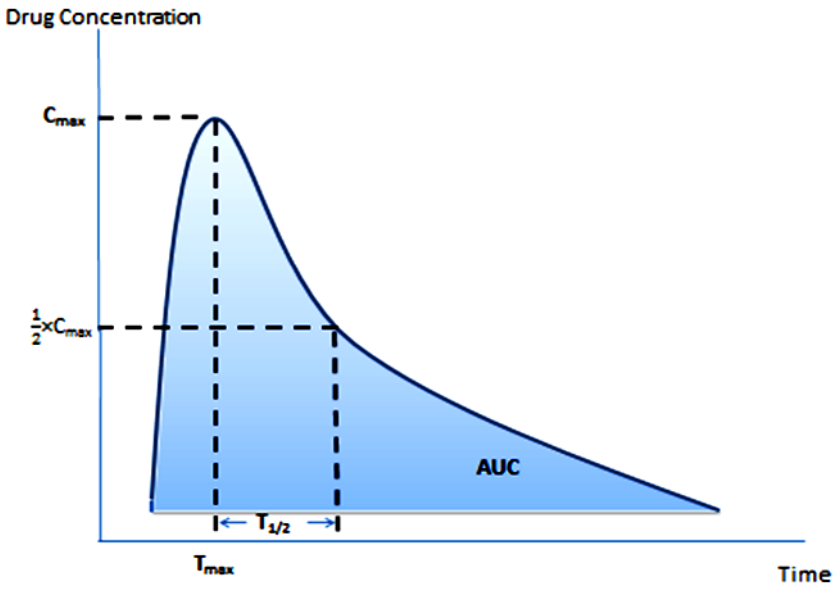

Figure 4-1, A typical pharmacokinetic drug concentration-time curve. *[C_{max}: the highest drug concentration after administration; T_{max}: the time point when C_{max} is achieved; $T_{1/2}$: the half-life of drug (using the decay from C_{max} as example); AUC: the area under the drug concentration-time curve.]*

AUC, short for area under the drug concentration-time curve, is mathematically expressed as the integral of the plasma drug concentration over an interval of

definite time. (212) (219) In clinical practice, the commonly used method to estimate a drug pharmacokinetic profile is to plot time and the plasma drug concentration measured at a number of discrete time points. (211) (212) (213) More sample measurements of plasma concentrations would be expected to increase the precision of the drug AUC value. (212) As shown in *Figure 4-1*, the AUC is dependent upon the achieved/maintained drug concentration and the length of the drug elimination time, which contributes to the drug exposure to the body's tissues and organs. (216) The efficacy and toxicity of a drug, as discussed above, are related to the degree of drug exposure in the systemic compartment of the patient body. (211) (212) As a result, AUC can be used as a surrogate for the total drug exposure over time and this information can facilitate various methods to increase the drug efficacy or reduce the toxicity, such as dosage adjustment, administration scheduling and formulation selection. (212) (219) In this study, five different administration schedules were evaluated and the AUC values were used in the data analysis as the key index to estimate and compare the therapeutic effect that these dosing methods would have on clinical outcomes.

4.1.2 Background to this study

4.1.2.1 PK characteristics for dosing methods selection

An *ex vivo* PK simulation model under laboratory conditions was designed and applied in this study to compare the key PK parameters from five different dosing methods, including Individual dosing, dose-banding with 5% and 10% deviation in each band, Logarithmic dose-banding and Flat-fixed dosing. These drug administration methods are all related to intravenous infusion but with different dose calculations and infusion administration systems.

In order to obtain a reliable comparison, drug infusions were prepared in similar volumes. Drug concentrations in the 'central compartment' were measured at 22 time points, to produce a drug concentration-time curve for each 'patient' with each administration method. The simulated PK parameters used for comparisons between the 5 different dosing methods were C_{max} and AUC. C_{max} indicates the highest drug concentration achieved in the central compartment (blood circulation), which, ideally would remain within the threshold concentrations for the drug efficacy and toxicity if a therapeutic window could be defined. AUC represents the total drug exposure in a 'patient's body', which is a key PK surrogate to estimate the drug efficacy and toxicity. As with C_{max}, AUC can be calculated from the drug concentration-time curve by using specialist PK curve-fitting software.

In clinical practice, the individual dosing scheme preparation according to patient's BSA (body surface area), is used for the routine administration of oxaliplatin in chemotherapy. This traditional BSA-based dosing method is believed to provide the safest and most effective dose algorithms for patients when receiving cytotoxic drugs with a narrow therapeutic window. (8) However, the individualized BSA-based dosing is not optimal in many cases, and alternative dosing methods should be considered to provide more effective chemotherapy administrations to patients. (221) Therefore, this study will compare the new drug dosing approaches, such as different dose-banding schemes and flat-fixed dosing, with the conventional individualised dosing method. To identify any differences in the availability of drug to the tissues in a patient's body between these different dosing systems, the comparison will focus on oxaliplatin PK parameters of AUC and C_{max}.

4.1.2.2 PK data from previous studies of oxaliplatin

Pharmacokinetic evaluation of oxaliplatin is a key part for the drug clinical development. Previously published PK studies on oxaliplatin are summarised in *Table 4-1* with key data for oxaliplatin pharmacokinetic parameters.

Since all these PK studies of oxaliplatin have been conducted under different laboratory conditions, there was a large variability between key PK parameters obtained by different studies. To ensure the referenced studies were of comparable quality, only the more robust studies were considered in this study, which met the following conditions:

- Samples from at least **10 patients** were taken, regardless of whether they received oxaliplatin in combination chemotherapy or as a single agent.
- The oxaliplatin dosage used in the study was within the range of **70 – 90 mg/m^2**, which was closer to the licenced dose of 85 mg/m^2 used in this study.
- Blood samples were taken at a minimum of **10 time points within 24 hours**.

Generally, it was considered that the higher sampling frequency would reduce the influence of any experimental errors and would add to the robustness of the concentration-time curve data obtained.

Differences among previous PK studies of oxaliplatin included different drug administration systems (e.g. pumps, giving sets), assay methods and different PK curve-fitting models used for data analysis. In addition to the use of oxaliplatin in different combination chemotherapy regimens, the lengths of intravenous infusion of oxaliplatin also varied between studies (such as 2 hours, 3 hours or 4 hours). Oxaliplatin assays in blood samples were different, but there were some common methods used in these studies including inductively coupled plasma mass spectrometer, flameless atomic absorption

spectrophotometry and liquid chromatography with post-column derivatization. The most common PK model used to analyse oxaliplatin PK data was the non-compartmental PK model, while 2-compartment and 3-compartment models were occasionally reported.

To assemble the *ex vivo* PK simulation model, the reference values of V_d and CL_{total} are required, which should be selected from previous PK studies on oxaliplatin. However, analysis of the available data identified significant differences between previous studies in key pharmacokinetic parameters, such as V_d and CL, (see *Table 4-1* below). Aside from the issues discussed above, these variations might also be attributed to the preparation and treatment of patient samples. In some studies patient samples were assayed directly as blood samples, whereas plasma and PUF samples were detected in other studies (shown in *Table 4-1*). To represent the distribution volume of total oxaliplatin in blood samples, V_d data demonstrated in three previous studies were selected for analysis, including *Takimoto (2007)* (129), *Ehrsson (2002)* (131) and *Delord (2002)* (222). Thus, the average value of V_d = 12.41 L/m^2 was used for model assembly in this *ex vivo* PK study. The total clearance (CL_{total}) of oxaliplatin referred in *ex vivo* simulations (2.88 L/h/m^2) was selected as the mean value of general oxaliplatin clearance obtained in previous studies listed in *Table 4-1* below with major outliers excluded.

For practical and financial purposes, it was necessary to scale down the distribution volume used in the *ex vivo* model from the normal range of physiological volumes of human patients. The scale-down system applied in this study is described in detail in the experimental section. (Section 4.3.1.1)

Table 4-1, Summary of published literature on oxaliplatin pharmacokinetics.

Resource	Dose (mg/m^2)	No. of Patients	Sample Type	AUC_{0-t} (μg*h/mL)	CL_{total} (L/h)	C_{max} (μg/mL)	V_d (L)	$T_{1/2}$ (h)	V_d (L/m^2) in whole blood	CL ($L/h/m^2$)
Claudia Burz, 2009 (3)	85	4	Plasma	51.4 (t=24h)	2.77	1.61	108	26.1	15.08	1.55
			UFT	4.8 (t=24h)	30.17	0.38	567	12.1	16.67	16.85
	130	4	Plasma	54.81 (t=24h)	3.81	2.35	132.6	24.2	18.52	2.47
			UFT	5.87 (t=24h)	35.24	0.86	469.25	9.32	13.8	19.69
Chris H. Takimoto, 2007 (129)	130	11	Plasma	89.5 (t=48h) 313 (t=Infinity)	0.214 (*1.73)	3.94	38.9 (*1.73)	-	9.73	0.214
			UFT	9.14 (t=48h)	4.2 (*1.73)	1.31	361 (*1.73)	-	19	4.2
Kuniaki Shirao, 2006 (223)	90	3	Plasma	244.3	0.37 (*1.73)	2.26	110.3 (*1.73)	7.6	27.58	0.37
			UFT	7.9	11.6 (*1.73)	0.96	1433.4 (*1.73)	0.18	75.44	11.6
	130	6	Plasma	258.3	0.52 (*1.73)	3.22	127.2 (*1.73)	7.8	31.8	0.52
			UFT	11.3	11.7 (*1.73)	1.45	1612.7 (*1.73)	0.17	84.88	11.7
Wolfgang Kern, 1999 (133)	130	12	Plasma	1593	1.56	3.11	179	1	25	0.87
			UFT	20.17	13.32	1.61	349	0.09	10.26	7.44
SPC from Aventis Pharma Limited, 2011 (122)	85	-	UFT	4.19 (t=48h)	17.4	0.814	440	0.43	12.94	9.72
	130		UFT	8.20 (t=48h)	10.1	1.21	582	0.28	17.11	5.64
Margaret E. Macy, 2013 (224)	85	4	UFT	6.29	12.02	0.288	-	116.7	-	6.72
	100	4	UFT	8.17	-	0.294	-	-	-	-
Paul L Mitchell, 2011 (143)	70	43	Plasma	87 (t=168h)	1.107	1.97	117.534	90	16.42	0.62
H. Ehrsson, 2002 (131)	85	4	UFT	2.83	32.1 (*1.73)	1.44	0.26 (L/kg)	0.235	10.28	32.1
		6								
J.P. Delord, 2002 (222)	130; 80; 100	40	UFT	-	18.7	-	40.8	-	10	10.45
			Blood	-	0.127	-	17.9	-		0.071
Claire Massari, 2000 (225)	130	13	Plasma	49.02	2.51	2.59	69.72	0.45	9.74	1.4
			UFT	5.21	25.7	1.09	338.38	0.42	9.95	14.36

Continued

Resource	Dose (mg/m^2)	No. of Patients	Drug Administration	Sample points	Assay Method	PK model
Claudia Burz, 2009	85	4	2-hour intravenous infusion in FOLFOX4	12 in 24 hours	Inductively Coupled Plasma Mass Spectrometer (ICP-MS)	non-compartment (WinNonLin, USA)
	130	4	4-hour intravenous infusion in XELOX			
Chris H. Takimoto, 2007	130	11	2-hour intravenous infusion with prophylactic antiemetic regimen	13 in 504 hours	Inductively Coupled Plasma Mass Spectrometer (ICP-MS)	non-compartment (WinNonLin 3.1, USA)
Kuniaki Shirao, 2006	90	3	2-hour intravenous infusion with 5-fluorouracil and leucovorin	13 in 338 hours	Inductively Coupled Plasma Mass Spectrometer (ICP-MS)	non-compartment (WinNonLin 3.1, USA)
	130	6				
Wolfgang Kern, 1999	130	12	4-hour intravenous infusion with 5-fluorouracil	13 in 8 hours	Flameless Atomic Absorption Spectrometry (GBC 904 AA, Maassen, Germany)	3-compartment (TOPFIT)
SPC from Aventis Pharma Limited, 2011	85	-	2-hour intravenous infusion	-	-	non-compartment
	130					
Margaret E. Macy, 2013	85	4	2-hour intravenous infusion in FOLFOX	4 in 48 hours	Flameless Atomic Absorption Spectrophotometry	non-compartment (SAAM II 2.0, The Epsilon Group, VA)
	100	4				
Paul L Mitchell, 2011	70	43	2-hour intravenous infusion with gemcitabine	10 in 24 hours	-	-
H. Ehrsson, 2002	85	4	2-hour intravenous infusion	9 in 12 hours	Liquid Chromatography with postcolumn derivatization	2-compartment
		6				non-compartment (WIN NONLIN 1.5 SCI)
J.-P. Delord, 2002	130; 80; 100	40	3-hour intravenous infusion with 5FU and FA	9 in 24 hours	Flameless Atomic Absorption Spectrophotometry	3-compartment (NONMEM)
Claire Massari, 2000	130	13	2-hour intravenous infusion	12 in 24 hours	Flameless Atomic Absorption Spectrophotometry (FAAS)	-

4.1.3 PK simulation under laboratory conditions

Although PK parameters are a potential surrogate for estimating the clinical effects and therapeutic outcomes obtained by switching from individual dosing to dose-banding (216), there is a problem in conducting PK studies with cytotoxic drugs. The acute and chronic toxicity, genotoxicity, potential mutagenic and carcinogenic effects of cytotoxic drugs, precludes the administration of this class of drugs to healthy volunteers who would normally provide subjects for PK studies. (3) This study explored the use of an *ex vivo* simulation model for the pharmacokinetic study on oxaliplatin in five different dosing strategies. Since oxaliplatin has a simple distribution procedure within the central blood circulation, only a single compartment needed to be simulated in the model. (109) (225) Additionally, because of the 100% renal excretion of oxaliplatin, the simulated elimination rate used in this system could be simply adjusted with the renal clearance rate. (11) (225) Therefore, a simply structured *ex vivo* simulation model could be constructed for oxaliplatin PK studies.

In this model, an infusion bag with 5% glucose was used to simulate the central compartment of the body, in which the volume represented the volume of distribution selected by the review of data from previous oxaliplatin PK studies. The renal drug elimination was replicated by the continuous outlet flow from the infusion bag (central compartment), the flow rate of which was scaled down from the total clearance (CL_{total}) of oxaliplatin. Moreover, to balance the loss of glucose solution from the infusion bag caused by simulated renal excretion, a compensation bag with 5% glucose was used to replace this loss and maintain a constant volume for the 'central compartment'. To apply the result from this study to general estimation of the oxaliplatin pharmacokinetics, the CL_{total}

values, referred for the outlet flowrate in this simulation system, were selected from previous PK studies on patients with normal renal and hepatic functions.

The main advantage of the *ex vivo* PK simulation approach was that it could avoid the need to request cancer patients to participate in a PK study. Generally, the clinical PK study would require up to 24 hours hospital stay of patients from post-infusion and blood samples would be taken invasively at many time points. Except for those inconveniences for patients, the clinical study involved with cancer patients would always require ethics approval and informed consent from patients and their oncologist.

Other advantages of the *ex vivo* PK simulation method also included:

- The simulation model was easy to build up and operate in laboratory conditions;
- This simulation model can also be adjusted to simulate the PK process for different drugs;
- The *ex vivo* PK simulation approach was cheaper and easier to obtain the PK data for cytotoxic drugs compared with *in vivo* clinical trials.
- This *ex vivo* PK simulation model could be used in pre-clinical studies for the novel drug development, such as to facilitate a drug bioactivity study and/or animal experiments.

However, there were certainly limitations of this *ex vivo* PK simulation system. As described above, only the central compartment of the body was simulated, thus the system cannot be applied to those drugs with more complicated distribution and elimination processes. Since this model simulated the human body with an infusion bag, the whole system with drug dose, distribution volume

and total body clearance must be scaled down to a practical, laboratory-friendly volume which might affect the accuracy of the PK result.

Considering all advantages and drawbacks, the *ex vivo* PK simulation model could be considered for future PK studies as an alternative approach to a study with real patients.

4.1.4 Dosing administration strategies

Since the 1950s, traditional dosing of chemotherapeutic drugs has normally been calculated from the body surface area (BSA). (226) (227) This approach results mainly from the use of BSA to scale doses between species to estimate the dose in different animals (mouse, dog) and thus determine a dose for phase I clinical studies in humans. (226) (227) As most cytotoxic agents have a relatively narrow therapeutic window this approach was considered the standard means of determining a “safe” dose close to the maximum tolerated dose (MTD) to provide optimal dose intensity of the drug. (221) (228) Meanwhile, patients with extreme body weight, such as the obese and children, can receive a suitably adjusted dosage to ensure the efficient and safe level of drug concentration *in vivo*. (226) The licenced dose for oxaliplatin was 85 mg/m^2 , with the body surface calculated from the BSA formula: (229)

$$BSA(m^2) = 0.007184 \times height(cm)^{0.725} \times weight(kg)^{0.425}.$$

However, there are several problems associated with the BSA-based individual dosing of chemotherapeutic drugs, including the increased waiting time for patients, drug calculation errors and working stress for pharmacists and nursing staff involved. (8) (9) Therefore, dose-banding schedules have been introduced as a new approach for chemotherapy administration, which could provide the pre-prepared standard doses in batch-scale aseptic infusions (in bags or

syringes). (8) Additionally, these standard doses are calculated based on patients' BSA. (8) (10) The dose-banding method is currently used for several chemotherapeutic drugs in the UK. (148) Compared to the individualized therapy, the dose-banding schemes allow the prospective quality control for pre-made infusions in the manufactory and prevent errors in dose calculation and drug administration. (8) (148) The dose-banding method could also reduce the waiting time for patients since the drug infusions are all pre-made and ready for administrations. (10) Therefore, a proper dose-banding scheme should be developed for the administration of oxaliplatin in cancer chemotherapies.

In this study, three different dose-banding schemes were tested on 10 'patients' which were a dose-banding scheme with the maximum 5% deviation in each band (5%DB), a dose-banding scheme with the maximum 10% deviation in each band (10%DB) and a logarithmic dose-banding scheme (LDB). Dose-banding with 5% deviation (5%DB) was the most acceptable dose-banding scheme for physicians, which has been applied to different chemotherapeutic drugs in clinical practice. (152)

Generally, the drug dose variations can be effected be several factors, such as the potential variation in drug stock vials; calculation errors on patients' BSA; administration errors by pharmacists or nurses; the drug infusion waste in infusion bags and tubes during the injection to patients. (221) Therefore, the normal administration of individualized dosing could possibly cause more than 10% drug dose errors in total, and 10% difference in the drug dosage should be theoretically acceptable. (9) Therefore, 10%DB with the dose variation of no more than 10% from the prescribed dose to the standard dose in each band should be considered as an optional dose-banding scheme for oxaliplatin administrations. Compared to 5%DB, 10%DB can be designed and operated

with less dose bands, which requires a lower number of pre-preparation infusion bags for standard doses in each band.

In general, dose-banding schemes are designed with a linear relationship in band ranges, such as 5%DB and 10%DB. (230) As a result, the dose variation in bands with higher doses is smaller than in those bands with lower doses. (230) To improve the accuracy and safety in use of dose-banding schemes, a logarithmic dose-banding (LDB) scheme has been introduced with logarithmic relationships in doses to give even dose variations (≤ 6%) between concurrent dose bands. (230) (231) With the LDB scheme, patients prescribed with lower doses could be treated with less potential risk on dose errors. (231) In addition, a single sequence of LDB could cover doses from 1 mg to 100000mg, which can be applied to all systematically delivered cytotoxic drugs for patients of all sizes. (230) (231) To compare the logarithmic dose-banding (LDB) with other dose-banding schemes and individual dosing method, the dosing system of LDB referred from *Geoff Hall* (232) was also tested in this study.

Previous literature discussed the flat-fixed dosing method and suggested to replace the current BSA-based dosing administration for some anticancer drugs, because of the weak relation found between BSA and the inter-individual PK variations. (221) (226) (233) However, the question of whether the analysis of such limited data can prove the safety and superiority of the flat-fixed dosing method has been controversial. (227)

In this study, the *ex vivo* PK simulation model was set up to determine the PK parameters with these five different dosing schedules (IND, 5%DB, 10%DB, LDB and FFD). In order to find the preferred dosing administration strategy for oxaliplatin infusions, the simulated AUC and C_{max} values and their inter-

individual variabilities were analysed and compared for different dosing schemes in this *ex vivo* study.

4.1.5 Purpose and objectives

- To discover the optimal dosing method for oxaliplatin administration in chemotherapies by comparing the AUC and C_{max} values simulated from the *ex vivo* PK model;
- To determine the differences in simulated PK parameters from various drug administration methods, including individual dosing method (IND), dose-banding scheme with the maximum 5% variation in each band (5%DB), dose-banding scheme with the maximum 10% variation in each band (10%DB), logarithmic dose-banding scheme (LDB) and flat-fixed dosing method (FFD);
- To evaluate the inter-individual variability of AUC and C_{max} for each patient group (n = 10) with different dosing schemes including IND, 5%DB, 10%DB, LDB and FFD.
- To validate the reliability and accuracy of the *ex vivo* PK simulation system.

4.2 Materials and methodology

4.2.1 Materials

4.2.1.1 Chemicals and reagents

Oxaliplatin concentrate solution 5 mg/mL, batch no. PP02066; expiry 10/2015 (Accord Healthcare Limited, UK)

Glucose 5% w/v Macoflex, Lot. 899914122H, expiry 09/2016 (Macopharma, UK)

Sodium phosphate monobasic monohydrate (ACS reagent, 98.0 - 102.0%) (Sigma-Aldrich, Japan)

Sodium phosphate dibasic (HPLC grade) (Sigma, USA)

Sodium hydroxide 1M; Acid ortho-phosphoric; Methanol; Hydrochloric acid 1M (HPLC grade, Fisher Scientific, UK)

Multifunction peroxide solution 3% (Specsavers, UK)

pH electrode storage solution; pH 4.01 Buffer solution; pH 7.00 Buffer solution (Thermo Fisher Scientific, USA)

Sterile water for irrigation and dilution, batch 12E22B28, expiry 04/2015 (distilled and de-ionised) (Baxter, UK)

4.2.1.2 Consumables

Luer-lock syringes in 2 mL and 5 mL (B. Braun Medical Ltd, UK)

Luer-lock syringes in 10 mL and 20 mL (Codan Medical ApS, UK)

0.8 mm × 40 mm needles, 21G × 11/2'', LOT 120507, expiry 04-2017 (BD Microlence, UK)

1.1 mm × 40 mm needles, 19G × 11/2'', LOT 120411, expiry 03-2017 (BD Microlance, UK)

Screw-cap polypropylene tubes 20 mL (Gosselin SAS, UK)

HPLC auto-sampler crew-cap vials 2 mL and caps (Chromacol Ltd., UK)

Cellulose acetate filters (pore size 0.45 µm) (Sartorius Stedium Biotech, Germany)

Disposable polypropylene pipettes 3 mL (Fisher Scientific, UK)

Micropipette tips 1000 µL (Gilson SAS, France)

Prep Pad, 70% Isopropyl Alcohol BP (A CliniMed Group Company, UK)

Colleague pump/gravity solution administration sets (Baxter, UK)

4.2.1.3 Equipment and instrumentation

Analytical balance: Sartorius Basic 100g - 0.0001g 4-figure balance (Sartorious Ltd., UK)

Balance: SG-601(600g ×0.1g) 3-figure (S/N: 8335436123, Ohaus Corporation, USA)

Colleague Volumetric Infusion Pump No. 1 – 4 (SN 9030134CK; SN 9030221CK; SN 9030207CK; SN 99BC2129AA, Baxter, UK)

Refrigerator at 2 – 8℃ (Fisher Scientific, UK)

Incubator at 25 ± 1℃ (Gallenkamp, Japan)

Digital thermometers (Fisher Scientific, UK)

Micropipette: Proline Plus 100 – 1000 µL (Biohit Limited, UK)

Micropipette: Proline Plus 10 – 100 µL (Biohit Limited, UK)

pH meter : Orion 5 Star Benchtop with glass combination electrode (Thermo Scientific, UK)

Magnetic stirrer : HI 190M (Hanna Instruments, UK)

Glass filter holder and flask (Emil company, UK)

Filter vacuum pump: FB 70155 (Fisher Scientific, UK)

Class Ⅱ safety cabinet: Biological Class Ⅱ containment (Bigneat Ltd., UK)

Gyro-rotatory shaker: Certomat M, Type 886008/4 (B. Braun Melsungen, AG, Germany)

Glassware: Grade A flasks, measuring cylinders and beakers (Fisher Scientific, UK)

HPLC Column: Water Spherisorb® (CNRP) 4.6 mm×250 mm stainless steel, packed with 5.0 µm bonded phase cyano CNRP Column (Waters Limited, UK)

HPLC System: (Jasco UK, Essex UK)

Intelligent Column Thermostat, CO-2060 plus

Intelligent Auto-sampler, AS 2057 plus

Quaternary Gradient Pump, PU-2089 plus

Multiwavelength Detector, FP 2020

LE-Net Ⅱ/ADC

Chrompass Software version 1.8.6.1

4.2.2 HPLC assay for oxaliplatin

A bonded-phase cyano column of Waters Spherisorb® (CNRP), 5.0 µm particle size and 4.6 mm × 250 mm, was used to separate oxaliplatin in the HPLC assay which was adapted and developed from the European Pharmacopoeia. (164) Separation was achieved under isocratic conditions with a mobile phase of 5 mM phosphate buffer in pH 6.5. The detector was UV diode-array detector using 210 nm wave length, and Jasco ChromPass version 1.8.6.1 was used to collect and analyse the chromatographic data. HPLC conditions were shown below:

Flow rate = 1.0 mL/min

Injection volume = 20 µL

Run time = 10 mins

Detection wave length = 210 nm

Temperature of column oven = 35℃

Column maintenance: The HPLC column was conditioned every 14 days with the following cycle of solvents run through the column at a flow rate of 1 mL/min: HPLC grade water for 20 minutes; methanol and water with 50 : 50 (v/v) for 20 minutes; 100% methanol for 20 minutes; methanol and water 50 : 50 (v/v) for 20 minutes; HPLC grade water for 20 minutes. In addition, daily storage of the column required flushing with HPLC grade water for 20 minutes followed by 50:50 methanol : water (v/v) for 20 minutes prior to shut down.

Details of validation tests for this oxaliplatin assay in HPLC system have been described in the previous stability study of oxaliplatin infusions in Chapter 2 (Section 2.2.2, page 110).

4.2.3 The *ex vivo* PK simulation model

This *ex vivo* system was designed for simulating the main pharmacokinetics behaviour of oxaliplatin in the central compartment of a human body.

4.2.3.1 Four main components

Reservoir A (central compartment) represented the central compartment of the patient's body by using a 5% glucose infusion bag in 500 mL/1000 mL size. The volume of these bags referred to the volume of drug distribution (V_d) in the patient's body which depended on the individual patient's BSA (body surface area). (Refer to Section 4.3.1)

Reservoir B (Compensation solution) was used to compensate the solution loss of the Reservoir A, in order to balance the volume of the simulated 'central compartment' of a patient's body. During the experiment, the solution in

Reservoir A continuously flowed out in the flowrate scaled down from CL_{total} to simulate the elimination process in the body. To maintain the 'body weight', different volumes of 5% glucose solution was infused to Reservoir A in different flowrate as the compensation for an individual 'patient', which volume (V_B) could be calculated following the equation: $V_B = R1 \times T1 + R1' \times T2$. *[R1: flow rate of pump 1(Reservoir A – Reservoir B) during infusion time; R1': flow rate of pump 1 after infusion time; T1: infusion time, 2 hours; T2: experimental time of post-infusion, 6 hours.]*. Considering various BSA values of the 10 'patients' selected in this study, 5% glucose bags in 1000 mL were used as single or the combination of two bags for different patients.

Reservoir C (Waste) was an empty bag used for collecting waste solutions from the 'central compartment', Reservoir A. This bag can be safely sealed and was in a large volume which could handle all waste solution produced from the 'patient' during the 8-hour experiment. The volume of waste solution can be approximately calculated from the following equation: $V_{waste} = CL \times T_{total}$. *[CL: the total clearance of oxaliplatin for individual 'patient'; T_{total}: the total experimental time for each runs, 8 hours.]*

Drug infusions (diluted with 5% glucose) were prepared in different size syringes following different schedules according to the dosing administration systems (individual dosing, dose-banding schemes and flat-fixed dosing). Preparation and administration schedules for different dosing methods are described in the experimental section (Section 4.3.2).

4.2.3.2 Pump function

To ensure the accuracy of the infusion flowrates in this study, three Baxter Colleague volumetric pumps were validated and used to connect Reservoir B,

Reservoir C and oxaliplatin infusions to Reservoir A with the chemotherapeutic drug administration sets. The flowrates of inlet/outlet solutions to/from Reservoir A were controlled by these pumps and were adjusted respectively to balance the volume of the 'central compartment" during each experimental run.

Since these pumps are specially designed for intravenous infusion in clinical practice, they could provide lower flowrates within the range of 1 – 1200 mL/hr to meet the requirements for this study. To determine the accuracy and precision of each pump, validation tests for four Colleague pumps were conducted and as described in the experimental section (Section 4.3.1.3.1).

The positions of each pump in the *ex vivo* simulation model are shown below:

- Pump 1: set up on the transfer tubing between Reservoir B and Y-set tubing to pump 5% glucose solutions into Reservoir A for compensation.
- Pump 2: set up on the transfer tubing between drug infusion syringes and Y-set tubing to pump oxaliplatin infusions into Reservoir A.
- Pump 3: set up on the transfer tubing between Y-set and Reservoir C to pump the waste solution from Reservoir A to Reservoir C.

Flowrates of these pumps needed to be re-set in different time periods: infusion time and post-infusion time.

- **F_1**: The flowrate of Pump 1 (F_1) to compensate the 'central compartment" with 5% glucose was adjusted to keep the constant weight/volume of Reservoir A.
- **F_2**: The flowrate of Pump 2 (F_2) was the drug infusion rate which depended on the dosage (drug concentration and volume) of oxaliplatin infusions.

- **F_3**: The flowrate of Pump 3 (F_3) was the drug elimination rate which was calculated from the statistical value of the total clearance (CL_{total}) for oxaliplatin published in previous literature. This flowrate was kept constant through the whole experiment period (including infusion time and post-infusion time).

As a result, these three flowrates were related as $F_1 + F_2 = F_3$ during the infusion time (when Pump 1, 2, 3 were working at the same time). After the oxaliplatin infusion was complete, Pump 2 was immediately stopped and the relation for the other two pump flowrates was $F_1 = F_3$. The adjustment schedule of pump flowrates for different dosing administration is described in detail in the experiment section below (Section 4.3.1.3.2).

4.2.3.3 System connections

Several different connecting components were involved in this simulation system (shown in *Figure 4-2*), including connection ports, Y-set extension line, needles, spikes, syringes and the solution administration sets specifically designed for the Colleague volumetric pumps.

Between main reservoirs, the Colleague pump/gravity solution administration sets were used where pumps were required. A Y-set extension line was used to connect each of the pump administration sets from Reservoir B and the drug infusions, and then combine the inlet flow into Reservoir A through a fine needle.

To control the outlet flow from Reservoir A, another Y-set extension line was used to connect Reservoir A to the waste bag (Reservoir C). One branch of the Y-set line was connected to a pump administration set leading to Reservoir C. The other branch of the Y-set line with a luer-lock end was used as a sample port and connected to a sampling syringe at each time point.

Oxaliplatin infusions were prepared in different size syringes following different schedules of each dosing method (Section 4.3.2). Since more than one syringe was used for each patient, manifold sets, allowing at least 4 syringes to be used in combination, were applied in this system to connect drug infusion syringes and the pump administration set.

Some solution transfer lines were adapted and remade to produce bespoke connections for the administration lines loaded into the pumping chambers of Pump 2 and Pump 3. For example, the administration set for Pump 2 (connecting drug infusions to Reservoir A) required replacing a spike connector with a male luer-lock connector. The connection sets used are shown in *Figure 4-2*.

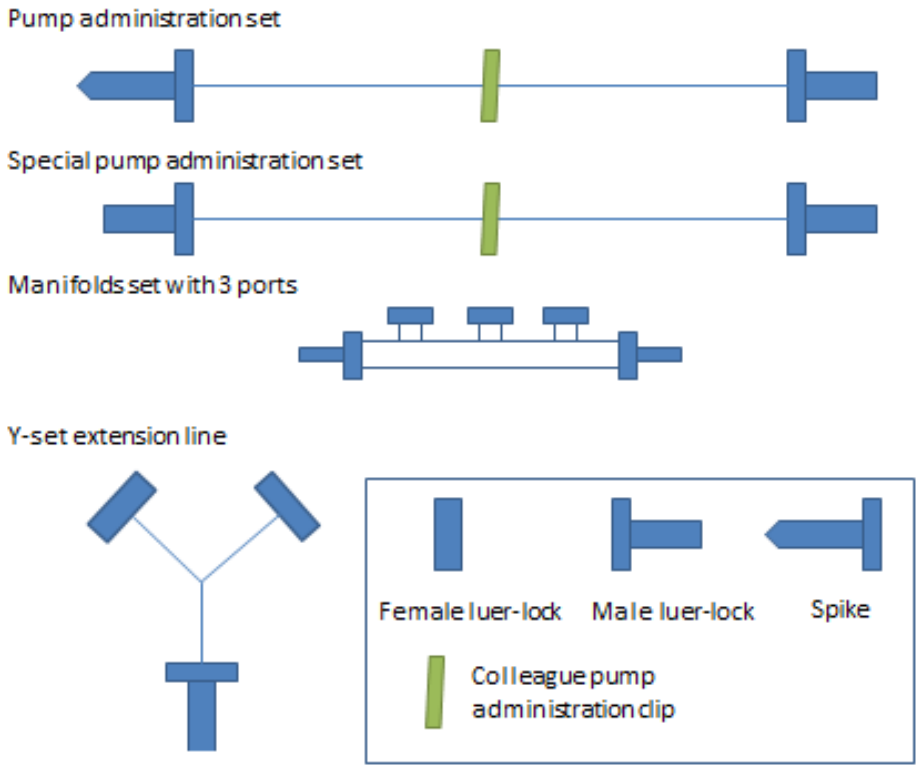

Figure 4-2, Connection components and tubing for the *ex vivo* PK simulation model, including pump administration set, special remade pump administration set, Y-set extension line and manifold set with 3 female luer-lock ports.

4.2.3.4 Whole system assembly

The *ex vivo* pharmacokinetic (PK) simulation system is shown in *Figure 4-3* below.

Reservoir A was located onto a tared analytical balance for monitoring the 'central compartment' bag weight. Meanwhile, to ensure the complete mixing of the drug infusion, compensation 5% glucose and the solutions in Reservoir A, the Reservoir A and balance assembly were placed on a gyro-rotatory shaker at the adjusted speed (130 oscillations per minute).

Reservoir B was hung up by an infusion bag holder higher than the table top level to give unrestricted input of 5% glucose solutions into the transfer line.

Reservoir C was sealed safely with the connected solution transfer line to avoid any leaking of cytotoxic solutions.

The transfer lines and connections between the oxaliplatin infusion syringes and Reservoir A were primed with the drug infusion to avoid any dead volume and ensure the accurate drug dose was delivered. Also when drug infusions were complete, the drug delivery line was flushed with 5% glucose to ensure all drug infusions could reach Reservoir A.

Samples were withdrawn from the Y-set line connecting Reservoir A and Reservoir C. Before 8 mL of samples was collected from Reservoir A using a luer-lock syringe, a blank volume of solution (2 mL) was withdraw and abandoned to remove the dead volume in the Y-set connection line. It could prevent the effect on determination of oxaliplatin concentrations from the dead volume in solution transferring lines.

Before each simulation, all lines and connections were checked carefully to make sure the whole system was sealed properly without any risk of cytotoxic leakage or contamination.

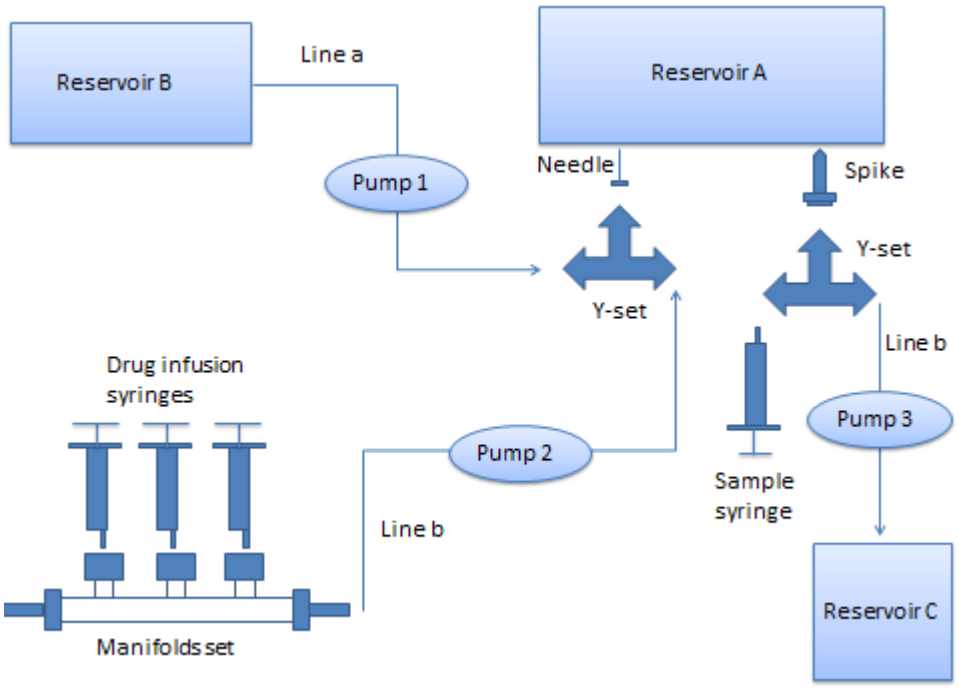

Figure 4-3, Whole system of the *ex vivo* PK simulation model. Reservoir A simulated the central compartment of the human body; Reservoir B was used to maintain the constant weight of Reservoir A by compensation with 5% glucose solution; Reservoir C was to collect waste solutions from Reservoir A. All connection components are as described in Figure 4-2. Line a: pump administration set; Line b: special pump administration set; all syringes used in this system are luer-lock, including drug infusion syringes and sample syringes.

4.2.4 pH and weight monitor

In the whole process of PK modelling, the solution volume of the infusion bag, representing the central compartment, was changed frequently with the withdrawal of samples for analysis, which could affect the oxaliplatin concentration in the infusion bag solution and in subsequent samples. Also, the change in composition could modify the pH in the solution and influence the chemical degradation of oxaliplatin. Therefore, the pH and weight of the 'central compartment' were monitored regularly throughout each simulation run.

An Orion 5 Star pH meter with glass electrode and automatic temperature compensation was used to measure the pH of samples at each time point for each patient. Triplicate measurements were used to gain the average pH value for the final pH for each sample. The pH meter was calibrated with reference buffer solutions at pH 4.01 and pH 7.00 each time before sampling. Between samples, distilled water was used to rinse the glass electrode.

A 5-figure analytical balance was placed under the Reservoir A (simulated central compartment) to assess the volume change for each patient at each sample point. Weight of the 'central compartment' was calculated and adjusted in the beginning of the process according to the V_d of patient. After the infusion was started, the weight of Reservoir A before and after sampling was recorded to monitor the volume change during the experiment for each patient. As there was a gyro-rotatory stirrer under the balance, the balance reading might vary; so before recording the weight the stirrer was stopped for a few seconds to allow the balance reading to become stabilized.

4.3 Experimental

4.3.1 The *ex vivo* PK simulation model

4.3.1.1 Scale-down schedule for the simulation model

To enable the practical working of the PK simulation model with realistic volumes and to ensure simulated pharmacokinetic parameters of oxaliplatin were representative for the *in vivo* situation, the *ex vivo* simulation model in this study was scaled down proportionately in accordance with published pharmacokinetic data from previous oxaliplatin studies.

The system characteristics were based on the following equations:

$$\mathrm{Rate_{in}} = \mathrm{Rate_{out}} \qquad \text{Equation 1}$$

$$\mathrm{Rate_{in}} = \frac{\mathrm{Dose} \times \mathrm{BSA}}{\mathrm{T}} \qquad \text{Equation 2}$$

$$\mathrm{Rate_{out}} = \mathrm{CL} \times \mathrm{BSA} \times \mathrm{C_{ss}} \qquad \text{Equation 3}$$

$$\mathrm{C_{ss}} = \frac{\mathrm{Dose}}{\mathrm{CL} \times \mathrm{T}} \qquad \text{Equation 4}$$

$$\mathrm{AUC} = \frac{\mathrm{Dose}}{\mathrm{CL}} \qquad \text{Equation 5}$$

$$\mathrm{K} = \frac{\mathrm{CL}}{\mathrm{V_d}} \qquad \text{Equation 6}$$

($Rate_{in}$: the rate of drug passing into the system; $Rate_{out}$: the rate of drug running out of the system; dose: drug dose in infusions; BSA: the body surface area of 'patient'; T: infusion time; CL: total clearance of the drug; C_{ss}: the drug steady state concentration (equal with C_{max}); AUC: the area under drug concentration-time curve; K: elimination rate constant; V_d: the volume of drug distribution.)

AUC (area under the drug concentration-time curve) is one of the key pharmacokinetic parameters, which could represent the exposure of tissues (normal and cancerous) to the drug in each patient. Therefore, to achieve AUC and K values that are physiologically and pharmacokinetic representative in different patients, the drug dose, V_d and CL must be reduced by the same factor in the scaling process for this simulation study.

Selection of the scaling factor was dependent on various practical considerations such as the practical administration drug dose, the limited volume of available containers for the reservoirs that simulate the central compartment of the human body and the working flowrate range of the infusion pumps. For example, the reduced V_d (used as the 'body volume' for Reservoir A in this simulation system) must be small enough to be provided by a commercially – available IV bag; and the infusion outlet flowrate of the pump

that determined by the scaled down CL (total clearance) must be within the working range of these Colleague volumetric pump [1 – 1200 mL/hr (± 1 mL/hr)]. Similarly, the drug dose of oxaliplatin simulated in this model had to reflect the acceptable infusion concentration. Meanwhile, the volume of those drug infusions must be workable at a flowrate within the working range of the Colleague pumps. Finally, the oxaliplatin concentration in the samples from Reservoir A must be within the analytical range validated for the HPLC assay. Considering all potentially influential factors in this *ex vivo* study, three characteristics were scaled down by a factor of 40 as follows for each individual simulation.

$$\mathrm{Dose} = 85\ \mathrm{mg/m^2} \times \mathrm{BSA}/40 \qquad \text{Equation 7}$$

$$\mathrm{V_d} = 12.41\ \mathrm{L/m^2} \times \mathrm{BSA}/40 \qquad \text{Equation 8}$$

$$\mathrm{CL} = 2.88\ \mathrm{L/h/m^2} \times \mathrm{BSA}/40 \qquad \text{Equation 9}$$

[The mean values of V_d (12.41 L/m^2) and CL(2.88 L/h/m^2) were selected from previous literatures as described in Section 4.1.2.2]

Ten nominal patients were randomly selected from actual patients with the BSA range of 1.12 m^2 - 2.70 m^2 by using Microsoft Excel 2010 software (Microsoft Corporation, USA). This BSA (body surface area) range included typical values of BSA for cancer patients, and the random selection produced patients in BSA values of: 2.70 m^2, 1.82 m^2, 1.29 m^2, 1.55 m^2, 2.20 m^2, 1.33 m^2, 1.99 m^2, 1.72 m^2, 2.52 m^2 and 2.04m^2.

The summarised information of these ‘patients’ selected for this simulation study is shown below in *Table 4-2*. The individual dosage, V_d (volume of drug distribution) and CL (the total clearance) were calculated based on the patients’ BSA and scaled down by a factor of 40 in accordance with equations 7, 8 and 9 shown above.

Table 4-2, Information of 10 'patients' selected for this PK simulation study and their PK characteristics (Dose, V_d and CL) of calculated values and scaled-down (factor of 40) values.

Patient	BSA (m^2)	Dose (mg)	Dose (mg)*	V_d (L)	V_d (L)*	CL (L/hr)	CL (L/hr)*
Patient 1	2.70	229.50	5.74	33.51	0.8377	7.78	0.1944
Patient 2	1.82	154.70	3.87	22.59	0.5647	5.24	0.1310
Patient 3	1.29	109.65	2.74	16.01	0.4002	3.72	0.0929
Patient 4	1.55	131.75	3.29	19.24	0.4809	4.46	0.1116
Patient 5	2.20	187.00	4.68	27.30	0.6826	6.34	0.1584
Patient 6	1.33	113.05	2.83	16.51	0.4126	3.83	0.0958
Patient 7	1.99	169.15	4.23	24.70	0.6174	5.73	0.1433
Patient 8	1.72	146.20	3.66	21.35	0.5336	4.95	0.1238
Patient 9	2.52	214.20	5.36	31.27	0.7818	7.26	0.1814
Patient 10	2.04	173.40	4.34	25.32	0.6329	5.88	0.1469

*The PK characteristics were calculated based on equations 7, 8 and 9 shown above. Dose: calculated with 85 mg/m^2 dose of oxaliplatin; V_d: volume of drug distribution in patient body; CL: the total clearance of oxaliplatin. [*] indicates scaled down values of PK characteristics.*

The scaled-down factor of 40 times was applied to all scenarios running on the *ex vivo* simulation including individual dosing, dose-banding schedules (5%DB, 10%DB and LDB) and flat-fixed dosing. The scaled-down dosing schemes are shown in Section 4.3.2.

4.3.1.2 Model assembly

Before the simulation system was assembled, three reservoirs were prepared in different ways referring to the 'patient' information shown in *Table 4-2.*

- Reservoir A: To represent the 'central compartment' of the patient's body, a 1000 mL or 500 mL 5% glucose IV infusion bag was adjusted into the volume of V_d required for the individual patient. For 'patients' with V_d values lower than 600 mL, a 500 mL glucose bag was used as Reservoir A. While the 1000 mL glucose bag was used for 'patients' with V_d greater than 600 mL.
- Reservoir B: For Reservoir B, 5% glucose solution was used to maintain the constant volume of Reservoir A in order to balance the elimination from the simulated 'central compartment'. The volume of 5% glucose

required for Reservoir B can be calculated as described in Section 4.2.3.1, which mostly depended on the flowrate of Pump 1. The relationship between flowrates of Pump 1, Pump 2 and Pump 3 is described in Section 4.3.1.3.2 below. For the selected 'patients', the largest volume of Reservoir B required was approximately 1500 mL which required two 1000 mL glucose bags, and the lowest volume required was about 700 mL for which only one 1000 mL glucose IV infusion bag was used.

- Reservoir C: An empty infusion bag in the size of 3 L was used as the waste container for the outlet flow from Reservoir A, the 'central compartment' of the patient's body. This size bag had the capacity to handle all waste solution from the range of 800 mL to 1600 mL (calculated from CL multiplied by the total experiment time of 8 hours). As the empty bag had three ports for connection but only one of them was used in this study, the other connection ports were also checked carefully before the experiments to ensure they were properly sealed to prevent cytotoxic containment.

An analytical balance reading to 0.1 mg was validated and adjusted on the gyro-rotatory shaker. For the 1000 mL glucose bag of Reservoir A, the balance top-plate was not big enough to hold the whole bag and so a small laboratory tray was placed on top of the balance. The shaker underneath was set at 130 oscillations/min speed during the experiment in order to mix the solution in Reservoir A.

After all main pieces were set, the connection components (shown in Section 4.2.1.3) were used to connect the whole system in accordance to the model design in *Figure 4-3*. To secure the insertion points to the Reservoir A bag, a

needle and a separated spike both with luer-lock ends were connected respectively to the inlet and outlet Y-set extension lines.

4.3.1.3 Pump adjustment

4.3.1.3.1 Validation of four volumetric pumps

To ensure the accuracy of the infusion flowrates, four Baxter Colleague volumetric infusion pumps were prepared in this study: one pump was for spare and the other three were used according to *Figure 4-3* (Section 4.2.3.4). Since these pumps were specially designed for intravenous infusion in clinical practice, they were expected to provide accurate volume delivery at lower flowrates to meet the requirements of this study.

Validation tests were implemented before starting the experiment to determine the accuracy and precision of each pump. To include all flowrates applied in the study, the flowrates inside the broad range of 1 – 1200 mL/hr were tested over 14 different values: 1, 2, 5, 8, 10, 15, 20, 50, 100, 200, 500, 800, 1000 and 1200 mL/hr. Deionized water was used to be pumped at these flowrates and collected at the following time intervals.

- For lower flowrates (1, 2, 5, 8, 10, 15, 20 mL/hr), the eluent was weighed every 30 minutes.
- For higher flowrates (20, 50, 100, 200, 500, 800 and 1000 mL/hr), the eluent weights were measured every 5 minutes.

Flowrates for each pump were calculated with the equation shown below:

$$\text{Flowrate (mL/hr)} = \frac{\text{weight (mg)}}{\text{D (mg/mL)} \times \text{time (h)}}$$

[D: density of water, ≈ 1g/m^3.]

For each pump, all flowrates were tested in triplicate and the average results were used for precision and accuracy analysis with the respective criteria of standard deviation ≤ 2.0% and ≤ 5%. (234) The validation results for these four pumps are shown with the mean values of each flowrate and standard deviation (%) of triplicate data in *Table 4-8* in Section 4.4.1.1.

4.3.1.3.2 Flowrate calculation and adjustment for different patient simulations

50 models in total with various pump flowrate settings were tested for 10 'patients' with 5 different dosing methods. Before the experiment of each model, some extra time was required to test and set up three working pumps, and the spare pump to be used when failure result occurred in any of these pumps.

Pump flowrates were adjusted following the operation steps described below:

1) Pump 3 (between Reservoir A and Reservoir C) used the scaled-down value of the total clearance (CL_{total}) of oxaliplatin for each 'patient' as the flowrate (F_3) during the whole experiment period of 8 hours;

2) The flowrate in Pump 2 (between Drug infusions and Reservoir A) depended on the oxaliplatin infusion volume, which was different for individual 'patients' with different dosing methods. The flowrate of Pump 2 (F_2) was only used during the infusion time of 2 hours and can be calculated with the equation:

$$F_2 = \frac{V_S}{T_1}$$

[F_2 (mL/hr): flowrate of Pump 2; V_S (mL): volume of oxaliplatin infusion syringe combination; T_1 (hr): infusion time, 2 hours.]

3) The flowrate of Pump 1 (between Reservoir B and Reservoir A) during the infusion time (2 hours) can be calculated as:

$$F_1 = F_3 - F_2$$

[F_1 (mL/hr): the flowrate of Pump 1 during 2-hour infusion time; F_2 (mL/hr): flowrate of Pump 2 during infusion time; F_3 (mL/hr): flowrate of Pump 3.]

For the post-infusion time of 6 hours, the flowrate of Pump 1 should be changed and adjusted again as showing below:

$$F_1' = F_3$$

[F_1' (mL/h): the flowrate of Pump 1 during 6-hour post-infusion time; F_3 (mL/h): flowrate of Pump 3.]

The pump administration set used for transferring drug infusions was primed with oxaliplatin infusions before the pump adjustment and experiment started.

Even though flowrates for the three working pumps were calculated theoretically, they still needed to be slightly adjusted during the experiment in order to balance the weight of Reservoir A. Variations of the 'central compartment' weight were controlled within ± 2% in this study.

4.3.1.4 Solution preparation

For different dosing methods, two oxaliplatin **stock solutions** at concentrations of 1 mg/mL and 5 mg/mL were used to prepare the drug infusion syringes. Oxaliplatin (5 mg/mL) was from the manufacturer original vial and stored in a refrigerator at 2 – 8℃. Oxaliplatin stock solution in 1 mg/mL was prepared by diluting 5 mg/mL oxaliplatin (2 mL) to 10 mL with 5% glucose in a 10 mL grade A volumetric flask.

The 1 mg/mL stock solution was used for the preparation of oxaliplatin infusion syringes for the individual dosing method. Oxaliplatin syringes for other dosing

methods were prepared from 5 mg/mL oxaliplatin solution following schedules presented in Section 4.3.2.2.

The **standard oxaliplatin solution** for HPLC assay was at the 10 µg/mL concentration, which was prepared by diluting 5 mg/mL oxaliplatin (0.01 mL) in a 5 mL volumetric flask with deionized water (HPLC grade). This concentration of 10 µg/mL has been selected especially considering the concentrations of all samples which were expected to be in the range of 2.0 µg/mL – 20.0 µg/mL.

4.3.2 Drug dosing administration

Five different dosing methods were tested in this study, including individual dosing method (IND), dose-banding scheme with 5% deviation (5%DB), dose-banding scheme with 10% deviation (10%DB), flat-fixed dosing method (FFD) and Logarithmic dose-banding scheme (LDB). Individual dosing is the most widely used dosing administration method for anticancer drugs, in which the drug dose is calculated based on the BSA value of individual patient. (8) It must be noted that the BSA-based individual dose is not necessarily the optimal dose for the patient. (8) (13) (235) As an alternative dosing approach, dose-banding has been introduced into clinical practice for over 15 years and is increasingly adopted for a number of anticancer drugs. (10) (152) To compare the efficacy of different dosing methods, dose-banding schemes with a ± 5% and/or 10% maximum deviation from the BSA-individualised dose were simulated in this study along with the individual dosing method. In addition, logarithmic dose-banding (LDB) has been introduced in some cancer centres. (230) The potential advantage of LDB is that it reduces the wide error bands at low doses seen in conventional dose-banding schemes. (230) (231) In recent years, the controversy about the application of flat-fixed dosing (FFD) to cytotoxic drugs,

which was recommended to replace the BSA-based dosing method, has been discussed in some related literatures. (13) (226) Therefore, the flat-fixed dosing was also included as a dosing scheme for oxaliplatin in this study.

According to the previous stability study (Chapter 2, page 101), the stability concentration range of oxaliplatin infusions in 5% glucose was 0.2 mg/mL – 0.7 mg/mL. Thus, in this simulation study oxaliplatin infusion syringes were prepared within this stability concentration range using 5% glucose. The preparation and administration of drug infusions are following the different dosing schedules listed in sections below.

4.3.2.1 Individual dosing method (IND)

Individual doses were calculated respectively by multiplying the licenced oxaliplatin dose of 85 mg/m^2 by each patient's BSA, and recording to 2 decimal places. (5) (174) To ensure comparisons between different dosing methods were realistic, drug infusions for different methods were prepared in similar volume by diluting oxaliplatin working stock with 5% glucose.

In this *ex vivo* simulation study, all oxaliplatin infusions for IND and FFD were freshly prepared on the day of the experiment and were produced according to the principles of GMP (Good Manufacturing Practice) for aseptically prepared infusions. (236) The schedule of individual dosing for each 'patient' is described in *Table 4-7* in Section 4.3.2.4 with other dosing methods.

4.3.2.2 Dose-banding schemes

With the BSA range of 1.12 m^2 – 2.70 m^2, different dose-banding schemes of oxaliplatin are shown in tables below based on the licenced dose of 85 mg/m^2. (5) Three different dose-banding schemes were designed and applied in this study, including dose-banding scheme with ≤5% maximum deviation (5%DB),

dose-banding scheme with ≤10% maximum deviation (10%DB) and logarithmic dose-banding scheme with ≤6% maximum deviation (LDB).

As discussed above, these dose-banding schemes were administered using the scaled-down standard dose that was prepared within the concentration range of 0.2 mg/mL – 0.7 mg/mL for each band. Therefore, all pre-made oxaliplatin syringes for dose-banding schemes were prepared in different infusion volumes to keep drug concentrations in the required range (Shown in *Table 4-3*). Oxaliplatin syringes required for each dose-banding scheme were freshly prepared before model simulations and were used within the defined shelf-life (refer to the extended stability study in Chapter 2).

Table 4-3, Preparation of oxaliplatin pre-made infusion syringes for dose-banding schemes (including 5%DB, 10%DB and LDB) in the *ex vivo* PK simulation study.

Oxaliplatin dose of pre-made infusion (mg)	Infusion syringe size (mL)	Volume of 5 mg/mL oxaliplatin used for dilution (µL)	Oxaliplatin concentration in the syringe after dilution with 5% glucose (mg/mL)
0.24	1.0	48	0.24
0.25	1.0	50	0.25
0.30	1.0	60	0.30
0.37	1.0	74	0.37
0.38	1.0	76	0.38
0.41	2.0	82	0.21
0.46	2.0	92	0.23
0.50	2.0	100	0.25
1.13	5.0	226	0.23
2.00	10.0	400	0.20
3.00	15.0	600	0.20

With the scaled-down drug doses, volumes of pre-made oxaliplatin infusions were too small to be prepared in infusion bags. Therefore, luer-lock syringes (in size of 1 mL, 2 mL, 5 mL, 10 mL and 15 mL) were used as containers for pre-made drug infusions that were prepared from 5 mg/mL oxaliplatin stock solution. The preparation schedule of pre-made infusion syringes for three dose-banding schemes is shown together in *Table 4-3.*

4.3.2.2.1 Dose-banding scheme with 5% deviation in each band (5%DB)

There were 12 dose bands in this dose-banding scheme covering oxaliplatin doses from 95 mg to 230 mg. The maximum deviation of the standard dose from the individual prescribed dose in each dose band was 5%. Standard doses were scaled down by a factor of 40 in each dose band. Pre-made syringes were prepared and used singly or in combinations at the following doses: 0.25 mg (1 mL), 0.5 mg (2 mL), 2.0 mg (10 mL) and 3.0 mg (15 mL).

The scaled-down dose-banding scheme with ≤5% deviation is shown in *Table 4-4*.

Table 4-4, Dose-banding scheme with ≤5% maximum deviation (5%DB) for oxaliplatin administration after scaled down by a factor of 40 for the *ex vivo* PK simulation study.

Dose band (mg)	Standard dose (mg)	Standard dose scaled down by 40 times (mg)	Max deviation (%)	Pre-made infusion syringes combination to deliver banded dose	No. of syringes*	Total volume of pre-made drug infusion (mL)
95-105	100	2.50	5.00	2mg+0.5mg	2	12.00
105-115	110	2.75	4.55	2mg+0.5mg+0.25mg	3	13.00
115-125	120	3.00	4.17	3mg	1	15.00
125-135	130	3.25	3.85	3mg+0.25mg	2	16.00
135-145	140	3.50	3.57	3mg+0.5mg	2	17.00
145-155	150	3.75	3.33	3mg+0.5mg+0.25mg	3	18.00
155-165	160	4.00	3.13	2mg+2mg	2	20.00
165-175	170	4.25	2.94	2mg+2mg+0.25mg	3	21.00
175-185	180	4.50	2.78	2mg+2mg+0.5mg	3	22.00
185-195	190	4.75	2.63	2mg+2mg+0.5mg+0.25mg	4	23.00
195-210	200	5.00	5.00	3mg+2mg	2	25.00
210-230	220	5.50	4.55	3mg+2mg+0.5mg	3	27.00

[*]: the syringes used for 5%DB scheme were those pre-made syringes prepared with the schedule shown in Table 4-3.

4.3.2.2.2 Dose-banding scheme with 10% deviation in each band (10%DB)

Seven dose bands were designed, which covered doses from 95 mg to 230 mg of oxaliplatin. The deviation of a standard dose from a prescribed dose was less

than 10% among all bands in this scheme. Pre-made syringes used in this scheme were in the same dose and concentration as those syringes used in the 5%DB scheme above.

For some 'patients' from the selected BSA range there would be an overlap of the 10%DB and 5% DB doses as their dose bands were close. Thus, the 10 'patients' used in this study were screened out to make sure the same standard dose would not be applied to the same 'patient' from these two different dose-banding schemes.

The scaled-down dose-banding scheme with ≤10% deviation is shown in *Table 4-5*.

Table 4-5, Dose-banding scheme with ≤10% deviation (10%DB) for oxaliplatin administration after scaled down by a factor of 40 for the *ex vivo* PK simulation study.

Dose band (mg)	Standard dose (mg)	Standard dose scaled down by 40 times (mg)	Max Deviation (%)	Pre-made infusion syringes combination to deliver banded dose	No. of syringes*	Total volume of pre-made drug infusion (mL)
90-110	100	2.50	10.00	2mg+0.5mg	2	12.00
110-130	120	3.00	8.33	3mg	1	15.00
130-150	140	3.50	7.14	3mg+0.5mg	2	17.00
150-170	160	4.00	6.25	2mg+2mg	2	20.00
170-190	180	4.50	5.56	2mg+2mg+0.5mg	3	22.00
190-210	200	5.00	5.00	3mg+2mg	2	25.00
210-250	230	5.75	9.52	3mg+2mg+0.5mg+0.25mg	3	28.00

[*]: the syringes used for 5%DB scheme were those pre-made syringes prepared with the schedule shown in Table 4-3.

4.3.2.2.3 Logarithmic dose-banding scheme (LDB)

The logarithmic dose-banding scheme applied in this study is shown in *Table 4-6* with 9 bands covering from 84.6 mg to 230.9 mg of oxaliplatin dose and the maximum dose variation was ≤6% for each band. The pre-made syringes for LDB contained some different doses from 5%DB and 10%DB syringes, including 0.24 mg (1 mL), 0.30 mg (1 mL), 0.37 mg (1 mL), 0.38 mg (1mL), 0.41 mg (2 mL), 0.46 mg (2 mL) and 1.13 mg (5 mL). Pre-made syringes in various

doses of LDB are shown in *Table 4-3* together with the infusion syringes prepared for 5%DB and 10%DB.

Table 4-6, Logarithmic dose-banding scheme (LDB) for oxaliplatin administration after scaled down by a factor of 40 for the *ex vivo* PK simulation study.

Dose band (mg)	Standard dose (mg)	Standard dose scaled down by 40 times (mg)	Max deviation (%)	Pre-made infusion syringes combination to deliver banded dose	No. of syringes	Total volume of pre-made drug infusion (mL)
84.6-94.6	89.4	2.24	5.82	2mg+0.24mg	2	11.00
94.6-105.7	100.0	2.50	5.70	2mg+0.5mg	2	12.00
105.7-118.2	111.8	2.80	5.72	2mg+0.5mg+0.3mg	3	13.00
118.2-132.2	125.0	3.13	5.76	2mg+1.13mg	2	15.00
132.2-147.8	139.8	3.50	5.72	3mg+0.5mg	2	17.00
147.8-165.2	156.3	3.91	5.69	3mg+0.5mg+0.41mg	3	19.00
165.2-184.7	174.7	4.37	5.72	2mg+2mg+0.37mg	3	21.00
184.7-206.5	195.3	4.88	5.73	2mg+2mg+0.5mg+0.38mg	4	23.00
206.5-230.9	218.4	5.46	5.72	3mg+2mg+0.46mg	4	27.00

[*]: the syringes used for 5%DB scheme were those pre-made syringes prepared with the schedule shown in Table 4-3.

4.3.2.3 Flat-fixed dosing schedule (FFD)

In the flat-fixed dosing method, a standardized dose was applied and used for all patients with different BSA. This fixed dose was 152.15 mg which was calculated by dose (mg) = 85mg/m^2 ×BSA (m^2) with the mean value of BSA 1.79 m^2. (157) To adjust to the scale-down system, the fixed dose was reduced by a factor of 40, which became approximately 4.00 mg. The flat-fixed dose can be prepared in the same size syringes (20 mL) for all 'patients'. The oxaliplatin concentration in these syringes was 0.2 mg/mL, which was within the stability-assessed concentration range (0.2 mg/mL – 0.7 mg/mL).

The flat-fixed dosing scheme for 10 selected 'patients' is shown together with the individual dosing schedule in *Table 4-7* in the following section.

4.3.2.4 Oxaliplatin administration for ‘patients’ with different dosing method

The oxaliplatin administration for each ‘patient’ is shown in *Table 4-7* with five different dosing methods after the appropriate scale-down procedure. BSA values of ten selected ‘patients’ are listed in this table, and for each dosing method three parts of information are included:

- **Drug dose** used for each ‘patient’ is provided, and for the same patient drug dose might be different with different dosing method;
- **Syringe operations** are described variously for different dosing methods. For individual dosing and flat-fixed dosing, the preparation of drug infusion syringes is shown in detail, including the required volume and concentration of oxaliplatin stock solution. For dose-banding schemes (5%DB, 10%DB and LDB), combinations of pre-made infusion syringes are described.
- **Total volume of oxaliplatin infusion** presented in this table can be used to calculate the flowrate of oxaliplatin infusion operated in the experiment.

Table 4-7, Doses and infusion combinations for each 'patient' with different dosing methods: individual dosing, flat-fixed dosing, dose-banding schemes [with maximum 5% (5%DB) and 10% (10%DB) deviation in each band] and logarithmic dose-banding.

Patient	BSA (m^2)	Individual dosing (IND)			Dose-Banding with ≤ 5% deviation (5%DB)			Dose-Banding with ≤ 10% deviation (10%DB)			Flat-fixed Dosing (FFD)			Logarithmic dose-Banding)LDB)		
		Dose (mg)	Drug Volume of Stock Solution	Infusion Volume (mL)	Dose (mg)	Syringe Combination	Pre-made Infusion Volume (mL)	Dose (mg)	Syringe Combination	Pre-made Infusion Volume (mL)	Dose (mg)	Drug Volume of Stock Solution	Infusion Volume (mL)	Dose (mg)	Syringe Combination	Pre-made Infusion Volume (mL)
Patient 1	2.70	5.74	5.74mL, 1mg/mL	20	5.50	3mg+2mg+0.5mg	27	5.75	3mg+2mg+0.5mg+0.25mg	28	4	0.8mL, 5mg/mL	20	5.46	3mg+2mg+0.46mg	27
Patient 2	1.82	3.87	3.87mL, 1mg/mL	20	3.75	3mg+0.5mg+0.25mg	18	4.00	2mg+2mg	20	4	0.8mL, 5mg/mL	20	3.91	3mg+0.5mg+0.41mg	19
Patient 3	1.29	2.74	2.74mL, 1mg/mL	20	2.75	2mg+0.5mg+0.25mg	13	2.50	2mg+0.5mg	12	4	0.8mL, 5mg/mL	20	2.80	2mg+0.5mg+0.3mg	13
Patient 4	1.55	3.29	3.29mL, 1mg/mL	20	3.25	3mg+0.25mg	16	3.50	3mg+0.5mg	17	4	0.8mL, 5mg/mL	20	3.13	2mg+1.13mg	15
Patient 5	2.20	4.68	4.68mL, 1mg/mL	20	4.75	2mg+2mg+0.5mg+0.25mg	23	4.50	2mg+2mg+0.5mg	22	4	0.8mL, 5mg/mL	20	4.88	2mg+2mg+0.5mg+0.38mg	23
Patient 6	1.33	2.83	2.83mL, 1mg/mL	20	2.75	2mg+0.5mg+0.25mg	13	3.00	3mg	15	4	0.8mL, 5mg/mL	20	2.80	2mg+0.5mg+0.3mg	13
Patient 7	1.99	4.23	4.23mL, 1mg/mL	20	4.25	2mg+2mg+0.25mg	21	4.00	2mg+2mg	20	4	0.8mL, 5mg/mL	20	4.37	2mg+2mg+0.37mg	21
Patient 8	1.72	3.66	3.66mL, 1mg/mL	20	3.75	3mg+0.5mg+0.25mg	18	3.50	3mg+0.5mg	17	4	0.8mL, 5mg/mL	20	3.50	3mg+0.5mg	17
Patient 9	2.52	5.36	5.36mL, 1mg/mL	20	5.50	3mg+2mg+0.5mg	27	5.75	3mg+2mg+0.5mg+0.25mg	28	4	0.8mL, 5mg/mL	20	5.46	3mg+2mg+0.46mg	27
Patient 10	2.04	4.34	4.34mL, 1mg/mL	20	4.25	2mg+2mg+0.25mg	21	4.50	2mg+2mg+0.5mg	22	4	0.8mL, 5mg/mL	20	4.37	2mg+2mg+0.37mg	21

4.3.3 Study procedure

4.3.3.1 Study period

According to the SPC (Summary of Product Characteristics), oxaliplatin infusion is generally used as 2-hour to 6-hour intravenous infusion in clinical practice. (122) However, as a part of the chemotherapy, oxaliplatin infusions are normally used to combine with other chemotherapeutic drugs. (237) In this case, the infusion time of oxaliplatin should be compromised with the chemotherapeutic procedure in clinical studies. (237) However, this *ex vivo* pharmacokinetics (PK) study was to simulate the oxaliplatin PK process in a 'patient' as a single agent without other anticancer drug combinations. Therefore 2-hour intravenous infusion time was applied to closely replicate the clinical situation in the simulation.

Most previous pharmacokinetics studies of oxaliplatin were operated with the total study period of 24 hours, while 12 hours and 48 hours were rarely used as the whole study time in a few studies. (11) However, these studies suggested that the key pharmacokinetic data of oxaliplatin can be collected within 6 hours from the beginning of infusion. (11) (133) A 6-hour trial run before this study was implemented, and on the basis of this the total study period was adjusted into 8 hours to obtain the valid pharmacokinetics data of oxaliplatin infusions.

During the 8-hour study, flowrates of all three pumps were adjusted following the schedule described in Section 4.3.1.3.2 above to ensure the constant weight of the body reservoir (Reservoir A). At the end of the infusion time (after 2 hours), the flowrate of Pump 1 was changed manually to balance the system for the post-infusion period. Meanwhile samples were taken at pre-defined time

points through the whole study period including 2-hour infusion time and a post-infusion time of 6 hours as shown below.

4.3.3.2 Sample schedule

In previous literature, samples were withdrawn in different schedules, among which an average of 11 samples were taken during the whole experiment period. (11) (223) To increase the accuracy and reliability of this study, 22 samples were taken and tested throughout the experiment. The sampling time schedules respectively for infusion and post-infusion periods are:

- Sampling time points during infusion time:

  0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 (hour)

- Sampling time points during post-infusion period:

  2.25, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 (hour)

Samples were withdrawn from one branch of the Y-set extension line connected with Reservoir A by using 20 mL syringes with luer-lock. Before each test sample was taken, 2 mL of blank solution from the connecting line was withdrawn to avoid the dead volume effect on sample tests. 8 mL of sample was taken and stored in a 20 mL pre-labelled polypropylene container. 1 – 2 mL samples were pipetted from the container for HPLC assay, and the remaining sample solution was used for pH measurement. Samples for HPLC assay and pH measurement were analysed undiluted.

4.3.3.3 Data analysis

Oxaliplatin concentrations in samples were detected by the validated HPLC assay as described in Chapter 2 (Section 2.2.2, page 108), and then used for PK analysis with a software package (PK Solutions, version 2.0, Summit

Research Services, Colorado, USA). As there were 5 different dosing methods applied in this study, the total 50 pharmacokinetic models were built in PK Solutions with the oxaliplatin data from each simulation, including scaled down oxaliplatin dose (μg), the weight of Reservoir A (kg) and oxaliplatin concentrations (μg/mL) of 22 samples at all sampling time points. Values of AUC (area under the drug concentration-time curve) and C_{max} were calculated from these pharmacokinetic models for each 'patient' with different dosing administration systems. Results of the PK parameters were then compared between the different dosing systems for oxaliplatin.

4.4 Results

4.4.1 System validation

4.4.1.1 Pump validation

Three Baxter Colleague volumetric infusion pumps were used in this study with one spare pump held in reserve. Each pump was tested at 14 different flowrates to obtain precision of volume delivery over the working range of 1 mL/hr – 1200 mL/hr. The validation was carried out in triplicate from which the standard deviation (%) was calculated for each flowrate. Mean values of calculated flowrates are shown in *Table 4-8* with the standard deviation.

Differences between the nominal flowrates and the mean values of flowrate from each test were within the range of 0.00% - 2.52%, which were in the acceptance criteria of ≤ 5%. (234) The relative standard deviation (RSD) values of triplicate runs for each flowrate were generally ≤ 2.85%, while higher RSD values appeared at low flowrate of 1 mL/hr and 2 mL/hr in Pump A as respectively 10% and 20%. The working range of the flowrate required in this

study was 5 mL/hr – 200 mL/hr. Within this working range, flowrate precisions for all four pumps were under 2.47%.

For all four pumps, the calibration plots of the nominal flowrate and test flowrate were linear over the flowrate range of 1.0 – 1200 mL/hr (n=14) in *Figure 4-4*. R^2 of 1.0000, 0.9999, 0.9999 and 1.0000 were respectively determined from the least-squares regression analysis of the plots and an even distribution of flowrates about the regression line can be inspected visually with all regression lines passed through the origin. Error bars of RSD from triplicate values for each flowrate are shown in these plots, which are not obvious since the RSD values were relatively small. Because the flowrate variations between test flowrate and the set flowrate were ≤ 2.52% in all four pumps, while the RSD values for triplicate tests in different flowrates were within 2.47%, the accuracy and precision of these pumps was considered acceptable for this study.

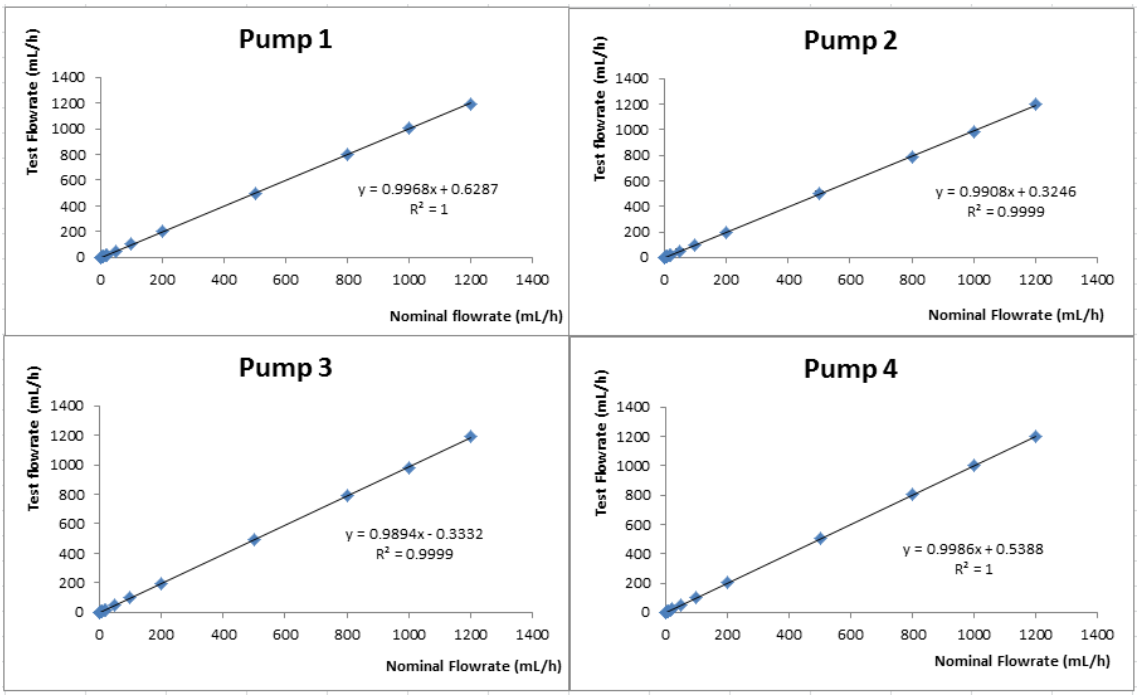

Figure 4-4, Flowrate linearity plot for Pump A, B, C and D, with actual flowrate (test) plotted against nominal or "set" flowrate for each pump. (n = 14)

Table 4-8, Validation results of four pumps (Pump A, B, C and D) at different flowrates with mean values and relative standard deviation (RSD) of triplicate tests for precision and accuracy tests.

Set or nominal flowrate (mL/hr)	Pump A		Pump B		Pump C		Pump D	
	Calculated flowrate [RSD%] (mL/hr)	Difference to set or nominal flowrate (%)	Calculated flowrate [RSD%] (mL/hr)	Difference to set or nominal flowrate (%)	Calculated flowrate [RSD%] (mL/hr)	Difference to set or nominal flowrate (%)	Calculated flowrate [RSD%] (mL/hr)	Difference to set or nominal flowrate (%)
1200	1192.4 [2.85]	0.63	1194.4 [0.15]	0.47	1193.6 [0.91]	0.53	1196.0 [0.21]	0.33
1000	1001.6 [1.02]	0.16	986.0 [0.19]	1.40	981.2 [0.46]	1.88	998.8 [1.08]	0.12
800	799.2 [0.65]	0.10	786.8 [0.92]	1.65	793.32 [1.56]	0.84	802.4 [1.73]	0.30
500	497.2 [0.91]	0.56	502.66 [1.64]	0.53	490.4 [0.51]	1.92	501.6 [0.24]	0.32
200	204.87 [1.98]	2.44	200.0 [0.35]	0.00	197.2 [0.93]	1.40	202.0 [2.47]	1.00
100	101.2 [0.68]	1.20	100.4 [1.83]	0.40	98.60 [0.93]	1.40	101.6 [2.46]	1.60
50	49.6 [1.40]	0.80	48.74 [1.05]	2.52	50.13 [1.61]	0.26	49.6 [1.40]	0.80
20	19.9 [2.10]	0.67	19.75 [1.27]	1.27	19.73 [0.59]	1.33	20.0 [0.00]	0.00
15	15.07 [0.77]	0.44	15.0 [1.33]	0.00	14.73 [1.57]	1.78	15.03 [2.06]	2.00
10	10.0 [0.00]	0.00	9.87 [1.17]	1.33	9.81 [1.84]	1.87	9.87 [2.34]	1.33
8	8.11 [0.62]	1.38	7.87 [1.47]	1.67	7.97 [1.92]	0.42	8.11 [1.92]	1.38
5	5.0 [0.00]	0.00	4.93 [2.34]	1.33	5.0 [0.00]	0.00	4.93 [2.34]	1.33
2	2.0 [10.00]	0.00	2.0 [0.00]	0.00	2.0 [0.00]	0.00	2.0 [0.00]	0.00
1	1.0 [20.00]	0.00	1.0 [0.00]	0.00	1.0 [0.00]	0.00	1.0 [0.00]	0.00

RSD, relative standard deviation; calculated flowrates are all in mean values of triplicate results (n=3).

4.4.1.2 Model precision

To validate the precision of this *ex vivo* pharmacokinetic simulation system, 5 test runs of a 'simulated patient' under identical conditions were undertaken over 5 successive days. The 'patient' simulated in this precision test was selected from the BSA range of 1.12 m^2 – 2.70 m^2 and other parameters were calculated by the equations described in the experimental section (Section 4.3.1.1). Information of the selected patient was: BSA = 1.84 m^2, dose = 3.91 mg, V_d = 570.9 mL, CL_{total} = 132 mL/h. The individual dosage, V_d and CL_{total} of oxaliplatin were scaled down according to the schedule described in Section 4.3.1.1 above.

Because the BSA-individualised dosing method (IND) is currently used in clinical practice as the routine administration method of oxaliplatin infusions, IND method was considered as a control group in this study. Thus, the precision of this PK simulation system was tested with the IND method. The precision tests were carried out under exactly the experimental conditions to be used, including model assembly, experiment time period and sampling schedule.

The concentration-time curves of oxaliplatin for this 'patient' in the series of 5 tests were all similar, as shown in *Figure 4-5* below. The values of C_{max}, T_{max} and AUC were calculated using the 'PK Solutions' software package and compared in *Table 4-9*, which also includes the relative standard deviation (RSD) of these 5 tests. As shown in *Table 4-9*, C_{max} of oxaliplatin were within the range of 4.5118 – 4.6875 μg/mL, and AUC values from 5 tests were between 19.6 – 22.1 μg-hr/mL. T_{max} of oxaliplatin in this model was 2 h, which mostly depended on the 2-hour intravenous infusion simulation. Meanwhile, values of RSD for C_{max}, T_{max} and AUC were 1.5625%, 0.00% and 4.8401%,

respectively, which indicated that the sensitivity and reproducibility of this *ex vivo* PK simulation system was satisfactory.

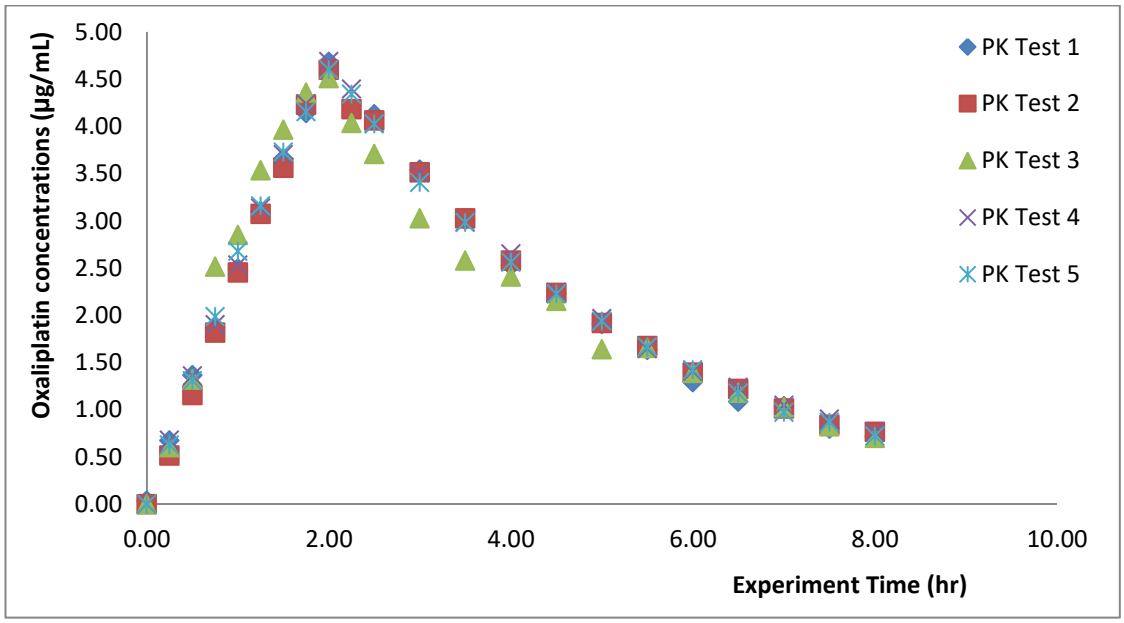

Figure 4-5, Concentration-time curves of 5 oxaliplatin PK tests on a 'simulated patient' with the BSA value of 1.84m^2 in the precision test.

Table 4-9, C_{max}, T_{max} and AUC∞ of oxaliplatin in precision test of 5 simulations on the selected 'patient' (BSA=1.84 m^2) with the relative standard deviation (RSD).

Test	C_{max} (µg/mL)	T_{max} (hour)	AUC∞ (µg-hr/mL)
Test 1	4.6826	2	21.1
Test 2	4.5996	2	22.1
Test 3	4.5118	2	19.6
Test 4	4.6875	2	20.5
Test 5	4.6038	2	19.9
RSD (%)	1.5625	0	4.8401

4.4.1.3 Oxaliplatin assay validation

Oxaliplatin concentrations were detected by the HPLC assay validated and used in the previous stability study in Chapter 2. Validation details of this assay were described in Chapter 2 (Section 2.2.2.2, page 110). However, the expected oxaliplatin concentrations in samples from PK simulations were in the range of 1 – 20 µg/mL, which were lower than those concentrations tested in the previous stability study. Therefore, a linearity validation test on oxaliplatin

solutions (in 5% glucose) was conducted in this PK study with concentrations of 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20 µg/mL.

The calibration plot was shown to be linear over the concentration range 0.5 – 20.0 µg/mL (n=7), with the equation of y = 1.0512x - 0.035 (R^2 = 0.9999) derived from the least-squares regression analysis, and an even distribution of the data on the regression line can be inspected visually as shown in *Figure 4-6*.

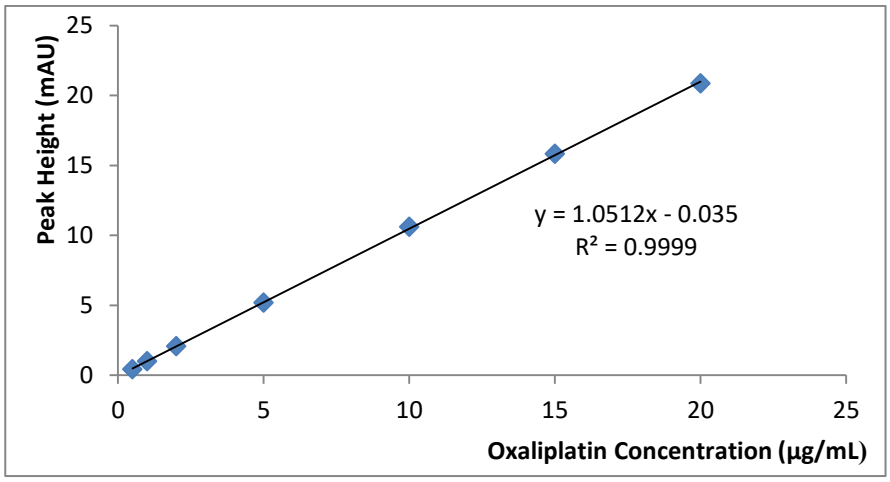

Figure 4-6, Oxaliplatin linearity of response plot with analytic concentrations and peak heights obtained with the HPLC assay in the concentration range of 0.5 – 20.0 µg/mL (n= 7).

4.4.2 The *ex vivo* PK simulation results for each 'patient'

4.4.2.1 pH monitor

Table 4-10 shows pH measurements for ten patients in different dosing methods with the relative standard deviations (RSD) of all 22 samples in each simulation experiment. The mean pH value for each 'patient' was within the range of 4.10 – 4.32, and the relative standard deviation values shown in *Table 4-10* were between 0.61% - 4.68%. pH change in samples can reflect the fluid composition in the system, which might influence oxaliplatin concentrations in the 'central compartment' of the patient's body (Reservoir A) and then result in the incorrect pharmacokinetic simulation of oxaliplatin. pH variation among the samples for each 'patient' simulation was less than 0.23 unit, which indicated no

significant chemical and/or physical change in the *ex vivo* PK simulation system throughout this study.

Table 4-10, Mean values of pH for ten 'patients' in different dosing methods (IND, 5%DB, 10%DB, FFD and LDB) with the relative standard deviations (RSD) from 22 measured pH values for each simulation.

Patient	Individual Dosing [RSD%]	Dose-banding with 5% deviation [RSD%]	Dose-banding with 10% deviation [RSD%]	Flat-fixed dosing [RSD%]	Log dose-banding [RSD%]
Patient 1	4.16 [3.94]	4.16 [2.37]	4.25 [2.45]	4.21 [0.61]	4.17 [1.08]
Patient 2	4.18 [1.27]	4.22 [2.80]	4.27 [2.80]	4.24 [1.05]	4.18 [1.56]
Patient 3	4.14 [1.29]	4.25 [1.18]	4.21 [3.40]	4.21 [1.82]	4.17 [1.42]
Patient 4	4.31 [2.56]	4.10 [4.50]	4.23 [2.46]	4.21 [0.89]	4.28 [2.66]
Patient 5	4.20 [1.30]	4.23 [4.68]	4.20 [0.99]	4.31 [2.20]	4.41 [1.25]
Patient 6	4.21 [2.37]	4.24 [1.16]	4.28 [2.46]	4.24 [3.38]	4.29 [0.79]
Patient 7	4.13 [4.57]	4.16 [3.10]	4.25 [1.47]	4.18 [1.33]	4.31 [1.71]
Patient 8	4.12 [3.05]	4.21 [4.17]	4.21 [1.19]	4.11 [1.65]	4.15 [1.09]
Patient 9	4.22 [1.87]	4.29 [2.20]	4.32 [1.96]	4.20 [1.91]	4.44 [2.74]
Patient 10	4.19 [3.20]	4.15 [1.98]	4.17 [0.66]	4.23 [2.05]	4.26 [1.47]

RSD: relative standard deviation.

4.4.2.2 Weight monitor

The weight of Reservoir A ('central compartment' of the patient's body) were measured and recorded before and after each sample time to monitor the change of 'body' weight and volume. Although flowrates of inlet and outlet solutions to/from Reservoir A were adjusted to balance the 'body' weight, the gain and/or loss of Reservoir A weight could be caused by other factors, such as reservoir bag position moved by the gyro-rotatory shaker, solution loss during sample withdrawal, dead volume in solution transfer tubing. Therefore, the weight monitoring applied in this study throughout the experiment was to ensure these potential effects on the weight change of Reservoir A would not artificially affect the determination of oxaliplatin concentrations.

Weight change within/between sample times of each 'patient' administered with different dosing methods were analysed and are shown in *Table 4-11*. Weight changes between/within sampling were compared to the weight of Reservoir A

at the end of each experiment, which gave the maximum percentage of weight gain/loss to Reservoir A weight.

- Weight change within sampling: At each sample time, the weight of Reservoir A was recorded before and after sample withdrawal, which were used to calculate the weight change during the sampling operation.
- Weight change between sampling: There were different time gaps between each sample point. To monitor the weight change occurred in those time gaps, the weight of Reservoir A measured before sample withdrawal was compared with the weight recorded after the last sampling time.

Any potential effect of weight change to the whole system, thereby, could be indicated by these percentage values. As shown in *Table 4-11*, the weight changes between/within sampling were all less than 1.0%, in the range of 0.0681% - 0.9968%. According to this result, the weight change of Reservoir A would not significantly influence the oxaliplatin concentration in the samples from the pharmacokinetic simulations.

Table 4-11, Weight changes of Reservoir A ('central compartment of patient body') between/within each sample time for ten simulated 'patients' in different dosing methods.

Patient	Individual dosing		Dose-banding with 5% deviation		Dose-banding with 10% deviation		Flat-fixed dosing		Log dose-banding	
	Weight change between sampling (%)	Weight change within sampling (%)	Weight change between sampling (%)	Weight change within sampling (%)	Weight change between sampling (%)	Weight change within sampling (%)	Weight change between sampling (%)	Weight change within sampling (%)	Weight change between sampling (%)	Weight change within sampling (%)
Patient 1	0.5008	0.1713	0.5105	0.1873	0.2300	0.1313	0.5951	0.2317	0.6661	0.3702
Patient 2	0.3655	0.2230	0.5465	0.1437	0.6139	0.3242	0.8275	0.1718	0.5951	0.1631
Patient 3	0.8441	0.4573	0.4923	0.2249	0.9499	0.1485	0.8071	0.2067	0.8966	0.3427
Patient 4	0.6183	0.3990	0.2381	0.1628	0.2379	0.1208	0.9078	0.1569	0.6442	0.1831
Patient 5	0.7815	0.1462	0.5961	0.2384	0.8711	0.2433	0.6944	0.3432	0.5753	0.1661
Patient 6	0.4540	0.6609	0.4886	0.1361	0.9087	0.1775	0.9968	0.1458	0.8878	0.2311
Patient 7	0.4790	0.1204	0.6891	0.1356	0.6970	0.3587	0.5693	0.0681	0.7974	0.1777
Patient 8	0.6033	0.3605	0.6049	0.2093	0.8628	0.6036	0.6246	0.1171	0.7283	0.1860
Patient 9	0.4121	0.1334	0.3016	0.2552	0.3718	0.1151	0.4572	0.1787	0.0455	0.4930
Patient 10	0.4460	0.1588	0.8407	0.3493	0.8098	0.1977	0.6084	0.1264	0.7799	0.0808

Weights of sample volumes (10 mL) withdrawn from Reservoir A were excluded from the 'weight change within sampling'.

4.4.2.3 Results of oxaliplatin PK simulations

At each sample time, concentrations of oxaliplatin were determined by the validated HPLC assay. Oxaliplatin concentrations calculated from chromatographic peak height were input to the PK Solutions software to create oxaliplatin concentration-time curves. From the concentration-time curve, the main oxaliplatin PK parameters were estimated by the 'PK Solutions' software package, including C_{max}, T_{max} and AUC. The estimated values of these PK parameters for each patient with different dosing methods are shown in *Table 4-12*.

Table 4-12, Oxaliplatin PK results from the *ex vivo* PK simulations, including C_{max}, T_{max} and AUC, for each patient in different dosing methods.

Patient 1	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	4.5521	3.8042	3.7242	2.509	3.9544
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	19.8	17.6	17.4	12.9	18.1

Patient 2	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	4.9723	4.5272	4.3002	4.4275	4.2346
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	20.7	18.0	18.4	17.0	17.9

Patient 3	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	5.52584	4.2217	4.7359	6.9103	4.5646
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	19.4	15.8	18.0	26.5	18.3

Patient 4	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	5.3099	4.6301	4.3712	5.3974	4.4593
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	20.5	18.1	18.4	22.5	17.3

Patient 5	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	4.472	4.2776	4.0688	3.9859	4.4491
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	19.5	18.9	17.2	19.0	20.7

Continued

Patient 6	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	5.2413	4.2497	4.5942	6.1034	4.3727
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	18.9	15.3	17.9	24.1	16.7

Patient 7	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	4.7062	3.9707	4.2534	4.2387	4.3543
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	19.8	17.3	16.4	18.7	19.5

Patient 8	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	4.7477	4.3622	4.1917	3.348	3.8551
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	19.0	17.7	16.9	13.8	15.5

Patient 9	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	4.2402	4.2977	4.1917	3.348	3.8551
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	19.1	19.7	17.9	14.1	20.2

Patient 10	IND	5%DB	10%DB	FFD	LDB
C_{max} (μg/mL)	4.6187	3.9431	3.8633	3.9268	3.8919
T_{max} (hr)	2.0	2.0	2.0	2.0	2.0
AUC (μg-hr/mL)	19.8	17.4	17.7	17.8	17.0

IND = Individual dosing; 5%DB = dose-banding scheme with 5% deviation; 10%DB = dose-banding scheme with 10% deviation; FFD = Flat-fixed dosing; LDB = Logarithmic dose-banding scheme; C_{max} = the maximum oxaliplatin concentration in 'body' after dose administration; T_{max} = the time of maximum oxaliplatin concentration; AUC = area under the oxaliplatin concentration-time curve.

4.4.3 Summary and comparisons of PK results

4.4.3.1 AUC distribution

To achieve the required therapeutic efficacy, AUC values for patients of different BSA should ideally cluster around a constant value, which can provide the safe (non-toxic) and effective drug exposure in the patient.

AUC values of each patient for different dosing methods are shown in *Figure 4-7*, in which the distributions of patients' AUC values are visualized. As shown in *Figure 4-7*, AUC values have a more compact distribution for individual dosing (IND) and the dose-banding scheme with 10% deviation (10%DB). While in the

dose-banding scheme with 5% deviation (5%DB) and logarithmic dose-banding scheme (LDB), the variation between patients is larger. AUC values for flat-fixed dosing (FFD) are distributed across the widest range compared to the other dosing methods.

Mean values of AUC for 10 patients in each dosing scheme are shown with standard deviations in *Figure 4-8*. Relative standard deviation (RSD) values for Individual dosing, 5% dose-banding scheme, 10% dose-banding scheme and Log dose-banding scheme are 0.6023%, 1.2985%, 0.6460% and 1.6254%, respectively. However, with the Flat-fixed dosing method, AUC values show more variations between patients with different BSA, giving an higher RSD of 4.5583%.

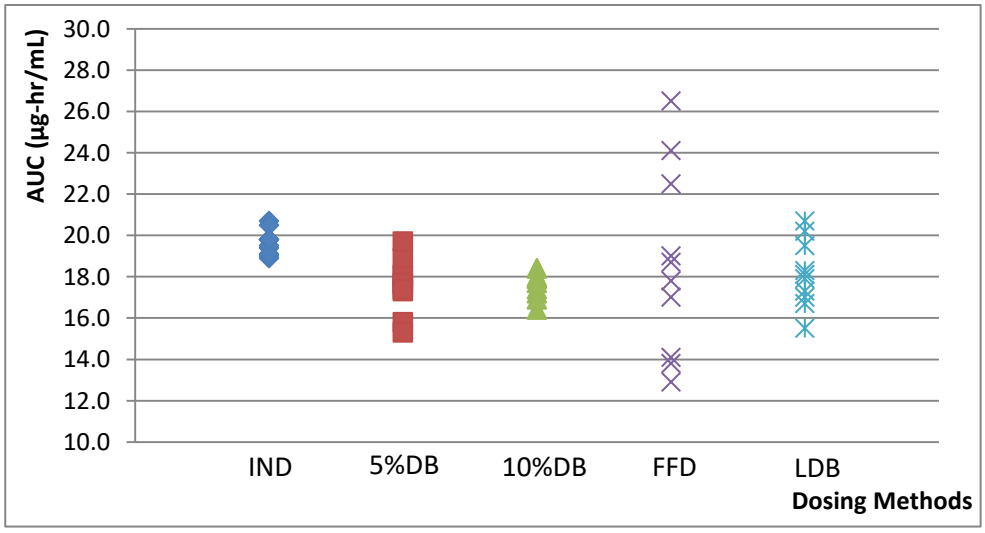

Figure 4-7, Distribution of 'patients' AUC values for five different dosing methods: Individual dosing (IND), dose-banding scheme with 5% deviation (5%DB), dose-banding scheme with 10% deviation (10%DB), Flat-fixed dosing (FFD) and Log dose-banding scheme (LDB).

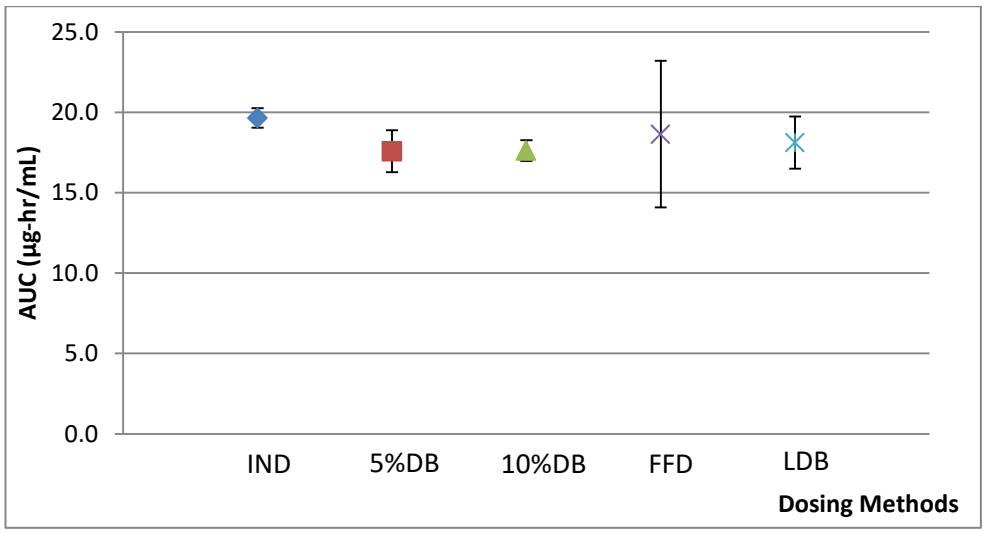

Figure 4-8, Mean values of 10 'patients' AUC with relative standard deviations (error bars) for five different dosing methods: Individual dosing (IND), dose-banding scheme with 5% deviation (5%DB), dose-banding scheme with 10% deviation (10%DB), Flat-fixed dosing (FFD) and Log dose-banding scheme (LDB).

4.4.3.2 Relationships between patient's BSA and AUC values for different dosing methods

Ten 'patients' with randomly selected BSA were included in this simulation study to estimate the inter-patient variability of oxaliplatin AUC values gained with different dosing administration methods.

The relationship between patients' BSA and the AUC, calculated from PK simulations, are shown in *Figures 4-9* to *4-13*: *Figure 4-9* represents the Individual dosing method (IND); *Figure 4-10* represents the dose-banding scheme with 5% deviation (5%DB); *Figure 4-11* represents the dose-banding scheme with 10% deviation (10%DB); *Figure 4-12* represents the Logarithmic dose-banding scheme (LDB) and *Figure 4-13* represents the Flat-fixed dosing (FFD).

With the IND dosing method, AUC values are all around the same level of 20.0 µg-hr/mL for ten 'patients' with different BSA and the rough trend line is flat as shown in *Figure 4-9*. *Figure 4-11* for 10%DB shows similar AUC-BSA relationship as IND. For dose-banding scheme with 5% deviation (5%DB), AUC

values slightly increase in patients with higher BSA. As shown in *Figure 4-12*, AUC values are more variable in Log dose-banding scheme (LDB), and also increase when BSA grows higher. The most dispersive AUC distribution for different BSA is presented in *Figure 4-13* with Flat-fixed dosing method (FFD). However, there is an obvious variation tendency in FFD plot which indicates AUC values reduce with BSA's increase.

Regardless of FFD, the inter-patient variabilities of AUC with IND and three dosing-banding schemes were relatively small.

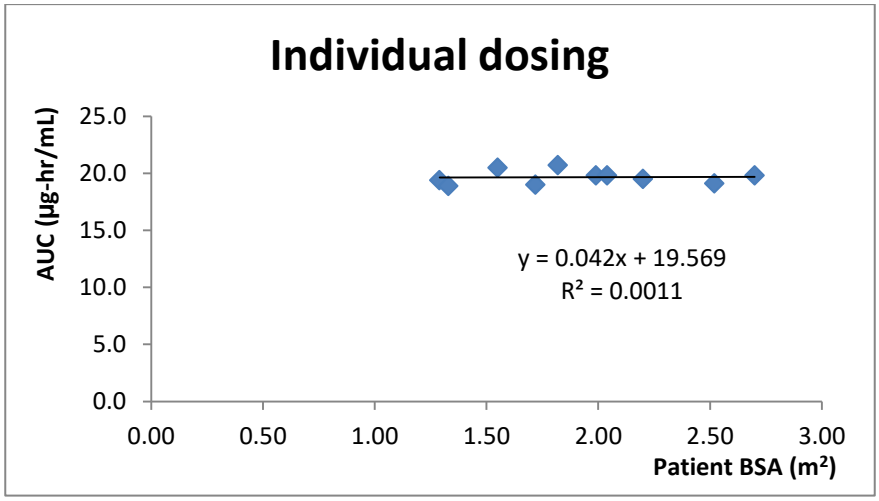

Figure 4-9, Relationship between patients' BSA and AUC from the *ex vivo* PK simulations in Individual dosing (IND).

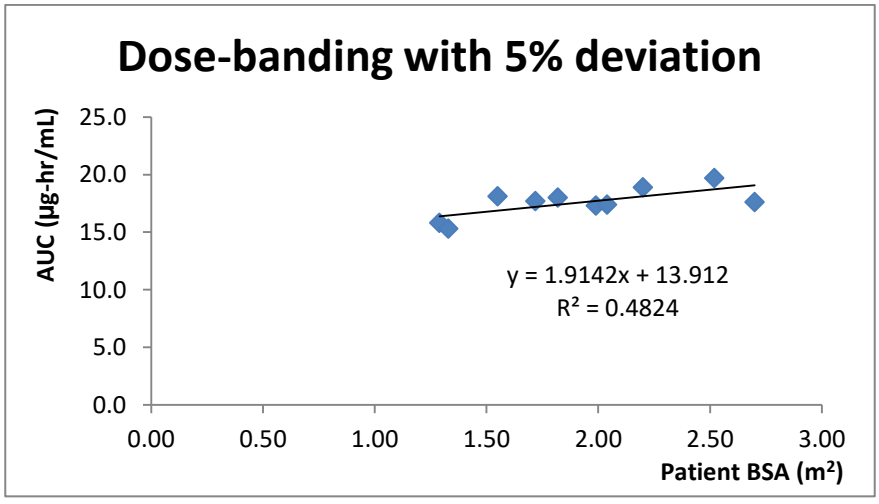

Figure 4-10, Relationship between patients' BSA and AUC from the *ex vivo* PK simulations in dose-banding scheme with 5% deviation (5%DB).

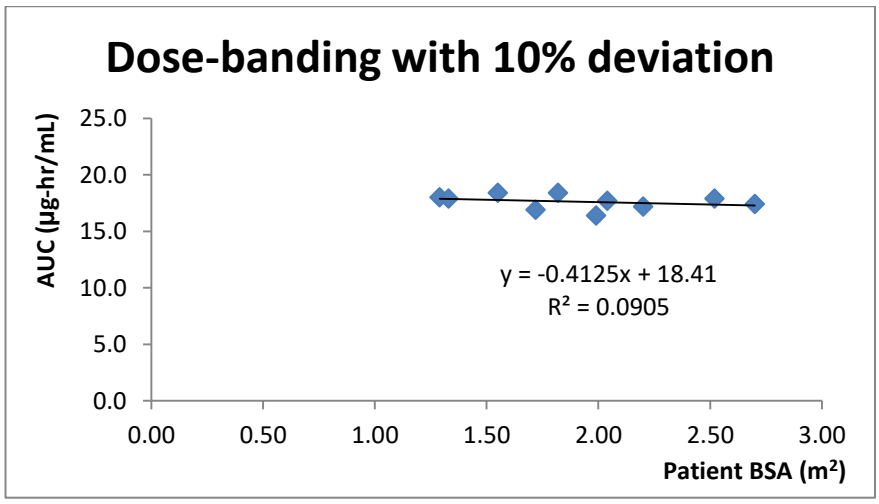

Figure 4-11, Relationship between patients' BSA and AUC from the *ex vivo* PK simulations in dose-banding scheme with 10% deviation (10%DB).

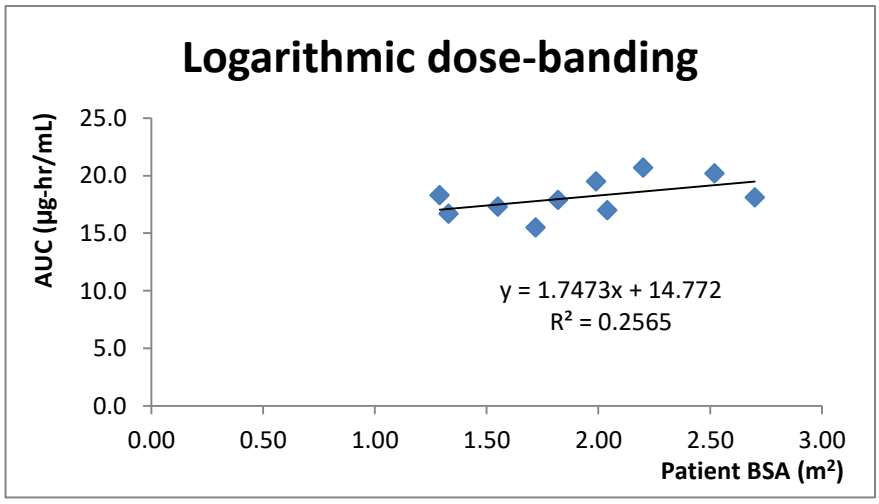

Figure 4-12, Relationship between patients' BSA and AUC from the *ex vivo* PK simulations in Logarithmic dose-banding scheme (LDB).

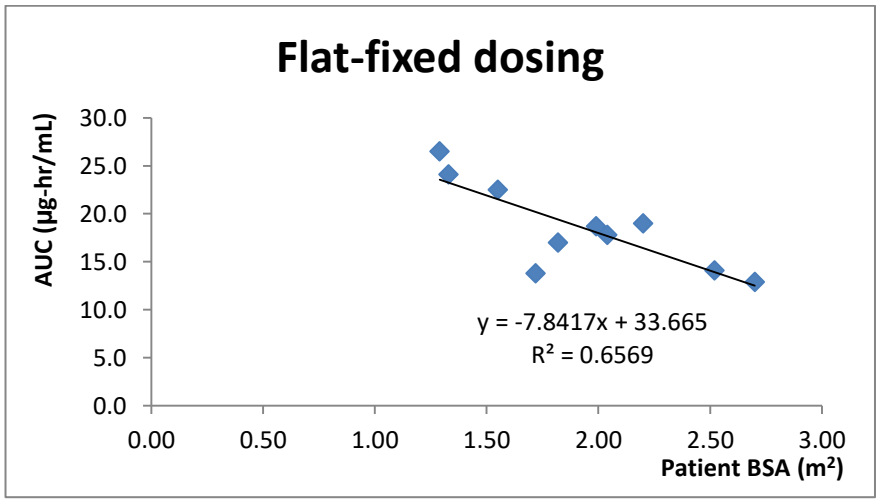

Figure 4-13, Relationship between patients' BSA and AUC from the *ex vivo* PK simulations in Flat-fixed dosing method (FFD).

4.4.3.3 Comparisons between different dosing strategies

Individual dosing calculated with BSA represents the current practice and was used as the control group for comparisons with other dosing schemes. As mentioned in the above sections, C_{max} and AUC are two important pharmacokinetic parameters, which could be surrogate measurements for the drug's efficacy and toxicity. In this section, C_{max} and AUC of IND (control) were compared to those results of other dosing methods as shown in *Table 4-13* and *Table 4-14*.

C_{max} values for each 'patient' with different dosing methods are shown in *Table 4-13*, which also contains C_{max} variations of each dosing scheme compared to IND. The mean value of C_{max} from 10 'patients' in IND is 4.8119 μg/mL (± 0.3682). With respect to the IND, the mean percentage of C_{max} values from simulations with 5%DB, 10%DB, LDB and FFD are respectively 12.13%, 12.26%, 11.13% and 8.53%. Although the C_{max} variation between FFD and IND is smaller than other dosing methods, the inter-patient difference of C_{max} values in FFD is much higher with the relative standard deviation (RSD) of 31.06% among 10 'simulated patients'.

Table 4-14 shows AUC values for ten 'patients' with each dosing method and the variations of AUC from 5%DB, 10%DB, FFD and LDB compared to IND. These AUC variations are visualized in *Figure 4-14* which presents the distribution of AUC variations (compared to IND) separately for each dosing scheme. For an individual 'patient' the AUC values simulated from dose-banding schemes (5%DB, 10%DB and LDB) are generally within 20% difference to the IND result. Compared with other dosing methods, the inter-patient variation of AUC in FFD is the largest with the RSD of 24.45%.

Table 4-13, C_{max} values for ten 'patients' with different dosing administration methods and comparisons of C_{max} between dosing schemes of 5%DB, 10%DB, FFD, LDB and the IND.

Patient	C_{max} (µg/mL)								
	Individual dosing (IND)	Dose-banding scheme with 5% deviation (5%DB)		Dose-banding scheme with 10% deviation (10%DB)		Flat-fixed dosing (FFD)		Logarithmic dose-banding scheme (LDB)	
		Conc. (µg/mL)	Compare to IND (%)	Conc. (µg/mL)	Compare to IND (%)	Conc. (µg/mL)	Compare to IND (%)	Conc. (µg/mL)	Compare to IND (%)
Patient 1	4.5521	3.8042	83.57	3.7242	81.81	2.5090	55.12	3.9544	86.87
Patient 2	4.9723	4.5272	91.05	4.3002	86.48	4.4275	89.04	4.2346	85.16
Patient 3	5.2584	4.2217	80.28	4.7359	90.06	6.9103	131.41	4.5646	86.81
Patient 4	5.3099	4.6301	87.20	4.3712	82.32	5.3974	101.65	4.4593	83.98
Patient 5	4.4720	4.2776	95.65	4.0688	90.98	3.9859	87.12	4.4491	99.49
Patient 6	5.2413	4.2497	81.81	4.5942	87.65	6.1034	116.45	4.3727	83.43
Patient 7	4.7062	3.9707	84.37	4.2534	90.38	4.2387	90.07	4.3543	92.52
Patient 8	4.7477	4.3622	91.88	4.1917	88.29	3.3480	70.52	3.8551	81.20
Patient 9	4.2402	4.2977	101.36	4.1182	97.12	3.1679	74.71	4.6267	109.12
Patient 10	4.6187	3.9431	85.37	3.8633	83.64	3.9268	85.02	3.8919	84.26
Mean values of C_{max} (SD)	4.8119 (0.37)	4.2284 (0.26)	88.25 (6.66)	4.2221 (0.31)	87.87 (4.64)	4.4015 (1.37)	90.11 (22.15)	4.2763 (0.28)	89.28 (8.76)
RSD (%)	7.65	6.13	7.55	7.25	5.28	31.06	24.58	6.59	9.81

Conc. = Concentration; Compare to IND (%): the percentage of C_{max} from one dosing scheme compared to C_{max} from Individual dosing for the same patient; RSD: relative standard deviation of C_{max} values among 10 'patients' in each dosing method.

Table 4-14, AUC data for ten ‘patients’ with different dosing administration methods and comparisons of AUC between dosing schemes of 5%DB, 10%DB, FFD, LDB and the IND.

Patient	AUC∞ (μg-hr/mL)								
	Individual dosing (IND)	Dose-banding scheme with 5% deviation (5%DB)		Dose-banding scheme with 10% deviation (10%DB)		Flat-fixed dosing (FFD)		Logarithm dose-banding (LDB)	
		AUC∞ (μg-hr/mL)	Compare to IND (%)	AUC∞ (μg-hr/mL)	Compare to IND (%)	AUC∞ (μg-hr/mL)	Compare to IND (%)	AUC∞ (μg-hr/mL)	Compare to IND (%)
Patient 1	19.8	17.6	88.89	17.4	87.88	12.9	65.15	18.1	91.41
Patient 2	20.7	18.0	86.96	18.4	88.89	17.0	82.13	17.9	86.47
Patient 3	19.4	15.8	81.44	18.0	92.78	26.5	136.60	18.3	94.33
Patient 4	20.5	18.1	88.29	18.4	89.76	22.5	109.76	17.3	84.39
Patient 5	19.5	18.9	96.92	17.2	88.21	19.0	97.44	20.7	106.20
Patient 6	18.9	15.3	80.95	17.9	94.71	24.1	127.51	16.7	88.36
Patient 7	19.8	17.3	87.37	16.4	82.83	18.7	94.44	19.5	98.48
Patient 8	19.0	17.7	93.16	16.9	88.95	13.8	72.63	15.5	81.58
Patient 9	19.1	19.7	103.14	17.9	93.72	14.1	73.82	20.2	105.76
Patient 10	19.8	17.4	87.88	17.7	89.40	17.8	89.90	17.0	85.86
Mean values of AUC (SD)	19.7 (0.60)	17.6 (1.30)	89.50 (6.73)	17.6 (0.65)	89.71 (3.41)	18.6 (4.56)	94.94 (23.67)	18.1 (1.63)	92.28 (8.73)
RSD (%)	3.07	7.39	7.52	3.67	3.80	24.45	24.93	8.97	9.46

AUC∞: the area under oxaliplatin concentration-time curve; Compare to IND (%): the percentage of AUC value from one dosing scheme compared to AUC from Individual dosing (IND) for the same patient; RSD: relative standard deviation of AUC values among 10 ‘patients’ in each dosing method.

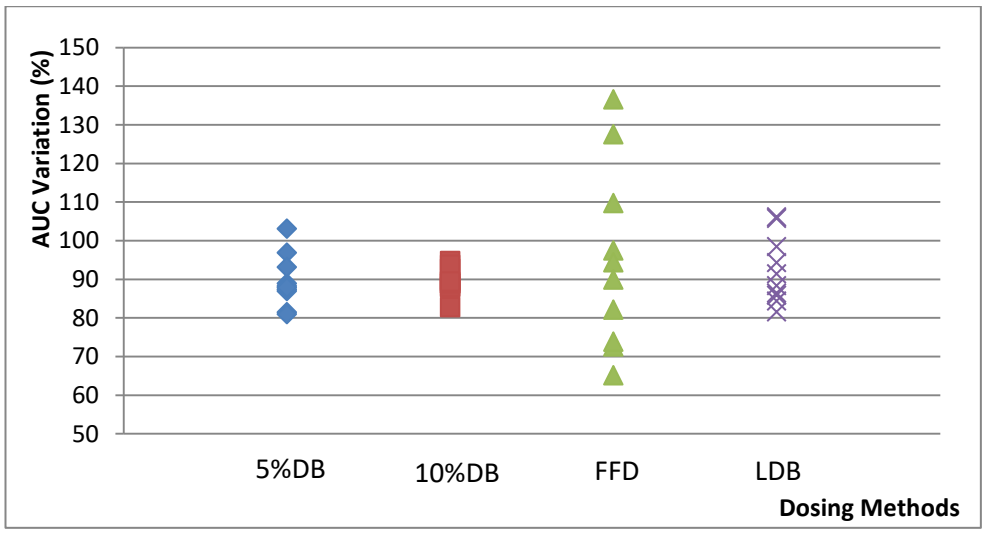

Figure 4-14, Distribution of AUC variations as % (compared to the AUC from IND for the same patient) for ten 'patients' with 5%DB, 10%DB, FFD and LDB dosing schemes, respectively.

In *Table 4-15*, AUC variations for the four alternative dosing schemes compared with the IND method have been divided into five bands which are ≤ ±5%, ≤ ±10%, ≤ ±15%, ≤ ±20% and > ±20%. For 5%DB, 10%DB and LDB, all 10 'patients' exhibit AUC variations of less than 20% from IND, while for FFD only 5 'patients' are within 20% AUC deviation. Simulations with 5%DB, 10%DB and LDB produced AUC variation ≤ ±15%, with respect to IND, for which 'patient' numbers were respectively 8, 9 and 8. As the AUC variations in 'patients' with FFD exhibited a wide range, only 4 'patients' are within the ≤ ±15% AUC variations. Therefore, for most patients the dose-banding schemes provide AUC values closer to IND than the FFD scheme.

Table 4-15, Number of 'patients' with different AUC variations (compared to IND) for each dosing method.

Dosing methods	AUC variation compared to Individual Dosing (IND)				
	≤ ±5%	≤ ±10%	≤ ±15%	≤ ±20%	> ±20%
Dose-banding scheme with 5% deviation (5%DB)	2	3	8	10	0
Dose-banding scheme with 10% deviation (10%DB)	0	3	9	10	0
Logarithmic dose-banding scheme (LDB)	1	5	8	10	0
Flat-fixed dosing (FFD)	1	3	4	5	5

4.5 Discussion

4.5.1 Functionality of the *ex vivo* PK simulation model

4.5.1.1 Model design

The pharmacokinetic simulation model created in this study has been described in detail in the methodology and experimental sections above. Some modifications were made to this model specific for oxaliplatin simulations.

I. 5% Glucose was used to simulate the 'body fluid' in Reservoir A and Reservoir B, which respectively represented the 'central compartment' of the patient's body and the compensation solution. Generally, 0.9% saline solution is the most commonly used solution for medical purposes, such as wound flush cleaning and intravenous injections. In this case, 0.9% saline solution was once considered for use in this PK simulation model. However, oxaliplatin is not stable in acid environment and will undergo nucleophilic substitution reactions with chloride ions in 0.9% saline solution. (127) As a result, in clinical practice oxaliplatin infusions are administered in 5% glucose solution instead of 0.9% saline solution. (124) (127) To prevent the un-necessary degradation of oxaliplatin during the experiment and balance the mixture of drug infusions (in 5% glucose) and solutions in other reservoirs, 5% glucose solution was selected to be used as the 'body fluid' in this study.

II. The high distribution volume of oxaliplatin after drug administration required the use of a 1 litre solution bag as the 'central compartment' of the patient's body in the scaled model. (11) (109) Due to the nature of the DACH (1,2-diaminocyclohexane) platinum complexes are lipophilic, oxaliplatin in

plasma ultra-filtrate (PUF) can widely distribute in the central blood system. (11) (131) In addition, oxaliplatin derivatives will irreversibly bind to proteins, DNA and/or other cellular macromolecules, which increases the apparent distribution volume of oxaliplatin. (11) (109) According to the literature, the high distribution volume of oxaliplatin is normally calculated from the determination of oxaliplatin in plasma ultra-filtrate rather than in plasma. (11) (129) (238) This may be because the analysis of filterable platinum is more straightforward than analysis of intake oxaliplatin in plasma. Since the Reservoir A in this model represents the centre blood system of a 'patient body', the distribution volume of oxaliplatin in plasma was modelled in this study. Following the scale-down schedule in the experiment section, the stated distribution volumes for the 10 selected 'patients' were within the range of 400.2 mL – 837.7 mL.

The infusion and clearance processes simulated in this PK model represented the addition of the drug to, and the removal of drug from, the central compartment of the patient's body. Since oxaliplatin has a simple distribution procedure without special preference for tissues or organs, (11) this *ex vivo* PK simulation system was assembled based on a single reservoir representing the whole 'body' compartment. Otherwise, more reservoirs might be adjusted and combined to represent the 'patient body' in order to simulate different distribution rates for diffusion to different tissues/organs in a human body.

III. Because the elimination of oxaliplatin from the human body is almost entirely via the kidney, the simulation system could be simplified by using single outlet tubing to replicate drug clearance. (11) (225) Only 2% of the administration oxaliplatin dose is excreted by faeces. (11) (109) The total

amount of oxaliplatin in plasma includes bound and un-bound platinum complexes, which can be cleared from plasma by binding to proteins or NDA and renal elimination. (11) (223) (225) The un-bound (free) platinum from oxaliplatin is rapidly excreted through urine after drug administration, with a half-life of approximate14 minutes. (109) (225) While total oxaliplatin in plasma normally is characterized by tri-phasic elimination, which has a short half-life Tα following a long terminal half-life Tγ (can be as much as 300 hours). (3) (11) (109) (129) In this study, there was no real plasma in Reservoir A and subsequently oxaliplatin in the simulated 'patient body' is all in un-bound form. Therefore, the total clearance of oxaliplatin in this study could be considered as the renal clearance: $\mathrm{CL}_{\mathrm{total}} \approx \mathrm{CL}_{\mathrm{renal}}$.

The single compartment PK model used in this study enables different dose systems to be compared but could not provide meaningful comparisons of key pharmacokinetic values, such as AUC and C_{max}, with the data obtained from the *in vivo* studies, see Section 4.5.1.2 below.

4.5.1.2 Simulation results of PK parameters

As described above, the PK simulation model for oxaliplatin was designed as a one-compartment system in 5% glucose solutions which cannot replicate the actual change of oxaliplatin in human plasma, such as protein binding and non-enzymic degradation/metabolism due to the nucleophilic attack by plasma electrolytes. (4) (124) Therefore, the simulated results of AUC and C_{max} from this study could not reflect the *in vivo* PK process of oxaliplatin.

However, the *in vivo* PK data of oxaliplatin obtained from previous clinical studies varied greatly. (11) (131) The *in vivo* studies on patients with cytotoxic drugs were always affected by difficulties, such as the subject patient non-

homogeneity; various chemotherapy combinations were administered to patients with oxaliplatin in different doses (mainly of 85 mg/m^2 and 130 mg/m^2); un-predictable patient numbers (not all enrolled patients would go through the whole study) in each study; oxaliplatin was administered in different infusion time length. (3) (11) (123) In addition, different types of sample were examined for oxaliplatin determination in these clinical studies, including whole blood, plasma, plasma ultra-filtrate (PUF) and urine samples. (131) (225)

With the limitation of having no comparability to *in vivo* studies, this PK simulation model, however, still allows the comparisons between the different dosing systems used for oxaliplatin administration in this *ex vivo* study.

4.5.2 Optimal dosing administration for oxaliplatin infusions

4.5.2.1 Traditional dosing method

When compared with regular medicines, cytotoxic drugs normally have a narrower therapeutic window, and thus it is believed that, the drug dose of cytotoxic agents need to be prepared and administered with more precision, as its precise dosing is related to drug therapeutic effect and toxicity. (239) Therefore, the individualized dosing method was applied to cytotoxic drugs to replace the initial dosing method of fixed dose in grams or milligrams. (36) The first introduction of BSA-based dosing method to clinical oncology was in 1958 by *Pinkel* (228), which was based on the theory that patient's elimination capacity is dependent on the body surface area (BSA) rather than the body weight. (13) (228) Since then, the BSA-based dosing is always considered as the standard drug administration method for most cytotoxic drugs. (226) (227) Pharmacokinetic investigations were constantly conducted in order to improve the therapeutic index of cytotoxic agents by using new-modified detection

methods and technics. (227) In recent studies, BSA-based dosing has been proven with no significant contribution to the reduction in variability of AUC. (227) (233) In addition, it has been indicated that the BSA value of patient was either poorly or not related to the PK characteristics of the drug, and generally could not affect the drug efficacy and toxicity. (13) As shown in *Figure 4-15*, the key PK parameter of AUC (area under the drug concentration-time curve) is significantly correlated with the elimination CL (total clearance). (211) But patient's BSA is not correlated with the CL for most cytotoxic drugs. (226) This resulted in the large inter-individual variability that has been observed with the BSA-based dosing administration in current clinical practice, which could be improved by using alternative dosing methods to patients, such as flat-fixed dosing (FFD). (13) (221)

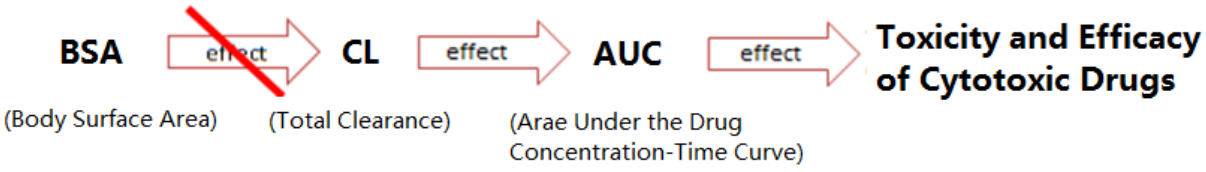

Figure 4-15, Effective factors to the therapeutic efficacy and toxicity of most cytotoxic drugs.

For most cytotoxic drugs currently under use, the knowledge of their metabolism mechanism is not clearly known, especially the characteristics that could affect drug clearance and AUC. (13) (221) If a relationship between BSA and drug exposure and/or clearance was established for a cytotoxic drug, the BSA-based dosing method could be the most appropriate dosing scheme for the administration of that drug. (221)

Dose-banding is a novel dosing administration strategy based on standardized dosages calculated with BSA in each band. (Refer to Section 1.4 in Chapter 1, page 88) Unlike the traditional BSA-based individual dosing method (IND), dose-banding schemes are operated with several pre-defined dose bands and

the patient can receive the standard dose from one of these bands which covers his/her prescribed dosage (calculated with BSA value). (8) Since the dose-banding scheme is also based on BSA calculations, it would not be expected to largely improve the drug efficacy compared with the IND. (13) Therefore, the primary concern for dose-banding studies should be proving that dose-banding schemes are not worse than IND in terms of drug exposure in the patient's body. (13) (152)

4.5.2.2 Comparisons of simulated AUC and C_{max} values for different dosing methods

In this study, the AUC and C_{max} values were simulated from the *ex vivo* PK model to investigate the difference between drug exposures in a 'central compartment' of the patient's body administered with different dosing schemes.

The percentage difference between the simulated AUC values of each dosing scheme and IND method are presented in *Table 4-16* below, which was calculated using the equation:

$$\text{Relative Difference}(\%) = \frac{\text{AUC}' - \text{AUC}_{\text{IND}}}{\text{AUC}_{\text{IND}}} \times 100$$

[AUC': AUC value from the simulation with one of the dosing methods among 5%DB, 10%DB, FFD and LDB; AUC_{IND}: AUC value from simulations with the individual dosing method].

In addition, the absolute value of AUC difference between dose-banding schemes or FFD and the IND was compared using the paired Student's *t*-tests, from which the calculated *P* (n = 10) values are also shown in *Table 4-16*.

Table 4-16, Differences of AUC values and the associated standard deviation (SD) from dosing schemes of 5%DB, 10%DB, FFD and LDB compared to IND.

Patient	AUC difference of 5%DB to IND (%)	AUC difference of 10%DB to IND (%)	AUC difference of LDB to IND (%)	AUC difference of FFD to IND (%)
Patient 1	-11.11	-12.12	-8.59	-34.85
Patient 2	-13.04	-11.11	-13.53	-17.87
Patient 3	-18.56	-7.22	-5.67	36.6
Patient 4	-11.71	-10.24	-15.61	9.76
Patient 5	-3.08	-11.79	6.2	-2.56
Patient 6	-19.05	-5.29	-11.64	27.51
Patient 7	-12.63	-17.17	-1.52	-5.56
Patient 8	-6.84	-11.05	-18.42	-27.37
Patient 9	3.14	-6.28	5.76	-26.18
Patient 10	-12.12	-10.6	-14.14	-10.1
Mean values of AUC difference (SD)	-10.50 (6.73)	-10.29 (3.41)	-7.72 (8.73)	-5.06 (23.67)
P (n = 10)	< 0.05	< 0.05	< 0.05	< 0.05

AUC difference to IND (%): the percentage of the AUC difference between each dosing scheme and individual dosing for the same patient; SD: standard deviation; RSD: relative standard deviation; IND = Individual dosing; 5%DB = dose-banding scheme with 5% deviation; 10%DB = dose-banding scheme with 10% deviation; FFD = Flat-fixed dosing; LDB = Logarithmic dose-banding scheme; P (n = 10): calculated from absolute values of AUC differences by the paired Student's t-tests.

Even though AUC (area under the drug concentration-time curve) can surrogate the drug exposure in the body, it could not represent the drug characteristics alone without considering other pharmacokinetic parameters, especially C_{max}. (13) Generally AUC values depend on the drug concentration-time curve; however it is possible for two different concentration-time curves to share the same AUC value as shown in *Figure 4-16*. There are two different concentration-time curves in this figure, named as curve A and curve B, of which AUC values are the same (AUC = AUC') but C_{max} values are significantly different ($C_{max} > C_{max}'$). Even though the drug exposures in a patient's body are identical for curve A and curve B, the therapeutic effect could be totally different. If the drug in question has a defined "therapeutic window", then there is a risk

that a low C_{max} value will not provide a therapeutic effect and, conversely, that a high C_{max} value would produce unacceptable toxicity.

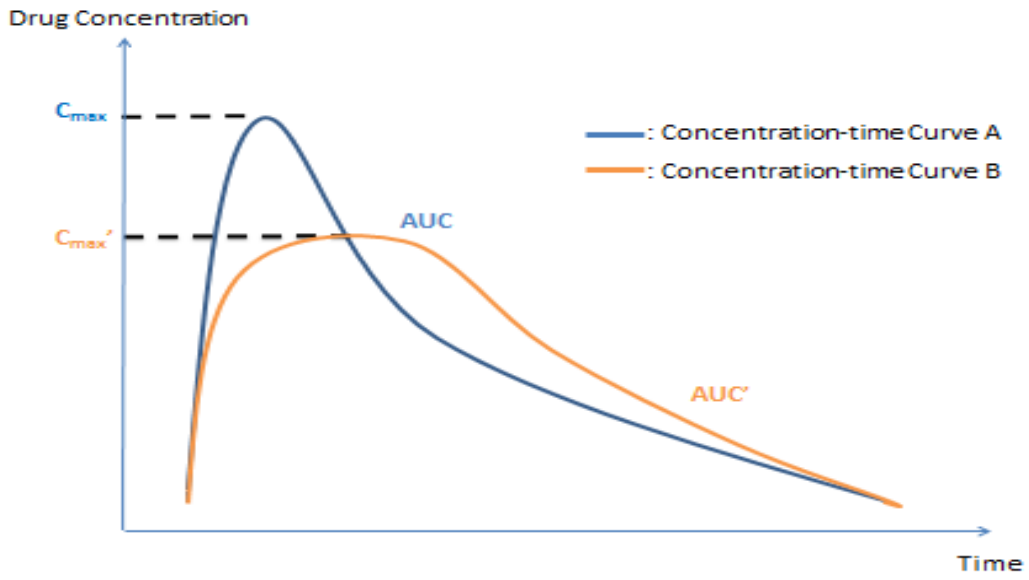

Figure 4-16, Example of two concentration-time curves for a theoretical drug with the same AUC value and different C_{max} for each curve. *[C_{max} and AUC are for Curve A; C_{max}' and AUC' are for Curve B]*

The C_{max} variations between simulation results of different dosing schemes and the IND are shown in *Table 4-17* below. Furthermore, absolute values of C_{max} differences in each dosing method were compared using the Student's *t*-test, for which *p* values are also shown in the table.

Among three different dose-banding schemes, the LDB exhibit the smallest AUC and C_{max} variation of -7.72% and -10.72% compared to IND. AUC and C_{max} variations for 5%DB are respectively similar to those values obtained from 10%DB (-10.29% and -12.13%), which are -10.50% and -11.75%. In this study, oxaliplatin concentration-time curves from simulations administered with different dosing methods were all of similar curve shapes with C_{max} obtained at 2.0 hour time point (T_{max}). (Shown in Appendix C) Furthermore, C_{max} differences between dose-banding schemes and the IND are within the range of -19.72% – +9.12%, which is similar to their AUC variation range (-19.05% – +5.76%). Therefore, the AUC values and differences (compared to the IND) could be

used to compare the simulated oxaliplatin PK characteristics for dose-banding schemes.

Table 4-17, Differences of C_{max} values and the associated standard deviation (SD) from dosing schemes of 5%DB, 10%DB, FFD and LDB compared to IND.

Patient	C_{max} difference of 5%DB to IND (%)	C_{max} difference of 10%DB to IND (%)	C_{max} difference of LDB to IND (%)	C_{max} difference of FFD to IND (%)
Patient 1	-16.43	-18.19	-13.13	-44.88
Patient 2	-8.95	-13.52	-14.84	-10.96
Patient 3	-19.72	-9.94	-13.19	31.41
Patient 4	-12.8	-17.68	-16.02	1.65
Patient 5	-4.35	-9.02	-0.51	-12.88
Patient 6	-18.19	-12.35	-16.57	16.45
Patient 7	-15.63	-9.62	-7.48	-9.93
Patient 8	-8.12	-11.71	-18.8	-29.48
Patient 9	1.36	-2.88	9.12	-25.29
Patient 10	-14.63	-16.36	-15.74	-14.98
Mean values of C_{max} difference (SD)	-11.75 (6.66)	-12.13 (4.64)	-10.72 (8.76)	-9.89 (22.15)
P (n = 10)	< 0.05	< 0.05	< 0.05	< 0.05

C_{max} difference to IND (%): the percentage of the C_{max} difference between one dosing scheme and individual dosing for the same patient; SD: standard deviation; RSD: relative standard deviation; IND = Individual dosing; 5%DB = dose-banding scheme with 5% deviation; 10%DB = dose-banding scheme with 10% deviation; FFD = Flat-fixed dosing; LDB = Logarithmic dose-banding scheme; P (n = 10): calculated from absolute values of C_{max} differences by the paired Student's t-tests.

As shown in *Table 4-18* below, the oxaliplatin doses administered with dose-banding schemes are mostly within 5% difference to the IND, while some of the doses used in 10%DB are within the 10% difference compared to IND. But these small variations on drug doses could not explain the larger differences found in simulated AUC values observed between dose-banding schemes and the IND.

For example, in 10%DB Patient 3 (1.29 m^2) was administered with 2.50 mg oxaliplatin, which was 8.76% (the largest dose difference among all dose-banding schemes) lower than the dose calculated for the IND. However, the AUC variation between 10%BD and IND for this patient was only -7.22% which

was even lower than the average value of AUC variations between 10%DB and IND. The highest AUC variation to the IND (-19.05%) was observed from the simulation of Patient 6 (1.33 m^2) administered with 5%DB using the dose of 2.75 mg, which was only 2.47% lower than individualized dosage. Therefore, the AUC variation simulated between dose-banding schemes and the IND did not directly correspond to the dose differences in drug administration. Hence, other effective factors should be considered for these AUC variations, including the technical difficulties with the administration of dose-banding schemes.

In this simulation study, as described in the experimental sections, pre-made drug infusions for dose-banding schemes (including 5%DB, 10%DB and LDB) were prepared in syringes with multiple sizes. The dose-banding schemes were administered with combinations of infusion syringes, which required more complex drug infusion administration sets to ensure all syringes were infused to 'patients' within the 2-hour infusion time. Compared with the single infusion syringe used in the IND method, the intricate administration of dose-banding schemes could result in a larger amount of drug loss in combination syringes (especially 2 mL and 5 mL syringes) and/or infusion tubing sets. Because all dosing schemes were scaled down by a factor of 40 in this simulation model, the small loss of drug infusions would cause effective changes in drug doses used in dose-banding schemes. This potential drug loss could also explain the lower values of simulated AUC and C_{max} in dose-banding schemes compared to the result from the IND method.

Table 4-18, Different doses administered to each simulated 'patient' with different dosing methods and the percentage difference of 'administered' dose to the individualized dosage (used in IND).

Patient	BSA (m^2)	Dose in IND (mg)	5%DB scheme		10%DB scheme		LDB scheme		FFD scheme	
			Dose (mg)	Dose Difference (%)	Dose (mg)	Dose Difference (%)	Dose (mg)	Dose Difference (%)	Dose (mg)	Dose Difference (%)
Patient 1	2.70	5.74	5.50	4.18	5.75	0.17	5.46	4.88	4.00	30.31
Patient 2	1.82	3.87	3.75	3.10	4.00	3.36	3.91	1.03	4.00	3.36
Patient 3	1.29	2.74	2.75	0.36	2.50	8.76	2.80	2.19	4.00	45.99
Patient 4	1.55	3.29	3.25	1.22	3.50	6.38	3.13	5.17	4.00	21.58
Patient 5	2.20	4.68	4.75	1.50	4.50	3.85	4.88	4.27	4.00	14.53
Patient 6	1.33	2.83	2.75	2.47	3.00	6.01	2.80	1.06	4.00	41.34
Patient 7	1.99	4.23	4.25	0.47	4.00	5.44	4.37	3.31	4.00	5.44
Patient 8	1.72	3.66	3.75	2.46	3.50	4.37	3.50	4.37	4.00	9.29
Patient 9	2.52	5.36	5.50	2.61	5.75	7.28	5.46	1.87	4.00	25.37
Patient 10	2.04	4.34	4.25	2.07	4.50	3.69	4.37	0.69	4.00	7.83

BSA: body surface area; IND: individual dosing method; 5%DB: dose-banding scheme with 5% deviation; 10%DB: dose-banding scheme with 10% deviation; FFD: flat-fixed dosing; LDB: logarithmic dose-banding scheme; dose difference: the percentage of dose difference between one dosing scheme and IND for the same patient,

$Dose\ difference\ (\%) = \frac{|Dose' - Dose|}{Dose} \times 100\%$ *[Dose' = drug dose in different dosing method; dose = drug dose in IND].*

As shown in *Table 4-16* and *Table 4-17* above, results of $p < 0.05$ from the paired Student's *t*-tests (n = 10) indicated the statistically significance of the differences in those simulated AUC values between three dose-banding schemes and the IND method. This value calculated based on the *ex vivo* PK simulations might not fully reflect the clinical situations, considering that those paired *t*-tests were based on a small sample group of 10 'patients'. In addition, a recent study from *Chatelut* (13) has indicated that for most studied drugs there exists no statistically significant difference of AUC values between the dose-banding scheme and the individual dosing method (IND). (13) In the *Chatelut* study, 6 drugs were studied in total on adult cancer patients and the significant difference ($P < 0.05$) of mean values of AUC only occurred for Paclitaxel between the dose-banding scheme and the IND method. (13) Furthermore, the dose-banding scheme used in the *Chatelut* study featured larger dose variations (-14% to +22%) in wider bands, of which three dose bands were defined as BSA < 1.7 m^2; 1.7 m^2 ≤ BSA < 1.9 m^2; BSA ≥ 1.9 m^2. (13) The evidence then suggests that, dose-banding schemes with lower dose variations, such as the 5%DB, 10%DB and LDB simulated in the *ex vivo* PK model within this study, should be able to provide the effective AUC in patients close to the values gained from the IND method.

In this simulation study, the flat-fixed dosing method (FFD) with the standardized 4 mg dose showed large variations on drug doses among all 10 'patients' compared to the IND, which was within the range of 3.36% - 45.99% as shown in *Table 4-18*. Even though the mean values of AUC and C_{max} variations between FFD and the IND were relatively small, the variation ranges were much wider than the dose-banding schemes, which were -34.85% to +36.6% and -44.88% to +31.41% respectively for AUC and C_{max} variations.

According to these results, the administration of FFD could potentially over- or under-dose patients. However, the estimation of clearance in this *ex vivo* PK model was only based on the BSA value of each 'patient'. Other factors that might influence the FFD dosing approach were not reflected in this simulation, such as renal function, liver function, plasma protein levels etc. (227)

Since the BSA-based dosing method started to be questioned, the flat-fixed dosing scheme was recommended by some published literature sources. (226) For drugs established with a broad therapeutic window and small inter-individual variability in limited toxicity, flat-fixed dosing is likely to be the best approach. (226) However, there is not enough evidence to support the application of FFD to cytotoxic drugs. (227) (233) A previous study on cisplatin has indicated that FFD could be used for cisplatin administration on well-defined homogenous patients without significant inter-individual variabilities. (240) But for patients with extreme BSA values, such as children and obese patients, the fixed dose has not been proven with acceptable efficacy and safety with cytotoxic drugs. (227) (241) In some clinical studies on actual patients, the flat-fixed dosing has also shown greater inter-individual variability on PK characteristics, such as AUC values, compared with the BSA-based dosing scheme. (13) (233) Similar to the results from previous clinical studies, in this *ex vivo* simulation study the flat-fixed dosing scheme has been shown to be worse than other BSA-based dosing methods, including individual dosing and dose-banding schemes.

4.5.2.3 Inter-patient variability of AUC and C_{max} from different dosing methods

To obtain the therapeutic effectiveness on more patients, the inter-individual pharmaceutical variability is a key parameter to investigate in drug therapies,

especially for cytotoxic drugs with a narrow therapeutic window. (218) (221) The significant PK inter-individual variability could potentially lead to unpredictable variety in clinical response and adverse effects. (218) (226) Some studies showed that the BSA-based dosing method did not significantly reduce the inter-individual variability for some drugs. (221) (226) However, for most cytotoxic drugs, other available dosing scheme, such as flat-fixed dosing, has not shown better results of lower inter-individual variability compared with the BSA-based individual dosing method. (13) (227) (233)

Therefore, the PK inter-individual variability from different dosing schemes was compared in this *ex vivo* simulation study. The inter-individual variability of AUC and C_{max} for 10 selected 'patients' was expressed as coefficient of variation (CV%) shown in *Table 4-19* below. These CV (%) values were calculated from the AUC and C_{max} values observed from PK simulations administered with different dosing methods, including individual dosing (IND), dose-banding scheme with 5% deviation in each band (5%DB), dose-banding scheme with 10% deviation in each band (10%DB), logarithmic dose-banding scheme (LDB) and flat-fixed dosing (FFD).

As shown in *Table 4-19*, the CV (%) values of C_{max} for three dose-banding schemes were close to the IND all around 7%. In terms to AUC, the CV (%) gained from the IND and 10%DB were respectively 3.0652% and 3.6664%, while for 5%DB and LDB the CV (%) values were slightly higher of 7.3865% and 8.9699%. According to the available literature, the inter-individual variability for cytotoxic drugs is normally around 25% considering all dosing schemes (including BSA-based dosing). (226) In this case, drug administration with dose-banding schemes has not significantly increased the inter-individual variability when compared with the IND method. While among all three dose-banding

schemes, 10%DB showed the lowest AUC variability in simulations among 10 'patients'.

Table 4-19, Mean values of AUC and C_{max} with coefficient of variation (CV) calculated from 10 simulated 'patients' with different dosing methods, including IND, 5%DB, 10%DB, LDB and FFD.

Dosing Methods	**AUC** (Area under the concentration-time curve)		**C_{max}** (The maximum drug concentration)	
	Mean values of AUC [SD] (µg-hr/mL)	CV (%)	Mean values of C_{max} [SD] (µg/mL)	CV (%)
Individual dosing (IND)	19.7 [0.60]	3.0652	4.8119 [0.37]	7.6514
Dose-banding scheme with 5% deviation (5%DB)	17.6 [1.30]	7.3865	4.2284 [0.26]	6.1267
Dose-banding scheme with 10% deviation (10%DB)	17.6 [0.65]	3.6664	4.2221 [0.31]	7.2514
Logarithmic dose-banding scheme (LDB)	18.1 [1.63]	8.9699	4.2763 [0.28]	6.5919
Flat-fixed dosing (FFD)	18.6 [4.56]	24.4545	4.4015 [1.37]	31.0601

SD: standard deviation; CV (%): coefficient of variation.

Compared with other dosing methods, PK simulations with flat-fixed dosing (FFD) resulted in the largest CV (%) values of 24.4545% and 31.0601% respectively for the AUC and C_{max}. These high CV values could indicate that compared with other dosing schemes, the flat-fixed dosing is less optimal for oxaliplatin administration since it might cause a greater inter-individual variability of drug exposure in real patients. According to those simulation results, the flat-fixed dosing scheme is not recommended for clinical use.

4.5.3 Clinical application

4.5.3.1 Dose-banding schemes (including 5%DB, 10%DB and LDB)

With a small number of clinical studies on cytotoxic drugs, there is only limited evidence to persuade oncologists to consider alternative dosing strategies which challenge the traditional individual dosing method (IND). (152) (227) At the present time, some clinicians, regulators and/or industrial drug developers

remain unconvinced by the arguments for change. (221) However, the dose-banding scheme with less than 5% deviation from the prescribed dose in each band (5%DB) has gained wide acceptance and has been applied to several widely-used anticancer drugs in clinical practice. (10) (152) It may be more difficult for the 10%DB with higher dose deviation to gain acceptance from physicians without reliable pharmacological and pharmacokinetic data to support the efficacy and safety of this dosing scheme. (152) However, the greater variation of administered dose possible with 10%DB should be considered in context of the complexities of cytotoxic drug administration, and whether the individualised dose calculated from BSA is ever administered accurately in clinical practice anyway. (221) (226) In fact, intravenous infusion dosing errors with the individual dosing method (IND) during the drug preparation and administration are very common with a reported incidence of up to 50%. (79) (221) These errors are normally caused by systematic errors (fault calculation), inaccurate height and weight information from patient, initial variation from stock vial of the drug and inevitable residual infusion waste in infusion administration tubing. (149) (221)

Therefore, 10% dose variation in the 10%DB scheme should not be considered excessive when compared with the high error incidence in current IND method for oxaliplatin. Moreover, 10%DB scheme can be operated with less dose bands compared with the other two dose-banding schemes. In this case fewer variants of standard dose, administered with the combination of pre-made infusions, would be required for each band, which is more practical and economical for both manufacturers and pharmacists (*Table 4-20*).

The pre-filled syringes in this simulation study were scaled down from actual drug doses to represent the pre-prepared drug infusion bags in practice, which

are one of the main error causes in drug preparation. In practice, the pre-prepared drug infusion bags could be prepared in more convenient doses for dose-banding schemes. As a result, 10%DB would only require 3 types of pre-made infusion bags with various doses which is less than 4 infusion bags required by 5%DB (Shown in *Table 4-20*).

Table 4-20, Comparison of pre-made infusions requirements for three dose-banding schemes of 5%DB, 10%DB and LDB in clinical practice.

Dose-banding schemes designed for oxaliplatin administration	Max dose deviation in each band	Number of dose bands	Number of pre-made infusions	Max number of syringes in a combination
Dose-banding scheme with 5% deviation (5%DB)	5%	12	4	4
Dose-banding scheme with 10% deviation (10%DB)	10%	7	3	4
Logarithmic dose-banding scheme (LDB)	6%	9	10	4

Max dose deviation: the maximum dose deviation between the standard dose and the prescribed dose in each band. Dose-banding schemes presented in this table are designed for oxaliplatin administrations to patients with BSA range from 1.12 m^2 to 2.70 m^2.

For the logarithmic dose-banding scheme (LDB), 10 infusion bags in different doses are required to be prepared for drug administration as shown in *Table 4-20*. In clinical practice, the more infusion bags produced and stored for the LBD scheme could result in greater potential waste of drug infusions compared with other dose-banding schemes. Furthermore, the standard dose in each band is more precise in the LDB, which raises the potential risk of calculation and preparation errors. Most importantly, few pharmacists and technicians are confident with their understanding or use of the LDB scheme in clinical practice. Even if administration doses of the LDB scheme are calculated by computers, serious medication errors could still occur since the pharmacist may not recognise potential errors caused by computer faults.

In summary, simulation results from this study indicated that the 10%DB scheme provided comparable pharmacokinetic performance to IND and other

dose-banding schemes in terms of AUC and C_{max}. Considering the advantages discussed above, the 10% DB scheme would be the preferred option for oxaliplatin administration compared to 5%DB and LDB. The low number of different pre-prepared drug infusions required with this scheme would make it more attractive for a pharmaceutical manufacturer to enter the market with a small range of licenced ready-to-use infusions, for which providing extended drug-stability periods of >12 months is achievable.

4.5.3.2 Flat-fixed dosing

As discussed in the above Section 4.5.2, the flat-fixed dosing scheme (FFD) was recommended to be applied for cytotoxic drug administration since the value of the BSA-based dosing method has been put into question. (226) (227)

Flat-fixed dosing refers to simply application with one fixed dose for all patients regardless to their various BSA values or other pharmacological differences, which is a widely used dosing strategy for the majority of drugs. (226) The highlight benefits of FFD are the positive economic implications and the lower risk in terms of dosing errors caused by dose calculation, preparation and administration. (226) (227) However, patients with extreme conditions, such as children and obese patients, might be easily over- and/or under-dosed by administration with the fixed dose. (227) (241) The drugs that are suitable for FFD administrations would have: 1) a broad therapeutic window; 2) lower inter-individual variability of PK parameters; 3) limited toxicity and adverse effects. (226)

In this *ex vivo* PK study, as discussed above, the simulation results on 10 'patients' (within the BSA range of 1.12 m^2 – 2.70 m^2) indicated the large variations of PK parameters (AUC and C_{max}) between FFD and the individual

dosing method. With a relatively high inter-individual variability for AUC/C_{max}, the flat-fixed dosing scheme is not recommended for oxaliplatin administration in clinical practice among the five dosing methods featured in this thesis.

4.5.4 Limitations of this study

Three main limitations of the *ex vivo* PK simulation model were identified during this study:

First, lack of reliable pharmacokinetic data on oxaliplatin has given limited parameterisation of this *ex vivo* PK simulation model. The drug total clearance (CL), has been selected and calculated from previous PK studies of oxaliplatin. Therefore, the CL value applied to each simulated 'patient' might not accurately reflect the actual individual patient without consideration of patient-specific clinical factors such as renal function. This gives rise to the real-life possibility of patients with the same BSA having different CL values. In clinical studies, patients are subjected to rigorous clinical monitoring and drug concentrations are measured in blood/plasma/ultra-filtrate plasma (PUF) samples from patients to obtain an oxaliplatin concentration-time curve for PK analysis. (225) In these cases, the PK result is more specific and accurate for each individual patient. The variability of AUC/C_{max}, compared to experiments on patients, was unavoidable in *ex vivo* simulations because those simulation settings relied on assumed values of patient parameters, such as CL and V_d. However, in clinical studies the intra-individual variability should be higher with real patients, as the patient's medical conditions are not stabilized all the time and the PK values may change during the course of chemotherapy.

Second, the scaled-down model required higher precision of drug preparation, model assembly and experiment processing. The purpose of the scaled-down

schedule in this study was to adjust this simulation model to suit the laboratory conditions. However, after the drug dose has been scaled down by a factor of 40, the drug preparation required accuracy of measurements of small volumes ranging from 0.01 mL to 1 mL. For example, using the same stock solution of 1 mg/mL, a patient with 2.20 m^2 BSA requires 187 mL stock drug infusion for dilution. In the scaled-down simulation model, 4.68 mL stock has to be diluted in 5% glucose and stored within a 20 mL volumetric syringe. A small inaccuracy with these low volumes can result in a high percentage error. For dose-banding schemes, the pre-filled oxaliplatin syringes were prepared 12 days in advance to simulate the practical situation. Some of those syringes were prepared in smaller volumes (the smallest syringe was in 2 mL) to ensure the oxaliplatin concentrations of infusions were within the stability range of 0.2 mg/mL – 0.7 mg/mL (Chapter 2, page 97). The dose error could be relatively high in small volumes since a single drop of infusion might cause a large variation within the syringe. Furthermore, even a small dead volume in the scaled-down simulation system might make a difference in the determination of oxaliplatin concentrations at sample times. These errors caused by the scale-down schedule could not be totally eliminated despite all of the precautions taken:

- Oxaliplatin stock solutions in different concentrations have been diluted in syringes to provide appropriate operable drug volumes;
- Pipettes with two different working ranges and precisions were used for transferring drug infusions in different volumes;
- All tubing in the simulation system was pre-filled with the correct solutions: tubing connected with drug syringes was pre-filled with oxaliplatin infusions from the syringe; tubing connected with

compensation reservoir (Reservoir B) and waste reservoir (Reservoir C) was pre-filled with 5% glucose.

Third, ‘patients’ with extreme BSA values were not included in this study. The BSA range selected in this study was 1.12 m^2 – 2.70 m^2, which included slightly higher BSA values than the average BSA range from the other literature. (157) (242) In consideration of the increasing trend of body weight within cancer patients, larger BSA values should be included in pre-clinical and clinical studies. However, extreme BSA values were not included in this simulation study because pharmacokinetic data could not have been validated on extreme ranges and these groups could also include patients with special conditions who would require specialised clinical evaluation prior to chemotherapy.

4.6 Conclusion

The *ex vivo* PK simulation model designed and used in this study was validated with acceptable accuracy and precision. Considering the difficulties surrounding clinical studies of cytotoxic drugs on real cancer patients, the *ex vivo* PK simulation is more economical and easier to operate, which should be considered as an alternative approach for PK studies of cytotoxic drugs.

Simulations of flat-fixed dosing (FFD) showed results with relatively large AUC variability among patients, which was not even comparable to the individual dosing method. However, all three dose-banding schemes demonstrated acceptable inter-individual variability on AUC and C_{max} values, including dose-banding scheme with maximum 5% deviation (5%DB), dose-banding scheme with maximum 10% deviation (10%DB) and logarithmic dose-banding scheme (LDB). Among the different dose-banding schemes, 10%DB has been proven to have more advantages in practice.

Overall, the *ex vivo* PK simulations for oxaliplatin infusions demonstrated that dose-banding scheme with 10% deviation (10%DB) would be the preferred dosing scheme for oxaliplatin administration compared with other studied dosing methods.

This simulation study could not fully reflect the clinical situations and PK characteristics of oxaliplatin when administered to real patients. But these simulation results confirmed the feasibility of future application of dose-banding schemes in clinical practice. Consequently, further clinical studies of dose-banding with assessment of drug toxicity and efficacy could facilitate the implementation of dose-banding schemes for oxaliplatin administration.

Chapter 5: Conclusion and Recommendations

5.1 Contribution to knowledge on dose-banding of cancer chemotherapy

The studies reported in this thesis have confirmed that implementation of dose-banding for oxaliplatin chemotherapy into clinical practice is feasible. Three key issues for the development of dose-banding scheme for oxaliplatin administration were investigated in studies presented in Chapter 2, Chapter 3 and Chapter 4 of this thesis.

Chapter 2 focused on the preparation and stability of standard oxaliplatin infusions. Dose-banding schemes require long-term physical and chemical stability (28 – 84 days) (149) of the infusion to enable advance batch preparation of standard infusions by hospital pharmacy compounding units or pharmaceutical manufacturers. (8) (159) The stability study described in Chapter 2 indicated the effective stability period (84 days) for oxaliplatin infusions over a concentration range of 0.2 mg/mL – 0.7 mg/mL, which is a significant increase in the previous shelf-life of oxaliplatin infusions claimed in the SPC (Summary of Product Characteristics) and available literature. (5) (243) (168) After confirming the stability of oxaliplatin infusions is sufficient for advance preparation for dose-banding schemes, the potential for oxaliplatin infusions subjected to long-term storage to contribute to patient toxicity as a result of small quantities of drug degradation products was explored with studies on oxalate, a potential degradation product of oxaliplatin, conducted in Chapter 3. Oxalate has been implicated in previous reports (12) (147) as being associated with the oxaliplatin-related peripheral neuropathy. This study found that a relatively small increase in oxalate concentration occurred in oxaliplatin infusions after long-term storage and also in human plasma containing

oxaliplatin. However, comparisons of these oxalate sources against the levels of endogenous oxalate suggest that oxalate from oxaliplatin infusions is unlikely to be the main cause of neurotoxicity associated with oxaliplatin treatments. (147) The combination of these two studies thus demonstrated that oxaliplatin infusions within a standard concentration range prepared and stored for dose-banding schemes are safe for patient administration. The final uncertainty which this thesis aimed to address was, whether or not the dose-banding scheme can provide acceptable drug efficacy and toxicity for patients compared with the individual dosing method based on BSA (Body Surface Area) that are currently used in routine practice. Clinical studies to answer this question were precluded on the basis of patient numbers, cost and the level of intervention necessary to accurately monitor disseminated disease. Therefore the AUC (area under the concentration-time curve) of plasma concentration-time curves for oxaliplatin was used as a surrogate for clinical measurement and the pharmacokinetic (PK) behaviour of oxaliplatin simulated in an *ex vivo* model was compared for dose-banding schemes and the individual dosing method, as shown in Chapter 4. Different dose-banding schemes with 5% and 10% maximum deviation from the prescribed dose both demonstrated an acceptable inter-individual variability of AUC values, and the mean values of simulated AUC and their standard deviation values were comparable to the individualised dosing. Although the *ex vivo* simulation has a number of limitations which are discussed in Chapter 4, the results of the dose-banding approach were comparable to individual dosing when evaluated under the same conditions. In addition, the dose-banding scheme with 10% maximum deviation from prescribed dose was as acceptable as the 5% variation dose-banding scheme. The 10% variation scheme requires fewer standard infusions and was recommended in this thesis as the best

option for oxaliplatin administration among all three dose-banding schemes. In summary, the results from studies reported in this thesis support the dose-banding of oxaliplatin administration in routine oncology practice using the scheme with 10% maximum deviation from the prescribed dose.

5.2 Advancing knowledge on oxaliplatin stability

The stability study on oxaliplatin infusions in Chapter 2 has set a benchmark for stability studies required by dose-banding schemes with the sequential temperature cycling design. It has not only extended the stability period of the drug up to 84 days, but also supports the storage and re-use of unused drug infusions that have been dispensed and returned from the clinic.

In clinical practice, the drug waste caused by change and/or cancellation of the chemotherapy treatment is thought to cost about 1% of a hospital cancer drug budget. (244) Since the stability of the re-issued oxaliplatin infusions was simulated in the stability study, the standard infusion bags for dose-banding can be safely re-used within 7 days of further refrigerated storage after being exposed to room temperature for up to 24 hours. The temporary storage conditions during transportation and in clinical areas must be monitored to ensure the conditions used in the sequential temperature cycling study (25℃) are not exceeded. In terms of wastage reduction, dose-banding offers a further advantage over individualised dosing in that batches of standard infusions for dose-banding schemes can be prepared in numbers that use an integral number of manufacturer's drug vials. This could reduce the drug wastage associated with part-used vials and circumvents the technical challenges of re-using part-vial contents.

These savings, although significant with conventional cytotoxic chemotherapy, are even more significant if the concept of dose-banding can be applied to high-cost targeted therapies with stability considerations permitting.

Another key issue arising from the stability study was defining the acceptance limits for variation of the oxaliplatin concentration. For long-term stability studies, it is difficult to track all components that may be present in the drug infusion, which could include diluent components, potentially leachable chemicals from the container, the drug and drug degradation products. The latter could be present in very low concentrations, which makes the resolution and quantification of degradation products very difficult. Furthermore, the drug degradation products may be transient species that would degrade further during the term of the stability study. For cytotoxic drugs, such as oxaliplatin, the information and knowledge about their degradation products are currently limited with unclear/unknown therapeutic and toxic effects. Drug degradation products arising in the drug infusion are not always the same as the related substances or impurities described in the Pharmacopoeia (e.g. European Pharmacopoeia). In this case, it is very difficult to measure, isolate and identify those drug-related complexes from a dilute infusion. A pragmatic approach to prevent any potential risk from those compounds is required and one practical approach is to use more restrictive acceptance criteria for the drug assay. The stability study in Chapter 2 used the acceptance criteria of ± 5% (instead of the ± 10% often used in previous studies) for concentration variations of oxaliplatin. With this ± 5% criteria for the drug change, concentrations of the drug degradation products or other drug-related complexes are limited, and so the risk of any potential toxicities to patients arising from these compounds would be reduced. (149)

In the UK, aseptic pharmaceuticals with shelf-life exceeding 7 days must be prepared in appropriately accredited hospital pharmacies or by manufacturers licenced by the Medicines and Healthcare products Regulatory Agency (MHRA). (159) The preparation procedure for those pharmaceuticals must be in compliance with the European guidelines on good pharmaceutical manufacturing practice (GMP). This ensures that pre-prepared drug infusions for dose-banding schemes will be manufactured with rigorous quality assurance and that extensive validation procedures will be carried out by appropriately qualified staff. (245) When compared with the traditional individualized dosing method that is normally prepared and administered in hospital/clinic, the industrial manufacture of drug infusions for dose-banding schemes, and the associated quality control (QC) procedures, should ensure that drug administration errors are minimised.

5.3 Findings related to the neurotoxicity of oxaliplatin

In Chapter 3, the knowledge of the potential contribution of oxalate to oxaliplatin-related neurotoxicity is enhanced, by the finding that the level of oxalate produced in oxaliplatin infusions after long-term storage and the amount of oxalate produced from oxaliplatin by non-enzymic transformation in human plasma (incubated at 37℃ to simulate the patient body) were within accepted safe levels in both cases. The study implies that it is highly unlikely that oxalate derived from administered doses of oxaliplatin is the main cause of neurotoxicity associated with oxaliplatin treatments.

In previous studies, the neurotoxicity of oxalate has been demonstrated by exposing the neuron cells to a high dose of oxalate. (147) (246) According to the findings from this thesis, however, the plasma oxalate increase caused by

oxaliplatin administration would not reach the toxic levels indicated in these previous studies. (145) (246) The role of oxalate in oxaliplatin side-effects can therefore be drawn into question. This leaves several alternative hypotheses surrounding the role of oxalate in the neurotoxicity of oxaliplatin:

1) Oxaliplatin might affect the neuro-sensitivity of the patient body to oxalate, meaning that oxalate can become neurotoxic at lower concentrations;
2) Oxaliplatin might influence the rate of oxalate uptake in humans from normal dietary/metabolic sources;
3) The oxaliplatin-related neurotoxicity might be due to other factors independent of oxalate.

The first two hypotheses could have potential ramifications in clinical practice, in that there might be potential benefits in modifying the patients' diet before and during oxaliplatin treatments to reduce the baseline concentration of plasma oxalate. Clearly further studies are required to clarify these hypotheses, including research into the effect of oxaliplatin on the absorption, transport and metabolism of oxalate. Such work is beyond the scope of this thesis.

5.4 An alternative approach to the understanding of PK characteristics of cytotoxic drugs

With the notable exception of carboplatin, for most cytotoxic drugs there are no clear relationships between blood-concentration and therapeutic effect or toxicity. (119) As such, pharmacokinetic-guided chemotherapy has been of little benefit in clinical practice. However, PK studies are of value in drawing comparisons between different drug administration systems including oral versus intra-venous dosing (e.g. methotrexate) (247), liposomal formulations

versus solutions for injection (e.g. epirubicin) (248) and dose-banding versus individualised chemotherapy. (13) The latter example has been used as a surrogate for clinical studies in efforts to establish that dose banding is both safe and effective. There is, however, a paucity of reported PK information for cytotoxic drugs. The limited oxaliplatin PK data in the published literature was identified in this thesis. Reasons for the limited PK experimentation and clinical studies on oxaliplatin are:

1) Oxaliplatin is a relatively new drug that was recently launched in the 1990s (1), and most studies and investigations on oxaliplatin PK characteristics have only been conducted since it was approved for clinical use (129);

2) There are unavoidable difficulties in general associated with clinical studies on cytotoxic drugs including oxaliplatin. These include the ethical barrier against administration of cytotoxic agents to healthy subjects, and the non-homogeneity of available test patients, which leads to significant differences of reported PK data between studies (11) (129).

In addition, variations of PK characteristics between previous studies in similar conditions were mostly affected by the form of oxaliplatin measured in samples. (11) (129) For example, the volume of distribution (V_d) estimated for the total amount of oxaliplatin was 35 L, whereas the V_d value tested for the un-bound oxaliplatin was 440 L. (11) (249) Furthermore, in previous clinical studies the concentration of un-bound (free) oxaliplatin determined in plasma was generally overestimated because the inactive oxaliplatin bound to amino acids and other small biomolecules in blood was also included in the plasma oxaliplatin. (11) Another issue contributing to the incomplete PK data of oxaliplatin and other anticancer drugs is that no data are available for drug behaviours in the healthy

human body, for the reason mentioned above where administration of cytotoxic drugs to healthy volunteers is not permitted.

The *ex vivo* PK simulation model developed in Chapter 4 is in need of further development and refinement (as discussed previously), but has potential value in comparing dose-banding, and the dose-banding variants against individualised dosing as it is possible to replicate the practical differences in the drug administration systems associated with these different schemes but without subjecting cancer patients to inconvenient and invasive clinical studies. This validated *ex vivo* model can be adjusted for different drugs to simulate their PK processes. Generally, drug PK models are built on specific simulation software parameterised on a large amount of available PK data. But this *ex vivo* PK model only requires the estimated values of CL_{total} (total clearance) and V_d (distribution volume) to set up, and then the drug concentration-time curve can be obtained and analysed to give the key PK parameters of AUC (area under the drug concentration-time curve), C_{max} (the maximum drug concentration) and T_{max} (the time when C_{max} is achieved).

Even though the *ex vivo* simulations might not be able to fully replicate the actual physiological process experienced *in vivo*, it is of utility in comparing drug administration systems and schemes (as described above) and also to simulate the drug PK profile in 'patients' with different conditions, such as patients with impaired renal function and patients with extreme BSA (body surface area) values.

The above considerations demonstrate that the *ex vivo* simulation model described in this thesis merits further development to facilitate ongoing research into dose-banding and other alternative dose-schemes.

5.5 Recommendations for the further implementation of dose-banding

Dose-banding, as a relatively new dosing scheme for cytotoxic drug administration, has been described and explained in previous chapters in this thesis, together with the benefits that dose-banding would bring to the clinical practice of chemotherapy.

This project on oxaliplatin, as described in each chapter, including extended stability studies, evaluation of potential toxicities that could be related to the drug, its degradation products or metabolites, and the use of a PK simulation, could all serve as a template for studies on the dose-banding of other cytotoxic drugs. The question for clinicians is not just a simple choice between individualised dosing and dose-banding, but a more detailed scrutiny of the different variants of dose-banding schemes available (5% or 10% maximum deviation from prescribed dose, logarithmic dose-banding). Clinical studies on other cytotoxic drugs have demonstrated that there was no significant difference between dose-banding and individual dosing on drug administration to cancer patients. (13) (152) Replicating such studies with all cytotoxic agents currently used in cancer chemotherapy is unrealistic and the pragmatic approach described in this thesis offers a way forward. It is important that clinicians have at least some evidence available to support their decisions on whether to implement new ideas such as dose-banding into practice. A national survey of oncologists by *Kaestner* and *Sewell* (152), indicated strong support for dose-banding but also the desire for further evidence, especially with new drugs. The work described in this thesis provides a means of securing evidence within a reasonable time-scale.

In principle, dose-banding schemes should enable the use of a limited range of defined standard infusions which, in turn, should support the development of licensed “ready-made/ready to administer” chemotherapy infusions by the pharmaceutical industry. Licensed pharmaceuticals produced by the pharmaceutical industry are generally accepted as the “gold-standard” for patient safety and drug efficacy. However, to encourage the pharmaceutical industry to launch a range of standard infusions for dose-banding of oxaliplatin, for example, one common standard dose-banding scheme would need to be adopted by all pharmacists for national or European use. The numbers of standard drug infusions required in this common schedule would then make it financially viable for the industry to launch the dose-banding product. Compared with 5%DB, fewer pre-made drug infusions would be required for 10%DB, which would be attractive for both the industry and pharmacists, from a manufacturing and storage perspective. In this context, the studies described in this thesis could have significant long-term impact.

The very first licensed dose-banding product with a range of standard cytotoxic infusions has just been launched in spring 2016. This was the Gemcitabine ready-to-go infusion bag by Sun Pharma. (250) All users could benefit from the convenience, quality control and regulatory protection offered by this licenced product. With this Gemcitabine product, the pharmaceutical industry wishes to convince all users, including pharmacists and oncology physicians, to accept and conform to their proposed dose-banding scheme. Clearly, there needs to be a harmonised approach to dose-banding schemes, not only within a country but across countries. It is hereby recommended that the dose-banding scheme with 10% deviation constructed in this thesis should be considered for oxaliplatin administration by both the pharmaceutical industry and hospital

pharmacists, which could be used as the standard schedule to avoid difficulties of future approaches caused by different administration schedules used between industry and clinical practice.

Furthermore, dose-banding schemes could also be adopted in non-oncological therapeutic areas for drugs that currently require individualized dosage (based on various rationales which include, but are not limited to, BSA) for patients, such as infectious diseases and IV antibiotics. To gain the quality and safety benefits from dose-banding, the drug used for non-cancer treatments and/or supportive therapies must be suitable for a dose-banding scheme in line with the approach outlined within this thesis for oxaliplatin. The drug must have an extended shelf-life (the minimum 28 days – 84 days) and should be assigned a harmonised scheme of standard infusions to support the pre-preparation procedure in either the pharmaceutical industry or hospital aseptic units. Clinical studies and/or PK studies of the target drug must indicate no significant difference on therapeutic effect and toxicity between the proposed dose-banding scheme and its in-use individualized dosing method.

In parallel with such developments, it is necessary to ensure that health-care professionals (pharmacists, physicians and nurses) receive continuing professional education on dose-banding from a reliable source. This is necessary to ensure the safe implementation of such schemes, and to ensure these new developments, with opportunities for improved patient experience and safety, are fully exploited in our healthcare systems. Considering the paucity of literature and studies on dose-banding schemes, the dose-banding section in the literature review of this thesis (Section 1.4, Chapter 1) could form the basis of two materials about dose-banding:

1) A review paper to update the current knowledge on dose-banding developments;
2) An information and education package could be produced from Chapter 1 (literature review) to introduce the dose-banding approach and its benefits for health professionals.

It is acknowledged that such developments would require a multi-disciplinary input from all key stakeholders.

References

1. Graham J, Mushin M, Kirkpatrick P. Oxaliplatin. Nat Rev Drug Discov. 2004 Jan;3(1):11–2.
2. Martelli L, Ragazzi E, Di Mario F, Basato M, Martelli M. Cisplatin and oxaliplatin cytotoxic effects in sensitive and cisplatin-resistant human cervical tumor cells: time and mode of application dependency. Anticancer Res. 2009 Oct;29(10):3931–7.
3. Burz C, Berindan-Neagoe IB-N, Balacescu O, Tanaselia C, Ursu M, Gog A, et al. Clinical and pharmacokinetics study of oxaliplatin in colon cancer patients. J Gastrointestin Liver Dis. 2009 Mar;18(1):39–43.
4. Raymond E, Faivre S, Chaney S, Woynarowski J, Cvitkovic E. Cellular and molecular pharmacology of oxaliplatin. Mol Cancer Ther. 2002;1(3):227–35.
5. Sanofi-Aventis. ELOXATIN® (oxaliplatin) Summary of Product Characteristics. Bridgewater, US: Sanofi-aventis; 2012. p. 1–14.
6. André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med. 2004;350(23):2343–51.
7. Jardim DL, Rodrigues C a., Novis Y a S, Rocha VG, Hoff PM. Oxaliplatin-related thrombocytopenia. Ann Oncol. 2012;23(8):1937–42.
8. Plumridge RJ, Sewell GJ. Dose-banding of cytotoxic drugs: a new concept in cancer chemotherapy. Am J Heal Pharm. 2001 Sep 15;58(18):1760–4.
9. G. Sewell and S. Kaestner. Dose-banding of cancer chemotherapy. In: Hospital Pharmacy Europe. 2010.
10. Sewell G. Dose banding: the present evidence base for and against. In: Packaging and Safety Symposium. 2011. p. 3–6.
11. Graham MA, Lockwood GF, Greenslade D, Brienza S, Bayssas M, Gamelin E. Clinical Pharmacokinetics of Oxaliplatin : A Critical Review. Clin Cancer Res. 2000;6:1205–18.

12. Pasetto LM, D'Andrea MR, Rossi E, Monfardini S. Oxaliplatin-related neurotoxicity: How and why? Crit Rev Oncol Hematol. 2006;59(2):159–68.

13. Chatelut E, White-Koning ML, Mathijssen RH, Puisset F, Baker SD, Sparreboom A. Dose banding as an alternative to body surface area-based dosing of chemotherapeutic agents. Br J Cancer. 2012 Sep 25;107(7):1100–6.

14. F. Macdonal, C. Ford AC. Molecular Biology of Cancer. Garland Science/BIOS Scientific Publishers; 2005.

15. G. M. Cooper. The cancer book: A guide to understanding the causes, prevention and treatment of cancer. Jones and Bartlett Publishers; 1993.

16. World health organization. National cancer control programmes: Policies and managerial guidelines. World health organization; 2002.

17. M. R. Alison CES. Understanding cancer: from basic science to clinical practice. Cambridge University Press; 1997.

18. B. W. Stewart. World cancer report. 2003.

19. Boyle P, Maisonneuve P. Lung cancer and tobacco smoking. Lung Cancer. 1995 Jun;12(3):167–81.

20. J. D. Potter. Food, Nutrition and the Prevention of Cancer: A Global Perspective. 1997.

21. Man-made Vitreous Fibres. In: Evaluation of Carcinogenic Risks to Humans. IARC (International Agency for Research on Cancer) Press; 2002. p. Vol.81.

22. L. Tomatis. Cancer: Causes, Occurence and Control. IARC Scientific Publication; 1990.

23. Food, Nutrition and the Prevention of Cancer: A Global Perspective. 1997.

24. G. Ress. Understanding cancer. Poole, Dorset: Family Doctor Publications; 2004.

25. E. C. Friedberg. Cancer answers: Encouraging answers to 25 questions you were always afraid to ask. W. H. Freeman and Company Ltd; 1993.

26. J. So. Solid tumours. In: Clinical pharmacy and therapeutics. Churchill Livingstone; 1999. p. 775–89.

27. Maclean M, Blake D. Lymphomas. In: Clinical pharmacy and therapeutics. Churchill Livingstone; 1999. p. 759–73.

28. Jackson G, Stark G. Leukaemia. In: Clinical pharmacy and therapeutics. Churchill Livingstone; 1999. p. 743–58.

29. Thomas H, Sikora K. Cancer: A positive approach. HarperCollins Publishers Limited; 1995.

30. J. Tobias. Cancer: What every patient needs to know. Bloomsbury Publishing Plc; 1995.

31. Robert G. McKineell. The biological Basis of Cancer. Cambridge University Press; 1998.

32. Rahman S, Ali M, Khalili Y, Ali A, Lal A, Teaching S, et al. Role of fine needle aspiration cytology in eveluating the breast lumps. Gomal J Med Sci. 2011;9(1):59–61.

33. Gospodarowicz MK, Miller D, Groome P a, Greene FL, Logan P a, Sobin LH. The process for continuous improvement of the TNM classification. Cancer. 2004 Jan 1;100(1):1–5.

34. Cassidy J, Bissett D, Spence RAJ, Payne M. Oxford Handbook of Oncology. Oxford University Press; 2010.

35. Neal AJ, Hoskin PJ. Clinical Oncology: A textbook for students. Edward Arnold; 1994.

36. T. J. Priestman. Cancer Chemotherapy: An Introduction (Third Edition). Springer-Verlag; 1989.

37. A. Horwich. Oncology: A multidisciplinary textbook. Chapman&Hall; 1995.

38. M. D. Micheal Colvin. Chapter 48, Alkylating agents and platinum antitumor compounds. In: Holland-Frei Cancer Medicine, 5th Edition. BC Decker; 2000.

39. Siddik ZH. Mechanisms of Action of Cancer Chemotherapeutic Agents : DNA-Interactive Alkylating Agents and Antitumour Platinum-Based Drugs. In: Alison MR, editor. The Cancer Handbook. 1st Editio. 2002.

40. Lee P, Kakadiya R, Su T, Lee T-C. Combination of Bifunctional Alkylating Agent and Arsenic Trioxide Synergistically Suppresses the Growth of

Drug-Resistant Tumor Cells. Neoplasia. 2010;12(5):376–87.

41. Z. H. Siddik. Mechanisms of action of cancer chemotherapeutic agents: DNA-Interactive alkylating agents and antitumour platinum-based drugs. In: The Cancer Handbook. John Wiley and Sons Ltd; 2005.

42. Ekhart C, Rodenhuis S, Beijnen JH, Huitema ADR. Carbamazepine induces bioactivation of cyclophosphamide and thiotepa. Cancer Chemother Pharmacol. 2009 Feb;63(3):543–7.

43. Pinto N, Ludeman SM, Dolan ME. Pharmacogenetic studies related to cyclophosphamide-based therapy. Pharmacogenomics. 2009;10(12):1897–903.

44. Mulder RL, Paulides M, Langer T, Kremer LC, Dalen EC van. Cyclophosphamide versus ifosfamide for paediatric and young adult bone and soft tissue sarcoma patients (Review). Cochrane Darabase Syst Rev. 2010;(2).

45. Hillmen P. Using the biology of chronic lymphocytic leukemia to choose treatment. Hematology Am Soc Hematol Educ Program. 2011 Jan;2011(Figure 1):104–9.

46. Shanafelt TD, Lin T, Geyer SM, Zent CS, Leung N, Kabat B, et al. Pentostatin, cyclophosphamide, and rituximab regimen in older patients with chronic lymphocytic leukemia. Cancer. 2007 Jun 1;109(11):2291–8.

47. Gisselbrecht C. Current approaches to the treatment of non-Hodgkin’s lymphoma. Hematol Rep. 2011 Oct 28;3(3s):e3.

48. Yao X, Hosenpud J, Chitambar CR, Charlson J, Cheng YC. A Phase II Study of Concurrent Docetaxel, Epirubicin and Cyclophosphamide as a Neoadjuvant Chemotherapy Regimen in Patients with Locally Advanced Breast Cancer. J Cancer. 2012 Jan;3:145–51.

49. Yingzi Ge, Christoph Domschke, Natalija Stoiber, Sarah Schott, Joerg Heil, Joachim Rom, Maria Blumenstein, Janina Thum, Christof Sohn, Andreas Schneeweiss, Philipp Beckhove FS. Metronomic cyclophosphamide treatment in metastasized breast cancer patients: immunological effects and clinical outcome. Cancer Immunol Immunother. 2012;61(3):353–62.

50. Annavarajula SK, Murty KVD, Prayaga A, Das U, Desai M, Narain C a. The outcome of proliferative lupus nephritis with pulse cyclophosphamide therapy. Indian J Nephrol. 2011 Jul;21(3):160–5.

51. Leoni LM. Bendamustine: rescue of an effective antineoplastic agent from the mid-twentieth century. Semin Hematol. 2011 Apr;48 Suppl 1(2):S4-11.

52. Annapurna MM, Pavani S, Anusha S, Mahanti H, Venkatesh B. New analytical methods for the determination of Bendamustine hydrochloride : An anti-neoplastic drug. J Chem Pharm Res. 2012;4(3):1696–701.

53. Plosker GL, Carter NJ. Bendamustine: A review of its use in the management of indolent non-hodgkin lymphoma. Adis drug Eval Drugs. 2008;68(18):2645–60.

54. Leoni LM, Bailey B, Reifert J, Bendall HH, Zeller RW, Corbeil J, et al. Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents. Clin Cancer Res. 2008 Jan 1;14(1):309–17.

55. Kalaycio M. Bendamustine: a new look at an old drug. Cancer. 2009 Feb 1;115(3):473–9.

56. Hannewald P, Maunit B MJ. Screening of DHFR-binding drugs by MALDI-TOFMS. Anal Bioanal Chem. 2008;392(7–8):1335–44.

57. Wen PY, Schiff D, Lec EQ. Neurologic complications of cancer therapy. Demos Medical, New York; 2012.

58. Gmeiner WH. Antimetabolite incorporation into DNA: structural and thermodynamic basis for anticancer activity. Biopolymers. 2002 Nov 5;65(3):180–9.

59. Golan DE, Tashjian AH, Armstrong EJ, Armstrong A. Principles of Pharmacology: The pathophysiologic basis of drug therapy. Lippincott Williams & Wilkins; 2011.

60. Parker WB. Enzymology of Purine and Pyrimidine Antimetabolites Used in the Treatment of Cancer. Chem Rev. 2010;109(7):2880–93.

61. Kinsella a R, Smith D. Tumor resistance to antimetabolites. Gen Pharmacol. 1998 May;30(5):623–6.

62. Kaye SB. New antimetabolites in cancer chemotherapy and their clinical impact. Br J Cancer. 1998 Jan;78 Suppl 3:1–7.

63. Walko CM, Lindley C. Capecitabine : A Review. Clin Ther. 2005;27(1):23–44.

64. Ayyappan S, Sundaraganesan N, Aroulmoji V, Murano E, Sebastian S. Molecular structure, vibrational spectra and DFT molecular orbital calculations (TD-DFT and NMR) of the antiproliferative drug Methotrexate. Spectrochim Acta A Mol Biomol Spectrosc. 2010 Sep 15;77(1):264–75.

65. el-Hady DA, el-Maali NA, Gotti R, Bertucci C, Mancini F, Andrisano V. Methotrexate determination in pharmaceuticals by enantioselective HPLC. J Pharm Biomed Anal. 2005 Apr 29;37(5):919–25.

66. Liekens S, Bronckaers A, Balzarini J. Improvement of purine and pyrimidine antimetabolite-based anticancer treatment by selective suppression of mycoplasma-encoded catabolic enzymes. Lancet Oncol. 2009 Jun;10(6):628–35.

67. Beaumais A de, Tiphaine, Jacqz-Aigrain E. Intracellular disposition of methotrexate in acute lymphoblastic leukemia in children. Curr Drug Metab. 2012;13(6):822–34.

68. Kang MH, Harutyunyan N, Hall CP, Papa RA, Lock RB. Methotrexate and aminopterin exhibit similar in vitro and in vivo preclinical activity against acute lymphoblastic leukaemia and lymphoma. Br J Haematol. 2009 May;145(3):389–93.

69. Thiel E, Korfel A, Martus P, Kanz L, Griesinger F, Rauch M, et al. High-dose methotrexate with or without whole brain radiotherapy for primary CNS lymphoma (G-PCNSL-SG-1): a phase 3, randomised, non-inferiority trial. Lancet Oncol. 2010 Nov;11(11):1036–47.

70. Kim A, Lee J-E, Jang W-S, Lee S-J, Park S, Kang HJ, et al. A combination of methotrexate and irradiation promotes cell death in NK/T-cell lymphoma cells via down-regulation of NF-κB signaling. Leuk Res. 2012 Mar;36(3):350–7.

71. Falchi L, Gunnellini M, Ferranti L, Liberati AM. High-dose methotrexate and temozolomide associated with intrathecal liposomal cytarabine for the

treatment of primary or secondary central nervous system lymphoma: a preliminary experience. Clin Neurol Neurosurg. 2012 Dec;114(10):1376–8.

72. Majumdar S, Anderson ME, Xu CR, Yakovleva T V, Gu LC, Malefyt TR, et al. Methotrexate (MTX)– cIBR Conjugate for Targeting MTX to Leukocytes : Conjugate Stability and In Vivo Efficacy in Suppressing Rheumatoid Arthritis. J Pharm Sci. 2012;1–17.

73. Srisuk P, Thongnopnua P, Raktanonchai U, Kanokpanont S. Physico-chemical characteristics of methotrexate-entrapped oleic acid-containing deformable liposomes for in vitro transepidermal delivery targeting psoriasis treatment. Int J Pharm. 2012 May 10;427(2):426–34.

74. Schmiegelow K. Advances in individual prediction of methotrexate toxicity: a review. Br J Haematol. 2009 Sep;146(5):489–503.

75. Mencia N, Selga E, Noé V, Ciudad CJ. Underexpression of miR-224 in methotrexate resistant human colon cancer cells. Biochem Pharmacol. 2011 Dec 1;82(11):1572–82.

76. Vogt FG, Vena J a., Chavda M, Clawson JS, Strohmeier M, Barnett ME. Structural analysis of 5-fluorouracil and thymine solid solutions. J Mol Struct. 2009 Aug;932(1–3):16–30.

77. Álvarez P1, Marchal JA, Boulaiz H, Carrillo E, Vélez C, Rodríguez-Serrano F, Melguizo C, Prados J, Madeddu R AA. 5-Fluorouracil derivatives: a patent review. Expert Opin Ther Pat. 2012;22(2):107–23.

78. Dr Robert B. Diasio BEH. Clinical pharmacology of 5-fluorouracil. Clin Pharmackinetics. 1989;16(4):215–37.

79. Saif MW, Choma A, Salamone SJ, Chu E. Pharmacokinetically guided dose adjustment of 5-fluorouracil: a rational approach to improving therapeutic outcomes. J Natl Cancer Inst. 2009 Nov 18;101(22):1543–52.

80. Amstutz U, Froehlich TK, Largiadèr CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. Pharmacogenomics. 2011 Sep;12(9):1321–36.

81. Fischer DS, Knobf MT, Durivage HJ. The cancer chemotherapy handbook. Mosby-Year Book; 1993.

82. Holmes S. Cancer Chemotherapy. Austen Cornish; 1990.

83. S. Corporation. Chemotherapy Handbook. Springhouse Corp; 1994.

84. Leonard RCF, Williams S, Tulpule a, Levine a M, Oliveros S. Improving the therapeutic index of anthracycline chemotherapy: focus on liposomal doxorubicin (Myocet). Breast. 2009 Aug;18(4):218–24.

85. Abraham SA1, Waterhouse DN, Mayer LD, Cullis PR, Madden TD BM. The liposomal formulation of doxorubicin. Methods Enzymol. 2005;391:71–97.

86. Wei G, Xiao S, Si D, Liu C. Improved HPLC method for doxorubicin quantification in rat plasma to study the pharmacokinetics of micelle-encapsulated and liposome-encapsulated doxorubicin formulations. Biomed Chromatogr. 2008;22:1252–8.

87. Zou S, Cao N, Cheng D, Zheng R, Wang J, Zhu K, et al. Enhanced apoptosis of ovarian cancer cells via nanocarrier-mediated codelivery of siRNA and doxorubicin. Int J Nanomedicine. 2012 Jan;7:3823–35.

88. Gilliam LAA, Ferreira LF, Bruton JD, Moylan JS, Westerblad H, Clair DKS, et al. Doxorubicin acts through tumor necrosis factor receptor subtype 1 to cause dysfunction of murine skeletal muscle. J Appl Physiol. 2009;107:1935–42.

89. Lee CJ, Kang JS, Kim MS, Lee KP, Lee MS. The Study of Doxorubicin and its Complex with DNA by SERS and UV-resonance Raman Spectroscopy. Bull Korean Chem Soc. 2004;25(8):1211–6.

90. Fazio S, Palmieri EA, Ferravante B, Bone F, Biondi B, Sacca L. Doxorubicin-Induced Cardiom yo pathy Treated with Carvedilol. Clin Cardiol. 1998;21:777–9.

91. Kaminskas LM, McLeod VM, Kelly BD, Sberna G, Boyd BJ, Williamson M, et al. A comparison of changes to doxorubicin pharmacokinetics, antitumor activity, and toxicity mediated by PEGylated dendrimer and PEGylated liposome drug delivery systems. Nanomedicine. 2012 Jan;8(1):103–11.

92. Riganti C, Voena C, Kopecka J, Corsetto PA, Montorfano G, Enrico E, et al. Liposome-encapsulated doxorubicin reverses drug resistance by inhibiting P-glycoprotein in human cancer cells. Mol Pharm. 2011 Jun

6;8(3):683–700.

93. Shen J, He Q, Gao Y, Shi J, Li Y. Mesoporous silica nanoparticles loading doxorubicin reverse multidrug resistance: performance and mechanism. Nanoscale. 2011 Oct 5;3(10):4314–22.

94. Moore A, Pinkerton R. Vincristine : Can Its Therapeutic Index Be Enhanced ? Pediatr Blood Cancer. 2009;53:1180–7.

95. Moore AS, Norris R, Price G, Nguyen T, Ni M, George R, et al. Vincristine pharmacodynamics and pharmacogenetics in children with cancer: a limited-sampling, population modelling approach. J Paediatr Child Health. 2011 Dec;47(12):875–82.

96. Dubrovay Z, Háda V, Béni Z, Szántay C. NMR and mass spectrometric characterization of vinblastine, vincristine and some new related impurities - Part I. J Pharm Biomed Anal. 2012 Aug 28;

97. R. T. Skeel. Handbook of Cancer Chemotherapy. Lippincott Williams & Wilkins; 2007.

98. Chen J, He H, Li S, Shen Q. An HPLC method for the pharmacokinetic study of vincristine sulfate-loaded PLGA-PEG nanoparticle formulations after injection to rats. J Chromatogr B Analyt Technol Biomed Life Sci. 2011 Jul 1;879(21):1967–72.

99. Dixit G, Dhingra A, Kaushal D. Vincristine induced cranial neuropathy. J Assoc Physicians India. 2012 Mar;60(mArch):56–8.

100. Naithani R, Dolai TK, Kumar R. Bilateral vocal cord paralysis following treatment with vincristine. Indian Pediatr. 2009 Jan;46(1):68–9.

101. Shabani M, Larizadeh MH, Parsania S, Asadi Shekaari M SN. Profound destructive effects of adolescent exposure to vincristine accompanied with some sex differences in motor and memory performance. Can J Physiol Pharmacol. 2012;90(4):379–86.

102. Sathiapalan RK, El-Solh H. Enhanced vincristine neurotoxicity from drug interactions: case report and review of literature. Pediatr Hematol Oncol. 2001 Dec;18(8):543–6.

103. Kelland LR, Clarke SJ, McKeage MJ. Advances in Platinum Complex Cancer Chernotheraw. Platin Met Rev. 1992;36(4):178–84.

104. Kelland LR, Sharp SY, O'Neill CF, Raynaud FI, Beale PJ, Judson IR. Mini-review: discovery and development of platinum complexes designed to circumvent cisplatin resistance. J Inorg Biochem. 1999 Oct;77(1–2):111–5.

105. Sadler SH van R and PJ. Current applications and future potential for bioinorganic chemistry in the development of anticancer drugs. Drug Discov Today. 2009 Dec;14(23–24):1089–97.

106. Kelland L. The resurgence of platinum-based cancer chemotherapy. Nat Rev Cancer. 2007 Aug;7(8):573–84.

107. Pérez JM, Fuertes M a, Alonso C, Navarro-Ranninger C. Current status of the development of trans-platinum antitumor drugs. Crit Rev Oncol Hematol. 2000 Aug;35(2):109–20.

108. Brabec V, Kasparkova J. Modifications of DNA by platinum complexes. Relation to resistance of tumors to platinum antitumor drugs. Drug Resist Updat. 2005 Jun;8(3):131–46.

109. Kweekel DM, Gelderblom H, Guchelaar H-J. Pharmacology of oxaliplatin and the use of pharmacogenomics to individualize therapy. Cancer Treat Rev. 2005 Apr;31(2):90–105.

110. Knox RJ, Friedlos F, Lydall DA, Roberts JJ. Mechanism of Cytotoxicity of Anticancer Platinum Drugs : Evidence and cis-Diammine Differ Only in the Kinetics of Their Interaction with DNA '. Cancer Res. 1986;46:1972–9.

111. Pace A, Giannarelli D, Galiè E, Savarese A, Carpano S, Della Giulia M, Pozzi A, Silvani A, Gaviani P, Scaioli V, Jandolo B, Bove L CF. Vitamin E neuroprotection for cisplatin neuropathy: a randomized, placebo-controlled trial. Neurology. 2010;74(9):762–6.

112. Jw A, Chaudhry V, Cavaletti G, Rc D. Interventions for preventing neuropathy caused by cisplatin and related compounds (Review). Cochrane Libr. 2011;(2).

113. Waissbluth S, Daniel SJ. Cisplatin-induced ototoxicity: transporters playing a role in cisplatin toxicity. Hear Res. 2013 May;299:37–45.

114. Lauren E. Wyatt and Mary Jayne Kennedy. Cisplatin-Induced ototoxicity and the Role of Pharmacogenetic Testing. J Pediatr Pharmacol Ther.

2012;17(4):395–9.

115. Ekhart C, Rodenhuis S, Schellens JHM, Beijnen JH, Huitema ADR. Carboplatin dosing in overweight and obese patients with normal renal function, does weight matter? Cancer Chemother Pharmacol. 2009 Jun;64(1):115–22.

116. Kaag D. Carboplatin dose calculation in lung cancer patients with low serum creatinine concentrations using CKD-EPI and Cockcroft-Gault with different weight descriptors. Lung Cancer. 2013 Jan;79(1):54–8.

117. Ando M, Minami H, Ando Y. Multi-Institutional Validation Study of Carboplatin Dosing Formula Using Adjusted Serum Creatinine Level. Clin Cancer Res. 2000;6:4733–8.

118. Bar-Sela G, Kaidar-Person O, Mari F, Assady S, Haim N. Evaluation of carboplatin dosing in non-small cell lung carcinoma patients using Calvert formula and Cockroft and Gault equation for glomerular filtration rate estimation. Oncol Lett. 2012 Nov;4(5):1072–6.

119. Jennings S, de Lemos ML, Levin A, Murray N. Evaluation of creatinine-based formulas in dosing adjustment of cancer drugs other than carboplatin. J Oncol Pharm Pract. 2010 Jun;16(2):113–9.

120. Public Assessment Report Decentralised Procedure: Cisplatin 1mg/ml concentration for solution for infusion. 2010.

121. MHRA. Carboplatin 10 mg/mL comcentrate for solution for infusion. 2011.

122. Sanofi. Summary of Product Characteristics: Oxaliplatin (Eloxatin). 2011.

123. Raymond E, Chaney SG, Taamma a, Cvitkovic E. Oxaliplatin: a review of preclinical and clinical studies. Ann Oncol. 1998 Oct;9(10):1053–71.

124. Jerremalm E, Wallin I, Yachnin J, Ehrsson H. Oxaliplatin degradation in the presence of important biological sulphur-containing compounds and plasma ultrafiltrate. Eur J Pharm Sci. 2006 Jul;28(4):278–83.

125. Elin Jerremalm, Inger Wallin HE. New insights into the biotransformation and pharmacokinetics of Oxaliplatin. J Pharm Sci. 2009;98(11):4215–27.

126. Jerremalm E, Videhult P, Alvelius G, Griffiths WJ, Bergman T, Eksborg S, et al. Alkaline hydrolysis of oxaliplatin--isolation and identification of the

oxalato monodentate intermediate. J Pharm Sci. 2002 Oct;91(10):2116–21.

127. Alberto ME, Lucas MF, Pavelka M, Russo N. The degradation pathways in chloride medium of the third generation anticancer drug oxaliplatin. J Phys Chem B. 2008 Sep 4;112(35):10765–8.

128. Anestål K, Arnér ESJ. Rapid induction of cell death by selenium-compromised thioredoxin reductase 1 but not by the fully active enzyme containing selenocysteine. J Biol Chem. 2003;278(18):15966–72.

129. Takimoto CH, Graham MA, Lockwood G, Ng CM, Goetz A, Greenslade D, et al. Oxaliplatin Pharmacokinetics and Pharmacodynamics in Adult Cancer Patients with Impaired Renal Function. Clin Cancer Res. 2007;13:4832–9.

130. Gabrielsson J, Weiner D. Non-compartmental analysis. Comput Toxicol. 2012;929:377–89.

131. Ehrsson H, Wallin I, Yachnin J. Pharmacokinetics of oxaliplatin in humans. Med Oncol. 2002;19(4):261–5.

132. Merkel U, Wedding U, Roskos M, Höffken K, Hoffmann A. Pharmacokinetics of oxaliplatin during chronomodulated infusion in metastatic gastrointestinal cancer patients: a pilot investigation with preliminary results. Exp Toxicol Pathol. 2003 Jun;54(5–6):475–9.

133. Kern W, Braess J, Böttger B, Bo B, Kaufmann CC, Hiddemann W, et al. Oxaliplatin Pharmacokinetics during a Four-Hour Infusion. Clin Cancer Res. 1999;5:761–5.

134. Zhang W, Seymour L, Chen EX. Determination of intact oxaliplatin in human plasma using high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2008 Dec 15;876(2):277–82.

135. Martin A. Graham. A clinical metabolism and pharmacokinetic study of oxaliplatin plus 5-fluorouracil in patients with advanced gastrointestinal cancer. Sanofi Research Report No. PKM2983 (part 2). 1998.

136. Brouwers EEM, Huitema ADR, Beijnen JH, Schellens JHM. Long-term platinum retention after treatment with cisplatin and oxaliplatin. BMC Clin

Pharmacol. 2008 Jan;8:7.

137. Kidani Y, Inagaki K, Masaaki, Hoshi A, Kuretani K. Antitumor activity of 1,2-diaminocyclohexane–platinum complexes against sarco-ma-180 ascites form. J Med Chem. 1978;21(12):1315–8.

138. Moyer M. Approved letter for Eloxatin by FDA (US). Vol. 7462. 2004.

139. Sanofi-Aventis. Eloxatin ® (oxaliplatin injection)-based chemotherapy sets new treatment benchmark in patients. 2007.

140. Delpeuch A, Leveque D, Rob L, Bergerat J-P. Off-label use of oxaliplatin in patients with metastatic breast cancer. Anticancer Res. 2011;31(5):1765–7.

141. Vici P, Sergi D, Pizzuti L, Mariani L, Arena M, Barba M, et al. Gemcitabine-oxaliplatin (GEMOX) as salvage treatment in pretreated epithelial ovarian cancer patients. J Exp Clin Cancer Res. 2013;32(1):49.

142. Zaanan A, Williet N, Hebbar M, Dabakuyo TS, Fartoux L, Mansourbakht T, et al. Gemcitabine plus oxaliplatin in advanced hepatocellular carcinoma: A large multicenter AGEO study. J Hepatol. 2013;58(1):81–8.

143. Mitchell PL, Broad A, Rosenthal M a, Galettis P, Abraham R, Burns I, et al. Randomized phase 2 sequencing and pharmacokinetic study of gemcitabine and oxaliplatin in advanced non-small cell lung cancer. Asia Pac J Clin Oncol. 2011 Dec;7(4):376–84.

144. Tsimberidou AM, Wierda WG, Wen S, Plunkett W, O'Brien S, Kipps TJ, et al. Phase I-II clinical trial of oxaliplatin, fludarabine, cytarabine, and rituximab therapy in aggressive relapsed/refractory chronic lymphocytic leukemia or Richter syndrome. Clin Lymphoma, Myeloma Leuk. 2013;13(5):568–74.

145. Broomand A, Jerremalm E, Yachnin J, Ehrsson H, Elinder F. Oxaliplatin neurotoxicity--no general ion channel surface-charge effect. J Negat Results Biomed. 2009 Jan;8(2).

146. Ta LE, Espeset L, Podratz J, Windebank AJ. Neurotoxicity of oxaliplatin and cisplatin for dorsal root ganglion neurons correlates with platinum-DNA binding. Neurotoxicology. 2006 Dec;27(6):992–1002.

147. Gamelin L, Capitain O, Morel A, Dumont A, Traore S, Anne LB, et al.

Predictive factors of oxaliplatin neurotoxicity: the involvement of the oxalate outcome pathway. Clin Cancer Res. 2007 Nov 1;13(21):6359–68.

148. Kaestner S a., Sewell GJ. Chemotherapy Dosing Part II: Alternative Approaches and Future Prospects. Clin Oncol. 2007 Mar;19(2):99–107.

149. Bardin C, Astier A, Vulto A, Sewell G, Vigneron J, Trittler R, et al. Guidelines for the practical stability studies of anticancer drugs: a European consensus conference. Ann Pharm françaises. 2011 Jul;69(4):221–31.

150. Provost K, Bouvet-Muller D, Crauste-Manciet S, Olivi L, Vlaic G, Michalowicz a. Degradation of platinum based anticancer drugs by methionine: An EXAFS study. J Phys Conf Ser. 2009 Nov 1;190:12206.

151. A. Junker, S. Roy, M.-C. Desroches, C. Moussay, M. Berhoune, A. Bellanger CF and RF. Stability of Oxaliplatin Solution. Ann Pharmacother. 2009;43:390–1.

152. Kaestner SA, Sewell GJ. A national survey investigating UK prescribers' opinions on chemotherapy dosing and "dose-banding". Clin Oncol (R Coll Radiol). 2009 May;21(4):320–8.

153. Jerremalm E, Eksborg S, Ehrsson H. Hydrolysis of oxaliplatin-evaluation of the acid dissociation constant for the oxalato monodentate complex. J Pharm Sci. 2003 Feb;92(2):436–8.

154. André P, Cisternino S, Roy A-L, Chiadmi F, Schlatter J, Agranat P, et al. Stability of oxaliplatin in infusion bags containing 5% dextrose injection. Am J Heal Pharm AJHP. 2007 Sep 15;64(18):1950–4.

155. André P, Cisternino S, Roy AL, Chiadmi F, Schlatter J, Agranat P, et al. Stability of oxaliplatin in infusion bags containing 5% dextrose injection. Am J Heal Pharm. 2007;64(18):1950–4.

156. Di Pasqua AJ, Kerwood DJ, Shi Y, Goodisman J, Dabrowiak JC. Stability of carboplatin and oxaliplatin in their infusion solutions is due to self-association. Dalton Trans. 2011 May 14;40(18):4821–5.

157. Sacco JJ, Botten J, Macbeth F, Bagust A, Clark P. The average body surface area of adult cancer patients in the UK: a multicentre retrospective study. PLoS One. 2010 Jan;5(1):e8933.

158. EP 5.0: Particulate contamination: sub-visible particles. In: European Pharmacopeia 50. 2005. p. 253–5.

159. Kaestner S, Sewell G. A sequential temperature cycling study for the investigation of carboplatin infusion stability to facilitate “dose-banding”. J Oncol Pharm Pract. 2007 Jun;13(2):119–26.

160. G.J.Sewell, A.E.Rigby-Jones, M.J.Priston. Statbility of intravesical epirubicin infusion: a sequential temperature study. J Clin Pharm Ther. 2003;28(5):349–53.

161. ICH. Validation of analytical procedures: Test and Methodology (Q2). Vol. 1994. 2005.

162. European pharmacopoeia 7.0: Index. Vol. 1. 2012.

163. Brümmer H. Particle characterisation in excipients, drug products and drug substances. In: Life Science: Technical Bulletin. 2008.

164. EP 6.0: Oxaliplatin. In: European Pharmacopoeia. 2008.

165. Appendix III D. Liquid Chromatography. In: British Pharmacopeia Volumn IV. 2012.

166. British pharmacopoeia commision. British Pharmacopoeia. The stationery office (TSO); 2012.

167. Oxaliplatin Injection. In: The United States Pharmacopeial Convention (Revision Bulletin). 2012.

168. Eiden C, Philibert L, Bekhtari K, Poujol S, Malosse F, Pinguet F. Physicochemical stability of oxaliplatin in 5% dextrose injection stored in polyvinyl chloride, polyethylene, and polypropylene infusion bags. Am J Heal Pharm AJHP. 2009 Nov 1;66(21):1929–33.

169. Committee for Veterinary Medicinal Products. Oxalic Acid Summary Report. 2003.

170. Material Safety Data: Oxalic Acid. 2008.

171. Benizri F, Bonan B, Ferrio A-L, Brandely M-L, Castagné V, Théou-Anton N, et al. Stability of antineoplastic agents in use for home-based intravenous chemotherapy. Pharm World Sci. 2009 Feb;31(1):1–13.

172. Larsson I, Kart T. Evaluation of sources to document extended shelf lives

of compounded cytostatics. J Oncol Pharm Pract. 2013 Dec 12;19(4):355–64.

173. Eidlhuber MC, Schnait H. Stability of Oxaliplatin “Ebewe” Lyophilisate after reconstitution. EBEWE Pharma. 2007.

174. Cayman Chemical. Product Information: Oxaliplatin. 2012.

175. NHS. Capecitabine and oxaliplatin in the adjuvant treatment of stage III (Dukes ’ C) colon cancer. 2009.

176. Yoshida Y. Oxaliplatin and pH. J Phys Chem Biophys. 2012;2(1):2161.

177. Kuwahara T, Asanami S, Kawauchi Y, Kubo S. Experimental Infusion Phlebitis: Tolerance pH of Peripheral Vein. J Toxicol Sci. 1999;24(2):113–21.

178. Final text for revision of the International Pharmacopoeia. 2012.

179. Madras G, McCoy BJ. Temperature effects during Ostwald ripening. J Chem Phys. 2003;119(3):1683–93.

180. Vyas N. Personal comments. 2012.

181. L. C. Hadaway. Infusion therapy equipment. In: Infusion Nursing: An evidence-based approach. Saunders Elsevier; 2010. p. 401.

182. Summary of Product Characteristics: Oxaliplatin. 2010.

183. McKeage MJ, Hsu T, Screnci D, Haddad G, Baguley BC. Nucleolar damage correlates with neurotoxicity induced by different platinum drugs. Br J Cancer. 2001;85(8):1219–25.

184. Merusi C, Corradini C, Cavazza A, Borromei C, Salvadeo P. Determination of nitrates, nitrites and oxalates in food products by capillary electrophoresis with pH-dependent electroosmotic flow reversal. Food Chem. 2010 May;120(2):615–20.

185. Xu H-W, Ji X-M, He Z-H, Shi W-P, Zhu G-H, Niu J-K, et al. Oxalate accumulation and regulation is independent of glycolate oxidase in rice leaves. J Exp Bot. 2006 Jan;57(9):1899–908.

186. Brzica H, Breljak D, Burckhardt BC, Burckhardt G, Sabolić I. Oxalate: from the environment to kidney stones. Arh Hig Rada Toksikol. 2013 Dec;64(4):609–30.

187. Adhirai M, Selvam R. Renal calcium oxalate binding protein: studies on its properties. Kidney Int. 1998 Jan;53(1):125–9.

188. Schepers MSJ, van Ballegooijen ES, Bangma CH, Verkoelen CF. Oxalate is toxic to renal tubular cells only at supraphysiologic concentrations. Kidney Int. 2005 Oct;68(4):1660–9.

189. Trinity Biotech. Oxalate Kit Instruction. 2013.

190. Petrarulo M, Cerelli E, Marangella M, Cosseddu D, Vitale C, Linari F. Assay of plasma oxalate with soluble oxalate oxidase. Clin Chem. 1994;40(11 I):2030–4.

191. Ladwig PM, Liedtke RR, Larson TS, Lieske JC. Sensitive spectrophotometric assay for plasma. Clin Chem. 2005 Dec;51(12):2377–80.

192. Kanno K. Technical Note: Analysis of Oxalic acid in food with amide column. 2011.

193. Heaton J, Smith NW. Advantages and Disadvantages of HILIC; a Brief Overview. Chromatogr Today. 2012;(May/June):44–7.

194. Kakde RB, Satone DD, Gadapayale KK, Kakde MG. Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Escitalopram Oxalate and Clonazepam. J Chromatogr Sci. 2012 Nov 7;1–6.

195. Patel VB, Dave JB, Patel CN. RP-HPLC Method for Simultaneous Estimation of Escitalopram oxalate and Etizolam in Bulk and Tablet Dosage Form. Am J Pharmtech Res AJHP. 2012;2(3).

196. Kaleemullah T, Ahmed M, Kumar H, Rajput P. Reverse Phase Liquid Chromatographic Method for the Quantification of Di-P-Toluoyl-D-Tartaric Acid in Escitalopram Oxalate Drug Substance. Eurasian J Anal Chem. 2011;6(3):197–205.

197. Machold T, Macedi E, Laird DW, May PM, Hefter GT. Decomposition of Bayer process organics: Low-molecular-weight carboxylates. Hydrometallurgy. 2009 Oct;99(1–2):51–7.

198. Murphy C, Murphy S, O'Brien F, O'Donoghue M, Boileau T, Sunvold G, et al. Metabolic activity of probiotics-oxalate degradation. Vet Microbiol. 2009 Apr 14;136(1–2):100–7.

199. Maalouf NM, Adams Huet B, Pasch A, Lieske JC, Asplin JR, Siener R, et al. Variability in urinary oxalate measurements between six international laboratories. Nephrol Dial Transplant. 2011 Dec;26(12):3954–9.

200. Evelyn A. Havir. Evidence for the presence in tobacco leaves of multiple enzymes for the oxidation of glycolate and glyoxylate. Plant Physiol. 1983;71:874–8.

201. Holloway WD, Argall ME, Jealous WT, Lee J a., Bradbury JH. Organic acids and calcium oxalate in tropical root crops. J Agric Food Chem. 1989 Mar;37(2):337–41.

202. Ross AB, Geoffrey PS, Martin RJ, Leo V. Oxalates in Oca (New Zealand Yam) (Oxalis tuberosa Mol.). J Agric Food Chem. 1999;47(12):5019–22.

203. Gekko K, Ohmae E, Kameyama K, Takagi T. Acetonitrile-protein interactions: Amino acid solubility and preferential solvation. Biochim Biophys Acta - Protein Struct Mol Enzymol. 1998;1387(1–2):195–205.

204. Meng M, Kuntz R, Fontanet A, Bennett PK. A Novel Approach to Quantify Unbound Cisplatin , Carboplatin , and Oxaliplatin in Human Plasma Ultrafiltrate by Measuring Platinum-DDTC Complex Using LC / MS / MS. In: ASMS (American Society for Mass Spectrometry) Comference. 2006.

205. EP: 2.2.46. chromatographic separation techniques. In: European Pharmacopeia 50. 2005. p. 69–73.

206. Costello J, Landwehr DM. Determination of oxalate concentration in blood. Clin Chem. 1988 Aug;34(8):1540–4.

207. Petrarulo M, Cerelli E, Marangella M, Cosseddu D, Vitale C, Linari F. Assay of plasma oxalate with soluble oxalate oxidase. Clin Chem. 1994 Nov;40(11 Pt 1):2030–4.

208. Starr C, Taggart R. The Unity and Diversity of Life. 5th ed. California: Wadsworth; 1989.

209. Ingraham RC, Lombard C, Visscher MB. The characteristics of ultrafiltrates of plasma. J Gen Physiol. 1933;16(4):637–55.

210. Kjellstrand P, Erixon M, Wieslander A, Linden T, Martinson E. Temperature : the Single Most Important Factor for. Perit Dial Int. 2004;24:385–91.

211. Gibaldi M, Perrier D. Pharmacokinetics. second edi. Drugs and the pharmaceutical sciences (A series of Textbooks and Monographs); 1989. Volume 15.

212. Kolthammer J. Pharmacokinetics: A beginner's guide. 1.0. The Institute of Clinical Research; 2007.

213. Pharmacokinetic studies in man. European Medicines' Agency. 1998.

214. Griffin JP. The Textbook of Pharmaceutical Medicine. 6th editio. New Jersey: BMJ Books.; 2009.

215. Gibaldi M, Koup J. Pharmacokinetic concepts - drug binding, apparent volume of distribution and clearance. Eur J Clin Pharmacol. 1981;20(4):299–305.

216. Winter ME. Basic Pharmacokinetics. In: Koda-Kimble MA, Young LY, editors. Basic clinical pharmacokinetics. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 20–3.

217. Davidian M. Introduction to statistical population modeling and analysis for pharmacokinetic data. InInvited white paper for the International Workshop on Uncertainty and Variability in Physiologically Based Pharmacokinetic (PBPK) Models. Retrieved from the Internet: URL: http://www. epa. gov/ncct/uvpkm/files/Calibration—PreMeeting—Draft. pdf (89 pages)[Retrieved on Jan. 29, 2014] 2006.

218. Lin JH, Lu AYH. Role of pharmacokinetics and metabolism in drug discovery and development. Pharmacol Rev. 1997;49(4):403–49.

219. Dhillon S, Gill K. Basic pharmacokinetics. In: Clinical pharmacokinetics. 2006. p. 1–44.

220. Benet LZ. Pharmacokinetic parameters: which are necessary to define a drug substance? Eur J Respir Dis. 1984;134:45–61.

221. Sparreboom A. BSA-based dosing and alternative approaches. Clin Adv Hematol Oncol. 2005;3(6):448–50.

222. Delord J-P, Umlil A, Guimbaud R, Gregoire N, Lafont T, Canal P, et al. Population pharmacokinetics of Oxaliplatin. Cancer Chemother Pharmacol. 2003;51(2):127–31.

223. Shirao K, Matsumura Y, Yamada Y, Muro K, Gotoh M, Boku N, et al. Phase I study of single-dose oxaliplatin in Japanese patients with malignant tumors. Jpn J Clin Oncol. 2006 May;36(5):295–300.

224. Macy ME, Duncan T, Whitlock J, Hunger SP, Boklan J, Narendren A, et al. A Multi-Center Phase Ib Study of Oxaliplatin (NSC # 266046) in Combination With Fluorouracil and Leucovorin in Pediatric Patients With Advanced Solid Tumors. Pediatr Blood Cancer. 2013;60:230–6.

225. Massari C, Brienza S, Rotarski M, Gastiaburu J, Misset J-L, Cupissol D, et al. Pharmacokinetics of oxaliplatin in patients with normal versus impaired renal function. Cancer Chemother Pharmacol. 2000;45(2):157–64.

226. Mathijssen RHJ, de Jong F a, Loos WJ, van der Bol JM, Verweij J, Sparreboom A. Flat-fixed dosing versus body surface area based dosing of anticancer drugs in adults: does it make a difference? Oncologist. 2007 Aug;12(8):913–23.

227. Hempel G, Boos J. Flat-fixed dosing versus body surface area based dosing of anticancer drugs: there is a difference. Oncologist. 2007 Aug;12(8):924–6.

228. Pinkel D. The Use of Body Surface Area as a Criterion of Drug Dosage in Cancer Chemotherapy The Use of Body Surface Area as a Criterion of Drug Dosage in Cancer Chemotherapy *. Cancer Res. 1958;18(3):853–6.

229. Du Bois D, Du Bois E. A formula to estimate the approximate surface area if height and weight be known.1916. Nutrition. 1989;5(5):303–11.

230. Zavery BB, Marsh G. Could logarithmic dosing change the way cytotoxics are prescribed ? Clin Pharm. 2011;3(April):116–8.

231. Hall G, Seligmann J, Marsh G, Zavery B, Twelves C. Logarithmic dose banding (LDB) of chemotherapy for cancer patients. In: 8th NCRI cancer comference. Liverpool; 2012.

232. Hall G. Conference Presentation: Logarithmic dose-banding (LDB) of chemotherapy. In: BOPA 15th Annual symposium. British Oncology Pharmacy Association; 2012.

233. Smorenburg CH. Randomized Cross-Over Evaluation of Body-Surface

Area-Based Dosing Versus Flat-Fixed Dosing of Paclitaxel. J Clin Oncol. 2003 Jan 15;21(2):197–202.

234. Baxter Healthcare Corporation. Colleague volumetric infusion pump Operator's Manual. 2002.

235. Leinwand JC, Bates GE, Allendorf JD, Chabot J a, Lewin SN, Taub RN. Body surface area predicts plasma oxaliplatin and pharmacokinetic advantage in hyperthermic intraoperative intraperitoneal chemotherapy. Ann Surg Oncol. 2013 Apr;20(4):1101–4.

236. EP 5.0: 5.1.4. Microbiological Quality of Pharmaceutical Preparations. In: European Pharmacopoeia 50. 2005. p. 449.

237. Peters GJ, van der Wilt CL, van Moorsel CJ, Kroep JR, Bergman a M, Ackland SP. Basis for effective combination cancer chemotherapy with antimetabolites. Pharmacol Ther. 2000;87(2–3):227–53.

238. Selig BP, Furr JR, Huey RW, Moran C, Alluri VN, Medders GR, et al. Cancer chemotherapeutic agents as human teratogens. Birth Defects Res A Clin Mol Teratol. 2012 Aug;94(8):626–50.

239. Metabolism and action of anti-cancer drugs. First Edition. Powis G, Prough RA, editors. Taylor & Francis Ltd.; 1987.

240. Jongh FE de, Verweij J, Loos WJ, Wit R de, Jonge MJA de, Planting AST, et al. Body-surface area-based dosing does not increase accuracy of predicting cisplatin exposure. J Clin Oncol. 2001;19(17):3733–9.

241. Loos WJ, Jongh FE de, Sparreboom A, Wit R de, Zomeren DM van B, Stoter G, et al. Evaluation of an alternate dosing strategy for cisplatin in patients with extreme body surface area values. J Clin Oncol. 2006;24(10):1499–506.

242. Verbraecken J, Van de Heyning P, De Backer W, Van Gaal L. Body surface area in normal-weight, overweight, and obese adults. A comparison study. Metab Clin Exp. 2006 Apr;55(4):515–24.

243. Junker A, Roy S, Desroches M-C, Moussay C, Berhoune M, Bellanger A, et al. Stability of oxaliplatin solution. Ann Pharmacother. 2009 Feb;43(2):390–1.

244. Berhoune M, Aboudagga H, Jacob A, Grandhaye J, Dayot C, Prognon P,

et al. Effect of requiring advance approval of chemotherapy on number and cost of ungiven doses. Am J Heal Pharm. 2011;68(7):557–8.

245. Rules and Guidance for Pharmaceutical Manufacturers and Distributors. MHRA (Medicines and Healthcare products Regulatory Agency); 2015.

246. Grolleau F, Gamelin L, Boisdron-Celle M, Lapied B, Pelhate M, Gamelin E, et al. A Possible Explanation for a Neurotoxic Effect of the Anticancer Agent Oxaliplatin on Neuronal Voltage-Gated Sodium Channels. J Neurophysiol. 2001;85(5):2293–7.

247. Hoekstra M, C Haaqsma C, Neef C, Proost J, Knuif A, Laar M van de. Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis. J rheumatol. 2004;31(4):645–8.

248. Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Matthay K, Huang SK, et al. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proceeding Natl Acad Sci. 1991;88(24):11460–4.

249. Liu J, Kraut E, Bender J, Brooks R, Balcerzak S, Grever M, et al. Pharmacokinetics of Oxaliplatin (NSC 266046) alone and in combination with paclitaxel in cancer patients. Cancer Chemother Pharmacol. 2002;49(5):367–74.

250. Sun Pharmaceuticals UK. Gemcitabine SUN 10 mg/mL Solution for infusion. Sun Pharmaceuticals UK; 2015.

Appendix: Data from the stability study, oxalate study and PK simulation study

Appendix A: The stability data for oxaliplatin infusions

In the extended stability study of oxaliplatin infusions in 5% glucose, each infusions group was stored and sampled following the sequential temperature cycling schedule mentioned in Chapter 2. Seven groups were included in this study, which were Group A, B, C, D, E, F and G. In each group, 6 oxaliplatin infusion bags were prepared in two concentrations, 3 bags were in 0.2 mg/mL and the other 3 bags were in 0.7 mg/mL. Each groups were sampled after preparation in the first day (T0) and then stored in the refrigerator at 2 - 8℃ for a certain period of time (which were 1, 3, 7, 14, 28, 56 and 84 days respectively for Group A to G). Samples were tested for chemical and physical stability from those infusions in each group after stored at 2 - 8℃ (T1), following which oxaliplatin infusions groups were kept at room temperature (25℃) for another 24 hours (T2) and then sampled again. Afterwards, those infusion groups were stored back in the refrigerator at 2 - 8℃ for another 7 days (T3) and samples were tested in the end of this storage period for chemical and physical stability of oxaliplatin infusions.

Results for all 6 oxaliplatin infusion bags in each group were shown below, including oxaliplatin concentrations in samples, pH measurements, weight monitor at each sample time, sub-visual particle counts (10 μm and 25 μm) and visual inspection.

Table A-1, Chemical and physical stability results in 6 oxaliplatin infusion bags from Group A at different sample time.

Sample time point	Infusion bag identifier in Group A	Nominal Conc. (mg/mL)	Oxaliplatin Conc. in samples (mg/mL)	pH	Bag Weight (g)		Particle Count		Visual Inspection
					Before Sampling	After Sampling	10 µm	25 µm	
D0T0	0.2A1	0.2	0.1855	4.44	273.8	264.6	6.0	0	clear, clean and colourless
	0.2A2	0.2	0.1848	4.35	275.0	266.0	2.5	0	clear, clean and colourless
	0.2A3	0.2	0.1817	4.36	274.8	265.6	2.5	0	clear, clean and colourless
	0.7A1	0.7	0.6499	4.41	274.2	265.0	6.0	0	clear, clean and colourless
	0.7A2	0.7	0.6499	4.41	273.8	264.7	15.0	0	clear, clean and colourless
	0.7A3	0.7	0.6471	4.41	273.9	264.6	7.0	0	clear, clean and colourless
D1T1 (stored at 2-8℃)	0.2A1	0.2	0.1813	4.46	264.6	255.3	1.5	0	clear, clean and colourless
	0.2A2	0.2	0.1795	4.40	266.0	256.8	2.5	0	clear, clean and colourless
	0.2A3	0.2	0.1832	4.41	265.6	256.5	2.5	0	clear, clean and colourless
	0.7A1	0.7	0.6410	4.47	265.0	255.9	3.0	0	clear, clean and colourless
	0.7A2	0.7	0.6435	4.47	264.7	255.6	3.5	0	clear, clean and colourless
	0.7A3	0.7	0.6412	4.48	264.7	255.5	2.5	0	clear, clean and colourless
D2T2 (stored at 25℃)	0.2A1	0.2	0.1804	4.44	255.3	246.1	4.5	0	clear, clean and colourless
	0.2A2	0.2	0.1814	4.37	256.8	247.6	7.5	0	clear, clean and colourless
	0.2A3	0.2	0.1812	4.37	256.5	247.4	3.5	0	clear, clean and colourless
	0.7A1	0.7	0.6396	4.43	255.9	246.6	0.5	0	clear, clean and colourless
	0.7A2	0.7	0.6347	4.44	255.5	246.4	3.0	0	clear, clean and colourless
	0.7A3	0.7	0.6331	4.43	255.5	246.3	0.0	0	clear, clean and colourless
D9T3 (stored at 2-8℃)	0.2A1	0.2	0.1870	4.44	246.0	236.8	2.5	0	clear, clean and colourless
	0.2A2	0.2	0.1866	4.39	247.6	238.3	2.5	0	clear, clean and colourless
	0.2A3	0.2	0.1883	4.41	247.3	238.2	0.5	0	clear, clean and colourless
	0.7A1	0.7	0.6625	4.46	246.6	237.4	1.0	0	clear, clean and colourless
	0.7A2	0.7	0.6621	4.47	246.4	237.1	1.5	0	clear, clean and colourless
	0.7A3	0.7	0.6620	4.48	246.3	237.0	1.0	0	clear, clean and colourless

Conc. = concentration; D0T0 = time zero, right after the infusion preparation; D1T1 = after stored at 2 - 8℃ for 1 day; D2T2 = after stored at 25℃ for 24 hours; D9T3 = after stored at 2 - 8℃ for 7 days following the 24-hour storage at 25℃.

Table A-2, Chemical and physical stability results in 6 oxaliplatin infusion bags from Group B at different sample time.

Sample time point	Infusion bag identifier in Group B	Nominal Conc. (mg/mL)	Oxaliplatin Conc. in samples (mg/mL)	pH	Bag Weight (g)		Particle Count		Visual Inspection
					Before Sampling	After Sampling	10 µm	25 µm	
D0T0	0.2B1	0.2	0.1893	4.28	275.1	265.9	5.5	0	clear, clean and colourless
	0.2B2	0.2	0.1868	4.28	275.0	265.7	4.0	0	clear, clean and colourless
	0.2B3	0.2	0.1902	4.28	275.2	266.0	4.0	0	clear, clean and colourless
	0.7B1	0.7	0.6726	4.36	273.9	264.8	4.0	0	clear, clean and colourless
	0.7B2	0.7	0.6664	4.37	274.9	265.8	6.5	0	clear, clean and colourless
	0.7B3	0.7	0.6609	4.36	274.8	265.6	6.5	0	clear, clean and colourless
D3T1 (stored at 2-8℃)	0.2B1	0.2	0.1911	4.37	266.0	256.8	6.5	0.5	clear, clean and colourless
	0.2B2	0.2	0.1862	4.37	265.7	256.5	2.0	0	clear, clean and colourless
	0.2B3	0.2	0.1894	4.35	266.0	256.8	1.0	0	clear, clean and colourless
	0.7B1	0.7	0.6720	4.45	264.9	255.6	1.0	0	clear, clean and colourless
	0.7B2	0.7	0.6631	4.45	265.9	256.6	0.0	0	clear, clean and colourless
	0.7B3	0.7	0.6651	4.45	265.7	256.4	2.0	0	clear, clean and colourless
D4T2 (stored at 25℃)	0.2B1	0.2	0.1878	4.40	256.7	247.5	1.5	0	clear, clean and colourless
	0.2B2	0.2	0.1859	4.38	256.4	247.2	0.5	0	clear, clean and colourless
	0.2B3	0.2	0.1879	4.38	256.7	247.4	4.5	0	clear, clean and colourless
	0.7B1	0.7	0.6680	4.47	255.5	246.3	2.0	0	clear, clean and colourless
	0.7B2	0.7	0.6650	4.46	256.5	247.3	1.0	0	clear, clean and colourless
	0.7B3	0.7	0.6590	4.46	256.4	247.1	0.5	0	clear, clean and colourless
D11T3 (stored at 2-8℃)	0.2B1	0.2	0.1886	4.39	247.5	238.3	1.5	0	clear, clean and colourless
	0.2B2	0.2	0.1895	4.38	247.3	238.1	3.0	0	clear, clean and colourless
	0.2B3	0.2	0.1861	4.38	247.5	238.2	2.5	0	clear, clean and colourless
	0.7B1	0.7	0.6752	4.46	246.3	237.1	1.0	0	clear, clean and colourless
	0.7B2	0.7	0.6617	4.46	247.3	238.1	1.0	0	clear, clean and colourless
	0.7B3	0.7	0.6646	4.46	247.1	237.9	1.5	0	clear, clean and colourless

Conc. = concentration; D0T0 = time zero, right after the infusion preparation; D3T1 = after stored at 2 - 8℃ for 3 day; D4T2 = after stored at 25℃ for 24 hours; D11T3 = after stored at 2 - 8℃ for 7 days following the 24-hour storage at 25℃.

Table A-3, Chemical and physical stability results in 6 oxaliplatin infusion bags from Group C at different sample time.

Sample time point	Infusion bag identifier in Group C	Nominal Conc. (mg/mL)	Oxaliplatin Conc. in samples (mg/mL)	pH	Bag Weight (g)		Particle Count		Visual Inspection
					Before Sampling	After Sampling	10 µm	25 µm	
D0T0	0.2C1	0.2	0.1915	4.28	274.9	265.6	1.5	0	clear, clean and colourless
	0.2C2	0.2	0.1893	4.28	274.6	265.3	2.0	0	clear, clean and colourless
	0.2C3	0.2	0.1899	4.25	275.1	265.9	3.0	0	clear, clean and colourless
	0.7C1	0.7	0.6609	4.33	273.7	264.5	5.5	0	clear, clean and colourless
	0.7C2	0.7	0.6647	4.34	273.9	264.8	9.0	0	clear, clean and colourless
	0.7C3	0.7	0.6669	4.32	274.4	265.2	4.0	0	clear, clean and colourless
D7T1 (stored at 2-8℃)	0.2C1	0.2	0.1871	4.41	265.7	256.4	4.0	0	clear, clean and colourless
	0.2C2	0.2	0.1874	4.42	265.4	256.1	2.5	0	clear, clean and colourless
	0.2C3	0.2	0.1881	4.40	265.9	256.6	0.5	0	clear, clean and colourless
	0.7C1	0.7	0.6540	4.47	264.5	255.2	2.0	0	clear, clean and colourless
	0.7C2	0.7	0.6561	4.48	264.9	255.6	2.5	0.5	clear, clean and colourless
	0.7C3	0.7	0.6551	4.45	265.3	256.0	3.0	0	clear, clean and colourless
D8T2 (stored at 25℃)	0.2C1	0.2	0.1885	4.43	256.4	247.0	3.0	0	clear, clean and colourless
	0.2C2	0.2	0.1856	4.43	256.1	246.8	1.0	0	clear, clean and colourless
	0.2C3	0.2	0.1857	4.42	256.6	247.5	1.0	0	clear, clean and colourless
	0.7C1	0.7	0.6595	4.48	255.2	245.9	2.0	0	clear, clean and colourless
	0.7C2	0.7	0.6543	4.49	255.6	246.3	2.5	0	clear, clean and colourless
	0.7C3	0.7	0.6546	4.48	256.0	246.7	0.5	0	clear, clean and colourless
D15T3 (stored at 2-8℃)	0.2C1	0.2	0.1887	4.45	247.0	237.9	2.0	0	clear, clean and colourless
	0.2C2	0.2	0.1903	4.42	246.9	237.6	2.5	0	clear, clean and colourless
	0.2C3	0.2	0.1887	4.39	247.6	238.3	3.5	0	clear, clean and colourless
	0.7C1	0.7	0.6650	4.47	246.0	236.8	5.5	0	clear, clean and colourless
	0.7C2	0.7	0.6624	4.47	246.3	237.1	1.5	0	clear, clean and colourless
	0.7C3	0.7	0.6682	4.44	246.8	237.6	2.5	0	clear, clean and colourless

Conc. = concentration; D0T0 = time zero, right after the infusion preparation; D7T1 = after stored at 2 - 8℃ for 7 day; D8T2 = after stored at 25℃ for 24 hours; D15T3 = after stored at 2 - 8℃ for 7 days following the 24-hour storage at 25℃.

Table A-4, Chemical and physical stability results in 6 oxaliplatin infusion bags from Group D at different sample time.

Sample time point	Infusion bag identifier in Group D	Nominal Conc. (mg/mL)	Oxaliplatin Conc. in samples (mg/mL)	pH	Bag Weight (g)		Particle Count		Visual Inspection
					Before Sampling	After Sampling	10 µm	25 µm	
D0T0	0.2D1	0.2	0.1894	4.38	275.1	265.9	2.5	0	clear, clean and colourless
	0.2D2	0.2	0.1880	4.37	274.9	265.6	2.0	0	clear, clean and colourless
	0.2D3	0.2	0.1902	4.38	274.0	264.7	3.0	0.5	clear, clean and colourless
	0.7D1	0.7	0.6637	4.43	275.0	265.8	17.5	0	clear, clean and colourless
	0.7D2	0.7	0.6665	4.45	274.2	265.0	5.5	0	clear, clean and colourless
	0.7D3	0.7	0.6624	4.43	275.1	265.8	23.5	0	clear, clean and colourless
D14T1 (stored at 2-8℃)	0.2D1	0.2	0.1894	4.35	265.9	256.6	3.5	0	clear, clean and colourless
	0.2D2	0.2	0.1905	4.36	265.6	256.5	1.0	0	clear, clean and colourless
	0.2D3	0.2	0.1908	4.38	264.7	255.5	2.0	0	clear, clean and colourless
	0.7D1	0.7	0.6625	4.43	265.8	256.5	0.5	0	clear, clean and colourless
	0.7D2	0.7	0.6657	4.44	264.9	255.8	1.0	0	clear, clean and colourless
	0.7D3	0.7	0.6651	4.43	265.8	256.6	1.5	0	clear, clean and colourless
D15T2 (stored at 25℃)	0.2D1	0.2	0.1880	4.37	256.6	247.4	4.5	0.5	clear, clean and colourless
	0.2D2	0.2	0.1874	4.36	256.5	247.3	1.5	0	clear, clean and colourless
	0.2D3	0.2	0.1882	4.38	255.5	246.3	1.5	0	clear, clean and colourless
	0.7D1	0.7	0.6562	4.43	256.5	247.3	1.0	0	clear, clean and colourless
	0.7D2	0.7	0.6688	4.44	255.8	246.5	2.5	0	clear, clean and colourless
	0.7D3	0.7	0.6606	4.43	256.6	247.4	4.5	0	clear, clean and colourless
D22T3 (stored at 2-8℃)	0.2D1	0.2	0.1837	4.38	247.3	238.1	3.5	0	clear, clean and colourless
	0.2D2	0.2	0.1855	4.38	247.2	238.0	3.0	0	clear, clean and colourless
	0.2D3	0.2	0.1848	4.40	246.2	237.0	5.0	0	clear, clean and colourless
	0.7D1	0.7	0.6527	4.45	247.3	238.1	3.0	0	clear, clean and colourless
	0.7D2	0.7	0.6565	4.48	246.5	237.2	4.5	0	clear, clean and colourless
	0.7D3	0.7	0.6500	4.46	247.3	238.1	2.5	0	clear, clean and colourless

Conc. = concentration; D0T0 = time zero, right after the infusion preparation; D14T1 = after stored at 2 - 8℃ for 14 day; D15T2 = after stored at 25℃ for 24 hours; D22T3 = after stored at 2 - 8℃ for 7 days following the 24-hour storage at 25℃.

Table A-5, Chemical and physical stability results in 6 oxaliplatin infusion bags from Group E at different sample time.

Sample time point	Infusion bag identifier in Group E	Nominal Conc. (mg/mL)	Oxaliplatin Conc. in samples (mg/mL)	pH	Bag Weight (g)		Particle Count		Visual Inspection
					Before Sampling	After Sampling	10 µm	25 µm	
D0T0	0.2 E1	0.2	0.1926	4.39	274.4	265.2	2.0	0	clear, clean and colourless
	0.2 E2	0.2	0.1910	4.42	274.4	265.2	7.5	0	clear, clean and colourless
	0.2 E3	0.2	0.1907	4.38	274.5	265.4	5.0	0	clear, clean and colourless
	0.7 E1	0.7	0.6744	4.43	274.6	265.3	5.0	0	clear, clean and colourless
	0.7 E2	0.7	0.6731	4.48	275.3	266.2	5.5	0	clear, clean and colourless
	0.7 E3	0.7	0.6761	4.45	275.1	265.9	5.0	0	clear, clean and colourless
D28T1 (stored at 2-8℃)	0.2 E1	0.2	0.1990	4.43	265.1	255.8	5.0	0	clear, clean and colourless
	0.2 E2	0.2	0.1951	4.45	265.1	255.8	4.0	0	clear, clean and colourless
	0.2 E3	0.2	0.1924	4.42	265.3	256.1	3.5	0.5	clear, clean and colourless
	0.7 E1	0.7	0.6845	4.46	265.2	256.0	4.0	0.5	clear, clean and colourless
	0.7 E2	0.7	0.6848	4.51	266.1	256.9	7.5	0.5	clear, clean and colourless
	0.7 E3	0.7	0.6839	4.48	265.8	256.7	7.0	0.5	clear, clean and colourless
D29T2 (stored at 25℃)	0.2 E1	0.2	0.1890	4.46	255.8	246.6	2.0	0	clear, clean and colourless
	0.2 E2	0.2	0.1869	4.45	255.7	246.6	9.0	0.5	clear, clean and colourless
	0.2 E3	0.2	0.1863	4.43	256.0	246.9	4.5	0	clear, clean and colourless
	0.7 E1	0.7	0.6565	4.48	255.9	246.7	5.0	0	clear, clean and colourless
	0.7 E2	0.7	0.6570	4.53	256.8	247.7	4.0	1.0	clear, clean and colourless
	0.7 E3	0.7	0.6564	4.49	256.6	247.5	6.5	0	clear, clean and colourless
D36T3 (stored at 2-8℃)	0.2 E1	0.2	0.1918	4.43	246.7	237.5	5.0	0	clear, clean and colourless
	0.2 E2	0.2	0.1910	4.44	246.7	237.5	9.5	0	clear, clean and colourless
	0.2 E3	0.2	0.1927	4.39	247.0	237.9	5.5	0	clear, clean and colourless
	0.7 E1	0.7	0.6816	4.45	246.8	237.7	18.5	0	clear, clean and colourless
	0.7 E2	0.7	0.6842	4.50	247.8	238.6	7.0	0.5	clear, clean and colourless
	0.7 E3	0.7	0.6856	4.47	247.6	238.5	4.5	0	clear, clean and colourless

Conc. = concentration; D0T0 = time zero, right after the infusion preparation; D28T1 = after stored at 2 - 8℃ for 28 day; D29T2 = after stored at 25℃ for 24 hours; D36T3 = after stored at 2 - 8℃ for 7 days following the 24-hour storage at 25℃.

Table A-6, Chemical and physical stability results in 6 oxaliplatin infusion bags from Group F at different sample time.

Sample time point	Infusion bag identifier in Group F	Nominal Conc. (mg/mL)	Oxaliplatin Conc. in samples (mg/mL)	pH	Bag Weight (g)		Particle Count		Visual Inspection
					Before Sampling	After Sampling	10 µm	25 µm	
D0T0	0.2 F1	0.2	0.1922	4.45	274.7	265.5	4.5	0	clear, clean and colourless
	0.2 F2	0.2	0.1934	4.39	274.5	265.3	2.0	0	clear, clean and colourless
	0.2 F3	0.2	0.1911	4.36	275.1	265.9	3.0	0	clear, clean and colourless
	0.7 F1	0.7	0.6634	4.45	274.5	265.4	3.0	0	clear, clean and colourless
	0.7 F2	0.7	0.6637	4.44	273.9	264.7	3.0	0.5	clear, clean and colourless
	0.7 F3	0.7	0.6639	4.43	275.0	265.9	3.0	0	clear, clean and colourless
D56T1 (stored at 2-8℃)	0.2 F1	0.2	0.1858	4.49	265.3	256.2	7.5	0	clear, clean and colourless
	0.2 F2	0.2	0.1860	4.33	265.1	256.0	5.5	0.5	clear, clean and colourless
	0.2 F3	0.2	0.1852	4.35	265.8	256.6	6.5	0	clear, clean and colourless
	0.7 F1	0.7	0.6467	4.40	265.3	256.0	2.5	0	clear, clean and colourless
	0.7 F2	0.7	0.6395	4.41	264.6	255.4	4.0	0	clear, clean and colourless
	0.7 F3	0.7	0.6496	4.44	265.7	256.6	3.0	0	clear, clean and colourless
D57T2 (stored at 25℃)	0.2 F1	0.2	0.1905	4.51	256.1	247.0	4.0	0	clear, clean and colourless
	0.2 F2	0.2	0.1913	4.39	256.0	246.8	6.0	0	clear, clean and colourless
	0.2 F3	0.2	0.1890	4.37	256.5	247.3	4.5	0	clear, clean and colourless
	0.7 F1	0.7	0.6617	4.45	256.0	246.9	3.0	0.5	clear, clean and colourless
	0.7 F2	0.7	0.6593	4.45	255.4	246.1	5.0	0	clear, clean and colourless
	0.7 F3	0.7	0.6562	4.46	256.6	247.4	4.0	0	clear, clean and colourless
D64T3 (stored at 2-8℃)	0.2 F1	0.2	0.1873	4.45	246.9	237.8	11.0	0.5	clear, clean and colourless
	0.2 F2	0.2	0.1903	4.37	246.7	237.5	3.0	0	clear, clean and colourless
	0.2 F3	0.2	0.1898	4.35	247.2	238.0	2.0	0	clear, clean and colourless
	0.7 F1	0.7	0.6578	4.43	246.9	237.7	3.5	0	clear, clean and colourless
	0.7 F2	0.7	0.6629	4.44	246.1	236.9	2.5	0	clear, clean and colourless
	0.7 F3	0.7	0.6729	4.43	247.3	238.1	4.5	0	clear, clean and colourless

Conc. = concentration; D0T0 = time zero, right after the infusion preparation; D56T1 = after stored at 2 - 8℃ for 56 day; D57T2 = after stored at 25℃ for 24 hours; D64T3 = after stored at 2 - 8℃ for 7 days following the 24-hour storage at 25℃.

Table A-7, Chemical and physical stability results in 6 oxaliplatin infusion bags from Group G at different sample time.

Sample time point	Infusion bag identifier in Group G	Nominal Conc. (mg/mL)	Oxaliplatin Conc. in samples (mg/mL)	pH	Bag Weight (g)		Particle Count		Visual Inspection
					Before Sampling	After Sampling	10 µm	25 µm	
D0T0	0.2 G1	0.2	0.1862	4.45	275.1	265.9	5.0	0	clear, clean and colourless
	0.2 G2	0.2	0.1873	4.38	275.0	265.9	1.0	0	clear, clean and colourless
	0.2 G3	0.2	0.1868	4.36	274.9	265.7	4.5	0	clear, clean and colourless
	0.7 G1	0.7	0.6579	4.47	273.6	264.3	4.5	0	clear, clean and colourless
	0.7 G2	0.7	0.6561	4.42	274.8	265.7	5.0	0	clear, clean and colourless
	0.7 G3	0.7	0.6559	4.44	274.8	265.5	13.0	0	clear, clean and colourless
D84T1 (stored at 2-8℃)	0.2 G1	0.2	0.1916	4.45	265.7	256.5	2.5	0	clear, clean and colourless
	0.2 G2	0.2	0.1944	4.36	265.7	256.5	8.5	0	clear, clean and colourless
	0.2 G3	0.2	0.1906	4.32	265.5	256.3	0.5	0	clear, clean and colourless
	0.7 G1	0.7	0.6760	4.41	264.1	254.9	0.5	0	clear, clean and colourless
	0.7 G2	0.7	0.6606	4.37	265.5	256.3	3.0	0	clear, clean and colourless
	0.7 G3	0.7	0.6619	4.39	265.4	256.2	2.0	0	clear, clean and colourless
D85T2 (stored at 25℃)	0.2 G1	0.2	0.1832	4.44	256.5	247.3	3.5	0	clear, clean and colourless
	0.2 G2	0.2	0.1922	4.39	256.5	247.4	7.0	0	clear, clean and colourless
	0.2 G3	0.2	0.1834	4.36	256.3	247.1	2.5	0	clear, clean and colourless
	0.7 G1	0.7	0.6666	4.46	254.9	245.8	10.5	0	clear, clean and colourless
	0.7 G2	0.7	0.6440	4.41	256.3	247.3	1.5	0	clear, clean and colourless
	0.7 G3	0.7	0.6525	4.43	256.1	247.0	2.5	0	clear, clean and colourless
D92T3 (stored at 2-8℃)	0.2 G1	0.2	0.1869	4.47	247.3	238.0	13.5	1.0	clear, clean and colourless
	0.2 G2	0.2	0.1850	4.38	247.3	238.1	6.5	0.5	clear, clean and colourless
	0.2 G3	0.2	0.1880	4.35	247.1	237.9	4.0	0	clear, clean and colourless
	0.7 G1	0.7	0.6531	4.44	245.7	236.6	4.0	0	clear, clean and colourless
	0.7 G2	0.7	0.6481	4.42	247.2	238.0	6.5	0	clear, clean and colourless
	0.7 G3	0.7	0.6419	4.42	246.9	237.8	4.5	0	clear, clean and colourless

Conc. = concentration; D0T0 = time zero, right after the infusion preparation; D84T1 = after stored at 2 - 8℃ for 84 day; D85T2 = after stored at 25℃ for 24 hours; D92T3 = after stored at 2 - 8℃ for 7 days following the 24-hour storage at 25℃.

Appendix B: Data from the oxalate study in plasma

The oxalate study described in Chapter 3 included the oxalate determination in human plasma after administration with oxaliplatin. Four groups of oxaliplatin solutions were prepared and detected for oxaliplatin and oxalate concentrations:

Group A (Control group) was prepared in 5% glucose, and results from this group could be used as the benchmark comparison reference for other groups;

Group B was prepared in human plasma and the total amount of oxaliplatin/oxalate (including fractions bound to plasma protein and un-bound fractions) in plasma was determined in this group.

Group C was also prepared in human plasma, but only concentrations of the un-bound (free) oxaliplatin/oxalate were detected in this group.

Group D was prepared in plasma ultra-filtrate (PUF) in order to test the concentration change of oxaliplatin/oxalate without interference from plasma proteins.

In order to simulate the degradation process of oxaliplatin in blood circulation of human bodies, all four groups were stored at 37℃ for 5 days to simulate the physiological temperature.

Results of oxaliplatin and oxalate concentrations for triplicate tests in Group A – D are shown in Table B-1 and Table B-2 below, together with the concentration variations relative to the result at time zero (first experiment day).

pH values for triplicate tests of each group were measured at each sampling day, which results and changes throughout the experiment are shown in Table B-3 below.

Table B-1, Oxaliplatin concentrations in triplicate sample solutions from Group A – D at each sample time, together with the concentration variations of oxaliplatin with respect to the initial values at T_0.

Group Identifier		Day0 (T0)	Day 1		Day 2		Day 3		Day 4		Day 5	
		Conc. (µg/mL)	Conc. (µg/mL)	Conc. Relative to T_0 (%)	Conc. (µg/mL)	Conc. Relative to T_0 (%)	Conc. (µg/mL)	Conc. Relative to T_0 (%)	Conc. (µg/mL)	Conc. Relative to T_0 (%)	Conc. (µg/mL)	Conc. Relative to T_0 (%)
A (Control)	A1	159.0030	142.2250	89.45	110.0380	69.20	106.9930	67.29	86.2220	54.23	74.5530	46.89
	A2	157.3870	133.2880	84.69	114.9010	73.01	105.7320	67.18	81.6440	51.87	76.3430	48.51
	A3	157.0970	146.3810	93.18	117.2760	74.65	107.0660	68.15	87.5690	55.74	74.3830	47.35
B	B1	68.2960	8.8280	12.93	10.5500	15.45	8.1600	11.95	8.1210	11.89	11.0840	16.23
	B2	65.6720	20.0050	30.46	22.1240	33.69	20.7410	31.58	18.4490	28.09	21.8180	33.22
	B3	66.5603	18.5930	27.93	22.3380	33.56	20.1750	30.31	16.6030	24.94	21.6120	32.47
C	C1	60.4530	4.2650	7.06	4.7920	7.93	6.4930	10.74	5.7510	9.51	6.4500	10.67
	C2	63.3940	4.4550	7.03	4.1660	5.92	6.4930	10.24	6.6490	10.49	5.7720	9.10
	C3	61.7920	4.3670	7.07	4.5500	7.36	5.1020	8.26	6.3740	10.32	6.5220	10.55
D	D1	111.5850	31.8480	28.54	9.1570	8.21	5.1020	4.57	6.0380	5.41	6.3890	5.73
	D2	119.0970	36.0450	30.27	9.6260	8.08	5.0150	4.21	5.4760	4.60	6.1230	5.14
	D3	118.2130	34.2120	28.94	9.8820	8.36	7.6960	6.51	5.9380	5.02	5.9290	5.02

Conc. : Concentration; Conc. Relative to T_0: the percentage of tested oxaliplatin concentration at each sample time compared with the initial values at time zero.

Table B-2, Oxalate concentrations in triplicate sample solutions from Group A – D, together with the variations of oxalate concentrations with respect to the initial values at T_0.

Group Identifier		Day0 (T0)	Day 1		Day 2		Day 3		Day 4		Day 5	
		Conc. (µg/mL)	Conc. (µg/mL)	Increased Conc. Relative to T_0 (µg/mL)	Conc. (µg/mL)	Increased Conc. Relative to T_0 (µg/mL)	Conc. (µg/mL)	Increased Conc. Relative to T_0 (µg/mL)	Conc. (µg/mL)	Increased Conc. Relative to T_0 (µg/mL)	Conc. (µg/mL)	Increased Conc. Relative to T_0 (µg/mL)
A (Control)	A1	0.0000	4.2339	4.2339	12.8223	12.8223	12.1131	12.1131	15.7895	15.7895	25.1705	25.1705
	A2	-1.8417	4.8387	6.6804	12.8223	14.6640	15.1413	16.9830	15.1822	17.0239	24.5566	26.3983
	A3	-3.6835	10.8871	14.5706	12.2117	15.8952	13.3244	17.0079	17.6113	21.2948	23.9427	27.6262
B	B1	9.8226	43.5484	33.7258	42.7408	32.9182	37.5505	27.7279	32.1862	22.3636	46.6576	36.8350
	B2	9.2087	40.5242	31.3155	42.7408	33.5321	36.3392	27.1305	38.8664	29.6577	46.6576	37.4489
	B3	10.4366	42.3387	31.9021	37.8562	27.4196	38.7618	28.3252	46.7611	36.3245	47.2715	36.8349
C	C1	8.5948	43.5484	34.9536	43.9620	35.3672	49.6635	41.0687	49.1903	40.5955	52.1828	43.5880
	C2	8.5948	41.1290	32.5342	42.7408	34.1460	49.6635	41.0687	49.1903	40.5955	52.1828	43.5880
	C3	9.8226	40.5242	30.7016	42.1303	32.3077	38.1561	28.3335	50.4049	40.5823	51.5689	41.7463
D	D1	3.6835	33.2661	29.5826	40.9091	37.2256	50.8748	47.1913	42.5101	38.8266	44.8158	41.1323
	D2	5.5252	39.3145	33.7893	40.2985	34.7733	50.8748	45.3496	43.7247	38.1995	46.0437	40.5185
	D3	4.2974	36.8952	32.5978	39.0773	34.7799	50.2692	45.9718	44.9393	40.6419	46.6575	42.3601

Conc. : Concentration.

Table B-3, pH measurements at each sample time for Group A – D with the variations compared to the initial value at T_0.

Group Identifier		Day0 (T0)	Day 1		Day 2		Day 3		Day 4		Day 5	
		pH	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)	pH	pH difference to T_0 (%)
A (Control)	A1	5.30	4.95	-0.35	4.84	-0.46	4.73	-0.57	4.60	-0.70	4.59	-0.71
	A2	5.21	4.93	-0.28	4.84	-0.37	4.77	-0.44	4.65	-0.56	4.61	-0.60
	A3	5.25	4.88	-0.37	4.83	-0.42	4.73	-0.52	4.64	-0.61	4.61	-0.64
B	B1	7.10	6.91	-0.19	7.20	0.10	7.05	-0.05	7.18	0.08	7.14	0.04
	B2	7.12	6.94	-0.18	7.22	0.10	7.15	0.03	7.20	0.08	7.14	0.02
	B3	7.12	6.98	-0.14	7.26	0.14	7.09	-0.03	7.23	0.11	7.26	0.14
C	C1	7.08	7.07	-0.01	7.22	0.14	7.24	0.16	7.33	0.25	7.06	-0.02
	C2	7.04	7.07	0.03	7.19	0.15	7.23	0.19	7.31	0.27	7.08	0.04
	C3	7.05	6.93	-0.12	7.17	0.12	7.21	0.16	7.34	0.29	7.04	-0.01
D	D1	7.04	7.11	0.07	7.12	0.08	7.31	0.27	7.21	0.17	7.34	0.30
	D2	7.16	7.13	-0.03	7.12	-0.04	7.17	0.01	7.28	0.12	7.36	0.20
	D3	7.08	7.08	0.00	7.18	0.10	7.35	0.27	7.25	0.17	7.33	0.25

pH difference to T_0: the percentage of pH changes at each sample time compared with the initial values at time zero.

Appendix C: Pharmacokinetics (PK) simulation data

In the *ex vivo* pharmacokinetic (PK) simulation study, 10 patients with different BSA (body surface area) values were selected and simulated with oxaliplatin administered with different dosing methods. 5 dosing methods were applied, including individual dosing scheme, dose-banding scheme with 5% deviation, dose-banding scheme with 10% deviation, logarithmic dose-banding scheme and flat-fixed dosing method. During the simulation, 22 samples were withdrawn from the 'body reservoir' following the study schedule and weight changes of the 'body reservoir' bag were monitored by recording the balance readings before and after sampling. Meanwhile, the concentration of oxaliplatin in each sample was detected by a validated HPLC assay. pH values for those 22 samples were also measured at each sample time point.

Drug doses applied for each dosing scheme were different:

Individual dosing method: the drug dose was calculated by the patient's BSA and 85 mg/m^2 oxaliplatin.

Dose-banding schemes (including all three schemes): the drug dose was used as the standard dose from the certain dose band that fitted the patient's individual dose, which should following the scheme schedules described in Chapter 4.

Flat-fixed dosing: the drug dose was selected to be fixed 4 mg for all patients.

Results for the total 50 simulation tests (10 'patients' × 5 dosing methods) are presented in tables below along with the illustration of oxaliplatin concentration – time curve for each patient in each dosing scheme.

C.1 Patient 1

The basic setting information for Patient 1 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 2.70 m^2; V_c = 837.7 mL; CL_{total} = 194.4 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

Table C-1, Results of the simulation test on Patient 1 with oxaliplatin administered in Individual dosing scheme with the drug dose of 5.74 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin Concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.34	837.3	827.4
S1T0.25	0.25	0.6113	4.27	829.1	817.7
S2T0.5	0.50	1.2824	4.21	821.6	810.4
S3T0.75	0.75	1.9043	4.22	811.5	801.9
S4T1.0	1.00	2.4641	4.20	804.1	793.8
S5T1.25	1.25	2.9902	4.19	796.9	786.1
S6T1.5	1.50	3.5416	4.18	789.9	779.9
S7T1.75	1.75	4.0069	4.17	781.7	771.5
S8T2.0	2.00	4.5521	4.17	774.2	762.1
S9T2.25	2.25	4.1682	4.16	762.7	755.5
S10T2.5	2.50	3.9333	4.17	756.8	746.4
S11T3.0	3.00	3.4238	4.18	742.9	731.1
S12T3.5	3.50	2.9774	4.16	742.3	730.9
S13T4.0	4.00	2.5811	4.14	738.7	726.4
S14T4.5	4.50	2.2228	4.14	728.8	717.8
S15T5.0	5.00	1.9366	4.14	725.9	714.5
S16T5.5	5.50	1.6799	4.15	722.5	710.7
S17T6.0	6.00	1.4349	4.13	718.2	706.2
S18T6.5	6.50	1.2478	4.11	709.2	702.1
S19T7.0	7.00	1.0719	4.11	707.6	698.3
S20T7.5	7.50	0.9411	4.12	700.7	691.2
S21T8.0	8.00	0.7724	4.13	696.8	688.9

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

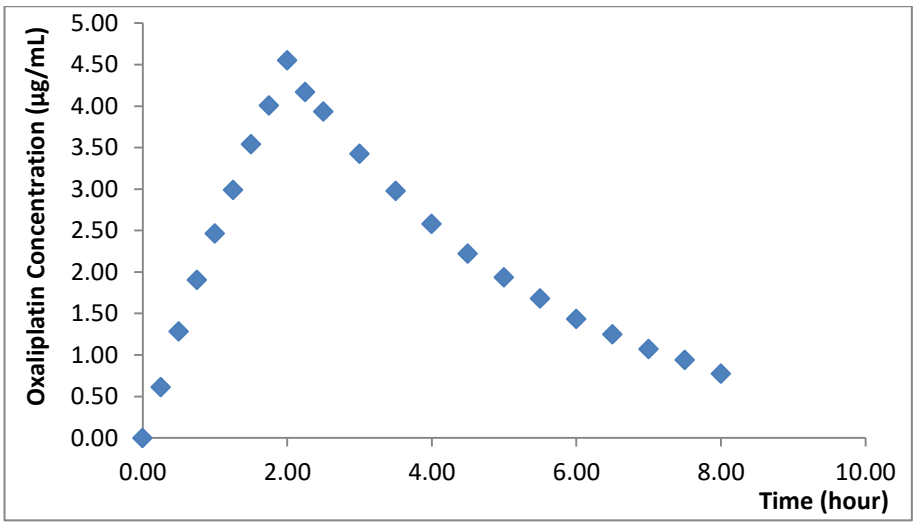

Figure C-1, Oxaliplatin concentration-time curve for Patient 1 with 2-hour infusions administered in Individual dosing scheme.

Table C-2, Results of the simulation test on Patient 1 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the standard dose of 5.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.22	838.1	827.6
S1T0.25	0.25	0.6986	4.19	833.8	820.9
S2T0.5	0.50	1.2066	4.18	825.1	814.1
S3T0.75	0.75	1.7703	4.18	818.2	805.9
S4T1.0	1.00	2.2789	4.17	809.3	799.7
S5T1.25	1.25	2.8054	4.17	804.1	795.3
S6T1.5	1.50	3.3075	4.16	792.9	781.3
S7T1.75	1.75	3.6074	4.16	785.7	776.3
S8T2.0	2.00	3.8042	4.16	777.6	765.4
S9T2.25	2.25	3.5746	4.15	768.3	759.4
S10T2.5	2.50	3.3186	4.15	767.1	787.1
S11T3.0	3.00	2.8990	4.15	759.1	751.5
S12T3.5	3.50	2.5206	4.15	755.1	748.4
S13T4.0	4.00	2.1787	4.14	750.1	740.9
S14T4.5	4.50	1.9066	4.15	739.4	728.3
S15T5.0	5.00	1.6197	4.14	738.0	728.9
S16T5.5	5.50	1.4347	4.16	731.7	721.9
S17T6.0	6.00	1.2522	4.14	728.9	718.0
S18T6.5	6.50	1.1006	4.13	722.1	712.4
S19T7.0	7.00	0.9507	4.13	709.1	697.3
S20T7.5	7.50	0.8260	4.12	705.4	696.5
S21T8.0	8.00	0.6994	4.12	698.2	688.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

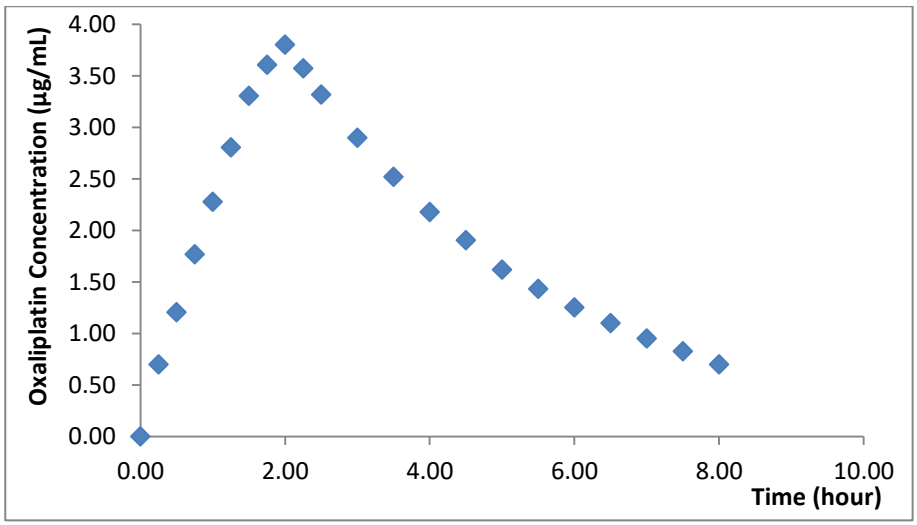

Figure C-2, Oxaliplatin concentration-time curve for Patient 1 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-3, Results of the simulation test on Patient 1 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the standard dose of 5.75 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.30	837.0	827.1
S1T0.25	0.25	0.6028	4.26	828.6	818.0
S2T0.5	0.50	1.1686	4.26	818.8	810.6
S3T0.75	0.75	1.7129	4.25	812.7	803.4
S4T1.0	1.00	2.0908	4.26	805.0	796.1
S5T1.25	1.25	2.5483	4.26	795.9	786.3
S6T1.5	1.50	3.0631	4.27	788.9	778.1
S7T1.75	1.75	3.4551	4.26	781.4	770.5
S8T2.0	2.00	3.7242	4.26	771.2	759.0
S9T2.25	2.25	3.5191	4.26	762.6	753.1
S10T2.5	2.50	3.2684	4.27	755.9	746.4
S11T3.0	3.00	2.8681	4.27	749.2	738.5
S12T3.5	3.50	2.5057	4.27	742.5	733.6
S13T4.0	4.00	2.1224	4.27	736.5	727.8
S14T4.5	4.50	1.8755	4.26	732.2	723.2
S15T5.0	5.00	1.5979	4.24	727.6	717.7
S16T5.5	5.50	1.3815	4.23	722.7	712.8
S17T6.0	6.00	1.2052	4.22	714.3	705.2
S18T6.5	6.50	1.0009	4.21	705.6	693.8
S19T7.0	7.00	0.9076	4.22	686.8	677.9
S20T7.5	7.50	0.7404	4.22	679.4	670.2
S21T8.0	8.00	0.6475	4.20	664.1	654.3

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

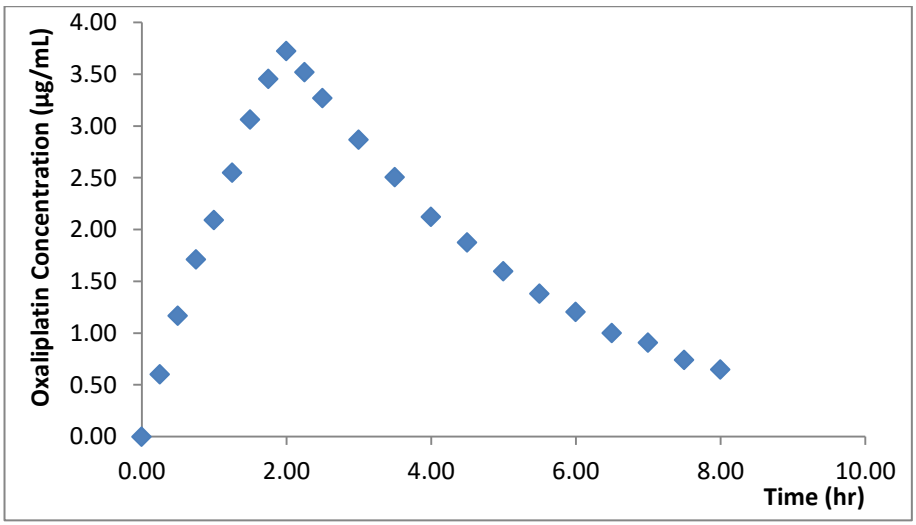

Figure C-3, Oxaliplatin concentration-time curve for Patient 1 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-4, Results of the simulation test on Patient 1 with oxaliplatin administered in Logarithmic Dose-banding scheme with the standard dose of 5.46 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.19	837.6	828.9
S1T0.25	0.25	0.6016	4.17	830.7	820.0
S2T0.5	0.50	1.1702	4.17	823.7	812.0
S3T0.75	0.75	1.7391	4.16	820.9	809.5
S4T1.0	1.00	2.2994	4.17	817.9	798.2
S5T1.25	1.25	2.7880	4.16	804.3	786.7
S6T1.5	1.50	3.2514	4.17	789.4	784.8
S7T1.75	1.75	3.7309	4.17	791.7	777.8
S8T2.0	2.00	3.9544	4.17	784.2	771.2
S9T2.25	2.25	3.7020	4.18	774.5	766.5
S10T2.5	2.50	3.4385	4.17	770.8	760.1
S11T3.0	3.00	3.0273	4.17	762.8	758.1
S12T3.5	3.50	2.6093	4.16	764.5	751.9
S13T4.0	4.00	2.2592	4.16	758.5	745.0
S14T4.5	4.50	1.9558	4.16	752.4	740.6
S15T5.0	5.00	1.7237	4.17	735.3	722.9
S16T5.5	5.50	1.5000	4.17	730.1	717.5
S17T6.0	6.00	1.3006	4.18	721.8	707.2
S18T6.5	6.50	1.1087	4.18	706.8	695.6
S19T7.0	7.00	0.9576	4.19	699.7	692.4
S20T7.5	7.50	0.8232	4.20	697.5	686.1
S21T8.0	8.00	0.7007	4.18	682.8	671.3

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

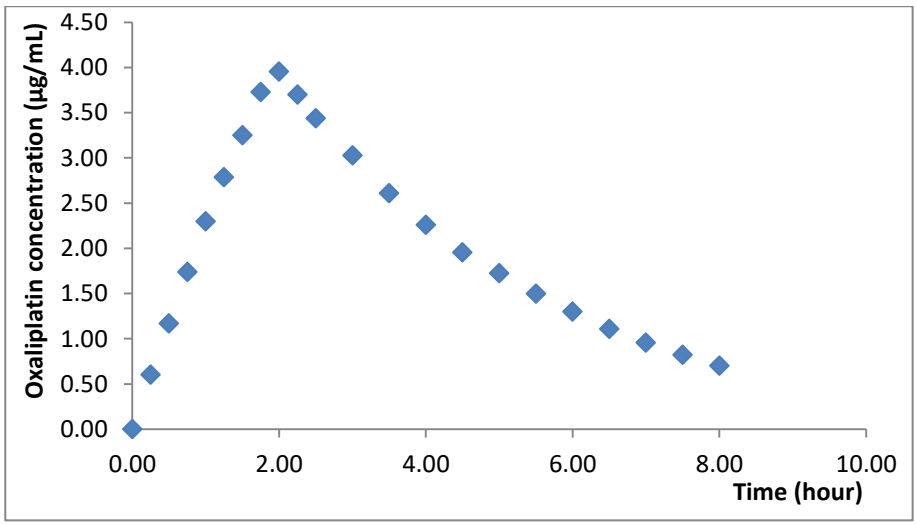

Figure C-4, Oxaliplatin concentration-time curve for Patient 1 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-5, Results of the simulation test on Patient 1 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.22	837.5	826.5
S1T0.25	0.25	0.3607	4.21	829.4	818.9
S2T0.5	0.50	0.7166	4.21	820.5	815.6
S3T0.75	0.75	1.0538	4.21	818.4	802.8
S4T1.0	1.00	1.3956	4.21	806.0	795.8
S5T1.25	1.25	1.7406	4.21	793.2	782.6
S6T1.5	1.50	2.0307	4.20	785.7	777.0
S7T1.75	1.75	2.2393	4.20	779.6	770.4
S8T2.0	2.00	2.5090	4.21	774.2	765.5
S9T2.25	2.25	2.3914	4.21	768.0	757.3
S10T2.5	2.50	2.3052	4.21	759.8	746.3
S11T3.0	3.00	1.9806	4.21	754.8	745.5
S12T3.5	3.50	1.7391	4.21	752.8	743.1
S13T4.0	4.00	1.5180	4.21	747.7	738.5
S14T4.5	4.50	1.3110	4.21	743.5	734.4
S15T5.0	5.00	1.1651	4.21	739.4	729.8
S16T5.5	5.50	1.0334	4.21	736.2	726.3
S17T6.0	6.00	0.8860	4.21	734.6	721.8
S18T6.5	6.50	0.7668	4.21	723.7	711.4
S19T7.0	7.00	0.6884	4.20	716.7	709.1
S20T7.5	7.50	0.5504	4.20	714.0	704.2
S21T8.0	8.00	0.4987	4.19	712.3	700.2

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

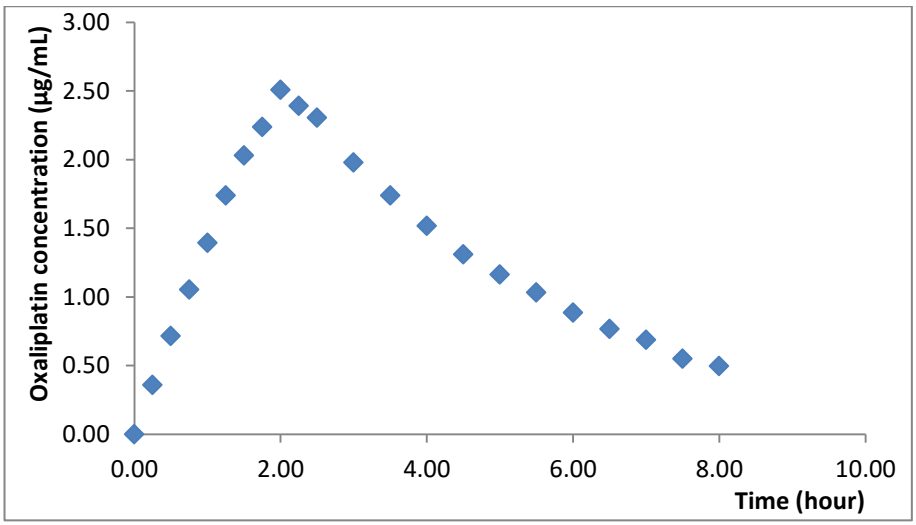

Figure C-5, Oxaliplatin concentration-time curve for Patient 1 with 2-hour infusions administered in Flat-fixed dosing method.

C.2 Patient 2

The basic setting information for Patient 2 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 1.82 m^2; V_c = 564.7 mL; CL_{total} = 131.0 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

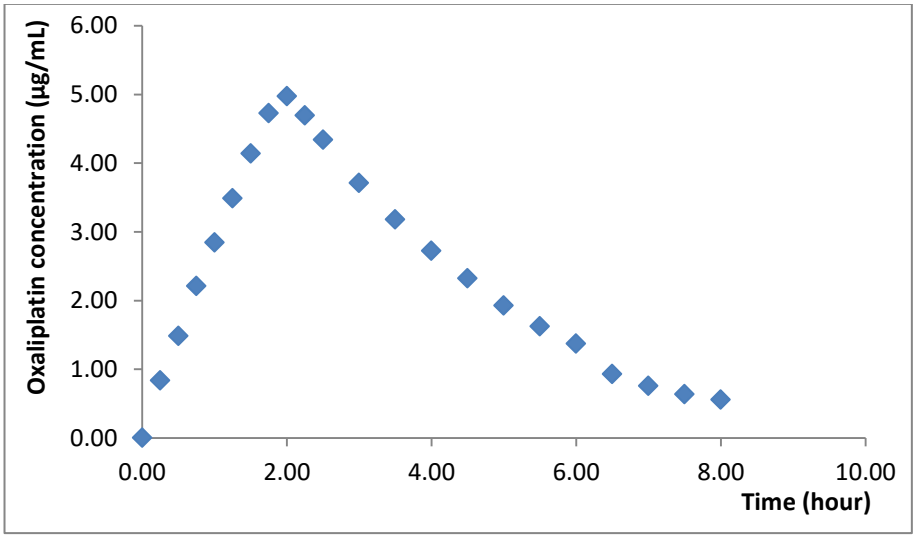

Figure C-6, Oxaliplatin concentration-time curve for Patient 2 with 2-hour infusions administered in Individual dosing scheme.

Table C-6, Results of the simulation test on Patient 2 with oxaliplatin administered in Individual dosing scheme with the drug dose of 3.87 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.22	564.2	554.5
S1T0.25	0.25	0.8351	4.19	553.3	544.6
S2T0.5	0.50	1.4855	4.19	543.4	534.0
S3T0.75	0.75	2.2115	4.19	536.1	525.8
S4T1.0	1.00	2.8460	4.19	526.1	516.9
S5T1.25	1.25	3.4865	4.19	518.0	507.6
S6T1.5	1.50	4.1378	4.19	507.5	497.6
S7T1.75	1.75	4.7249	4.18	504.7	495.5
S8T2.0	2.00	4.9723	4.18	488.6	478.7
S9T2.25	2.25	4.6924	4.18	481.4	471.9
S10T2.5	2.50	4.3415	4.18	472.6	462.8
S11T3.0	3.00	3.7106	4.18	464.8	458.2
S12T3.5	3.50	3.1772	4.17	451.6	439.3
S13T4.0	4.00	2.7216	4.17	446.8	438.5
S14T4.5	4.50	2.3234	4.17	439.9	430.0
S15T5.0	5.00	1.9243	4.18	430.8	420.8
S16T5.5	5.50	1.6231	4.17	422.6	411.1
S17T6.0	6.00	1.3705	4.17	420.0	408.6
S18T6.5	6.50	0.9302	4.17	411.1	402.0
S19T7.0	7.00	0.7588	4.17	405.5	395.4
S20T7.5	7.50	0.6369	4.17	398.8	388.4
S21T8.0	8.00	0.5558	4.16	387.7	379.1

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

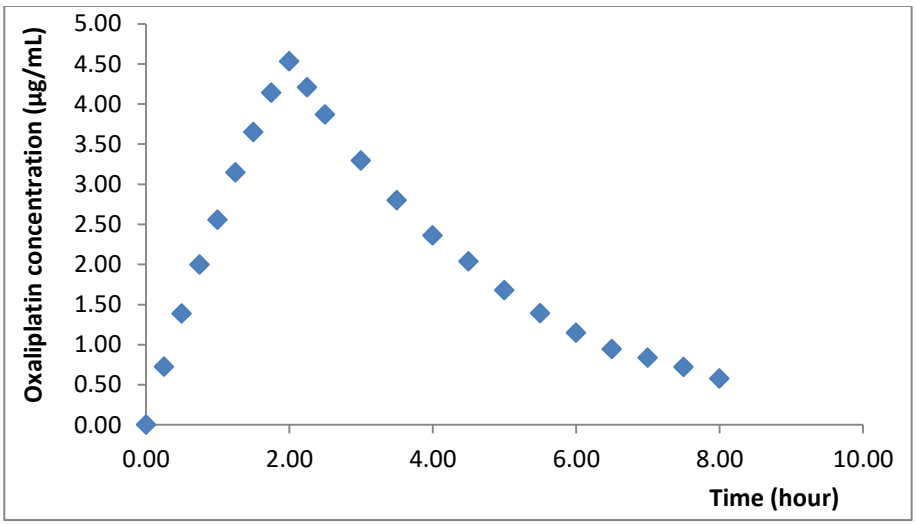

Figure C-7, Oxaliplatin concentration-time curve for Patient 2 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-7, Results of the simulation test on Patient 2 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 3.75 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.29	566.3	556.1
S1T0.25	0.25	0.7207	4.27	558.5	547.6
S2T0.5	0.50	1.3854	4.24	544.8	535.5
S3T0.75	0.75	1.9967	4.22	535.7	527.2
S4T1.0	1.00	2.5540	4.23	528.4	518.5
S5T1.25	1.25	3.1436	4.22	519.3	510.6
S6T1.5	1.50	3.6471	4.22	513.4	503.7
S7T1.75	1.75	4.1373	4.21	504.4	495.6
S8T2.0	2.00	4.5272	4.20	497.8	487.1
S9T2.25	2.25	4.2069	4.21	489.0	478.9
S10T2.5	2.50	3.8650	4.21	480.5	470.5
S11T3.0	3.00	3.2915	4.22	473.5	464.2
S12T3.5	3.50	2.7978	4.22	467.5	456.4
S13T4.0	4.00	2.3590	4.22	459.7	450.2
S14T4.5	4.50	2.0335	4.23	452.8	442.6
S15T5.0	5.00	1.6769	4.22	445.8	435.5
S16T5.5	5.50	1.3887	4.21	439.3	429.2
S17T6.0	6.00	1.1461	4.22	432.4	422.8
S18T6.5	6.50	0.9398	4.22	426.1	415.4
S19T7.0	7.00	0.8321	4.22	417.1	406.9
S20T7.5	7.50	0.7163	4.21	409.5	399.9
S21T8.0	8.00	0.5732	4.20	402.9	392.1

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

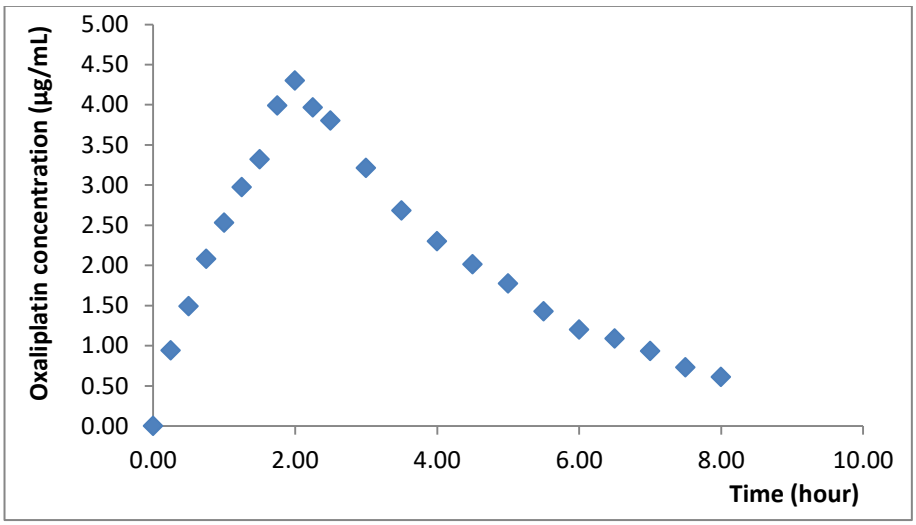

Figure C-8, Oxaliplatin concentration-time curve for Patient 2 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-8, Results of the simulation test on Patient 2 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.22	546.9	554.2
S1T0.25	0.25	0.9396	4.19	553.2	544.1
S2T0.5	0.50	1.4874	4.20	543.6	535.4
S3T0.75	0.75	2.0788	4.18	535.2	525.1
S4T1.0	1.00	2.5306	4.18	526.0	517.2
S5T1.25	1.25	2.9704	4.18	517.7	508.4
S6T1.5	1.50	3.3186	4.18	508.6	499.2
S7T1.75	1.75	3.9880	4.18	499.6	490.7
S8T2.0	2.00	4.3002	4.18	486.0	479.6
S9T2.25	2.25	3.9640	4.19	485.5	475.9
S10T2.5	2.50	3.8004	4.17	476.6	466.7
S11T3.0	3.00	3.2105	4.17	472.6	464.3
S12T3.5	3.50	2.6822	4.17	470.9	458.6
S13T4.0	4.00	2.2979	4.16	461.2	451.2
S14T4.5	4.50	2.0098	4.16	455.3	445.3
S15T5.0	5.00	1.7741	4.16	447.7	436.3
S16T5.5	5.50	1.4259	4.14	443.2	429.8
S17T6.0	6.00	1.1977	4.14	437.7	424.6
S18T6.5	6.50	1.0852	4.13	431.0	420.3
S19T7.0	7.00	0.9306	4.13	424.2	414.6
S20T7.5	7.50	0.7265	4.11	416.7	409.2
S21T8.0	8.00	0.6094	4.11	408.3	399.5

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

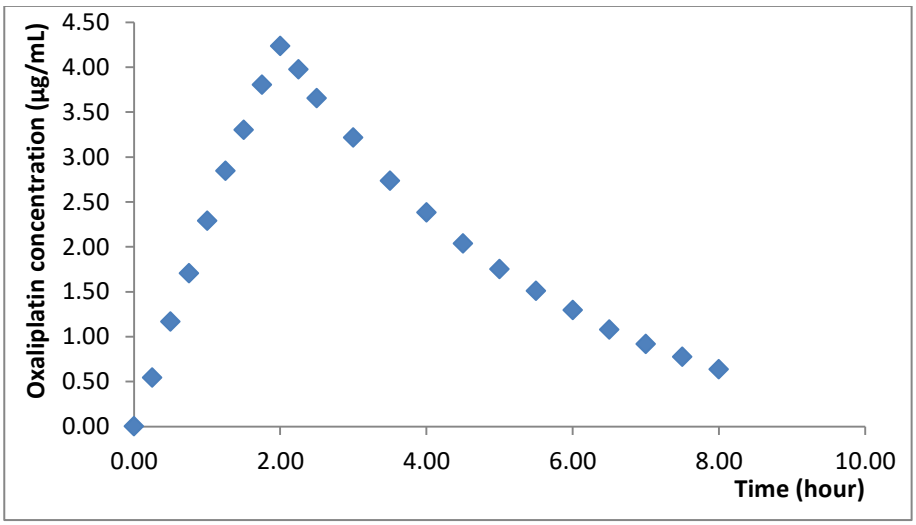

Figure C-9, Oxaliplatin concentration-time curve for Patient 2 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-9, Results of the simulation test on Patient 2 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 3.91 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.22	564.7	553.6
S1T0.25	0.25	0.5426	4.20	557.6	547.5
S2T0.5	0.50	1.1643	4.19	549.0	539.8
S3T0.75	0.75	1.7035	4.17	540.9	530.2
S4T1.0	1.00	2.2898	4.18	533.3	523.8
S5T1.25	1.25	2.8439	4.18	525.2	515.8
S6T1.5	1.50	3.3016	4.18	517.1	507.5
S7T1.75	1.75	3.8028	4.18	508.6	499.0
S8T2.0	2.00	4.2346	4.18	501.0	492.0
S9T2.25	2.25	3.9741	4.18	493.0	483.7
S10T2.5	2.50	3.6533	4.17	484.9	475.9
S11T3.0	3.00	3.2140	4.17	478.1	468.7
S12T3.5	3.50	2.7328	4.17	471.8	462.2
S13T4.0	4.00	2.3821	4.17	465.3	455.8
S14T4.5	4.50	2.0350	4.16	459.2	449.6
S15T5.0	5.00	1.7503	4.17	453.3	444.4
S16T5.5	5.50	1.5067	4.18	446.1	436.7
S17T6.0	6.00	1.2940	4.19	439.5	429.5
S18T6.5	6.50	1.0756	4.19	433.3	424.3
S19T7.0	7.00	0.9147	4.19	427.2	417.6
S20T7.5	7.50	0.7743	4.20	420.9	412.2
S21T8.0	8.00	0.6346	4.22	415.0	404.1

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

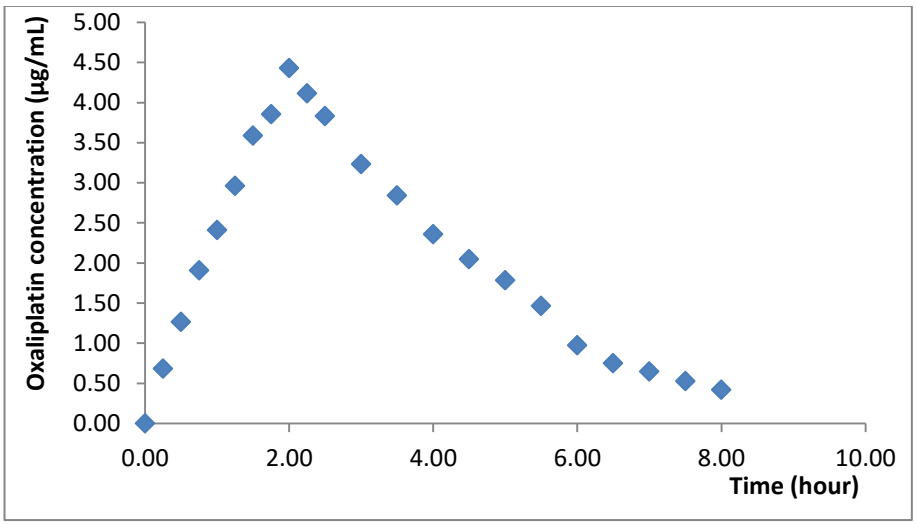

Figure C-10, Oxaliplatin concentration-time curve for Patient 2 with 2-hour infusions administered in Flat-fixed dosing method.

Table C-10, Results of the simulation test on Patient 2 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.27	564.5	553.8
S1T0.25	0.25	0.6807	4.22	552.6	540.8
S2T0.5	0.50	1.2613	4.24	543.3	532.6
S3T0.75	0.75	1.9050	4.23	538.2	529.5
S4T1.0	1.00	2.4101	4.23	530.6	521.9
S5T1.25	1.25	2.9575	4.24	523.5	513.7
S6T1.5	1.50	3.5851	4.23	516.6	505.8
S7T1.75	1.75	3.8515	4.24	507.6	497.0
S8T2.0	2.00	4.4275	4.24	499.6	483.4
S9T2.25	2.25	4.1103	4.24	491.9	479.8
S10T2.5	2.50	3.8269	4.24	482.6	473.8
S11T3.0	3.00	3.2294	4.24	477.8	467.7
S12T3.5	3.50	2.8382	4.23	470.9	460.2
S13T4.0	4.00	2.3577	4.24	462.8	453.3
S14T4.5	4.50	2.0451	4.25	457.2	447.0
S15T5.0	5.00	1.7833	4.23	449.9	441.2
S16T5.5	5.50	1.4645	4.24	444.0	434.1
S17T6.0	6.00	0.9717	4.24	435.3	425.8
S18T6.5	6.50	0.7484	4.23	430.0	420.1
S19T7.0	7.00	0.6453	4.22	425.9	415.5
S20T7.5	7.50	0.5236	4.23	420.9	410.8
S21T8.0	8.00	0.4158	4.23	417.4	407.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

C.3 Patient 3

The basic setting information for Patient 3 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 1.29 m^2; V_c = 400.2 mL; CL_{total} = 92.9 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

Table C-11, Results of the simulation test on Patient 3 with oxaliplatin administered in Individual dosing scheme with the drug dose of 2.74 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.18	400.6	391.7
S1T0.25	0.25	0.6975	4.15	392.5	383.3
S2T0.5	0.50	1.4649	4.15	383.7	373.4
S3T0.75	0.75	2.1951	4.16	374.9	365.5
S4T1.0	1.00	2.9157	4.14	366.4	357.1
S5T1.25	1.25	3.5895	4.15	357.8	349.1
S6T1.5	1.50	4.2533	4.16	350.1	340.0
S7T1.75	1.75	4.8382	4.15	340.5	331.5
S8T2.0	2.00	5.2584	4.15	333.0	323.1
S9T2.25	2.25	4.8554	4.15	321.8	314.4
S10T2.5	2.50	4.4369	4.14	310.8	293.3
S11T3.0	3.00	3.7699	4.14	300.2	292.4
S12T3.5	3.50	3.1757	4.14	292.4	283.8
S13T4.0	4.00	2.6474	4.14	295.5	284.2
S14T4.5	4.50	2.2173	4.14	288.0	280.2
S15T5.0	5.00	1.7775	4.13	281.8	272.4
S16T5.5	5.50	1.4676	4.13	274.6	264.5
S17T6.0	6.00	1.1898	4.13	265.9	257.6
S18T6.5	6.50	0.9702	4.13	259.2	250.6
S19T7.0	7.00	0.8021	4.13	253.7	244.3
S20T7.5	7.50	0.6554	4.13	247.5	237.9
S21T8.0	8.00	0.4812	4.13	240.7	229.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

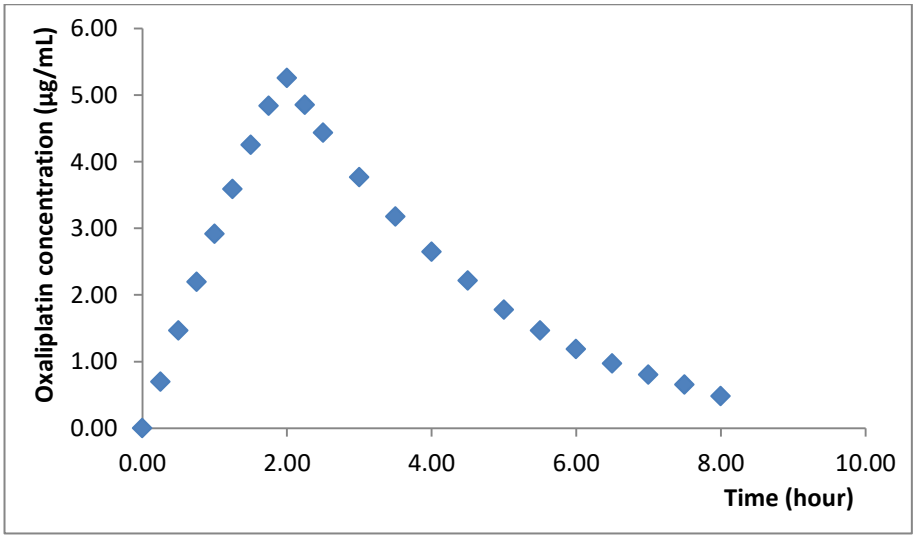

Figure C-11, Oxaliplatin concentration-time curve for Patient 3 with 2-hour infusions administered in Individual dosing scheme.

Table C-12, Results of the simulation test on Patient 3 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 2.75 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.29	401.5	392.2
S1T0.25	0.25	0.9373	4.27	393.0	384.8
S2T0.5	0.50	1.4646	4.26	385.0	374.9
S3T0.75	0.75	2.0083	4.25	375.8	365.5
S4T1.0	1.00	2.5576	4.24	366.0	355.7
S5T1.25	1.25	3.0355	4.26	356.1	347.0
S6T1.5	1.50	3.5308	4.24	346.7	337.3
S7T1.75	1.75	3.9402	4.24	337.9	327.5
S8T2.0	2.00	4.2217	4.24	328.4	318.5
S9T2.25	2.25	3.8772	4.24	318.6	308.4
S10T2.5	2.50	3.5229	4.25	310.0	299.5
S11T3.0	3.00	2.9904	4.25	301.9	292.8
S12T3.5	3.50	2.5029	4.25	294.5	284.3
S13T4.0	4.00	2.0239	4.25	286.2	275.7
S14T4.5	4.50	1.7083	4.25	277.8	267.1
S15T5.0	5.00	1.4001	4.24	268.3	257.6
S16T5.5	5.50	1.0974	4.25	259.7	249.6
S17T6.0	6.00	0.9163	4.25	251.4	241.7
S18T6.5	6.50	0.7509	4.25	243.0	233.4
S19T7.0	7.00	0.5933	4.26	234.7	224.4
S20T7.5	7.50	0.4533	4.26	226.2	216.3
S21T8.0	8.00	0.3555	4.25	214.1	204.1

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

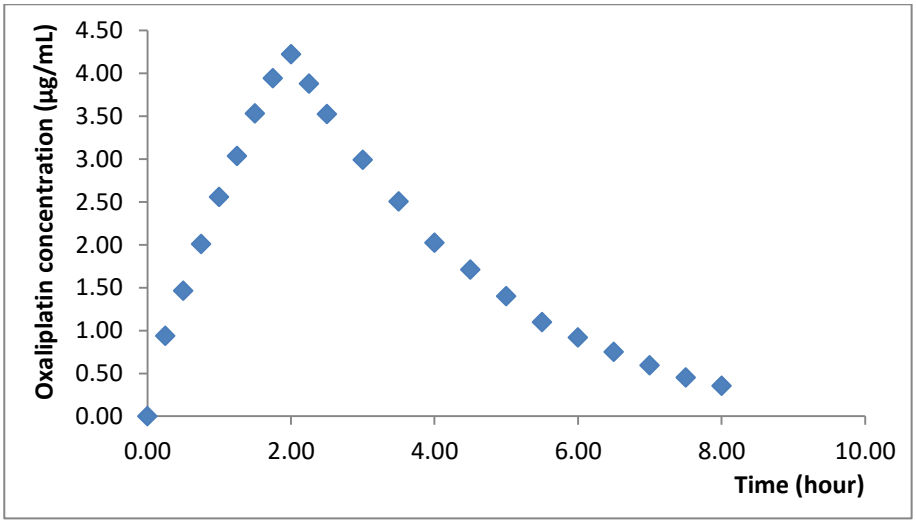

Figure C-12, Oxaliplatin concentration-time curve for Patient 3 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-13, Results of the simulation test on Patient 3 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 2.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.21	400.4	390.2
S1T0.25	0.25	1.0742	4.18	392.2	382.9
S2T0.5	0.50	1.8013	4.18	384.4	374.6
S3T0.75	0.75	2.0669	4.17	376.4	366.6
S4T1.0	1.00	2.8496	4.18	368.3	358.6
S5T1.25	1.25	3.5472	4.18	360.2	350.6
S6T1.5	1.50	4.0096	4.19	352.0	342.4
S7T1.75	1.75	4.7330	4.18	343.6	333.9
S8T2.0	2.00	4.7359	4.17	334.8	325.0
S9T2.25	2.25	4.3373	4.20	325.4	315.7
S10T2.5	2.50	4.0075	4.19	317.0	307.4
S11T3.0	3.00	3.5190	4.20	310.6	300.7
S12T3.5	3.50	2.9033	4.21	304.3	294.6
S13T4.0	4.00	2.4871	4.22	298.3	288.6
S14T4.5	4.50	2.1012	4.23	292.3	282.5
S15T5.0	5.00	1.7298	4.24	285.7	276.3
S16T5.5	5.50	1.4387	4.25	278.8	269.8
S17T6.0	6.00	1.1580	4.25	272.8	263.1
S18T6.5	6.50	0.7416	4.26	266.6	257.1
S19T7.0	7.00	0.5156	4.26	260.5	251.1
S20T7.5	7.50	0.4015	4.27	254.5	245.0
S21T8.0	8.00	0.3121	4.26	248.5	238.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

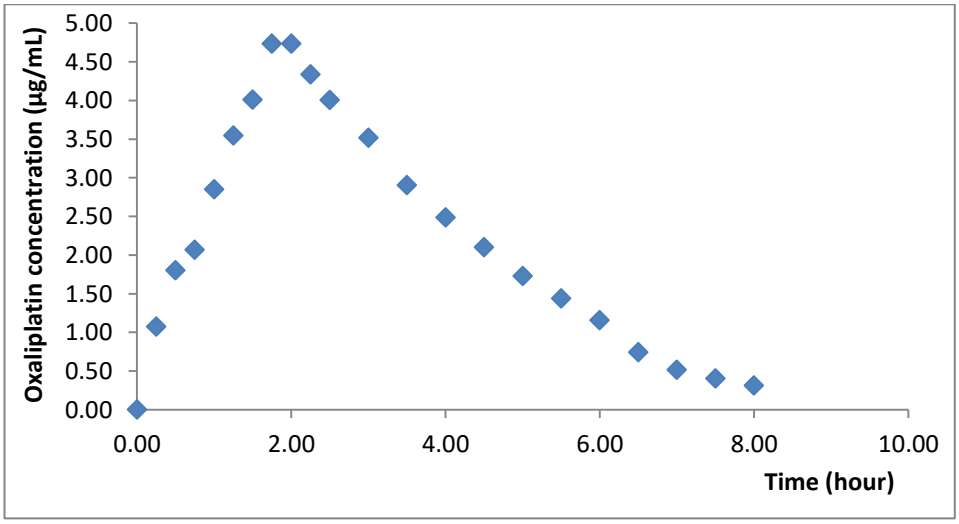

Figure C-13, Oxaliplatin concentration-time curve for Patient 3 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-14, Results of the simulation test on Patient 3 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 2.8 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.18	401.2	388.9
S1T0.25	0.25	0.7747	4.14	391.2	382.9
S2T0.5	0.50	1.5022	4.16	384.4	375.2
S3T0.75	0.75	2.0260	4.17	376.9	367.2
S4T1.0	1.00	2.7284	4.15	368.0	359.0
S5T1.25	1.25	3.2128	4.17	360.3	350.9
S6T1.5	1.50	3.7788	4.17	351.6	341.2
S7T1.75	1.75	4.2394	4.18	342.8	334.4
S8T2.0	2.00	4.5646	4.17	336.1	326.4
S9T2.25	2.25	4.1454	4.18	328.3	318.6
S10T2.5	2.50	3.8481	4.17	320.5	309.8
S11T3.0	3.00	3.2690	4.17	312.3	304.0
S12T3.5	3.50	2.8535	4.16	307.8	297.0
S13T4.0	4.00	2.3393	4.18	300.5	291.9
S14T4.5	4.50	1.8966	4.18	294.6	284.7
S15T5.0	5.00	1.6218	4.17	288.4	279.5
S16T5.5	5.50	1.3478	4.16	281.2	271.6
S17T6.0	6.00	1.1075	4.14	274.1	265.0
S18T6.5	6.50	0.9187	4.14	267.5	257.6
S19T7.0	7.00	0.7720	4.17	261.7	251.8
S20T7.5	7.50	0.6019	4.19	253.5	244.8
S21T8.0	8.00	0.5152	4.18	247.4	237.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

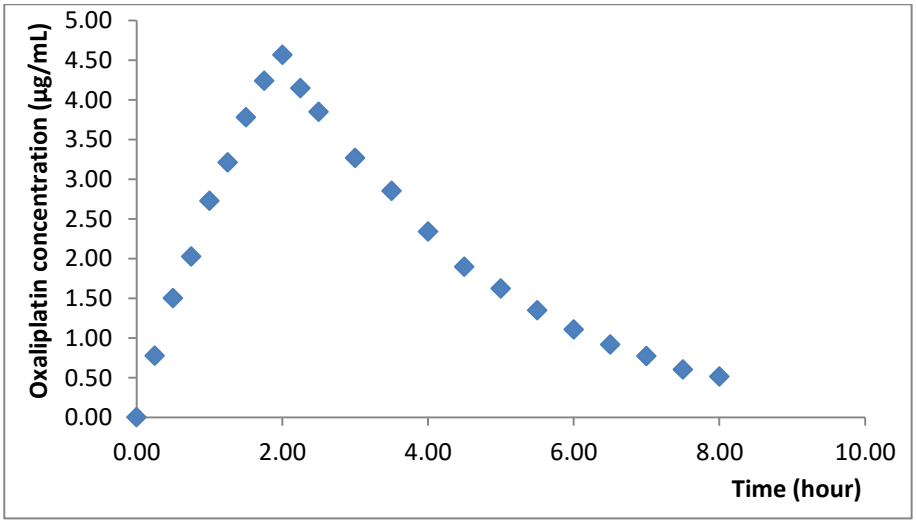

Figure C-14, Oxaliplatin concentration-time curve for Patient 3 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-15, Results of the simulation test on Patient 3 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.23	400.3	390.4
S1T0.25	0.25	0.8864	4.18	391.8	381.6
S2T0.5	0.50	1.8380	4.19	382.8	372.2
S3T0.75	0.75	2.7590	4.19	373.5	363.3
S4T1.0	1.00	3.6580	4.20	365.0	354.5
S5T1.25	1.25	4.6133	4.20	356.4	346.9
S6T1.5	1.50	5.4098	4.19	348.3	339.3
S7T1.75	1.75	6.2397	4.20	340.8	329.0
S8T2.0	2.00	6.9103	4.21	330.5	319.8
S9T2.25	2.25	6.4137	4.21	320.8	312.2
S10T2.5	2.50	5.9227	4.21	312.7	302.9
S11T3.0	3.00	5.0283	4.21	305.6	295.8
S12T3.5	3.50	4.3090	4.21	298.4	287.5
S13T4.0	4.00	3.5343	4.22	290.1	280.2
S14T4.5	4.50	2.9951	4.23	282.6	272.4
S15T5.0	5.00	2.4782	4.23	275.0	265.1
S16T5.5	5.50	2.0646	4.23	266.9	256.8
S17T6.0	6.00	1.7106	4.24	258.4	248.5
S18T6.5	6.50	1.3939	4.22	250.8	241.2
S19T7.0	7.00	1.1777	4.24	243.1	233.3
S20T7.5	7.50	0.9553	4.23	235.5	225.9
S21T8.0	8.00	0.7521	4.24	227.7	217.7

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

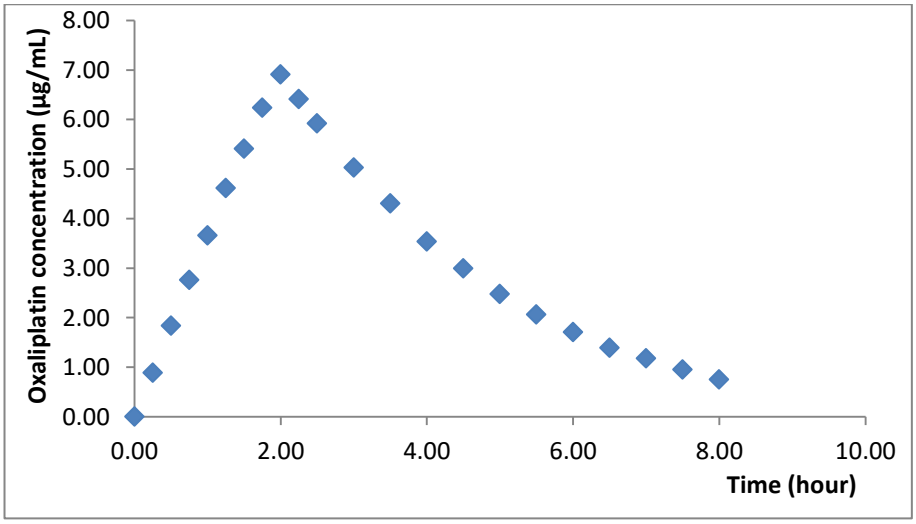

Figure C-15, Oxaliplatin concentration-time curve for Patient 3 with 2-hour infusions administered in Flat-fixed dosing method.

C.4 Patient 4

The basic setting information for Patient 4 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 1.55 m^2; V_c = 480.9 mL; CL_{total} = 111.6 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

Table C-16, Results of the simulation test on Patient 4 with oxaliplatin administered in Individual dosing scheme with the drug dose of 3.29 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.36	481.0	471.6
S1T0.25	0.25	0.7600	4.32	472.2	462.4
S2T0.5	0.50	1.5478	4.31	463.8	455.2
S3T0.75	0.75	2.2017	4.31	456.4	447.4
S4T1.0	1.00	2.9174	4.31	448.1	439.5
S5T1.25	1.25	3.5806	4.27	441.0	432.1
S6T1.5	1.50	4.2536	4.27	431.5	416.5
S7T1.75	1.75	4.7211	4.27	417.9	408.8
S8T2.0	2.00	5.3099	4.27	409.3	399.2
S9T2.25	2.25	4.9380	4.27	397.1	391.7
S10T2.5	2.50	4.5386	4.29	394.5	383.8
S11T3.0	3.00	3.8509	4.29	389.2	377.2
S12T3.5	3.50	3.2051	4.30	379.8	368.3
S13T4.0	4.00	2.6733	4.31	372.1	360.6
S14T4.5	4.50	2.2650	4.31	364.6	355.5
S15T5.0	5.00	1.8885	4.32	358.2	347.2
S16T5.5	5.50	1.5272	4.33	351.0	340.8
S17T6.0	6.00	1.2829	4.33	344.2	333.9
S18T6.5	6.50	1.0795	4.35	332.2	322.4
S19T7.0	7.00	0.8414	4.32	325.2	315.4
S20T7.5	7.50	0.7142	4.31	318.1	307.4
S21T8.0	8.00	0.5847	4.30	310.4	299.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

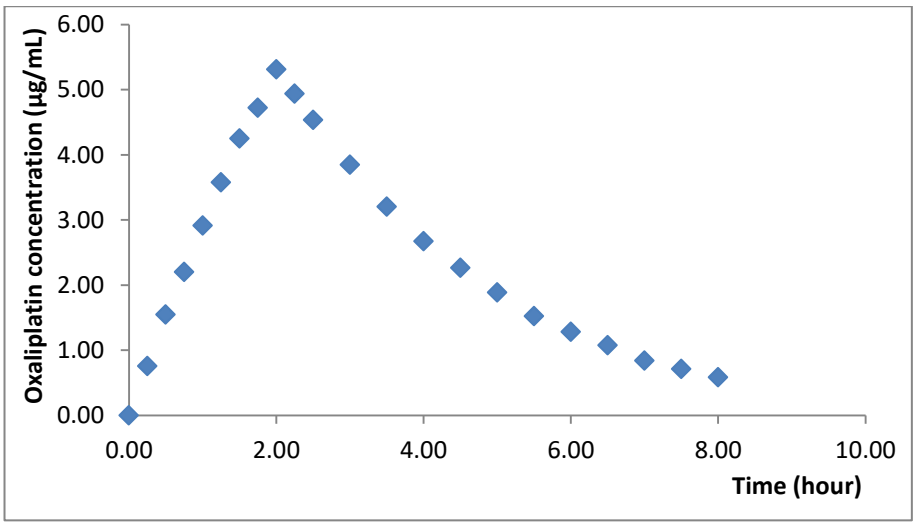

Figure C-16, Oxaliplatin concentration-time curve for Patient 4 with 2-hour infusions administered in Individual dosing scheme.

Table C-17, Results of the simulation test on Patient 4 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 3.25 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.12	481.2	470.6
S1T0.25	0.25	0.5385	4.07	469.5	460.1
S2T0.5	0.50	1.3128	4.04	459.7	449.9
S3T0.75	0.75	2.2207	4.06	449.8	440.3
S4T1.0	1.00	2.6911	4.06	440.5	431.0
S5T1.25	1.25	3.2784	4.06	431.1	421.0
S6T1.5	1.50	3.6568	4.06	421.3	411.5
S7T1.75	1.75	4.2633	4.07	412.0	402.9
S8T2.0	2.00	4.6301	4.06	403.0	393.5
S9T2.25	2.25	4.0541	4.07	393.6	384.4
S10T2.5	2.50	3.9544	4.10	385.3	374.8
S11T3.0	3.00	3.3231	4.10	375.2	366.4
S12T3.5	3.50	2.8103	4.10	366.5	356.8
S13T4.0	4.00	2.4234	4.11	358.6	349.0
S14T4.5	4.50	2.0745	4.13	350.0	340.2
S15T5.0	5.00	1.6942	4.13	341.8	332.2
S16T5.5	5.50	1.4345	4.15	333.6	323.3
S17T6.0	6.00	1.1683	4.17	325.6	316.4
S18T6.5	6.50	0.9380	4.18	317.6	307.5
S19T7.0	7.00	0.7640	4.19	308.7	299.4
S20T7.5	7.50	0.6570	4.20	300.2	290.4
S21T8.0	8.00	0.5258	4.20	292.1	282.0

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

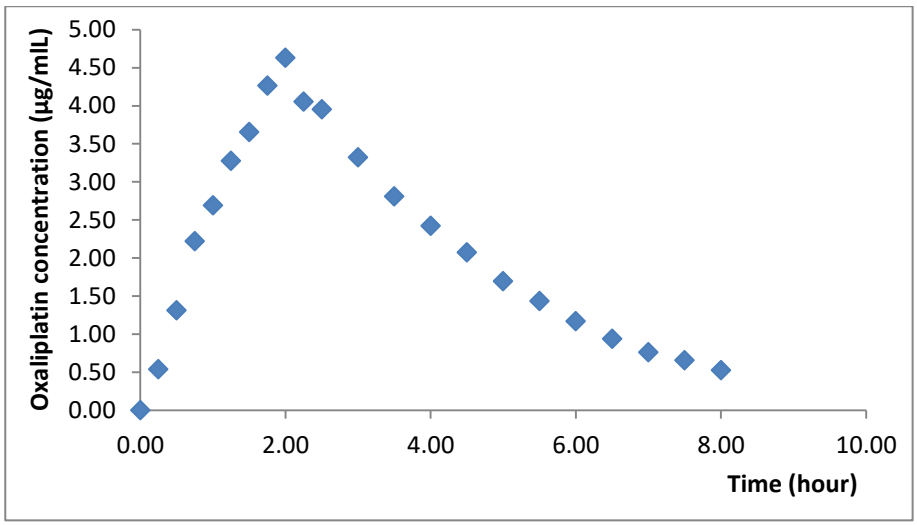

Figure C-17, Oxaliplatin concentration-time curve for Patient 4 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-18, Results of the simulation test on Patient 4 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 3.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.25	481.0	471.2
S1T0.25	0.25	0.3102	4.20	469.9	459.8
S2T0.5	0.50	1.2048	4.21	459.9	450.4
S3T0.75	0.75	1.6559	4.20	450.4	440.4
S4T1.0	1.00	2.2944	4.21	440.4	431.0
S5T1.25	1.25	3.0272	4.21	430.8	421.3
S6T1.5	1.50	3.4285	4.21	421.2	411.6
S7T1.75	1.75	4.1582	4.21	411.6	402.3
S8T2.0	2.00	4.3712	4.21	402.4	392.9
S9T2.25	2.25	3.9714	4.22	393.6	384.0
S10T2.5	2.50	3.8049	4.22	384.6	374.8
S11T3.0	3.00	3.1619	4.23	376.4	366.9
S12T3.5	3.50	2.7623	4.23	368.0	358.4
S13T4.0	4.00	2.2899	4.25	360.2	350.5
S14T4.5	4.50	2.0102	4.25	352.7	343.1
S15T5.0	5.00	1.6454	4.25	344.8	335.0
S16T5.5	5.50	1.3693	4.25	336.4	326.7
S17T6.0	6.00	1.2402	4.26	328.4	318.8
S18T6.5	6.50	1.0253	4.26	319.4	309.8
S19T7.0	7.00	0.8958	4.27	310.3	300.2
S20T7.5	7.50	0.7229	4.27	301.8	291.9
S21T8.0	8.00	0.6257	4.27	291.9	282.2

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

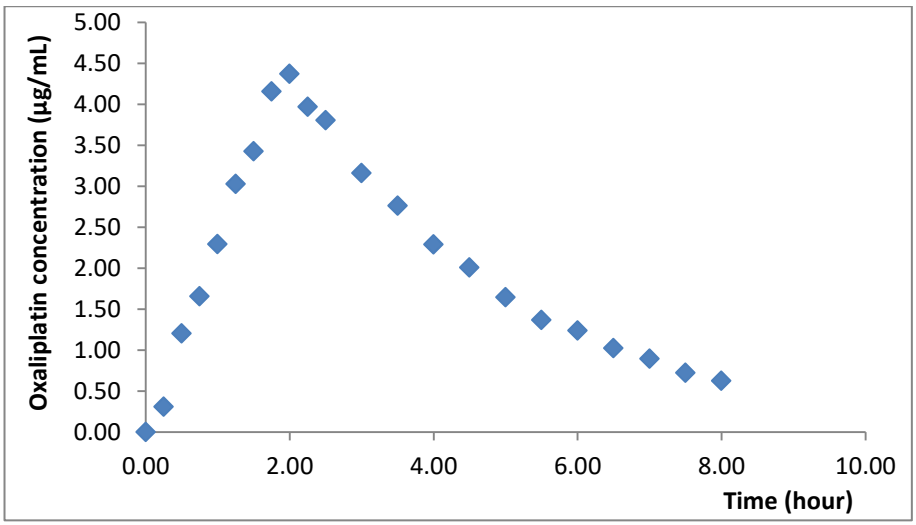

Figure C-18, Oxaliplatin concentration-time curve for Patient 4 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-19, Results of the simulation test on Patient 4 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 3.13 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.25	480.8	467.0
S1T0.25	0.25	0.3220	4.24	466.9	457.3
S2T0.5	0.50	1.0807	4.24	457.3	447.9
S3T0.75	0.75	1.6900	4.25	448.2	438.8
S4T1.0	1.00	2.3577	4.26	438.9	429.0
S5T1.25	1.25	2.7305	4.26	430.2	420.3
S6T1.5	1.50	3.4348	4.26	421.3	411.7
S7T1.75	1.75	4.2809	4.28	412.8	403.0
S8T2.0	2.00	4.4593	4.27	404.5	395.1
S9T2.25	2.25	3.9447	4.27	395.9	386.1
S10T2.5	2.50	3.6214	4.28	387.7	378.3
S11T3.0	3.00	3.0886	4.29	380.7	370.3
S12T3.5	3.50	2.7041	4.30	373.5	364.0
S13T4.0	4.00	2.2616	4.30	367.3	357.8
S14T4.5	4.50	1.9097	4.31	361.1	351.4
S15T5.0	5.00	1.4999	4.31	354.3	344.7
S16T5.5	5.50	1.4153	4.32	347.8	338.7
S17T6.0	6.00	1.1612	4.31	341.6	332.4
S18T6.5	6.50	0.9377	4.31	335.8	326.0
S19T7.0	7.00	0.8220	4.31	330.0	320.3
S20T7.5	7.50	0.6705	4.31	323.6	313.5
S21T8.0	8.00	0.5589	4.31	315.6	305.3

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

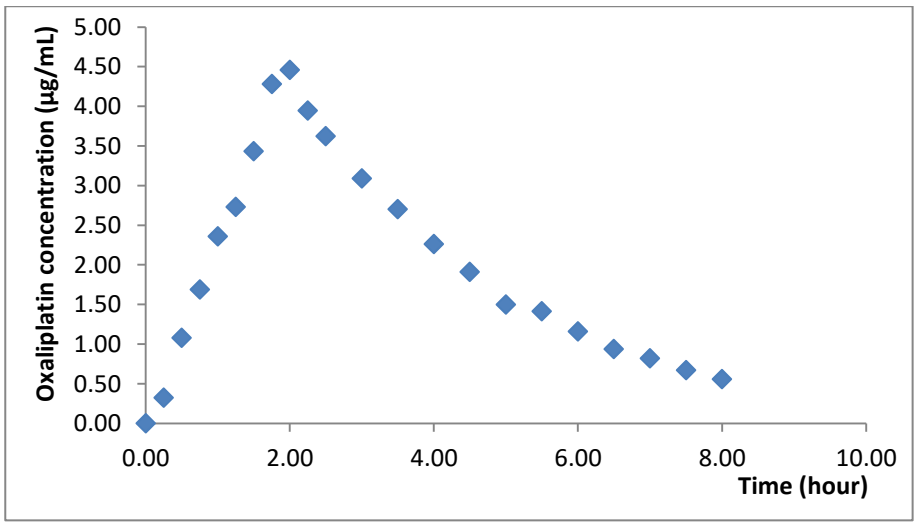

Figure C-19, Oxaliplatin concentration-time curve for Patient 4 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-20, Results of the simulation test on Patient 4 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.22	480.9	471.1
S1T0.25	0.25	0.5836	4.20	473.0	462.7
S2T0.5	0.50	1.5475	4.21	464.8	454.5
S3T0.75	0.75	2.2670	4.21	456.6	446.5
S4T1.0	1.00	3.0404	4.20	450.1	440.9
S5T1.25	1.25	3.6745	4.21	443.7	432.6
S6T1.5	1.50	4.4418	4.21	434.8	425.0
S7T1.75	1.75	4.8977	4.22	426.1	415.4
S8T2.0	2.00	5.3974	4.24	417.0	406.7
S9T2.25	2.25	4.8952	4.22	408.9	398.4
S10T2.5	2.50	4.6380	4.21	400.1	389.7
S11T3.0	3.00	4.0212	4.21	394.0	383.7
S12T3.5	3.50	3.4345	4.21	387.3	378.0
S13T4.0	4.00	2.9324	4.21	380.2	370.8
S14T4.5	4.50	2.5078	4.21	375.0	364.8
S15T5.0	5.00	2.1380	4.22	368.0	357.4
S16T5.5	5.50	1.7980	4.22	362.2	352.1
S17T6.0	6.00	1.5165	4.22	351.4	340.3
S18T6.5	6.50	1.2643	4.21	346.7	334.6
S19T7.0	7.00	1.0686	4.20	337.3	327.1
S20T7.5	7.50	0.8857	4.21	331.4	321.5
S21T8.0	8.00	0.7444	4.22	325.8	315.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

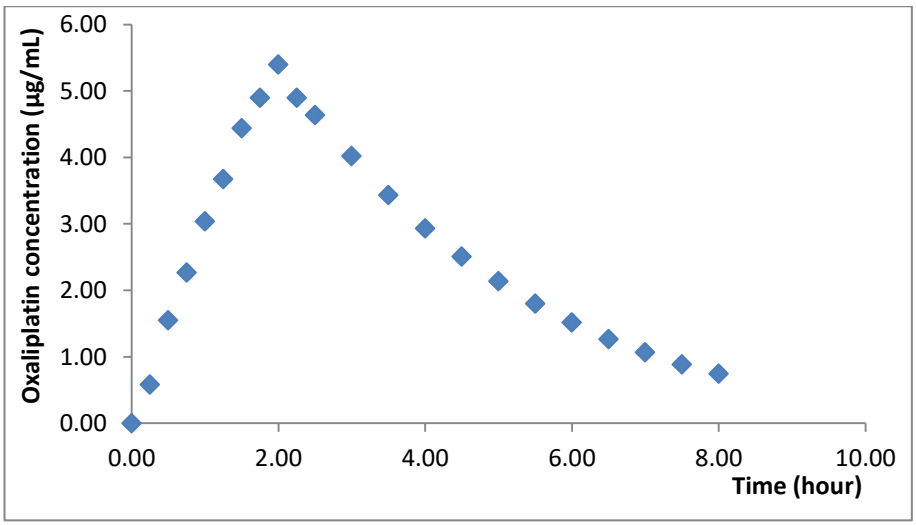

Figure C-20, Oxaliplatin concentration-time curve for Patient 4 with 2-hour infusions administered in Flat-fixed dosing method.

C.5 Patient 5

The basic setting information for Patient 5 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 2.20 m^2; V_c = 682.6 mL; CL_{total} = 158.4 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

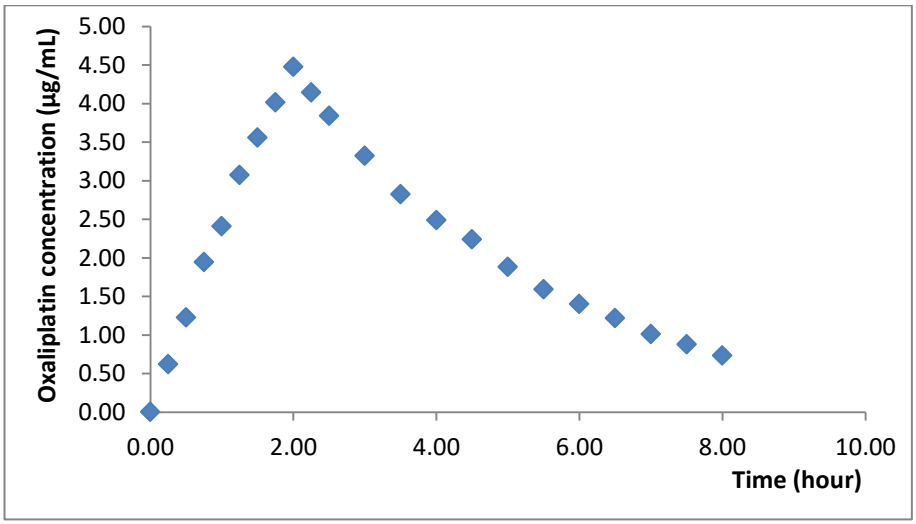

Figure C-21, Oxaliplatin concentration-time curve for Patient 5 with 2-hour infusions administered in Individual dosing scheme.

Table C-21, Results of the simulation test on Patient 5 with oxaliplatin administered in Individual dosing scheme with the drug dose of 4.68 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.24	682.5	672.2
S1T0.25	0.25	0.6216	4.20	674.8	665.0
S2T0.5	0.50	1.2251	4.21	669.2	658.4
S3T0.75	0.75	1.9427	4.20	658.4	648.4
S4T1.0	1.00	2.4086	4.20	651.3	641.0
S5T1.25	1.25	3.0705	4.20	644.1	635.5
S6T1.5	1.50	3.5557	4.19	640.8	627.3
S7T1.75	1.75	4.0128	4.20	629.8	619.7
S8T2.0	2.00	4.4720	4.19	624.8	615.6
S9T2.25	2.25	4.1414	4.20	621.9	609.3
S10T2.5	2.50	3.8399	4.18	613.4	603.6
S11T3.0	3.00	3.3201	4.18	608.4	597.7
S12T3.5	3.50	2.8215	4.19	603.8	594.0
S13T4.0	4.00	2.4850	4.19	600.5	589.1
S14T4.5	4.50	2.2362	4.20	595.8	586.3
S15T5.0	5.00	1.8813	4.19	593.0	582.9
S16T5.5	5.50	1.5892	4.18	586.9	576.8
S17T6.0	6.00	1.3982	4.19	581.1	571.3
S18T6.5	6.50	1.2179	4.18	575.4	563.4
S19T7.0	7.00	1.0113	4.20	566.3	556.8
S20T7.5	7.50	0.8750	4.20	560.2	551.7
S21T8.0	8.00	0.7338	4.20	557.0	547.2

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

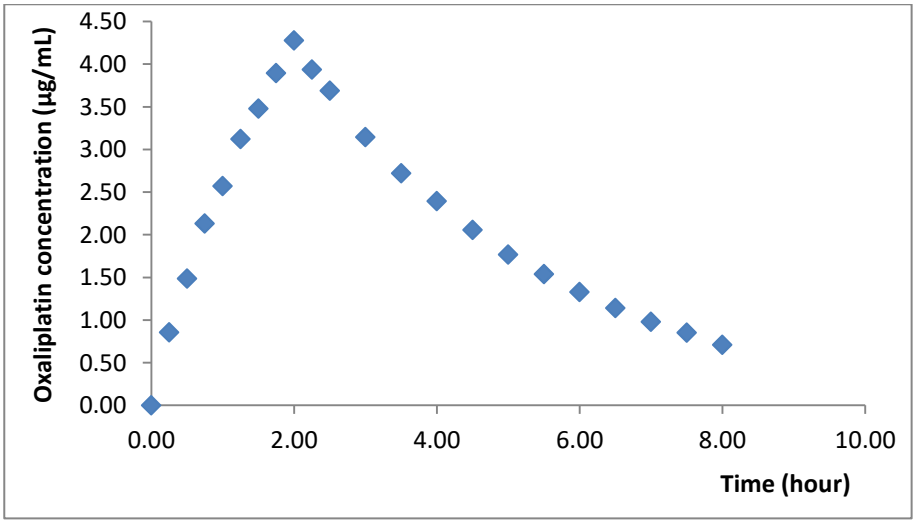

Figure C-22, Oxaliplatin concentration-time curve for Patient 5 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-22, Results of the simulation test on Patient 5 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 4.75 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.37	682.9	671.3
S1T0.25	0.25	0.8564	4.34	673.9	663.2
S2T0.5	0.50	1.4846	4.32	663.6	654.7
S3T0.75	0.75	2.1310	4.30	657.5	648.9
S4T1.0	1.00	2.5713	4.27	650.8	639.6
S5T1.25	1.25	3.1217	4.27	641.3	628.6
S6T1.5	1.50	3.4765	4.25	638.0	627.2
S7T1.75	1.75	3.8962	4.22	629.9	620.5
S8T2.0	2.00	4.2776	4.22	621.7	613.5
S9T2.25	2.25	3.9349	4.21	616.8	605.7
S10T2.5	2.50	3.6891	4.21	609.5	600.4
S11T3.0	3.00	3.1431	4.20	605.7	595.7
S12T3.5	3.50	2.7201	4.18	601.4	590.8
S13T4.0	4.00	2.3943	4.18	594.2	583.9
S14T4.5	4.50	2.0542	4.19	591.3	582.2
S15T5.0	5.00	1.7669	4.21	588.7	580.0
S16T5.5	5.50	1.5392	4.21	585.3	575.2
S17T6.0	6.00	1.3291	4.22	573.8	559.6
S18T6.5	6.50	1.1408	4.21	567.8	554.7
S19T7.0	7.00	0.9798	4.21	557.5	548.5
S20T7.5	7.50	0.8505	4.18	547.3	537.6
S21T8.0	8.00	0.7102	4.18	530.3	522.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

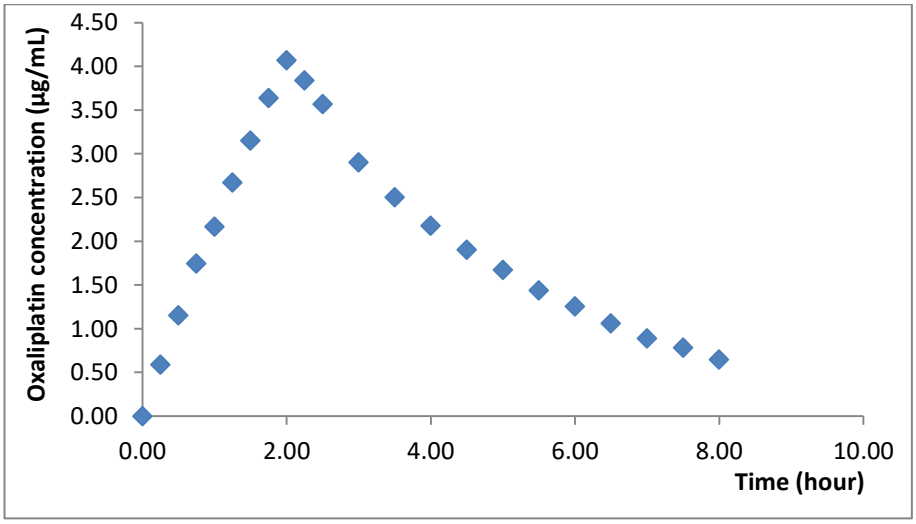

Figure C-23, Oxaliplatin concentration-time curve for Patient 5 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-23, Results of the simulation test on Patient 5 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 4.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.23	682.2	671.7
S1T0.25	0.25	0.5860	4.20	674.3	661.9
S2T0.5	0.50	1.1525	4.19	666.3	655.9
S3T0.75	0.75	1.7453	4.19	658.6	649.5
S4T1.0	1.00	2.1658	4.20	652.1	639.6
S5T1.25	1.25	2.6700	4.19	643.4	634.2
S6T1.5	1.50	3.1512	4.20	636.7	625.5
S7T1.75	1.75	3.6356	4.20	628.2	618.6
S8T2.0	2.00	4.0688	4.18	622.3	610.6
S9T2.25	2.25	3.8367	4.19	614.6	604.3
S10T2.5	2.50	3.5652	4.20	607.9	597.6
S11T3.0	3.00	2.9002	4.19	603.7	592.2
S12T3.5	3.50	2.5029	4.19	598.6	585.5
S13T4.0	4.00	2.1778	4.20	592.1	581.3
S14T4.5	4.50	1.9018	4.19	587.9	571.5
S15T5.0	5.00	1.6710	4.19	578.3	568.2
S16T5.5	5.50	1.4379	4.20	575.1	565.0
S17T6.0	6.00	1.2527	4.20	569.9	561.3
S18T6.5	6.50	1.0607	4.20	566.7	554.7
S19T7.0	7.00	0.8863	4.20	560.2	551.4
S20T7.5	7.50	0.7815	4.19	556.7	546.0
S21T8.0	8.00	0.6458	4.21	552.0	541.7

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

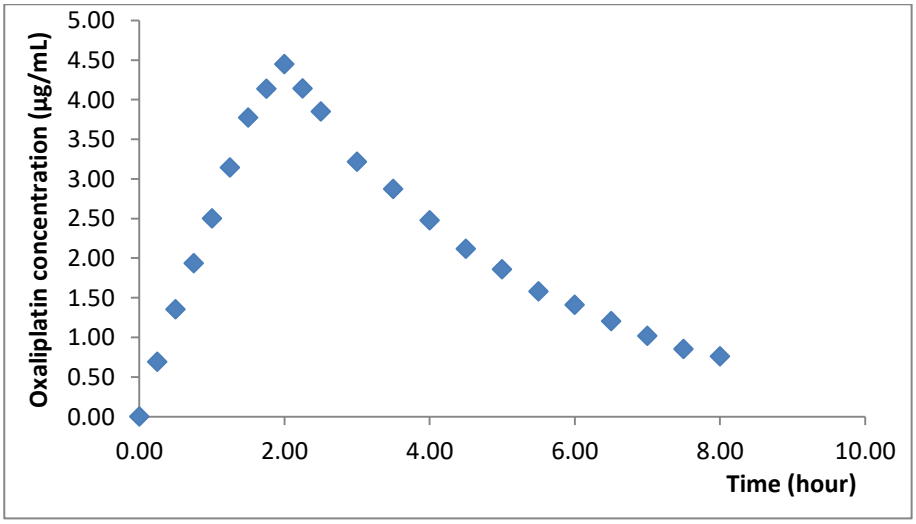

Figure C-24, Oxaliplatin concentration-time curve for Patient 5 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-24, Results of the simulation test on Patient 5 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 4.88 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.44	682.5	671.5
S1T0.25	0.25	0.6930	4.42	674.9	662.9
S2T0.5	0.50	1.3549	4.41	665.2	652.7
S3T0.75	0.75	1.9360	4.42	654.0	643.4
S4T1.0	1.00	2.5026	4.42	646.3	634.7
S5T1.25	1.25	3.1445	4.41	638.3	625.4
S6T1.5	1.50	3.7744	4.40	629.7	620.4
S7T1.75	1.75	4.1370	4.42	623.3	613.2
S8T2.0	2.00	4.4491	4.42	612.8	603.8
S9T2.25	2.25	4.1403	4.41	605.5	596.7
S10T2.5	2.50	3.8499	4.41	597.0	587.3
S11T3.0	3.00	3.2140	4.41	590.3	579.2
S12T3.5	3.50	2.8707	4.41	583.4	573.7
S13T4.0	4.00	2.4749	4.40	577.5	567.9
S14T4.5	4.50	2.1183	4.41	572.2	560.9
S15T5.0	5.00	1.8579	4.41	564.7	554.6
S16T5.5	5.50	1.5779	4.40	559.1	550.0
S17T6.0	6.00	1.4114	4.40	552.6	543.2
S18T6.5	6.50	1.2051	4.39	548.8	538.7
S19T7.0	7.00	1.0175	4.40	541.6	531.7
S20T7.5	7.50	0.8519	4.40	535.8	525.8
S21T8.0	8.00	0.7579	4.38	527.2	517.3

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

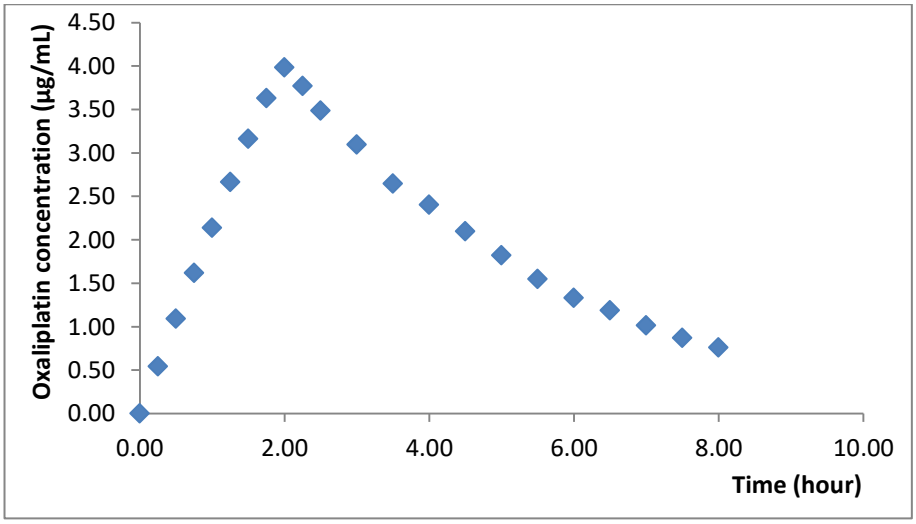

Figure C-25, Oxaliplatin concentration-time curve for Patient 5 with 2-hour infusions administered in Flat-fixed dosing method.

Table C-25, Results of the simulation test on Patient 5 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.35	682.0	667.7
S1T0.25	0.25	0.5432	4.28	674.2	661.8
S2T0.5	0.50	1.0926	4.27	666.5	653.3
S3T0.75	0.75	1.6200	4.29	656.9	644.6
S4T1.0	1.00	2.1388	4.28	647.3	636.5
S5T1.25	1.25	2.6651	4.27	639.0	626.2
S6T1.5	1.50	3.1622	4.29	629.1	618.4
S7T1.75	1.75	3.6315	4.30	619.9	608.6
S8T2.0	2.00	3.9859	4.31	614.6	601.6
S9T2.25	2.25	3.7723	4.30	602.8	591.0
S10T2.5	2.50	3.4866	4.30	593.9	583.8
S11T3.0	3.00	3.0977	4.31	594.4	581.0
S12T3.5	3.50	2.6466	4.32	587.9	575.9
S13T4.0	4.00	2.4036	4.31	581.5	568.9
S14T4.5	4.50	2.0979	4.31	574.4	562.2
S15T5.0	5.00	1.8229	4.32	570.4	560.6
S16T5.5	5.50	1.5473	4.32	562.7	553.2
S17T6.0	6.00	1.3307	4.32	554.2	543.1
S18T6.5	6.50	1.1873	4.32	543.5	532.7
S19T7.0	7.00	1.0148	4.35	537.3	526.3
S20T7.5	7.50	0.8695	4.33	526.5	517.5
S21T8.0	8.00	0.7614	4.32	510.9	500.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

C.6 Patient 6

The basic setting information for Patient 6 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 1.33 m^2; V_c = 412.6 mL; CL_{total} = 95.8 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

Table C-26, Results of the simulation test on Patient 6 with oxaliplatin administered in Individual dosing scheme with the drug dose of 2.83 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.28	412.6	403.3
S1T0.25	0.25	0.7300	4.19	400.8	392.5
S2T0.5	0.50	1.5026	4.18	390.5	381.1
S3T0.75	0.75	2.2440	4.19	380.6	370.8
S4T1.0	1.00	2.9795	4.19	374.6	366.0
S5T1.25	1.25	3.5859	4.20	365.0	354.6
S6T1.5	1.50	4.2538	4.19	357.0	346.1
S7T1.75	1.75	4.9113	4.20	345.1	335.6
S8T2.0	2.00	5.2413	4.21	343.0	334.1
S9T2.25	2.25	4.8283	4.21	334.8	323.1
S10T2.5	2.50	4.4176	4.21	321.8	313.1
S11T3.0	3.00	3.6283	4.21	316.5	306.5
S12T3.5	3.50	3.0294	4.22	306.5	299.5
S13T4.0	4.00	2.5511	4.23	300.6	292.1
S14T4.5	4.50	2.0594	4.22	292.2	284.2
S15T5.0	5.00	1.6769	4.22	283.4	275.3
S16T5.5	5.50	1.3672	4.23	276.4	268.4
S17T6.0	6.00	1.0893	4.24	271.5	267.9
S18T6.5	6.50	0.8702	4.23	269.5	256.8
S19T7.0	7.00	0.6884	4.20	261.8	251.5
S20T7.5	7.50	0.5591	4.20	250.2	238.5
S21T8.0	8.00	0.4333	4.17	241.1	229.7

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

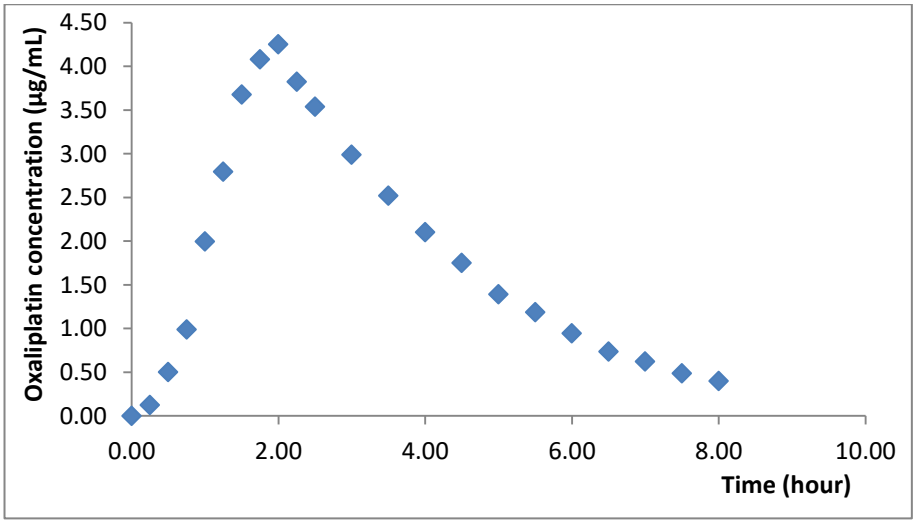

Figure C-26, Oxaliplatin concentration-time curve for Patient 6 with 2-hour infusions administered in Individual dosing scheme.

Table C-27, Results of the simulation test on Patient 6 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 2.75 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.27	411.6	401.6
S1T0.25	0.25	0.1229	4.25	402.2	392.3
S2T0.5	0.50	0.5001	4.24	393.3	383.3
S3T0.75	0.75	0.9866	4.24	384.3	374.3
S4T1.0	1.00	1.9954	4.24	374.5	364.7
S5T1.25	1.25	2.7944	4.25	365.0	354.3
S6T1.5	1.50	3.6776	4.25	355.9	346.0
S7T1.75	1.75	4.0790	4.26	346.5	336.7
S8T2.0	2.00	4.2497	4.25	337.4	327.3
S9T2.25	2.25	3.8239	4.26	328.2	317.3
S10T2.5	2.50	3.5370	4.25	319.0	309.0
S11T3.0	3.00	2.9876	4.25	310.8	300.6
S12T3.5	3.50	2.5183	4.24	302.5	292.2
S13T4.0	4.00	2.1029	4.23	294.3	283.4
S14T4.5	4.50	1.7502	4.23	285.6	275.8
S15T5.0	5.00	1.3904	4.23	277.5	267.4
S16T5.5	5.50	1.1853	4.23	269.4	258.6
S17T6.0	6.00	0.9440	4.23	260.9	250.8
S18T6.5	6.50	0.7359	4.24	252.4	242.6
S19T7.0	7.00	0.6228	4.23	242.9	233.5
S20T7.5	7.50	0.4881	4.24	234.4	224.0
S21T8.0	8.00	0.3981	4.23	220.3	210.5

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

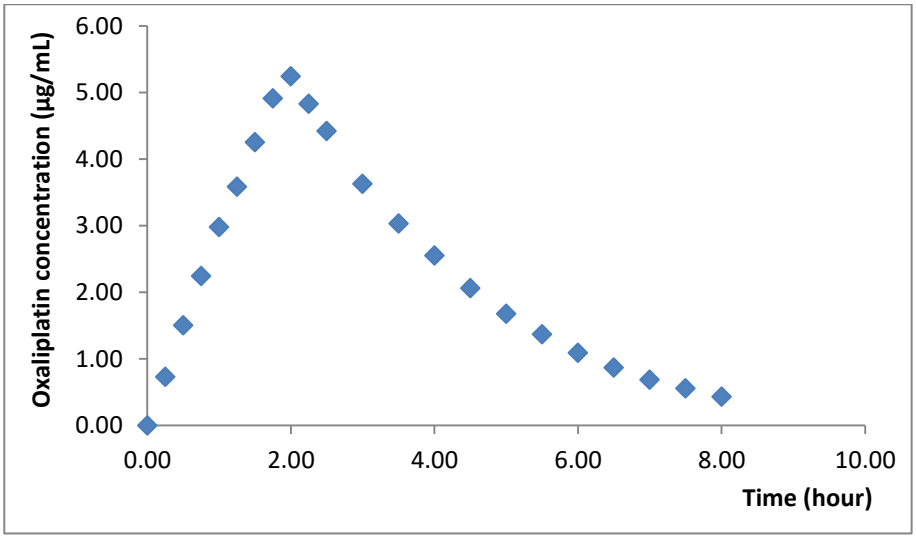

Figure C-27, Oxaliplatin concentration-time curve for Patient 6 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-28, Results of the simulation test on Patient 6 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 3.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.29	412.7	402.7
S1T0.25	0.25	0.2472	4.24	403.1	395.1
S2T0.5	0.50	0.8506	4.25	394.7	384.5
S3T0.75	0.75	1.5381	4.25	386.2	376.5
S4T1.0	1.00	2.4892	4.24	377.7	368.1
S5T1.25	1.25	3.0885	4.26	369.4	359.4
S6T1.5	1.50	3.6707	4.25	360.7	351.1
S7T1.75	1.75	4.3343	4.26	352.5	342.5
S8T2.0	2.00	4.5942	4.27	343.8	333.8
S9T2.25	2.25	4.2131	4.28	335.4	325.7
S10T2.5	2.50	3.8791	4.28	326.9	317.3
S11T3.0	3.00	3.3145	4.28	320.3	310.0
S12T3.5	3.50	2.8266	4.30	313.6	304.4
S13T4.0	4.00	2.4358	4.30	307.6	297.4
S14T4.5	4.50	2.0531	4.31	301.2	291.5
S15T5.0	5.00	1.7425	4.32	294.5	285.2
S16T5.5	5.50	1.4730	4.31	289.2	279.9
S17T6.0	6.00	1.2145	4.31	283.3	274.2
S18T6.5	6.50	1.0152	4.29	277.7	268.4
S19T7.0	7.00	0.8455	4.29	271.6	261.6
S20T7.5	7.50	0.7324	4.29	264.5	255.4
S21T8.0	8.00	0.5782	4.30	258.2	248.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

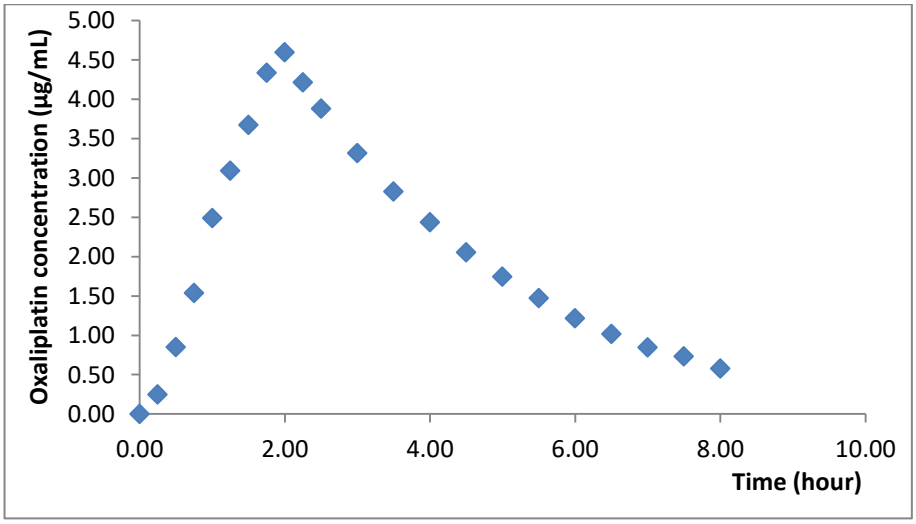

Figure C-28, Oxaliplatin concentration-time curve for Patient 6 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-29, Results of the simulation test on Patient 6 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 2.8 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.28	412.4	402.1
S1T0.25	0.25	0.6180	4.29	403.9	394.8
S2T0.5	0.50	1.4927	4.30	395.2	385.1
S3T0.75	0.75	2.0839	4.30	386.4	378.8
S4T1.0	1.00	2.7181	4.30	380.6	370.5
S5T1.25	1.25	2.8951	4.30	371.0	360.6
S6T1.5	1.50	3.5291	4.30	362.4	353.5
S7T1.75	1.75	3.9844	4.30	353.6	344.2
S8T2.0	2.00	4.3727	4.29	346.7	336.6
S9T2.25	2.25	3.9826	4.28	338.9	329.6
S10T2.5	2.50	3.6199	4.28	331.2	321.2
S11T3.0	3.00	3.0437	4.29	323.6	314.8
S12T3.5	3.50	2.5720	4.29	317.9	308.3
S13T4.0	4.00	2.1189	4.29	311.1	301.9
S14T4.5	4.50	1.7608	4.29	304.8	295.4
S15T5.0	5.00	1.4670	4.30	298.9	289.5
S16T5.5	5.50	1.0680	4.29	292.3	281.6
S17T6.0	6.00	0.8080	4.29	283.6	274.0
S18T6.5	6.50	0.6870	4.28	276.7	267.1
S19T7.0	7.00	0.5593	4.28	270.2	260.4
S20T7.5	7.50	0.4429	4.28	264.2	254.6
S21T8.0	8.00	0.3921	4.29	257.6	247.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

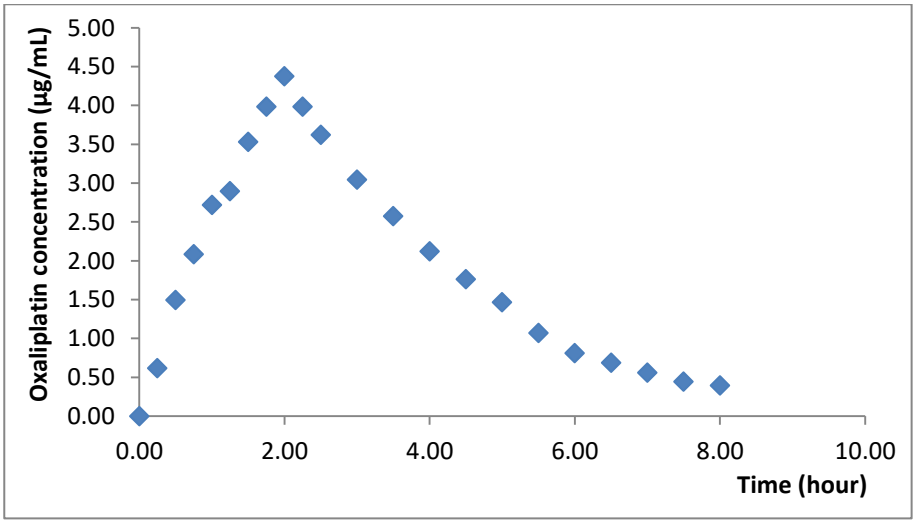

Figure C-29, Oxaliplatin concentration-time curve for Patient 6 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-30, Results of the simulation test on Patient 6 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.37	412.4	403.2
S1T0.25	0.25	0.8850	4.26	405.6	396.3
S2T0.5	0.50	1.6466	4.26	398.6	388.4
S3T0.75	0.75	2.5550	4.26	392.3	382.2
S4T1.0	1.00	3.6221	4.24	385.0	375.3
S5T1.25	1.25	4.5456	4.24	378.3	368.8
S6T1.5	1.50	4.7759	4.24	371.2	361.2
S7T1.75	1.75	5.5781	4.23	363.8	353.2
S8T2.0	2.00	6.1034	4.25	356.8	346.3
S9T2.25	2.25	5.3578	4.24	347.6	336.9
S10T2.5	2.50	5.1012	4.23	337.5	328.0
S11T3.0	3.00	4.3757	4.23	329.9	319.4
S12T3.5	3.50	3.7766	4.23	322.7	313.0
S13T4.0	4.00	3.2118	4.23	316.2	306.0
S14T4.5	4.50	2.7327	4.22	309.1	299.6
S15T5.0	5.00	2.3058	4.22	301.6	291.9
S16T5.5	5.50	1.9232	4.22	294.1	284.2
S17T6.0	6.00	1.6365	4.21	286.7	276.6
S18T6.5	6.50	1.3360	4.22	279.2	268.8
S19T7.0	7.00	1.1001	4.22	271.3	261.5
S20T7.5	7.50	0.9233	4.20	264.4	254.6
S21T8.0	8.00	0.7314	4.21	256.5	246.3

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

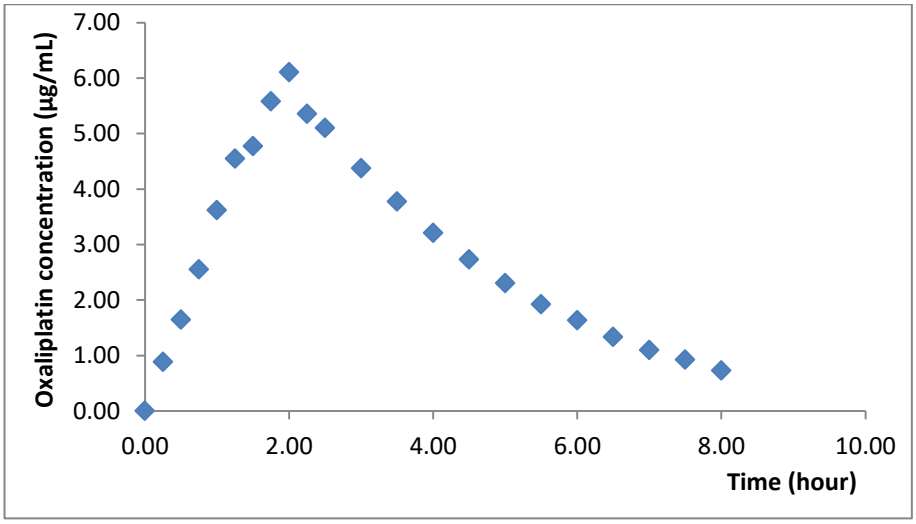

Figure C-30, Oxaliplatin concentration-time curve for Patient 6 with 2-hour infusions administered in Flat-fixed dosing method.

C.7 Patient 7

The basic setting information for Patient 7 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 1.99 m^2; V_c = 617.4 mL; CL_{total} = 143.3 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

Table C-31, Results of the simulation test on Patient 7 with oxaliplatin administered in Individual dosing scheme with the drug dose of 4.23 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.29	617.6	607.3
S1T0.25	0.25	0.7784	4.12	607.8	597.5
S2T0.5	0.50	1.4378	4.11	598.2	588.6
S3T0.75	0.75	2.1934	4.10	589.5	578.8
S4T1.0	1.00	2.9226	4.11	579.9	569.7
S5T1.25	1.25	3.4829	4.10	571.4	561.4
S6T1.5	1.50	3.9512	4.10	562.8	553.1
S7T1.75	1.75	4.3470	4.10	554.6	545.6
S8T2.0	2.00	4.7062	4.10	547.0	537.2
S9T2.25	2.25	4.3651	4.10	538.5	528.1
S10T2.5	2.50	4.0904	4.10	530.6	520.6
S11T3.0	3.00	3.5074	4.11	524.4	515.2
S12T3.5	3.50	3.0378	4.08	518.5	507.5
S13T4.0	4.00	2.5863	4.10	510.1	500.2
S14T4.5	4.50	2.1912	4.12	502.1	492.2
S15T5.0	5.00	1.8681	4.13	494.1	484.3
S16T5.5	5.50	1.5671	4.14	487.3	476.5
S17T6.0	6.00	1.3192	4.16	480.0	469.3
S18T6.5	6.50	1.1092	4.20	473.2	465.2
S19T7.0	7.00	0.9278	4.16	470.2	460.1
S20T7.5	7.50	0.7758	4.15	462.9	454.7
S21T8.0	8.00	0.6373	4.13	455.8	445.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

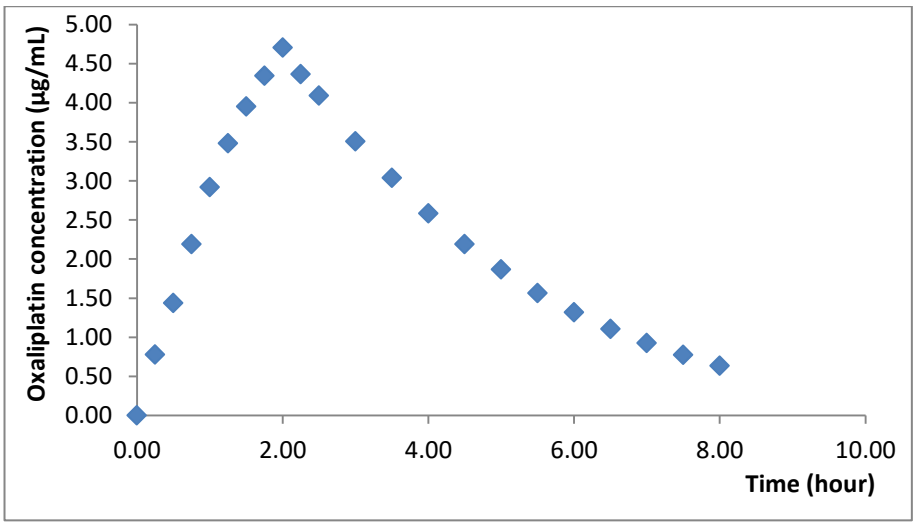

Figure C-31, Oxaliplatin concentration-time curve for Patient 7 with 2-hour infusions administered in Individual dosing scheme.

Table C-32, Results of the simulation test on Patient 7 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 4.25 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.22	616.1	596.0
S1T0.25	0.25	0.8018	4.19	599.1	589.0
S2T0.5	0.50	1.2091	4.19	585.9	573.8
S3T0.75	0.75	1.8157	4.19	577.1	566.1
S4T1.0	1.00	2.3655	4.19	569.6	559.9
S5T1.25	1.25	2.7377	4.19	562.3	551.6
S6T1.5	1.50	3.2164	4.17	553.8	543.9
S7T1.75	1.75	3.5326	4.16	545.4	536.6
S8T2.0	2.00	3.9707	4.17	538.6	527.7
S9T2.25	2.25	3.6321	4.14	531.4	520.9
S10T2.5	2.50	3.3792	4.14	522.6	512.7
S11T3.0	3.00	2.9084	4.13	516.9	506.2
S12T3.5	3.50	2.5079	4.13	510.1	500.6
S13T4.0	4.00	2.1261	4.13	505.1	494.5
S14T4.5	4.50	1.8102	4.16	499.0	489.0
S15T5.0	5.00	1.5502	4.15	492.9	483.6
S16T5.5	5.50	1.2883	4.16	487.8	477.1
S17T6.0	6.00	1.1032	4.15	480.8	470.3
S18T6.5	6.50	0.9485	4.13	474.4	463.8
S19T7.0	7.00	0.7642	4.11	468.1	457.9
S20T7.5	7.50	0.6460	4.12	461.7	452.3
S21T8.0	8.00	0.5682	4.10	455.4	445.7

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

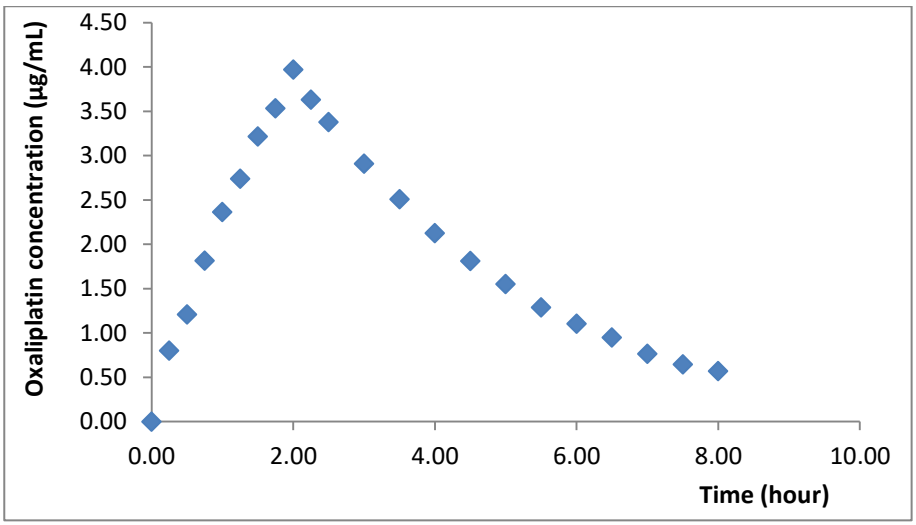

Figure C-32, Oxaliplatin concentration-time curve for Patient 7 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-33, Results of the simulation test on Patient 7 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.26	617.3	603.8
S1T0.25	0.25	0.7673	4.23	604.3	594.0
S2T0.5	0.50	1.4682	4.24	596.8	586.7
S3T0.75	0.75	2.1427	4.24	589.4	578.5
S4T1.0	1.00	2.6998	4.24	584.0	570.2
S5T1.25	1.25	3.1525	4.25	572.8	563.8
S6T1.5	1.50	3.5631	4.24	567.1	554.4
S7T1.75	1.75	3.9610	4.24	558.3	546.5
S8T2.0	2.00	4.2534	4.24	547.6	539.7
S9T2.25	2.25	3.7479	4.25	540.7	531.9
S10T2.5	2.50	3.2385	4.25	535.2	525.5
S11T3.0	3.00	2.7715	4.25	529.5	520.9
S12T3.5	3.50	2.2821	4.25	524.3	516.3
S13T4.0	4.00	1.7486	4.26	519.9	510.6
S14T4.5	4.50	1.5623	4.28	514.4	505.7
S15T5.0	5.00	1.2628	4.28	510.7	501.6
S16T5.5	5.50	1.1056	4.26	507.5	498.9
S17T6.0	6.00	0.9329	4.26	502.1	493.8
S18T6.5	6.50	0.8365	4.27	497.0	489.4
S19T7.0	7.00	0.7337	4.26	492.8	484.9
S20T7.5	7.50	0.7044	4.27	487.4	480.0
S21T8.0	8.00	0.5907	4.28	484.4	471.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

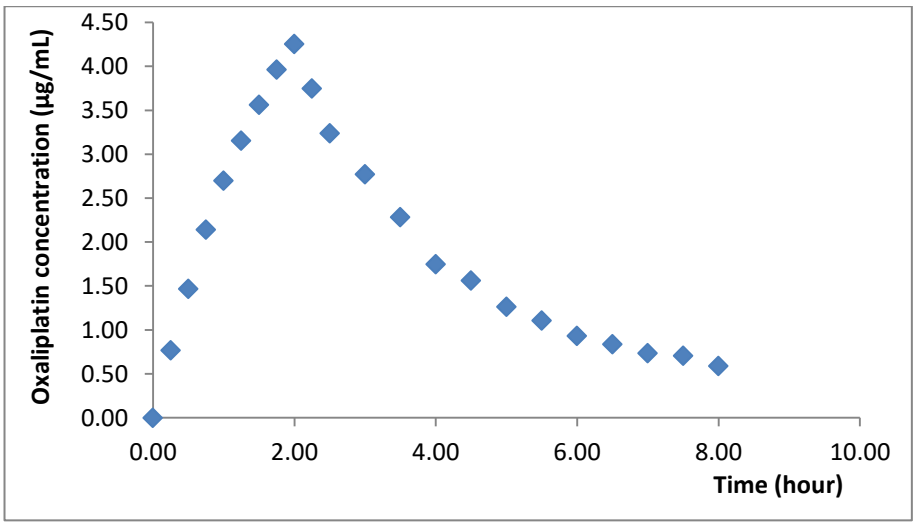

Figure C-33, Oxaliplatin concentration-time curve for Patient 7 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-34, Results of the simulation test on Patient 7 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 4.37 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.35	617.1	605.7
S1T0.25	0.25	0.7039	4.31	610.9	598.4
S2T0.5	0.50	1.3603	4.31	601.6	589.4
S3T0.75	0.75	1.9712	4.31	591.4	580.9
S4T1.0	1.00	2.5512	4.31	582.4	571.6
S5T1.25	1.25	3.0873	4.32	574.0	562.2
S6T1.5	1.50	3.5903	4.31	564.3	554.9
S7T1.75	1.75	4.1263	4.31	555.8	546.2
S8T2.0	2.00	4.3543	4.31	548.3	536.7
S9T2.25	2.25	4.0318	4.29	538.8	528.4
S10T2.5	2.50	3.6842	4.35	531.5	520.6
S11T3.0	3.00	3.2184	4.31	525.2	514.9
S12T3.5	3.50	2.7930	4.30	520.9	510.3
S13T4.0	4.00	2.3838	4.30	514.7	504.5
S14T4.5	4.50	2.0977	4.30	509.4	499.2
S15T5.0	5.00	1.8067	4.31	504.1	493.8
S16T5.5	5.50	1.5584	4.28	498.8	488.8
S17T6.0	6.00	1.3270	4.29	494.3	484.0
S18T6.5	6.50	1.0794	4.29	489.8	480.9
S19T7.0	7.00	0.9672	4.30	483.0	472.1
S20T7.5	7.50	0.8175	4.30	476.7	466.6
S21T8.0	8.00	0.7073	4.31	471.3	460.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

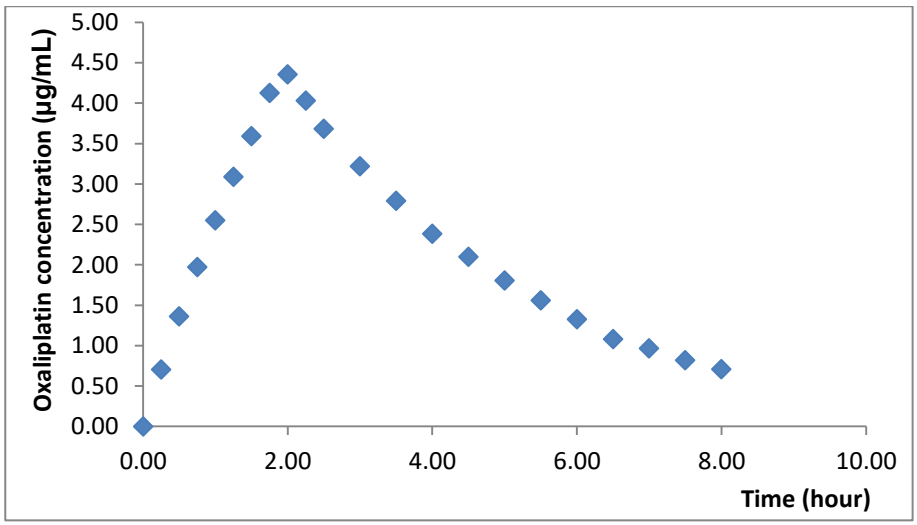

Figure C-34, Oxaliplatin concentration-time curve for Patient 7 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-35, Results of the simulation test on Patient 7 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.19	616.3	604.9
S1T0.25	0.25	0.5969	4.18	614.8	604.3
S2T0.5	0.50	1.1297	4.17	606.7	596.8
S3T0.75	0.75	1.7327	4.18	598.4	588.3
S4T1.0	1.00	2.2981	4.15	590.2	579.7
S5T1.25	1.25	2.9056	4.16	582.1	571.8
S6T1.5	1.50	3.3126	4.17	573.7	564.0
S7T1.75	1.75	3.8159	4.18	565.3	555.3
S8T2.0	2.00	4.2387	4.18	555.0	544.5
S9T2.25	2.25	3.9624	4.18	546.8	536.6
S10T2.5	2.50	3.6992	4.22	538.9	528.8
S11T3.0	3.00	3.2139	4.19	532.7	522.7
S12T3.5	3.50	2.8027	4.19	526.6	516.1
S13T4.0	4.00	2.4539	4.19	519.8	509.8
S14T4.5	4.50	2.1229	4.19	512.6	502.8
S15T5.0	5.00	1.7855	4.18	505.9	495.7
S16T5.5	5.50	1.5725	4.18	498.6	488.7
S17T6.0	6.00	1.3863	4.18	491.6	481.9
S18T6.5	6.50	1.1564	4.18	484.3	474.4
S19T7.0	7.00	1.0155	4.18	477.6	468.0
S20T7.5	7.50	0.8764	4.19	472.3	462.2
S21T8.0	8.00	0.7287	4.19	465.0	454.1

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

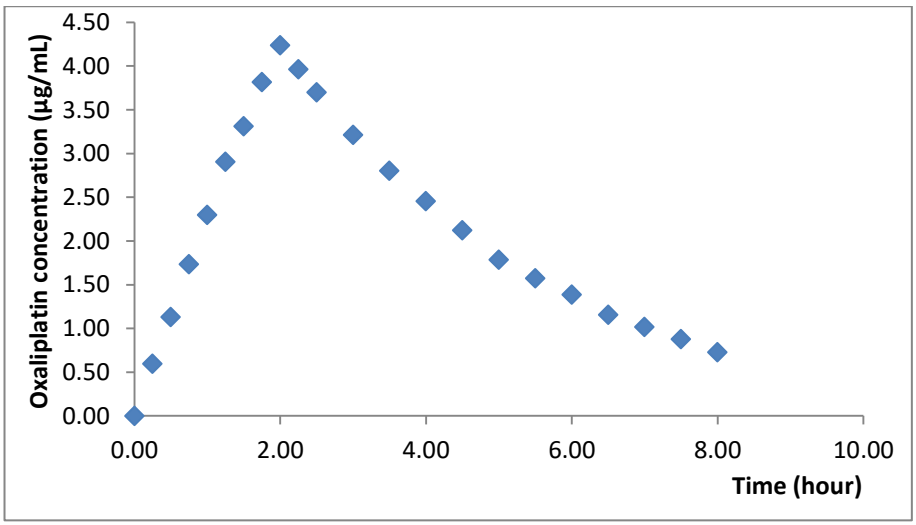

Figure C-35, Oxaliplatin concentration-time curve for Patient 7 with 2-hour infusions administered in Flat-fixed dosing method.

C.8 Patient 8

The basic setting information for Patient 8 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 1.72 m^2; V_c = 533.6 mL; CL_{total} = 124.0 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

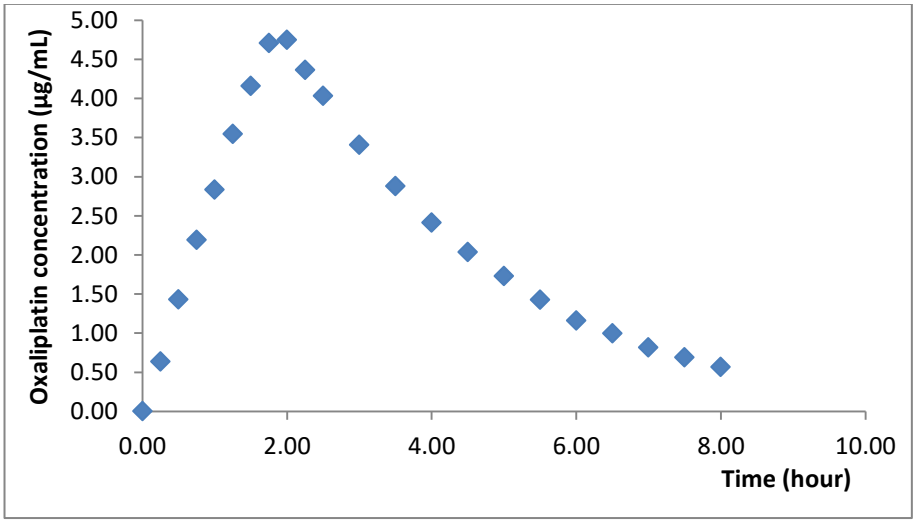

Figure C-36, Oxaliplatin concentration-time curve for Patient 8 with 2-hour infusions administered in Individual dosing scheme.

Table C-36, Results of the simulation test on Patient 8 with oxaliplatin administered in Individual dosing scheme with the drug dose of 3.66 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.21	533.6	524.4
S1T0.25	0.25	0.6364	4.15	528.8	514.4
S2T0.5	0.50	1.4302	4.15	516.1	505.7
S3T0.75	0.75	2.1922	4.13	507.7	498.1
S4T1.0	1.00	2.8343	4.13	499.4	489.8
S5T1.25	1.25	3.5461	4.12	492.0	482.8
S6T1.5	1.50	4.1577	4.13	484.5	474.5
S7T1.75	1.75	4.7059	4.12	466.9	458.3
S8T2.0	2.00	4.7477	4.10	456.0	445.0
S9T2.25	2.25	4.3648	4.09	452.1	440.9
S10T2.5	2.50	4.0303	4.08	442.4	432.6
S11T3.0	3.00	3.4042	4.08	437.1	424.6
S12T3.5	3.50	2.8763	4.08	431.2	421.6
S13T4.0	4.00	2.4096	4.09	424.7	414.8
S14T4.5	4.50	2.0347	4.10	421.1	410.1
S15T5.0	5.00	1.7269	4.12	411.6	404.7
S16T5.5	5.50	1.4240	4.13	406.4	397.7
S17T6.0	6.00	1.1590	4.13	397.7	387.6
S18T6.5	6.50	0.9965	4.14	386.3	377.2
S19T7.0	7.00	0.8169	4.15	377.8	368.9
S20T7.5	7.50	0.6878	4.12	371.7	361.4
S21T8.0	8.00	0.5656	4.10	371.3	356.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

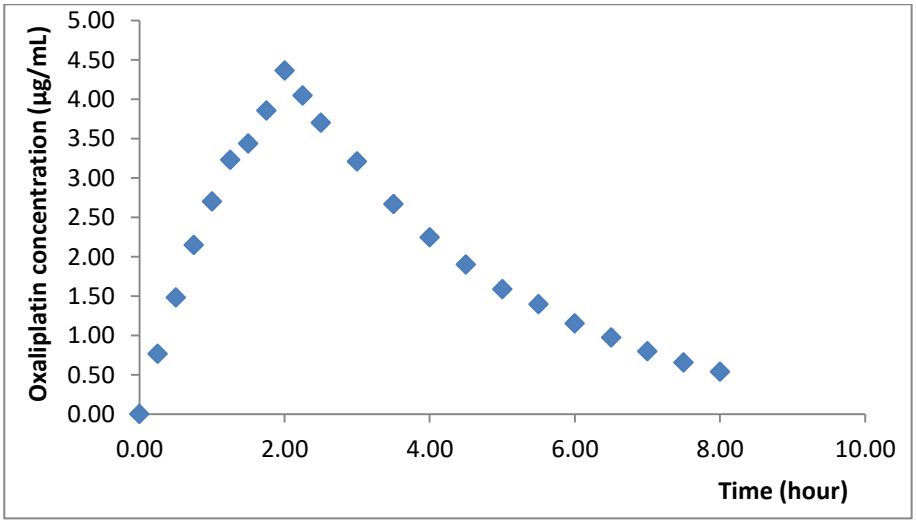

Figure C-37, Oxaliplatin concentration-time curve for Patient 8 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-37, Results of the simulation test on Patient 8 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 3.75 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.30	533.4	523.4
S1T0.25	0.25	0.7656	4.27	524.5	514.7
S2T0.5	0.50	1.4817	4.26	515.3	506.3
S3T0.75	0.75	2.1458	4.25	507.3	499.3
S4T1.0	1.00	2.7018	4.24	501.8	489.8
S5T1.25	1.25	3.2299	4.24	494.2	481.5
S6T1.5	1.50	3.4368	4.23	485.1	475.5
S7T1.75	1.75	3.8551	4.22	477.9	468.0
S8T2.0	2.00	4.3622	4.22	470.2	458.7
S9T2.25	2.25	4.0461	4.21	459.1	448.2
S10T2.5	2.50	3.7019	4.21	450.2	438.7
S11T3.0	3.00	3.2070	4.20	443.3	433.3
S12T3.5	3.50	2.6661	4.19	437.5	428.6
S13T4.0	4.00	2.2457	4.18	430.9	420.2
S14T4.5	4.50	1.9014	4.18	422.9	413.1
S15T5.0	5.00	1.5854	4.18	415.3	405.2
S16T5.5	5.50	1.3961	4.17	407.5	396.7
S17T6.0	6.00	1.1510	4.17	399.4	389.4
S18T6.5	6.50	0.9713	4.16	392.3	382.3
S19T7.0	7.00	0.8001	4.16	384.8	374.8
S20T7.5	7.50	0.6558	4.15	370.5	359.5
S21T8.0	8.00	0.5386	4.15	361.9	351.9

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

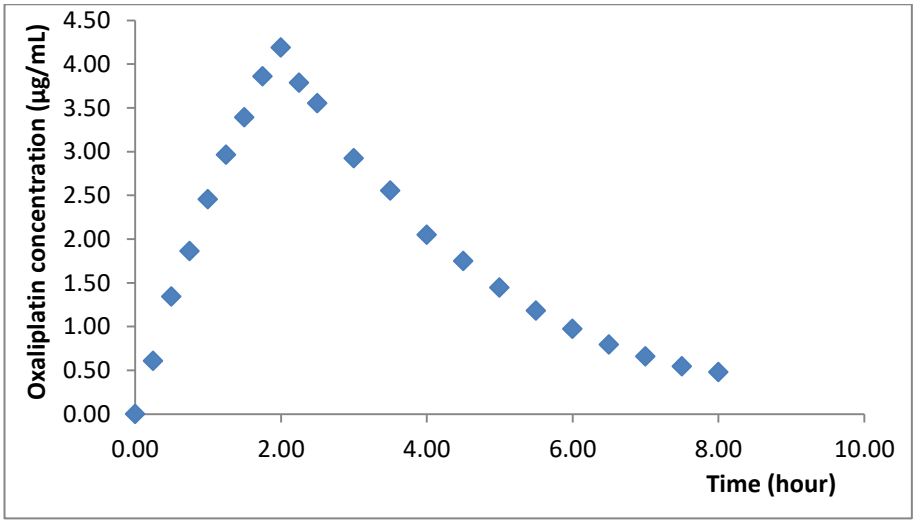

Figure C-38, Oxaliplatin concentration-time curve for Patient 8 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-38, Results of the simulation test on Patient 8 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 3.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.25	533.7	520.0
S1T0.25	0.25	0.6081	4.22	524.0	510.0
S2T0.5	0.50	1.3431	4.22	514.3	502.9
S3T0.75	0.75	1.8641	4.20	506.0	494.6
S4T1.0	1.00	2.4562	4.21	496.3	486.3
S5T1.25	1.25	2.9645	4.22	487.8	478.0
S6T1.5	1.50	3.3913	4.22	482.1	469.2
S7T1.75	1.75	3.8597	4.21	473.0	458.3
S8T2.0	2.00	4.1917	4.21	463.5	440.0
S9T2.25	2.25	3.7883	4.20	453.5	440.0
S10T2.5	2.50	3.5542	4.21	443.9	427.9
S11T3.0	3.00	2.9227	4.20	431.6	421.4
S12T3.5	3.50	2.5527	4.20	425.2	417.0
S13T4.0	4.00	2.0489	4.21	420.5	410.5
S14T4.5	4.50	1.7487	4.20	413.4	403.2
S15T5.0	5.00	1.4459	4.21	406.4	397.4
S16T5.5	5.50	1.1831	4.19	399.3	390.4
S17T6.0	6.00	0.9740	4.22	391.2	382.8
S18T6.5	6.50	0.7934	4.21	384.8	375.2
S19T7.0	7.00	0.6581	4.21	375.0	369.5
S20T7.5	7.50	0.5437	4.21	372.6	360.6
S21T8.0	8.00	0.4795	4.21	365.7	355.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

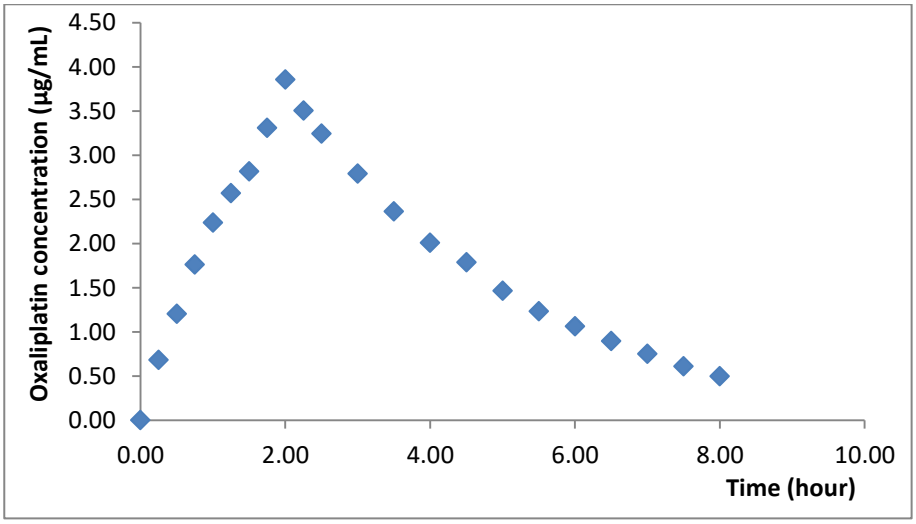

Figure C-39, Oxaliplatin concentration-time curve for Patient 8 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-39, Results of the simulation test on Patient 8 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 3.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.19	533.6	523.9
S1T0.25	0.25	0.6810	4.16	524.3	514.8
S2T0.5	0.50	1.2028	4.15	515.0	505.4
S3T0.75	0.75	1.7627	4.16	506.1	496.5
S4T1.0	1.00	2.2370	4.15	497.2	487.1
S5T1.25	1.25	2.5712	4.14	488.1	478.1
S6T1.5	1.50	2.8169	4.15	478.6	469.7
S7T1.75	1.75	3.3099	4.15	470.2	460.5
S8T2.0	2.00	3.8551	4.15	461.0	449.8
S9T2.25	2.25	3.5048	4.15	450.6	440.4
S10T2.5	2.50	3.2443	4.14	441.2	432.0
S11T3.0	3.00	2.7910	4.14	434.7	425.2
S12T3.5	3.50	2.3636	4.15	430.1	420.6
S13T4.0	4.00	2.0098	4.15	426.4	416.9
S14T4.5	4.50	1.7884	4.15	422.5	406.9
S15T5.0	5.00	1.4646	4.15	410.6	401.0
S16T5.5	5.50	1.2332	4.15	405.3	395.5
S17T6.0	6.00	1.0625	4.15	400.7	391.0
S18T6.5	6.50	0.8953	4.15	396.3	387.1
S19T7.0	7.00	0.7512	4.16	391.9	382.2
S20T7.5	7.50	0.6116	4.17	386.9	377.5
S21T8.0	8.00	0.4973	4.16	381.2	371.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

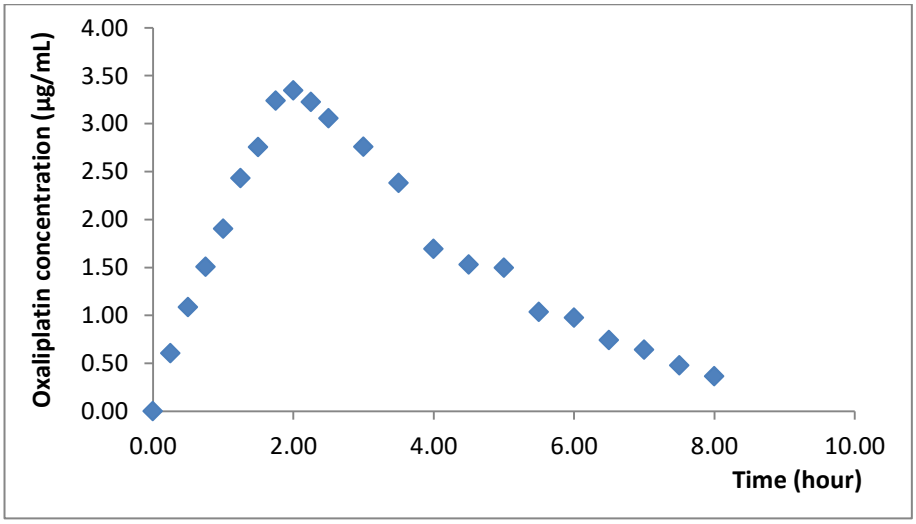

Figure C-40, Oxaliplatin concentration-time curve for Patient 8 with 2-hour infusions administered in Flat-fixed dosing method.

Table C-40, Results of the simulation test on Patient 8 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.16	533.8	524.0
S1T0.25	0.25	0.6040	4.09	525.1	514.8
S2T0.5	0.50	1.0877	4.10	515.9	506.5
S3T0.75	0.75	1.5074	4.09	507.9	497.9
S4T1.0	1.00	1.9049	4.09	498.3	488.9
S5T1.25	1.25	2.4305	4.09	489.8	479.8
S6T1.5	1.50	2.7557	4.10	481.2	471.2
S7T1.75	1.75	3.2408	4.10	472.6	463.0
S8T2.0	2.00	3.3480	4.09	464.5	454.5
S9T2.25	2.25	3.2247	4.10	456.0	446.3
S10T2.5	2.50	3.0543	4.10	447.8	438.0
S11T3.0	3.00	2.7588	4.11	439.5	429.6
S12T3.5	3.50	2.3827	4.11	432.7	424.1
S13T4.0	4.00	1.6947	4.10	428.5	418.9
S14T4.5	4.50	1.5297	4.10	422.2	412.8
S15T5.0	5.00	1.4981	4.10	416.5	406.5
S16T5.5	5.50	1.0347	4.12	409.9	400.7
S17T6.0	6.00	0.9754	4.12	404.9	395.2
S18T6.5	6.50	0.7408	4.10	399.0	388.9
S19T7.0	7.00	0.6414	4.13	393.4	384.6
S20T7.5	7.50	0.4779	4.12	389.1	380.6
S21T8.0	8.00	0.3667	4.11	383.2	372.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

C.9 Patient 9

The basic setting information for Patient 9 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 2.52 m^2; V_c = 781.8 mL; CL_{total} = 181.4 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

Table C-41, Results of the simulation test on Patient 9 with oxaliplatin administered in Individual dosing scheme with the drug dose of 5.36 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.26	781.2	773.2
S1T0.25	0.25	0.7069	4.24	793.2	784.0
S2T0.5	0.50	1.2968	4.24	785.8	774.9
S3T0.75	0.75	1.8192	4.23	777.8	768.0
S4T1.0	1.00	2.4378	4.22	770.4	761.1
S5T1.25	1.25	2.9138	4.22	763.2	753.5
S6T1.5	1.50	3.4722	4.22	756.2	746.9
S7T1.75	1.75	4.0254	4.22	749.6	740.4
S8T2.0	2.00	4.2402	4.22	742.2	732.2
S9T2.25	2.25	3.9252	4.23	734.4	723.8
S10T2.5	2.50	3.6784	4.23	727.5	717.9
S11T3.0	3.00	3.1830	4.23	723.6	714.3
S12T3.5	3.50	2.7402	4.24	719.4	709.7
S13T4.0	4.00	2.4111	4.24	711.5	702.0
S14T4.5	4.50	2.1093	4.24	707.5	697.8
S15T5.0	5.00	1.8276	4.24	703.9	694.1
S16T5.5	5.50	1.6008	4.22	696.1	687.6
S17T6.0	6.00	1.3749	4.21	691.6	682.5
S18T6.5	6.50	1.1602	4.19	688.7	679.5
S19T7.0	7.00	1.0147	4.19	682.7	674.4
S20T7.5	7.50	0.8667	4.20	677.6	667.8
S21T8.0	8.00	0.7351	4.19	658.3	654.0

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

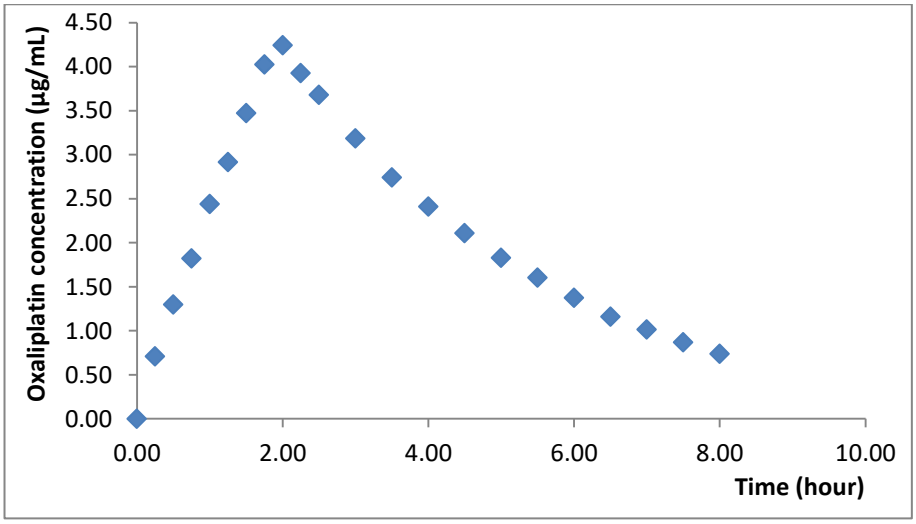

Figure C-41, Oxaliplatin concentration-time curve for Patient 9 with 2-hour infusions administered in Individual dosing scheme.

Table C-42, Results of the simulation test on Patient 9 with oxaliplatin administered in Dose-banding with 5% deviation with the drug dose of 5.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.36	781.9	774.0
S1T0.25	0.25	0.8305	4.32	779.0	768.2
S2T0.5	0.50	1.2594	4.31	768.8	756.5
S3T0.75	0.75	1.8844	4.30	758.8	748.5
S4T1.0	1.00	2.3579	4.30	751.6	743.1
S5T1.25	1.25	2.9216	4.29	743.3	733.8
S6T1.5	1.50	3.4738	4.29	737.4	728.8
S7T1.75	1.75	3.9538	4.29	733.1	721.3
S8T2.0	2.00	4.2977	4.28	723.2	714.5
S9T2.25	2.25	3.9954	4.29	713.1	703.3
S10T2.5	2.50	3.7361	4.29	705.4	695.3
S11T3.0	3.00	3.2235	4.27	702.5	694.8
S12T3.5	3.50	2.8351	4.28	697.2	688.8
S13T4.0	4.00	2.4524	4.28	689.7	678.7
S14T4.5	4.50	2.1317	4.29	685.7	681.4
S15T5.0	5.00	1.8245	4.29	678.2	669.6
S16T5.5	5.50	1.5576	4.29	675.2	671.8
S17T6.0	6.00	1.3570	4.28	669.3	658.2
S18T6.5	6.50	1.2071	4.28	663.6	658.2
S19T7.0	7.00	1.0383	4.27	656.7	648.0
S20T7.5	7.50	0.8841	4.25	652.0	642.9
S21T8.0	8.00	0.7619	4.26	635.5	625.2

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

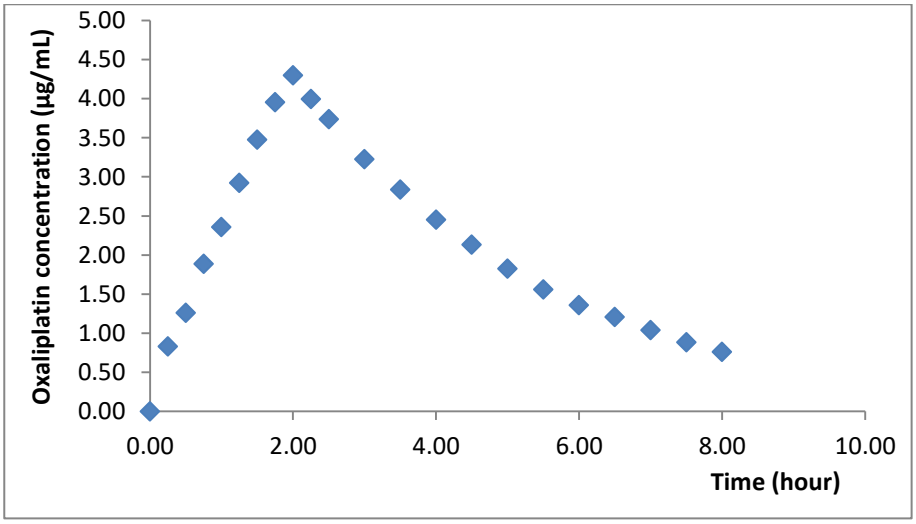

Figure C-42, Oxaliplatin concentration-time curve for Patient 9 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-43, Results of the simulation test on Patient 9 with oxaliplatin administered in Dose-banding with 10% deviation with the drug dose of 5.75 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.32	782.5	771.3
S1T0.25	0.25	0.6183	4.30	774.3	765.6
S2T0.5	0.50	1.3274	4.29	766.6	755.8
S3T0.75	0.75	1.9448	4.29	757.5	748.6
S4T1.0	1.00	2.6048	4.29	749.2	738.6
S5T1.25	1.25	3.0321	4.30	739.4	729.3
S6T1.5	1.50	3.4235	4.30	731.5	721.4
S7T1.75	1.75	3.9454	4.30	722.3	711.4
S8T2.0	2.00	4.1182	4.30	710.7	699.5
S9T2.25	2.25	3.7884	4.31	699.5	689.7
S10T2.5	2.50	3.5208	4.32	693.5	681.3
S11T3.0	3.00	3.0417	4.32	688.5	678.6
S12T3.5	3.50	2.6919	4.33	681.4	670.6
S13T4.0	4.00	2.3302	4.33	674.1	664.0
S14T4.5	4.50	2.0068	4.34	667.0	657.0
S15T5.0	5.00	1.7429	4.34	659.1	650.2
S16T5.5	5.50	1.4667	4.34	652.4	641.7
S17T6.0	6.00	1.2787	4.35	645.0	636.0
S18T6.5	6.50	1.0683	4.34	638.7	629.4
S19T7.0	7.00	0.8898	4.34	631.6	621.6
S20T7.5	7.50	0.8163	4.33	624.3	615.5
S21T8.0	8.00	0.6580	4.33	618.4	608.4

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

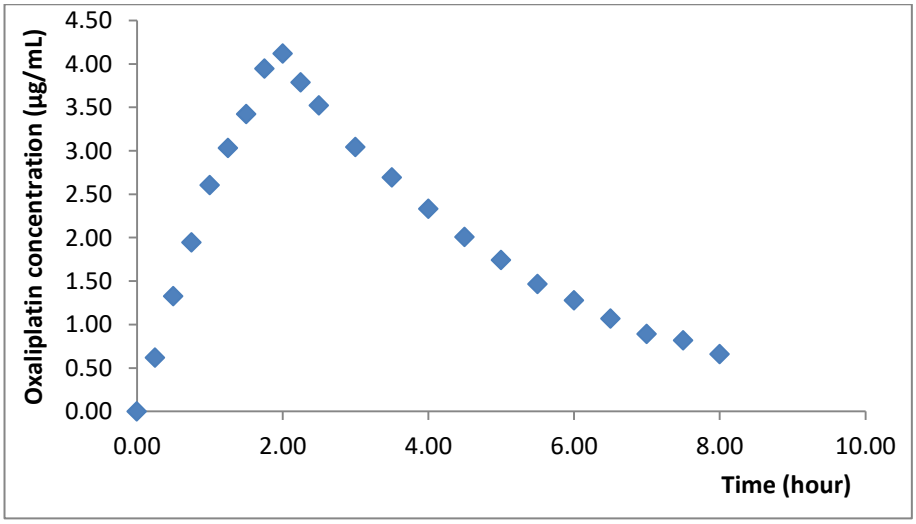

Figure C-43, Oxaliplatin concentration-time curve for Patient 9 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-44, Results of the simulation test on Patient 9 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 5.46 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.50	778.5	767.3
S1T0.25	0.25	1.0263	4.46	762.8	753.2
S2T0.5	0.50	1.3962	4.45	754.8	740.7
S3T0.75	0.75	2.0020	4.46	741.0	724.8
S4T1.0	1.00	2.6081	4.47	724.9	719.4
S5T1.25	1.25	3.1317	4.45	718.5	707.2
S6T1.5	1.50	3.6732	4.46	707.3	692.8
S7T1.75	1.75	4.1828	4.47	698.1	684.2
S8T2.0	2.00	4.6267	4.46	686.3	674.5
S9T2.25	2.25	4.3297	4.47	676.8	662.6
S10T2.5	2.50	3.9930	4.45	662.3	653.2
S11T3.0	3.00	3.4816	4.45	654.6	643.0
S12T3.5	3.50	3.0309	4.43	644.5	631.1
S13T4.0	4.00	2.6473	4.43	631.0	623.3
S14T4.5	4.50	2.2570	4.42	619.6	610.3
S15T5.0	5.00	1.9208	4.41	609.5	598.8
S16T5.5	5.50	1.6462	4.40	597.2	585.4
S17T6.0	6.00	1.4144	4.41	583.7	572.6
S18T6.5	6.50	1.1908	4.41	571.1	553.7
S19T7.0	7.00	1.0453	4.43	550.7	538.5
S20T7.5	7.50	0.8483	4.40	535.0	524.4
S21T8.0	8.00	0.7114	4.41	521.8	512.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

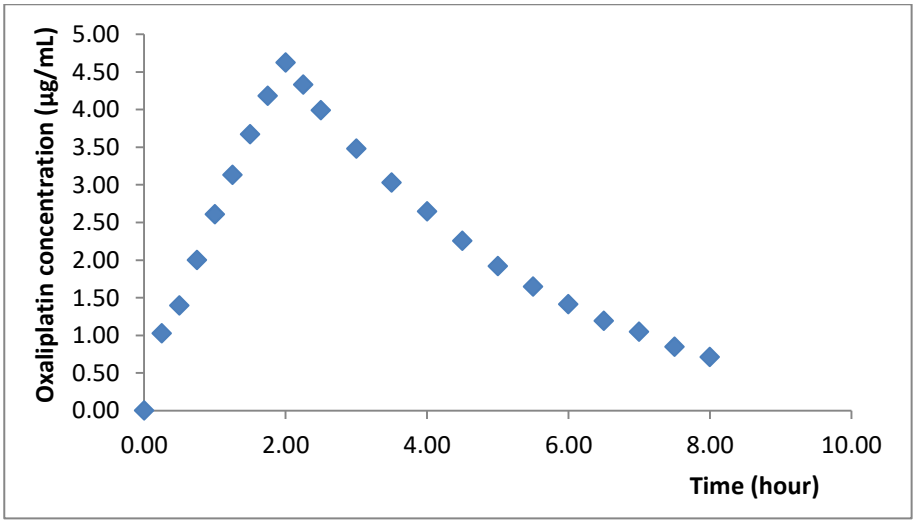

Figure C-44, Oxaliplatin concentration-time curve for Patient 9 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-45, Results of the simulation test on Patient 9 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.25	781.8	774.6
S1T0.25	0.25	0.5839	4.21	776.0	764.6
S2T0.5	0.50	0.8787	4.22	764.8	756.2
S3T0.75	0.75	1.3952	4.20	758.7	748.6
S4T1.0	1.00	1.8009	4.20	748.8	739.7
S5T1.25	1.25	2.2217	4.20	739.2	728.5
S6T1.5	1.50	2.5466	4.20	730.9	720.4
S7T1.75	1.75	2.9371	4.20	721.1	707.3
S8T2.0	2.00	3.1679	4.26	708.9	698.4
S9T2.25	2.25	3.0612	4.20	701.3	690.8
S10T2.5	2.50	2.8221	4.21	691.5	684.2
S11T3.0	3.00	2.4955	4.19	688.3	678.7
S12T3.5	3.50	2.1252	4.19	680.5	668.9
S13T4.0	4.00	1.8300	4.19	678.7	669.4
S14T4.5	4.50	1.6029	4.19	673.3	663.6
S15T5.0	5.00	1.3401	4.20	668.3	657.7
S16T5.5	5.50	1.1392	4.20	663.1	654.8
S17T6.0	6.00	1.0048	4.20	656.9	643.8
S18T6.5	6.50	0.8473	4.19	648.2	638.1
S19T7.0	7.00	0.7367	4.19	643.8	633.7
S20T7.5	7.50	0.6104	4.19	636.1	626.9
S21T8.0	8.00	0.5093	4.18	630.1	620.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

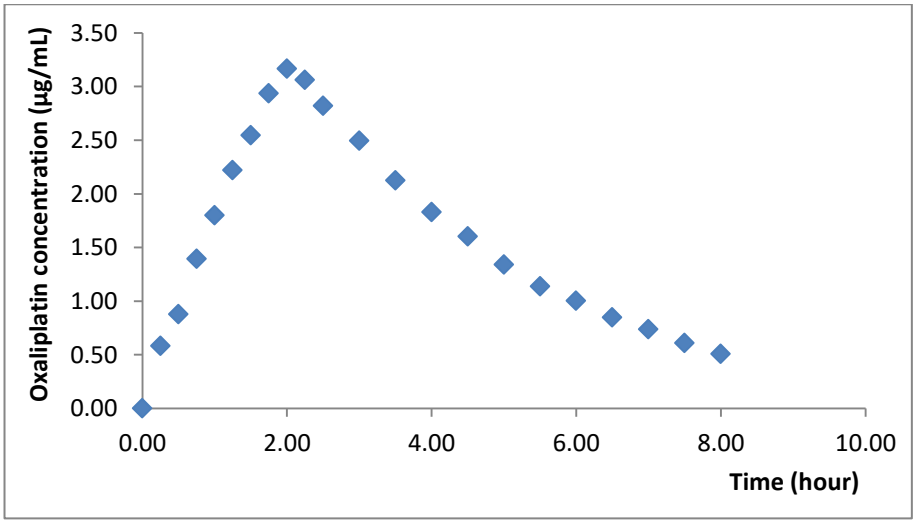

Figure C-45, Oxaliplatin concentration-time curve for Patient 9 with 2-hour infusions administered in Flat-fixed dosing method.

C.10 Patient 10

The basic setting information for Patient 10 was calculated based on the study schedule mentioned in Chapter 4, which was listed below:

BSA = 2.04 m^2; V_c = 632.9 mL; CL_{total} = 146.9 mL/h.

[V_c: distribution volume of oxaliplatin; CL_{total}: the total clearance of oxaliplatin.]

Table C-46, Results of the simulation test on Patient 10 with oxaliplatin administered in Individual dosing scheme with the drug dose of 4.34 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (μg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.29	631.7	622.3
S1T0.25	0.25	0.8716	4.23	624.0	613.9
S2T0.5	0.50	1.5275	4.22	614.5	606.4
S3T0.75	0.75	2.1684	4.21	608.4	596.8
S4T1.0	1.00	2.8103	4.21	597.8	588.0
S5T1.25	1.25	3.4091	4.20	589.0	578.8
S6T1.5	1.50	3.9923	4.20	580.7	570.1
S7T1.75	1.75	4.2616	4.18	571.5	561.5
S8T2.0	2.00	4.6187	4.19	559.1	547.7
S9T2.25	2.25	4.3476	4.18	553.6	545.1
S10T2.5	2.50	4.0392	4.16	544.7	538.7
S11T3.0	3.00	3.4737	4.18	540.7	531.4
S12T3.5	3.50	3.0115	4.17	536.5	526.9
S13T4.0	4.00	2.5495	4.18	532.2	522.3
S14T4.5	4.50	2.1599	4.19	527.6	518.4
S15T5.0	5.00	1.8208	4.21	522.5	512.6
S16T5.5	5.50	1.5756	4.22	516.9	506.7
S17T6.0	6.00	1.3321	4.20	511.7	501.4
S18T6.5	6.50	1.1400	4.18	498.7	487.9
S19T7.0	7.00	0.9483	4.16	493.4	484.3
S20T7.5	7.50	0.8349	4.14	479.0	469.6
S21T8.0	8.00	0.6845	4.15	476.3	466.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

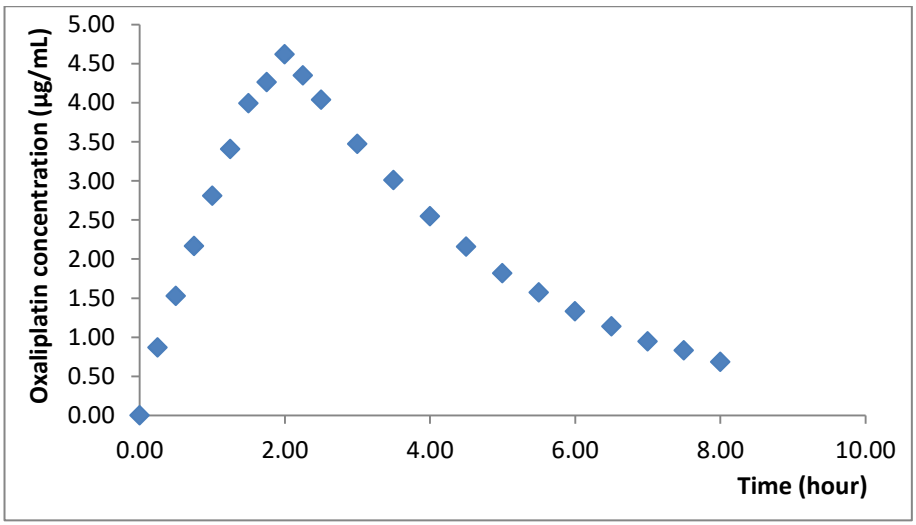

Figure C-46, Oxaliplatin concentration-time curve for Patient 10 with 2-hour infusions administered in Individual dosing scheme.

Table C-47, Results of the simulation test on Patient 10 with oxaliplatin administered in Dose-banding scheme with 5% deviation with the drug dose of 4.25 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.21	633.2	621.1
S1T0.25	0.25	0.6325	4.17	620.6	609.0
S2T0.5	0.50	1.2130	4.16	614.1	603.5
S3T0.75	0.75	1.8145	4.16	606.9	594.9
S4T1.0	1.00	2.3507	4.16	599.6	590.8
S5T1.25	1.25	2.8517	4.15	594.4	584.8
S6T1.5	1.50	3.2702	4.15	589.2	577.2
S7T1.75	1.75	3.7176	4.15	582.3	570.2
S8T2.0	2.00	3.9431	4.15	572.7	560.2
S9T2.25	2.25	3.6723	4.16	567.1	550.0
S10T2.5	2.50	3.4298	4.14	555.8	546.5
S11T3.0	3.00	2.8992	4.13	556.7	548.0
S12T3.5	3.50	2.4980	4.12	552.3	543.4
S13T4.0	4.00	2.1743	4.12	547.3	532.4
S14T4.5	4.50	1.8015	4.12	538.1	527.9
S15T5.0	5.00	1.6052	4.13	533.1	522.3
S16T5.5	5.50	1.3721	4.14	521.8	510.8
S17T6.0	6.00	1.1648	4.15	514.4	504.5
S18T6.5	6.50	0.9749	4.16	507.3	496.3
S19T7.0	7.00	0.8396	4.15	500.5	489.6
S20T7.5	7.50	0.7286	4.15	493.1	479.5
S21T8.0	8.00	0.6274	4.14	488.1	477.6

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

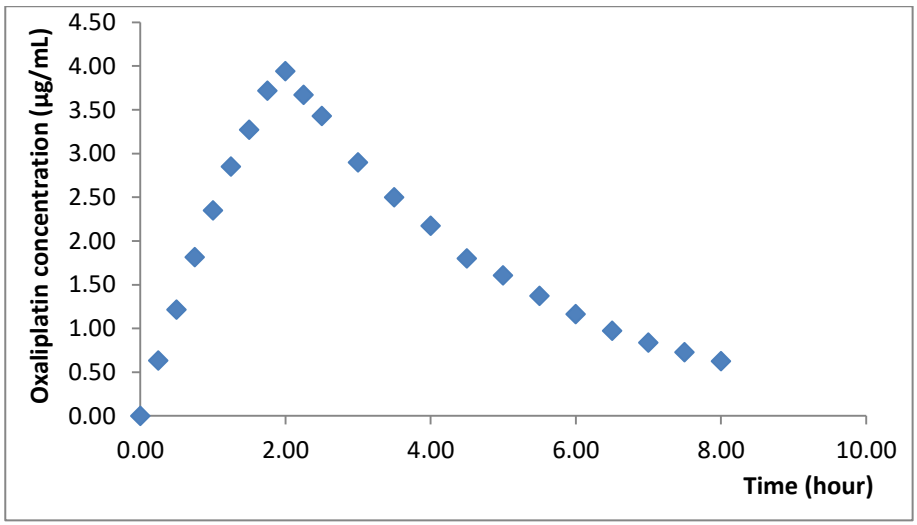

Figure C-47, Oxaliplatin concentration-time curve for Patient 10 with 2-hour infusions administered in Dose-banding scheme with 5% deviation.

Table C-48, Results of the simulation test on Patient 10 with oxaliplatin administered in Dose-banding scheme with 10% deviation with the drug dose of 4.5 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.19	632.7	622.4
S1T0.25	0.25	0.6617	4.17	623.9	614.5
S2T0.5	0.50	1.2355	4.18	617.8	609.0
S3T0.75	0.75	1.8200	4.18	610.2	598.4
S4T1.0	1.00	2.1583	4.18	602.8	591.0
S5T1.25	1.25	2.7784	4.18	594.3	584.9
S6T1.5	1.50	3.2296	4.17	592.2	579.8
S7T1.75	1.75	3.7441	4.18	580.6	569.5
S8T2.0	2.00	3.8633	4.18	570.4	560.9
S9T2.25	2.25	3.5348	4.17	564.6	553.6
S10T2.5	2.50	3.4114	4.17	556.9	545.9
S11T3.0	3.00	2.9851	4.17	551.5	540.6
S12T3.5	3.50	2.6670	4.18	544.8	534.0
S13T4.0	4.00	2.3217	4.17	538.5	527.3
S14T4.5	4.50	1.8731	4.16	532.4	521.7
S15T5.0	5.00	1.6386	4.17	526.8	515.2
S16T5.5	5.50	1.5034	4.17	520.2	510.7
S17T6.0	6.00	1.2890	4.17	514.3	504.3
S18T6.5	6.50	1.0909	4.17	508.8	499.5
S19T7.0	7.00	0.9467	4.17	504.8	493.3
S20T7.5	7.50	0.7833	4.17	497.6	487.9
S21T8.0	8.00	0.6594	4.17	493.1	482.8

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

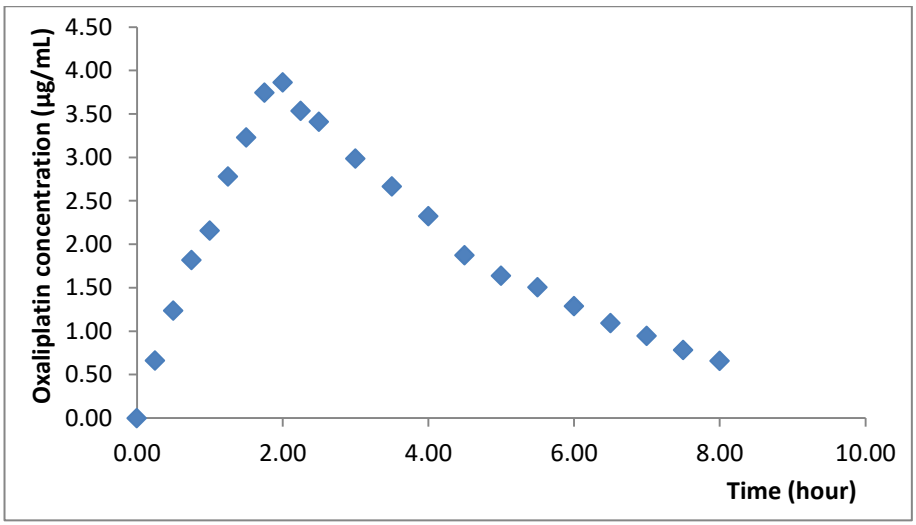

Figure C-48, Oxaliplatin concentration-time curve for Patient 10 with 2-hour infusions administered in Dose-banding scheme with 10% deviation.

Table C-49, Results of the simulation test on Patient 10 with oxaliplatin administered in Logarithmic Dose-banding scheme with the drug dose of 4.37 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.25	632.9	622.7
S1T0.25	0.25	0.6118	4.24	625.8	616.2
S2T0.5	0.50	1.2108	4.25	618.6	608.8
S3T0.75	0.75	1.7186	4.25	612.1	600.9
S4T1.0	1.00	2.1818	4.25	603.0	593.6
S5T1.25	1.25	2.5384	4.25	595.5	585.6
S6T1.5	1.50	2.9547	4.25	587.0	577.5
S7T1.75	1.75	3.4216	4.24	579.8	570.0
S8T2.0	2.00	3.8919	4.25	572.3	561.2
S9T2.25	2.25	3.5188	4.26	564.7	554.3
S10T2.5	2.50	3.2979	4.25	557.4	547.2
S11T3.0	3.00	2.8366	4.26	552.9	543.4
S12T3.5	3.50	2.4848	4.27	549.8	539.9
S13T4.0	4.00	2.1763	4.26	544.7	535.0
S14T4.5	4.50	1.9002	4.28	539.3	529.5
S15T5.0	5.00	1.6230	4.27	534.5	524.4
S16T5.5	5.50	1.4129	4.28	530.0	520.3
S17T6.0	6.00	1.2108	4.28	525.2	515.8
S18T6.5	6.50	1.0608	4.28	521.8	511.9
S19T7.0	7.00	0.8988	4.28	516.6	507.0
S20T7.5	7.50	0.7801	4.28	511.5	502.0
S21T8.0	8.00	0.6557	4.28	505.8	495.2

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

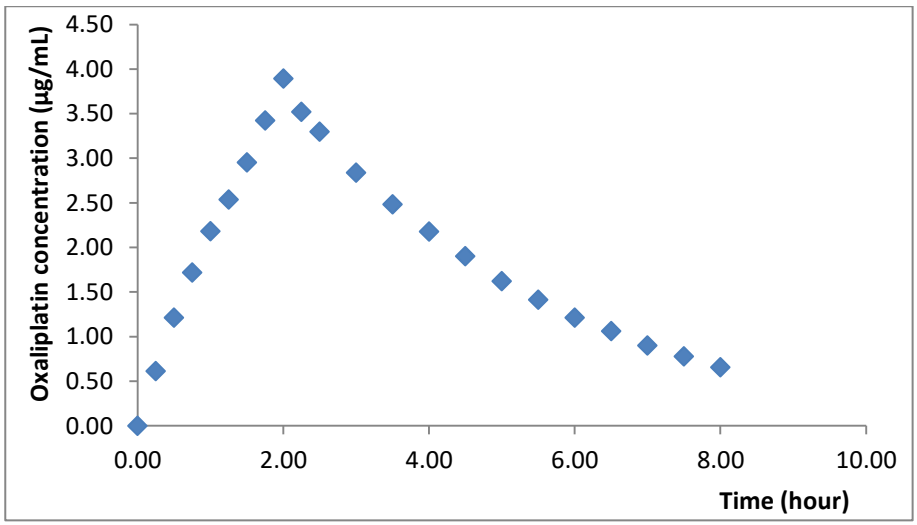

Figure C-49, Oxaliplatin concentration-time curve for Patient 10 with 2-hour infusions administered in Logarithmic Dose-banding scheme.

Table C-50, Results of the simulation test on Patient 10 with oxaliplatin administered in Flat-fixed dosing method with the drug dose of 4.0 mg.

Sample Identifier	Sample Time (hour)	Oxaliplatin concentration (µg/mL)	pH	Weight (g)	
				Before sampling	After sampling
S0T0	0.00	0.0000	4.28	632.6	621.2
S1T0.25	0.25	0.4876	4.25	624.0	613.8
S2T0.5	0.50	1.0135	4.27	615.1	604.8
S3T0.75	0.75	1.5849	4.25	606.3	596.4
S4T1.0	1.00	2.1352	4.25	598.0	587.4
S5T1.25	1.25	2.6707	4.24	588.9	578.7
S6T1.5	1.50	3.1345	4.24	580.2	569.6
S7T1.75	1.75	3.5536	4.25	573.7	564.8
S8T2.0	2.00	3.9268	4.24	565.1	552.8
S9T2.25	2.25	3.7328	4.24	555.3	545.6
S10T2.5	2.50	3.5011	4.23	547.6	536.7
S11T3.0	3.00	2.9587	4.22	540.8	530.4
S12T3.5	3.50	2.6339	4.22	535.2	524.6
S13T4.0	4.00	2.2793	4.22	529.3	519.6
S14T4.5	4.50	1.9462	4.22	523.5	514.0
S15T5.0	5.00	1.6847	4.22	517.5	507.4
S16T5.5	5.50	1.4326	4.22	511.0	500.6
S17T6.0	6.00	1.2506	4.21	504.9	494.5
S18T6.5	6.50	1.0719	4.22	498.4	488.1
S19T7.0	7.00	0.9196	4.21	490.8	480.6
S20T7.5	7.50	0.7777	4.20	483.8	474.0
S21T8.0	8.00	0.6708	4.21	475.6	464.1

Sample identifier: presented as the sample number and time points in hour; for example, S9T2.25 means the number 9 sample which was withdrawn at the 2.25 hour (2 hours and 15 minutes) after the start of the infusion. Weight: weight monitor of Reservoir A, the 'body reservoir'.

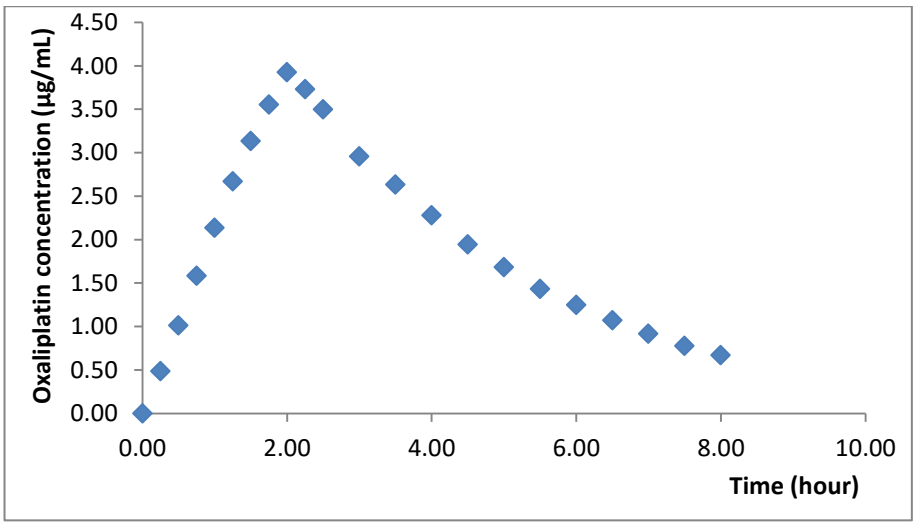

Figure C-50, Oxaliplatin concentration-time curve for Patient 10 with 2-hour infusions administered in Flat-fixed dosing method.