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DETERMINATION OF ADDITIVES IN FUELS USING AUTOMATED FLOW INJECTION ANALYSIS WITH CHEMILUMINESCENCE DETECTION

FLETCHER, PHILIP JAMES

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**DETERMINATION OF ADDITIVES IN FUELS USING AUTOMATED FLOW
INJECTION ANALYSIS WITH CHEMILUMINESCENCE DETECTION**

By

PHILIP JAMES FLETCHER

A thesis submitted to the University of Plymouth

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ABSTRACT

DETERMINATION OF ADDITIVES IN FUELS USING AUTOMATED FLOW INJECTION ANALYSIS WITH CHEMILUMINESCENCE DETECTION

Philip James Fletcher

The overall objective of this thesis was to develop field deployable instrumentation for the selective, sensitive determination of additives in diesel fuels using flow injection with chemiluminescence detection. The target analytes were the detergent dodecylamine and the lubricity additive P655.

Chapter One describes the types of additives that are used in fully formulated diesel fuels in order to improve performance and outlines the need for robust analytical methods to be able to detect their presence / absences in fuels at the point of distribution, *i.e.* at the petrol pump. Flow injection (FI), and chemiluminescence (CL) are described as suitable techniques for sample preparation and detection respectively. The application of FI-CL for the quantitative determination of various analytes is reviewed, with the focus on real sample matrices. Finally the technique of solid phase extraction is discussed as a means of selective analyte preconcentration / matrix removal prior to FI-CL detection

Chapter Two describes the development and optimisation (both univariate and simplex) of an FI-CL method for the determination of dodecylamine in acetonitrile / water mixtures using the catalytic effect of amines on the peroxyoxalate / sulphorhodamine 101 CL reaction. The linear range for dodecylamine was 0 – 50 mg L⁻¹ with a detection limit of 190 µg L⁻¹ and RSDs typically < 4 %. The effect of indigenous diesel compounds on the CL response is also investigated.

Chapter Three investigates the applicability of the method developed in Chapter Two to determine dodecylamine in diesel fuels. Solid phase extraction was needed prior to analysis by FI-CL. The development of a solid phase extraction that is compatible with the FI-CL system is detailed. GC-NPD and GC-MS analysis are used in order to validate the solid phase extraction procedure. A range of diesel fuels have been spiked with an additive package containing dodecylamine and have been analysed off-line using FI-CL. Recoveries for all diesel fuels analysed were < 72 % and all fuels could be identified from the corresponding base fuel.

Chapter Four describes the design and construction of a fully automated on-line solid phase extraction flow injection chemiluminescence analyser for the determination of dodecylamine in diesel fuel. Details of the automation and programming using LabVIEW™ are described. Results obtained using the automated on-line system are compared with results obtained using off-line SPE with FI-CL detection from Chapter Three. Recoveries for all fuels except SNV were < 71 %, and all fuels except SNV could be positively identified from the corresponding base fuels. No significant differences were found between the on-line and off-line results (within 95 % confidence limits).

Chapter Five investigates the feasibility of determining the lubricity additive P655 in diesel fuel using FI-CL. The optimisation and development of a method using the competing reactions of periodate with alcohols and periodate with the CL oxidation reaction with pyrogallol is discussed, and the development of a solid phase extraction procedure for the extraction of P655 from an organic matrix is described. The limit of detection for P655 using SPE without preconcentration was 860 mg L⁻¹ and was linear in the range 0 – 10000 mg L⁻¹ ($R^2 = 0.9965$).

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AUTHOR'S DECLARATION

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The work described in this thesis has been entirely carried out by the author. Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultation purposes and several papers were prepared for publication, as listed below.

Fletcher, P., Andrew, K. N., Calokerinos, A. C., Forbes, S. and Worsfold, P. J., Analytical Applications of Flow Injection With Chemiluminescence Detection - a Review, Luminescence, 2001, 16, 1-23.

Hanrahan, G., Gledhill, M., Fletcher, P. J. and Worsfold, P. J., High Temporal Resolution Field Monitoring of Phosphate in the River Frome Using Flow Injection With Diode Array Detection, Analytica Chimica Acta, 2001, 440, 55-62.

2000, Royal Society of Chemistry, Research and Development Topics conference, UMIST, Manchester, poster presentation entitled 'Automated flow injection-solid phase extraction-chemiluminescence analyser for the determination of dodecylamine in diesel fuels'.

1999, Symposium of Analytical Chemistry '99 conference, Dublin City University, Dublin, Ireland, oral and poster presentation entitled 'Field instrumentation for organic marker compounds in fuels'.

1999, Royal Society of Chemistry, Research and Development Topics conference, University of Greenwich, London, poster presentation entitled 'Field instrumentation for organic Marker compounds in fuels'.

1998, Royal Society of Chemistry, Research and Development Topics conference, University of Durham, poster presentation . 'Solid phase extraction and flow injection chemiluminescent detection of organic marker compounds in fuels'.

Regular lecture presentations were given, both at the University of Plymouth research seminars and at Shell Global Solutions, Cheshire Innovation Park, Chester.

External contact: Dr. Stuart Forbes, Analytical Services Business Group, Shell Global Solutions, Cheshire Innovation Park, P.O. Box 1, Chester, CH1 3SH.

Signed... *Philip J. Fletcher*

Date... *3/3/2002*

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Chapter 1

Introduction

1 INTRODUCTION

1.1 DETERMINATION OF ADDITIVES IN DIESEL FUELS

1.1.1 COMPOSITION AND SOURCES OF DIESEL FUELS

Crude oil has many uses. When refined, crude can be used to produce many products from fuel gas to coke and asphalt. A summary of the refining products of crude is shown in Fig. 1.1. One of the products derived from crude is diesel fuel which is used for a significant

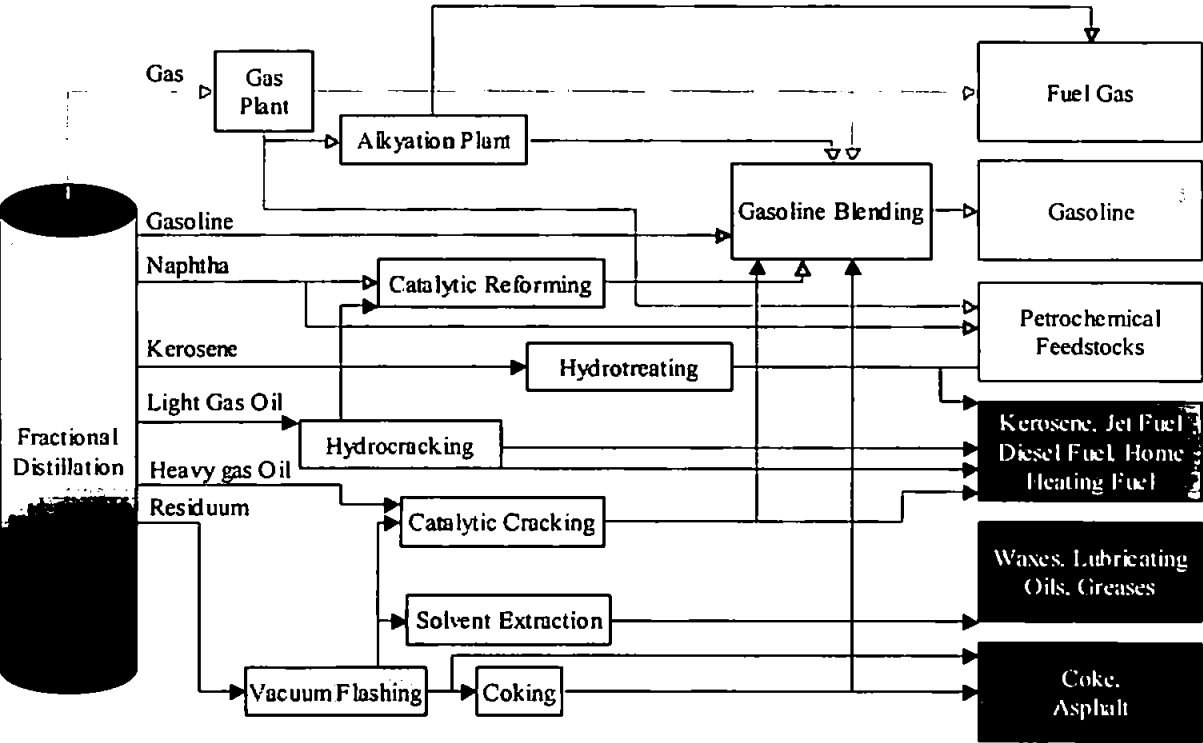


Figure 1.1: Distillation of crude oil.

proportion of motor cars (22 % in western Europe in 1998, (Davies and Weston, 1998)) and generators, with 56 % of the middle distillate of crude being used in the production of diesel fuel. Diesel is blended from a range of boiling point mixtures (usually in the range 120-400 °C) and may contain many thousands of compounds, but the main constituents are

hydrocarbons with up to 30 % of the fuel consisting of mono-, di- and tri-aromatics. Blending is necessary in order to achieve the desired cetane number (EU minimum of 51) (EU, 1998). Cetane number is a measure of the ignition quality of a diesel fuel. If the cetane number of a fuel is too low, then there will be a long delay between fuel injection and combustion, causing increased engine noise and roughness of operation. Fuels with high cetane numbers will give better ignition quality by reducing the ignition delay period. Cetane numbers can also be altered by the inclusion of particular additives in fuels. Examples of common cetane improvers are 2-ethylhexyl nitrate and tetraethylene glycol dinitrate (Suppes et al., 1997).

1.1.2 FUEL ADDITIVES

Cetane improvers are not the only additives used to enhance the properties of fuels, and a summary of the key classes of additives used is shown in Table 1.1.

Table 1.1: Common diesel fuel additive types and their purposes.

Additive	Purpose
Detergents	Control the formation of deposits on the fuel injectors, and clean off existing deposits.
Ignition improvers	(Cetane improvers) increase the cetane number of diesel fuel.
Anti-foaming agents	Reduce the amount of foam produced when a tank is filled allowing quicker and cleaner refuelling.
Corrosion inhibitors	Surfactants which prevent rust from forming.
Dehazers	Accelerate the rate at which water droplets clear, restoring the fuel's 'clear and bright' appearance.
Re-odourants	Give the fuel a more acceptable odour.
Lubricity improvers	Added to reduce the pump wear on fuels with low sulphur content.
Flow improvers	Improve cold weather starting ability by interacting with the waxes that separate from diesel fuel as it cools.
Antioxidants	Prevent fuel from degrading
Drag-reducers	Reduce turbulence in flowing fuel, so it can be pumped at a faster rate through pipelines joining refineries and depots.
Dispersant additives	Reduce electrostatic build-up to avoid the risk of explosion when the fuel is being pumped at high speed.
Biocides	Prevent growth of bacteria and fungi in fuel tanks.

Compounds used as fuel additives often differ from one manufacturer to another, and can therefore be used to differentiate different manufacturer's products. Analysis of additives is very important in order to maintain product quality throughout the distribution chain of a fuel. At present, analysis is performed by taking samples from different points of the distribution chain (distribution terminals, garage forecourts etc.) and sending them to a laboratory. This can incur a lengthy and potentially costly delay between sampling and analysis. Analysis at the point of distribution would avoid this delay and, in some parts of the world where counterfeiting is commonplace, help to ensure that the product on sale is genuine.

Diesel is a very complex and variable matrix. A gas chromatogram of a typical diesel fuel is shown in Fig. 1.2. The composition depends on the source of crude oil and also the manufacturing process used to produce the fuel. A straight run distillate will have a very different composition to that of a heavily catalytically cracked fuel. This presents a problem with analysis, as most simple analytical techniques require standards to be prepared in a matrix of very similar composition to that of the sample to be analysed.

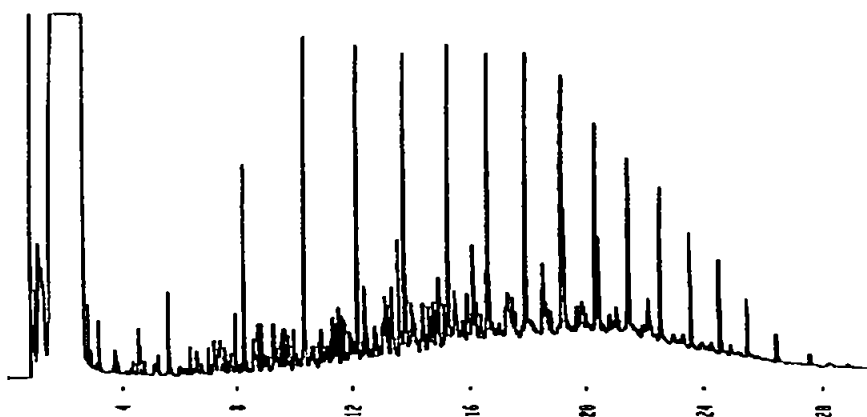


Figure 1.2: Gas chromatogram of 0.1 % diesel in heptane. The large number of components present can cause problems with analysis.

Fuel additives are usually added at relatively low concentrations (typically 10-500 mg L⁻¹). Analysis of compounds present at low concentrations is likely to be difficult, as more abundant matrix compounds will interfere. Another analytical problem to overcome is that many diesel additives are not distinct chemical entities. Most additives consist of a mixture of a number of compounds with broadly similar structures, however, they may have a range of molecular masses. Two classes of additives which are of particular importance to the consumer are detergents and lubricants, which are discussed in more detail below.

1.1.3 DETERGENTS

Detergents are used in fuels to control the formation of deposits in the engine, helping to prevent problems caused by the build up of gum, lacquer and carbonaceous deposits. Gum deposits are caused by the oxidation of diesel fuel and may lead to the sticking of injector needles, engine misfiring, loss of power and increased smoke emissions. Lacquer and carbonaceous deposits on fuel injectors can affect the amount of fuel injected and the spray pattern, leading to loss of power, poor starting, misfiring, irregular and noisy combustion, increased fuel consumption, exhaust odour and increased exhaust emissions.

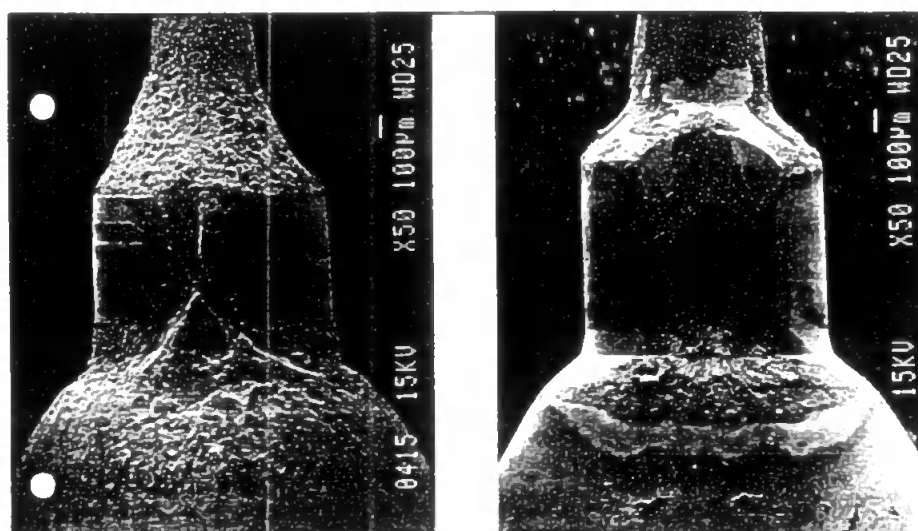


Figure 1.3: Fuel injector needles after 3000 km (magnified), using fuel without (left) and with (right) a detergent (Forbes, 2000).

Photographs of fuel injectors, both with and without detergent additives, are shown in Fig. 1.3. A large build up can be seen on the injector from the engine that was run without detergent in the fuel. Detergent additives for diesel fuel are usually surfactants and function by forming a film on metal surfaces which prevents deposits from forming. Detergents can also gradually remove deposits which have already formed on the metal, give some protection against rust and are sometimes used in conjunction with dispersants to help disperse particulate matter.

Fuels with a low sulphur content often require different detergent chemistry from fuels with a high sulphur content. Additives which function well in one type of fuel may be much less effective in other types. The concentration needed for an additive to perform its task effectively is known as the dose rate for that additive. Typical dose rates for detergent additives are 40-200 mg L⁻¹. The choice of additive and treatment rate are determined by the characteristics of fuel and whether the aim is to maintain clean injectors or to clean up dirty ones.

Detergent additives may be distinct chemical entities *e.g.* dodecylamine (see Fig. 1.4) or can be polymeric containing many compounds of similar functionality *e.g.* mono and bis succinimide (see Fig. 1.5), which have molecular weights in the range 1100 to 2700 g mol⁻¹.

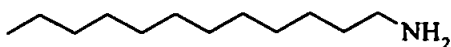


Figure 1.4: Structure of detergent additive dodecylamine.

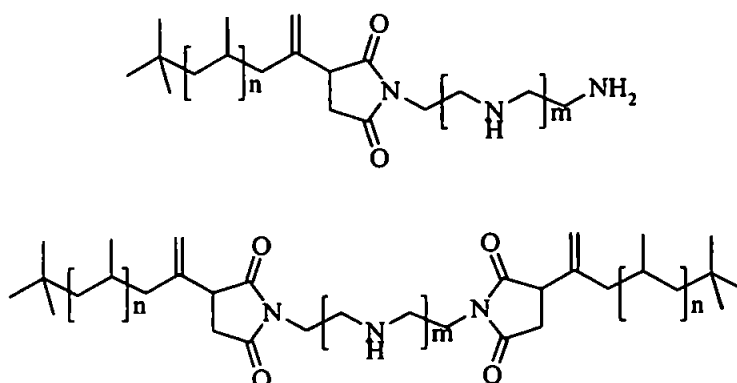


Figure 1.5: Structure of detergent additive mono and bis succinimide.

1.1.4 LUBRICANTS

Sulphur compounds present in diesel fuel act as natural lubricants for fuel system components such as fuel pumps and injectors. When sulphur is removed from diesel fuel the natural lubricity of the fuel is reduced. Prior to the year 2000, European Union fuel specifications allowed a maximum of 500 mg L⁻¹ sulphur. Since 2000 the maximum sulphur content allowed by the EU has been reduced to 350 mg L⁻¹ (Directive 98/70/EC) (EU, 1998) and this will be further reduced to 50 mg L⁻¹ in 2005. Poor lubricity in fuel can lead to excessive wearing of the rotary injection pumps, which rely on the fuel for lubrication. Other disadvantages caused by poor lubricity are unreliable fuel delivery, increased emissions, increased fuel consumption and pump failure. To ensure that this does not cause problems, low sulphur diesel fuel is manufactured with some residual sulphur compounds. Lubricity is measured by additional testing during the manufacturing process and if lubricity does not meet accepted international standards then it is treated with an additive (usually at the refinery).

Typical dose rates are from 20-500 mg L⁻¹ depending on the base fuel and the additive used. As with detergent additives, lubricity additives often consist of a mixture of

compounds, for example P655 (see Fig. 1.6), which is a mixture of mono-, di- and tri-esters of glycerol and linoleic acid with molecular weights in the range 354-878 g mol⁻¹.

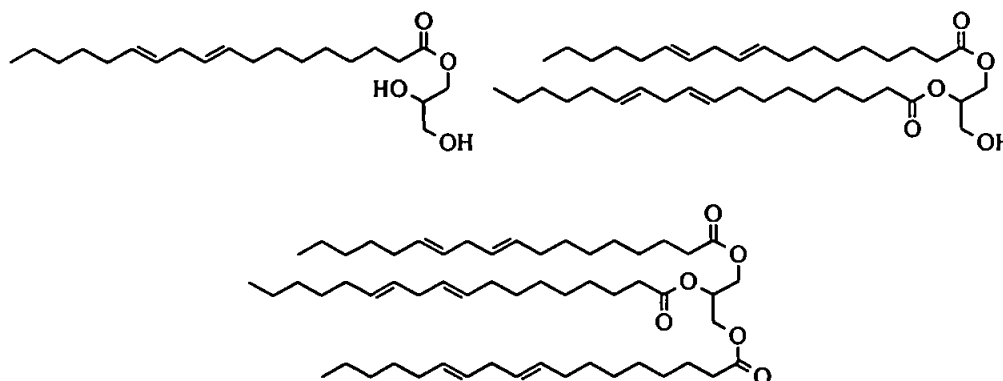


Figure 1.6: Structures of mono-, di- and tri-esters of glycerol and linoleic acid which comprise the additive Paramin 655 (P655).

1.1.5 THE NEED FOR RAPID DETERMINATION OF FUEL ADDITIVES

Current methods for the determination of fuel additives all require samples to be analysed in a laboratory environment. Examples of current methods used in fuel additive analysis are; HPLC with IR detection for the detection of alkyl nitrate cetane improvers (Schabron and Fuller, 1982); online HPLC-GC for the analysis of vegetable oil methyl esters used as lubricants (Plank and Lorbeer, 1994); and trifluoroacetylation derivatisation prior to analysis by GC-MS for the detection of amines in petroleum (Thomson et al., 1994). All of these methods require a laboratory for analysis. Rapid, portable, low cost instrumentation that could be deployed at the point of distribution would be very desirable because the lengthy and potentially costly delay incurred whilst sending samples to the laboratory could be eliminated.

1.2 CHEMILUMINESCENCE

1.2.1 OVERVIEW OF LUMINESCENCE TECHNIQUES

Luminescence spectroscopy encompasses a wide range of analytical techniques, which are often used in analytical chemistry. All luminescence techniques involve the emission of ultraviolet (UV), visible (Vis) or near-infrared (NIR) radiation from an excited electronic state. An overview of analytical luminescence techniques is shown in Table 1.2.

Table 1.2: Summary of luminescence techniques.

Effect	Cause
Luminescence	The emission of ultraviolet (UV), visible or near infrared (NIR) radiation from a molecule or an atom resulting from the transition of an electronically excited state to a lower energy state (usually the ground state).
Fluorescence	Short-lived luminescence arising from an 'allowed' transition (usually singlet to singlet). Lifetimes for fluorescence are 10^{-9} - 10^{-7} s
Phosphorescence	Long-lived luminescence arising from a 'forbidden' electronic transition between excited and ground states with different spin multiplicities (usually triplet to singlet). Lifetimes for phosphorescence are 10^{-4} -10 s.
Photoluminescence	Luminescence produced from the absorption of ultraviolet, visible or near infrared light.
Anodoluminescence	Luminescence arising from irradiation by α -particles.
Cathodoluminescence	Luminescence arising from irradiation by β -particles.
Radioluminescence	Luminescence arising from irradiation by X- or γ - rays.
Thermoluminescence	Luminescence arising from solids on mild heating.
Electroluminescence	Luminescence arising from electronically excited states produced by the presence on an electric field.
Sonoluminescence	Luminescence arising from the passage of intense sound waves through a liquid formed by electric discharges in the residual gas of cavities formed by the acoustic energy in the liquid.
Triboluminescence	Luminescence arising from the rubbing together of certain solids, for example when solids are crushed.
Crystalloluminescence	Luminescence produced by the crystallisation of certain crystal, thought to be due to the cleavages occurring during the growth of individual crystals.
Lyoluminescence	Luminescence produced when certain crystals dissolve.
Chemiluminescence	Emission of light from electronically excited states produced in a chemical reaction.
Bioluminescence	Chemiluminescence from a biological system.
Electrogenerated chemiluminescence	Luminescence produced by chemical reactions of chemiluminescent species produced during electrolysis in solution.

Luminescence techniques have many advantages over competitive techniques such as UV/Vis absorption spectroscopy. Luminescence is inherently more sensitive than absorption spectroscopy. Absorption techniques rely upon the ratio of transmitted to incident light as shown by the Beer-Lambert law (see Equation 1.1).

$$A = \epsilon bc = \text{Log} \frac{I_0}{I} \quad (1.1)$$

Where A is the absorption, ϵ is the molar absorptivity, c is the concentration, b is the path length, I_0 is the intensity of incident light and I is the intensity of transmitted light. Luminescence techniques measure emitted photons and so do not rely on the ratio of absorbed to incident light. Detectors that are used in luminescence have the ability to respond to very low levels of light and also have very low background signals, making luminescence techniques very sensitive. Luminescence techniques are generally linear over several orders of magnitude and are usually very selective. The selectivity of a luminescence technique can also be a disadvantage, as there is a limited number of species that show luminescence. In order to extend the range applications, derivatisation is often used to produce a fluorescent derivative of a non-fluorescent analyte however, the presence of excess derivatising reagents can also cause interferences.

Suppression of luminescence can also be used to detect analytes that cannot be directly detected using luminescence. The analyte of interest can react with one of the luminescent reagents providing a competing reaction, thus reducing the luminescence emission.

A potential energy diagram showing the electronic transitions: absorption, fluorescence and phosphorescence is shown in Fig. 1.7.

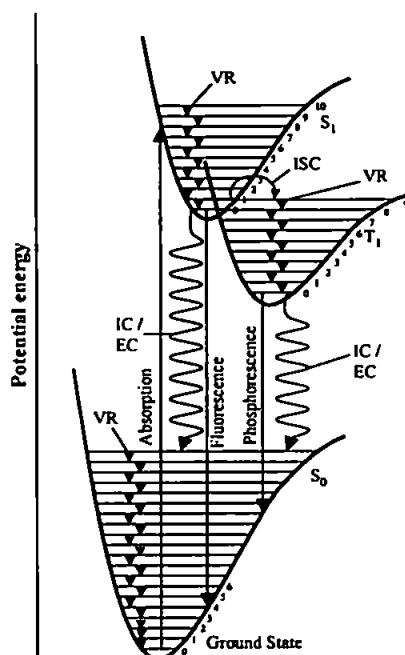


Figure 1.7: Potential energy diagram showing the processes of absorption, fluorescence and phosphorescence (IC is internal conversion, EC is external conversion, ISC is intersystem crossing and VR is vibrational relaxation).

Most organic compounds have a pair of electrons in the ground state. According to the Pauli exclusion principle, the electrons will have opposing spins, as shown in Fig. 1.8. This state is known as a singlet ground state (S_0). On absorption of UV or visible radiation an electron can be raised to an excited singlet state.

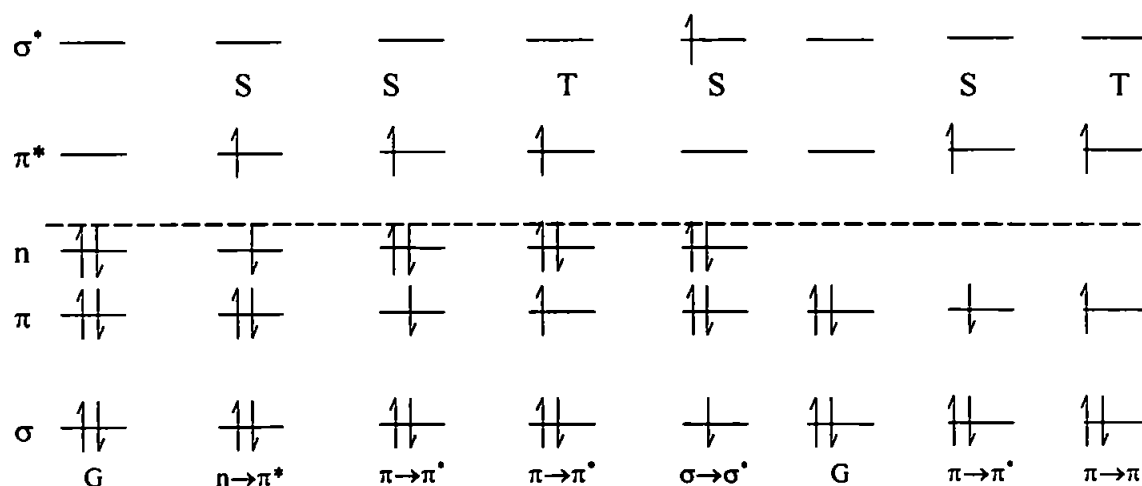


Figure 1.8: Diagram showing some examples of electronic transitions from the ground state G. Singlet states are shown as S, and triplet states as T (σ is a sigma bonding orbital, π is a pi bonding orbital and n is a non-bonding orbital).

When a molecule had absorbed light and formed an excited singlet state, then it can undergo non-radiative (internal / external conversion or intersystem crossing) or radiative (fluorescence or phosphorescence) pathways to relax. The quantum yield (efficiency) of a process is defined as shown in Equation 1.2.

$$\Phi = \frac{\text{number of events}}{\text{number of photons absorbed}} \quad (1.2)$$

In the absence of any photochemical reactions, the sum of the quantum efficiencies for fluorescence (Φ_F), phosphorescence (Φ_P) and non-radiative processes (Φ_I) is 1 (see Equation 1.3).

$$\Phi_F + \Phi_P + \Phi_I = 1 \quad (1.3)$$

Non radiative processes

Non radiative processes are vibrational relaxation, internal conversion, external conversion and intersystem crossing. Vibrational relaxation is the deactivation of vibrational energy levels within a given electronic state. Internal conversion (IC) is a radiationless transition between states with the same spin quantum numbers (*e.g.* S_1 to S_0). This occurs when the two energy levels are close enough for the vibrational energy levels to overlap allowing vibrational relaxation. External conversion (or energy transfer) is the transfer of electronic energy from one molecule to another. Intersystem crossing (ISC) is a radiationless transition between states with different spin quantum numbers (*e.g.* T_1 to S_0).

Fluorescence

For fluorescence to take place the excited molecule must relax to the lowest vibrational energy level within the excited singlet state. The molecule can relax to any of the vibrational energy levels in the ground singlet state with the emission of a photon with the

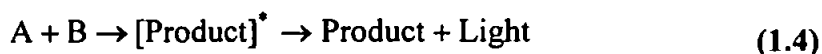
corresponding amount of energy. No change of spin is needed for fluorescence and so the transition is very rapid ($10^{-9} - 10^{-7}$ s). Transitions that involve no change of spin are termed 'allowed'.

Phosphorescence

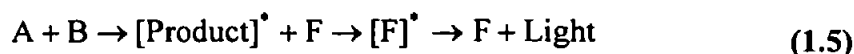
Phosphorescence emission involves the change of spin multiplicity prior to emission. Because of the change of spin needed for phosphorescence, the transition is termed 'forbidden' and is a much slower process than fluorescence (10^{-4} -10 s). The change of spin is usually from an excited singlet state to a triplet state. Non-radiative processes are very fast in relation to phosphorescence, and so phosphorescence is rarely observed.

1.2.2 CHEMILUMINESCENCE THEORY

Chemiluminescence (CL) reactions are chemical reactions where one of the products is light. The most familiar examples of CL reactions are the peroxyoxalate CL reaction, which is used in light sticks, and the light produced by fireflies. CL reactions exist in the gas, liquid and solid phases. The simplest form of chemiluminescence is direct CL, in which two chemicals are mixed to produce a product in an excited state (Product^{*}) which can subsequently relax to the ground state with the emission of a photon (see Equation 1.4).



In certain cases the excited molecule is a weak or non-emitting species. In this case, the energy may be passed to another species F (known as a sensitiser or fluorophore) which can go on to emit light. This is known as indirect chemiluminescence (see Equation 1.5).



The intensity of chemiluminescence is related to the quantum yield for chemiluminescence as shown by Equation 1.6.

$$I_{\text{CL}} = \Phi_{\text{CL}} \frac{dP}{dt} = \Phi_{\text{EX}} \Phi_{\text{EM}} \frac{dP}{dt} \quad (1.6)$$

Where I_{CL} is the intensity of CL emission (photons per second), Φ_{CL} is the quantum yield for chemiluminescence (number of photons emitted per molecule reacted), dP/dt is chemical reaction rate (molecules reacting per second), Φ_{EX} is the quantum yield for excitation (excited states produced per molecule reacted) and Φ_{EM} is the quantum yield for emission (photons emitted per excited state).

1.2.3 INSTRUMENTATION FOR CL DETECTION

Detection systems in chemiluminescence consist of two major parts; a flow cell and a device for quantifying the CL emission.

1.2.3.1 Flow cells

A flow cell must have a large area in contact with the detector in order to maximise the amount of light that is received by the detector. A spiral flow cell is the most common design to allow a large emission area, however other designs such as zigzag flow cells have been utilised (see Fig. 1.9). A typical flow cell is 10-15 cm long with a volume >100 μL .

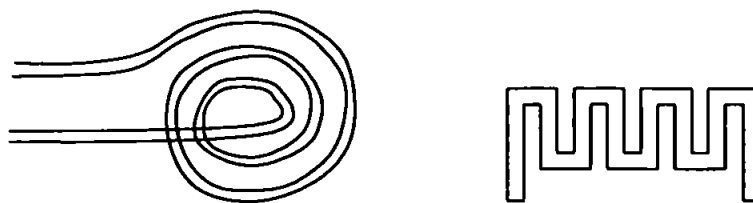


Figure 1.9: Spiral flow cell (left) and zigzag flow cell (right) designs.

1.2.3.2 Detectors

Photodiodes

Photodiodes are solid state devices. Silicon photodiodes are constructed from single crystal silicon wafers with 'N' type silicon used as the starting material. A thin layer is formed on the front surface of the device by thermal diffusion or ion implantation of the appropriate doping material known as the 'p' layer. The interface between the 'p' layer and the 'n' silicon is known as a pn junction. A cross section of a typical silicon photodiode is shown in Fig. 1.10. The thickness of the 'p' layer determines the wavelength of radiation to be detected. The silicon near the pn junction becomes depleted of electrical charges and this is known as the "depletion region". The depth of the depletion region can be varied by applying a reverse bias voltage across the junction. When light hits the pn junction free electrons are formed producing a current that is proportional to the intensity of light. Examples of photodiodes used for chemiluminescence detection are the determinations of H_2O_2 and l-lactate (Hayashi et al., 1996; Hemmi et al., 1995). Photodiodes are small and relatively inexpensive and have low power requirements, operating at $< 15 \text{ V}$.

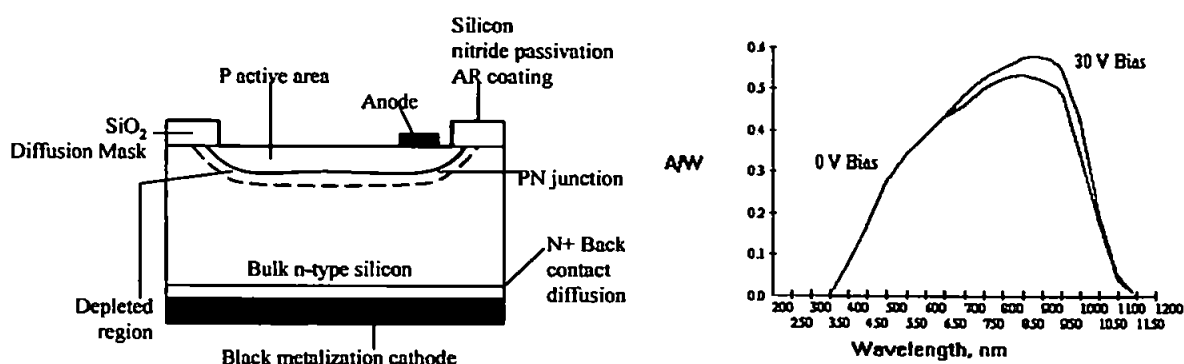


Figure 1.10: Cross section of a typical silicon photodiode (left), and a typical spectral response profile for a photodiode (right) (Centrovision Inc., 2001)

Photomultiplier tube (PMT)

A schematic diagram of a PMT is shown in Fig. 1.11. When a photon of light hits the photocathode, an electron is emitted. This electron is accelerated towards a dynode which is at a positive potential.

The dynode then emits a number of electrons that are accelerated to the second dynode, which is at a higher potential. The second dynode consequently emits a number of electrons for each electron that hits it, and this process is sequentially repeated until the electrons reach the last dynode, which is at a very high potential. The resulting current is proportional to the amount of light reaching the photocathode. Photomultiplier tubes have fast response times (typically nanoseconds), very low noise, and good linearity. Wavelength ranges are between 150 and 900 nm. A typical wavelength response profile for a PMT is shown in Fig. 1.12.

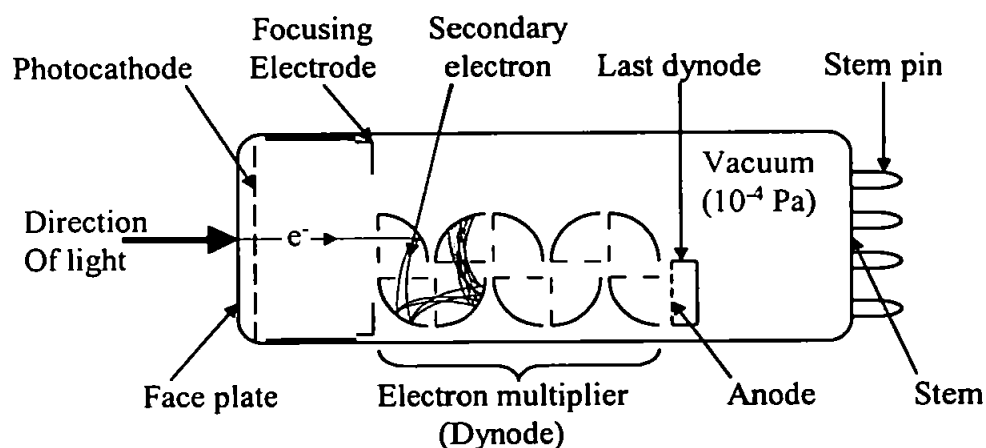


Figure 1.11: A schematic of an end window photomultiplier tube.

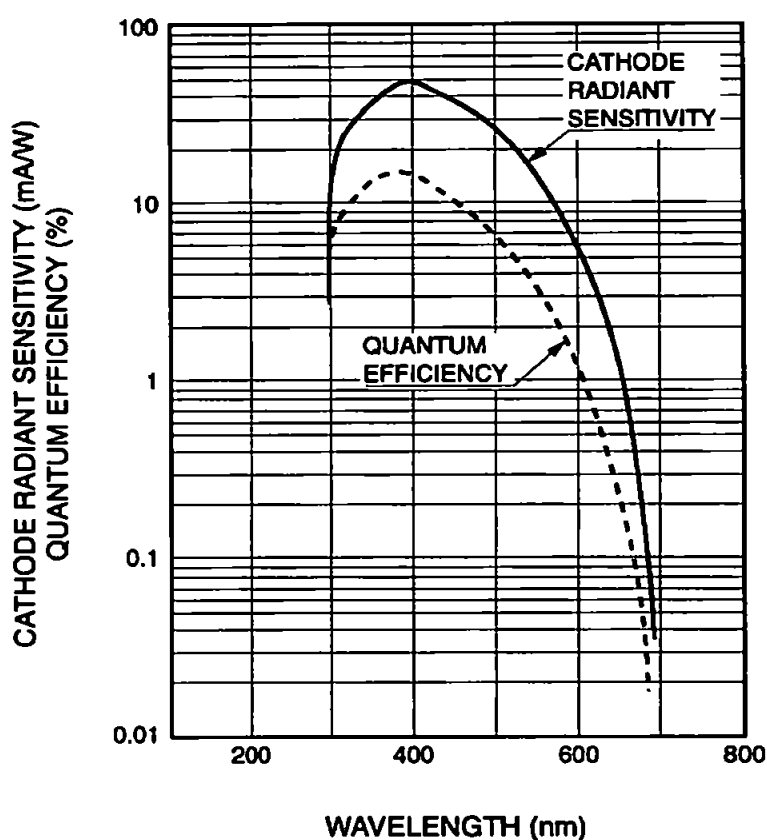


Figure 1.12: Typical photomultiplier tube response profile (Hamamatsu Photonics K.K., 1998).

The limitations of PMTs are that they are fairly fragile and require a high voltage power supply (>1 kV) to power them, although PMTs with built in power supplies can be obtained, which operate at 12 V.

1.2.4 COMMON LIQUID PHASE CL REACTIONS

A number of chemiluminescence reactions are available in analytical chemistry. The most common reactions (luminol, lucigenin, tris(2,2'-bipyridine)ruthenium(II), peroxyoxalate and pyrogallol) are discussed below.

1.2.4.1 Luminol

The luminol CL reaction involves the oxidation of luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) to produce an excited molecule of 3-aminophthalate (see Fig. 1.13). Emission is in the blue region (425 nm) with a quantum yield of 0.01 in aqueous alkali and blue-green emission (480-502 nm) with a quantum yield of 0.05 in dimethyl sulphoxide.

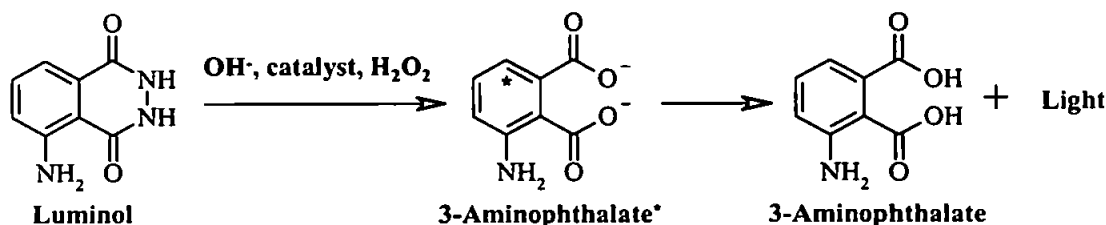


Figure 1.13: Luminol chemiluminescence reaction (Robards and Worsfold, 1992).

The reaction is catalysed in the presence of a number of metal cations and by haem containing enzymes.

1.2.4.2 Lucigenin

Lucigenin (*N,N'*-dimethyl-9,9'-diacridinium nitrate) undergoes oxidation to produce an excited molecule of *N*-methylnacridone, which can relax to produce light in the blue-green region (440 nm) as shown in Fig. 1.14.

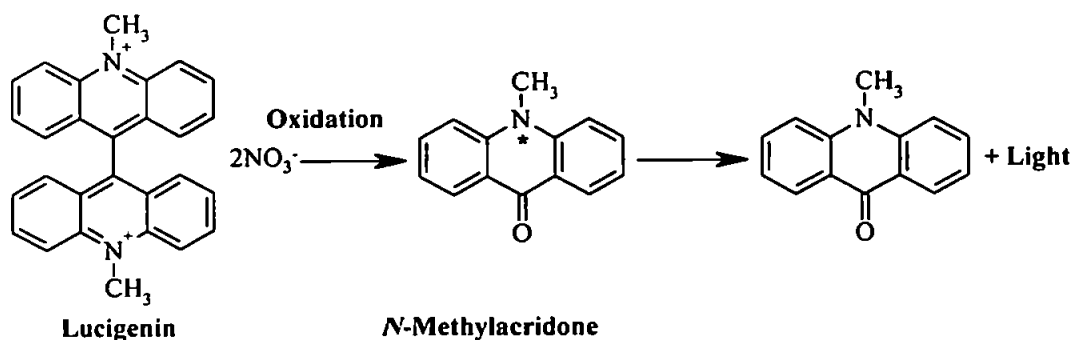


Figure 1.14: Lucigenin chemiluminescence reaction (Birks et al., 1989).

The quantum yield for this reaction is 0.016 (Robards and Worsfold, 1992). The lucigenin reaction (as with luminol) is catalysed by a number of metal cations, as well as some cations, *e.g.* Bi(III), that do not catalyse the luminol reaction.

1.2.4.3 *Tris(2,2'-bipyridine)ruthenium(II)*

In the tris(2,2'-bipyridine)ruthenium(II) reaction, $\text{Ru}(\text{bpy})_3^+$ and $\text{Ru}(\text{bpy})_3^{3+}$ react yielding the excited state molecule $\text{Ru}(\text{bpy})_3^{2*}$ which relaxes with the emission of orange light.

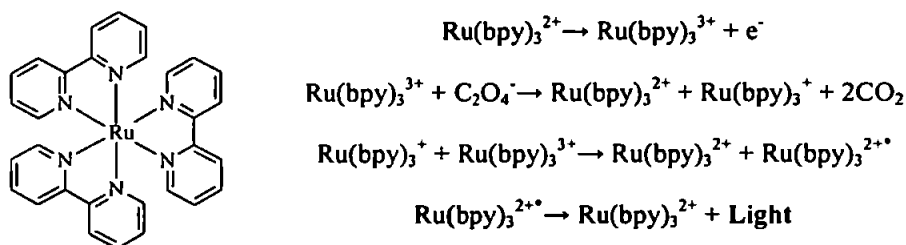


Figure 1.15: Tris(2,2'-bipyridine)ruthenium(II) chemiluminescence reaction with oxalate (Birks et al., 1989)

$\text{Ru}(\text{bpy})_3^+$ and $\text{Ru}(\text{bpy})_3^{3+}$ can both be electrogenerated or an appropriate oxidant or reductant (*e.g.* oxalate) can be used to chemically generate these oxidation states. This reaction is particularly useful for the detection of amines (Noffsinger and Danielson, 1987).

1.2.4.4 Peroxyoxalate

Peroxyoxalate chemiluminescence involves the oxidation of an aryl oxalate ester (usually with hydrogen peroxide) as shown in Fig. 1.16. This reaction is an example of indirect CL, whereby emission is not from the CL reagents, but from a fluorophore. The emission wavelength, therefore, depends on the fluorophore used.

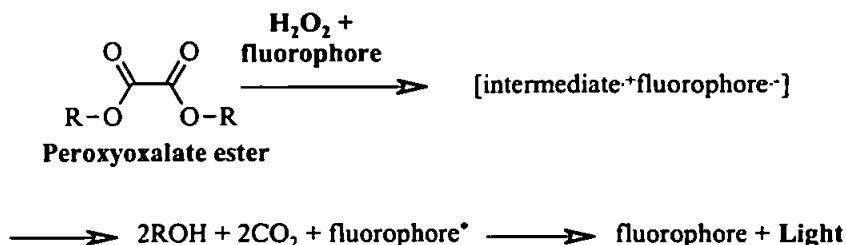


Figure 1.16: Peroxyoxalate chemiluminescence reaction (Rauhut et al., 1967).

Quantum yields for the peroxyoxalate reaction are relatively high (up to 0.5). The peroxyoxalate reaction is discussed in more detail in Chapter 2.

1.2.4.5 Pyrogallol

The oxidation of pyrogallol with periodate will produce weak chemiluminescence emission. Hydroxylamine is thought to react with pyrogallol to produce a sensitizer for this reaction resulting in a stronger CL emission. The emission from the pyrogallol reaction is in the reddish pink region (630 nm) in alkaline hydrogen peroxide. A more detailed discussion of the pyrogallol CL reaction is presented in Chapter 5.

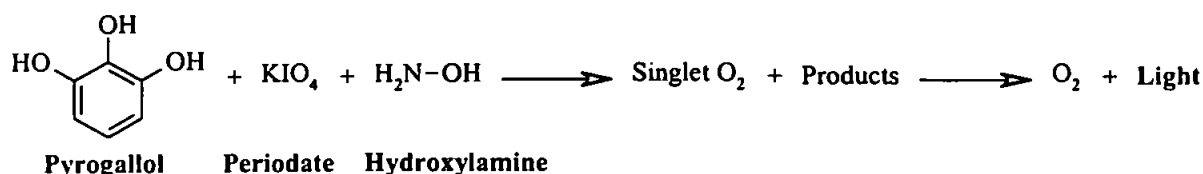


Figure 1.17: Pyrogallol chemiluminescence reaction (Evmiridis, 1987).

1.2.5 ADVANTAGES OF CHEMILUMINESCENCE

Chemiluminescence has certain advantages over other flow through detection systems. Most CL reactions are highly selective for a particular class of compounds which means that an analyte can often be measured without requiring any separation from the sample matrix. Chemiluminescence is also very sensitive compared with other analytical techniques. Instrumentation needed for chemiluminescence is very simple and relatively inexpensive, resulting in the need for less sophisticated instrumentation than that needed for fluorescence or spectrophotometric techniques that also require a light source and monochromator. This also makes chemiluminescence a potentially more portable detection technique.

1.3 FLOW INJECTION

Flow injection analysis (FIA) was first described in 1975 (Ruzicka and Hansen, 1975). This technique involves the injection of a highly precise volume of liquid sample into a continuously flowing reagent stream and, as is shown below, is an excellent method for automating wet chemical reactions.

1.3.1 BASIC PRINCIPLES

The key components of a flow injection system are a propulsion device to cause the liquid to flow, an injector to introduce a known volume of sample into the flowing stream, and a flow-through detection system to measure the resulting chemical reaction. A schematic of a basic FI system is shown in Fig. 1.18.

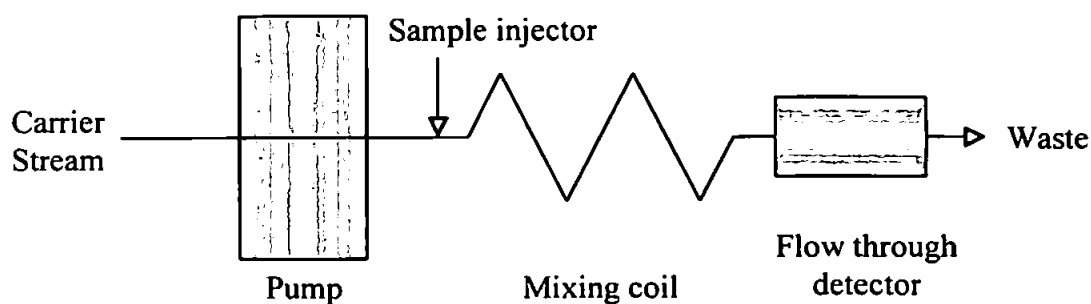


Figure 1.18: Schematic diagram of a basic flow injection manifold.

A key principle of flow injection is 'controlled dispersion' of the sample into the carrier stream. Dispersion must be controlled in order to obtain reproducible results. Dispersion is the process by which a sample appears to spread out or disperse after injection. An example of this is shown in Fig. 1.19.

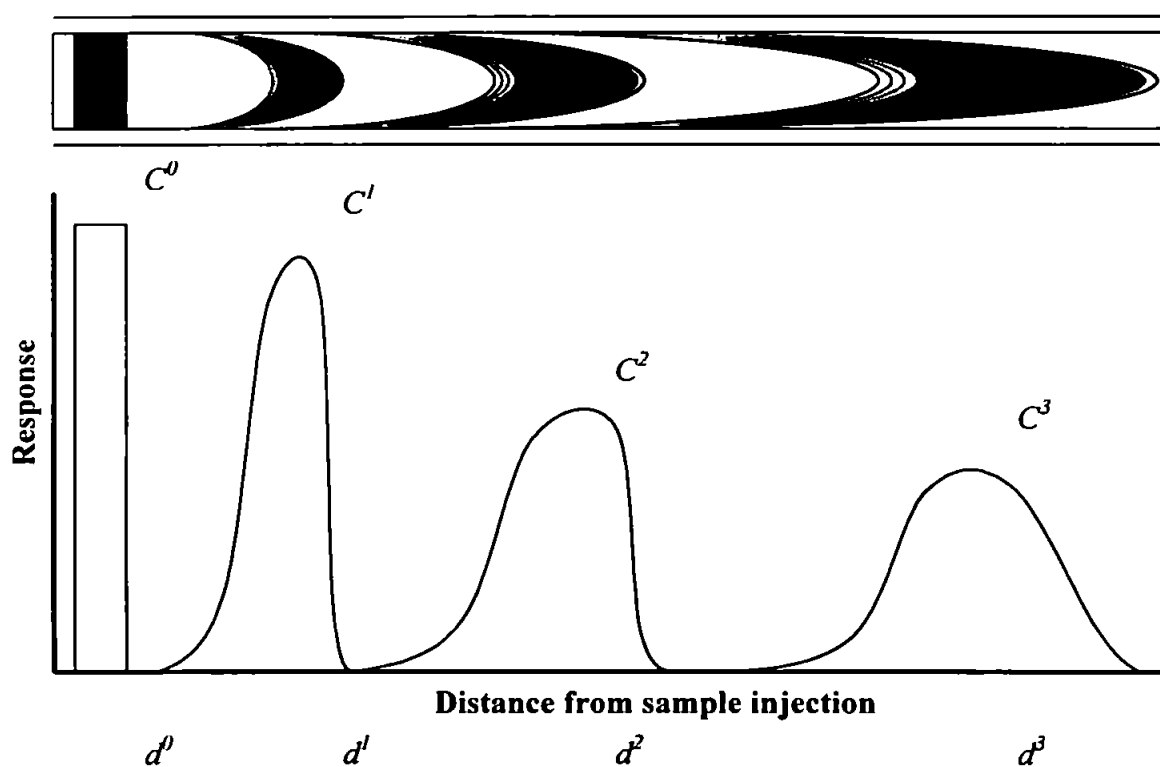


Figure 1.19: Dispersion process of an injected fluid zone in flow injection. Modified from (Fang, 1995) where C^n is the measured concentration at distance d^n from the injection valve.

The two processes that cause dispersion are molecular diffusion and convection. Convection effects are the most dominant of these. A measure of dispersion is defined by the dispersion coefficient as shown in Equation 1.7

$$D = \frac{C^0}{C''} \quad (1.7)$$

D is the dispersion coefficient, C^0 is the measured concentration before dispersion, and C'' is the concentration after dispersion at a distance d'' (see Fig. 1.19) from the injection. Dispersion coefficients can be less than 1 if preconcentration is used. Typical values are 1-3 for selective detector manifolds (*i.e.* no sample conversion) and 3-10 for manifolds with chemical derivatisation.

1.3.1.1 Pumps

Various systems have been used for pumping in flow injection, each of which have various advantages and disadvantages associated with them.

HPLC piston pumps can be used for flow injection, however they are very expensive, prone to blocking, not very portable and are only single channel, so multiple pumps are needed to pump multiple carrier / reagent streams. The flow from HPLC is also pulsed, resulting in variations in background signals.

Gas pressure FI relies on a stream of compressed gas to force reagents out of reagent reservoirs. The resulting stream is very smooth, but flow rates are not constant due to back pressure. A compressed gas cylinder and pressure regulators are needed which mean this system cannot be used for portable instrumentation.

Gravity fed FI, like gas pressure FI, offers smooth delivery of reagents. A large height difference is needed to produce relatively small hydrostatic pressure resulting in low flow rates and cumbersome instrumentation. This system is therefore also not readily portable.

Solenoid operated micro-pumps have been used in flow injection (Coles et al., 2000). Computer controlled solenoids are used to pump liquids, with flow rates proportional to solenoid switching rates. These pumps offer excellent portability, are low in cost, can be battery operated and are very small. They are, however, prone to blockages and have limited suction power, which means they cannot pump viscous liquids such as diesel fuel. Pumping from solenoid valves is also very pulsed, resulting in large fluctuations in baseline signals. Each pump is only single channel, meaning that a separate pump is needed for each reagent stream.

Peristaltic pumps are the most commonly used pumps in flow injection. A number of rotating rollers force liquid to travel along a compressible tube, by squeezing small aliquots of liquid between the rollers as they rotate. An example of a peristaltic pump is shown in Fig. 1.20. Flow rate can be altered by changing either the rotation speed of the rollers or by changing the internal diameter of the peristaltic pump tubing. The flow resulting from a peristaltic pump is pulsed, although careful selection of the correct internal diameter tubing and rotations speed of the rollers can reduce the pulsing. These pumps are small, portable, low cost and may be battery operated and are multiple channel, so often only one peristaltic pump is needed for a flow injection system.

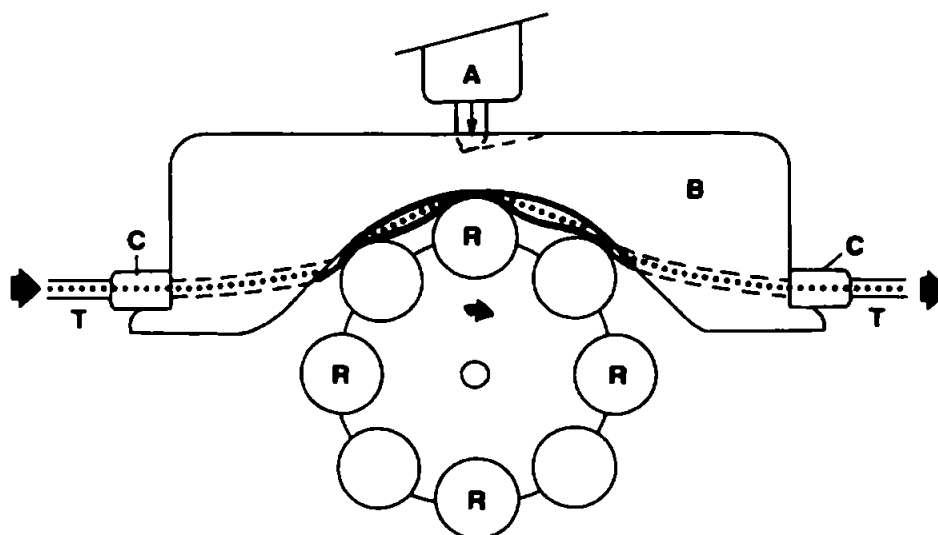


Figure 1.20: Schematic diagram of a peristaltic pump. (Fang, 1995). R, rollers; B, compression block; A, pressure adjustment screw; T, peristaltic pump tube; C, tube collars.

1.3.1.2 Injectors

The most common injection system used in flow injection is the 6 port, 2 position rotary injection valve. A schematic diagram of which is shown Fig. 1.21. Position A shows the sample loading. Sample is pumped through the sample loop to waste whilst the reagents are continually pumped through to the detector. Position B shows the sample injection. In this case reagents are directed through the sample loop, pushing the sample in the sample loop through to the flow manifold.

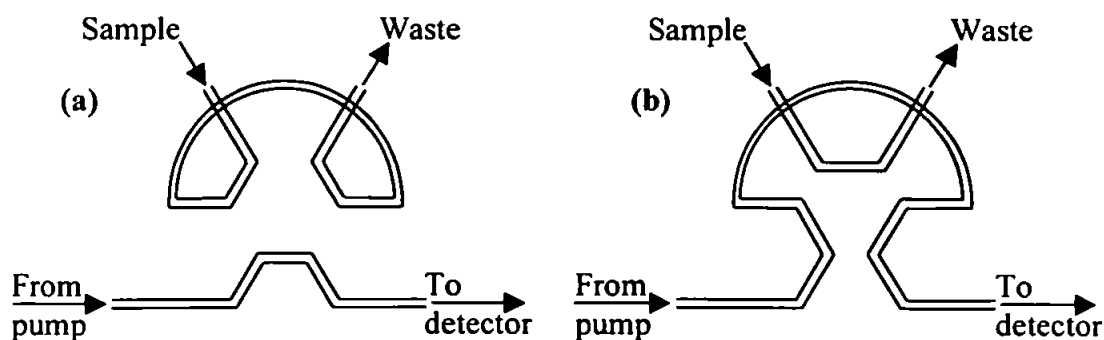


Figure 1.21: Flow injection sample injection valve: a) Sample loading; b) sample injection.

1.3.1.3 Flow injection connections

Not all flow injection manifolds contain just one line (as shown in Fig. 1.18). They may contain many different reagent or sample lines that need to be joined. This usually takes place using a T-piece, as shown in Fig. 1.22, although other junctions such as Y-pieces can also be used. The most common devices for connecting flow injection lines are $\frac{1}{4}$ "-28 flange fittings (Fig. 1.22) and $\frac{5}{16}$ "-PTFE cone fittings (Fig. .23).

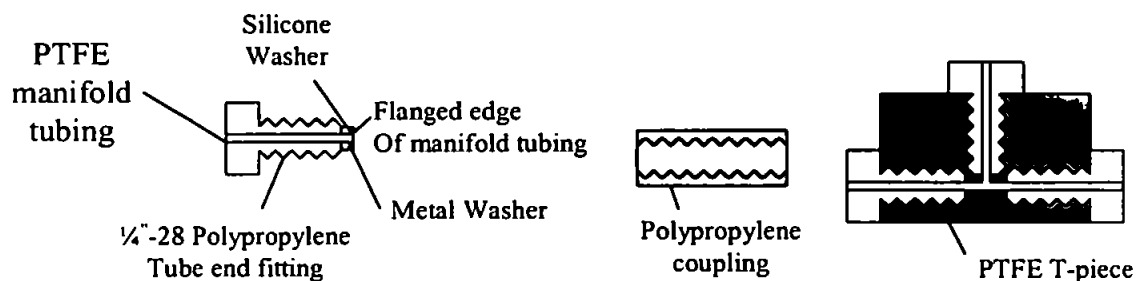


Figure 1.22: $\frac{1}{4}$ "-28 Flange fitting (left), flange coupling fitting (middle) and flange T-piece (right)

Flange fittings require the PTFE manifold tubing to be melted in order to produce a flat end on the tubing which is used to hold the flange fitting in place. A metal washer and/or a silicone washer are often used as well to help seal the connection.

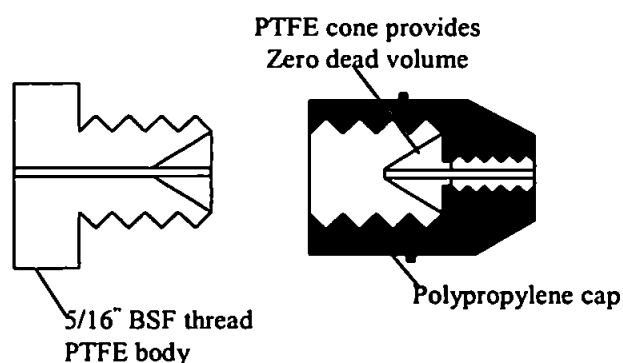


Figure 1.23: $\frac{5}{16}$ "-PTFE cone fittings.

PTFE cone fittings require a PTFE cone to be fitted to the end of the manifold tubing. These connections are simpler than flange fittings because the tubing does not have to be melted.

Reproducible mixing is very important in flow injection. Many different techniques can be deployed to ensure that good mixing happens. Straight tubing will not promote the radial redistribution of fluid that is required for good mixing. In order to promote this radial redistribution, the tube must contain bends. The most common methods used are mixing coils and knitted tubing (see Fig. 1.24). A mixing coil has the advantage of simplicity and does not require very much space within the manifold. Knitted tubing will result in greater radial redistribution due to the tubing being bent in many different directions, however knitted tubing takes up a lot more space.



Figure 1.24: A mixing coil (left) and a length of knitted tubing (right).

1.3.1.4 *Flow injection detection systems*

A large range of detection systems including all HPLC detectors are applicable to flow injection. The key requirement for an FI detector is fast response time. FI peaks generally have a width of less than a minute, therefore the detector must be able to respond faster than this in order to detect any peaks. Data acquisition rates in FI are generally in the range 0.1 –10 s. The most common detectors in FI are based on molecular spectroscopic techniques such as UV/visible spectrophotometry (Purohit and Devi, 1997), fluorescence (Guo et al., 2000), chemiluminescence (see Section 1.4) and bioluminescence (Gamborg and Hansen, 1994). Atomic spectroscopic techniques such as ICP-MS (Elwaer et al., 2000) and ICP-AES (Alonso et al., 1995) are also routinely used. Less common detection

techniques are FT-IR (Cassella et al., 2000), biosensors (Adelaju et al., 1996), optical (Scudder et al., 1992) and fluorescence microscopy (Scampavia et al., 1999), mass spectrometry (Morand et al., 2001), electrochemical techniques such as voltammetry (Fogg et al., 1983) and ion-selective electrodes (Najib and Othman, 1992) and finally radiochemistry (Grate and Egorov, 1998)

1.3.2 ADVANTAGES OF FLOW INJECTION ANALYSIS

FIA has many advantages over tradition 'batch' methods for chemical analysis. Sample analysis can be very rapid (up to 120 samples h^{-1}). Human error is reduced, as sample volumes are measured out using sample loops, thereby eliminating manual pipetting errors. Reagents are continuously flowing and therefore reagent mixing is always constant. This also means that analysis is very reproducible (typically $< 5\%$ RSD). The chance of contamination from the surrounding environment is thus greatly reduced. Instrumentation is relatively low cost compared with other analytical techniques. FI instrumentation is generally simple and robust, and therefore lends itself to automation and is suitable for field deployment.

1.4 FLOW INJECTION WITH CHEMILUMINESCENCE DETECTION

Chemiluminescence reactions are usually very rapid and therefore require rapid, reproducible mixing prior to detection. Flow injection can provide the necessary rapid, reproducible mixing required for chemiluminescence. FI provides portable, low cost, automated reactions, whilst chemiluminescence provides rapid, sensitive and selective detection using simple instrumentation. All of these features are desirable for field

deployable instrumentation. FI-CL has many analytical applications, a summary of which follows.

1.4.1 APPLICATIONS OF FI-CL

1.4.1.1 Pharmaceutical applications

The high sensitivity of CL due to the dark current principle of the technique in combination with the versatility of FIA has attracted considerable effort on the development of chemiluminogenic reactions, which can be applied in pharmaceutical samples. A variety of oxidants have been developed for this area of application. The luminol – hydrogen peroxide reaction without and with $K_7Cu(IO_6)_2$ added to luminol has been used for the measurement of ascorbic acid in tablets with good agreement with iodimetry (Feng et al., 1996; Shen et al., 1997), morphine, sinomenine and codeine in tablets and injection solutions with recoveries of 96-106% (Li and Lu, 1997b), pyridoxine in some dietary sources, e.g. peanut, yeast, lemon, tomato and apple (Alwarthan and Aly, 1998), nitroprusside in injection solutions with recovery of 98.5-101% and good agreement with official methods (Wang et al., 1997b) and rutin in traditional Chinese medicines (He et al., 1999a). Some catecholamines (dopamine, adrenaline, isoprenaline) have been measured by the same reaction after treatment with Reinecke's salt (Li and Lu, 1997a). Potassium periodate added to the luminol stream has been proposed for the determination of ascorbic acid (Wu et al., 1999b) or isoniazid (Zhao et al., 1997a) in preparations with good agreement with official methods or glucose after treatment with glucose oxidase and measurement of the hydrogen peroxide evolved (Zhou et al., 1999). Ascorbic acid has also been determined by the luminol – hydrogen peroxide reaction in the presence of Fe(II) in vitamin C tablets, multivitamin capsules, mung bean sprouts, tomato and cucumber skin, with recoveries of 96-105% (Chen et al., 1997). The presence of hexacyanoferrate in the

chemical reaction allowed measurement of beta-lactam antibiotics via generation of hydroxy and superoxide radicals (Kubo et al., 1999). Ascorbic acid has been also determined by using hexacyanoferrate(III) instead of hydrogen peroxide (Yang et al., 1996). Inhibition of luminol CL has been applied to the determination of ascorbic acid in vitamin pills and tablets of vegetable extracts with good agreement with iodimetry (Zhang and Qin, 1996) or isoniazid (Alapont et al., 1998) or paracetamol (Alapont et al., 1999). Tannic acid in Chinese gall has been determined by the inhibition effect on the luminol-hydrogen peroxide reaction catalysed by copper(II) (Cui et al., 1998).

A variety of other oxidants in acidic or alkaline media has been investigated within the area of drug analysis by CL detection. Potassium permanganate in acidic medium with or without CL enhancers or promoters has been proposed for the determination of amidopyrine (He et al., 1999c; He et al., 1999b), benzocaine, procaine and other local anaesthetics (Zhang et al., 1995c), cefadroxil (Aly et al., 1998a), codeine (Christie et al., 1995), imipramine (LopezPaz and Townshend, 1996; Xue et al., 1999b), levodopa (Yang et al., 1998), medazepam (Sultan et al., 1998), methotrexate (He et al., 1998a), naltrexone (Campiglio, 1998a), perphenazine (Sultan et al., 1999), promethazine (Xue et al., 1999a), reserpine (Li et al., 1998a), salicylamide (Mestre et al., 1999), tetracyclines (Li et al., 1997d) and tetrahydropalmatine (Li et al., 1997a) in pharmaceutical preparations with good recovery values and agreement with official or accepted analytical methods. Initiation of CL reactions in acidic media has also be achieved by using cerium(IV) for analgin (Huang et al., 1999d), captopril (Zhang et al., 1996b), furosemide (Rao et al., 1999), hydrochlothiazide (Ouyang et al., 1998), naproxen (Campiglio, 1998b), penicillamine (Zhang et al., 1995d; Zhang et al., 1996a), phenothiazines (Aly et al., 1998b), tetracyclines (Zhang et al., 1995b) and tiopronin (PerezRuiz et al., 1998; Zhao et al., 1997b).

The CL reaction of cerium(IV) with sulphite has been used for excitation by energy transfer to ciprofloxacin (Liang et al., 1997) and prednisone acetate (Liang et al., 1998). CL also occurs if sulphite is substituted by a mercapto-compound such as glutathione or cysteine and sensitisation by hydrocortisone (Li and Ci, 1997b) while the reaction with glutathione has been used for excitation of progesterone or hydrocortisone which was measured in human serum by means of the standard addition procedure (Li and Ci, 1997a). The release of sulphite from menadione sodium bisulphite has been utilised for measurement of the analyte by cerium(IV) CL in injection solutions with good agreement with the spectrophotometric method (Huang et al., 1999a). Similarly the reaction of permanganate with dithionite has been used for exciting riboflavine (Li et al., 1997b) or with thiosulphate for vitamin B6 (Li et al., 1998b).

Potassium hexacyanoferrate(III) in alkaline solutions has been used for the determination of ergonovine maleate in synthetic pharmaceutical preparations with good agreement with the official method (FusterMestre et al., 1999) or thiamine (Ishii and Kawashima, 1998). Alternatively, sodium hypochlorite can be used for initiation of CL reactions in alkaline media and the assay of persatin in injection and tablets with good agreement with the official method (Nie et al., 1997). Rutin has been measured successfully in Chinese traditional medicines with good agreement with the official method by the hypochlorite-semicarbazide CL chemical system (Nie et al., 1999).

The CL autooxidation of analgin in Tween 80 in the presence of rhodamine 6G as sensitiser allows measurement of the analyte in pharmaceutical preparations (Huang et al., 1999b). Lucigenin CL has been employed for measuring ascorbic acid (Hasebe and Kawashima, 1996) while inhibition of the reaction catalysed by iron(II) allows measurement of dopamine (Zhang et al., 1999).

Table 1.3: Pharmaceutical applications.

Analyte	Sample matrix	Reaction	L.O.D.	Reference
Amidopyrine	Injection solutions	Formaldehyde / acidified potassium permanganate chemiluminescence.	$3 \times 10^{-5} \text{ mol dm}^{-3}$	(He et al., 1999c; He et al., 1999b)
Analgin	Pharmaceutical preparations	Auto-oxidation of analgin in the presence of Tween 80 with rhodamine 6G as a sensitiser immobilised on a cation-exchange column.	0.15 mg L^{-1}	(Huang et al., 1999b)
Analgin	Tablets	Ce(IV) / sulphuric acid chemiluminescence with rhodamine 6G as a sensitiser.	$0.02 \text{ } \mu\text{g mL}^{-1}$	(Huang et al., 1999d)
Ascorbic acid	Vitamin C tablets and multivitamin capsules	Luminol / Fe(II) / $\text{Na}_2\text{B}_4\text{O}_7$ / potassium hydroxide chemiluminescence.	0.2 ng mL^{-1}	(Chen et al., 1997)
Ascorbic acid	Pharmaceutical samples and tablets	Luminol / $\text{K}_2[\text{Cu}(\text{IO}_6)_2]$ / potassium hydroxide chemiluminescence.	$1.5 \times 10^{-8} \text{ mol dm}^{-3}$	(Feng et al., 1996)
Ascorbic acid	Pharmaceutical samples	Lucigenin chemiluminescence with ascorbic acid in a basic medium, enhanced with iron(III) and Brij 35.	$2 \times 10^{-9} \text{ mol dm}^{-3}$	(Hasebe and Kawashima, 1996)
Ascorbic acid	Tablets	Luminol / sodium hydroxide / hydrogen peroxide chemiluminescence.	$8.6 \times 10^{-9} \text{ mol dm}^{-3}$	(Shen et al., 1997)
Ascorbic acid	Pharmaceutical preparations	Luminol / potassium periodate / ascorbic acid chemiluminescence.	0.8 ng mL^{-1}	(Wu et al., 1999b)
Ascorbic acid	Pharmaceuticals and blood	Potassium hexacyanoferrate(III) / luminol chemiluminescence.	$4 \times 10^{-8} \text{ mol dm}^{-3}$	(Yang et al., 1996)
Ascorbic acid	Tablets	Inhibition of luminol / ferricyanide chemiluminescence (immobilised on an anion-exchange resin column, elution with sodium phosphate).	$5.5 \times 10^{-3} \text{ } \mu\text{g mL}^{-1}$	(Zhang and Qin, 1996)
Aztreonam, penicillin G, cephalothin, 6-aminopenicillanic acid, 7-aminocephalosporanic acid, panipenem, latamoxef and faropenem	Aqueous	Luminol / hydrogen peroxide chemiluminescence with hexacyanoferrate(III) and hexacyanoferrate(II) as catalysts.	100, 60, 40, 20, 4, 2, 1 and 1 ng respectively (5 μL injection)	(Kubo et al., 1999)
Benzocaine, butacaine, butoform, procaine, tetracaine.	Pharmaceutical preparations	Acidic permanganate chemiluminescence.	30 ng mL^{-1} , 20 ng mL^{-1} , 30 ng mL^{-1} , 40 ng mL^{-1} , and 3 ng mL^{-1} respectively.	(Zhang et al., 1995c)
Captopril	Pharmaceutical preparations	Cerium (IV) / sulphuric acid chemiluminescence.	$2 \times 10^{-7} \text{ mol dm}^{-3}$	(Zhang et al., 1996b)
Cefadroxil monohydrate	Pharmaceutical preparations and biological fluids	Potassium permanganate / sulphuric acid chemiluminescence with quinine as a sensitiser.	$0.05 \text{ } \mu\text{g mL}^{-1}$	(Aly et al., 1998a)
Ciprofloxacin hydrochloride	Tablets and capsules	Cerium sulphate / sulphuric acid / Na_2SO_3 chemiluminescence.	0.27 mg L^{-1}	(Liang et al., 1997)
Co(II)	Eye lotions	Lophine / Co(II) / hydrogen peroxide chemiluminescence enhanced with hydroxylammonium chloride.	$4.5 \times 10^{-8} \text{ mol dm}^{-3}$	(Nakashima et al., 1997)
Codeine	Pharmaceutical samples	Permanganate / polyphosphoric acid chemiluminescence.	$2 \times 10^{-7} \text{ mol dm}^{-3}$	(Christie et al., 1995)
Dopamine	Aqueous	Inhibition of the lucigenin / Fe(II) / Brij 35 chemiluminescence reaction	$2 \times 10^{-9} \text{ mol dm}^{-3}$	(Zhang et al., 1999)
Dopamine, adrenaline and isoprenaline	Injection solutions	Treatment with EDTA / Reineche's salt. Detection using luminol / hydrogen peroxide chemiluminescence.	4 ng mL^{-1} , 20 ng mL^{-1} and 16 ng mL^{-1} respectively	(Li and Lu, 1997a)
Ergonovine maleate	Pharmaceutical preparations	Potassium ferricyanide / sodium hydroxide chemiluminescence enhanced using hexadecylpyridinium chloride.	$0.07 \text{ } \mu\text{g L}^{-1}$	(FusterMestre et al., 1999)

Table 1.3: Pharmaceutical applications (continued).

Furosemide	Tablets	Ce(IV) / sulphuric acid chemiluminescence, sensitised by rhodamine 6G	2.2×10^{-7} mol dm ⁻³	(Rao et al., 1999)
Glutathione and cysteine	Aqueous	Ce(IV) / hydrocortisone chemiluminescence.	2×10^{-7} mol dm ⁻³ and 1.4×10^{-6} mol dm ⁻³ respectively	(Li and Ci, 1997b)
Hydrochlorothiazide	Pharmaceutical preparations and tablets.	Ce(IV) / sulphuric acid chemiluminescence, sensitised by rhodamine 6G.	1.5×10^{-7} mol dm ⁻³	(Ouyang et al., 1998)
Hydrogen peroxide, glucose and periodate	Pharmaceuticals	Luminol / hydrogen peroxide / potassium periodate chemiluminescence (indirect CL using glucose oxidase for the detection of glucose)	3×10^{-4} mol dm ⁻³ , 0.08 µg mL ⁻¹ and 6×10^{-4} mol dm ⁻³ respectively	(Zhou et al., 1999)
Imipramine	Tablets	Imipramine / glyoxal / potassium permanganate chemiluminescence	12 ng mL ⁻¹	(Xue et al., 1999b)
Imipramine and chlorpromazine	Urine	Acidified permanganate chemiluminescence	5×10^{-5} mol dm ⁻³ and 2×10^{-5} mol dm ⁻³ respectively	(LopezPaz and Townshend, 1996)
Isoniazid	Pharmaceutical formulations	Inhibition of the luminol / hydrogen peroxide / potassium hexacyanoferrate(III) reaction.	5 mg L ⁻¹	(Alapont et al., 1998)
Isoniazid	Pharmaceuticals	Mn(II) / luminol / potassium periodate chemiluminescence.	30 ng mL ⁻¹	(Zhao et al., 1997a)
Levodopa	Tablets	Acidified permanganate chemiluminescence.	62 µg L ⁻¹	(Yang et al., 1998)
Medazepam	Drug formulations	Potassium permanganate / sulphuric acid chemiluminescence	1.85×10^{-5} mol dm ⁻³	(Sultan et al., 1998)
Menadione sodium bisulphite	Injection solutions	Ce(IV) / menadione sodium bisulphite chemiluminescence.	2×10^{-3} µg mL ⁻¹	(Huang et al., 1999a)
Methotrexate	Injection solutions and tablets	Permanganate / H ₂ SO ₄ / formaldehyde chemiluminescence.	3.4×10^{-9} mol dm ⁻³	(He et al., 1998a)
Morphine, sinomenine and codeine	Pharmaceutical preparations and tablets	Treated with EDTA / Reinecke's salt. Detected with luminol / hydrogen peroxide chemiluminescence.	60 ng mL ⁻¹ , 70 mg mL ⁻¹ and 600 ng mL ⁻¹ respectively	(Li and Lu, 1997b)
Naltrexone	Pharmaceutical preparations	Acidified permanganate chemiluminescence.	2.5 ng mL ⁻¹	(Campiglio, 1998a)
Naproxen	Pharmaceutical preparations	Ce(IV) / sulphuric acid chemiluminescence.	15 ng mL ⁻¹	(Campiglio, 1998b)
Paracetamol	Pharmaceutical formulations	Inhibition of the luminol / hydrogen peroxide / potassium hexacyanoferrate(III) reaction.	2.5 µg mL ⁻¹	(Alapont et al., 1999)
Penicillamine	Pharmaceutical preparations	Cerium (IV) / sulphuric acid chemiluminescence with quinine as a sensitiser.	15 pmol (50 µl injection)	(Zhang et al., 1995d; Zhang et al., 1996a)
Perphenazine	Drug formulations	Acidified permanganate chemiluminescence	50 mg L ⁻¹	(Sultan et al., 1999)
Persantin	Pharmaceutical preparations and tablets	Sodium hypochlorite chemiluminescence with Triton X-100 as an enhancer.	11 ng mL ⁻¹	(Nie et al., 1997)
Phenothiazines	Pharmaceutical preparations and biological fluids	Cerium(IV) / acid chemiluminescence with rhodamine B as a sensitiser.	0.01 - 0.1 µg mL ⁻¹	(Aly et al., 1998b)
Prednisone acetate	Tablets	Na ₂ SO ₃ / ammonium ceric sulphate / sulphuric acid chemiluminescence.	31 µg L ⁻¹	(Liang et al., 1998)
Progesterone and hydrocortisones	Aqueous	Cerium(IV) / sulphuric acid chemiluminescence sensitised with the mercapto-group (-SH) of the analyte.	0.10 µg mL ⁻¹	(Li and Ci, 1997a)

Table 1.3: Pharmaceutical applications (continued).

Promethazine hydrochloride	Tablets	Potassium permanganate / oxalic acid chemiluminescence.	$3.5 \times 10^{-8} \text{ g mL}^{-1}$	(Xue et al., 1999a)
Pyridoxine hydrochloride	Tablets	Luminol / hydrogen peroxide chemiluminescence	$6 \mu\text{g mL}^{-1}$	(Alwarthan and Aly, 1998)
Reserpine	Injection solutions	Permanganate / hydrogen peroxide / $\text{H}_6\text{P}_4\text{O}_{13}$ chemiluminescence.	$0.3 \mu\text{g mL}^{-1}$	(Li et al., 1998a)
Riboflavin	Injection solutions and tablets	Acidified potassium permanganate / sodium dithionite chemiluminescence.	62 ng mL^{-1}	(Li et al., 1997b)
Rutin	Sophora japonica L (traditional Chinese medicine)	Luminol / potassium hexacyanoferrate chemiluminescence.	$6.7 \times 10^{-9} \text{ g mL}^{-1}$	(He et al., 1999a)
Rutin	Traditional Chinese medicines.	Sodium hypochlorite / rutin / semicarbazide hydrochloride chemiluminescence.	13 pg L^{-1}	(Nie et al., 1999)
Salicylamide	Human urine and pharmaceutical formulations	Acidified potassium permanganate chemiluminescence.	30 ng mL^{-1}	(Mestre et al., 1999)
Sodium nitroprusside	Pharmaceuticals	Luminol / hydrogen peroxide chemiluminescence	$9 \times 10^{-9} \text{ mol dm}^{-3}$	(Wang et al., 1997b)
Tannic acid	Chinese gall (traditional medicine)	Inhibition of luminol / hydrogen peroxide / Cu(II) chemiluminescence	$9 \times 10^{-9} \text{ mol dm}^{-3}$	(Cui et al., 1998)
Tetracyclines	Commercial formulations	Acidic permanganate chemiluminescence with octylphenyl polyglycol ether as a sensitiser.	$0.4 - 0.6 \mu\text{g mL}^{-1}$	(Li et al., 1997d)
Tetracyclines	Pharmaceutical preparations	Cerium (IV) / sulphuric acid chemiluminescence with quinine as a sensitiser.	$0.025 - 0.25 \text{ nmol (50 } \mu\text{L injection)}$	(Zhang et al., 1995b)
Tetra-hydropalmatine	Pharmaceutical preparations and tablets	Acidified potassium permanganate / sodium dithionite chemiluminescence.	3.2 ng mL^{-1}	(Li et al., 1997a)
Thiamine nitrate	Tablets	Potassium hexacyanoferrate(III)/ sodium hydroxide / uranine chemiluminescence.	$2.0 \times 10^{-5} \text{ mol dm}^{-3}$	(Ishii and Kawashima, 1998)
Tiopronin	Pharmaceuticals	Ce(IV) / sulphuric acid chemiluminescence using rhodamine 6G and quinine as fluorophores.	$1 \times 10^{-7} \text{ mol dm}^{-3}$	(PerezRuiz et al., 1998)
Tiopronin	Pharmaceuticals	Cerium(IV) / sulphuric acid chemiluminescence with quinine as a sensitiser.	$3.4 \times 10^{-7} \text{ mol dm}^{-3}$	(Zhao et al., 1997b)
Vitamin B6	Injection solutions and tablets	$\text{Na}_2\text{S}_2\text{O}_3$ / potassium permanganate / sodium polyphosphate chemiluminescence.	58 ng mL^{-1}	(Li et al., 1998b)

1.4.1.2 Environmental applications

FI-CL methodologies have been applied to wide ranging analyses of environmental samples, as detailed in Table 2. Here these are categorised according to the type of sample matrix concerned, which include aquatic (natural waters, drinking water and wastewaters), geological, atmospheric and biological (human hair) matrices.

Natural, potable and waste waters

Metal ion determinations comprise the majority of published FI-CL applications for aqueous environmental samples. Many of these are based on the luminol reaction, which is catalysed by certain metal ions. Examples include determinations of cobalt(II) to sub-picomolar levels in river, sea and tap water (Lan and Mottola, 1996) and of copper(II) at nanomolar levels in natural water samples (Liu et al., 1996). Luminol CL has been used to detect iron(II) and iron(III) at nanomolar or sub-nanomolar levels in seawater (Bowie et al., 1998; deJong et al., 1998), natural freshwaters (Emmenegger et al., 1998; Qin et al., 1998b; Saitoh et al., 1998), groundwater (Zhou and Zhu, 1997) and treated waters (Zhou et al., 1997). Similar methods have measured chromium(III) at part-per-billion levels in tap water (Economou et al., 1998), and both chromium(III) and chromium(IV) at nanomolar levels in wastewaters (Escobar et al., 1995; Zhang et al., 1995a; Zhang et al., 1995e). Modified luminol-based methods have also been used to determine mercury(II) at part-per-billion levels in natural waters (Zhao et al., 1996), manganese(II) at sub-nanomolar levels in seawater (Okamura et al., 1998), lead(II) at part-per-trillion levels in wastewaters (Gong and Yu, 1995) and vanadium(IV) at nanomolar levels in tap water (Li et al., 1998d). The chemiluminescent reaction between 1,10-phenanthroline and hydrogen peroxide has been applied to the determination of copper complexation in seawater (Zamzow et al., 1998), while cobalt(II) in seawater has been determined using methods involving both gallic acid/hydrogen peroxide (Hirata et al., 1996) and quercetin/hydrogen peroxide/potassium hydroxide (Li et al., 1999a). The latter reaction has also been applied to determinations of chromium(III) and chromium(IV) in natural waters (Han et al., 1998). Iron(II) in rainwater has been determined using peroxyoxalate CL (Quass and Klockow, 1995), and iron(II)/total iron have been measured in both river and seawater using brilliant sulfoflavine/hydrogen peroxide CL (Hirata et al., 1999). A CL method based on the oxidation of 7,7,8,8-tetracyanoquinodimethane has been used to measure manganese(II) in

drinking water (Bowie et al., 1995), and the reaction between lucigenin, hydrogen peroxide and sodium hydroxide has been applied to the determination of lead(II) in natural waters.

As shown previously, a number of CL reactions are based on the oxidative properties of hydrogen peroxide. FI-CL methods have therefore proved highly suitable for determining low levels of dissolved hydrogen peroxide in natural waters. Nanomolar levels have been detected in rainwater using methods based on either octylphenyl polyglycol ether/potassium permanganate (Feng et al., 1997b) or cobalt(II)/luminol (Qin et al., 1997a; Qin et al., 1998a) reactions. The latter method has also been applied to nanomolar and sub-nanomolar determinations of hydrogen peroxide in seawater (Price et al., 1998; Yuan and Shiller, 1999), while a periodate/potassium carbonate CL method has been used to measure nanomolar levels in melted snow (Lin et al., 1998).

FI-CL methods have been used to determine a range of dissolved inorganic molecular and anionic species in aqueous samples. Free chlorine has been measured at part-per-billion levels in tap water using a luminol CL method (Liu et al., 1996), and modified luminol methods have been used to determine cyanide at nanomolar levels in river (Ikebukuro et al., 1996b), tap and waste waters (Lu et al., 1995). Sub-micromolar levels of hydrazine in drinking water have been determined with a CL method based on n-bromosuccinimide (Safavi and Baezzat, 1998). Hypochlorite ions have been measured in tap water using CL generated by the oxidation of indole by hydrogen peroxide (Cheregi et al., 1999). Variants of the luminol reaction have been applied to the determination of nanomolar levels of nitrite in natural (Mikuska et al., 1995) and potable (Yang et al., 1997) waters, and to the determination of phosphorus and phosphates at micro-nanomolar levels in natural freshwaters (Ikebukuro et al., 1996a; Ikebukuro et al., 1996c; Jiang et al., 1997; Nakamura et al., 1999a; Nakamura et al., 1999b; Noguchi et al., 1995) and drinking water (Nakamura

et al., 1997). Sulphite in tap water has been measured using an auto-oxidative CL method, sensitised with rhodamine 6G (Huang et al., 1999c). Dissolved oxygen has been quantified at part-per-million levels in river and tap waters using a luminol/sodium hydroxide CL method (Shen et al., 1999), while a gas diffusion method based on luminol/ozone CL has been applied to the measurement of part-per-billion levels of dissolved ozone in treated waters (Mcgowan and Pacey, 1995).

Determinations of a small number of organic/biological parameters using FI-CL methods have been reported. Acetaldehyde has been measured at part-per-billion levels in both natural and waste waters using a gallic acid/hydrogen peroxide CL method (Yao et al., 1997). Similar levels of volatile phenols present in polluted waters have been determined using a hydrogen peroxide/p-chlorobezenediazonium fluoroborate CL reaction (Zhuang et al., 1995). FI-CL methods have also been used to identify the presence of two strains of red tide phytoplankton in natural waters (Asai et al., 1998; Asai et al., 1999), with a CL reaction based on 2-methyl-6-(p-methoxyphenyl)-3,7-dihydroimidazo(1,2- α)-pyrazin-3-one/ superoxide used in each case.

Geological samples

FI-CL methods have been reported for the determinations of five elements in a variety of geological/geochemical samples. Trace levels of arsenic(III) in geochemical solutions have been determined using a luminol/hydrogen peroxide CL reaction (Jia and Zhou, 1995), while a similar method incorporating prior reduction of arsenic(V) to arsenic(III) has been applied to the measurement of total As in acidic extracts of rocks and ores (Jia et al., 1998). Picogram levels of iridium(IV) have been determined using a hydrogen peroxide/potassium hydroxide CL reaction (Han et al., 1997), and a luminol method incorporating potassium dichromate and sodium hydroxide has been applied to the

measurement of part-per-billion levels of antimony(III) in mineral extracts (Li et al., 1997c). Variants of the luminol reaction have also been applied to the determinations of vanadium(V) and zirconium(IV) in geochemical samples. The former uses luminol and hexacyanoferrate(II) as immobilised reagents on an anion-exchange column (Qin et al., 1997b) while the latter uses the frequently applied luminol/hydrogen peroxide reaction (Li et al., 1998c).

Atmospheric samples

Four FI-CL applications have been reported for the determination of micromolar – sub-nanomolar levels of sulphur dioxide in air and of sulphite in aqueous solution. Three of these methods use triethanolamine for initial adsorption, followed by CL reactions involving either ruthenium (2,2'-bipyridyl)₃/potassium persulphate (He et al., 1998b), tris(1,10-phenanthroline) ruthenium/potassium periodate (He et al., 1999d) or ruthenium (2,2'-bipyridyl)₃/potassium permanganate (Meng et al., 1999). The fourth method is based on luminol CL, with luminol initially immobilised on an anion exchange column, then eluted by hydrolysis (Qin et al., 1998d). Trace levels of hydrazine in air have been determined using a CL method based on the luminol/potassium periodate/sodium hydroxide reaction, following initial absorption of hydrazine in sulphuric acid solution (Wu et al., 1997). Part-per-billion levels of gaseous hydrogen peroxide have been measured with a method based on 1,1'-oxalyldi-imidazole/peroxyoxalate CL, and incorporating a diffusion scrubber (Stigbrand et al., 1996). The peroxyoxalate/hydrogen peroxide CL reaction has also been employed in the determination of a range of polycyclic aromatic hydrocarbons (PAHs) at part-per-billion levels in synthetic hexane and acetonitrile solutions (Andrew et al., 1997). This method of detection, in combination with chromatographic separation, would also prove suitable for the analysis of PAHs in solvent-extracted atmospheric particulate samples.

Human hair

Analysis of human hair samples can often provide useful indications of the body's intake of a range of chemical species, either through diet or by environmental exposure. Analyses of human hair samples using FI-CL methods have been reported, all of which focus on the determination of trace metal levels. Part-per-billion levels of cobalt and nickel have been measured in microwave-digested samples by utilising their catalytic effect in the CL reaction of alizarin purple/ethanol cetyltrimethylammonium bromide/potassium hydroxide (Fang and Liu, 1996). Similar levels of copper(II) have been determined using the luminol/potassium permanganate reaction, with these reagents initially immobilised on an anion-exchange resin, then eluted with sodium hydroxide (Chen and Qin, 1997). Iron(II) in hair has been determined using both the lucigenin/sodium hydroxide (Zhu and Lu, 1998) and the luminol/hydrogen peroxide (Saitoh et al., 1998) CL reactions, either of which are capable of nanomolar/part-per-trillion detection limits when sensitised with cationic surfactants. Manganese has been measured at part-per-billion levels using an iodine/luminol CL method (Shen et al., 1998), and part-per-million levels of vanadium(V) have been determined with a CL method based on the luminol/hexacyanoferrate(II) reaction (Qin et al., 1997b).

Table 1.4: Environmental applications.

Analyte	Sample matrix	Reaction	L.O.D.	Reference
Acetaldehyde	River / waste water	Gallic acid / hydrogen peroxide / sodium hydroxide chemiluminescence.	0.31 ng mL ⁻¹	(Yao et al., 1997)
As	Ores and rocks	Dissolution with HCl. Reduction of As(V) to As(III) using potassium iodide / thiourea followed by luminol / hydrogen peroxide / Cr(III) chemiluminescence detection.	3.4x10 ⁻⁵ mol dm ⁻³	(Jia et al., 1998)
As(III)	Geochemical samples	Sample mixed with K ₂ Cr ₂ O ₇ / H ₂ SO ₄ followed by luminol / hydrogen peroxide chemiluminescence.	1x10 ⁻¹⁰ mol dm ⁻³	(Jia and Zhou, 1995)
Chattonella antiqua (red tide phytoplankton)	Aqueous	2-Methyl-6-(p- methoxyphenyl)-3,7-dihydroimidazo[1,2- α]-pyrazin-3-one / superoxide chemiluminescence.	2x10 ³ cells mL ⁻¹	(Asai et al., 1998)
Chlorine	Tap water	Luminol is immobilised on an anion exchange resin column. Sodium hydroxide is passed through the column to elute luminol which is mixed with a sample stream to produce CL.	8x10 ⁻⁹ g mL ⁻¹	(Liu et al., 1996)

Table 1.4: Environmental applications (continued).

Co(II)	Seawater	Preconcentration using 8-quinolinol immobilised on 8HQ-MAF and chemiluminescence detection using gallic acid / hydrogen peroxide.	0.62 ng L ⁻¹	(Hirata et al., 1996)
Co(II)	River, sea and tap water	Luminol chemiluminescence enhanced with CO _{2(g)} .	5x10 ⁻¹³ mol dm ⁻³	(Lan and Mottola, 1996)
Co(II)	Seawater and river water	Quercetin / hydrogen peroxide / potassium hydroxide reversed flow injection chemiluminescence.	0.1 ng mL ⁻¹	(Li et al., 1999a)
Co(II) and Cr(III)	Natural waters	Cetyltrimethylammonium bromide / hydrogen peroxide / luminol chemiluminescence.	10 pg mL ⁻¹ and 12 pg mL ⁻¹ respectively	(Fang and Wang, 1997)
Co(II) and Ni(II)	Hair	Microwave digestion in HNO ₃ and hydrogen peroxide. Detection using alizarin purple / ethanol cetyltrimethylammonium bromide / potassium hydroxide.	0.1 µg L ⁻¹	(Fang and Liu, 1996)
Cr(III)	Tap water	Hydrogen peroxide / luminol chemiluminescence.	0.5 µg L ⁻¹	(Economou et al., 1998)
Cr(III) and Cr(VI)	Waste water	Reduction to Cr(III) using hydrogen peroxide followed by detection with luminol / hydrogen peroxide chemiluminescence.	< 10 ⁻⁹ mol dm ⁻³	(Escobar et al., 1995)
Cr(III) and Cr(VI)	Natural waters	Online oxidation of Cr(III) to Cr(VI) on a PbO ₂ column followed by quercetin / hydrogen peroxide / potassium hydroxide chemiluminescence.	1 ng mL ⁻¹	(Han et al., 1998)
Cr(III) and Cr(VI)	Waste water	Reduction using copper coated zinc followed by luminol / hydrogen peroxide chemiluminescence detection.	2.3x10 ⁻⁸ mol dm ⁻³	(Zhang et al., 1995a)
Cr(VI)	Waste water	Chemiluminescence produced by luminol and hexacyanoferrate(II) (immobilised on an anion-exchange resin column, eluted with sodium phosphate).	0.014 µg mL ⁻¹	(Zhang et al., 1995c)
Cu (complexed)	Seawater	1,10-Phenanthroline / hydrogen peroxide chemiluminescence.	1x10 ⁻¹⁰ mol dm ⁻³	(Zamzow et al., 1998)
Cu(II)	Hair	Luminol / potassium permanganate immobilised onto D201*7 anion-exchange resin. Elution with sodium hydroxide to react with Cu(II) to produce chemiluminescence.	0.2 ng mL ⁻¹	(Chen and Qin, 1997)
Cu(II)	Natural waters	Immobilised luminol / cyanide are eluted with Na ₃ PO ₄ and mixed with the sample / sodium hydroxide for chemiluminescence detection.	1.3x10 ⁻⁹ mol dm ⁻³	(Liu et al., 1998)
Cyanide	River water	Sulphite generated by the reaction of cyanide and sodium thiosulphate catalysed by immobilised rhodanese reacts with immobilised sulphite oxidase and produces sulphate and hydrogen peroxide which is detected with luminol and peroxidase.	1.2x10 ⁻⁸ mol dm ⁻³	(Ikebukuro et al., 1996b)
Cyanide	Tap and waste water	Luminol immobilised on Amberlyst resin with copper immobilised on D151 resin. Chemiluminescence is produced with cyanide.	2x10 ⁻⁹ g mL ⁻¹	(Lu et al., 1995)
Fe (dissolved)	Seawater	Preconcentration with TSK-8HQ followed by detection with luminol / hydrogen peroxide chemiluminescence.	2.2x10 ⁻¹¹ mol dm ⁻³	(deJong et al., 1998)
Fe and Mn(dissolved)	Underground water	Luminol / potassium periodate chemiluminescence.	3x10 ⁻⁶ µg mL ⁻¹ and 5x10 ⁻⁶ µg mL ⁻¹ respectively	(Zhou and Zhu, 1997)
Fe(II)	Natural waters	Luminol / hydrogen peroxide chemiluminescence.	2x10 ⁻⁹ mol dm ⁻³	(Emmenegger et al., 1998)
Fe(II)	Treated waters	O-phenanthroline / luminol / potassium periodate chemiluminescence (reversed flow-injection).	3 ng L ⁻¹	(Zhou et al., 1997)
Fe(II)	Hair	Lucigenin / sodium hydroxide chemiluminescence with cetyltrimethylammonium bromide as a sensitiser.	2 pg mL ⁻¹	(Zhu and Lu, 1998)
Fe(II) and Fe(III)	Seawater	Preconcentration with 8-HQ followed by detection with luminol chemiluminescence	4x10 ⁻¹¹ mol dm ⁻³	(Bowie et al., 1998)
Fe(II) and Fe(III)	Natural waters	Fe(III) reduced to Fe(II) with Cu coated Zn. Luminol immobilised on an anion-exchange resin. Eluted with sodium hydroxide for chemiluminescence detection.	0.4 ng L ⁻¹	(Qin et al., 1998b)
Fe(II) and hydrogen peroxide	Rain water	Oxygen / peroxyoxalate chemiluminescence.	<1x10 ⁻⁷ mol dm ⁻³	(Quass and Klockow, 1995)

Table 1.4: Environmental applications (continued).

Fe(II) and total Fe	River and seawater	Preconcentration on amberlite XAD-4 functionalised by N-hydroxyethylthylenediamine groups followed by brilliant sulfoflavine / hydrogen peroxide chemiluminescence detection.	0.80 nmol L ⁻¹ and 0.36 nmol L ⁻¹ respectively	(Hirata et al., 1999)
Fe(II) and total Fe	Human hair and natural waters	Luminol / hydrogen peroxide chemiluminescence enhanced with cationic surfactant tetradecyltrimethylammonium bromide.	2x10 ⁻⁹ mol dm ⁻³ and 1x10 ⁻⁹ mol dm ⁻³ respectively	(Saitoh et al., 1998)
Heterosigma carterae (red tide phytoplankton)	Aqueous	2-Methyl-6-(p-methoxyphenyl)-3,7-dihydroimidazo[1,2- α]-pyrazin-3-one (MCLA) / superoxide chemiluminescence.	1x10 ² cells mL ⁻¹	(Asai et al., 1999)
Hg(II)	Environmental waters	Cu(II) / luminol / sodium hydroxide / potassium hexacyanoferrate(II) chemiluminescence.	0.33 μ g L ⁻¹	(Zhao et al., 1996)
Hydrazine	Drinking water	Chemiluminescence produced from N-bromosuccinimide in alkaline medium with dichlorofluorescein as sensitizer.	5x10 ⁻⁷ mol dm ⁻³	(Safavi and Baezzat, 1998)
Hydrazine	Air	Absorption in sulphuric acid followed by luminol / potassium periodate / sodium hydroxide chemiluminescence	2 μ g m ⁻³	(Wu et al., 1997)
Hydrogen peroxide	Rainwater	Octylphenyl polyglycol ether / acidic potassium permanganate chemiluminescence.	6.0x10 ⁻⁹ mol dm ⁻³	(Feng et al., 1997b)
Hydrogen peroxide	Snow-water	Potassium periodate / potassium carbonate chemiluminescence.	5x10 ⁻⁹ mol dm ⁻³	(Lin et al., 1998)
Hydrogen peroxide	Seawater	Co(II) / luminol chemiluminescence	1.06x10 ⁻⁸ mol dm ⁻³	(Price et al., 1998)
Hydrogen peroxide	Rainwater	Immobilised Co(II) and luminol are eluted with NaSO ₄ . Chemiluminescence is produced in the presence of hydrogen peroxide	3.5x10 ⁻⁸ mol dm ⁻³	(Qin et al., 1997a)
Hydrogen peroxide	Rain water	Luminol and Co(II) are immobilised on a strongly basic anion-exchange resin and a weakly acid cation-exchange resin, respectively. Reagents are eluted by hydrolysis for chemiluminescence detection.	1.2x10 ⁻⁸ mol dm ⁻³	(Qin et al., 1998a)
Hydrogen peroxide	Air	Hydrogen peroxide diffusion scrubbed. Detection with 1,1'-oxalyldi-imidazole / peroxyoxalate chemiluminescence	3.4 ppbv	(Stigbrand et al., 1996)
Hydrogen peroxide	Seawater	Co(II) / hydrogen peroxide / luminol chemiluminescence.	4.2x10 ⁻¹⁰ mol dm ⁻³	(Yuan and Shiller, 1999)
Hypochlorite	Tap water	Oxidation of indole in propan-2-ol with hydrogen peroxide.	5 μ g mL ⁻¹	(Cheregi et al., 1999)
Ir(IV)	Ores and rocks	Potassium hydroxide / hydrogen peroxide / Tween-80 chemiluminescence.	11 pg L ⁻¹	(Han et al., 1997)
Mn	Human hair	Sample ashed and dissolved in acid. detected using I ₂ / luminol chemiluminescence	0.1 ng mL ⁻¹	(Shen et al., 1998)
Mn(II)	Potable water	7,7,8,8- Tetracyanoquinodimethane oxidation with Eosin Y as a sensitizer.	4.5 μ g L ⁻¹	(Bowie et al., 1995)
Mn(II)	Natural waters	Immobilised luminol and IO ₄ ⁻ eluted with Na ₃ PO ₄ for chemiluminescence with Mn(II).	1x10 ⁻⁹ g mL ⁻¹	(Lu and Zhang, 1995)
Mn(II)	Seawater	Luminol / hydrogen peroxide chemiluminescence.	2.9x10 ⁻¹¹ mol dm ⁻³	(Okamura et al., 1998)
Nitrite	Natural waters	Nitrite reacts with hydrogen peroxide to form peroxynitrite which produces chemiluminescence with luminol.	1x10 ⁻⁹ mol dm ⁻³	(Mikuska et al., 1995)
Nitrite	Drinking water and food	Luminol / I ₂ (produced from potassium iodide in acid) chemiluminescence.	1.6 ng mL ⁻¹	(Yang et al., 1997)
Oxygen (dissolved)	River and tap water	Reaction with MnSO ₄ / iodine / potassium iodide / ammonia to form a precipitate that is dissolved in H ₂ SO ₄ / H ₃ PO ₄ . Detection with luminol / sodium hydroxide.	0.412 mg L ⁻¹	(Shen et al., 1999)
Ozone	Treated waters	Luminol / ozone chemiluminescence using gas diffusion FIA.	8 μ g O ₃ L ⁻¹	(Mcgowan and Pacey, 1995)
Pb(II)	Natural waters	Lucigenin / hydrogen peroxide / sodium hydroxide chemiluminescence enhanced with ethanol.	0.1 μ g mL ⁻¹	(Gong and Yu, 1995)

Table 1.4: Environmental applications (continued).

Pb(II)	Waste water	Pb(II) replaces Fe(II) from an EDTA complex. Fe(II) reacts with luminol / sodium hydroxide to produce chemiluminescence.	20 ng L ⁻¹	(Li et al., 1998e)
Phosphate	Natural waters	Hydrogen peroxide produced from the reaction of immobilised pyruvate oxidase with phosphate is detected using luminol / horseradish peroxidase chemiluminescence.	7.4x10 ⁻⁸ mol dm ⁻³	(Ikebukuro et al., 1996c; Ikebukuro et al., 1996a)
Phosphate	Drinking water	Phosphate ion-dependent pyruvate oxidase reaction produces hydrogen peroxide which is detected using luminol chemiluminescence catalysed by arthromyces ramosus peroxidase.	1.6x10 ⁻⁷ mol dm ⁻³	(Nakamura et al., 1997)
Phosphate	River water	Immobilised pyruvate oxidase G producing hydrogen peroxide for luminol chemiluminescence detection.	9.6x10 ⁻⁸ mol dm ⁻³	(Nakamura et al., 1999b)
Phosphate	River water	Maltose phosphorylase, mutarotase, and glucose-oxidase immobilised on N-hydroxysuccinimide beads with arthromyces ramosus peroxidase / luminol chemiluminescence detection.	1x10 ⁻⁶ mol dm ⁻³	(Nakamura et al., 1999a)
Phosphate	Natural waters	Purine nucleoside phosphorylase and xanthine oxidase immobilised on aminopropyl-controlled pore glass beads convert phosphate to hydrogen peroxide for peroxyoxalate / rhodamine B chemiluminescence detection.	3.9x10 ⁻⁸ mol dm ⁻³	(Noguchi et al., 1995)
Phosphorous	Natural waters	Conversion to phosphomolybdic acid using HClO ₄ / ammonium molybdate with Luminol / sodium hydroxide chemiluminescence detection.	36 µg L ⁻¹	(Jiang et al., 1997)
Polyaromatic hydrocarbons	Organic (hexane or acetonitrile)	Peroxyoxalate / hydrogen peroxide chemiluminescence.	0.6-79 µg L ⁻¹	(Andrew et al., 1997)
Sb(III)	Ores and rocks	K ₂ Cr ₂ O ₇ / luminol / sodium hydroxide chemiluminescence.	0.1 ng mL ⁻¹	(Li et al., 1997c)
Sulphite	Tap water	Auto- oxidation sensitised by rhodamine 6G (immobilised on cation exchange resin) enhanced with Tween 80.	0.01 mg L ⁻¹	(Huang et al., 1999c)
Sulphite and sulphur dioxide	Water and air	Luminol immobilised on an anion exchange column. Luminol is eluted by hydrolysis for chemiluminescence detection.	1x10 ⁻⁷ mol dm ⁻³	(Qin et al., 1998d)
Sulphur dioxide	Air	Adsorption using triethanolamine followed by Ru(2,2'-bipyridyl) ₃ ²⁺ / SO ₃ ²⁻ / K ₂ S ₂ O ₈ chemiluminescence.	4.1x10 ⁻⁸ mol dm ⁻³	(He et al., 1998b)
Sulphur dioxide	Air	Absorption on triethanolamine followed by Tris(1,10-phenanthroline)ruthenium / potassium periodate chemiluminescence.	7x10 ⁻¹⁰ mol dm ⁻³	(He et al., 1999d)
Sulphur dioxide	Air	Adsorption using triethanolamine followed by Ru(2,2'-bipyridyl) ₃ ²⁺ / SO ₃ ²⁻ / potassium permanganate chemiluminescence	2.5x10 ⁻⁸ mol dm ⁻³	(Meng et al., 1999)
V(IV)	Tap water	Potassium dichromate / potassium iodide / sodium hydroxide / luminol chemiluminescence.	7x10 ⁻¹⁰ mol dm ⁻³	(Li et al., 1998d)
V(V)	Geochemical and hair samples	Luminol and hexacyanoferrate(II), are both immobilised on an anion-exchange resin column, and are eluted with phosphoric acid to produce chemiluminescence.	5.4x10 ⁻³ µg cm ⁻³	(Qin et al., 1997b)
Volatile Phenols	Polluted waters	Quenched p-chlorobenzenediazonium fluoroborate / hydrogen peroxide chemiluminescence.	0.015-0.03 µg mL ⁻¹	(Zhuang et al., 1995)
Zr(IV)	Rocks and ores	Luminol / hydrogen peroxide chemiluminescence.	30 pg L ⁻¹	(Li et al., 1998c)

1.4.1.3 Food and beverage applications

This has been an area of considerable development in recent years. The bioluminescence reaction involving firefly luciferase has been used to monitor bacterial contamination of foods but CL reactions have also been used to quantify species such as ascorbic acid, sulphite and carbohydrates in alcoholic beverages and a variety of foods.

Ascorbic acid

Various FI-CL systems have been used to determine ascorbic acid in vegetables and non-alcoholic beverages with detection limits in the range $0.2 \mu\text{g l}^{-1}$ - 5.5 mg l^{-1} . The oxidation of ascorbic acid by permanganate produced CL (Agater and Jewsbury, 1997) and results compared well with spectrofluorimetric and titrimetric methods but transition metals, sorbitol and mannitol interfered. The inhibition of the CL reaction between ferricyanide and luminol can also be used for the detection of ascorbic acid. This reaction can be performed with (Zhang and Qin, 1996) or without (Chen et al., 1997) the reagent being immobilised on an anion exchange column. The CL reaction between luminol, permanganate and sodium hydroxide has also been used for the determination of ascorbic acid and, as with the previous reaction, the reagent can be used with (Wang et al., 1997a) or without (Ye and Wang, 1998) reagent immobilisation on an anion exchange column. Mn(II) and Cu(II) interfered when present at 5-fold excess.

Sulphite

A variety of CL reactions have been used for the determination of sulphite in diverse matrices, including sugar, beers and wines with sub micro-molar limits of detection. Chemiluminescence produced from tri(2,2'-bipyridyl)ruthenium(II) in the presence of either $\text{K}_2\text{S}_2\text{O}_8$ (He et al., 1998b) or KMnO_4 (Meng et al., 1999) was used to quantify sulphite in sugar and sulphur dioxide in air (absorbed in triethanolamine) with interference from Cu(II) and EDTA. Sulphite (detection limit $30 \mu\text{g ml}^{-1}$) has also been determined in beers and wines using the auto-oxidation of sulphite sensitised by rhodamine 6G in the presence of Tween 80 surfactant micelles (Huang et al., 1999e), with interference from S^{2-} , NO_2^- , and ascorbic acid. This is a good example of the increasing trend to add surfactants to enhance CL emission by providing a protective environment for the reaction. FI-CL has been used to determine sulphite in wines using the NaHCO_3 / sodium carbonate / Cu(II)

CL reaction (Lin and Hobo, 1996). The manifold incorporated a gas diffusion module to enhance selectivity and remove physical interferences but iodide, Co(II), Ni(II), and S²⁻ interfered at 2-fold excess.

Carbohydrates

Chemiluminescence is produced when carbohydrates are oxidised by acidified potassium permanganate (Agater et al., 1996). Glucose, galactose, fructose, arabinose, xylose, lactose and sucrose have all been detected over the linear range 10^{-4} - 10^{-1} mol dm⁻³. The oxidation of pyrogallol by periodate has been used for the detection of carbohydrates (Evmiridis et al., 1999) with a detection limit for hexose of 20 µg. An interesting aspect of this method was the use of differential CL reaction kinetics to allow the resolution of binary mixtures of glucose and fructose.

Other analytes

Luminol chemiluminescence has been used for a variety of other analytes in food and beverages including 3,4-dihydroxybenzoic acid (Cui et al., 1999), choline (Yaqoob et al., 1997a), citric acid (Li and Zhuang, 1999), ethanol (Danet et al., 1997), malate (Kiba et al., 1995a), glycerol (Kiba et al., 1996), lactate (Hemmi et al., 1995), nitrite (Yang et al., 1997) and tannic acid (Cui et al., 1998). 3,4-Dihydroxybenzoic acid (protocatechuric acid) (Cui et al., 1999) has been measured in wines using CL produced by the oxidation of luminol with hydrogen peroxide with Co(II) as a catalyst. Citric acid in orange drinks (Li and Zhuang, 1999) has also been determined using the luminol reaction. Fe(III) is reduced by citric acid to Fe(II), which can then be detected with luminol but ascorbic acid interfered. Ethanol in beer can be indirectly determined by the enzymatic generation of hydrogen peroxide using alcohol oxidase (Danet et al., 1997). Cysteine interference was removed by prior

complexation with Cu(II). Malate (Kiba et al., 1995a), glycerol (Kiba et al., 1996) and lactate (Hemmi et al., 1995) have all been indirectly detected using immobilised enzyme reactors to produce hydrogen peroxide, followed by luminol detection. Nitrite can be detected in foods by reaction with KI (Yang et al., 1997) to produce I_2 which then reacted with luminol. Fe(II), Cu(II), AsO_4^{3-} and SbO_4^{3-} interfered. Tannic acid (Cui et al., 1998) has been determined in hop pellets using the luminol / peroxide reaction with a Cu(II) catalyst and hydrogen peroxide has been measured in fermented liquors (Hasebe et al., 1996) using bis-(2,4,6- trichlorophenyl)oxalate and perylene chemiluminescence. The latter method has been applied to the determination of glutamic acid in culture media. Acetaldehyde has been determined using gallic acid / hydrogen peroxide / sodium hydroxide chemiluminescence (Yao et al., 1997) but formaldehyde, Co(II), Mn(II), Ag(I), Cd(II), Pb(II) and permanganate were all found to interfere. Amino acids have been detected in food (lysine) and serum (phenylalanine) using both the luminol / hydrogen peroxide and o-phenanthroline / hydrogen peroxide reactions (Zhang et al., 1996c).

Table 1.5: Food and beverage applications.

Analyte	Sample matrix	Reactions	L.O.D.	Reference
3, 4-Dihydroxybenzoic acid	Wines	Inhibition of the luminol / hydrogen peroxide / Co(II) chemiluminescence.	2.7×10^{-7} mol dm ⁻³	(Cui et al., 1999)
Acetaldehyde	Alcoholic beverages	Gallic acid / hydrogen peroxide / sodium hydroxide chemiluminescence.	0.31 ng mL ⁻¹	(Yao et al., 1997)
Amino acids	Food	Cu(II) amino acid complex formation catalysis of the luminol / hydrogen peroxide and o-phenanthroline / hydrogen peroxide chemiluminescence systems	Pmol	(Zhang et al., 1996c)
Ascorbic acid	Beverages	Oxidation with permanganate / acid.	5×10^{-7} mol dm ⁻³	(Agater and Jewsbury, 1997)
Ascorbic acid	Mung bean sprouts, tomato and cucumber skins.	Luminol / Fe(II) / $Na_2B_4O_7$ / potassium hydroxide chemiluminescence.	0.2 ng mL ⁻¹	(Chen et al., 1997)
Ascorbic acid	Fruit juices	Luminol and potassium permanganate immobilised on resins in a glass column. Eluent mixed with sodium hydroxide to produce chemiluminescence.	5 µg L ⁻¹	(Wang et al., 1997a)
Ascorbic acid	Vegetables	Acidified permanganate / luminol chemiluminescence	0.1 µg mL ⁻¹	(Ye and Wang, 1998)
Ascorbic acid	Vegetables	Inhibition of the chemiluminescence produced from luminol and ferricyanide (immobilised on an anion-exchange resin column, eluted with sodium phosphate).	5.5×10^{-3} µg mL ⁻¹	(Zhang and Qin, 1996)
Carbohydrates	Aqueous	Acidified permanganate / Mn(II) chemiluminescence.	1×10^{-4} mol dm ⁻³	(Agater et al., 1996)

Table 1.5: Food and beverage applications (continued).

Choline	Cabbage	Luminol / Co(II) detection of hydrogen peroxide produced from an immobilised choline oxidase column.	1×10^{-7} mol dm ⁻³	(Yaqoob et al., 1997a)
Citric acid	Non-alcoholic beverages	Reduction of Fe(III) to Fe(II) with citric acid followed by luminol chemiluminescence detection.	0.1 µg mL ⁻¹	(Li and Zhuang, 1999)
Ethanol	Beer	Production of hydrogen peroxide using alcohol oxidase followed by luminol chemiluminescence.	0.01% (v/v)	(Danet et al., 1997)
Free L-malate	Wines	Malate dehydrogenase/reduced nicotinamide adenine dinucleotide oxidase co-immobilised on polymer beads to produce hydrogen peroxide for detection using luminol / hexacyanoferrate(III) chemiluminescence.	8×10^{-8} mol dm ⁻³	(Kiba et al., 1995a)
Glucose and fructose	Aqueous	Pyrogallol / hydroxylamine hydrochloride / periodate chemiluminescence.	Not reported	(Evmiridis et al., 1999)
Glycerol	Wines	Glycerol dehydrogenase and NADH oxidase are co-immobilised on poly (vinyl alcohol) beads to produce hydrogen peroxide which was detected using luminol / hexacyanoferrate (III) chemiluminescence.	7×10^{-8} mol dm ⁻³	(Kiba et al., 1996)
Hydrogen peroxide	Fermented liquors	Peroxyoxalate chemiluminescence in an emulsion of ethyl acetate, non-ionic surfactant polyoxyethylene(20) sorbitane monolaurate(Tween 20) and water.	1×10^{-6} mol dm ⁻³	(Hasebe et al., 1996)
L-lactate	Food samples	Immobilised L-lactate oxidase to produce hydrogen peroxide for luminol / horseradish peroxidase / luminol chemiluminescence detection.	1×10^{-7} mol dm ⁻³	(Hemmi et al., 1995)
Nitrite	Water and food	Luminol / I ₂ (produced from potassium iodide in acid) chemiluminescence.	1.6 ng mL ⁻¹	(Yang et al., 1997)
Sulphite	Sugar	Ru(2,2'-bipyridyl) ₃ ²⁺ / SO ₃ ²⁻ / K ₂ S ₂ O ₈ chemiluminescence.	4.1×10^{-4} mol dm ⁻³	(He et al., 1998b)
Sulphite	Beers and wines	Auto- oxidation sensitised by rhodamine 6G (immobilised on cation exchange resin) enhanced with Tween 80.	0.03 mg L ⁻¹	(Huang et al., 1999e)
Sulphite	Wines	Na ₂ CO ₃ / NaHCO ₃ / Cu(II) chemiluminescence with a gas diffusion module.	5×10^{-7} mol dm ⁻³	(Lin and Hobo, 1996)
Sulphite	Sugar	Ru(2,2'-bipyridyl) ₃ ²⁺ / SO ₃ ²⁻ / potassium peroxide chemiluminescence	2.5×10^{-8} mol dm ⁻³	(Meng et al., 1999)
Tannic acid	Hop pellets samples	Inhibition of luminol / hydrogen peroxide / Cu(II) chemiluminescence	9×10^{-9} mol dm ⁻³	(Cui et al., 1998)

1.4.1.4 Biomedical applications

A particularly wide variety of analytes have been determined by enzymatic conversion to produce hydrogen peroxide which is then quantitatively detected by the luminol or peroxyoxalate reactions.

The luminol reaction has been used for the measurement of hydrogen peroxide generated from acetylcholine and choline after passing the sample through two consecutive columns containing immobilised acetylcholinesterase and choline oxidase (Fan and Zhang, 1996; Hasebe et al., 1997). Similarly ATP has been determined with immobilised alkaline phosphatase (Fujiwara et al., 1997) and glycerol-3-phosphate with immobilised glycerol-3-phosphate oxidase (Yaqoob et al., 1997b). Branched-chain l-amino-acids have been

determined in human plasma with recoveries in the range 98-102% by passing the analyte solution through an enzyme reactor column containing leucine dehydrogenase and NADH oxidase (Kiba et al., 1995b) or by introducing the solution mixed with luminol and NAD^+ into a spiral flow cell onto which leucine hydrogenase, NADH oxidase and peroxidase have been immobilised (Kiba et al., 1998). Cholesterol has been monitored by passing the sample through immobilised cholesterol oxidase (Huang et al., 1999f; Nabi et al., 1996) and L-alanine, alpha-ketoglutarate and L-glutamate determined with immobilised alanine aminotransferase and glutamate oxidase (Janasek and Spohn, 1999). Glucose has been monitored in subcutaneous tissue fluid and blood of rabbits after passing through immobilised glucose oxidase (Fang et al., 1997), in plasma by flowing through immobilised pyranose oxidase (Kiba et al., 1997), in mixtures with 3-hydroxybutyrate by passing through two NADH oxidase enzyme reactors containing glucose dehydrogenase and 3-hydroxybutyrate dehydrogenase (Kiba et al., 1995c) and in mixtures with lysine by passing through immobilised glucose oxidase to measure glucose and through lysine oxidase to measure lysine (Almuaibed and Townshend, 1997). The same principle has been applied to the measurement of lysine and ornithine (Almuaibed and Townshend, 1999). On line co-immobilised 3-hydroxybutyrate dehydrogenase and NADH oxidase has been used for the measurement of 3-hydroxybutyrate in serum with very good agreement with other accepted analytical methods (Tabata and Totani, 1995). Hydrogen peroxide can also be produced from sulphated bile acids by passing through a column of bile acid sulphate sulphotase and 3beta-hydroxysteroid dehydrogenase (Gao et al., 1997). Alpha-chymotrypsin, trypsin and a commercial protease have been determined by passing through a mini-column containing immobilised isoluminol (Edwards et al., 1995).

Catecholamines can be measured in plasma by imidazole conversion to hydrogen peroxide prior to the peroxyoxalate (PO) CL reaction (Nozaki et al., 1999). D-amino acids in plasma

have been determined with good agreement with a colorimetric method by flowing through a specific enzyme reactor containing D-amino acid oxidase before PO CL detection of hydrogen peroxide (Wada et al., 1997). Incubation of dopamine with imidazole at 60 °C for 30 min in the dark generated hydrogen peroxide which was then introduced into a FI analyser for PO-CL detection (Nozaki et al., 1996). Glucose or choline and acetylcholine, separated by a cation exchange column, have been determined in urine by conversion to hydrogen peroxide via a glucose oxidase reactor or a choline oxidase/acetylcholine esterase reactor respectively (Emteborg et al., 1997). Compounds containing an alcoholic or phenolic hydroxyl group (polyphenols, monophenols and sugars) have been determined by mixing with imidazole and heating to 80 °C prior to PO detection of hydrogen peroxide (Nozaki et al., 1995).

Several other reactions have also been used for biomedical applications of FI-CL. Amino acids have been determined by complexation with cobalt(II) and inhibition of the lucigenin CL reaction (Chen et al., 1996a), by the inhibiting effect of cobalt(II) complexes or enhancing effect of copper(II) complexes on the ninhydrin-hydrogen peroxide CL reaction (Chen et al., 1996b) and by their effect on the 1,10-phenanthroline-copper(II)-hydrogen peroxide CL reaction (Li et al., 1995). Chlorotetracycline has been assayed in urine, with recoveries of 98.8-101.1% by its effect on the copper(II)-ammonium carbonate-cetyltrimethylammonium bromide-hydrogen peroxide CL reaction (Li and Wang, 1998). Proteins have been monitored via the catalytic effect of copper(II) complexes on the luminol (Li et al., 1999b) or phenanthroline (Ping et al., 1998)-hydrogen peroxide CL reaction and porphyrins have been measured in urine via the PO-hydrogen peroxide reaction (Lin and Huie, 1997).

Chromium(III) has been determined in urine, blood serum and hair by its catalytic effect on the luminol-hydrogen peroxide CL. The method was validated by analysing a certified reference material and recoveries were within the range of 89-115% (Escobar et al., 1998). Copper(II) in serum has been determined with recoveries of 94-97% by its effect on the hydroxylamine-fluorescein CL reaction (Lin and Hobo, 1995) and iron(III) in blood has been monitored by its effect on the luminol and the results showed good agreement with those obtained by flame AAS (Qin et al., 1998c). Iodide in urine can be monitored after oxidation to iodine with dichromate and measurement by the cobalt(II) catalysed luminol reaction (Burguera et al., 1996). The luminol reaction with $K_7[Cu(IO_6)]_2$ has been used for determining glucose (Huang et al., 1997) or uric acid (Feng et al., 1997a) while vanilmandelic acid has been determined by its enhancement effect on the luminol-hexacyanoferrate(III) CL reaction, with an excellent detection limit (Barnett et al., 1999), and uric acid has been measured in urine by its inhibiting effect on the luminol-periodate reaction with results comparable with those obtained by spectrophotometry (Wu et al., 1999a).

Serum glucose has been assayed by the CL reaction of hydrogen peroxide with 3-propyl-7,8-dihydropyridazino-[4,5-g]quinoxaline-2,6,9(1 H)-trione, which is a luminol related compound (Ishida et al., 1995). Acidic permanganate has been used as a chemiluminogenic reagent for the determination of pyruvate in serum (Feng et al., 1999), serotonin and related indoles (Barnett et al., 1998) and uric acid (Li et al., 1998f). Tryptophan has been found to exhibit CL by the action of cerium(IV) (Alwarthan, 1995) while bilirubin generates CL by reaction with N-bromosuccinimide or sodium hypochlorite (Palilis et al., 1996).

Table 1.6: Biomedical applications.

Analyte	Sample matrix	Reaction	L.O.D.	Reference
Acetylcholine and choline	Rat brain tissue	Following sample preparation, injection into stream, passed through two reactors containing acetylcholinesterase and choline oxidase immobilised on glass beads. Detection using Co(II) / luminol chemiluminescence.	600 and 500 fmol respectively	(Fan and Zhang, 1996)
Acetylcholine and choline	Culture media	Production of hydrogen peroxide using an immobilised enzyme reactor followed by detection with luminol / Co(II) chemiluminescence enhanced using sodium dodecylsulphate.	1×10^{-6} mol dm ⁻³	(Hasebe et al., 1997)
Adenosine-5'-triphosphate	Aqueous	Alkaline phosphatase from <i>Escherichia coli</i> (immobilised) is used to produce hydrogen peroxide for detection with luminol / heteropoly acid.	1×10^{-8} mol dm ⁻³	(Fujiwara et al., 1997)
Alpha-chymotrypsin, trypsin and a commercial protease	Aqueous	Immobilised tripeptide and isoluminol / Co(II) / hydrogen peroxide chemiluminescence.	2.7×10^{-4} mg L ⁻¹ , 4×10^{-2} mg L ⁻¹ and 2×10^{-3} mg L ⁻¹ respectively	(Edwards et al., 1995)
Amino acids	Aqueous	Inhibition of the lucigenin / Co(II) chemiluminescence reaction	1×10^{-9} - 2×10^{-7} mol dm ⁻³	(Chen et al., 1996a)
Amino acids	Aqueous	Ninhydrin / hydrogen peroxide / Cu(II) or Co(II) chemiluminescence.	4.2×10^{-7} - 9.2×10^{-6} mol dm ⁻³	(Chen et al., 1996b)
Amino acids, polyamines, and salicylic acids	Aqueous	Unsaturated complex of Cu(II) and organic ligands enhanced 1,10-phenanthroline / hydrogen peroxide / CTMAB chemiluminescence	2.7-90 pmol	(Li et al., 1995)
Bilirubin	Aqueous	N-bromosuccinimide / sodium hypochlorite chemiluminescence.	1.75 µg mL ⁻¹	(Palilis et al., 1996)
Branched-chain l-amino-acids	Plasma	Leucine dehydrogenase and NADH oxidase are co-immobilised on aminated poly(vinyl alcohol) beads to produce hydrogen peroxide which is detected using luminol / hexacyanoferrate (III) chemiluminescence.	3×10^{-7} mol dm ⁻³	(Kiba et al., 1995b)
Catecholamines	Plasma	Imidazole catalysed decomposition by catecholamines producing hydrogen peroxide which is detected using peroxyoxalate chemiluminescence.	Not reported	(Nozaki et al., 1999)
Chlorotetracycline	Urine	Copper sulphate / hydrogen peroxide / ammonium carbonate / cetyltrimethylammonium bromide chemiluminescence.	4×10^{-8} mol dm ⁻³	(Li and Wang, 1998)
Cholesterol	Human serum	Cholesterol oxidase immobilised on amine-modified silica gel in a column is used to produce hydrogen peroxide. Luminol and ferrieyanide are co-immobilised on an anion-exchange column for chemiluminescence detection.	5×10^{-6} g mL ⁻¹	(Huang et al., 1999f)
Cholesterol	Blood serum	Hydrogen peroxide produced from an immobilised cholesterol oxidase column is detected using luminol / Co(II) chemiluminescence.	0.1 mg L ⁻¹	(Nabi et al., 1996)
Cr(III)	Urine	Luminol / hydrogen peroxide chemiluminescence.	0.01 µg L ⁻¹	(Escobar et al., 1998)
Cu(II)	Serum	Hydroxylamine / fluorescein / hydroxide chemiluminescence.	0.5 µg L ⁻¹	(Lin and Hobo, 1995)
D-amino acids	Human plasma	Immobilised enzyme column reactor with peroxyoxalate chemiluminescence detection.	0.4 to 30 pmol (10 µL injection)	(Wada et al., 1997)
D-glucose and 3-hydroxybutyrate	Serum	2 Enzyme reactors, one containing glucose dehydrogenase and NADH oxidase the other containing 3-hydroxybutyrate dehydrogenase and NADH oxidase co-immobilised on beads to produce hydrogen peroxide for detection using luminol / hexacyanoferrate(III) chemiluminescence.	1×10^{-5} mol dm ⁻³ and 1×10^{-6} mol dm ⁻³ respectively	(Kiba et al., 1995c)
Dopamine	Biochemical samples	Imidazole / peroxyoxalate chemiluminescence.	10 nmol (20 µL injection)	(Nozaki et al., 1996)
Fe(III)	Blood	Luminol and potassium hexacyanoferrate(II), are immobilised on a D201 anion exchange column. Elution with NaSO ₄ . Detection with sodium hydroxide.	7 µg L ⁻¹	(Qin et al., 1998c)

Table 1.6: Biomedical applications (continued).

Glucose	Rabbit fluid and blood	Micro-dialysis followed by reaction in an immobilised glucose oxidase reactor to produce hydrogen peroxide, detected with luminol / hexacyanoferrate chemiluminescence	$1 \times 10^{-5} \text{ mol dm}^{-3}$	(Fang et al., 1997)
Glucose	Biochemical samples	$\text{K}_2[\text{Cu}(\text{IO}_6)]_2$ / luminol / sodium hydroxide chemiluminescence.	0.18 ng mL^{-1}	(Huang et al., 1997)
Glucose	Plasma	Immobilised pyranose oxidase produced hydrogen peroxide for reaction with luminol in a flow cell containing immobilised peroxidase.	$3 \times 10^{-9} \text{ mol dm}^{-3}$	(Kiba et al., 1997)
Glucose acetylcholine and choline	Urine	Production of hydrogen peroxide using immobilised glucose oxidase or acetylcholine esterase / choline oxidase followed by peroxyoxalate chemiluminescence detection.	$3 \times 10^{-9} \text{ mol dm}^{-3}$ and $5 \times 10^{-8} \text{ mol dm}^{-3}$ respectively	(Emteborg et al., 1997)
Glycerol-3-phosphate	Aqueous	Glycerol-3-phosphate oxidase immobilised on controlled pore glass. Detection with luminol / Co(II) chemiluminescence.	$5 \times 10^{-7} \text{ mol dm}^{-3}$	(Yaqoob et al., 1997b)
Hydrogen peroxide	Human serum	3-Propyl-7,8-dihydropyridazino[4,5-g]quinoxaline-2,6,9(1H)-trione	1.3 pmol (100 μL injection)	(Ishida et al., 1995)
Iodide	Urine	Conversion to iodine by potassium dichromate followed by detection with luminol / Co(II) chemiluminescence.	$10 \mu\text{g L}^{-1}$	(Burguera et al., 1996)
L-alanine, 2-oxoglutarate and L-glutamate	Cell cultivation media of mammalian cells	Sample passed through reactor containing alanine aminotransferase and glutamate oxidase immobilised on sieved porous glass beads before passing into a flow cell containing NaHCO_3 / luminol / Co(II) immobilised peroxidase from <i>Arthromyces ramosus</i> for chemiluminescence detection.	$2 \times 10^{-6} \text{ mol dm}^{-3}$, $5 \times 10^{-6} \text{ mol dm}^{-3}$ and $1 \times 10^{-6} \text{ mol dm}^{-3}$ respectively	(Janasek and Spohn, 1999)
L-valine, L-leucine and L-isoleucine	Plasma	Leucine dehydrogenase, NADH oxidase and peroxidase are co-immobilised covalently on tresylate-hydrophilic vinyl polymer beads in a flow cell. NAD^+ and luminol used to produce chemiluminescence.	$1 \times 10^{-8} \text{ mol dm}^{-3}$	(Kiba et al., 1998)
Lysine and glucose	Aqueous	Immobilised lysine oxidase and glucose oxidase producing hydrogen peroxide with luminol / Co(II) chemiluminescence detection.	4×10^{-8} and $7 \times 10^{-7} \text{ mol dm}^{-3}$ respectively	(Almuaibed and Townshend, 1997)
Lysine and ornithine	Aqueous	Immobilised lysine oxidase producing hydrogen peroxide with luminol / Co(II) chemiluminescence detection.	$4 \times 10^{-7} \text{ mol dm}^{-3}$	(Almuaibed and Townshend, 1999)
Polyphenols, monophenols and sugars	Not reported	Imidazole / peroxyoxalate chemiluminescence.	Not reported	(Nozaki et al., 1995)
Porphyrins	Aqueous	Peroxyoxalate / hydrogen peroxide chemiluminescence.	$0.1 \mu\text{g L}^{-1}$	(Lin and Huie, 1997)
Proteins	bovine serum albumin, human serum albumin, human gamma-globulin, and egg albumin	Cu(II) - proteins complexes catalyse luminol / hydrogen peroxide chemiluminescence.	$0.03 - 0.05 \mu\text{g mL}^{-1}$	(Li et al., 1999b)
Proteins	Bovine serum albumin, human serum albumin, gamma-globulin, and egg albumin	1,10-Phenanthroline / hydrogen peroxide / Cu(II) cetyltrimethylammonium bromide / chemiluminescence.	$0.02 \mu\text{g mL}^{-1}$	(Ping et al., 1998)
Pyruvate	Blood serum	Acidified permanganate / quinine chemiluminescence.	$0.8 \mu\text{g mL}^{-1}$	(Feng et al., 1999)
Serotonin and related indoles	Aqueous	Potassium permanganate / sulphuric acid chemiluminescence.	$2 \times 10^{-9} - 1.5 \times 10^{-8} \text{ mol dm}^{-3}$	(Barnett et al., 1998)
Serum 3-hydroxybutyrate	Serum	Two immobilised enzymes, 3-hydroxybutyrate dehydrogenase and NADH oxidase producing hydrogen peroxide, detection with luminol / hexacyanoferrate chemiluminescence.	Not reported	(Tabata and Totani, 1995)

Table 1.6: Biomedical applications (continued).

Sulphated bile acids	Urine	Production of hydrogen peroxide using multistep enzymatic reactions with detection using luminol chemiluminescence.	$1 \times 10^{-7} \text{ mol dm}^{-3}$	(Gao et al., 1997)
Tryptophan	Tissue	Cerium(IV) / sulphuric acid chemiluminescence	$0.1 \text{ } \mu\text{g mL}^{-1}$	(Alwarthan, 1995)
Uric acid	Urine	Luminol / $\text{K}_2[\text{Cu}(\text{IO}_6)_2]$ / potassium hydroxide chemiluminescence enhanced with polyhydroxy compounds.	$7.2 \times 10^{-9} \text{ mol dm}^{-3}$	(Feng et al., 1997a)
Uric acid	Urine	Acidic permanganate chemiluminescence with octylphenyl polyglycol ether as a sensitiser.	$0.055 \text{ } \mu\text{g mL}^{-1}$	(Li et al., 1998f)
Uric acid	Aqueous	Inhibition of luminol / potassium periodate / Mn(II) chemiluminescence.	$1.8 \times 10^{-9} \text{ g mL}^{-1}$	(Wu et al., 1999a)
Vanilmandelic acid	Urine	Luminol / hexacyanoferrate(III) chemiluminescence.	$2 \times 10^{-8} \text{ mol dm}^{-3}$	(Barnett et al., 1999)

1.5 SOLID PHASE EXTRACTION

Solid phase extraction (SPE) is a method of sample preparation for the preconcentration and/or clean up of analytes prior to detection. SPE was introduced in the early 1970s as an alternative to liquid-liquid extractions (Burnham et al., 1972) however the name solid phase extraction was not introduced until much later (Zief et al., 1982). SPE is much more rapid than traditional liquid-liquid extractions, eliminates emulsions, requires much less solvent and hence saves money and is more environmentally friendly. Recoveries using SPE are usually high (>90 % is possible once a separation has been optimised).

SPE can be used in two different ways. The simplest method involves passing the sample through an SPE cartridge or column (See Fig. 1.25). Interfering compounds are retained on the sorbent bed allowing the cleaned up analyte to pass through unretained.

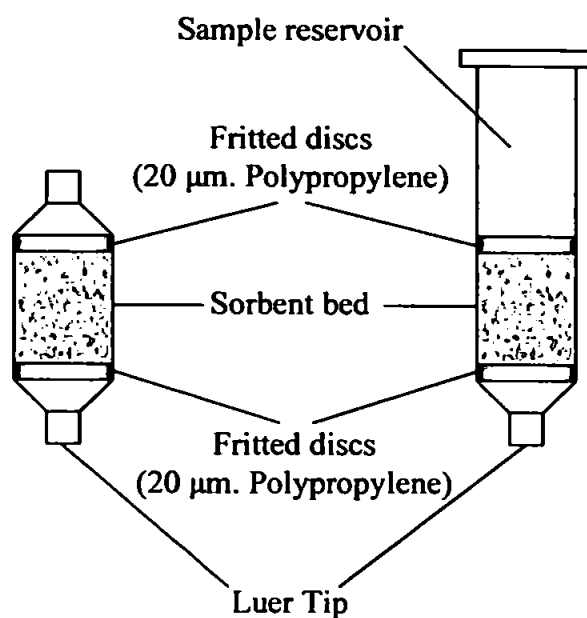


Figure 1.25: Solid phase extraction cartridge (left) and column (right).

The second (and most common) method of solid phase extraction is a four step procedure. First the sorbent is conditioned, then the sample is loaded (retaining the analyte and some interferences on the sorbent). Next, the interference compounds are washed off, and finally the desired analyte is eluted (allowing preconcentration). A diagram showing the four step procedure is shown in Fig. 1.26.

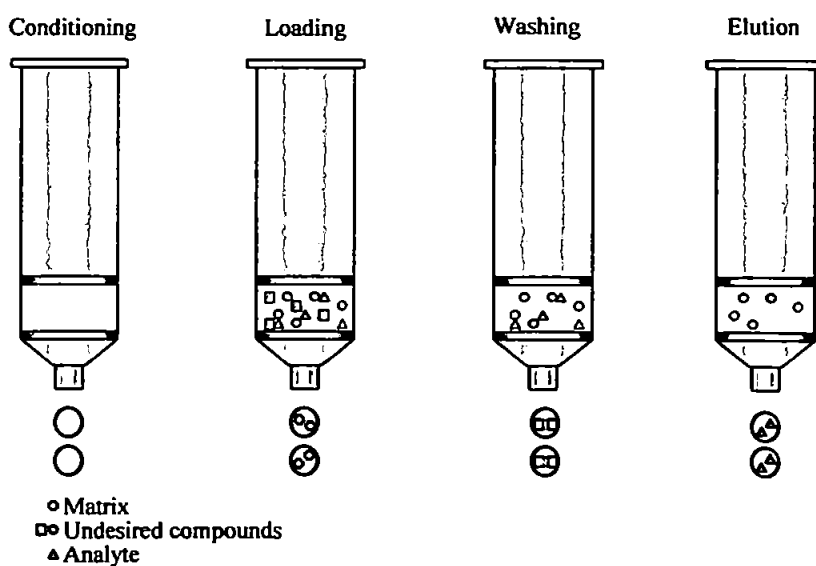


Figure 1.26: Four step solid phase extraction procedure.

Solid phase extraction cartridges and columns are usually disposable. Sorbent material is comprised of particles typically with an average diameter of 40-60 μm and an average pore size of 50-60 \AA .

1.5.1 PRINCIPLES OF SPE

Conditioning

An SPE cartridge is first conditioned with a solvent that is stronger than the final elution solvent in order to remove impurities present on the column, ensuring that the final eluent is free from impurities from the column. Conditioning is also needed to activate the sorbent. Diagrams of unconditioned and conditioned sorbents are shown in Fig. 1.27. The final conditioning solvent must be of a similar polarity (see Table 1.7) to that of the sample matrix to ensure that the maximum retention is obtained.

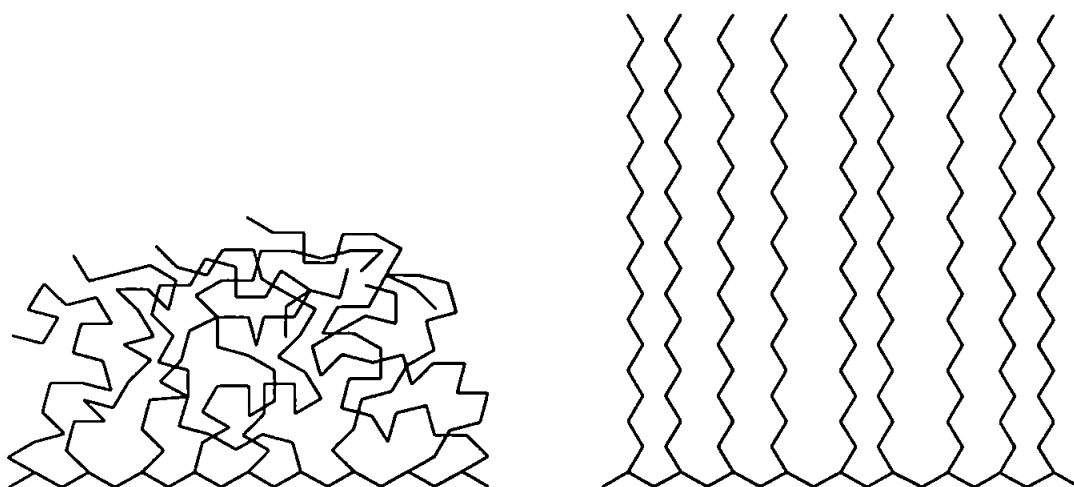


Figure 1.27: Unconditioned (left) and conditioned SPE phases.

Table 1.7: Solid phase extraction solvent strength.

Reverse phase solvent strength ↑	Solvent	Solvent Polarity $\epsilon^0(\text{Al}_2\text{O}_3)$	Normal phase solvent strength ↓
	n-Hexane	0.00	
	Toluene	0.29	
	Dichloromethane	0.39	
	Chloroform	0.40	
	Ethylmethyl ketone	0.51	
	Acetone	0.56	
	Tetrahydrofuran	0.62	
	Tert-Butylmethyl ether	0.62	
	Acetonitrile	0.65	
	Iso- + n-propanol	0.78	
	Ethanol	0.88	
	Methanol	0.95	
	Water	High	

Loading

When the sample is passed through the conditioned sorbent bed, the analyte (and possibly some interfering compounds) are retained. The matrix will pass straight through the sorbent and not be retained.

Washing

The column is washed with a solvent that is strong enough to remove impurities that have been retained along with the analyte, but not strong enough to elute the analyte.

Elution

Finally a solvent with enough strength to remove the analyte of interest is used. However if a solvent that is too strong is used then other impurities that have been retained on the column will also be eluted.

An example of a typical reverse phase SPE procedure is the extraction of sulcofuron and flucofuron from river water using C_{18} cartridges shown in Table 1.8 (Hancock et al., 1998).

Table 1.8: SPE procedure for the extraction of sulcofuron and flucofuron from river water.

Conditioning	Washing
5 mL acetone	No washing step required
5 mL methanol	
5 mL water	
Loading	Eluting
1000 mL water sample	3 x 2 mL aliquots of methanol

1.5.2 ANALYTE-SORBENT INTERACTIONS

Solid phase extraction utilises the same interactions that are used in high performance liquid chromatography. Normal phase, reverse phase, anion exchange and cation exchange are the most common interactions used in SPE.

Reversed phase sorbents (See Fig. 1.28) are more hydrophobic than the sample matrix, and are used predominantly when the sample matrix is aqueous. Van der Waals forces are the mechanism of primary interactions, although secondary interactions utilising free silanol (Si-OH) groups must be taken into account. An example of reversed phase SPE is the isolation of metal-organic complexes in ocean waters (Donat et al., 1986). Common reverse SPE phases are phenyl (Si-(CH₂)₃-C₆H₅), octyl (Si-(CH₂)₇-CH₃) and octadecyl (Si-(CH₂)₁₇-CH₃).

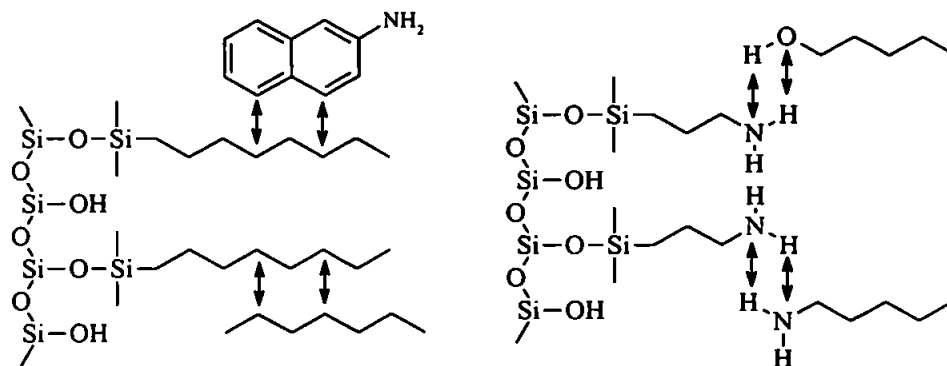


Figure 1.28: Left, interactions; Right, normal phase interactions.

Normal phase sorbents consist of a stationary phase that is more polar than the sample. Hydrogen bonding and dipole-dipole interactions are the mechanisms utilised in normal phase SPE. Aqueous samples are not usually used with normal phase SPE, as water is too strong a solvent for analytes to be retained. An example of normal phase SPE is the enrichment of polyunsaturated fatty-acids in hexane (Wilson et al., 1993). Common normal SPE phases are aminopropyl ($\text{Si}-(\text{CH}_2)_3\text{-NH}_2$), diol ($\text{Si}-(\text{CH}_2)_3\text{-O-CH}_2\text{-CH(OH)-CH}_2\text{-OH}$), cyano ($\text{Si}-(\text{CH}_2)_3\text{-CN}$), silica (Si-OH) and primary / secondary amine ($\text{Si}-(\text{CH}_2)_3\text{-NH-CH}_2\text{-CH}_2\text{-NH}_2$).

Electrostatic interactions occur between charged analytes and sorbents with the opposing electrostatic charge. Anion exchange uses negatively charged sorbent to attract positively charged analytes whilst cationic exchange uses positively charged sorbent to attract negatively charged species (see Fig. 1.29).

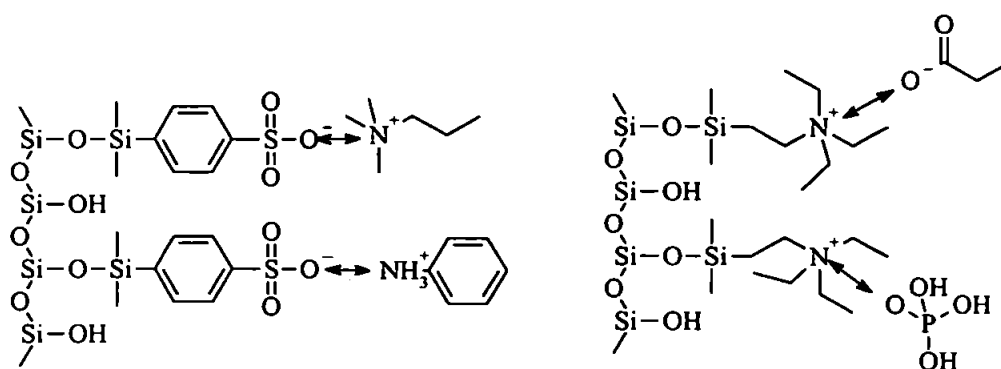


Figure 1.29: Electrostatic interactions (left, cationic exchange and right, anionic exchange).

Common cation exchange phases are benzenesulphonic acid ($\text{Si}-(\text{CH}_2)_3-\text{C}_6\text{H}_4-\text{SO}_3^-\text{H}^+$), carboxylic acid ($\text{Si}-(\text{CH}_2)_3-\text{CO}_2\text{H}$), propylsulphonic acid ($\text{Si}-(\text{CH}_2)_3-\text{SO}_3^-\text{H}^+$) and silica ($\text{Si}-\text{OH}$). An example of cation exchange SPE is the isolation of drug and drug metabolites from urine (Logan et al., 1990). Common anion exchange phases are quaternary amine ($\text{Si}-(\text{CH}_2)_3-\text{N}^+(\text{CH}_3)_3-\text{Cl}^-$), aminopropyl ($\text{Si}-(\text{CH}_2)_3-\text{NH}_2$) and primary / secondary amine ($\text{Si}-(\text{CH}_2)_3-\text{NH}-\text{CH}_2-\text{CH}_2-\text{NH}_2$). An example of anion exchange SPE is the preconcentration of urinary organic acids from urine (Verhaeghe et al., 1988).

1.5.3 SOLID PHASE EXTRACTION APPARATUS

When using a large sorbent bed it may not be possible for a sample to pass through a column using gravity flow alone. In order to assist the flow (and hence speed up extraction time) external pressure is often applied. This can either be positive pressure (applied from the top of the column) or negative pressure (a vacuum applied to the bottom of the column). Positive pressure is usually applied using a syringe. A vacuum can be applied to an individual cartridge or column (using a trap to collect the sample / solvent). When a number of samples are to be extracted, the most common method is to use an SPE vacuum manifold (see Fig. 1.30), which can accommodate 12 samples simultaneously.

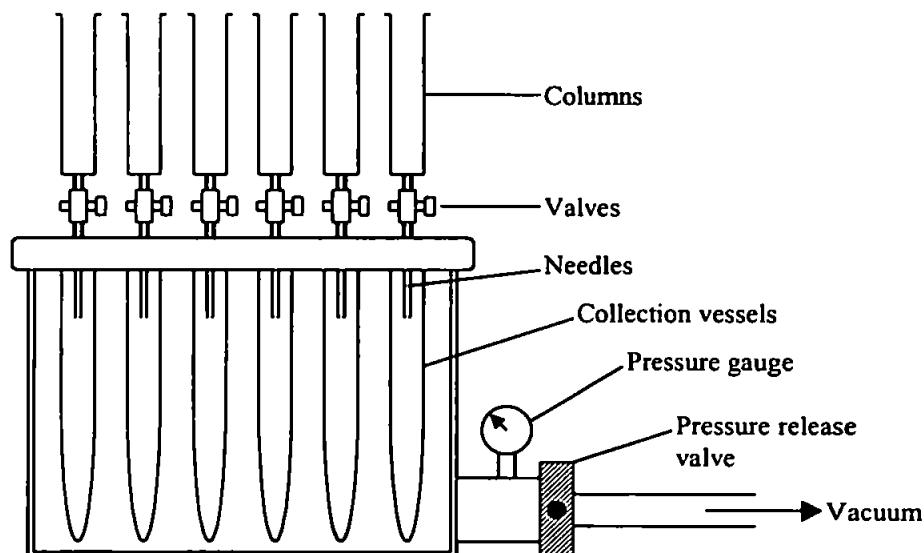


Figure 1.30: Solid phase extraction manifold.

1.5.4 AUTOMATED SPE

Automated SPE has many advantages over manually operated SPE. Automation results in better precision as gross operator errors (e.g. pipetting errors) are reduced and there is less chance of contamination.

Three common methods are used for the automation of solid phase extraction procedures. These are semiautomatic (96 well plates), workstations and on-line solid phase extraction.

1.5.4.1 Semiautomatic SPE

96 well plates consist of 96 solid phase extraction columns in 12 rows of 8. An array of 8 automatic pipettes applies samples to the columns one row at a time. This type of system is appropriate if a large number of samples need to be extracted in parallel, as all 96 samples can be processed in less than an hour instead of taking up a whole working day. A diagram and photograph of a 96 well system is shown in Fig. 1.31.



Figure 1.31: Diagram (3M, 1998) and photograph (Varian, 2000) of a 96 well plate.

The disadvantage of this type of system is that a large number of samples is needed in order for the system to become economical (each plate costs around £100). In addition, the system must be accommodated in a laboratory and is not suitable for field deployment.

1.5.4.2 Workstations

These instruments can perform multiple functions using dedicated computer software to control hardware incorporating a certain amount of robotics to perform a set of predefined operations. Workstations can perform a host of laboratory functions including addition of internal standards, filtration, mixing, evaporation and derivatisation, and can be operated in both batch and serial modes. Various commercial workstation systems are available with prices in the range £15,000 to £40,000. The most common systems are ASPEC XL (Gilson Medical Electronics, Inc.) (Langen et al., 2000), Microlab SPE (Hamilton) (Whitter et al., 1999), Prepstation (HP) (Hankemeier et al., 1999), Prospekt (Jones Chromatography) (Marchese et al., 1998), AutoTrace (Tekmar) (Gerwinski and Schmidt, 1998) and BenchMate (Zymark) (Hamlin, 2000). A photograph and diagram of the Prospekt system are shown in Fig. 1.32.

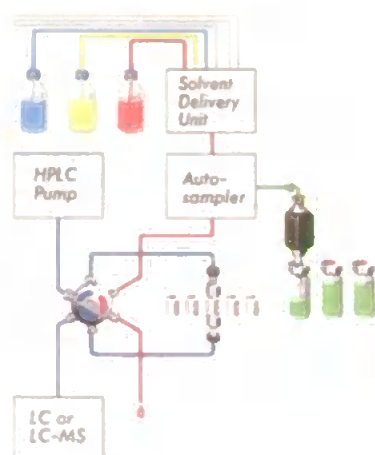


Figure 1.32: Prospekt automated SPE system. (Techlab, 2001)

As in the case of 96 well plates, workstations require laboratory conditions for successful operation and are therefore also inappropriate for field monitoring.

1.5.4.3 On-line SPE

The third approach is on-line SPE which involves placing a column into a flow system (see Fig. 1.33). The sample is preconcentrated onto the column and the column is then injected directly into the flowing stream, leading to the detector. On-line solid phase extraction has many advantages, including reduced solvent usage, lower detection limits due to the whole sample being analysed and faster analysis. On-line SPE is discussed in more detail in Chapter 4.

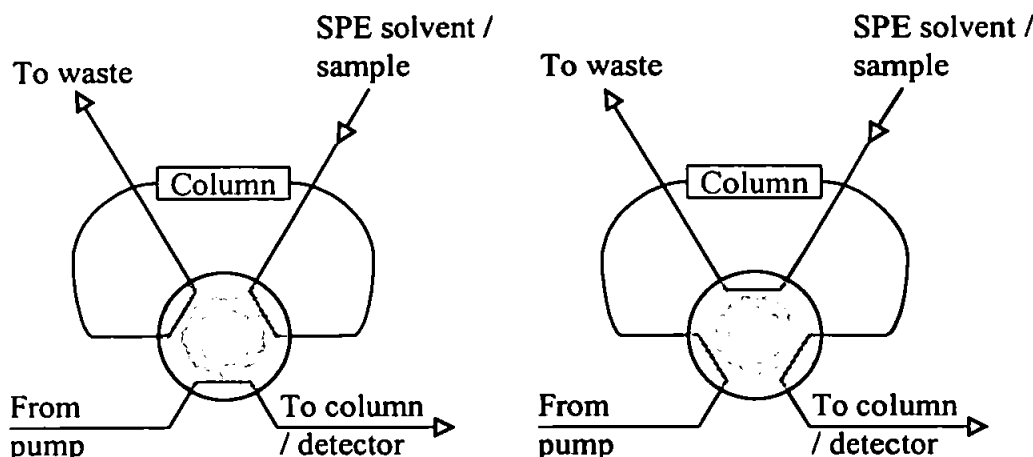


Figure 1.33: On-line solid phase extraction. Left conditioning / loading / washing, right elution.

1.6 AIMS OF PROJECT

The primary objective of this research was to develop field deployable instrumentation for the analysis of fuel additives in diesel fuel.

The specific objectives of this research were:

1. To develop and optimise a flow injection-chemiluminescence method for the determination of dodecylamine in acetonitrile / water mixtures and to investigate the effects indigenous compounds present in diesel fuel would have on this method.
2. To evaluate the potential of the FI-CL manifold for determination of dodecylamine in diesel fuels.
3. To develop solid phase extraction procedures for the extraction of dodecylamine from diesel fuel into a matrix compatible with FI-CL system.
4. To develop a reference method using GC-NPD / GC-MS for the determination of dodecylamine in solid phase extracts in order to validate the SPE procedure.

5. To construct an on-line FI-SPE-CL manifold for determination of DDA in diesel fuels and to fully automate the on-line manifold.
6. To compare results obtained from on-line SPE with off-line SPE results.
7. To develop and optimise an FI-CL method, based on the oxidation reaction of pyrogallol by periodate, for the detection of lubricant additive P655
8. To develop an SPE method for extraction of P655 from organic matrices.
9. To analyse P655 extracts using the FI-CL method developed.

Chapter 2

Determination Of Dodecylamine Using Flow Injection With Chemiluminescence Detection (FI-CL)

2. DETERMINATION OF DODECYLAMINE USING FLOW INJECTION WITH CHEMILUMINESCENCE DETECTION (FI-CL)

2.1 INTRODUCTION

This chapter describes the modification and the univariate and simplex optimisation of a flow injection peroxyoxalate / sulphorhodamine 101 chemiluminescence reaction (Katayama et al., 1993; Katayama et al., 1994) for the determination of dodecylamine (a detergent used in diesel fuels) in acetonitrile / water matrices. Dodecylamine (DDA) is typically present in fuels at a concentration of 40 mg L^{-1} . Detection limits must therefore be lower than this value in order for the method to be applicable to commercial diesel fuels. Interferences from indigenous compounds present in diesel fuels are evaluated and the effect of different solvents on the sensitivity of detection have been investigated. The analytical figures of merit for the FI-CL manifold are also reported.

2.2 EXPERIMENTAL

2.2.1 REAGENTS AND STANDARDS

Dodecylamine (98%) and sulphorhodamine 101 hydrate (~95%) were obtained from Aldrich (Gillingham, Dorset, U.K.). Bis(2,4-dinitrophenyl) oxalate (DNPO) (>97%), aniline (puriss grade), carbazole (purum grade), p-cresol (puriss grade), hexanoic acid (purum grade), indole (puriss grade), and phenol (puriss grade) were obtained from Fluka (Gillingham, Dorset, U.K.). Methanol (Hipersolve grade), propan-2-ol (AnalaR grade) and 30% (w/v) stabilised hydrogen peroxide were obtained from Merck (Poole, Dorset, U.K.).

Acetonitrile (HPLC grade) was purchased from Rathburns (Walkerburn, U.K.). Water used was analytical reagent grade ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) deionised water from an Elgastat UHQ II system (Elga Ltd., High Wycombe, Bucks., U.K.).

$1 \times 10^{-3} \text{ M}$ solutions of DNPO were prepared by dissolving 0.1056 g in 250 mL of acetonitrile (for the optimisation, a $1 \times 10^{-2} \text{ M}$ stock solution was prepared). Stock solutions of $1 \times 10^{-5} \text{ M}$ sulphorhodamine 101 were prepared by dissolving 0.0015 g in 250 mL of acetonitrile. Stock solutions of 0.1 M hydrogen peroxide were prepared by dissolving 2.58 mL in 250 mL of water (for the optimisation, a 1 M stock solution was prepared). Stock solutions of dodecylamine (400 mg L^{-1}) were prepared by dissolving 40 mg in 100 mL of 90 % acetonitrile / 10 % water. All standards and reagents were prepared by serial dilutions of the above stock solutions. Solutions of DNPO, hydrogen peroxide and sulphorhodamine 101 were stored in the dark when they were not being used. Sulphorhodamine stock solutions were found to be stable for months when kept in the dark. DNPO and peroxide solutions were stable for 1 week.

1000 mg L^{-1} stock solutions of interfering compounds (aniline, *p*-cresol, carbazole, hexanoic acid, indole and phenol) were prepared by weighing out 100 mg into 100 mL volumetric flasks and making them up to the mark with 90 % acetonitrile / 10 % water. Serial dilutions were made to produce lower concentrations as required.

All solutions were prepared in grade A glass volumetric flasks. All glassware was soaked overnight in 5% Decon 90 solution (Fisher Scientific) then rinsed with copious amounts of ultrapure water and left to air dry.

2.2.2 INSTRUMENTATION

Chemiluminescence emission was measured using a CamSpec CL-2 detector (Cambridge Instruments, Cambridge, U.K.) containing a low power, 12 V Hamamatsu photomultiplier tube with a quartz flow cell (emission length of 12 cm, with a volume of 120 μ L). The 0-10 V analogue output from the detector was acquired using a multifunctional DAQ-700 data acquisition / digital I/O card (National Instruments, Berkshire, U.K.) attached to a laptop computer. Software written in LabVIEW (National Instruments) was used to record and process the signal (see Chapter 4). The reagent pump used was a variable speed Gilson Minipuls 3 peristaltic pump (Gilson, Villiers-le-Bel, France). The sample pump used was a fixed speed Ismatec peristaltic pump (Ismatec, Weston-super-Mare, U.K.). Ismaprene (Ismatec) pump tubing was used (1.02 mm i.d. for DNPO, sulphorhodamine and sample, and 0.5 mm i.d. for H_2O_2 and carrier). A Rheodyne (Rheodyne, California, U.S.A.) 6 port, 2 position rotary injection valve (part no. 5020) was used to inject the sample into the carrier stream. All manifold tubing was 0.75 mm i.d. PTFE tubing (Fisher Scientific, Loughborough, U.K.). Poly(tetrafluoroethylene) T-pieces (Omnifit Limited, Cambridge, U.K.) were used to merge reagent streams. All other connections were made using $\frac{1}{4}$ "-28 flanged polypropylene FI fittings (Omnifit).

Fluorescence spectra were measured using a Hitachi F4500 fluorescence spectrometer with a photomultiplier voltage of 400 V and slit widths of 5 nm for excitation and emission. A charge coupled device (CCD) with a liquid nitrogen cooled (256 x 1024 pixels) chip and a 270M imaging spectrograph (Instruments SA) were used to record CL emission spectra. The injection valve was removed and the system was used in a continuous flow mode to produce a constant CL emission. The spectrum of sulphorhodamine 101 was obtained using a 1×10^{-5} M solution. 150 nm pieces of the spectrum were normalised and 'glued' together using Microsoft Excel 2000.

2.2.3 PROCEDURES

2.2.3.1 *Peak evaluation / limit of detection calculations*

Fig. 2.1 shows an example flow injection-chemiluminescence peak. Definitions of the terms peak height, start/end of peak, upper/lower noise limits and mean noise are all shown in this figure. Equation 2.1 gives a definition of how signal/noise has been measured.

$$\text{Signal/noise} = \frac{\text{Peak height}}{\text{Upper noise level} - \text{Lower noise level}} \quad (2.1)$$

In all cases where signal/noise has been measured, the limit of detection has been calculated as the point on the calibration graph where the signal/noise = 3. The magnitude of the mean peak-to-peak noise (0.415 in the Fig. 2.1) is caused by background CL emission from the reagents when they are mixed in the absence of sample.

$$\int_{\text{End of peak}}^{\text{Start of peak}} (\text{Signal} - \text{Mean noise}) \quad (2.2)$$

Equation 2.2 shows the method used for integrating peaks. The trapezoidal rule method for integration has been used in all cases. Limits of detection for calibration graphs using peak area have been defined as three times the standard deviation of the area of the blank ($3s_b$) + the intercept of the calibration graph on the y-axis (y_b) i.e. L.O.D. = $y_b + 3s_b$ (Miller and Miller, 1993).

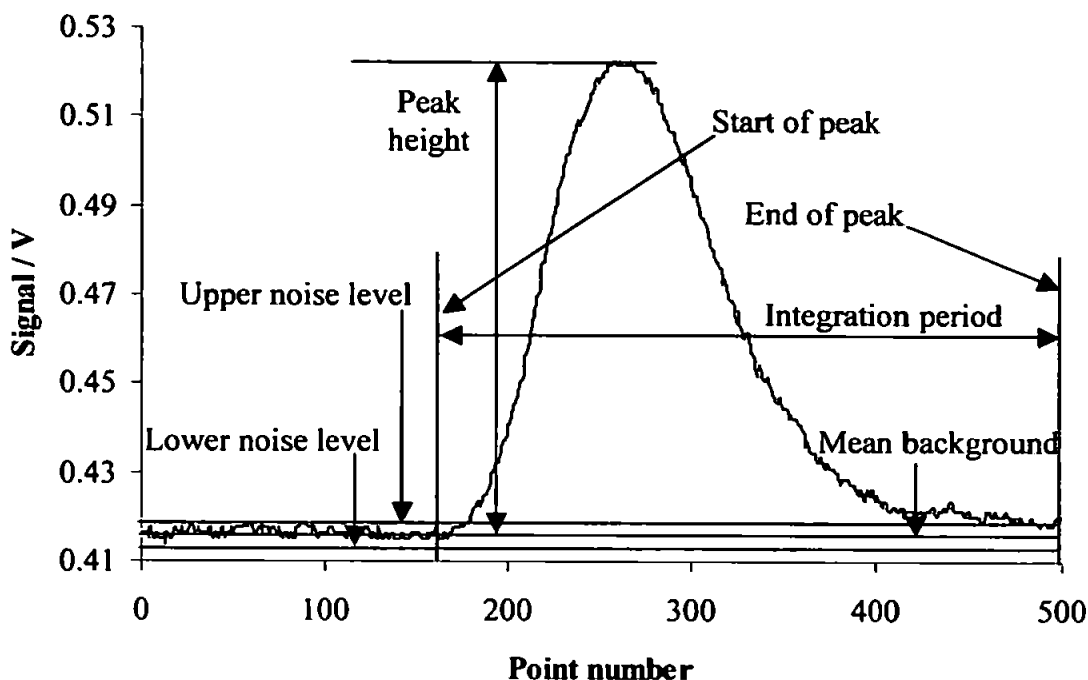


Figure 2.1: Example definitions of peak dimensions used in calculations.

2.2.3.2 Evaluation of the CL manifold

The manifold shown in Fig. 2.2 (Katayama et al., 1993) was evaluated to determine whether it was suitable for the detection of dodecylamine in acetonitrile / water mixtures.

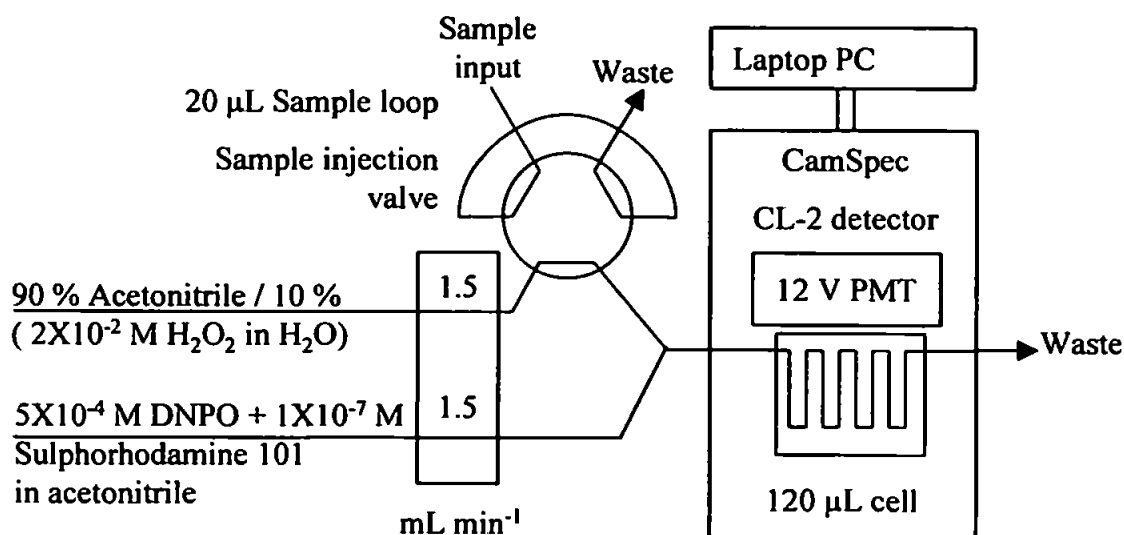


Figure 2.2: CL manifold for the detection of amines (Katayama et al., 1993).

2.2.3.3 *Univariate optimisation*

The manifold shown in Fig. 2.2 was adapted by the inclusion of a separate DNPO and sulphorhodamine streams to increase reagent stability. A 2 m mixing coil was introduced to ensure good mixing of the sulphorhodamine and DNPO. A separate carrier stream was also introduced so that the peroxide stream was not disrupted when samples were introduced. The adapted manifold shown in Fig. 2.3 was then univariately optimised for the parameters listed in Table 2.1. A 10 mg L⁻¹ solution of DDA in 90/10 acetonitrile / water was injected into the carrier stream, and the signal was measured. The baseline was recorded for 2 minutes, and the peak to peak noise was used to determine the noise level. The sample was injected 5 times for each experiment.

Table 2.1: Parameters used in the univariate optimisation of the FI-CL manifold shown in Fig 2.3 for the determination of dodecylamine.

Parameter	Lower limit	Upper limit	Interval
DNPO concentration / mol L ⁻¹	4X10 ⁻⁴	1.4X10 ⁻³	2x10 ⁻⁴
Sulphorhodamine 101 concentration / mL min ⁻¹	0	1X10 ⁻⁶	2X10 ⁻⁷
H ₂ O ₂ concentration / mol L ⁻¹	4X10 ⁻²	1.4X10 ⁻¹	2X10 ⁻³
Percentage of water in acetonitrile carrier stream / %	5	25	5
H ₂ O ₂ + carrier flow rate / mL min ⁻¹	1.53	6.9	0.75
DNPO + sulphorhodamine flow rate / mL min ⁻¹	0.67	3.2	0.33

2.2.3.4 *Simplex optimisation*

A simplex optimisation of the parameters (DNPO + sulphorhodamine 101) flow rate, (hydrogen peroxide + carrier) flow rate and the mixing coil length was performed. A 10 mg L⁻¹ DDA solution in 90 % acetonitrile / 10 % water was injected 5 times, and the average peak height was taken as the signal. The peak-to-peak noise from 2 min of baseline was calculated by subtracting the minimum noise value from the maximum noise value from the 2 minute period. Optimisation experiments were performed until there was

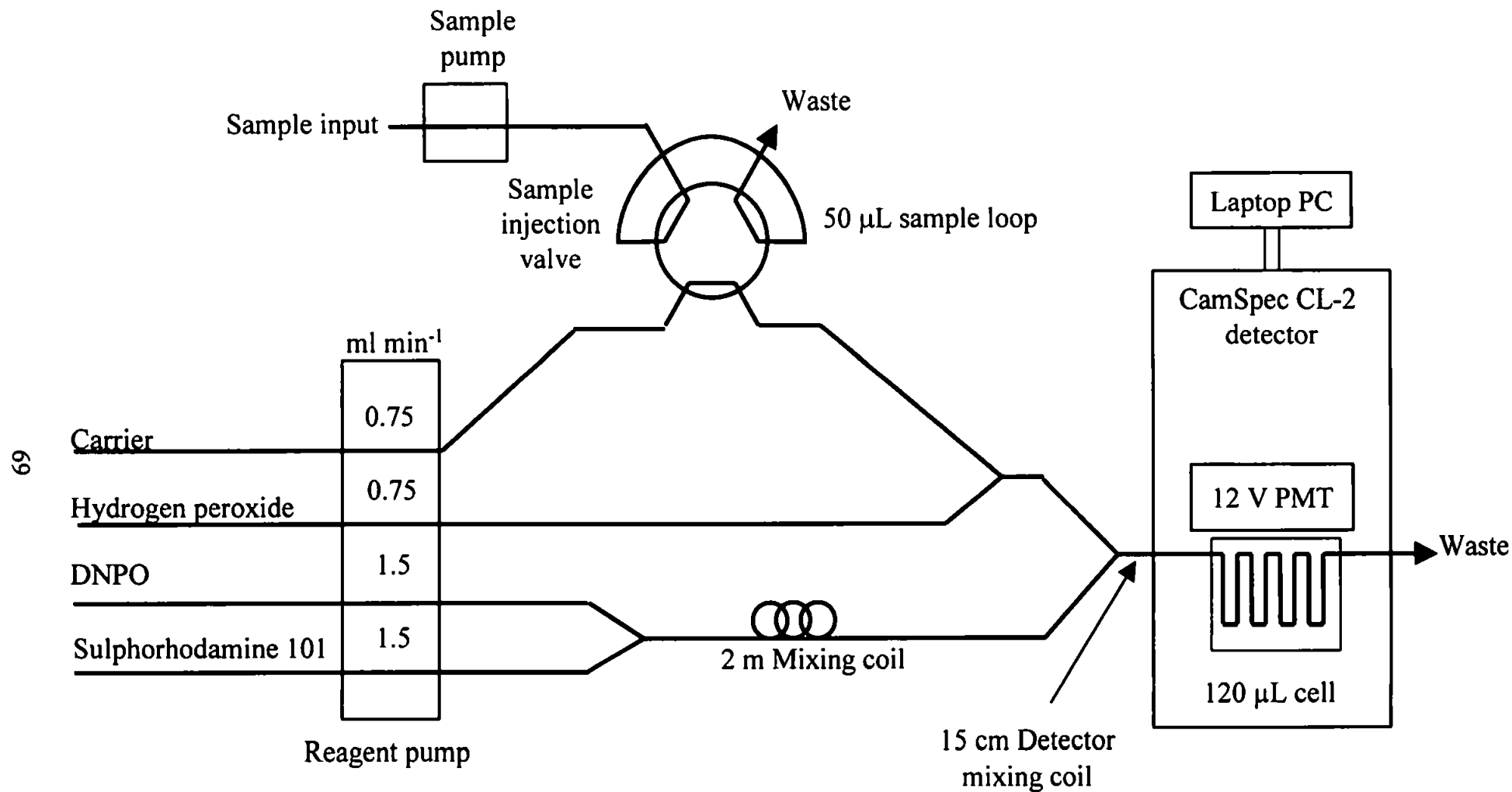


Figure 2.3: Adapted flow injection chemiluminescence manifold for the determination of dodecylamine.

no increase in the signal/noise for 5 experiments. The conditions used in the simplex optimisation are shown below in Table 2.2.

Table 2.2: Parameters used in the simplex optimisation of the FI-CL manifold for the detection of DDA.

Parameter	Starting condition	Lower limit	Upper limit	Interval	Simplex size
DNPO + sulphorhodamine flow rate / mL min ⁻¹	3	1.5	4.5	0.5	1.5
H ₂ O ₂ + carrier flow rate / mL min ⁻¹	1.25	0.75	1.75	0.25	0.6
Mixing coil length / cm	15	15	30	5	10

2.2.3.5 *Solvent effects*

Different ratios of acetonitrile / water from 50% to 100% acetonitrile were used as carrier solvents using the FI-CL manifold shown in Fig. 2.3. Standards prepared in the same solvents (0 to 10 mg L⁻¹) were analysed to determine which solvent ratio gave the best signal/noise ratio (S/N). Methanol and propan-2-ol (IPA) were also used as carrier solvents, once again using a matched solvent for the standard solutions.

2.2.3.6 *Interferences*

Samples containing 40 mg L⁻¹ DDA in 90% acetonitrile / 10% water (v/v) were spiked with varying concentrations (from 1 to 1000 mg L⁻¹) of the following compounds: aniline, carbazole, *p*-cresol, hexanoic acid, indole and phenol (structural formulae shown in Fig. 2.3) in order to determine the lowest concentration at which each caused an interference. The FI-CL manifold shown in Fig. 2.3 was used with 90% acetonitrile / 10% water as the carrier solvent. The fluorescence spectrum of a 1000 mg L⁻¹ solution of each interferent was measured to determine if the native fluorescence of the compound was causing an interference by energy transfer.

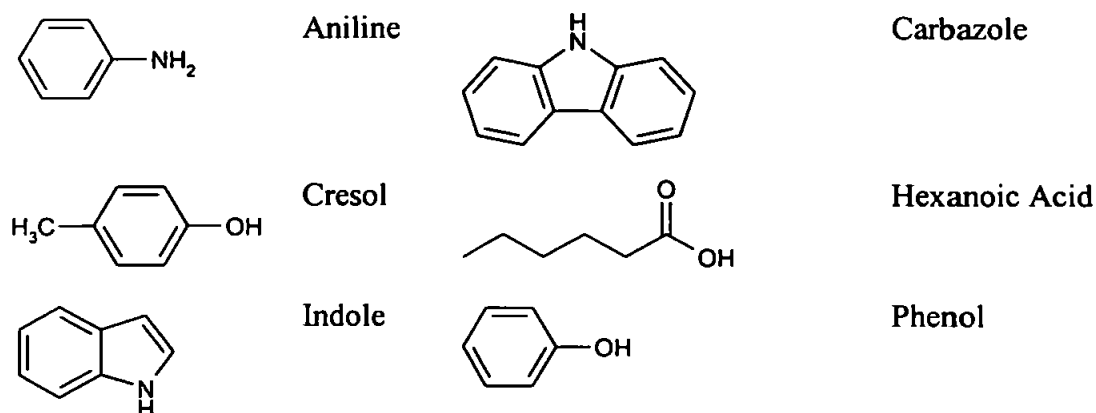


Figure 2.4: Structures of classes of indigenous diesel compounds.

2.3 RESULTS AND DISCUSSION

2.3.1 PEROXYOXALATE CHEMILUMINESCENCE

The peroxyoxalate chemiluminescence (PO-CL) reaction was discovered in 1963 (Chandross, 1963) by reacting oxalylchloride with hydrogen peroxide in the presence of fluorescent compounds. Chandross suggested that the compound $\text{HO}_2\text{COCOC}\text{Cl}$ was formed. When this molecule collided with another molecule A, the products HCl , 2CO , $^3\text{O}_2$ and $^3\text{A}^*$ were formed. The excited state triplet A species lost vibrational energy and crossed over to the fluorescent lowest excited singlet state ($^1\text{A}^*$), which emitted visible radiation on decay to the ground state. An alternative mechanism was proposed for the reaction (Rauhut et al., 1967) involving the formation of the peroxyacid oxalate ester which then formed the unstable intermediate 1,2-dioxetanedione as shown in Fig. 2.5.

The 1,2-dioxetanedione intermediate produces a charge-transfer complex with a fluorophore. As the complex breaks up to produce two molecules of carbon dioxide, the

fluorophore becomes sensitised and emits light in the visible region upon decay to the ground state.

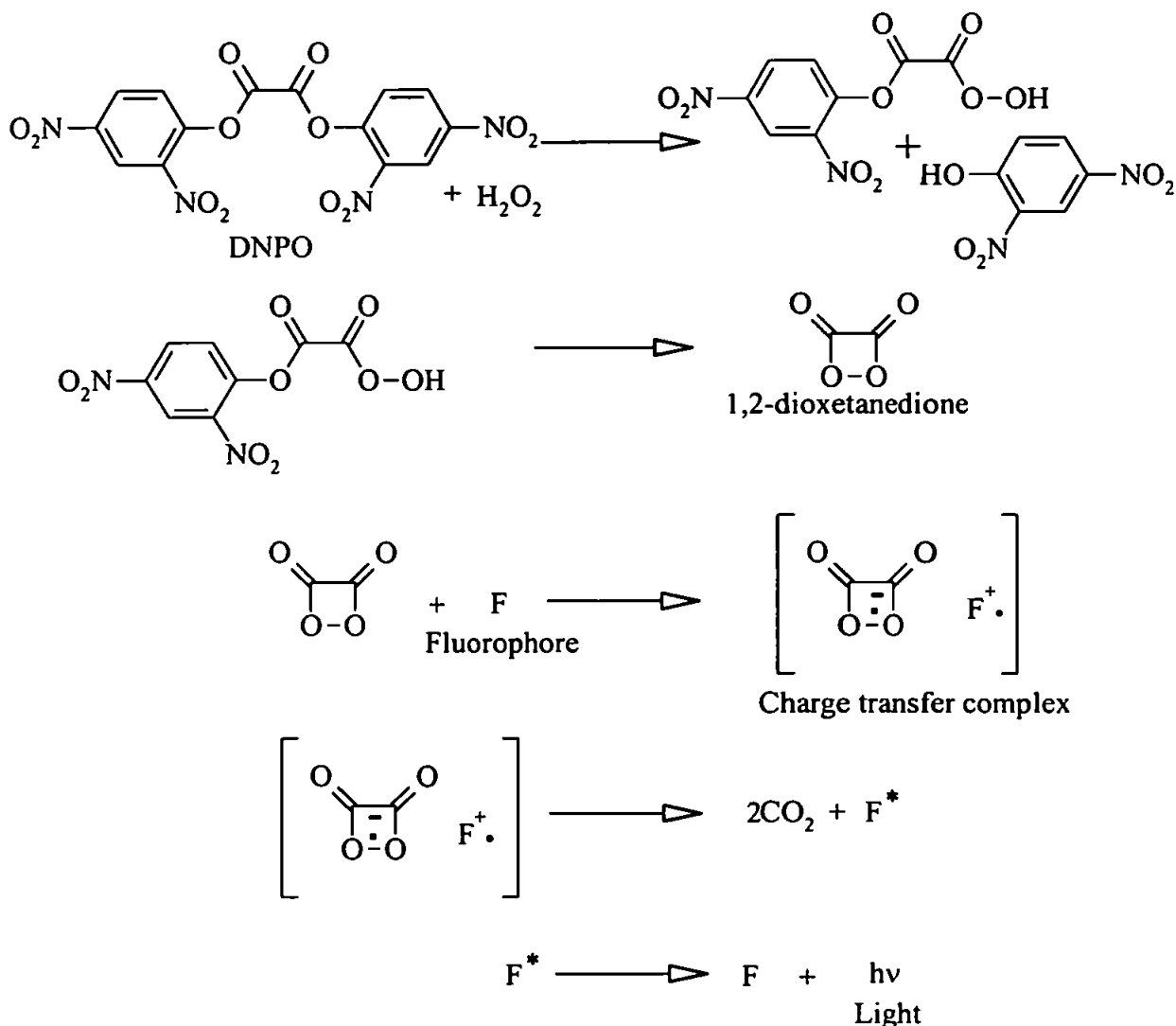


Figure 2.5: A schematic diagram of the Rauhut *et. al.* mechanism for the peroxyoxalate chemiluminescence reaction (Rauhut et al., 1967).

Many different fluorescent compounds have been used as fluorophores in order to produce visible emission from the PO-CL reaction such as aminopyrene (Weinberger et al., 1984), perylene, 1-aminoanthracene and anthracene (Lee et al., 1996). These reactions form the basis of many commercially available 'light sticks', with different fluorophores used to generate different coloured CL emission. Most PO-CL methods for the determination of amines require the chemical derivatisation of the amino group using compounds such as

dansyl chloride (Fu et al., 1993) or naphthalene-2,3-dialdehyde / anthracene-2,3-dialdehyde (Kwakman et al., 1990) but these steps are relatively time consuming. The sulphorhodamine 101 reaction (Katayama et al., 1993) does not require any derivatisation and has been shown to be a very sensitive method for the detection of primary, secondary and tertiary amines in acetonitrile / water mixture. For this reason, sulphorhodamine 101, the structure of which is shown in Fig. 2.6, has been used as the fluorophore for the detection of dodecylamine in this work.

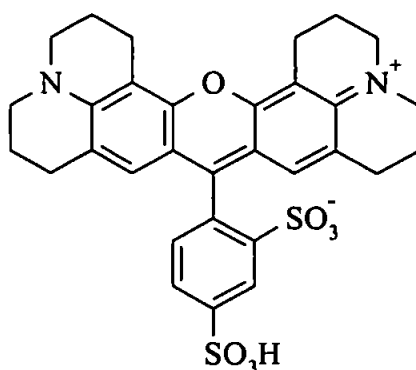


Figure 2.6: Structure of the fluorophore sulphorhodamine 101.

A postulated mechanism (Jonsson and Irgum, 1999) to explain why the PO-CL reaction is catalysed in the presence of strong nucleophiles such as amines is shown in Fig. 2.7.

The nucleophile (in this case DDA) attacks the peroxyoxalate **I** forming an amide **II**. This step is repeated to produce the disubstituted diamide **III**. A hydrogen peroxide anion attacks the carbonyl group producing the amide peroxyacid **IV**. The peroxyacid group can then intramolecularly cyclise, producing the 1,2-dioxetanedione excited state.

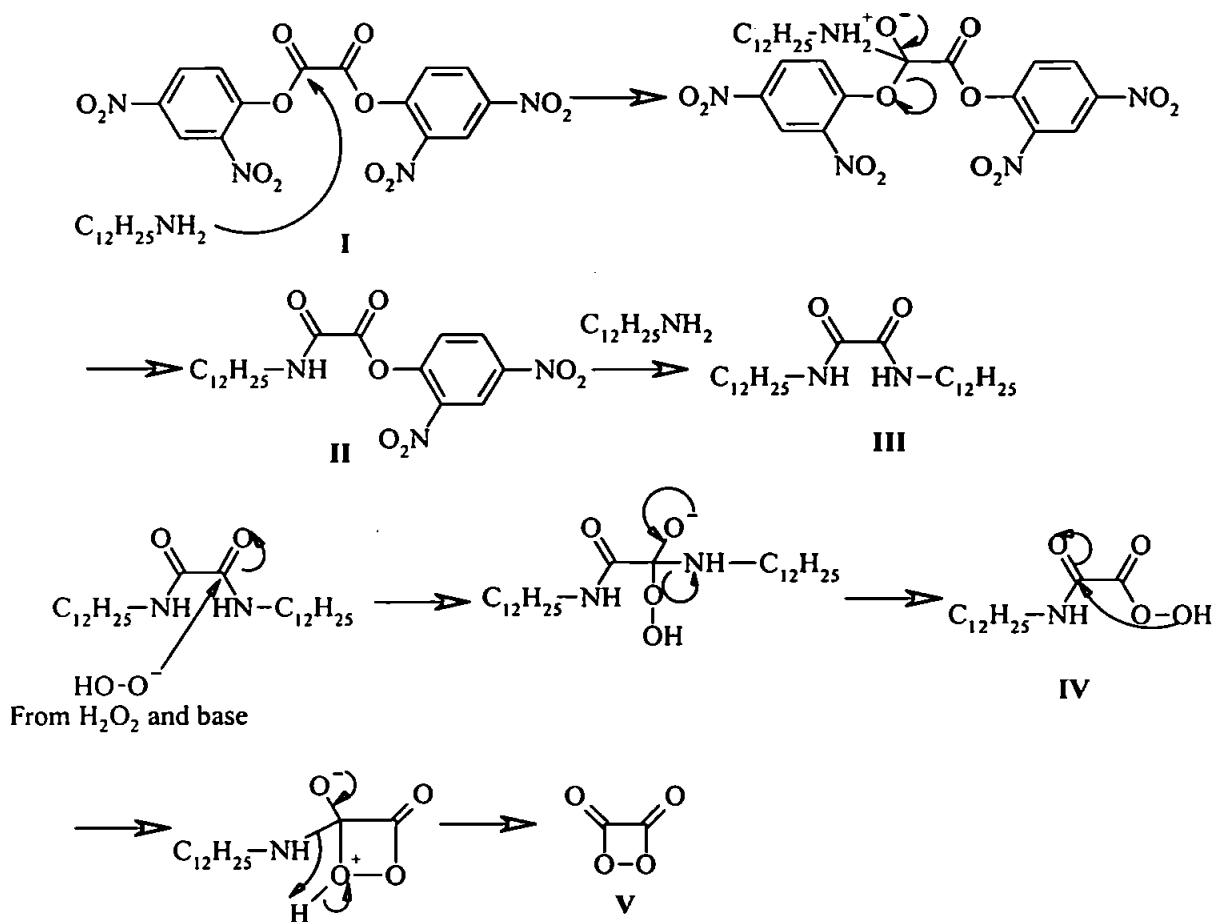


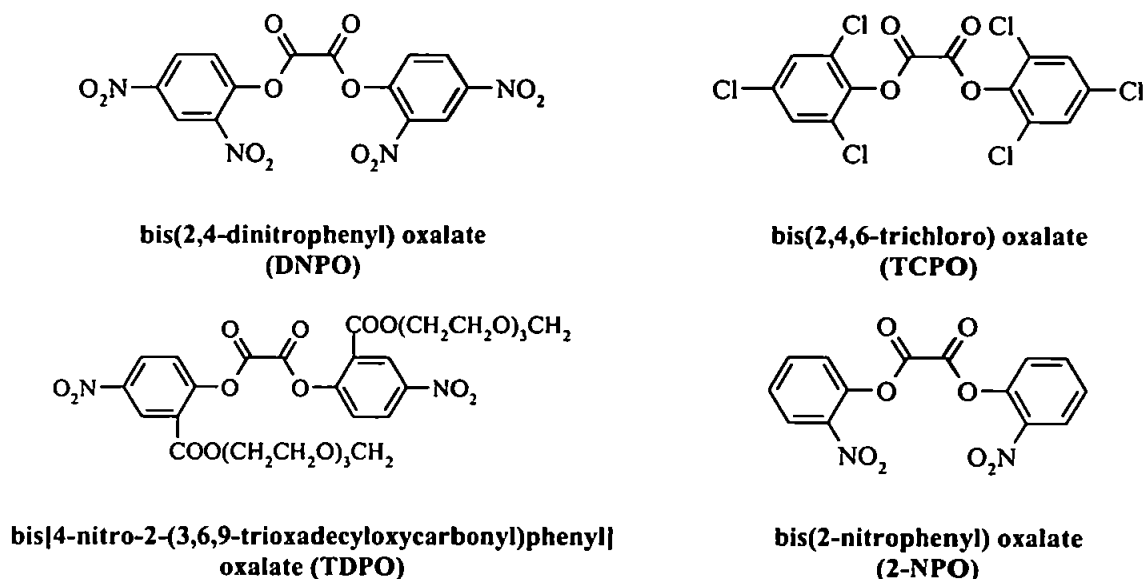
Figure 2.7: Postulated amine catalysed peroxyoxalate chemiluminescence reaction mechanism (Jonsson and Irgum, 1999) as applied to dodecylamine.

A number of different peroxyoxalate compounds have been used to produce CL, the most common of which are shown in Table 2.3.

Of the various peroxyoxalate compounds that have been used for chemiluminescence, the most popular are TCPO and DNPO. All of the common peroxyoxalates have associated relative advantages and disadvantages. TCPO and 2-NPO are stable in the presence of hydrogen peroxide, but these compounds are relatively insoluble in common LC solvents such as acetonitrile and methanol (Kwakman and Brinkman, 1992). The kinetics of DNPO are more rapid than for TCPO due to the presence of nitro substituents which are more electron withdrawing than chlorine groups. This means that although DNPO has a higher background signal than TCPO, it is more sensitive because CL intensity is a function of

reaction rate rather than a steady state measurement. TDPO is also soluble in acetonitrile and methanol, however it is not commercially available in the U.K. Other peroxyoxalate compounds that are water soluble have been synthesised (Barnett et al., 1998) for systems that require a purely aqueous environment.

Table 2.3: Common peroxyoxalate compounds used in chemiluminescence reactions.



2.3.2 DETERMINATION OF DDA USING THE FI-CL MANIFOLD

The manifold used by Katayama (Fig. 2.2) was initially evaluated for the detection of dodecylamine in 90 % acetonitrile / 10% water. It was found that within minutes of starting the experiments, the peaks heights of sequential injections of a 10 mg L⁻¹ standard solution of DDA dropped significantly, as shown in Fig. 2.8.

This drop in peak intensity was thought to be due to small amounts of amine present in the solvent (acetonitrile) reacting with the mixture of DNPO and sulphorhodamine. Also, when the solutions were premixed, they were found to have decomposed within 24 h (a complete colour change from light pink to yellow was observed). However, without premixing the DNPO and sulphorhodamine 101, peak heights were found to be constant, and the solutions were stable individually for at least one week.

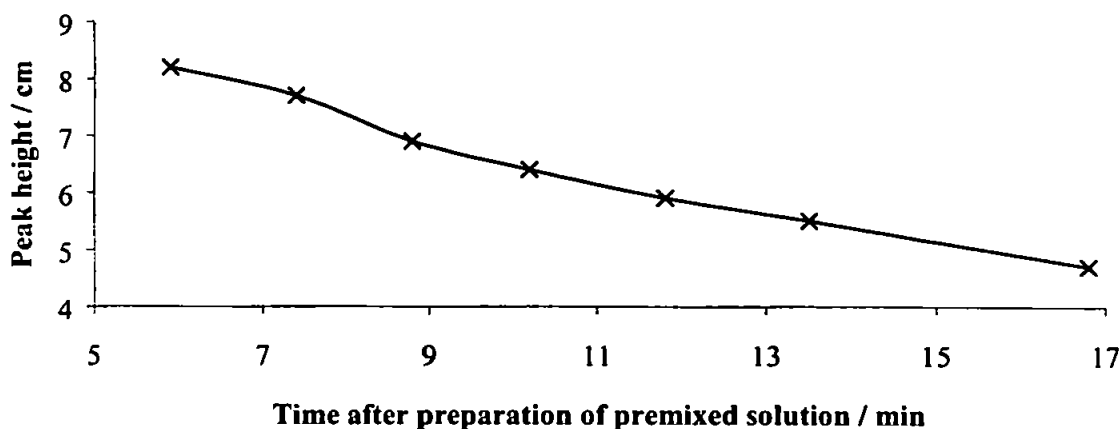


Figure 2.8: Peak heights of sequential injections of a 10 mg L⁻¹ standard solution of DDA in 90% ACN / 10% H₂O. DNPO and sulphorhodamine 101 solutions were premixed.

A 2 m mixing coil was therefore inserted to ensure the two reagents (pumped separately) were thoroughly mixed before reacting with hydrogen peroxide. The limit of detection decreased when the sample loop was increased from 20 to 50 μ L, however, when the larger sample volume was injected directly into the peroxide, split peaks resulted. This was because of the incomplete dispersion of the sample in the peroxide stream, causing a lower (rate limiting) peroxide concentration in the middle of the sample zone. The addition of an extra carrier line overcame this problem by providing a constant stream of peroxide to merge with the sample. The modifications stated above (separation of DNPO and sulphorhodamine, 2 m mixing coil for DNPO and sulphorhodamine, introduction of separate carrier and peroxide streams, and larger injection volume) are shown in the adapted manifold diagram (Fig. 2.3).

2.3.3 LUMINESCENCE SPECTRAL PROFILES FOR SULPHORHODAMINE 101

The fluorescence excitation and emission and the chemiluminescence emission of a 1×10^{-5} M solution of sulphorhodamine 101 are shown in Fig. 2.9. Fluorescence measurements were carried out in acetonitrile, whilst chemiluminescence measurements were carried out

using the FI-CL manifold shown in Fig. 2.3, and so contained some water in the reaction mixture.

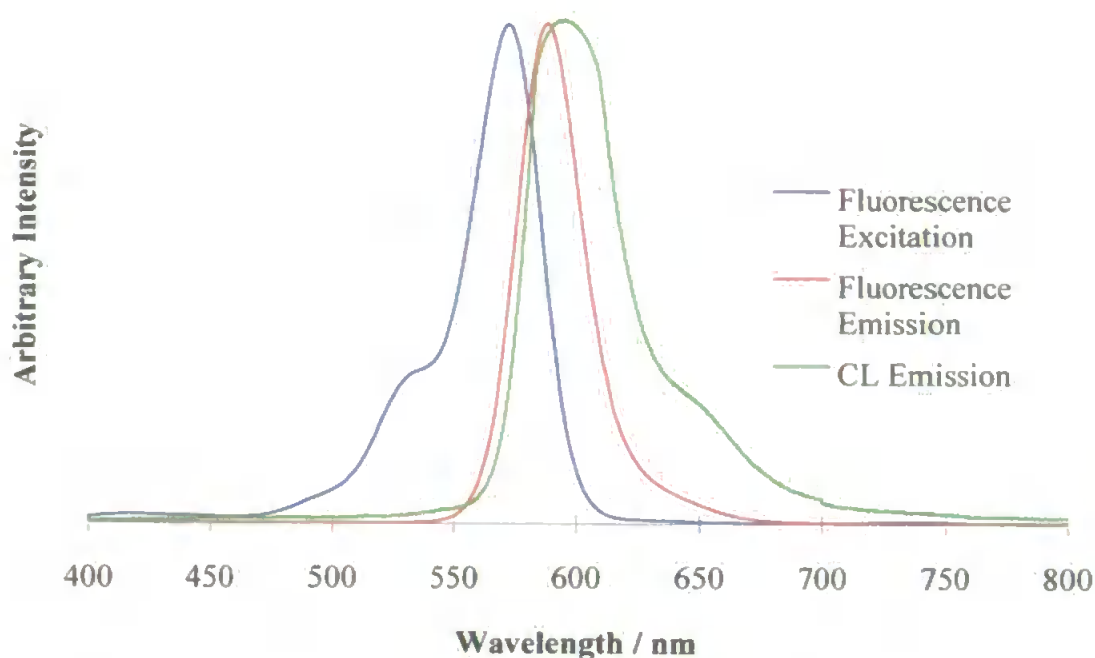


Figure 2.9: Normalised spectral profile for the fluorescence excitation and emission and chemiluminescence emission of sulphorhodamine 101.

The λ_{max} values for CL and fluorescence emission were found to be 598 and 592 nm respectively. The similarity of the emission profiles is consistent with the postulated charge transfer mechanism (Rauhut et al., 1967) shown earlier in Fig. 2.5 which suggests that CL emission is produced by the fluorescent sensitizer molecule (in this case sulphorhodamine 101). The CL emission broadening and the small bathochromic shift with chemiluminescence as compared with fluorescence are due to the presence of water creating a more polar environment.

2.3.4 UNIVARIATE OPTIMISATION

Univariate optimisation graphs for the 6 parameters DNPO, sulphorhodamine 101 and hydrogen peroxide concentrations, water content (in the carrier stream and sample matrix)

and reagent flow rates are shown in Fig. 2.10, with a summary of the optimum conditions shown in Table 2.4.

Table 2.4: Optimum FI-CL parameters for the detection of dodecylamine.

Parameter	Optimum Condition
DNPO concentration	$1.0 \times 10^{-3} \text{ mol L}^{-1}$
Sulphorhodamine 101 concentration	$8 \times 10^{-7} \text{ mol L}^{-1}$
Hydrogen peroxide concentration	0.1 mol L^{-1}
Water content in acetonitrile stream	10 %
Carrier + peroxide flow rate	1.25 mL min^{-1}
Sulphorhodamine + DNPO flow rate	3 mL min^{-1}

Fig. 2.10(a) shows the effect of altering the DNPO concentration in terms of the CL response. A maximum CL response was obtained using $1 \times 10^{-3} \text{ M}$ DNPO. At concentrations greater than this the noise level is increased due to the presence of higher concentrations of the product 2,4-dinitrophenol (Baeyens et al., 1990).

The effect of sulphorhodamine 101 concentration on the CL response is shown in Fig. 2.10(b). An emission maximum was seen using a concentration of $8 \times 10^{-7} \text{ M}$. At concentrations greater than this no increase was seen in the signal, however the noise level continued to increase.

Fig. 2.10(c) shows the effect of hydrogen peroxide concentration on the CL response. A maximum CL response was seen using an H_2O_2 concentration of 0.1 M . It has been shown (Hanaoka et al., 1988) that increasing the concentration of hydrogen peroxide increases the rate of reaction for peroxyoxalate chemiluminescence, and the decrease in CL response seen for concentrations of peroxide greater than 0.1 M is therefore likely to be due to the maximum CL intensity occurring before the sample arrives at the flow cell.

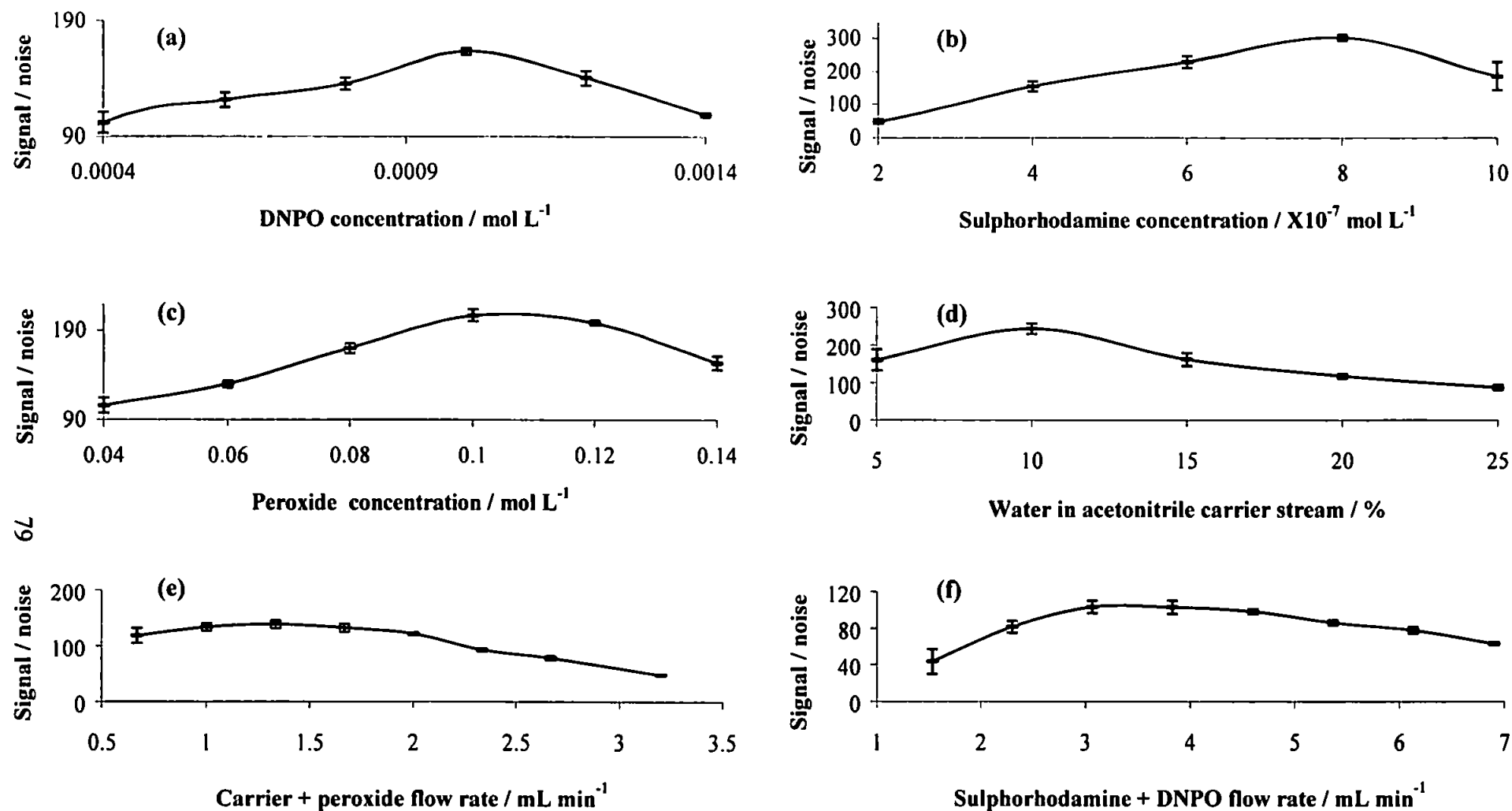
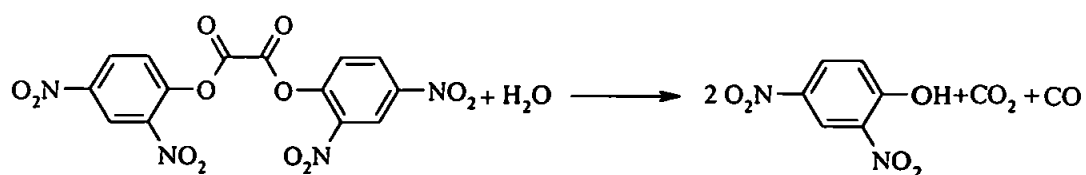


Figure 2.10: Univariate optimisation of the FI-CL manifold with the following FI parameters: a) DNPO concentration, b) sulphorhodamine 101 concentration, c) peroxide concentration, d) percentage of H₂O in the peroxide, e) carrier and peroxide flow rate, and f) sulphorhodamine and DNPO flow rate. (Error bars are 3s, n=5).

Fig. 2.10(d)v shows the effect of the water content in the carrier stream. A maximum was observed using 10 % water in 90 % acetonitrile. The hydrolysis of peroxyoxalate compounds has been shown to produce carbon dioxide and carbon monoxide as shown below (Orosz and Dudar, 1991).



Therefore if a high concentration of water is present, any DNPO will become hydrolysed and will not take part in the CL reaction.

Fig. 2.10(e) shows the effect of the carrier and peroxide stream flow rate on the chemiluminescence intensity. A plateau was observed between 0.7 and 2 mL min⁻¹ with a slight maximum at 1.25 mL min⁻¹. Fig 2.10(f) shows the effect of the sulphorhodamine and DNPO flow rate on the CL response. A plateau was observed between 3 and 4.5 mL⁻¹ with slight maximum at 3 mL min⁻¹. The effect of flow rates on the intensity can be described using the 'time windows' concept as shown in Fig. 2.11. If the flow rate is too great, then the point of maximum emission will occur after the sample has passed through the flow cell and conversely, if the flow rates are too low, then the point of maximum emission will occur before the sample reaches the flow cell.

If the detector mixing coil length had been changed, a different result may have been obtained for the flow rates. For this reason, a multivariate 'simplex' approach had also been used to optimise the flow rates and the detector mixing coil length.

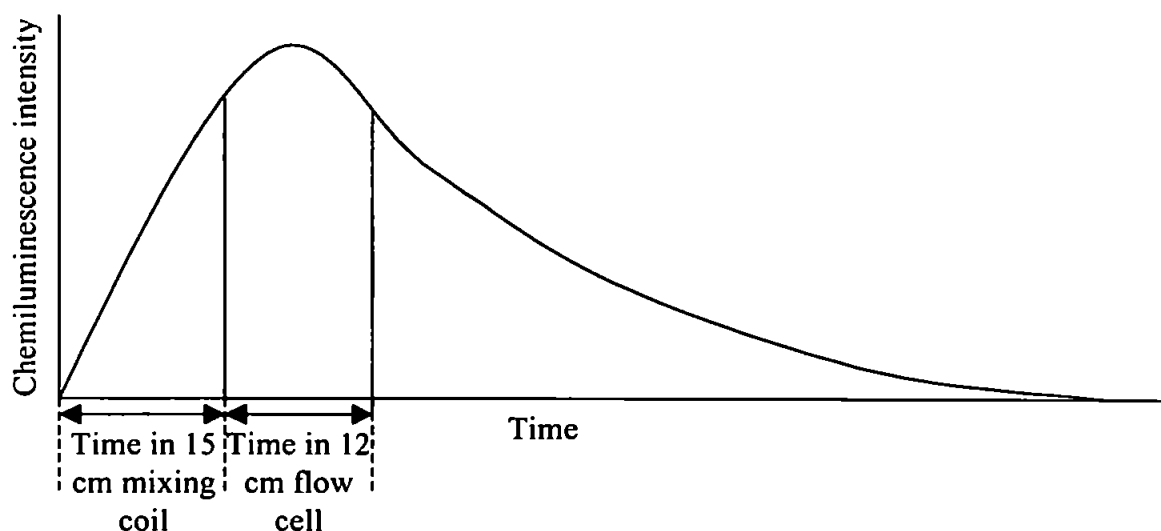


Figure 2.11: Time / intensity profile showing the 'time window' concept for CL detection.

2.3.5 SIMPLEX OPTIMISATION

Fig. 2.12 shows a schematic univariate optimisation of a two variable system. If variable 1 is optimised first using the starting conditions shown for variable 2, then the optimum value found univariately for variable 1 will not necessarily be the true optimum value. When variable 2 is optimised after this using the optimum value found for variable 1, then a univariate optimum for variable 2 is found. This however will also not necessarily be the optimum value for variable 2, as this approach does not take account of any synergistic interaction between the variables.

An alternative approach is to map the entire response surface by taking measurements using several combinations of values for all variables. This approach however, may result in many hundreds of measurements being required and is therefore not practical for systems that contain more than two experimental variables.

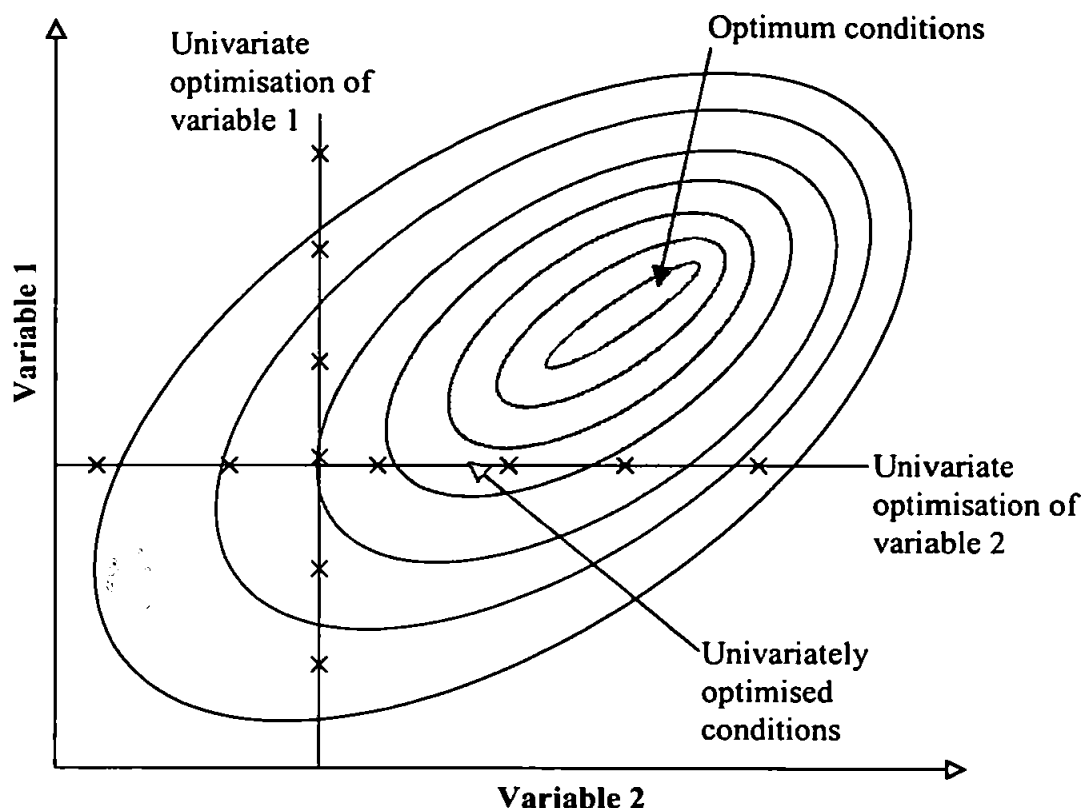


Figure 2.12: Univariate optimisation.

In contrast to the above, Multivariate optimisation techniques allow two or more variables to be altered simultaneously in a structured way which means that fewer experiments are needed to find optimum conditions. Examples of multivariate methods include: simplex optimisation, steepest ascent optimisation and pattern recognition techniques such as principle components analysis. Of these, simplex optimisation is relatively simple and reliable and has therefore been used for the optimisation of this reaction.

A simplex is a geometrical figure with one more vertex than the number of variables to be optimised (three vertices for a 2 variable system, 4 vertices for a 3 variable system *etc.*). An example for the two variable system is shown in Fig. 2.13. The starting conditions for the two variables are entered into the simplex, as shown in the diagram (experiment 1). The next two experiments (2 and 3) complete the first simplex triangle. Experiment 1

shows the worst result, and so would be rejected. A new simplex is then formed by projecting the initial simplex through the two points that gave the greater response (the triangle 2,3,4). This process is repeated again and again until the simplex does not produce any greater response. This is shown as experiment 13 in the diagram. More experiments may be needed to ensure that this is the true optimum (experiment 14) and not a local minimum. A refinement of this approach is the variable size simplex where the magnitude of the variable being projected can be increased (to speed up) or decreased (to provide better resolution) as required.

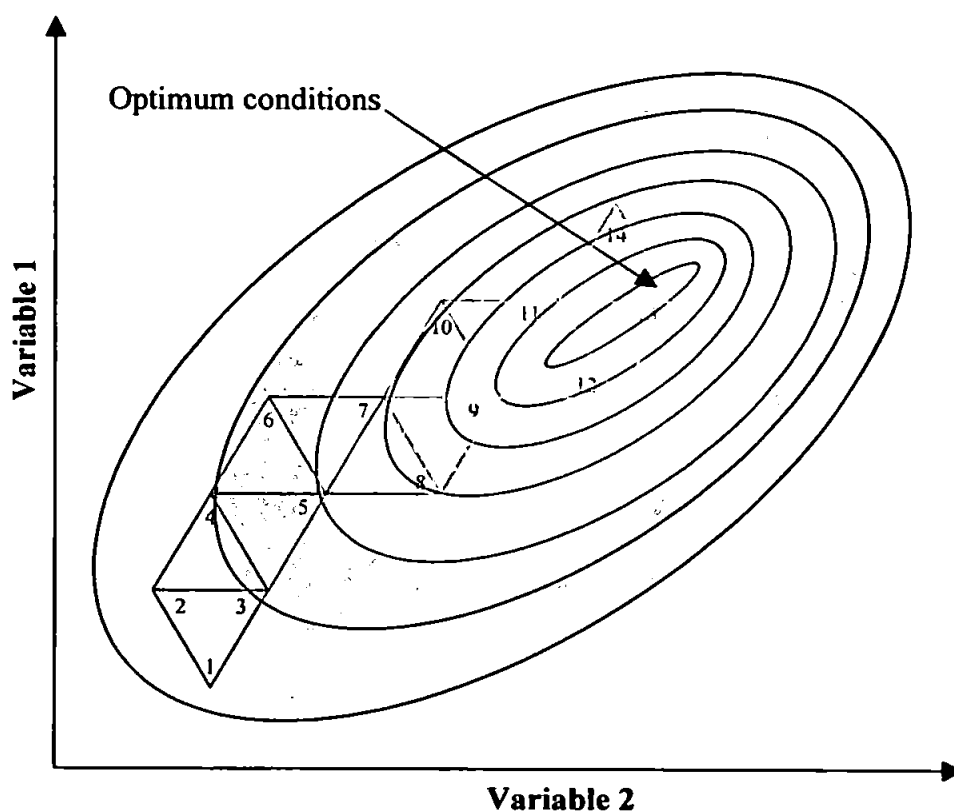


Figure 2.13: A typical two variable simplex optimisation.

For the detection of dodecylamine in 90 / 10 acetonitrile / water, using FI-CL, the variables H_2O_2 + carrier flow rate, DNPO + sulphorhodamine flow rate and detector mixing coil length, were identified as being highly dependent on each other. A simplex optimisation

was carried out using the results obtained from the univariate optimisation as a starting point. The starting conditions, limits and ranges of the variables used in the simplex optimisation are shown in Table 2.2. The conditions and response for each of the simplex experiments are shown in Fig. 2.14 and in Table 2.5. The signal/noise was improved from 112 (arbitrary units) after the univariate optimisation to 148 after the simplex optimisation (a 32% increase). The optimum conditions were DNPO + sulphorhodamine flow rate of 3.0 mL min^{-1} , H_2O_2 + carrier flow rate of 0.75 mL min^{-1} and a mixing coil length of 30 cm. Further simplex optimisation of the reagent concentrations may have increased the response still further, however the limit of detection was already well below the target needed for the determination of dodecylamine in diesel fuels.

Table 2.5: Simplex experimental parameters and the corresponding CL response.

Simplex experiment	DNPO + sulphorhodamine flow rate/ mL min^{-1}	H_2O_2 + carrier flow rate / mL min^{-1}	Detector mixing coil length / cm	Signal	Noise	S/N
1	3	1.25	15	0.4163	0.0037	113
2	4.5	1.25	15	0.3131	0.0037	85
3	3.5	1.75	15	0.3284	0.0037	89
4	3.5	1.5	25	0.4322	0.0037	117
5	2	1.75	20	0.1868	0.0024	78
6	3.5	1.25	15	0.4297	0.0049	88
7	3.5	1.25	15	0.476	0.0049	97
8	3.5	1.25	20	0.5286	0.0036	147
9	3	1	25	0.5225	0.0037	141
10	3	0.75	30	0.5481	0.0037	148
11	3	1	30	0.5188	0.0049	106
12	2.5	1	30	0.5017	0.0037	136
13	3.5	0.75	30	0.5481	0.0048	114
14	3	0.75	30	0.4638	0.0049	95
15	3.5	1.25	25	0.4931	0.0049	101

2.3.6 SOLVENT EFFECTS

The effect of changing the carrier / sample solvent was evaluated and the results are shown in Fig. 2.15 and in Table 2.6. The sensitivity was very poor for methanol, with a better sensitivity with propan-2-ol, and the greatest sensitivity for acetonitrile / water mixtures

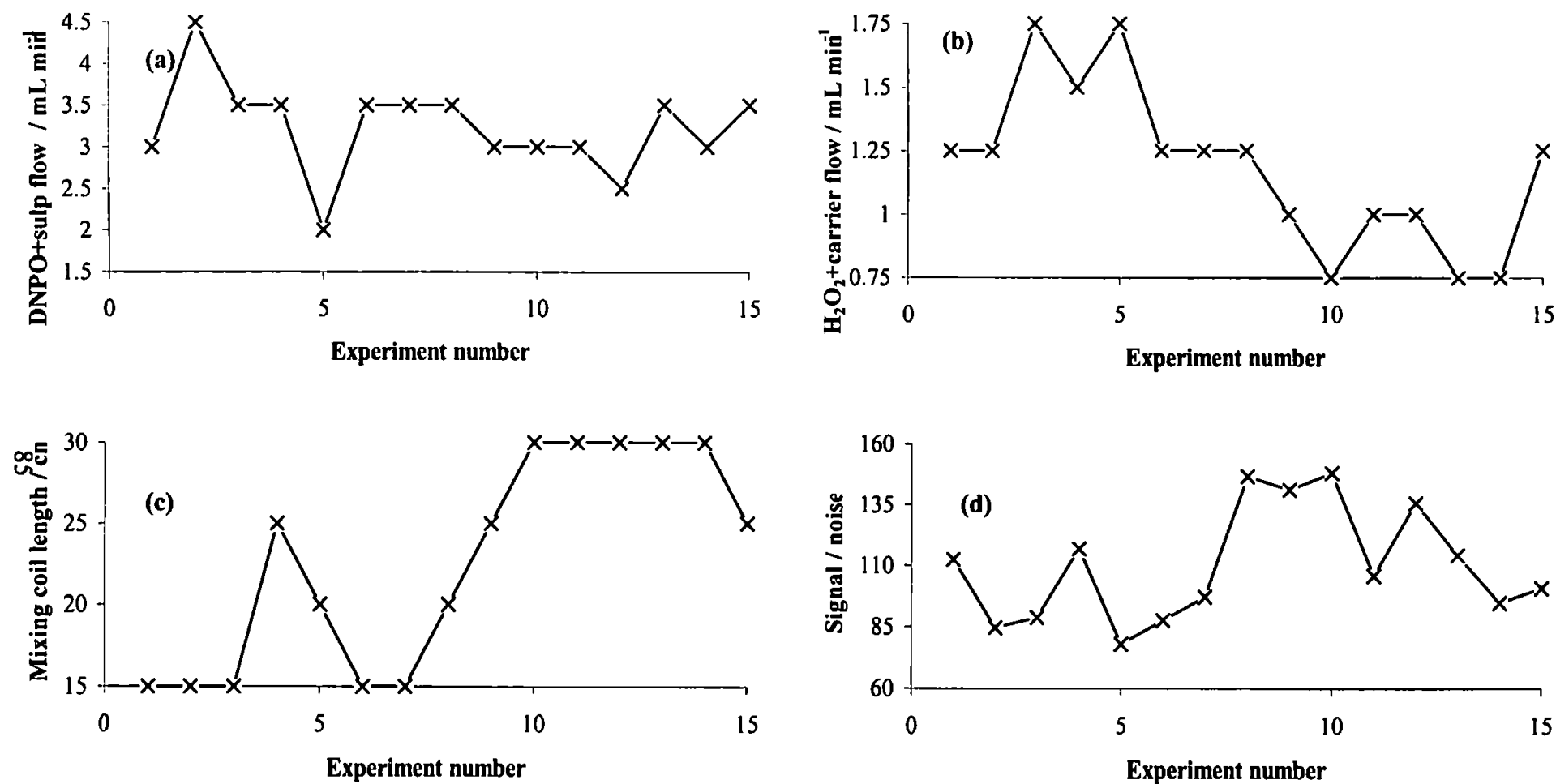


Figure 2.14: Simplex optimisation charts. Average taken from the 3 measurements.

with 90% or more acetonitrile.

The postulated peroxyoxalate chemiluminescence reaction mechanism (Fig 2.7) involves nucleophilic attack of H_2O_2 on the oxalate carbon. Nucleophilic solvents such as water and methanol can also attack the oxalate consuming it, resulting in non-chemiluminescent side reactions. It has been reported that 1 % methanol can result in the chemiluminescence intensity being halved (Hanaoka et al., 1988). A further reason for the decrease in sensitivity seen with methanol and propan-2-ol is the reaction rates. Kinetic studies using methanol, propan-2-ol and acetonitrile as mobile phases (Weinberger, 1984) have shown that the kinetics of the peroxyoxalate CL reaction are considerably faster in methanol and propan-2-ol than in acetonitrile and so a system optimised for the relatively slow acetonitrile kinetics would not give the greatest sensitivity for other solvents with different reaction rates. The system would need to be re-optimised using the simplex approach.

Table 2.6: Summary of the effects of different carrier / sample solvents on the chemiluminescence sensitivity.

Solvent	L.O.D.(S/N=3) / mg L^{-1}	S/N at 10 mg L^{-1}	RSD at 10 mg L^{-1} / % (n=5)
50/50 Acetonitrile / H_2O	4.3	9	3.3
60/40 Acetonitrile / H_2O	2.4	31	1.3
70/30 Acetonitrile / H_2O	1.5	86	1.9
80/20 Acetonitrile / H_2O	0.4	270	0.5
90/10 Acetonitrile / H_2O	0.6	1100	4.1
100/0 Acetonitrile / H_2O	0.6	1000	4.5
Methanol	1.3	26	3.5
Propan-2-ol (IPA)	0.2	180	4.4

The reason for the large decrease in the sensitivity between 90 % acetonitrile and 80 % acetonitrile is due to the increase in hydrolysis of the peroxyoxalate, which leads to the removal of DNPO via a non chemiluminescent pathway, as discussed in Section 2.3.4.

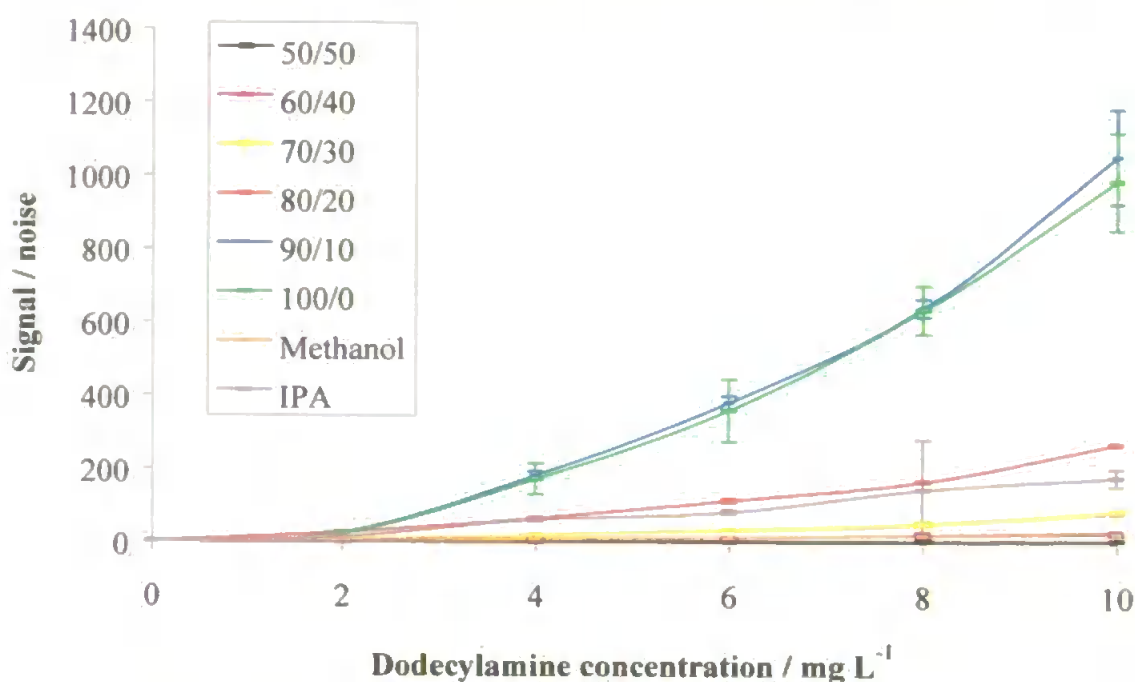


Figure 2.15: Effect of different acetonitrile / water ratios and methanol and IPA, used as carrier / sample solvents on the CL response.

2.3.7 INTERFERENCES

The effect of classes of compounds indigenous in diesel fuel on the peroxyoxalate chemiluminescence reaction was evaluated. Aniline, carbazole, *p*-cresol, hexanoic acid, indole and phenol were selected as representative compounds, and each evaluated separately by injection into the manifold shown in Fig 2.3 in order to determine their effects on the CL emission intensity. A summary of the interferences caused by these compounds is shown in Table 2.7, and calibration graphs for the most significant interferences are shown in Fig. 2.16

Aniline and indole were found to interfere with the peroxyoxalate reaction at relatively low concentrations (sub mg L⁻¹). This is due to their quenching effects (Vanzoonen et al., 1986). Quenching (Vanzoonen et al., 1987) takes place after the reaction between the

Table 2.7: Interfering effects of indigenous compounds found in diesel fuel.
***Concentration required to alter the measured concentration of a 40 mg L⁻¹ dodecylamine solution by ± 5 mg L⁻¹.**

Interference	Concentration causing interference*	Effect	Maximum fluorescence intensity
Aniline	< 1 mg L ⁻¹	Suppression	0
Indole	< 1 mg L ⁻¹	Suppression	273
p-Cresol	≥ 300 mg L ⁻¹	Suppression	241
Hexanoic acid	≥ 100 mg L ⁻¹	Enhancement	69
Carbazole	≥ 800 mg L ⁻¹	Enhancement	177
Phenol	≥ 1000 mg L ⁻¹	Suppression	194

peroxyoxalate and the hydrogen peroxide and also after the complex formation between the intermediate and the fluorophore. The quencher causes radiationless deactivation of the charge transfer complex $[F^+I^-]$ by donating an electron to the charge transfer complex. Aniline and indole both have delocalised structures with the capacity to stabilise a positive charge making them excellent quenchers. *p*-Cresol was found to suppress at much lower levels compared to phenol (300 mg L⁻¹ compared with > 1000 mg L⁻¹ for phenol). This is consistent with the above theory, as the electron donating methyl group present in cresol makes the molecule a better electron donor than phenol, which has no electron donating groups present. Carbazole has no electron donating groups present, and any stabilisation of an electron being donated from the nitrogen would result in disruption of the delocalisation present in carbazole. The slight enhancement caused by carbazole is likely therefore to be produced by fluorescent emission induced by the same charge transfer mechanism as for sulphorhodamine emission.

Hexanoic acid was found to cause a slight enhancement on the CL signal at concentrations over 100 mg L⁻¹, however hexanoic acid does not have a very intense fluorescence spectrum, and so the enhancement was more likely due to the small change in pH caused

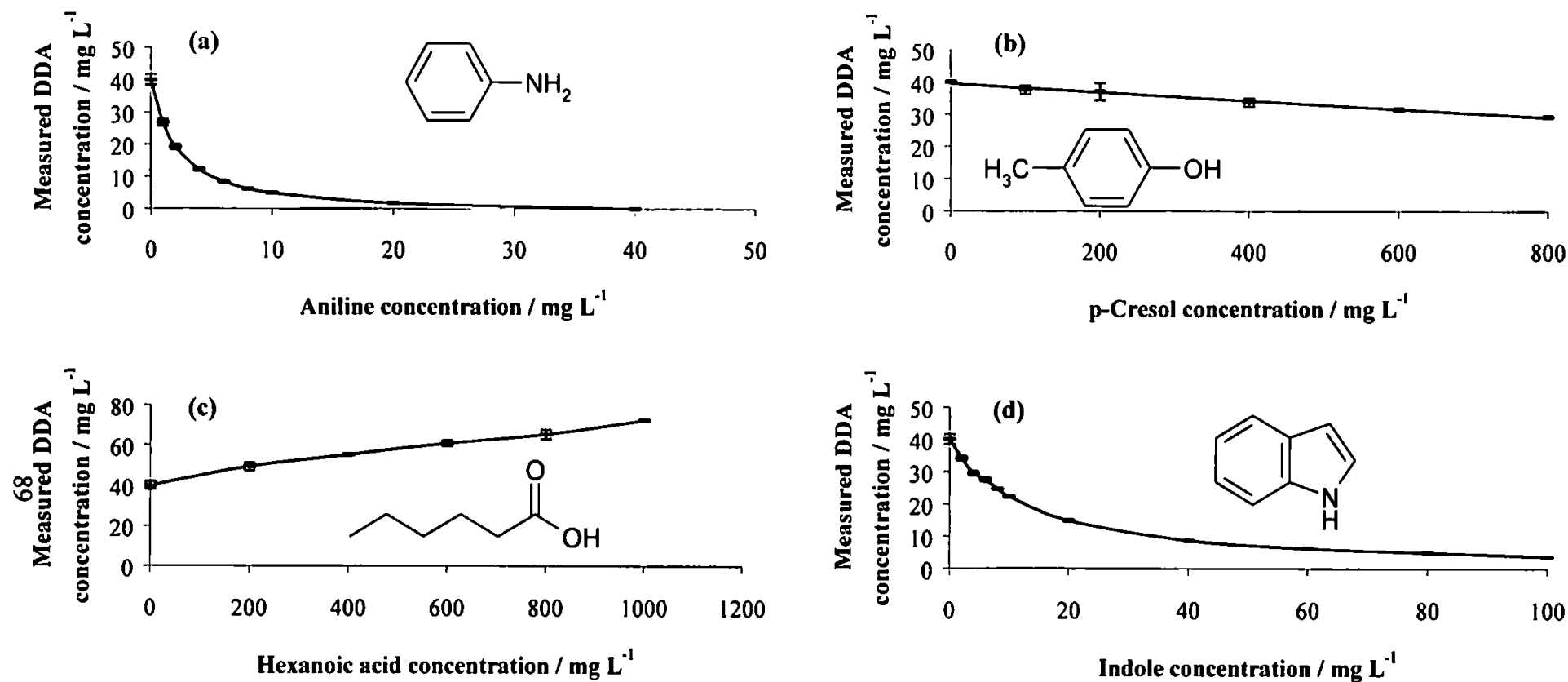


Figure 2.16: Effect of interfering compounds on the CL response for a 40 mg L⁻¹ DDA solution in acetonitrile: a) aniline, b) p-cresol, c) hexanoic acid, and d) indole (error bars 3s, n=3).

by the acid. The optimum pH using DNPO has been reported to be 6.0 (Honda et al., 1983), so a small increase in the acidity from a neutral pH is likely to have a small enhancement on the chemiluminescence response.

2.3.8 ANALYTICAL FIGURES OF MERIT

An example calibration set is shown in Fig. 2.17, which demonstrates the high sample throughput and good precision that are characteristic of FI-CL methods. Up to 80 injections can be made per hour using the adapted FI-CL manifold. A comparison of peak height and peak area calibrations (see Fig. 2.18) shows that they have similar precision but the peak area method has a lower detection limit due to the lower contribution of the blank.

Table 2.8 shows the analytical figures of merit for peak height and for peak area. Peak area was found to give a better straight line regression (0.9970 compared with 0.9959) and also a significantly lower limit of detection ($190 \mu\text{g L}^{-1}$ compared with $280 \mu\text{g L}^{-1}$). For this reason, the peak area was used for all subsequent measurements. A reported value for the limit of detection of dodecylamine using the manifold prior to the modifications is $9.8 \times 10^{-6} \text{ M}$ (1.8 mg L^{-1}) (Sanders, 1999). The adapted manifold gave a limit of detection of $1 \times 10^{-6} \text{ M}$ which is a significant improvement (one order of magnitude) in the sensitivity for dodecylamine.

Table 2.8: Analytical figures of merit.

	n	Gradient / L mg^{-1}	Intercept	L.O.D. / $\mu\text{g L}^{-1}$	L.O.D. / mol L^{-1}	R²
Peak height (signal / noise)	10	14.56	-1.018	280	1.5×10^{-6}	0.9959
Peak area	10	3.346	-0.1058	190	1.0×10^{-6}	0.9970

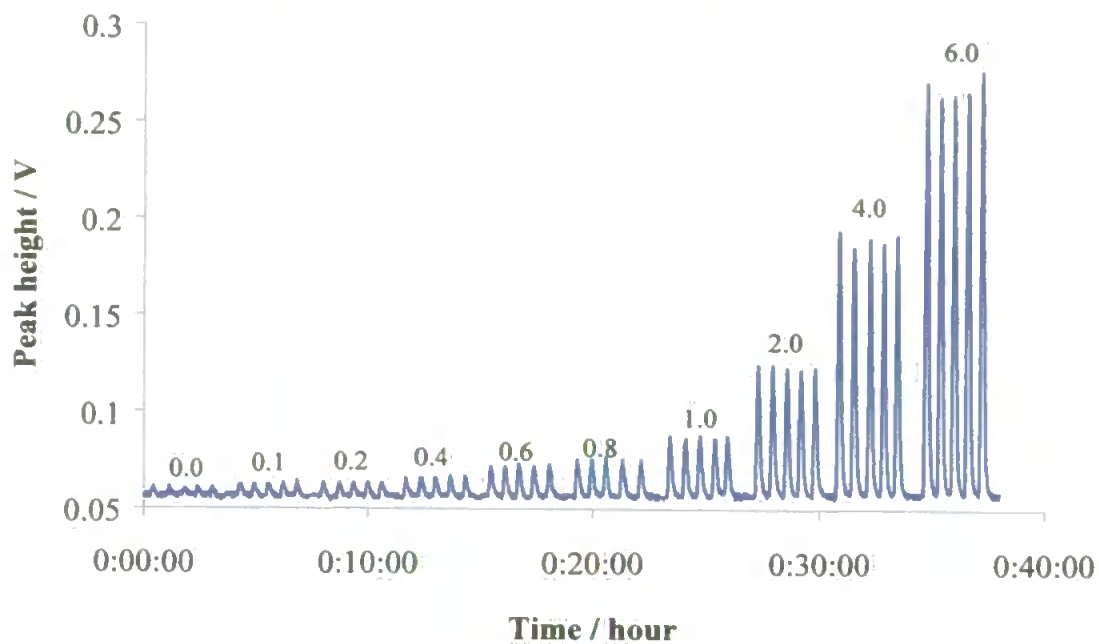


Figure 2.17: Example DDA peaks in the range 0 to 6 mg L⁻¹.

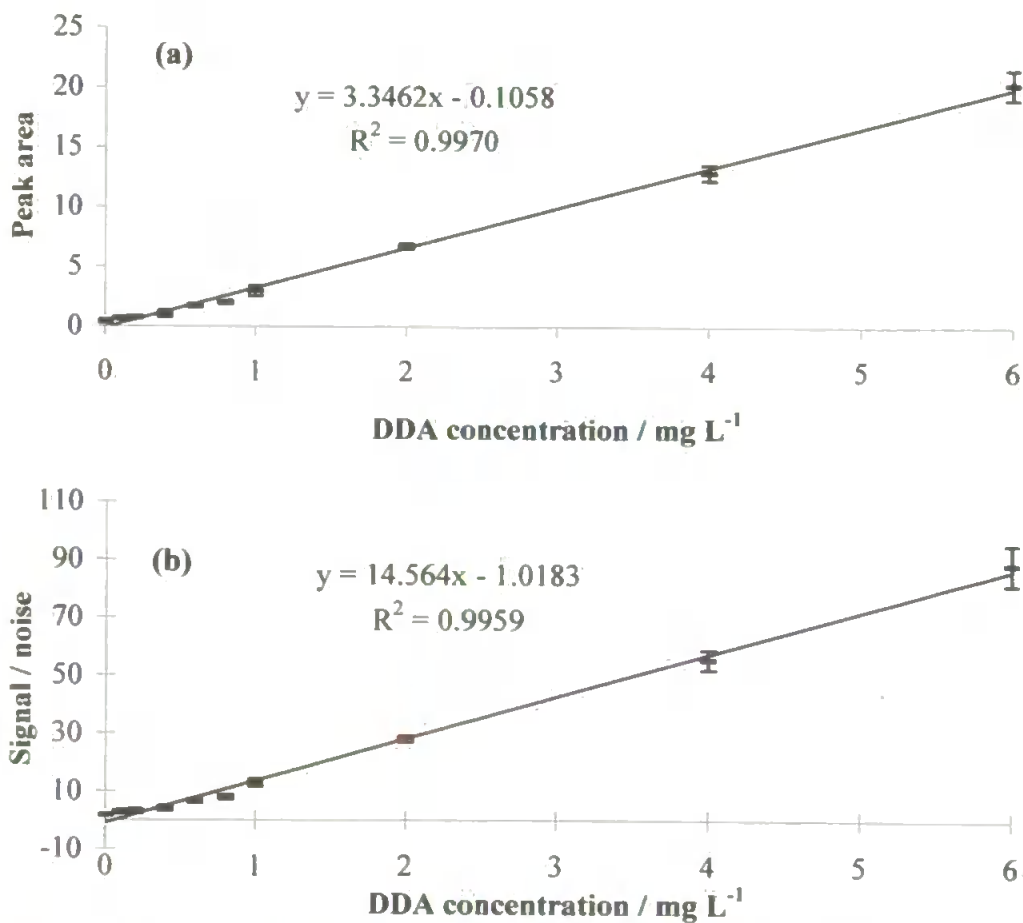


Figure 2.18: Calibration graphs for DDA using (a) peak area and (b) peak height / noise. (error bars 3s, n=5)

Fig 2.19 shows the linear range for DDA using the optimised manifold. The calibration graph is linear over the range 0 to 40 mg L⁻¹. At concentrations greater the 40 mg L⁻¹ the graph levels off due to depletion of the reagents which ultimately becomes rate limiting.

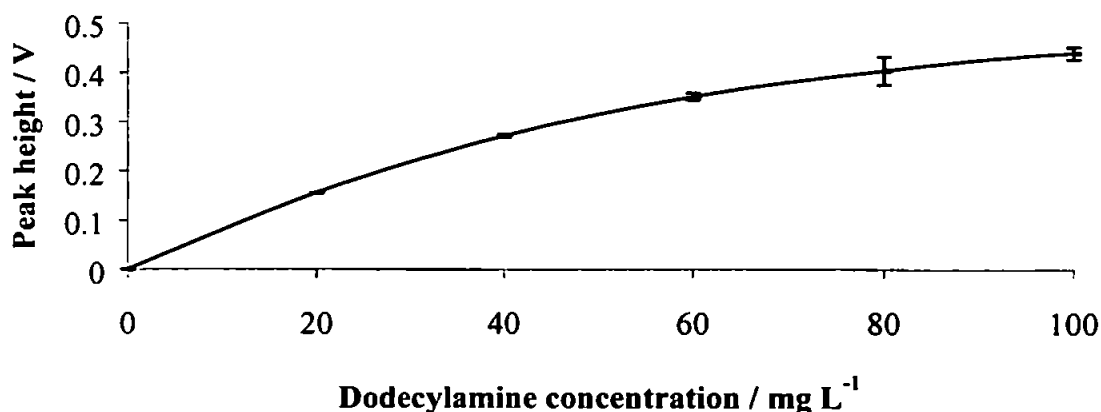


Figure 2.19: Linear range for DDA.

2.4 CONCLUSIONS

The specific conclusions from the research discussed in this chapter are:

1. Flow injection analysis with chemiluminescence detection can be utilised to provide rapid, reproducible, sensitive analysis. The sulphorhodamine 101 / peroxyoxalate reaction can be used in an FI-CL manifold to determine amines in various solvents. With a carrier solvent of 90% acetonitrile / 10% water, dodecylamine could be measured with a detection limit of 190 µg L⁻¹ and a linear range of 0 – 50 mg L⁻¹ ($R^2=0.9979$, $y= 3.34x - 0.106$). RSDs are typically <4% in the range 1-50 mg L⁻¹.
2. The emission from the peroxyoxalate / sulphorhodamine 101 reaction was found to have a λ_{max} of 598 nm for chemiluminescence and 592 nm for fluorescence.

3. The chemiluminescence reagents DNPO and sulphorhodamine 101 were found to be unstable when present together. Separating the streams increased stability allowing solutions to be kept for more than 24 hours.
4. Univariate optimisation of the manifold parameters determined that a maximum CL response was obtained with DNPO concentration of 1×10^{-3} M, sulphorhodamine 101 concentration of 8×10^{-7} M, hydrogen peroxide concentration of 0.1 M, 90% acetonitrile / 10 % water in the carrier stream and sample matrix, carrier + peroxide flow rate of 1.25 mL min^{-1} and sulphorhodamine 101 + DNPO flow rate of 3 mL min^{-1} .
5. A simplex optimisation of flow rates and detector coil length gave an increase of 32 % in the signal / noise ratio in comparison with that obtained using the univariately optimised conditions. In this case the optimum conditions were found to be DNPO + sulphorhodamine 101 flow rate of 3 mL min^{-1} , carrier + peroxide flow rate of 0.75 mL min^{-1} and a detector mixing coil of 30 cm.
6. The indigenous diesel compounds aniline, carbazole, *p*-cresol, hexanoic acid, indole and phenol were found to cause a significant change in the measured dodecylamine concentration when present at <1, 800, 300, <1, 100, 1000 mg L^{-1} respectively.

Chapter 3

Combination Of Off-Line Solid Phase Extraction(SPE) With FI-CL For the Determination of Dodecylamine In Diesel Fuels

3 COMBINATION OF OFF-LINE SOLID PHASE EXTRACTION (SPE) WITH FI-CL FOR THE DETERMINATION OF DODECYLAMINE IN DIESEL FUELS

3.1 INTRODUCTION

This chapter describes the analysis of diesel fuels spiked with dodecylamine directly (without any extraction), using the FI-CL manifold developed in Chapter 2. It also describes the development and optimisation of a solid phase extraction procedure to extract dodecylamine from diesel matrices into a solvent that is compatible with the FI-CL system described in Chapter 2. Gas chromatographic reference methods (with nitrogen phosphorus detection and mass spectrometric detection) have also been developed to validate the SPE procedures and are described in this chapter. Extracts of a number of diesel samples have been analysed by FI-CL and the results compared with those obtained using GC analysis.

3.2 EXPERIMENTAL

3.2.1 REAGENTS AND SAMPLES

All chemiluminescence reagents (DNPO, sulphorhodamine 101 and H_2O_2) were prepared as described in Section 2.2.1. Diesel fuels (Shell Haven, Swedish, Stanlow, Hamburg, SNV and Brazilian) and a diesel fuel additive package were obtained from Shell Global Solutions (Cheshire Innovation Park, Chester, U.K.). 400 mg L^{-1} spiked diesel stock solutions were prepared daily by dissolving 400 mg of dodecylamine in 100 mL of diesel fuel or heptane. 40 mg L^{-1} samples were prepared by serial dilution. Fully formulated fuel samples (containing the additive package) were prepared by dissolving 85 mg of the

additive package in 100 mL of diesel fuel to give the required 40 mg L⁻¹ dodecylamine concentration in the fuel (dodecylamine is present at a concentration of 4.7 % (m/v) in the additive package, therefore spiking at a concentration of 85 mg / 100 mL is equivalent to 40 mg L⁻¹ dodecylamine).

3.2.2 INSTRUMENTATION

All flow injection instrumentation was as described in Section 2.2.2 and the manifold used is shown in Fig. 2.3. Two different detection systems were used in conjunction with gas chromatography; nitrogen-phosphorus detection (NPD) and mass spectrometric detection (MS) and the gas chromatographic parameters for the different instruments used are shown in Table 3.1.

Table 3.1: Gas chromatographic conditions for the determination of dodecylamine in diesel fuel.

	GC-NPD	GC-MS
Chromatograph	Varian 3600 gas chromatograph	GC-MSD
Column	Hewlett Packard capillary HP-1	J&W Scientific DB-5
Injector	Autosampler, septum injection with a split of 10:1	Autosampler, septum injection with a split of 10:1
Injection volume	2 µL	1 µL
Injection temperature	70°C, held for 1 minute, 100°C / minute to 320°C, held for 1 minute	280°C
Initial oven temperature	70°C, held for 5 minutes	60°C held for 5 minutes
Temperature ramp	7°C / minute	10°C / minute
Final	325°C, held for 5 minutes	300°C, held for 10 minutes
Total run time	46 minutes	39 minutes
Detector	Nitrogen / phosphorus detection (detector temperature: 340°C, bead current: 3.30 A)	Mass spectrometric detector

Solid phase extraction columns used were 200 mg solid phase 3 mL, reservoir Aminopropyl Isolute SPE cartridges, purchased from International Sorbent Technology (Mid Glamorgan , U.K.). The manifold used for all solid phase extractions procedures was a 12-port vacuum SPE manifold purchased from Whatman (U.K.).

3.2.3 PROCEDURES

3.2.3.1 *Direct FI-CL analysis of diesel fuels without SPE*

Heptane and diesel samples (unspiked, spiked with 40 mg L⁻¹ dodecylamine and spiked with the additive package containing 40 mg L⁻¹ DDA) were diluted to 25 % (v/v) in propan-2-ol (IPA). These samples were analysed using the manifold shown in Fig. 2.3 using a carrier stream consisting of 25 % heptane / 75 % IPA. The chemiluminescence response for each was recorded.

3.2.3.2 *Extraction of dodecylamine from heptane with GC-NPD detection*

In order to develop an SPE method for the extraction of 40 mg L⁻¹ DDA from heptane, the procedure shown in Table 3.2 was used. All eluents were analysed using GC-NPD and recoveries were calculated by comparing peak areas with those obtained from standards.

3.2.3.3 *Extraction of dodecylamine from Swedish base fuel with GC-NPD detection*

The same procedure that was used for the extraction of DDA from heptane was used for the extraction of 40 mg L⁻¹ DDA from Swedish base fuel. Again, eluents were analysed by GC-NPD. This experiment was carried out in triplicate.

Table 3.2: Solid phase extraction procedure used to determine the solvent needed to elute 40 mg L⁻¹ DDA from an aminopropyl SPE column.

	Step	Volume / mL	Solvent	Polarity / $\epsilon^0(\text{Al}_2\text{O}_3)$
Conditioning	1	4	Methanol	0.95
	2	4	Toluene	0.29
	3	4	Heptane	0
Loading	4	10	40 mg L ⁻¹ DDA in heptane	0
Eluting	5	3	Heptane	0
	6	3	Toluene	0.29
	7	3	Dichloromethane	0.39
	8	3	Acetonitrile	0.65
	9	3	90 / 10 acetonitrile / water	>0.65
	10	3	80 / 20 acetonitrile / water	>>0.65

3.2.3.4 SPE elution volume optimisation for the extraction of DDA from heptane

To optimise the elution volume needed to elute DDA from the SPE cartridge, the procedure shown in Table 3.3 was used. Successive 0.5 mL aliquots of 90 / 10 acetonitrile / water were used to elute DDA from the column. Eluents were analysed using the FI-CL manifold developed in Chapter 2. Samples were diluted in order to bring them into the linear range of the FI-CL calibration.

Table 3.3: Solid phase extraction procedure used to optimise the volume of 90 / 10 acetonitrile / water needed to elute dodecylamine.

	Step	Volume / mL	Solvent
Conditioning	1	5	Methanol
	2	5	Toluene
	3	5	Heptane
Loading	4	4	40 mg L ⁻¹ dodecylamine in heptane
Washing	5	2	Heptane
	6	2	Toluene
	7	2	Dichloromethane
	8	0.5	Successive 0.5 mL aliquots of 90 / 10 acetonitrile / water

3.2.4 GC-MS OF BASE FUEL EXTRACTS

Heptane and each of the base fuels were spiked, both with 40 mg L⁻¹ DDA and with the additive package. Each sample was diluted to 25 % in heptane in order to reduce the viscosity of the samples. The samples were extracted as shown in Table 3.4.

Table 3.4: Optimised solid phase extraction procedure for the extraction of 40 mg L⁻¹ DDA from diesel fuels.

	Step	Volume / mL	Solvent
Conditioning	1	5	Methanol
	2	5	Toluene
	3	5	Heptane
Loading	4	10	25 % sample / 75 % heptane
Washing	5	2	Heptane
	6	2	Toluene
	7	2	Dichloromethane
Eluting	8	2.5	90 / 10 acetonitrile / water

The solvent was evaporated off of all the samples under a stream of nitrogen, and samples were resolubilised in dichloromethane prior to analysis by GC-MS.

3.2.5 FI-CL ANALYSIS OF EXTRACTS OF SPIKED FUELS FOR THE DETECTION OF DODECYLAMINE

The FI-CL manifold and all reagents used were as described in Chapter 2. A 40 mg L⁻¹ DDA standard was injected after every 5 samples to compensate for any drift in the CL response during the analysis. The solid phase extraction procedure used was as shown above in Table 3.4.

3.3 RESULTS AND DISCUSSION

3.3.1 DIRECT FI-CL ANALYSIS OF DIESEL FUELS WITHOUT SPE

In order to determine the effect of the diesel fuel matrix on the CL response, spiked and unspiked diesel samples were analysed using the FI-CL manifold developed in Chapter 2. All samples and the carrier stream were diluted to 25 % by adding 75 % (v/v) IPA, in order to ensure that the diesel fuel was totally miscible with the polar reagent streams (acetonitrile and water) required for the FI-CL manifold. The chemiluminescence responses for heptane, Shell Haven base fuel and Swedish base fuel samples are shown in Fig. 3.1 and the responses (relative to the peak height of a 40 mg L⁻¹ DDA in heptane sample) are plotted in Fig. 3.2. Fully formulated fuels are base fuels that have been spiked with an additive package containing, amongst other compounds, 40 mg L⁻¹ dodecylamine.

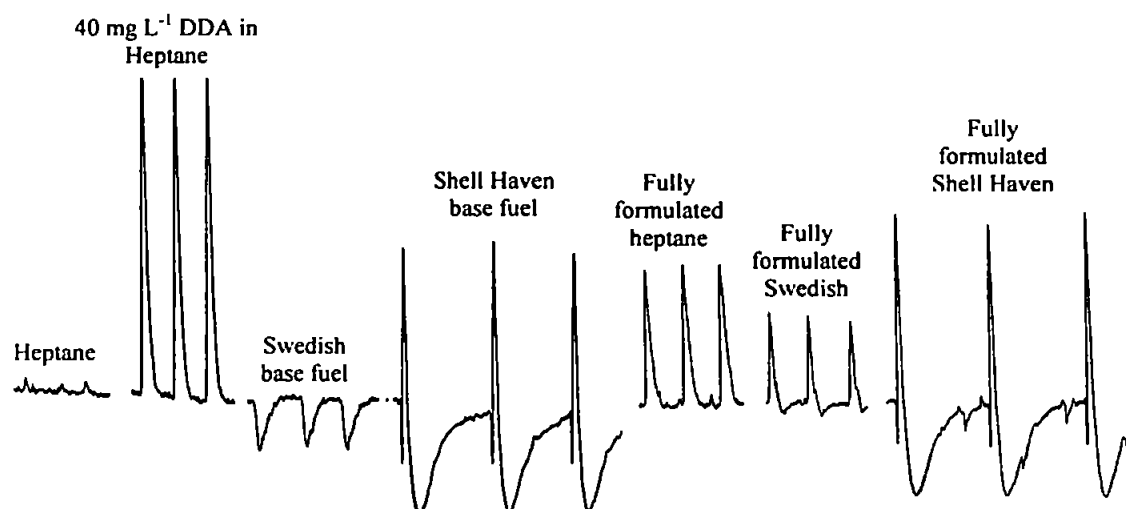


Figure 3.1: FI-CL peaks from heptane, heptane spiked with 40 mg L⁻¹, diesel base fuels, heptane and fuels spiked with an additive package containing 40 mg L⁻¹ dodecylamine.

There have been many papers published on the development of FI-CL methods for a range of analytes (See Chapter 1) (Fletcher et al., 2001). However, Fig. 3.1 highlights the potential difficulty in using such methods for the analysis of real sample matrices. Other

compounds in the matrix can either enhance the signal by reacting directly with CL reagents, acting as a sensitiser, or suppressing the CL signal by providing non-radiative relaxation pathways for the excited state or alternative, non-chemiluminescent reaction pathways.

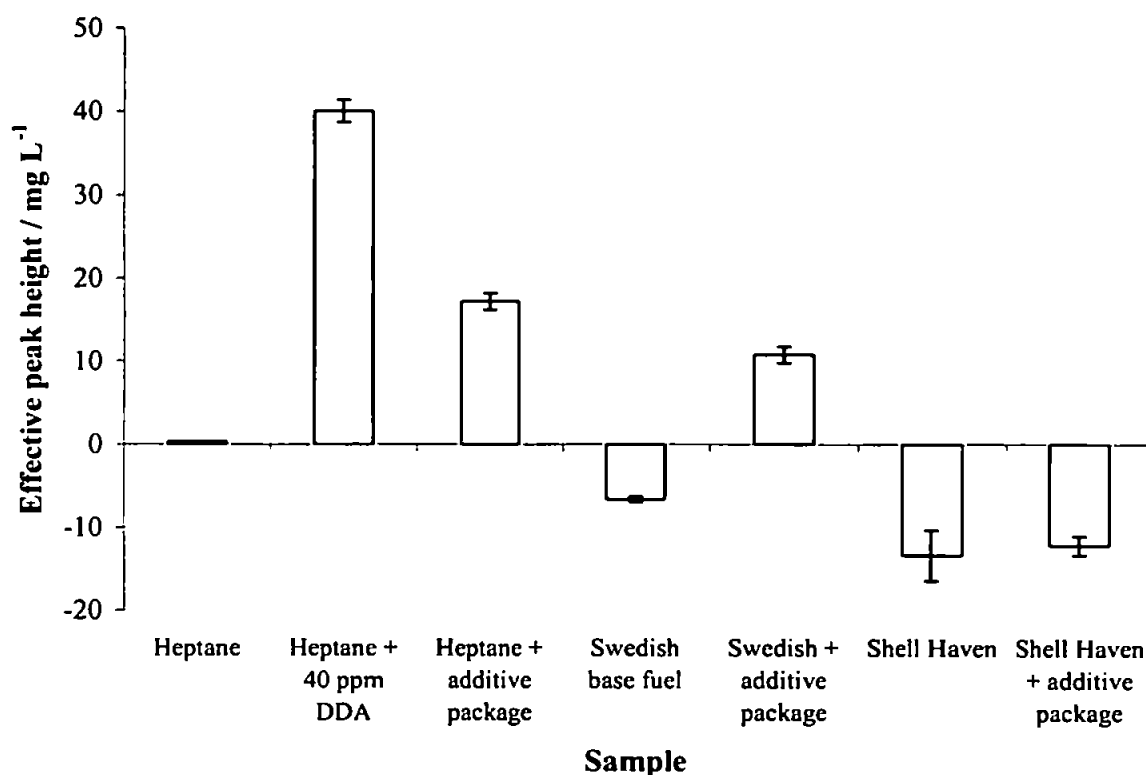


Figure 3.2: CL response for diesel samples injected directly into the FI-CL system with no SPE. Intensities shown are relative to the response from a 40 mg L⁻¹ DDA standard in 25% Heptane / 75% propan-2-ol. All samples were diluted to 25% with propan-2-ol. Error bars represent 3s (for n = 3).

In this case it can clearly be seen that heptane provides a stable baseline and that 40 mg L⁻¹ DDA provides a good CL response with a well defined peak shape. However, in the presence of the full additive package, the DDA response is significantly suppressed (by approximately 50 % in terms of peak height). The situation with the Swedish and Shell Haven fuels is markedly different. The Swedish base fuel contains components that suppress the CL response from the sulphorhodamine reaction, resulting in a reduction in the baseline (background CL) when injected. The fully formulated package generates a

good peak profile, the height of which (relative to the base fuel) is equivalent to that obtained with heptane. The Shell Haven base fuel gives a sharp positive peak followed by a broad negative peak for both the base fuel and the fully formulated fuel. This is due to the more viscous nature of the Shell Haven fuel which leads to poor mixing of the sample with the reagent stream. The CL suppression from both of the base fuel samples is most likely due to sulphur compounds in the fuel which are well known to have effects on a range of CL reactions (Galan et al., 1997). Selected properties of the diesel fuels used in this chapter are shown in Table 3.5.

Table 3.5: Sulphur and aromatic content of diesel fuels evaluated. (n.d. = not determined)

Fuel	Sulphur content / mg kg⁻¹	Mono aromatic content / % (m/v)	Di- aromatic content / % (m/v)	Tri aromatic content / % (m/v)	Total aromatic content / % (m/v)
Heptane	0	0	0	0	0
Swedish	<5	3.7	0.1	0.1	3.8
Stanlow	300	21.5	2.7	0.5	24.7
Hamburg	375	n.d.	n.d.	n.d.	n.d.
Shell Haven	400	19.0	4.6	0.5	24.7
SNV	600	21.5	3.9	0.6	26.0
Brazilian	3100	18.1	6.5	1.4	26

3.3.2 SOLID PHASE EXTRACTION

Solid phase extraction (SPE) is a technique that has predominantly been used for the selective extraction of analytes from aqueous samples and a number of reviews are available on this subject (Liska et al., 1989; Mills et al., 1992; Soriano et al., 2001). SPE has been widely used in the areas of pharmaceutical analysis and drug screening (Andersson, 2000; Scheurer and Moore, 1992), but to date, however, there have only been a limited number of papers describing its use for the extraction of components from fuels

and oils. A summary of these is presented in Table 3.6, which highlights the fact that most applications have involved edible and industrial oils rather than fuels. Also most applications have targeted classes of compounds rather than specific analytes or marker compounds. The aim of the methodology reported in this section is therefore to design a suitable SPE procedure for the selective and quantitative isolation of dodecylamine from a variety of real diesel fuels.

3.3.3 OPTIMISATION OF SPE PROTOCOL FOR THE EXTRACTION OF DDA FROM DIESEL FUELS

Diesel contains many different classes of compounds, such as aliphatics, aromatics (predominantly mono, di and tri-aromatics) and polar compounds (*e.g.* indoles, carbazoles, phenols, carboxylic acids, anilines and sulphur containing compounds such as sulphonic acids). Interference studies in Chapter 2 have shown that most of these interfere to a lesser or greater extent with the FI-CL system that has been developed. Studies have shown certain classes of compounds could be fractionated from crude oil using silica columns (Theobald, 1988). An ideal SPE procedure for the extraction of dodecylamine would therefore remove all classes of matrix components whilst leaving only dodecylamine on the column.

It has been reported that silica as a solid phase sorbent has problems with moisture (Burke and Calverley, 1998). Variable recoveries will result unless silica has been dried prior to use. To fractionate diesel fuel using silica it has been reported that the silica must be dried at 140°C for 1 hour for reproducible results. If a system is to be deployed in the field, as is the aim with this project, then having to dry sorbent prior to analysis is a distinct disadvantage. Aminopropyl has a similar sorbent strength to silica, however it does not

Table 3.6 Solid phase extraction procedures for analytes in non-aqueous solutions.

Analyte / Fraction	Matrix	Cartridge	Conditioning	Loading	Washing	Eluting	Reference
Fractionation of aliphatics, monoaromatics, diaromatics, polyaromatics, and polar compounds.	Diesel fuel	Self prepared silica gel in borosilicate glass.	Heated at 140°C for 1 h before use, followed by a 4-5 mL n-pentane wash at 30°C.	200 µL of 10 % diesel in n-pentane	2 mL n-pentane	Aliphatics – 4 mL n-pentane Monoaromatics - 8.5 mL 5 % DCM in n-pentane Diaromatics – 5 mL 10 % DCM in n-pentane Polyaromatics – 5 mL 60 % DCM in n-pentane Polar compounds – 5 mL 25 % acetic acid in methanol	(Bundt et al., 1991)
Alkylphenols	Crude oil	Jones Isolute C ₁₈ and C ₈ non end capped and C ₁₈ end capped.	3 mL Petroleum Ether.	0.5 mL of crude oil.	4.5 mL petroleum ether.	5 mL Methanol / water (1:1)	(Bennett et al., 1996)
Phospholipids	Sunflower oil	Diol (J.T. Baker 500mg)	2mL MeOH, 2mL chloroform, 4mL hexane.	50-150 mg oil in chloroform	2.5 mL chloroform	7 mL MeOH containing 0.5 mL / 100 mL 25 % ammonia solution.	(Carelli et al., 1997)
Fractionation of components	Crude oil	Baker 3ml silica	10mL DCM, 10mL hexane	100 µL hexane containing 10 µg of each compound.	None.	Alkanes / alkenes 2 mL hexane Benzenes 2mL 10% DCM in hexane. 2-3 Ring aromatics 2 mL 10% DCM in hexane. 4-6 Ring aromatics 2 mL 20% DCM in hexane. Cholesterol / fatty acids / phthalates 2 mL DCM.	(Theobald, 1988)
Nonpolar / polar Lipid Classes	Edible oils	Amino and silica (Analytichem)	None	20-60 mg of oil.	None.	Non-polar fraction, 20mL hexane / diethyl ether (9:1) Polar fraction, 10 mL chloroform / methanol (2:1) and 10 mL methanol / acetic acid (98:2)	(Hopia et al., 1992)

Table 3.6 Solid phase extraction procedures for analytes in non-aqueous solutions (continued).

Analyte / Fraction	Matrix	Cartridge	Conditioning	Loading	Washing	Eluting	Reference
Polar Compounds	Oils and fats	1000mg Sep-Pak Silica (Waters).	10mL 9:1 light petroleum / diethyl ether.	2 mL of light petroleum containing 50 mg of oil.	None.	Non-polar fraction, 15 mL of light petroleum / diethyl ether (9:1). Polar fraction, 15 mL diethyl ether	(Marquezr uiz et al., 1996)
Diacylglycerol isomers	Vegetable oils	3 mL Diol (Supelco)	4 mL Hexane.	200 μ L of 0.2 mg mL ⁻¹ oil in hexane.	6 mL hexane / DCM / methanol (89:10:1)	4 mL Chloroform / methanol (2:1).	(Perezcamino et al., 1996)
Phenolic Compounds	Virgin olive oils.	Alltech 500 mg 3.5 mL C ₈	10 mL n-hexane, 10 mL acetonitrile	1 g of oil in 10 mL n-hexane.	10 mL n-hexane / cyclohexane (1:1)	2.5 mL Acetonitrile.	(Pirisi et al., 1997)
Bitter taste components	Virgin olive oils.	J.T.Baker 6 mL C ₁₈	6 mL Methanol, 6 mL hexane.	1 g of oil in 4 mL hexane.	10 mL hexane	25 mL Methanol / water (1:1)	(Gutierrez Rosales et al., 1992)
Acylglycerols	Soybean oil.	3 mL 200 mg Silica (Bond Elut)	None.	15 – 20 mg oil in 0.5 mL hexane.	None.	Triacylglycerol: 5 mL diethyl ether / hexane (10:90). Fatty acids: 18 mL diethyl ether / hexane (10:90). Diacylglycerol: 6 mL diethyl ether / hexane / Acetic acid (50:50:1). Monoacylglycerol: 4 mL methanol.	(Neff et al., 1992)
Sulphides and Thiols	Petroleum	5 g Alumina (ICN Biomedical)	400°C for 4 hours, DCM / pentane (2.5:97.5).	10 mL of distillate.	None.	Hydrocarbons, thiophenes: 20 mL DCM / pentane (2.5:97.5). Sulphides, thiols: 20 mL methyl t-butyl ether. Phenols, polar compounds: methanol / methyl t-butyl ether (10:90).	(Thomson et al., 1997)

need to be dried prior to extraction. It was therefore decided to be used as the sorbent to extract dodecylamine from fuels.

3.3.3.1 Extraction of dodecylamine from heptane with GC-NPD detection

In order to extract dodecylamine from heptane, it must be determined which solvent has sufficient polarity to remove the analyte from the SPE column. A solid phase extraction procedure to determine the elution solvent needed to extract dodecylamine from heptane is shown in Table 3.2. Methanol was chosen as the first conditioning solvent as it is very polar and therefore was able to remove any impurities present on the column. For maximum retention, the final conditioning solvent should be as close to the sample matrix as possible, therefore heptane was chosen for this purpose. Heptane and methanol are immiscible and so a solvent of intermediate polarity (toluene) was used as an intermediate conditioning solvent. A sample of 10 mL 40 mg L⁻¹ DDA in heptane was passed through a 200 mg aminopropyl SPE column and the heptane leaving the column was collected for analysis to ensure that the dodecylamine had been fully retained by the sorbent. A vacuum solid phase extraction manifold was used for the extraction, with the pressure kept to below 15 cm Hg. Subsequent 3 mL aliquots of heptane, toluene, dichloromethane (DCM), acetonitrile, 90 / 10 acetonitrile / water, and 80 / 20 acetonitrile / water were passed through the column and collected for analysis by GC with nitrogen-phosphorus detection.

The nitrogen-phosphorus detector (NPD) uses a bead of alkali metal salt (often Rubidium) that is electrically heated, generating a stable population of alkali metal ions. Nitrogen and phosphorus compounds interact with the alkali ions by a series of complex reactions that produce thermionic electrons. These electrons are collected, giving rise to an increase in current proportional to the analyte concentration. Hydrogen burns on the surface of the bead producing a plasma. Nitrogen compounds produce CN radicals by pyrolysis, which

react with alkali atoms in the plasma to form an ion pair. The cyanide ions migrate to the collector electrode to form the detector signal. An NPD is 50 times more sensitive to nitrogen and 500 times more sensitive to phosphorus than a flame ionisation detector (FID). Normal FID type response is suppressed so compounds that do not contain nitrogen or phosphorus do not produce a measurable signal. This enhanced sensitivity to nitrogen makes the NPD ideal for method development of solid phase extraction procedures to isolate dodecylamine from diesel fuel. The GC-NPD results for the solid phase eluents are shown in Fig. 3.3.

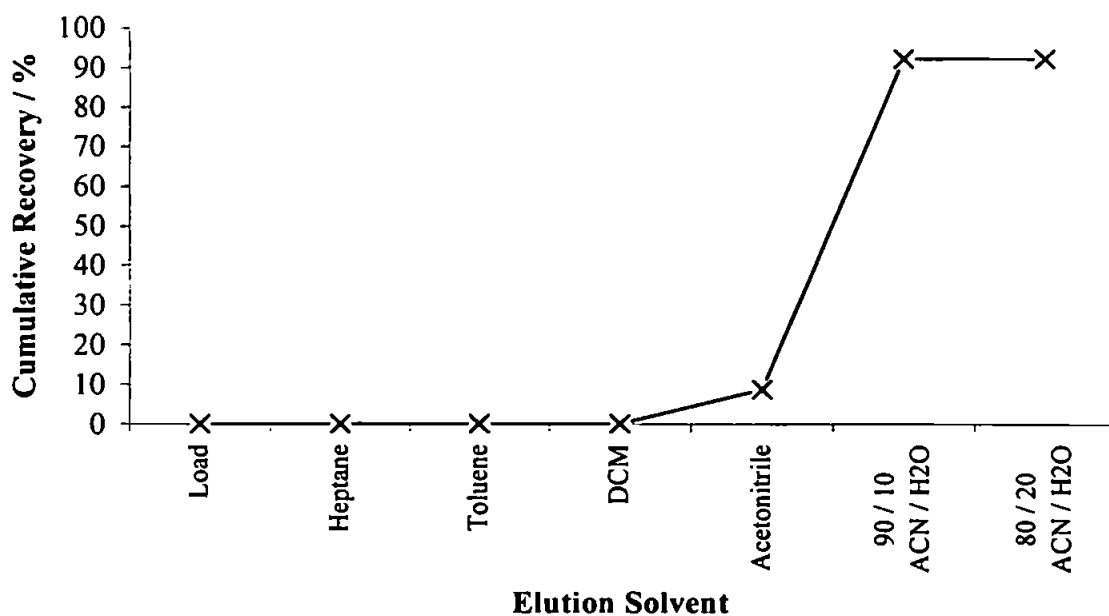


Figure 3.3: Elution profile from a 40 mg L⁻¹ dodecylamine sample in heptane. Analysis by GC-NPD.

No dodecylamine was measured in the loaded solution after it had passed through the column, therefore showing that all of the dodecylamine had been retained. The heptane, toluene and dichloromethane extracts were also free of dodecylamine, showing that they are suitable as wash solvents to remove impurities without eluting dodecylamine. 3 mL of acetonitrile eluted 9 % of the dodecylamine. Acetonitrile was therefore strong enough to

elute dodecylamine, however a large elution volume would be needed to elute all of the dodecylamine. A robust SPE procedure should be able to extract into a small volume, and therefore 90 / 10 acetonitrile / water was used. 92 % (cumulative) of the dodecylamine was extracted into this solvent, and using a stronger solvent (80 / 20 acetonitrile / water) eluted no more dodecylamine. 90 / 10 acetonitrile / water was therefore chosen as the appropriate elution solvent for dodecylamine.

Acetonitrile / water mixtures were chosen as elution solvents in preference to unmixed solvents (such as methanol) due to the compatibility of acetonitrile / water mixtures with the FI-CL manifold developed in Chapter 2.

3.3.3.2 *Extraction of dodecylamine from Swedish base fuel with GC-NPD detection*

The procedure that was used for the extraction of dodecylamine from heptane was also used for the extraction of 40 mg L⁻¹ dodecylamine from a commercial diesel fuel (Swedish base fuel) to determine whether similar results could be obtained. The spiked diesel fuel was loaded directly (without dilution). GC-NPD chromatograms of successive extracts from spiked Swedish base fuel are shown in Fig. 3.4 and the cumulative recoveries are plotted in Fig. 3.5.

As with heptane, the dichloromethane eluent contained no dodecylamine proving that it was a suitable wash solvent for the extraction of dodecylamine. The acetonitrile extract recovered 40 % of the dodecylamine, further showing that it was not a robust elution solvent (not reproducible). 90 / 10 acetonitrile / water recovered 84 % of the dodecylamine (cumulative) and the 80 / 20 acetonitrile / water extract again recovered no further

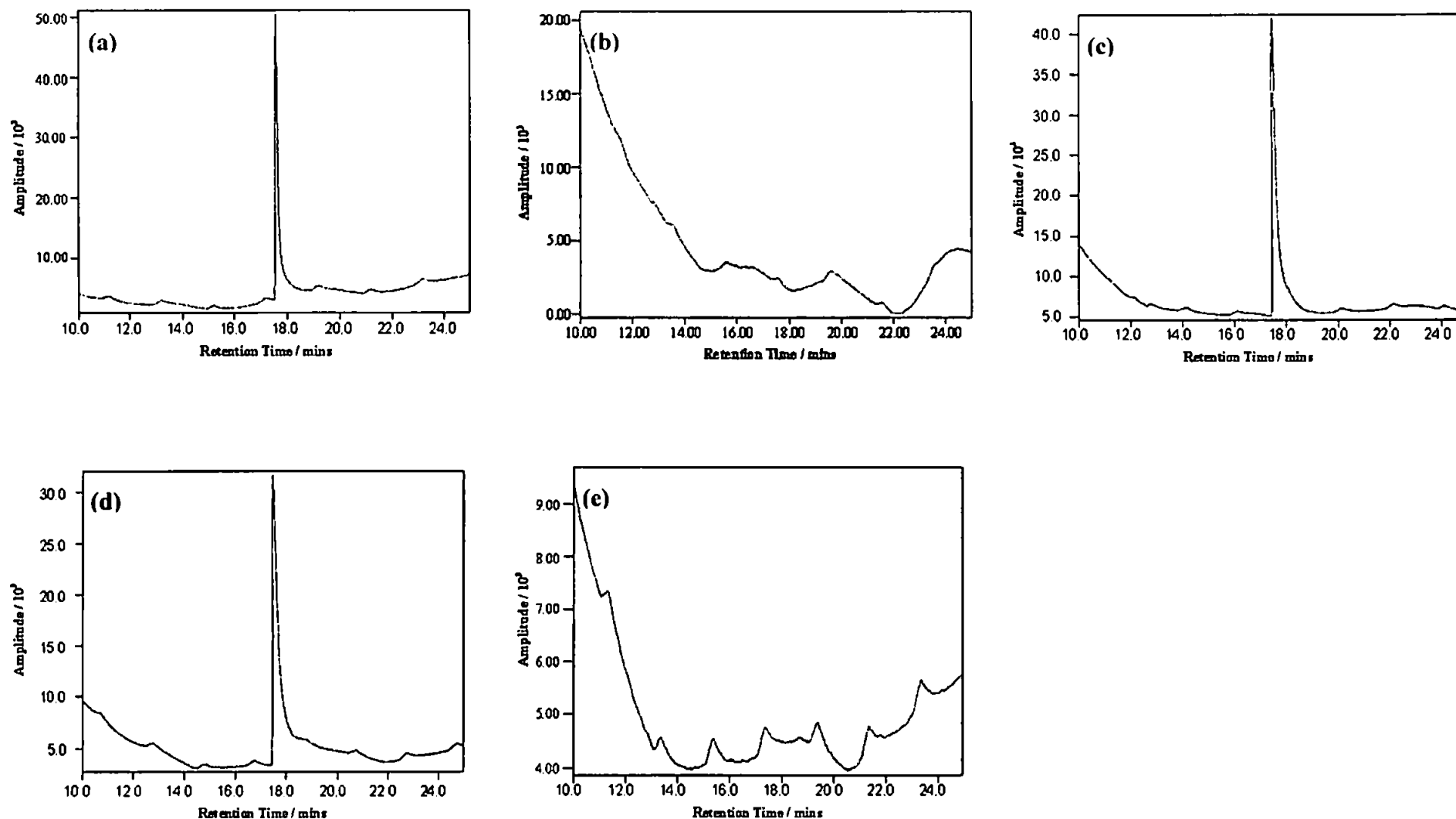


Figure 3.4: GC-NPD chromatograms of: (a) 40 mg L⁻¹ dodecylamine in heptane, (b) dichloromethane diesel extract, (c) acetonitrile diesel extract, (d) 90 / 10 acetonitrile / water diesel extract, (e) 80 / 20 acetonitrile / water diesel extract.

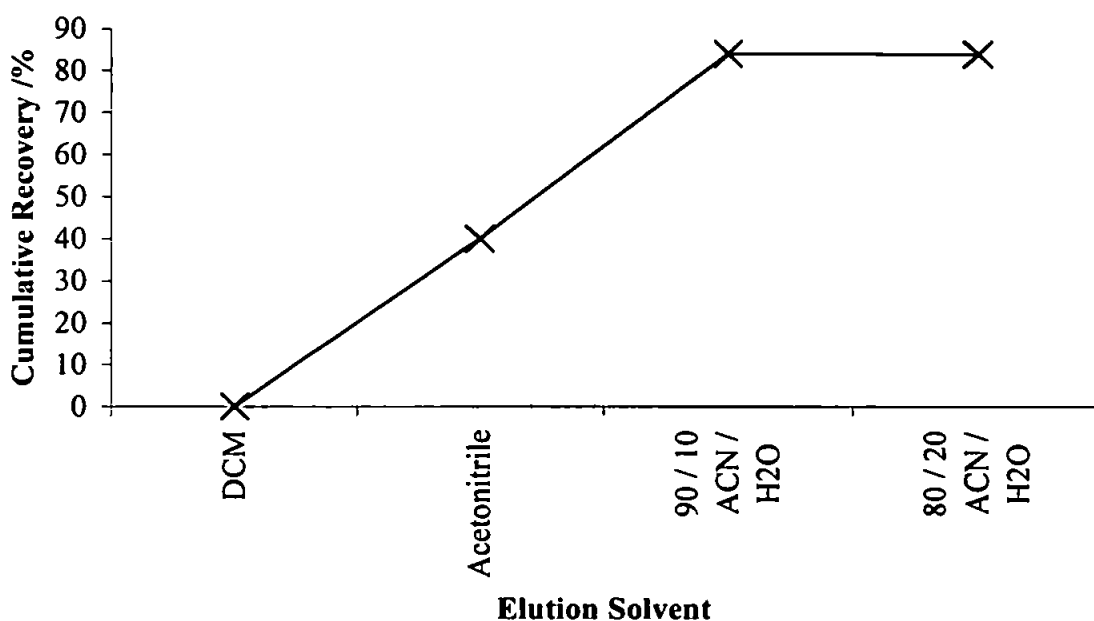


Figure 3.5: Elution profile from a 40 mg L⁻¹ dodecylamine sample in Swedish base fuel analysis by GC-NPD.

dodecylamine. This demonstrated that 90 / 10 acetonitrile / water was a suitable elution solvent for diesel samples as well as for heptane. The extraction procedure was performed in triplicate and reproducibility was shown to be good (RSD of 6.4 %, n=3). The reduced recovery was likely to be due to the viscosity of the diesel fuel, which has been reported to decrease analyte recovery (Porsch, 1994) by reducing the analyte retention. All subsequent diesel samples analysed were therefore diluted to 25 % in heptane to reduce the viscosity of samples prior to SPE.

3.3.3.3 Elution volume optimisation

In order to determine the optimum elution volume for the extraction of dodecylamine from heptane using 90 / 10 acetonitrile / water as the elution solvent, sequential 0.5 mL aliquots of 90 / 10 acetonitrile / water were passed through a column using the procedure described in Table 3.3. The resulting aliquots were analysed using the FI-CL manifold described in Chapter 2. Dilutions

of the aliquots were made when required to bring the concentration into the linear range of the FI-CL calibration. Recoveries were calculated by comparing peak heights with dodecylamine standards also prepared in 90 / 10 acetonitrile / water. A cumulative recovery graph for the sequential aliquots is shown in Fig. 3.6. 61 % of the dodecylamine eluted in the first 0.5 mL, and after 1.5 mL had passed through the column, 94 % of the dodecylamine had been eluted and further aliquots did not elute significantly more dodecylamine. 1.5 mL was therefore shown to be the ideal elution volume for dodecylamine using 90 / 10 acetonitrile / water.

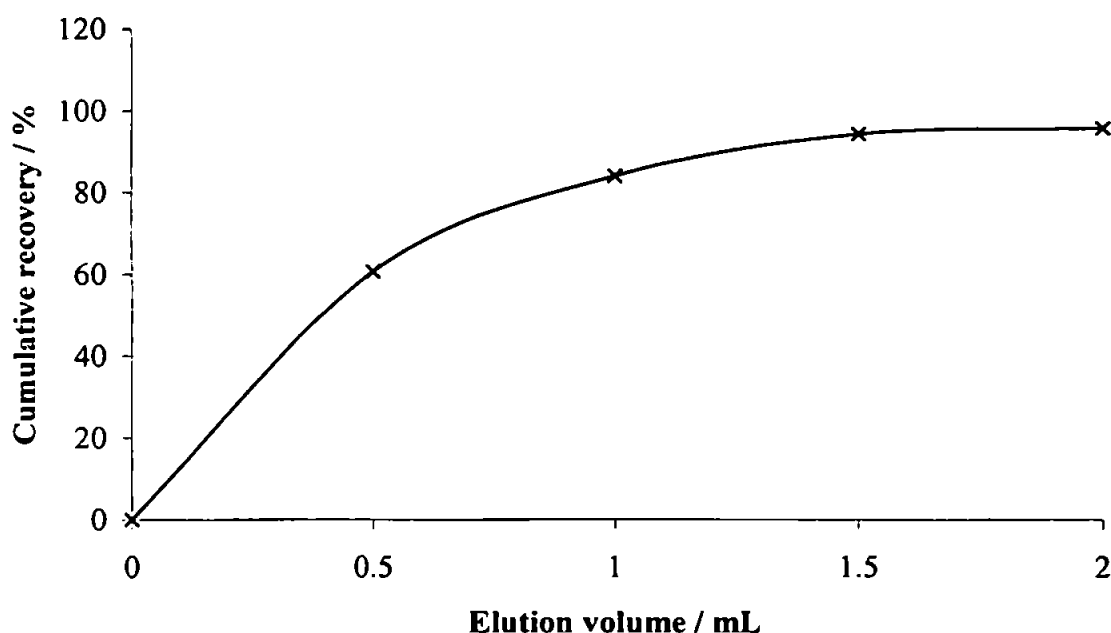


Figure 3.6: Elution profile for dodecylamine from a 200 mg aminopropyl SPE cartridge using 90 / 10 acetonitrile / water as the elution solvent.

3.3.4 GC-MS OF BASE FUEL EXTRACTS

In order to determine which compounds would be co-eluted with dodecylamine into 90 / 10 acetonitrile / water, the solid phase extraction procedure was carried out using only the base fuels. The chromatograms of the extracts from each base fuel are shown in Fig. 3.7. All of the peaks that could be identified are tabulated in Table 3.7.

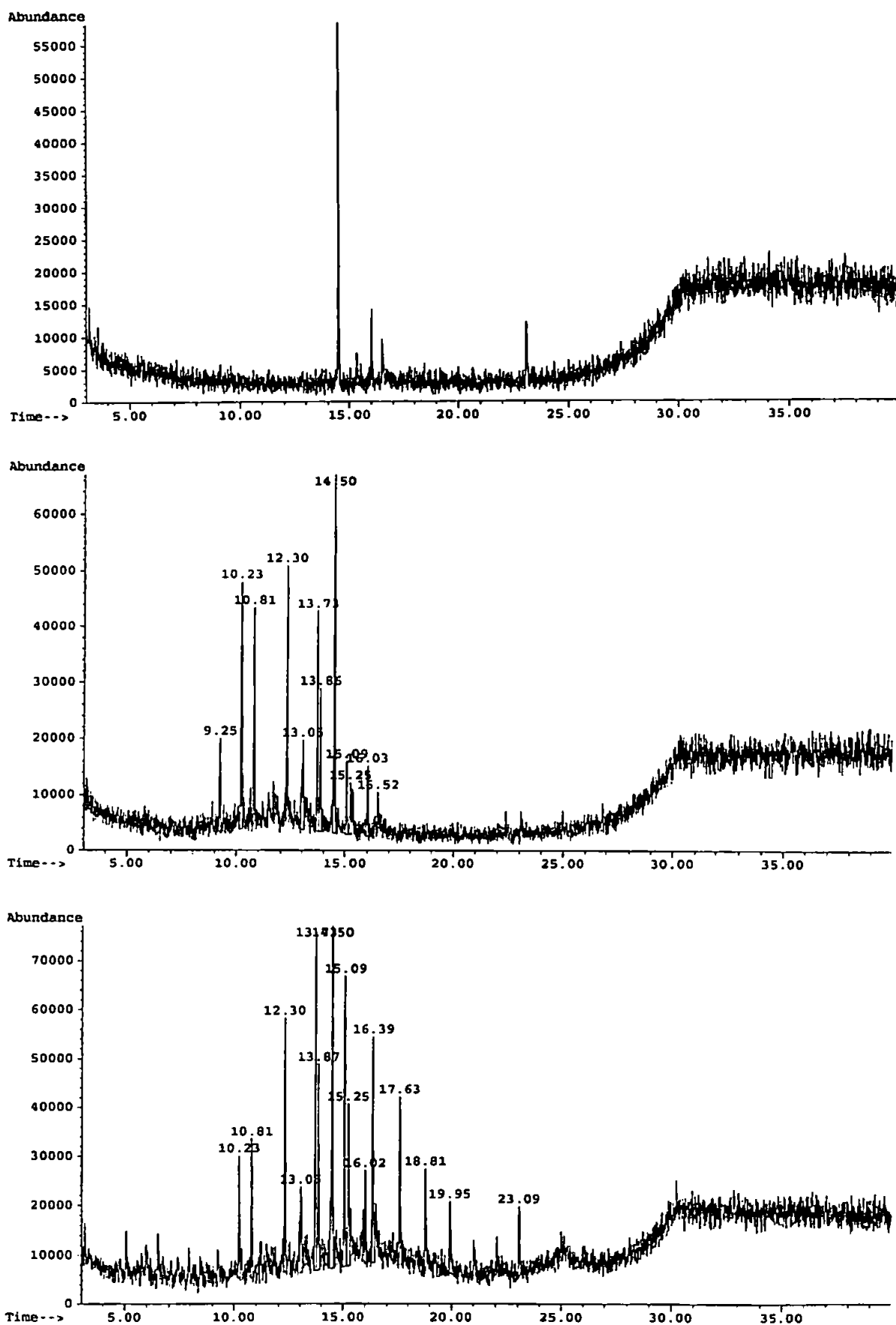


Figure 3.7: GC-MS chromatograms of extracts of the base fuels; (a) heptane, (b) Swedish and (c) Stanlow.

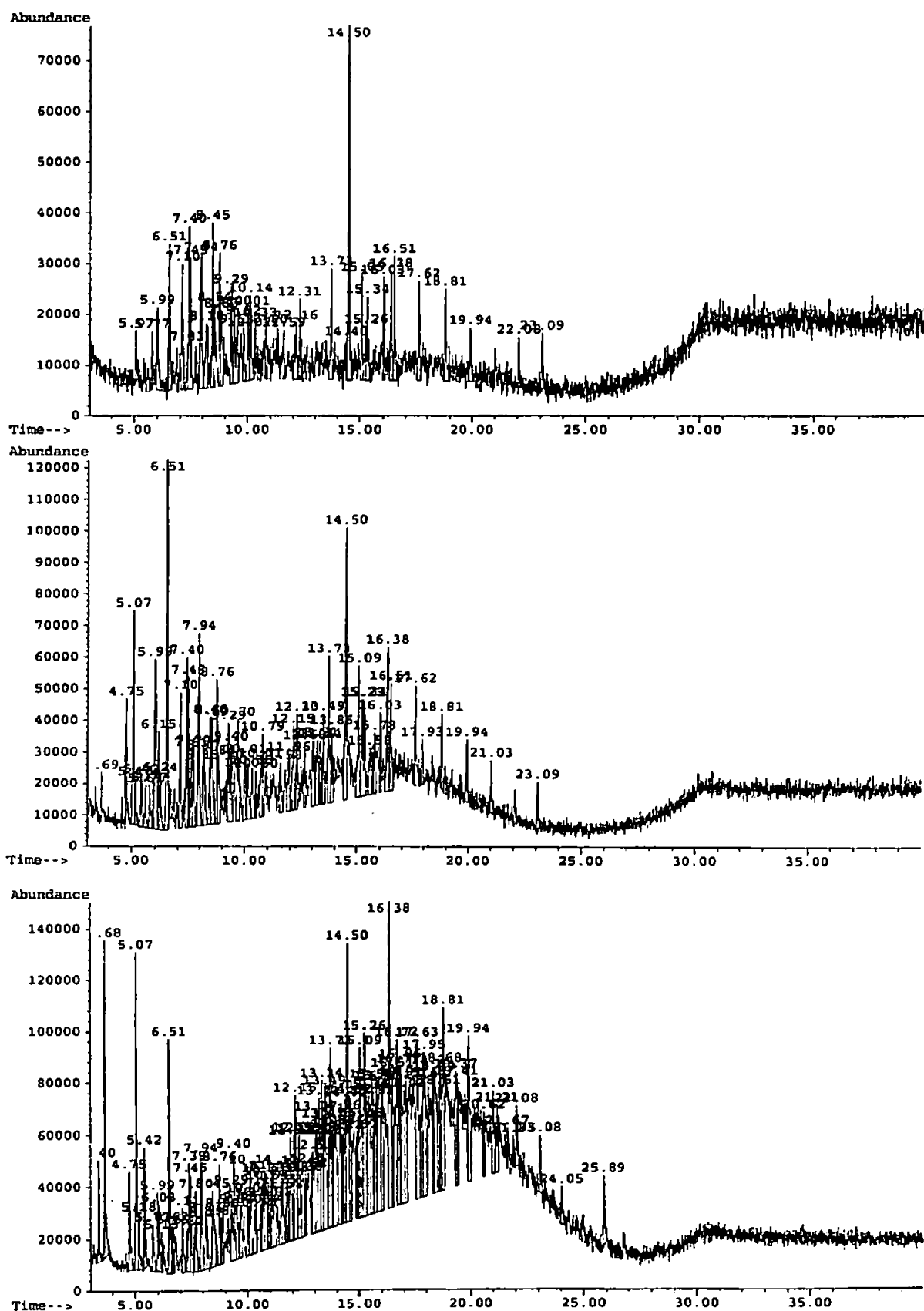


Figure 3.7: GC-MS chromatograms of extracts of the base fuels; (d) SNV, (e) Hamburg and (f) Brazilian.

Table 3.7: Identified compounds present in 90 / 10 acetonitrile / water extracts from base fuels(retention times in minutes are in brackets).

Swedish	Stanlow	Hamburg	SNV	Brazilian
undecane (9.3)	2,4-bis(1-methylethyl)-phenol (10.2)	3-methylphenol (3.9)	2,6-dimethylphenol (5.1)	4-methylphenol (3.7)
4- (1,1-dimethylethyl) –benzoic acid (10.2)	12-undecyl-pentacosane (10.8)	2,5-dimethylphenol (4.7)	3-(1-methylethyl)phenol (6.0)	2-methylphenol (3.4)
3-ethyl-2,7-dimethyloctane (10.8)	hexadecane (12.3)	3-ethylphenol (5.1)	2-ethyl-4-methylphenol (6.5)	2,5-dimethylphenol (5.1)
2,6,10,15-tetramethylheptadecane (12.3)	heptadecane (13.7)	2-ethyl-6-methylphenol (6.0)	2-ethyl-4,5-dimethylphenol (7.1)	3,4,5-trimethylphenol (6.5)
2,3-dimethylundecane (13.7)	pentadecane (13.9)	2,3,6-trimethylphenol (6.5)	4-(1-methylpropyl)phenol (7.4)	3-methyl-6-propylphenol (7.9)
5-ethyl-5-methyldecane (13.9)	octadecane (15.1)	2,3,5,6-tetramethylphenol (7.1)	2-ethyl-4,5-dimethylphenol (7.5)	1,2-diethyl-3,4-dimethylbenzene (10.1)
7, 9-dimethylhexadecane (15.1)	6-propyltridecane (15.3)	2-methyl-6-propylphenol (7.9)	1-(4-methoxyphenyl)-1-propanone (8.5)	4-cyclohexylphenol (12.1)
	9-octylheptadecane (16.4)	octacosane (12.3)	2,5-bis(1-methylethyl)phenol (10.1)	heptadecane (13.7)
	2-methylhexadecane (17.6)	2-methyl-tridecane (13.7)	5-methyl-pentadecane (12.3)	2-methylpentadecane (16.4)
	3-methylnonadecane(18.8)	2-methylhexadecane (15.1)	9-ocylheptadecane (13.7)	heptadecane (18.8)
		nonadecane (17.6)	Octadecane (15.1)	dodecane (19.9)
		4-ethyltetradecane (18.9)	4-methylheptadecane (17.6)	1-methoxy-3-pentylbenzene (25.9)

As expected, the chromatogram of the extract from heptane is very clean, showing that nothing is leaching from the column. The only peaks identifiable from the heptane chromatogram are plasticisers from the sample vial.

The only classes of compounds that were present in the base fuel extracts were hydrocarbons such as undecane and substituted phenols (and cresols). Phenols and cresols have been shown to produce a suppression of the sulphorhodamine 101 peroxyoxalate reaction (see Chapter 2) at relatively high concentrations ($>300 \text{ mg L}^{-1}$). The Brazilian base fuel extract contained the most components, with a characteristic diesel 'hump' still being present. However these compounds are unlikely to produce a significant interference at the concentration in which they are present in the extracts.

3.3.5 FI-CL ANALYSIS OF EXTRACTS OF SPIKED FUELS FOR THE DETECTION OF DODECYLAMINE

Samples of each base fuel and heptane (unspiked, spiked with 40 mg L^{-1} DDA and spiked with the additive package) were prepared and extracted into 90 / 10 acetonitrile / water using the procedure shown in Table 3.4. Each of the extracts was analysed using the FI-CL manifold described in Chapter 2. Each sample was extracted in triplicate (except the samples spiked with 40 mg L^{-1} which were only extracted once). The results of the FI-CL analysis are shown in Fig. 3.8. "FF" samples refer to fully formulated fuels (spiked with the additive package to produce a DDA concentration of 40 mg L^{-1}), while "40" samples refer to samples that have been spiked with 40 mg L^{-1} DDA without the additive package. "0" samples refer to base fuels containing no DDA or other additives.

All of the base fuels analysed gave a measured dodecylamine content of less than 6 mg L^{-1} , which is acceptably low for positive identification of the additive at 40 mg L^{-1} . Heptane had

no compounds present in the GC-MS chromatogram, so this enhancement of the CL response for heptane cannot be due to eluted interfering compounds. It is most likely due to small amounts of the wash solvent, dichloromethane, being present in the eluent. The suppression seen for Swedish, Hamburg and SNV base fuels is most likely due to the phenols and cresols that are co-eluted in the extracts. All of the fully formulated fuels gave measured DDA concentrations that were greater than 29 mg L⁻¹ with RSDs < 12% (n=3). It has been reported (Forbes, 2000) that sulphur compounds (present as sulphonic acids)

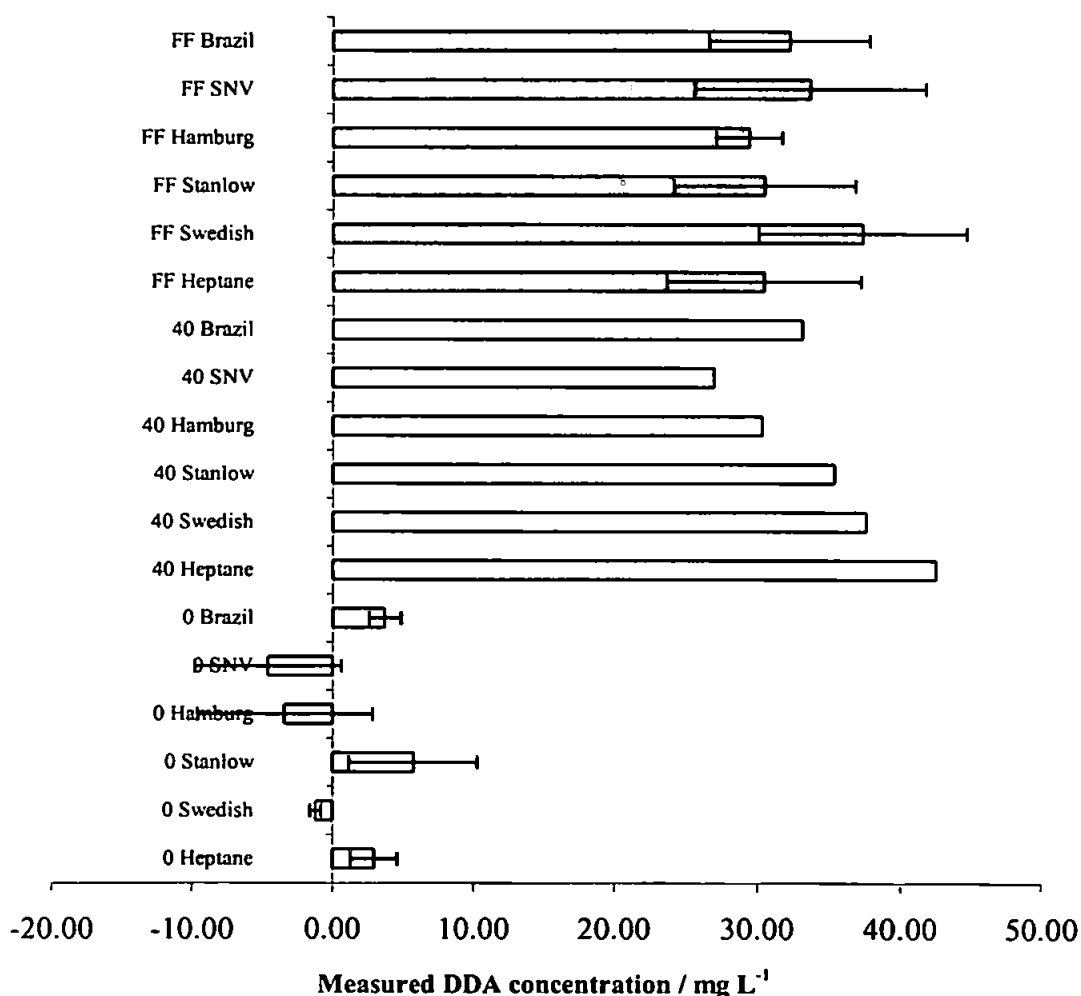


Figure 3.8: FI-CL results for the analysis of diesel fuel extracts (error bars represent 2s for the solid phase extraction (n=3)).

form dimers with dodecylamine. If the dodecylamine becomes bound up with the sulphur compounds then it will form neutral species that will not be retained on the SPE column,

therefore the fuels with the highest sulphur contents would be expected to have the lowest recoveries (see Table 3.5 for the sulphur content of the fuels analysed).

The fuels spiked with DDA show a different profile to those spiked with DDA in the additive package. This suggests that some components of the additive package are not being completely removed from the matrix by the procedure. Fuels containing dodecylamine however can clearly be distinguished from fuels without dodecylamine. In order to prove the results for the fully formulated fuels were significantly different from those of the base fuels, a *t*-test was performed (see Equations 3.1 and 3.2). A pooled estimate of the standard deviations was calculated (Equation 3.1) and the *t* value was then calculated using the pooled estimate (Equation 3.2)

$$s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \quad 3.1$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{1/n_1 + 1/n_2}} \quad 3.2$$

A table of the calculated *s* and *t* values are shown in Table 3.3. Each measurement was made in triplicate, so the number of degrees of freedom was (*n*₁+*n*₂ – 2) *i.e.* 3 + 3 – 2 = 4.

Table 3.8: Calculated *t*-values for the FI-CL results obtained for fully formulated fuels in comparison with their respective bases.

Fuel	Pooled estimate of the standard deviation (<i>s</i>) / mg L⁻¹	Calculated <i> t </i>
Fully formulated heptane	2.46	11.16
Fully formulated Swedish	2.58	14.94
Fully formulated Stanlow	2.76	8.97
Fully formulated Hamburg	2.35	13.99
Fully formulate SNV	3.40	11.26
Fully formulate Brazilian	2.03	14.03

The critical t value for this number of degrees of freedom is 4.3 (with 95 % confidence), and all of the calculated values were greater than this, therefore proving the results for the fully formulated fuels were significantly higher than those of the respective base fuels. This method can therefore be used to identify the presence or absence of this particular additive in diesel fuel. FI-CL with off-line SPE therefore represents a rapid, low cost approach to investigate fuel authenticity.

3.4 CONCLUSIONS

The specific conclusions of the work carried out in this chapter are as follows:

1. Dodecylamine could not be determined directly in diesel fuel samples using FI-CL without prior sample pre-treatment because the sample matrix and additive package components interfered with the chemiluminescence response.
2. Dodecylamine was quantitatively (> 92 %) extracted into 90 / 10 acetonitrile / water from a heptane matrix using an amino SPE cartridge. The optimum conditions were: conditioning with 5 mL of each of methanol, toluene and heptane, loading with 25 % sample in heptane, washing with 2 mL of each of heptane, toluene and dichloromethane and elution with 2.5 mL of 90 / 10 acetonitrile / water.
3. GC-MS analysis of solid phase extracts of base fuels showed the presence of phenols, cresols and hydrocarbons at low concentrations.
4. FI-CL analyses of five base fuel extracts (Swedish, Stanlow, Hamburg, SNV and Brazilian) gave CL responses less than that the equivalent of a 6 mg L⁻¹ DDA

sample. All five fully formulated fuels (containing 40 mg L⁻¹ DDA) gave a CL response equivalent to a measured DDA concentration of > 29 mg L⁻¹ (>72 % recovery) with RSDs < 12 %. A t-test proved that the results from all five of the fully formulated were significantly different to those of the base fuels (with 95 % confidence). This method is therefore suitable for determining the authenticity of fully formulated diesel fuels that should contain DDA in the additive package.

Chapter 4

Design And Performance Of An Automated FI-CL System With On-Line SPE

4 DESIGN AND PERFORMANCE OF AN AUTOMATED FI-CL SYSTEM WITH ON-LINE SPE

4.1 INTRODUCTION

Automation of chemical procedures has many desirable advantages. For example, analysis times can be reduced, precision can be improved, operator intervention can be minimised and results can be made available in real time. This chapter describes the design and construction of both a manual and a fully automated on-line SPE-FI-CL manifold for the determination of dodecylamine in diesel fuels. The objective was to design a prototype system that could be deployed in the field (*e.g.* at a filling station). Results for the determination of dodecylamine from a variety of diesel fuels using the on-line system are presented and compared with results obtained using off-line SPE with FI-CL detection (as described in Chapter 3).

4.2 EXPERIMENTAL

4.2.1 REAGENTS AND SAMPLES

All chemiluminescence reagents were prepared as described in Section 2.2.1. Diesel samples were prepared as described in Section 3.1.1.

4.2.2 INSTRUMENTATION

4.2.2.1 *Manual on-line-SPE-FI-CL manifold*

The manifold developed for the manual on-line SPE-FI-CL procedure is shown in Fig. 4.1. Both the sample and reagent pumps used were Minipuls 3 (Gilson, Villiers-le-Bel, France) peristaltic pumps. Ismaprene pump tubing (Ismatec) was used to propel all reagents and

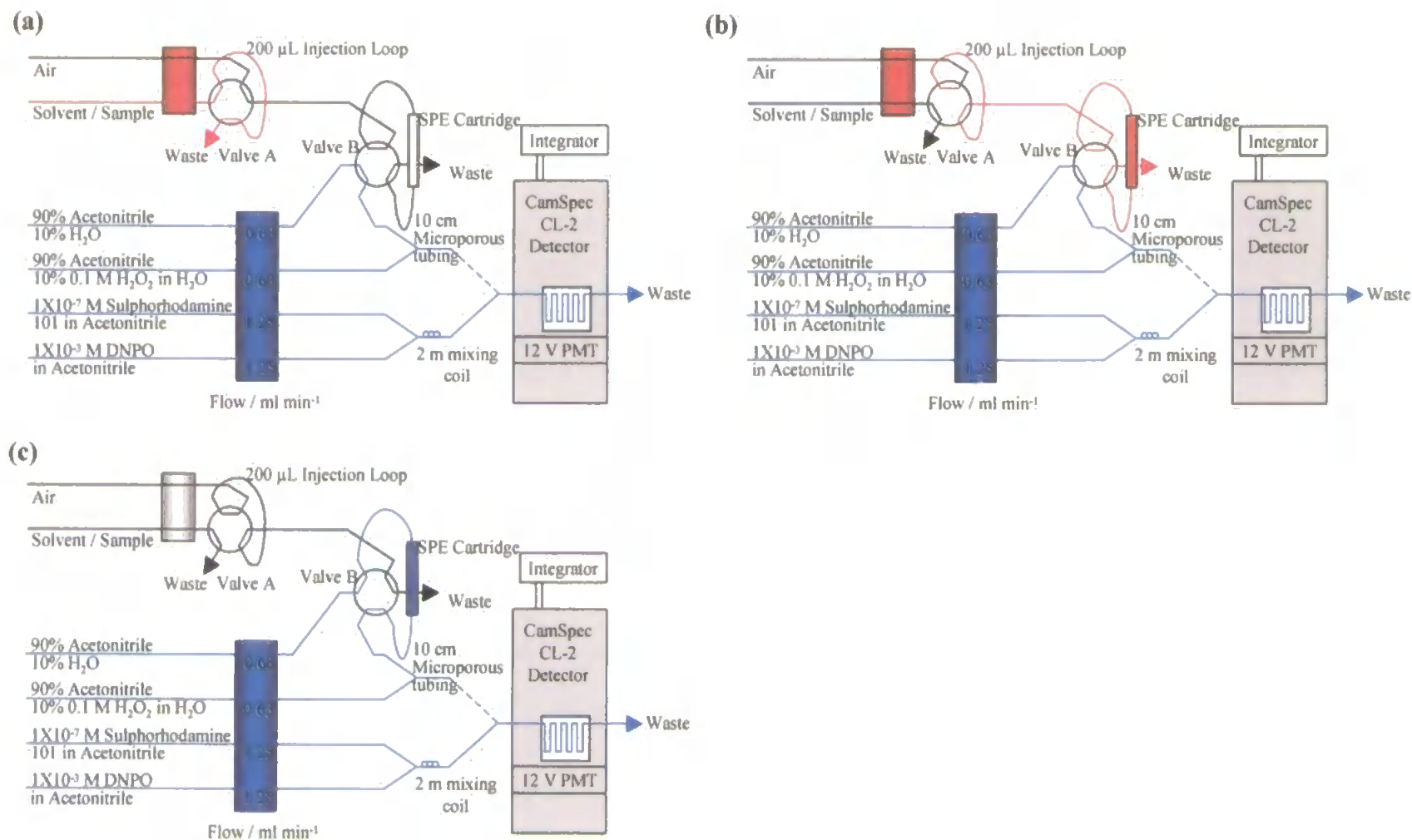


Figure 4.1: A schematic diagram of the manual SPE-FI-CL manifold: (a) measuring conditioning / loading / washing solvent volume, (b) conditioning / loading / washing and (c) eluting from the column.

carrier streams (0.5 mm i.d. for peroxide / carrier, and 1.0 mm i.d. for sulphorhodamine 101 and DNPO lines). 0.75 mm i.d. (PTFE) manifold tubing and T-pieces (Omnifit, Cambridge, U.K.) were used to merge reagent streams. Microporous tubing (W.I. Gore Ltd) was used to remove air bubbles from the dead volume of the SPE column. The solid phase extraction was achieved using a self packed Omnifit chromatography column (length 50 mm; diameter 5 mm). The column was packed with 50 mg of Isolute aminopropyl solid phase extraction sorbent (International Sorbent Technology, Mid Glamorgan, U.K.). The SPE column was attached to a Rheodyne two position, six port sample injection valve [5020] (Rheodyne, Bensheim, Germany) and another valve of the same type was used as a sample injection valve. Chemiluminescence emission was measured using a CamSpec CL-2 detector (Cambridge Instruments, Cambridge, U.K.) containing a low power, 12 V side window Hamamatsu photomultiplier tube with a quartz flow cell (length of 12 cm, with a volume of 120 μ L). The signal from the detector was acquired using a multifunctional DAQ-700 data acquisition / digital I/O card (National Instruments, Berkshire, U.K.) attached to a laptop computer. Peaks were integrated using self written analysis software (see Analysis2.VI details in Fig. 4.27).

4.2.2.2 *Automated on-line SPE-FI-CL manifold*

Reagent and SPE solvent pumps were Minipuls 3 (Gilson, Villiers-le-Bel, France) peristaltic pumps and a 12 V peristaltic pump [MS/CA4-E/8-100C] (Ismatec, Weston-super-Mare, U.K.) was used as a sample pump. Ismaprene pump tubing (Ismatec) was used to propel all reagents and carrier streams. 0.75 mm i.d. poly(tetrafluoroethylene) manifold tubing (Fisher Scientific, Loughborough, U.K.) was used, and T-pieces (Omnifit Limited, Cambridge, U.K.) were used to merge reagent streams. The autosampler was a 5 port, 4 position electric rotary valve (11526) purchased from Omnifit Limited (Cambridge, U.K.). The sample injection valve (6 port, 2 position [C22-3716EH]), SPE solvent selector valve

(11 port, 10 position [C25-3710EMH]), and column selector valve (10 port 2 position [C22-3716EH]) were Cheminert microactuated valves (Valco Instruments, Switzerland). On-line solid phase extraction columns comprised of 5 mm i.d. chromatography columns (Omnifit Limited, Cambridge, U.K.) packed with 50 mg of Isolute aminopropyl SPE sorbent (International Sorbent Technology). All pumps and valves were switched using TTL logic controlled with the DAQ-700 card. LabVIEW™ software (National Instruments) was used to provide a graphic user interface for the data acquisition and TTL input/output as described in Section 4.3.2. For a schematic diagram and a description of the system see Section 4.2.3.2.

4.2.2.3 *Electronics*

A photograph of the internal wiring for the automated system is shown in Fig. 4.2, and an electronic circuit diagram is shown in Fig. 4.3.

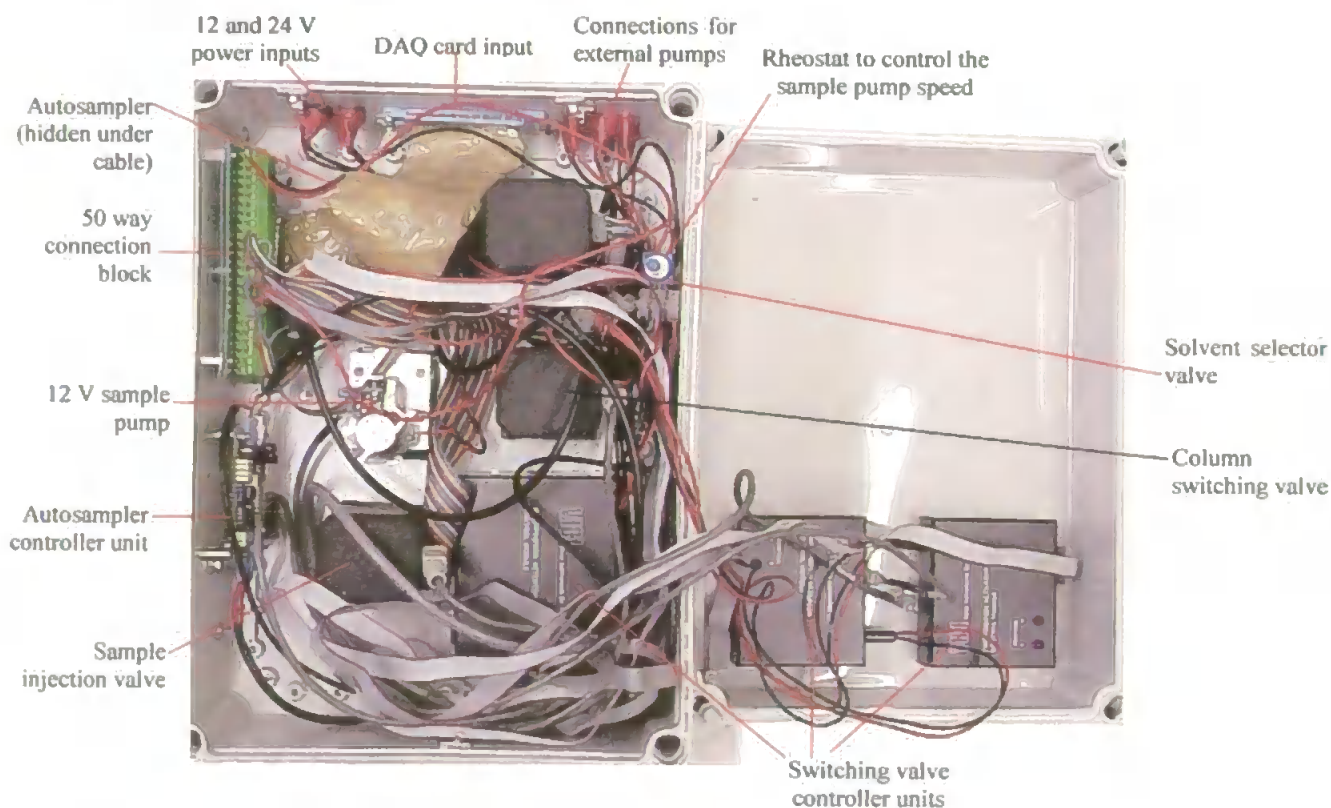


Figure 4.2: Interior of the on-line SPE-FI-CL system.

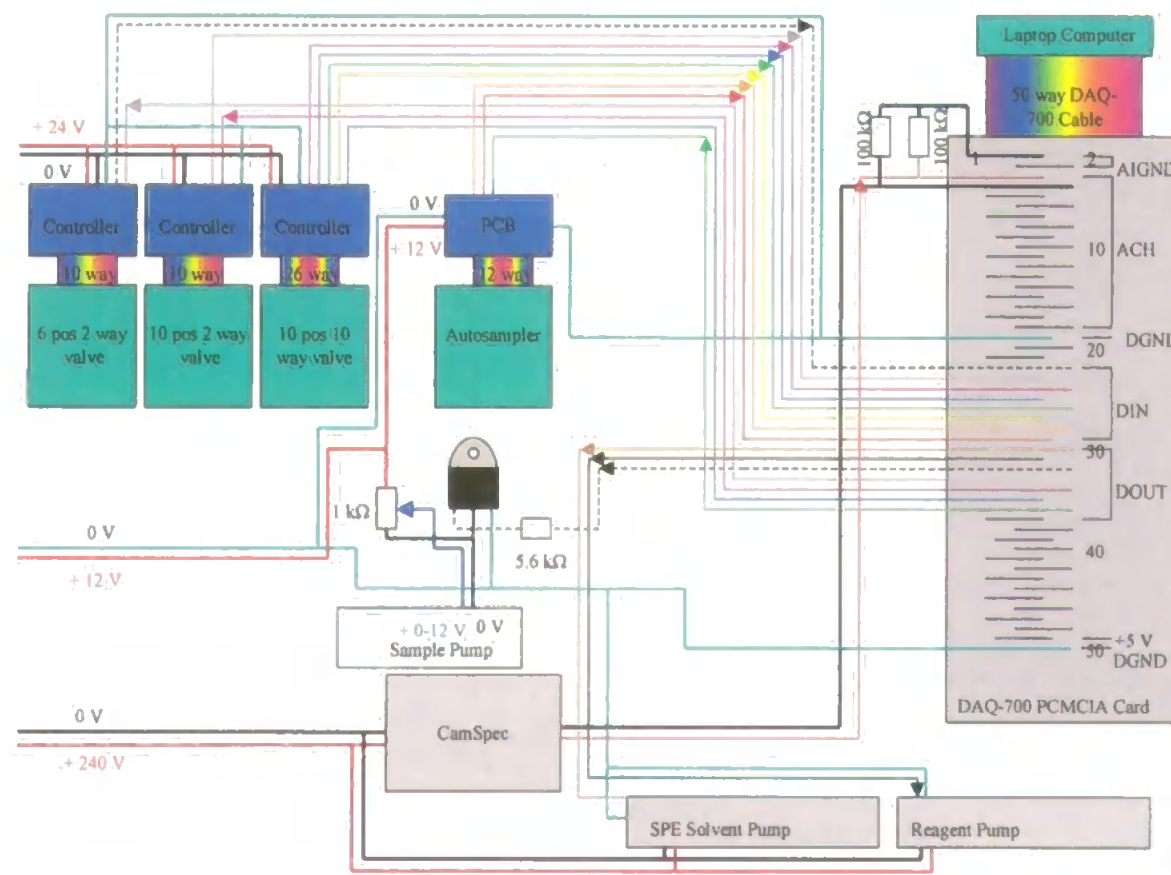


Figure 4.3: Electronics diagram for the automated on-line SPE-FI-CL diagram. Thin lines represent 5 V TTL lines. Thick lines represent power lines (either 12, 24, or 240 V), where AIGND is analogue input ground, ACH is analogue channel, DGND is digital ground, DIN is digital input and DOUT is digital output).

All devices (except the sample pump) were controlled directly using 5 V, 1 mA TTL lines on the DAQ card. The sample pump, however, was not TTL compatible, therefore a Darlington pair transistor (TIP141) was used in order to draw current from an external power source, in order to enable control using TTL logic (see Fig. 4.4) . A rheostat was also introduced in order to allow the speed of the pump to be controlled.

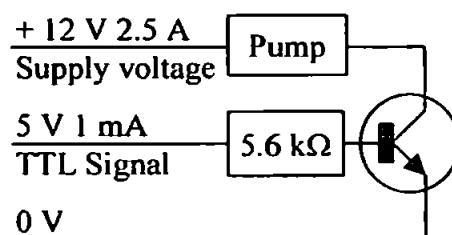


Figure 4.4: Circuit for a Darlington pair transistor to control the sample pump using TTL logic.

4.2.3 PROCEDURES

4.2.3.1 *Manual on-line SPE-FI-CL procedure*

A schematic diagram of the manual SPE-FI-CL manifold designed is shown in Fig. 4.1.

All chemiluminescence reagents were pumped continuously.

Conditioning / Washing

Valve A is used to measure the required volume of conditioning solvent being pumped by pump 1. Valve A is then switched and an air stream attached to pump 1 is used to propel the solvent through the SPE column attached to valve B. This procedure is repeated for all the conditioning / washing solvents.

Loading

The sample (diluted to 25% with heptane) is loaded in the same way as the conditioning solvent. This process is repeated 4 times in order to preconcentrate the required amount of sample onto the column.

Elution

When valve B is switched the carrier stream (90 / 10 acetonitrile / water) travels through the SPE column to elute the desired analyte. 10 cm of microporous tubing is used to prevent the air bubble that is present in the injection valve from entering the detector.

Table 4.1: Manual on-line solid phase extraction parameters.

Conditioning	Washing
2 X 200 μ L methanol	200 μ L heptane
2 X 200 μ L toluene	200 μ L toluene
2 X 200 μ L heptane	200 μ L dichloromethane
Loading	Elution
4 x 200 μ L (25 % dodecylamine in sample / 75 % heptane)	10% water / 90% acetonitrile

4.2.3.2 *Automated on-line SPE-FI-CL procedure*

A schematic diagram of the four steps involved in the automated on-line SPE-FI-CL is shown in Fig. 4.5. A description of each step in the automated on-line SPE-FI-CL procedure is given below.

Step 1: Sample loop filling / column 1 conditioning / column 2 eluting

Initially the autosampler is switched to the position of the sample to be analysed and the sample injection valve is set to the load position. The sample pump is then activated to fill the sample loop. When the loop is completely filled with sample, the pump is switched off. Whilst this is happening, each of the conditioning solvents is pumped through the solvent selector valve for a predefined length of time in order to condition the SPE sorbent. Column 2 is being eluted in parallel with column 1 conditioning in order to reduce the time required to perform a series of analyses

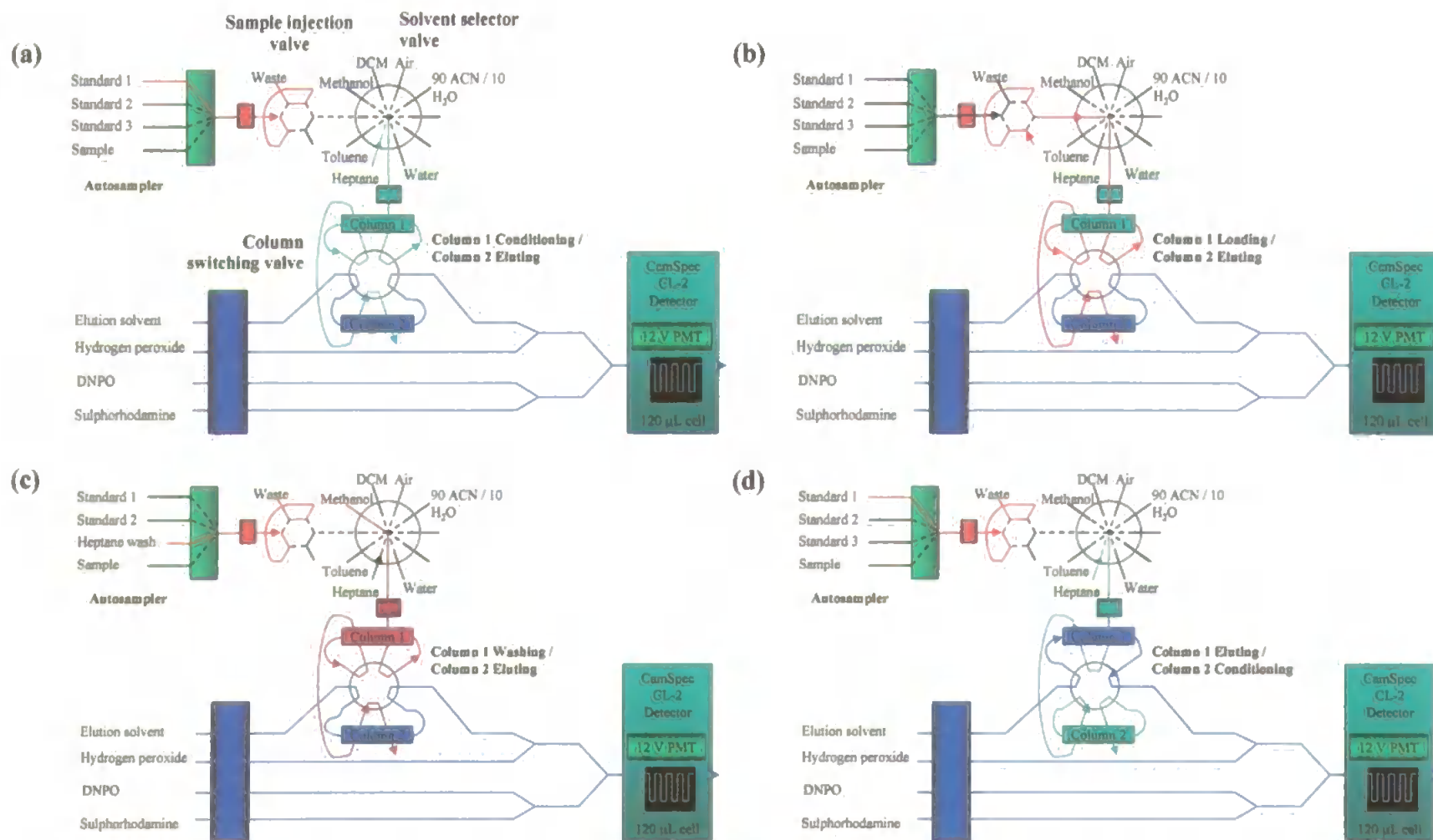


Figure 4.5: Four steps involved in the automated on-line SPE-FI-CL manifold. (a) Sample loop is filled with sample whilst column 1 is being conditioned, and column 2 is eluting. (b) Column 1 is loaded with sample whilst column 2 is eluting. (c) Column 1 is washed whilst column 2 is eluting. (d) Sample loop is filled with sample whilst column 2 is conditioned and column 1 is eluting. Sample pump is shown in red, SPE solvent pump in green, and reagent pump in blue.

Step 2: Column 1 loading / column 2 eluting

The solvent selector valve is switched to the sample position and the SPE solvent pump is activated forcing the sample in the sample loop through column 1. As with step 1, column 2 is still eluting.

Step 3: Column 1 washing / column 2 eluting

Each of the wash solvents is pumped through column 1 for a predefined length of time using the solvent pump. Whilst this is happening the sample loop is being washed with heptane.

Step 4: Column 1 eluting / column 2 conditioning

The column switching valve is now switched, resulting in column 1 undergoing elution, and column 2 commencing its conditioning, loading and washing cycle. The timing of each of these steps is shown in Table 4.2.

Table 4.2: Timing of the automated SPE events.

Time / s	Process
0	Switch SPE solvent selector valve to position 10 (water) Switch autosampler to position 1 (heptane) Switch sample injection valve from inject to load Turn on sample pump Turn on SPE solvent pump
18	Turn off sample pump Switch autosampler to desired sample position Turn on sample pump
34	Turn off sample pump Switch sample injection valve from load to inject
60	Switch SPE solvent selector valve to position 1 (methanol)
120	Switch SPE solvent selector valve to position 2 (toluene)
132	Switch SPE solvent selector valve to position 3 (heptane)
144	Switch SPE solvent selector valve to position 4 (sample)
189	Turn off SPE solvent pump Switch SPE solvent selector valve to position 3 (heptane) Turn on SPE solvent pump Switch sample injection valve from inject to load
204	Turn off SPE solvent pump Switch SPE solvent selector valve to position 2 (toluene) Turn on SPE solvent pump
219	Turn off SPE solvent pump Switch SPE solvent selector valve to position 5 (dichloromethane) Turn on SPE solvent pump
234	Switch SPE solvent selector valve to position 6 (air)
294	Switch column selector valve Turn off SPE solvent pump
Repeat all steps for the next sample	

4.3 RESULTS AND DISCUSSION

4.3.1 ON-LINE SOLID PHASE EXTRACTION

On-line solid phase extraction has many advantages over off-line SPE. The primary reason for incorporating SPE on-line is the ability to fully automate the analytical process. Automated off-line systems exist, however automation is generally achieved by the use of robots (Hoffman et al., 1996) which are very expensive and not readily portable. Another advantage of on-line SPE is that the entire extract is analysed, whereas with off-line SPE a sub-sample of the extract is taken for analysis. The sensitivity of analysis will be greater if the entire extract is used. With an automated on-line SPE system, small volumes can be measured out very accurately. An SPE procedure can therefore be scaled down by using a smaller sorbent bed and less solvent, thus reducing the cost of extraction and reducing the amount of waste that is generated. Flow injection with on-line solid phase extraction has been applied to various detection systems, including the analysis of pseudoephedrine in aqueous samples using capillary electrophoresis (Chen and Fang, 1997), the determination of lead in water samples by flame atomic absorption spectrometry (Naghmush et al., 1995; Sooksamiti et al., 1996), the detection of phenols in waters and waste waters using spectrophotometric detection (Song et al., 1997), Fourier transform infrared determination of caffeine in soft drinks (Daghbouche et al., 1997) and the determination of bromofenoxim in water samples using electrochemical detection (Svegl et al., 1996). All of these techniques using flow injection with on-line solid phase extraction have been applied to relatively simple aqueous matrices. As yet there are no publications describing flow injection with on-line SPE being applied to more complex organic matrices such as diesel fuels.

4.3.2 CONSTRUCTION OF A MANUAL SPE-FI-CL MANIFOLD FOR THE DETECTION OF DODECYLAMINE IN AN ORGANIC MATRIX

In order to determine whether flow injection with on-line SPE was a viable possibility for the extraction of dodecylamine from non-aqueous matrices, a manual on-line SPE system was constructed as shown in Fig. 4.1. In terms of chemiluminescence chemistry, the manifold was essentially the same as that used in Chapter 2. However in this case the sample loop in the 6 port injection valve was replaced with a self packed SPE column containing 50 mg of aminopropyl SPE sorbent. A second injection valve was used to measure precise volumes of SPE solvent as described in Section 4.2.2.1. Table 4.2 describes the solid phase extraction procedure used to extract dodecylamine from heptane. Peaks generated from the manual on-line SPE-FI-CL system are shown in Fig 4.6 and a calibration graph is shown in Fig 4.7. As with off-line solid phase extraction with FI-CL detection, heptane blanks gave a small positive CL response. This is again presumably caused by small amounts of the wash solvents present in the eluent. However, since all samples and standards are extracted using the same procedure, this systematic error has no

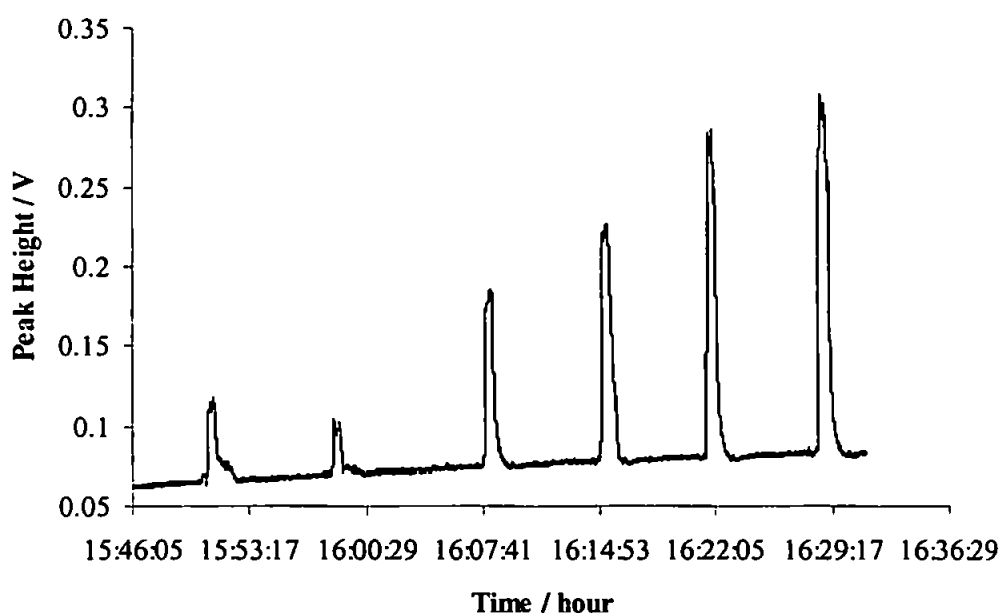


Figure 4.6: Example peaks for manual on-line SPE-FI-CL manifold (left to right = 10, 0, 20, 30, 40, 50 mg L⁻¹ DDA in heptane).

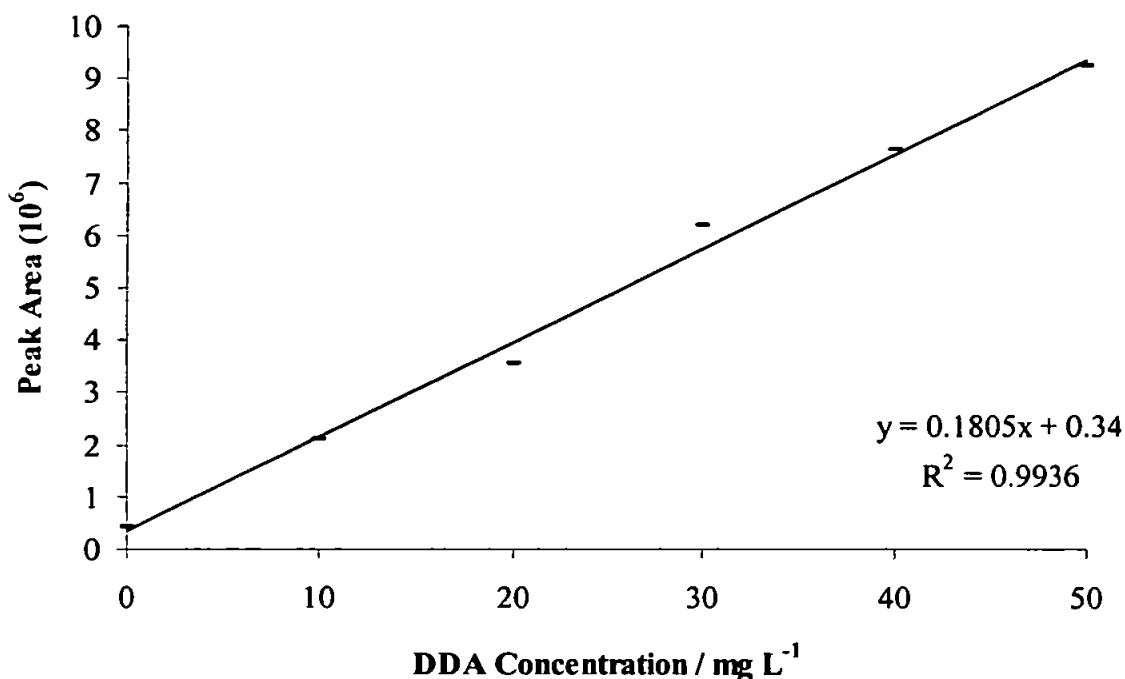


Figure 4.7: Calibration graph for the manual on-line SPE-FI-CL manifold (DDA samples were prepared in heptane).

effect on quantification. The peak shapes obtained were unusual (see Fig 4.6). This is due to channelling of the sorbent resulting in multiple paths for the eluent to pass through. For this reason peak area, and not peak height was used for the response. A good linear response was obtained ($R^2 = 0.994$) for dodecylamine samples in heptane in the range 0 – 50 mg L⁻¹. The reproducibility was also good (RSD of 8.5% (n=3) for a 40 mg L⁻¹ sample). Each sample required 7.5 minutes to undergo extraction and analysis, therefore 8 samples h⁻¹ could be analysed.

The complexity of the manual system demanded considerable care on the part of the operator. A large number of time critical operations were needed to complete each extraction and an error in any one of them resulted in the entire extraction having to be repeated. A fully automated system was therefore designed and constructed to eliminate such demands and potential errors.

4.3.3 DESIGN OF THE AUTOMATED SPE-FI-CL MANIFOLD

A manifold diagram for the automated on-line system is shown in Fig. 4.5 and photographs of the manifold and the entire system are shown in Figs. 4.8 and 4.9. When designing the automated on-line system, two columns were incorporated in a ten port valve instead of the single column in a six port valve that was used in the manual system. This had the advantage that one column could be eluting whilst the other was loading, thus doubling the rate of sample throughput.

As previously stated, automation allows very accurate and precise volumes to be pumped. For this reason it was decided that instead of measuring the volumes of conditioning and washing solvents using the sample loop (as had been done for the manual system), the conditioning and washing solvent were pumped for a known length of time that was calibrated for the desired volume of solvent, *i.e.* time based rather than volume introduction of solid phase extraction solvents.

In the manual system the samples were eluted by forward flushing the column, however, when this was tried using the automated system with real diesel samples, the columns were found to be insufficiently clean after each extraction procedure. An example of this is shown in Fig. 4.10. The baseline was observed to increase after each injection and the analyte peak therefore was masked by components from previous injections being eluted together with the dodecylamine. To solve this, the system was reconfigured so that the columns were backflushed during elution. An aqueous conditioning step was also introduced to ensure as much of the diesel matrix was removed between each injection as possible. Example peaks incorporating backflushing and the aqueous conditioning step are shown in Fig 4.11. A steady baseline was obtained and regular, reproducible peak shapes (and areas) resulted.

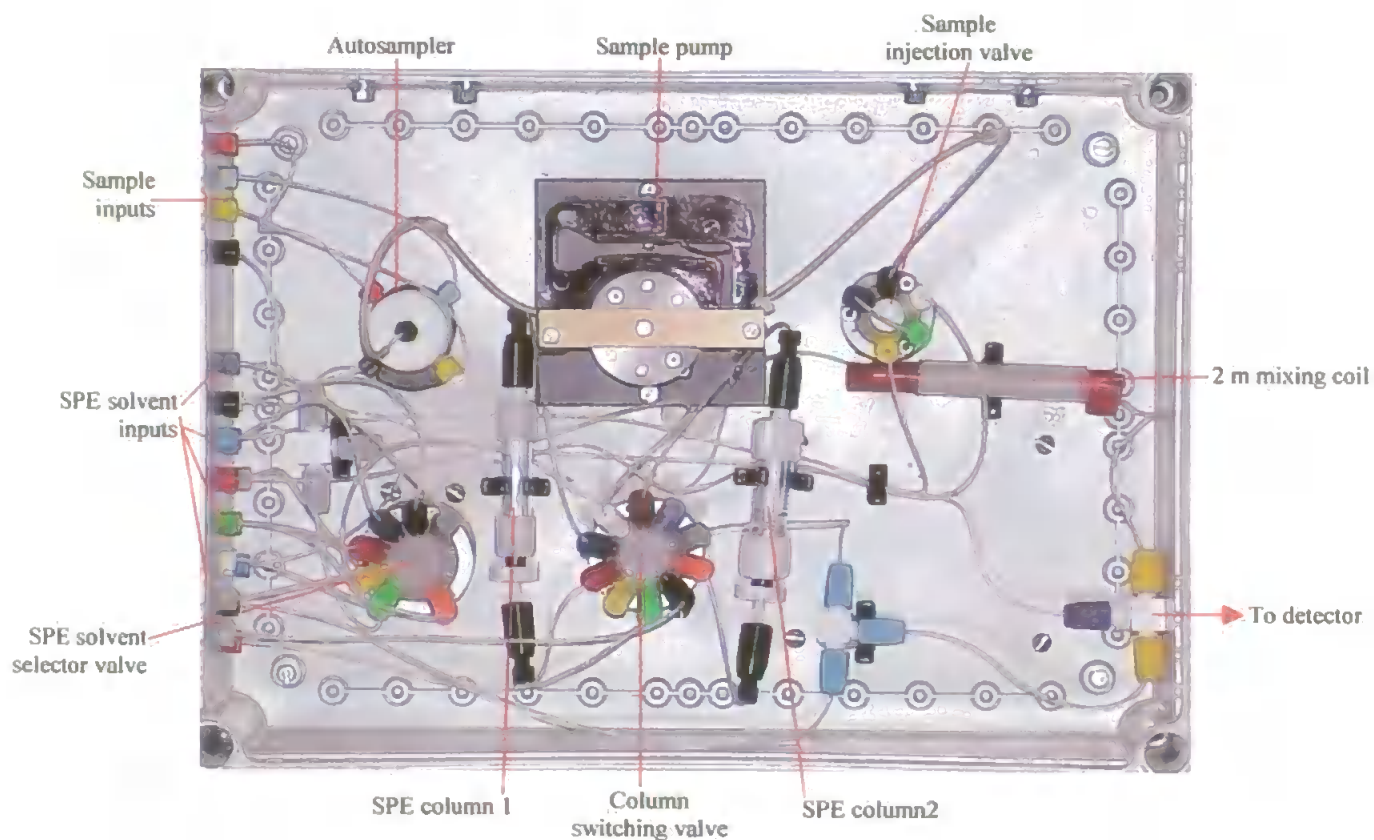


Figure 4.8: A photograph of the on-line SPE-FI-CL Manifold.



Figure 4.9: A photograph of entire on-line SPE-FI-CL monitor.

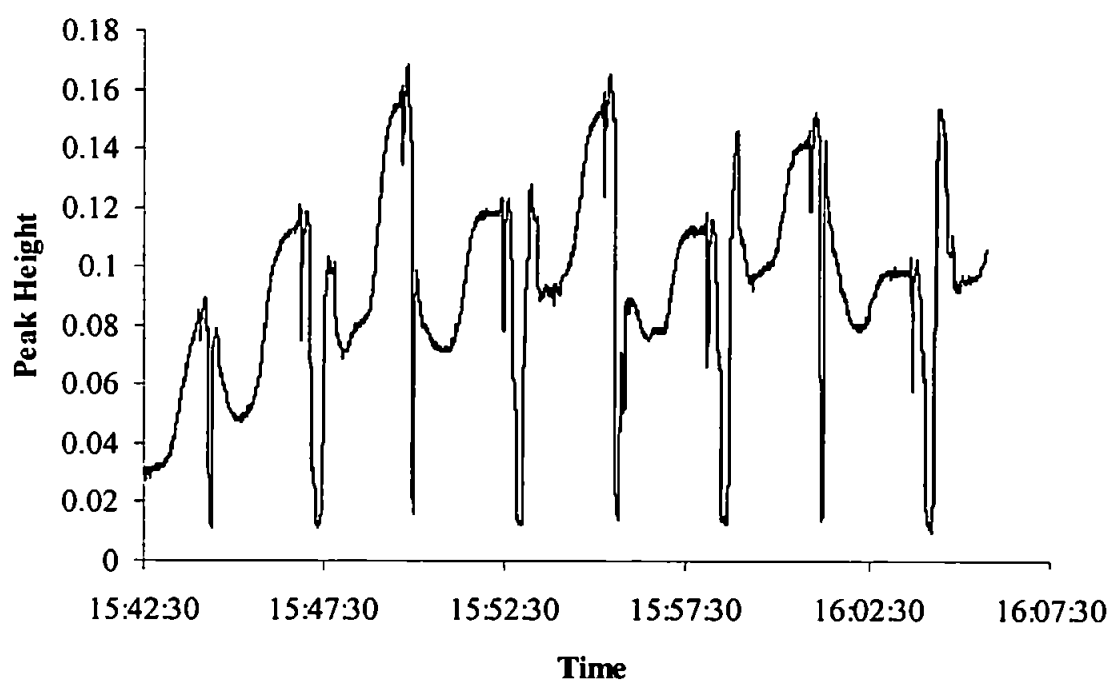


Figure 4.10: Brazilian diesel fuel peaks with columns forward flushing and no aqueous column conditioning.

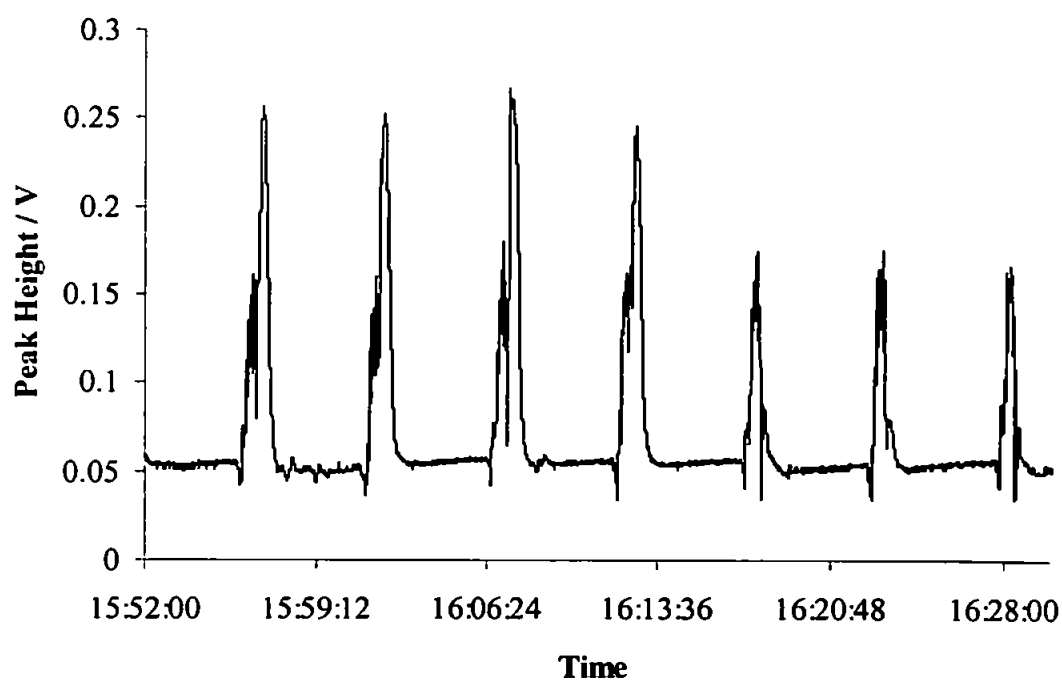


Figure 4.11: Brazilian diesel fuel peaks with back flushing.

After the column had been loaded and washed, air was pumped through the column in order to remove as much of the wash solvent from the column as possible. When the elution solvent was pumped through the column the air was pushed out and travelled through the detector, resulting in a disturbance in the CL response prior to each sample being detected as shown in Fig 4.12. This air bubble disruption was however the same size for each sample and could therefore be distinguished from the analyte peak by ignoring the first 67.5 seconds of the sample elution during data acquisition.

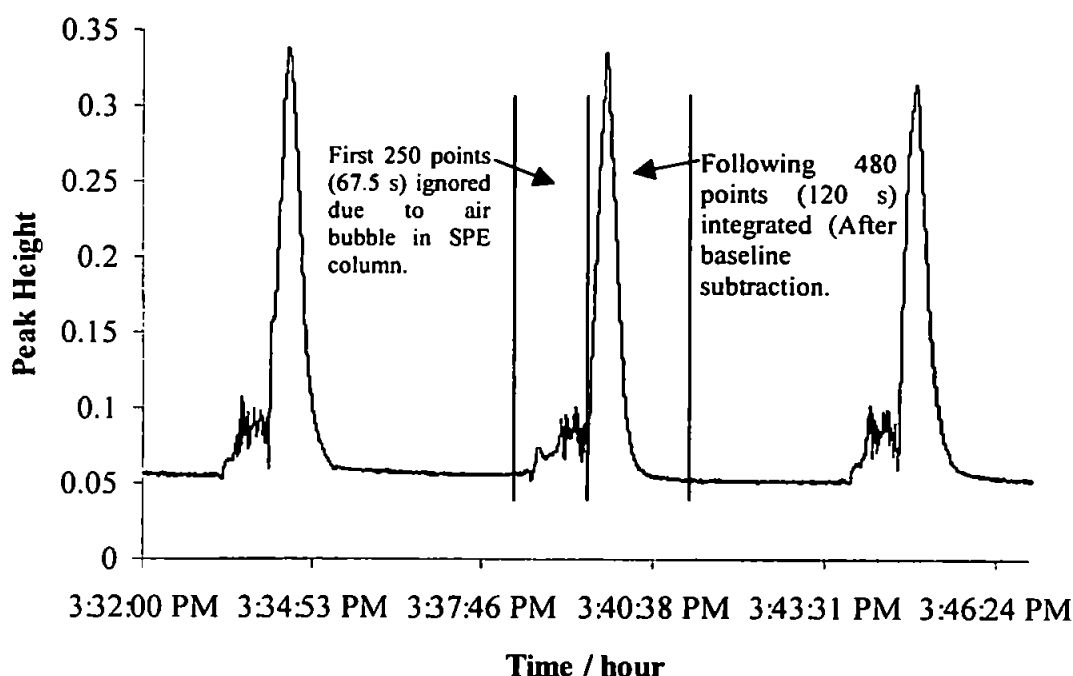


Figure 4.12: Typical peaks from the automated on-line SPE-FI-CL system.

In order to automate the system a complex programme was required to control the timing of each switching event and to acquire data. LabVIEW was chosen for the reasons discussed below.

4.3.3.1 *LabVIEW overview*

LabVIEW graphical programming language is similar to other high level windows programming environments such as BASIC or Pascal, but with one major difference in that LabVIEW has no written text-based code. All programs are created in block diagram form in the graphical language G. A program written in LabVIEW is called a virtual instrument (VI), and may contain many sub-VIs which are analogous to procedures in BASIC or Pascal.

LabVIEW VIs have two parts, the first of which is the front panel, which is the interactive part of the program. Virtual controls, switches and indicators are displayed on the front panel, which should mimic actual controls on a real instrument. The second part of a VI is the block diagram which contains the underlying program structure. Controls, indicators and sub-VIs are all linked together using “wires” to indicate data flow within the program.

Much like a traditional programming language, LabVIEW contains libraries and examples for all simple tasks, such as basic mathematics or file input / output. LabVIEW, however, also includes extensive libraries for more complicated tasks such as data acquisition, serial communication and data analysis. Amongst the data analysis libraries included in LabVIEW are some useful scientific analysis tools, for example, fast Fourier transforms, signal smoothing, curve fitting, graph drawing and peak recognition. LabVIEW also has the advantage of being able to be run on a number of different platforms, meaning that programmes written in the Windows version of LabVIEW should be transferable to the DOS or Macintosh versions.

LabVIEW's wide functionality has resulted in it becoming widely used in analytical chemistry. The most common use has been for data acquisition and logging, which is a

very complex task in a traditional programming language such as C or Pascal. LabVIEW has built in code for connection to a wide range of external interfaces such as serial, parallel, GPIB and national instrument DAQ interfaces (Borer et al., 1992; Krauss et al., 1999). Data has been acquired from a range of analytical instruments with LabVIEW including NMR (Belmonte et al., 1998), near infrared spectrophotometers (Claps et al., 2001), GC-MS and flame AAS (Burden and Petzold, 1999; Zhou et al., 1998). LabVIEW can also output to all of the different types of interface, meaning instrument control and automation can become simple tasks. Flow injection is very amenable to automation, and a number of LabVIEW automated FI systems have been developed. One such system involved the control of on-line ultrasonic filtration with charge coupled device (CCD) detection (Wang et al., 2000). Automated FI systems with UV detection have been developed for both nitrate (Coles et al., 2000) and phosphate (Hanrahan et al., 2001) monitoring in rivers.

Another useful feature of LabVIEW available to the analytical chemist is image recognition. Image recognition has been used to provide non-subjective colorimetric analysis of 96-well microplates using a CCD camera to acquire the images for analysis (Byrne et al., 2000).

Mathematical algorithms that are embedded into LabVIEW help to make programming more simple. The fast Hartley transform has been used for the deconvolution of analytical peaks in electroanalytical data (Economou et al., 1996). Multivariate curve resolution in LabVIEW has been applied to the identification of explosives with ion mobility spectrometry (Buxton and Harrington, 2001).

4.3.3.2

VI Programming Glossary



Controls correspond to front panel objects (e.g. switches) that can be altered on the front panel.



Indicators do not have the thick surround. They are used to display the values of variables.



Thick green data lines contain path information for file input / output.



Signed integers are denoted by I followed by the number of bits contained in the variable. An 8 bit variable can have any value between -128 and +127.



Unsigned integers do not have any magnitude. An 8 bit variable would therefore contain values between 0 and 255.



Boolean variables can only have two values. They are either true or false.



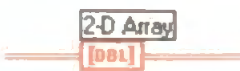
Single or double variables are floating point numbers, and must be used if a variable is not an integer.



Strings contain alpha-numeric information that cannot be stored in numeric variables, for example file names.



One dimensional arrays are denoted by thick lines. Multi-dimensional arrays are denoted by two thin lines.



Arrays are used to bundle data of the same type together.



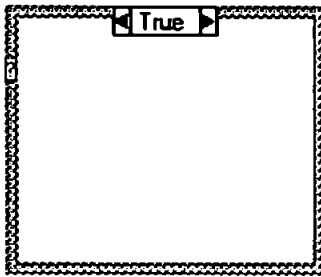
Clusters of mixed data types are denoted by thick pink dashed lines.



Local variables are variables that can be written to, or read from anywhere in a particular VI without the need for wiring.

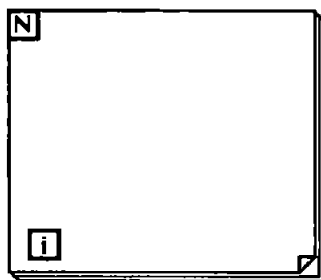


Global variables are variables that have the same properties as local variables, however they can be read or written to from more than one VI.



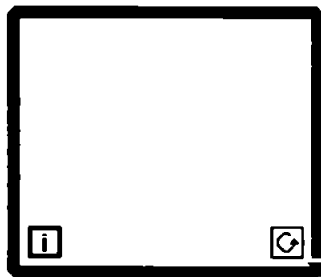
Case Structure

The case structure is equivalent to an If...Then...Else statement in a 'traditional' programming language. The question mark on the left must be wired to a comparison function such as $>$ $<$ $=$ *etc.* If the comparison is true, then everything in the true box is executed. If the case is not true, then everything in the false box is executed.



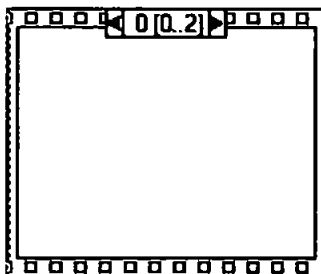
For Loop

The for loop is a structure that will execute everything in the structure a predefined number of times. The number of iterations of the loop is defined by the number that is wired to the N in the top corner of the loop. The number of the current iteration of the loop can be read from the I in the bottom left hand corner of the loop.



While Loop

The while loop executes the code in the loop while a condition is true. Conditions commonly used are $<$ $>$ $=$ \geq \leq *etc.* and must be wired to the green circular arrow in the bottom right hand corner of the loop. The iteration number of the loop can be read from the I in the bottom left hand corner of the loop.



Sequence Structure

Conventional programming languages execute code line by line in sequence. LabVIEW executes all code together. Sequences are used to allow parts of a program to be executed in a particular order and contain frames. Each frame contains code, and each frame is executed in sequence.

4.3.4 DESCRIPTION OF THE SUBROUTINES FOR CONTROL AND DATA CAPTURE

For this work, the program written to control the system contains many levels of sub VIs. A simple way of visualising all of the sub VIs used in the program is by viewing the VI hierarchy window as shown in Fig. 4.13. Each box corresponds to a separate VI. The links indicate where a sub VI is called from another VI.

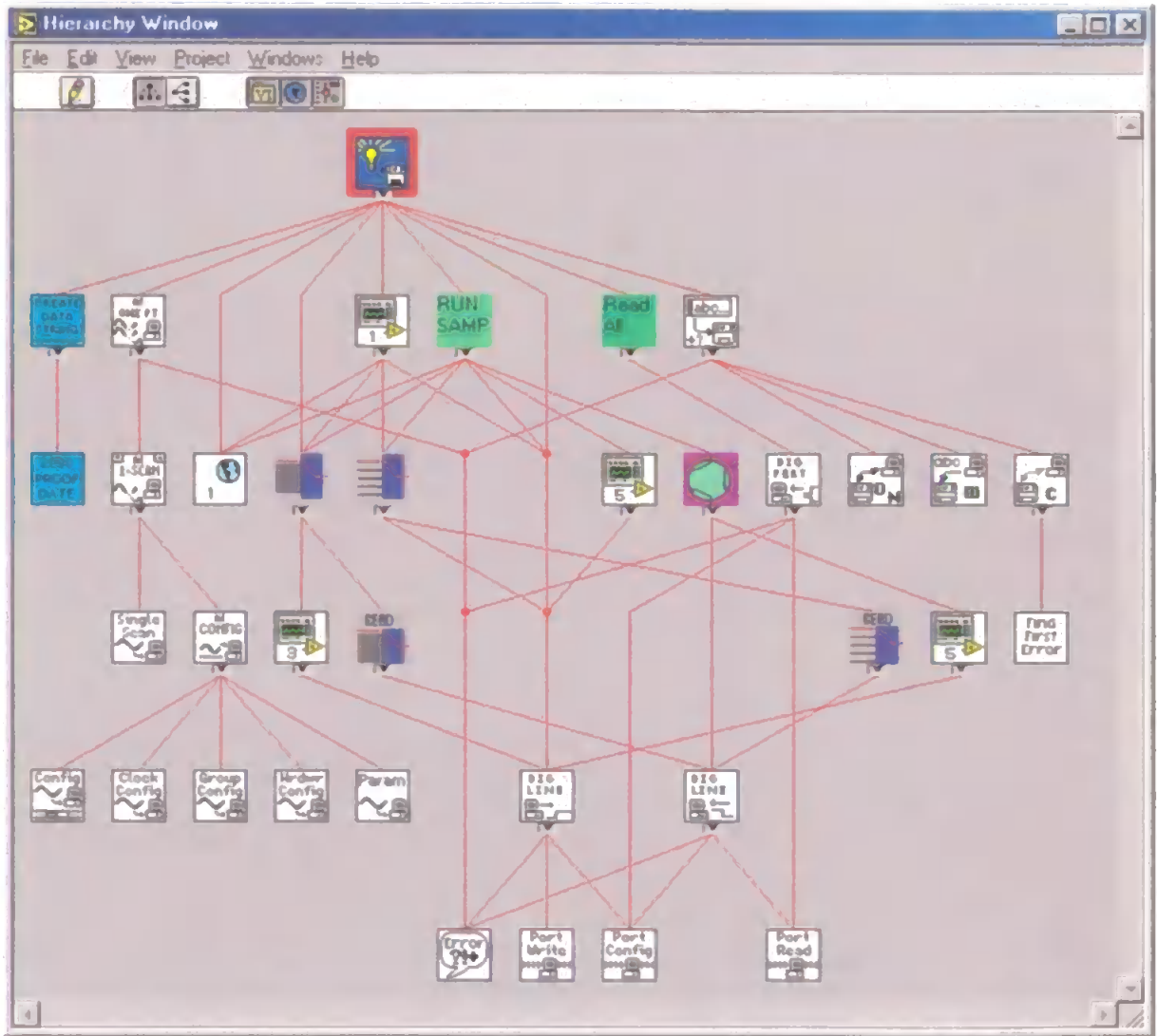


Figure 4.13: VI Hierarchy

The front and back panels are shown in Figs. 4.14 and 4.15 respectively. Each of the switching valves and pumps shown on the front panel corresponds to real valves and

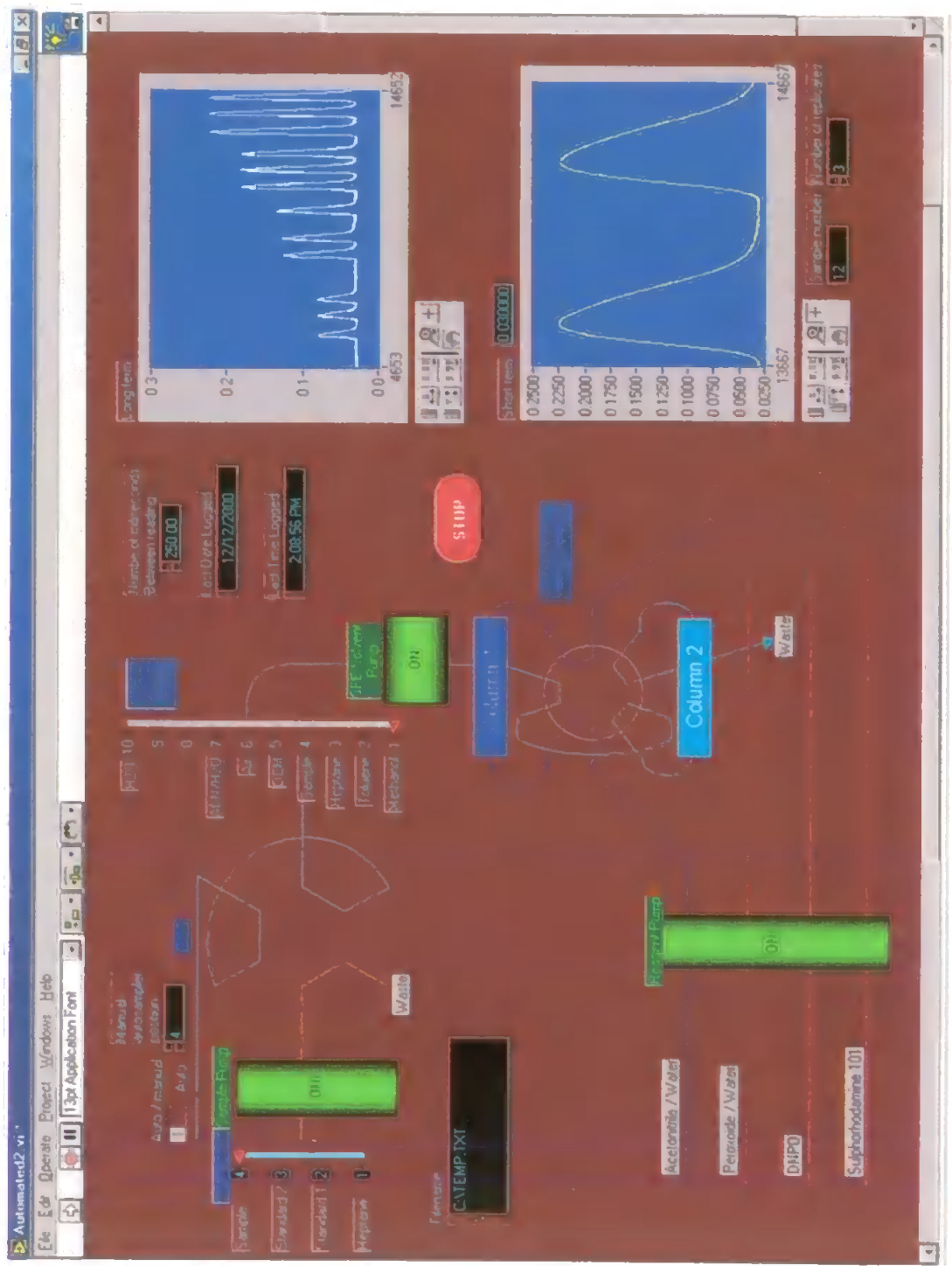


Figure 4.14: LabVIEW front panel for on-line SPE-FI-CL monitor.

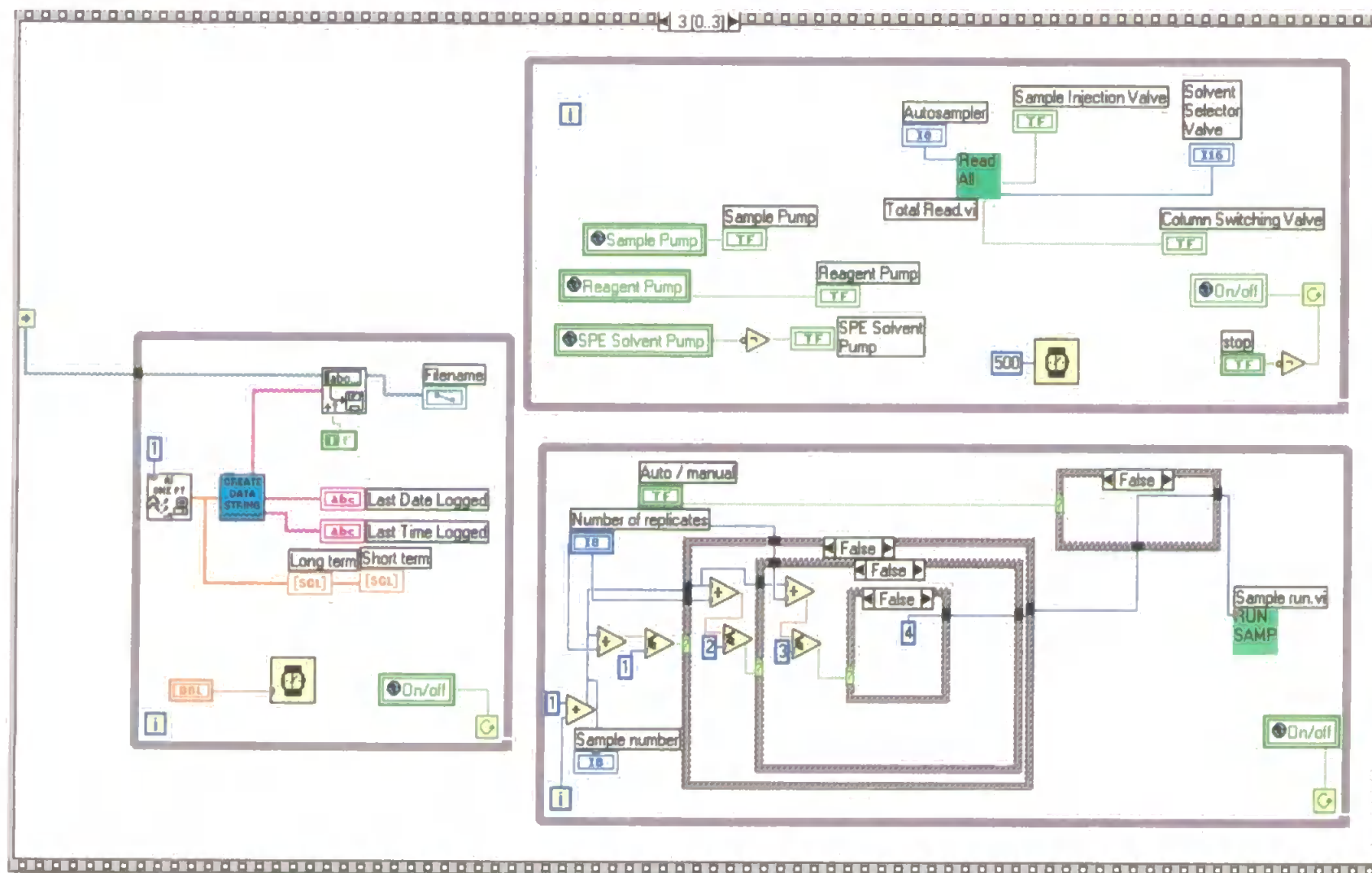


Figure 4.15: LabVIEW back panel (diagram) for monitor.

pumps in the automated system. When the real objects are switched, the corresponding virtual objects are also switched to show what position each object is in.

“Read Autosampler.VI” (see Fig 4.16) is used to read the position of the autosampler valve. Digital input lines 6 and 7 on the DAQ card are read by the VI. The autosampler position is reported in binary coded decimal (BCD) with line 6 being the least significant bit. Each autosampler position is correctly identified except position 4, which is identified as position 0 due its BCD code being 100, and since only 2 input lines are being used this must therefore be corrected.

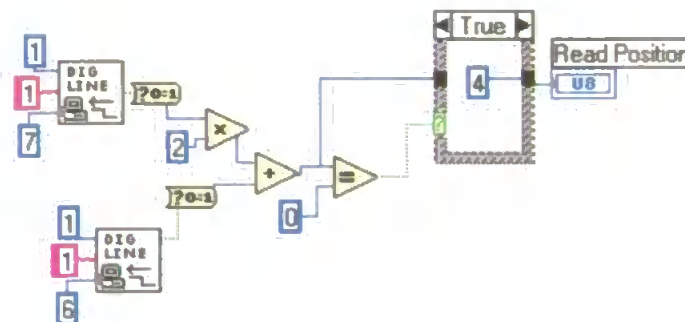


Figure 4.16: Read Autosampler.VI

“Switch Autosampler.VI” (see Fig 4.17) reads the position of the autosampler valve using “Read Autosampler.VI” and compares it with the desired position. If the two values are the same, then the VI ends. If they are not the same, then the TTL output from the DAQ card to the valve is switched from high to low, then back to high causing the valve to step one position. This process is repeated until the read position matches the desired position. A 400 ms delay is incorporated in the VI to give the program time to read the TTL lines.

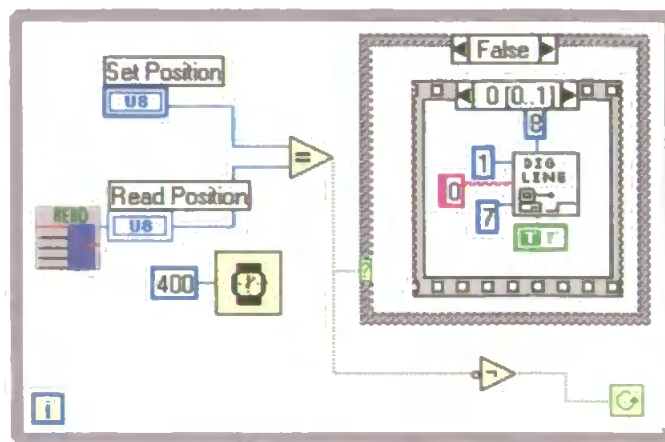


Figure 4.17: Switch Autosampler.VI

“Switch 6 or 10 way valve.VI” (see Fig 4.18) is used to switch either the 10 port column selector valve or to switch the 6 port sample injection valve. The desired position (write position) is compared with the current position of the valve (read from line 0 for 10 port valve, or from line 1 for the 6 port valve). If the two values are not the same, then TTL line (6 for the 10 port, and 5 for the 6 port) is switched from low to high for 50 ms and then switched back to low. A 500 ms delay is incorporated to allow time for the computer to read the valve position.

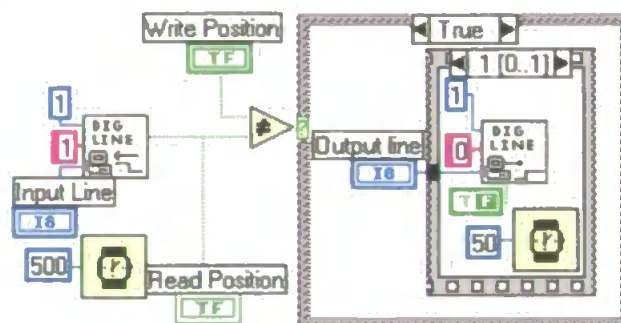


Figure 4.18: Switch 6 or 10 way valve.VI

“Read multiport valve.VI” (see Fig 4.19) reads the position of the 10 port valve in BCD from lines 2 to 5 of the DAQ card. Line 2 is the least significant bit. Each multiport valve position is correctly identified except position 10, which is identified as position 0 due to

its BCD code being 10000 and only 4 input lines are being used. This must therefore be corrected.

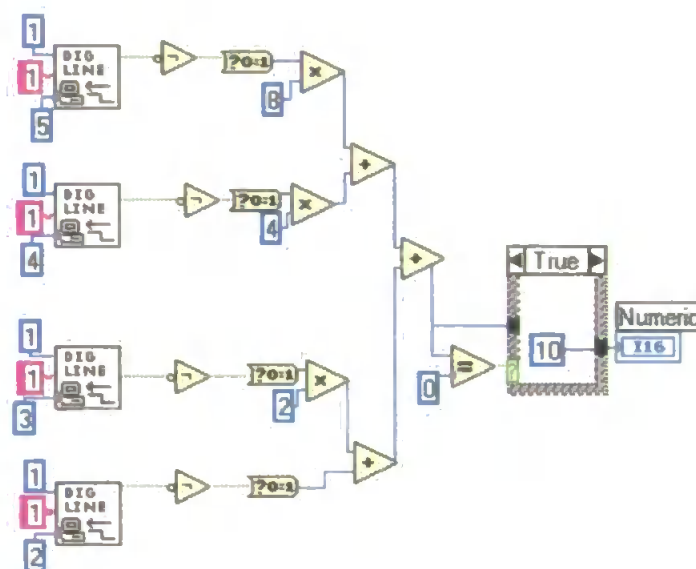


Figure 4.19: Read multiport valve.VI

“Switch multiport valve.VI” (see Fig 4.20) reads the position of the valve using “Read multiport valve.VI”, and compares it with the desired value. If the values are not the same then TTL output line 4 is switched from low to high and held for 50 ms, then returned to low. A 150 ms delay is incorporated to allow time for the computer to read the TTL line.

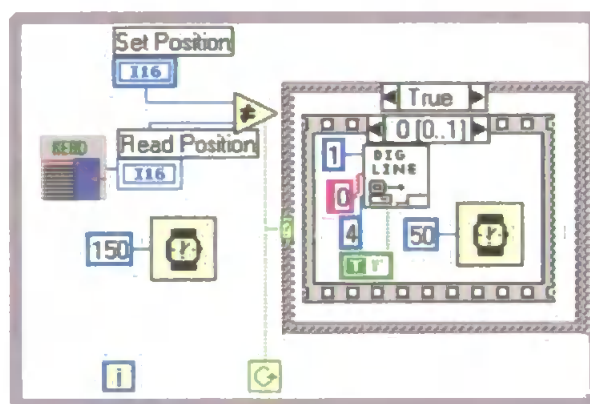


Figure 4.20: Switch multiport valve.VI

“Total read.VI” (see Fig 4.21) is used to display the position of the column switching valve, the sample injection valve, the multiport (solvent selector) valve and the

autosampler. This VI works in the same way as all the other read VIs except that in this case the whole input port is read, and each valve position is calculated from this.

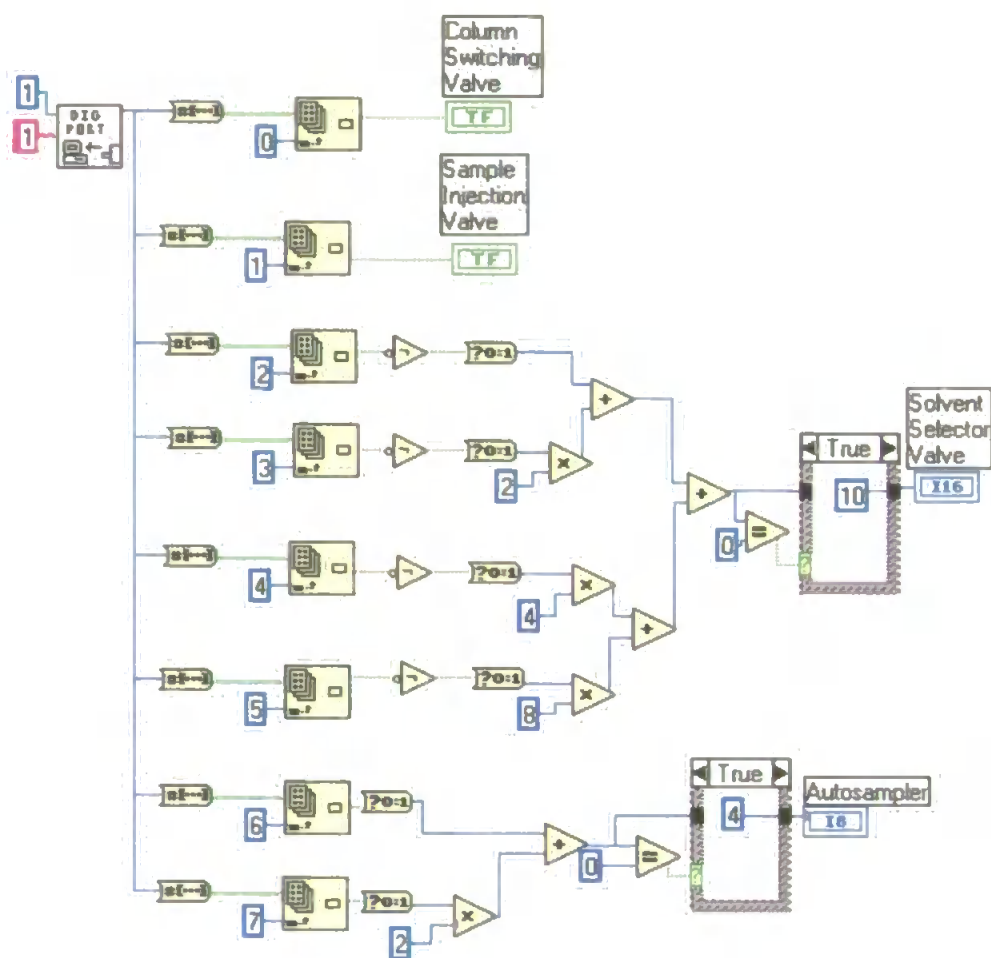


Figure 4.21: Total Read.VI

“Sample run.VI” (see Fig 4.22) introduces timed events into the program. When the time (set on the front panel of “Sample run.VI”) for a particular event is equal to the elapsed time then that part of the VI is executed. All events and their corresponding times are shown in Table 4.2.

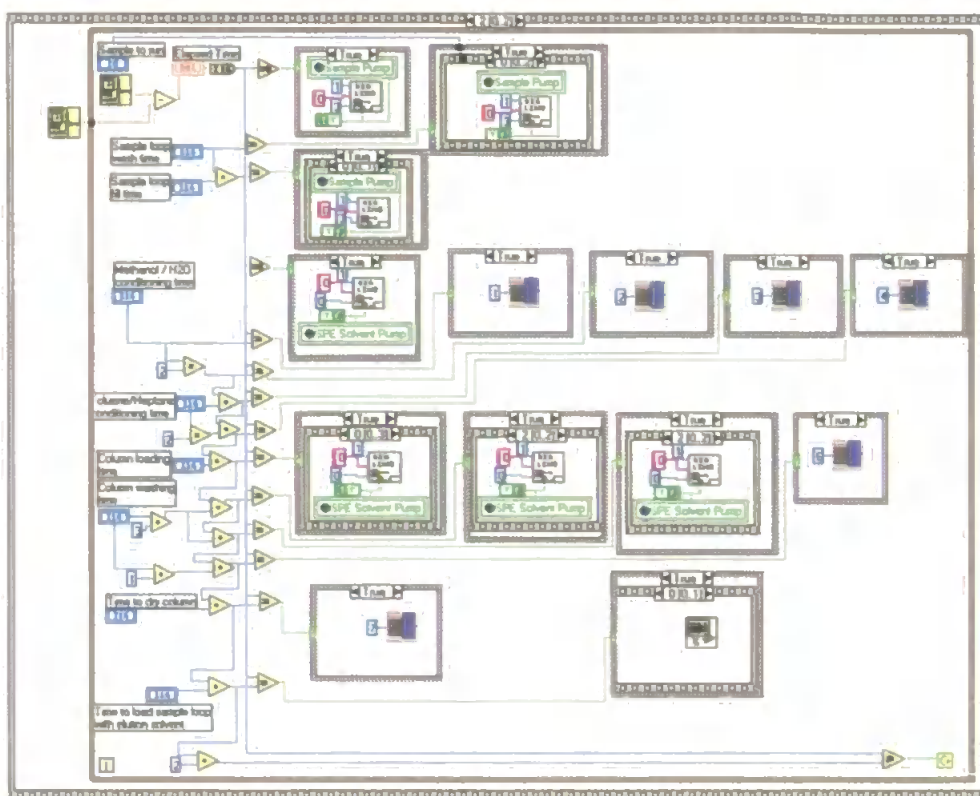


Figure 4.22: Sample run.VI

The section of the main VI shown in Fig 4.23 is used to update all of the display indicators on the front panel. “Total read.vi” is used to read the positions of all of the valves, and global variables are read to identify whether pumps are switched on or off. The front panel objects are updated every 500 ms.

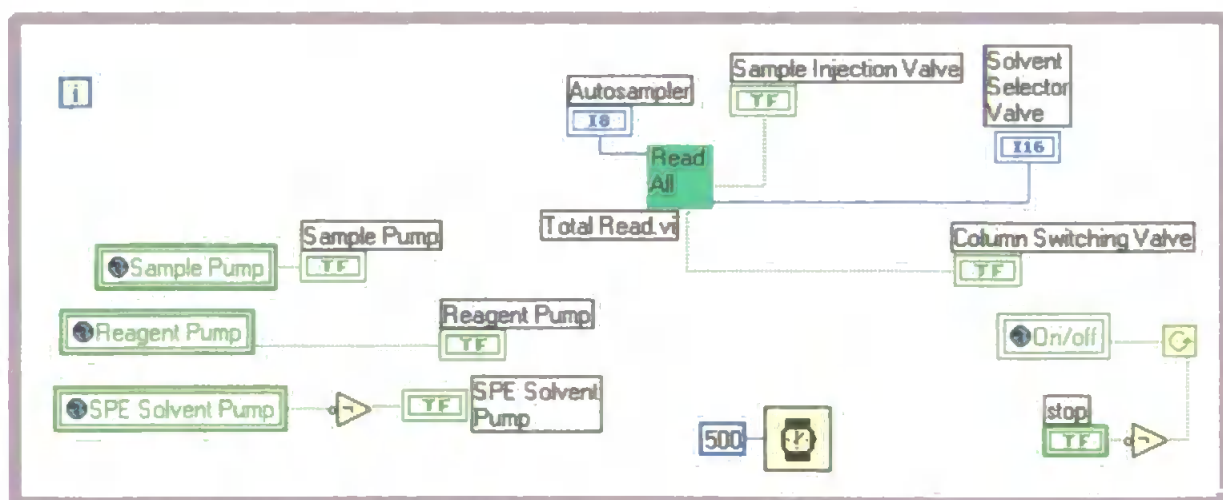


Figure 4.23: Front panel display update.

The autosampler position is set with the part of the main VI shown in Fig 4.24. If the autosampler mode is set to manual, then the front panel control 'manual autosampler position' will control the autosampler position. However, if the autosampler mode is Auto, then an injection is made with the autosampler position in position 1. This is repeated for the required number of replicates as specified on the front panel. Positions 2 and 3 are then injected with the required number of replicates. Position 4 (sample) is continually injected until the stop button is pressed.

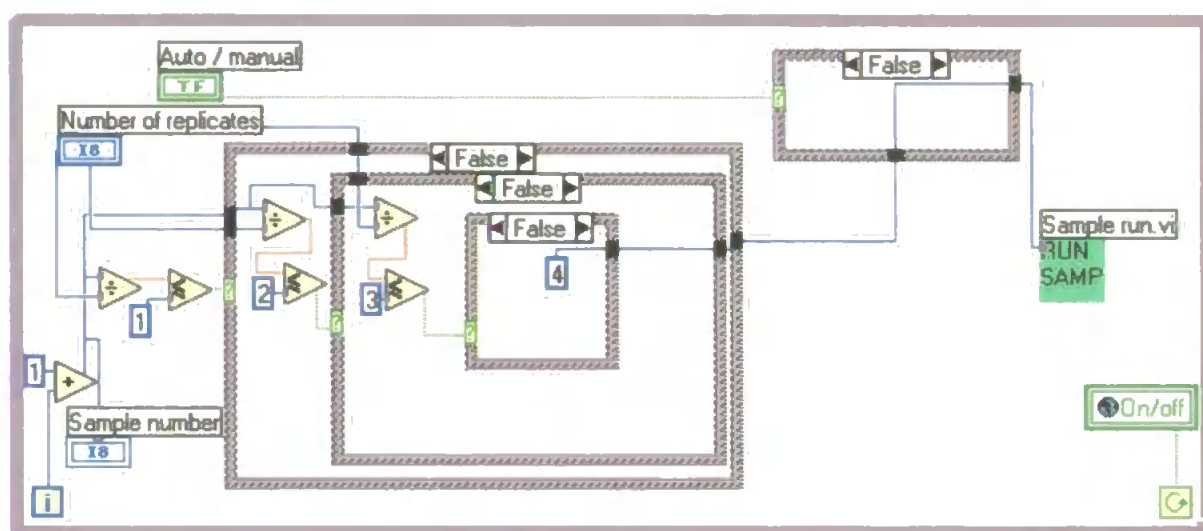


Figure 4.24: Autosampler program

The data collection function of the main VI, shown in Fig 4.25 is used for data acquisition and file output. The LabVIEW built in VI, "AI sample channels.VI" is used to capture the analogue signal from the PMT. This is then displayed on the short and long term displays. Create data string VI takes this signal and adds the date and time to it and converts this to a tabulated string followed by a carriage return. This string is written to a file using the LabVIEW pre-prepared VI, "Write Characters to File.VI". This is continually repeated with a delay that is set on the front panel to allow the time between reads to be varied. An example of data generated in this way is shown in Table 4.3.

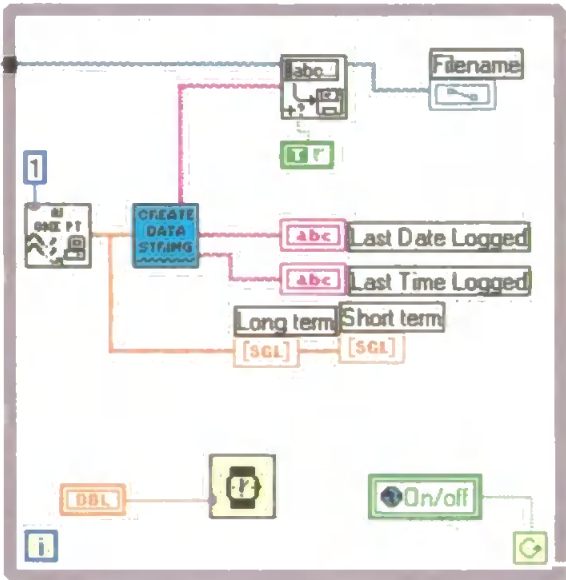


Figure 4.25: Data collection

Table 4.3: Example of data output format.

Date	Time	Output Voltage
21/09/2000	9:26:47 PM	0.080566
21/09/2000	9:26:47 PM	0.06958
21/09/2000	9:26:47 PM	0.062256
21/09/2000	9:26:47 PM	0.058594
21/09/2000	9:26:48 PM	0.056152
21/09/2000	9:26:48 PM	0.054932
21/09/2000	9:26:48 PM	0.05249
21/09/2000	9:26:48 PM	0.050049

“Purge.VI” (see Fig 4.26) is the first sub VI to be run. The user is prompted as to whether the solvent lines need to be purged (to remove air bubbles). If purging is required, then each of the sample lines is purged in sequence, and also each of the SPE solvent lines is purged in sequence.

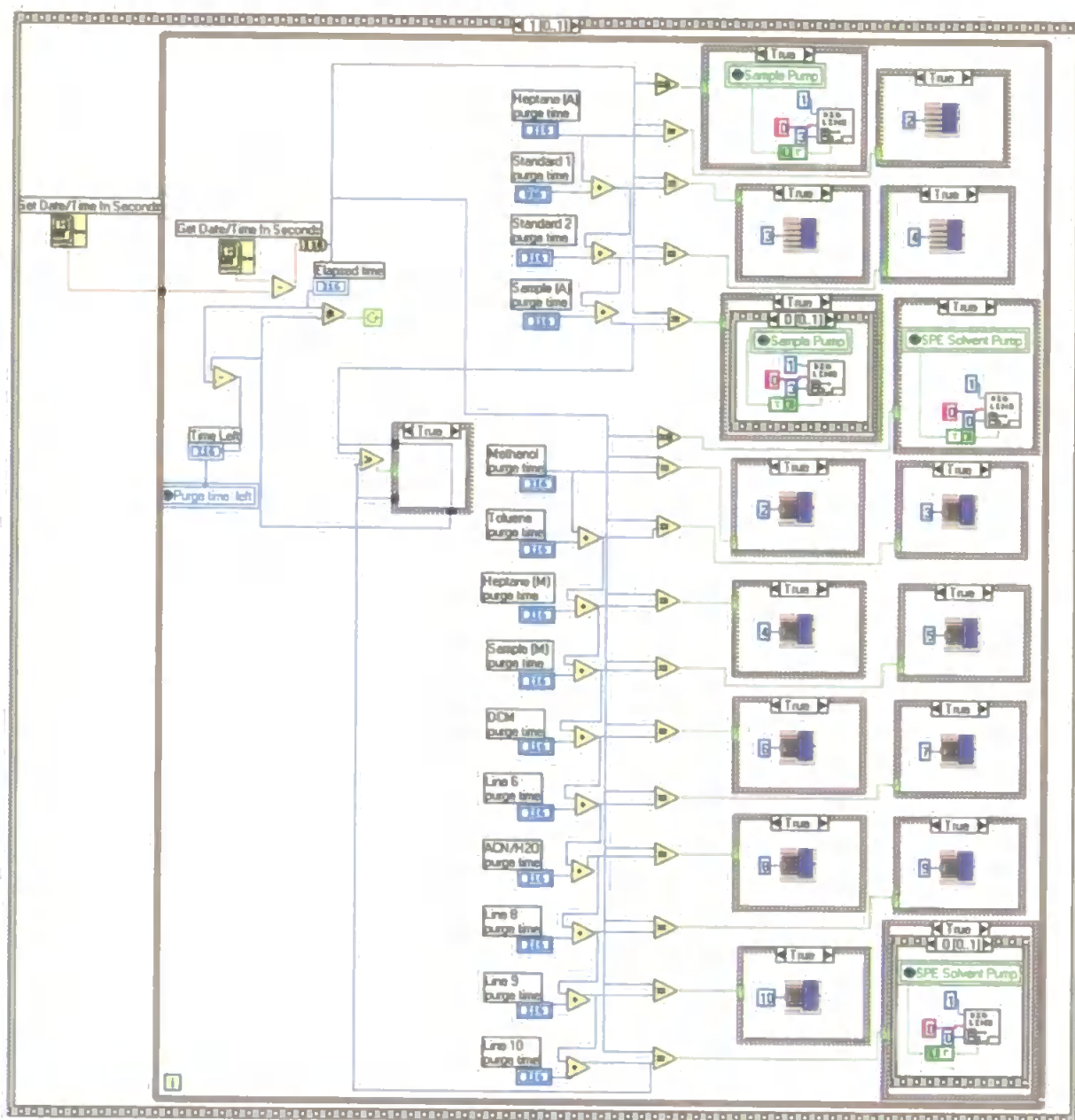


Figure 4.26: Purge.VI

Analysis2.VI (see Fig 4.27) loads data from files saved with the automated system and put the data into an array. The user of the program defines the starting point (red cursor) and end point (yellow) for integration as well as the position of the baseline (blue). A new array is then created containing only the data to be integrated, and the baseline value is subtracted from all of the elements in the array prior to integration.

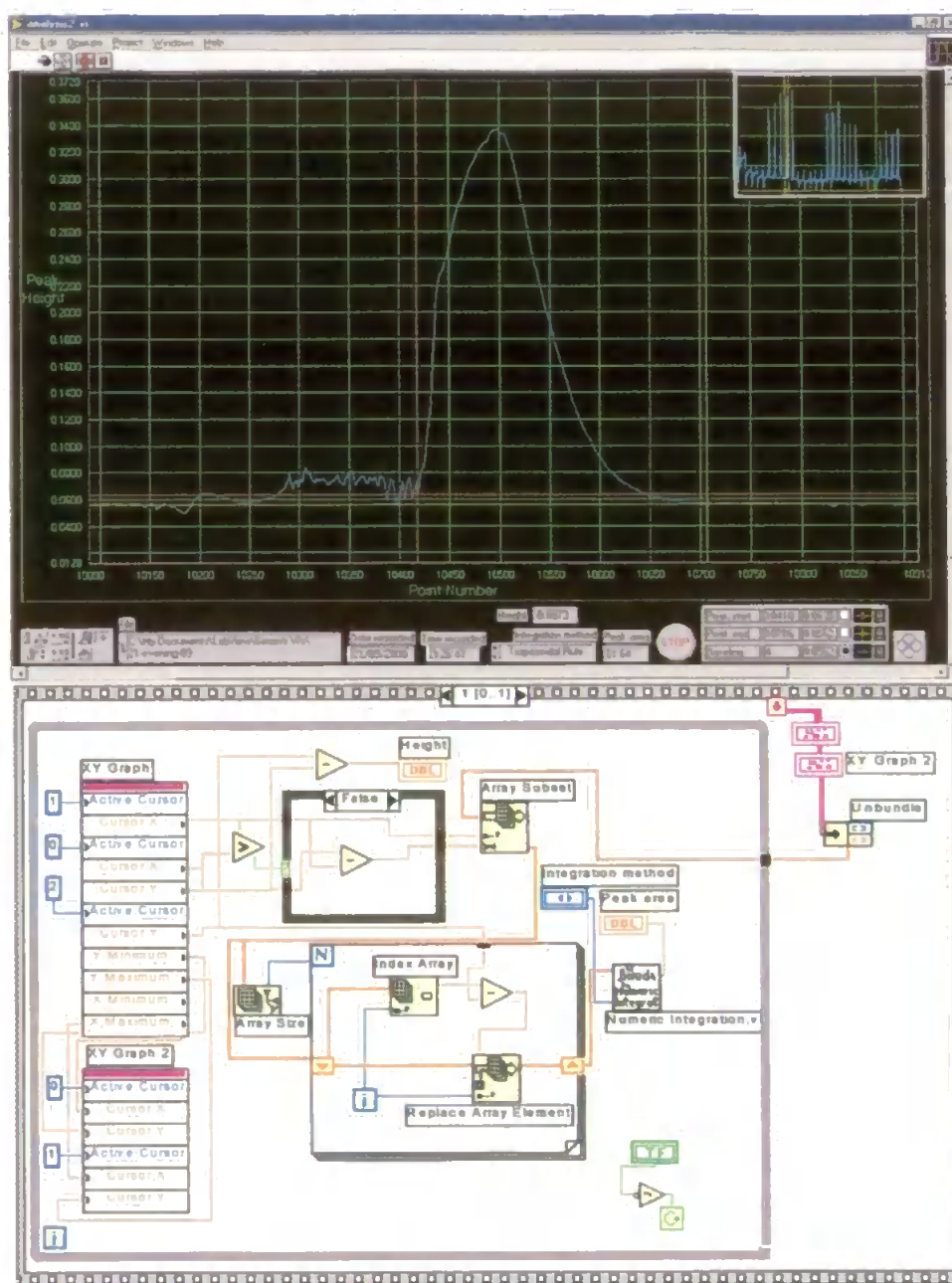


Figure 4.27: Analysis2.VI front and back panels.

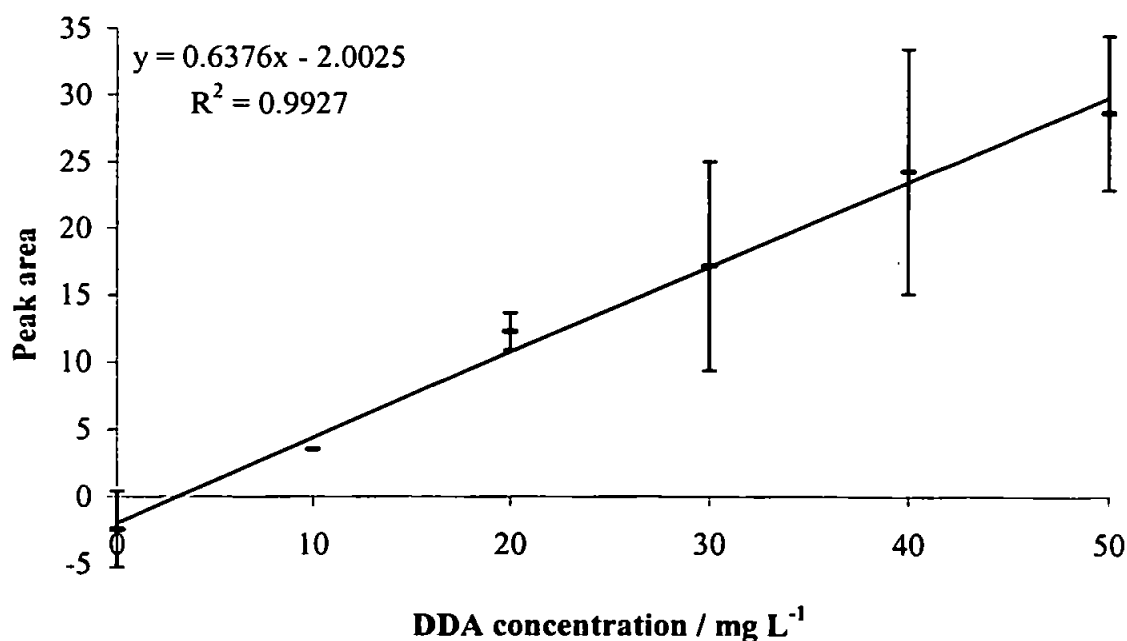
4.3.5 ANALYTICAL PERFORMANCE OF THE AUTOMATED ON-LINE SPE-FI-CL SYSTEM

The solid phase extraction procedure used in the on-line system is shown in Table 4.4. The volumes of the conditioning solvents were increased, as real diesel samples were to be analysed. All samples were diluted to 25 % in heptane to reduce their viscosity.

Table 4.4: Automated on-line solid phase extraction parameters.

Conditioning		Washing	
1 mL H ₂ O	60 s	0.25 mL heptane	15 s
1 mL methanol	60 s	0.25 mL toluene	15 s
0.25 mL toluene	15 s	0.25 mL dichloromethane	15 s
0.25 mL heptane	15 s	Drying	60 s
Loading		Eluting	
800 µL (25% Diesel or heptane / 75% Heptane) 45 s		10% Water / 90% Acetonitrile	

A calibration in the range 0-50 mg L⁻¹ DDA in heptane was carried out using the automated on-line system and the calibration graph is shown in Fig 4.28. Similar results were obtained for the automated system as for the manual system (R^2 of 0.9936 and 0.9927 respectively). The errors were slightly larger in the automated system (RSD of 13 % compared to 8.5 % for a 40 mg L⁻¹ DDA solution) which was probably due to the extra conditioning step with water which was introduced in order to ensure the column was fully cleaned between each sample injection.

**Figure 4.28: Automated on-line SPE FI-CL calibration graph (errors = 3s).**

The limit of detection for dodecylamine was 2.9 mg L^{-1} (measured using 3 times the error in the blank, $n=3$). This was well below the target of being able to determine concentrations of 40 mg L^{-1} . The heptane blank gave produced a slight negative peak, suggesting that all of the wash solvent had been removed by pumping air through the column for 60 s before elution. 13 samples h^{-1} could be analysed, giving a marked improvement in throughput compared with the manual system.

4.3.6 DETERMINATION OF DODECYLAMINE IN DIESEL FUELS USING THE AUTOMATED ON-LINE SPE-FI-CL SYSTEM

Each of the diesel samples (Brazilian, SNV, Hamburg, Stanlow and Swedish) and heptane that were analysed in Chapter 2 were analysed again using the automated on-line SPE-FI-CL system. The solid phase extraction procedure was as described in Table 4.4. A standard 40 mg L^{-1} DDA sample was injected after every six samples to ensure the CL response was stable. As in Chapter 3, “FF” samples refer to fully formulated fuels (spiked with the additive package with a DDA concentration of 40 mg L^{-1}), “40” samples refer to samples that have been spiked with 40 mg L^{-1} DDA without the additive package, and “0” samples refer to base fuels containing no DDA or additives.

The results for the on-line samples are shown in Fig. 4.29. All of the base fuels gave CL responses equivalent to $< 8 \text{ mg L}^{-1}$ DDA, and all of the spiked samples gave responses equivalent to $> 17.7 \text{ mg L}^{-1}$ DDA. The samples spiked with 40 mg L^{-1} DDA and the additive package displayed a similar trend with the recovery decreasing as the sulphur level increased. The exception was SNV which had less sulphur than the Brazilian samples, but lower recoveries, displaying the same trend as the off-line results. The reason for the lower recovery for SNV was probably due to more of its sulphur content being

present as sulphonic acids, which are most effective at binding to the dodecylamine, as described in chapter 3.

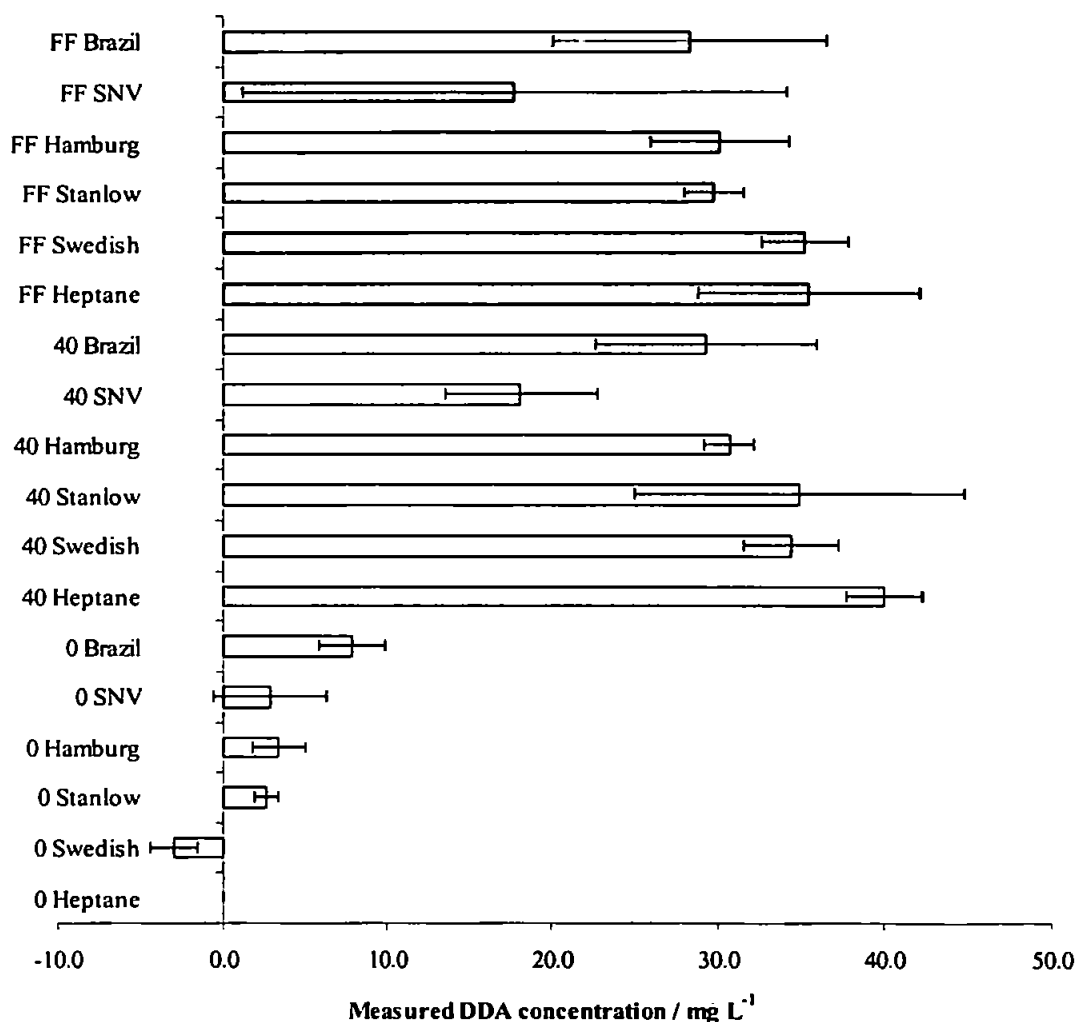


Figure 4.29: Results for automated on-line SPE (error bars represent $\pm 2s$ ($n=3$)).

4.3.7 COMPARISON OF RESULTS OBTAINED USING OFF-LINE SPE WITH RESULTS OBTAINED USING THE AUTOMATED ON-LINE SYSTEM

Results obtained using the on-line and off-line systems are compared in Figure 4.30 and a linear regression plot is shown in Fig 4.31. A reasonable correlation was obtained between the on-line and off-line results (R^2 of 0.8104). The recoveries were generally slightly

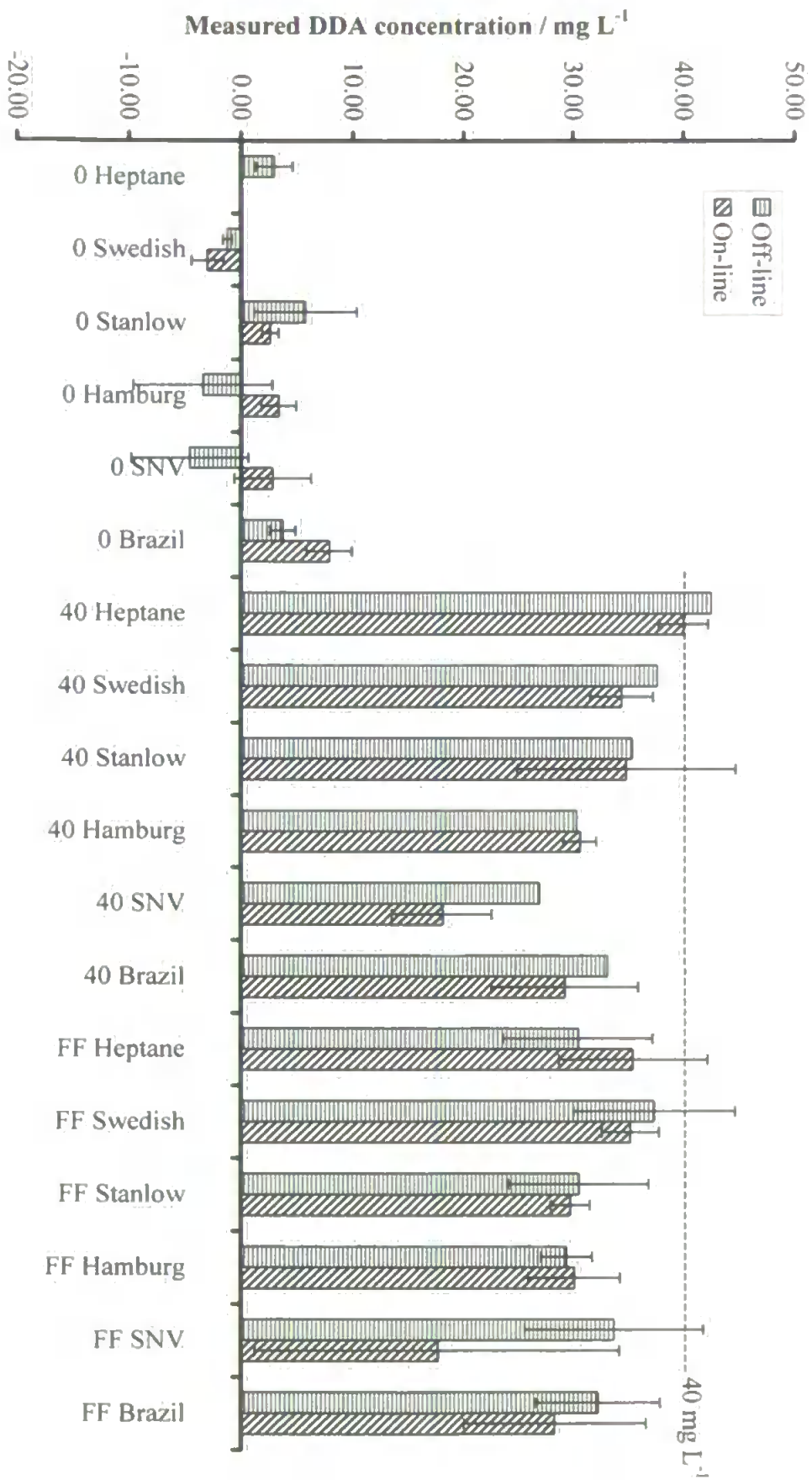


Figure 4.30: Comparison of results from off-line SPE followed by FI-CL detection with results obtained using the automated on-line SPE-FI-CL manifold.

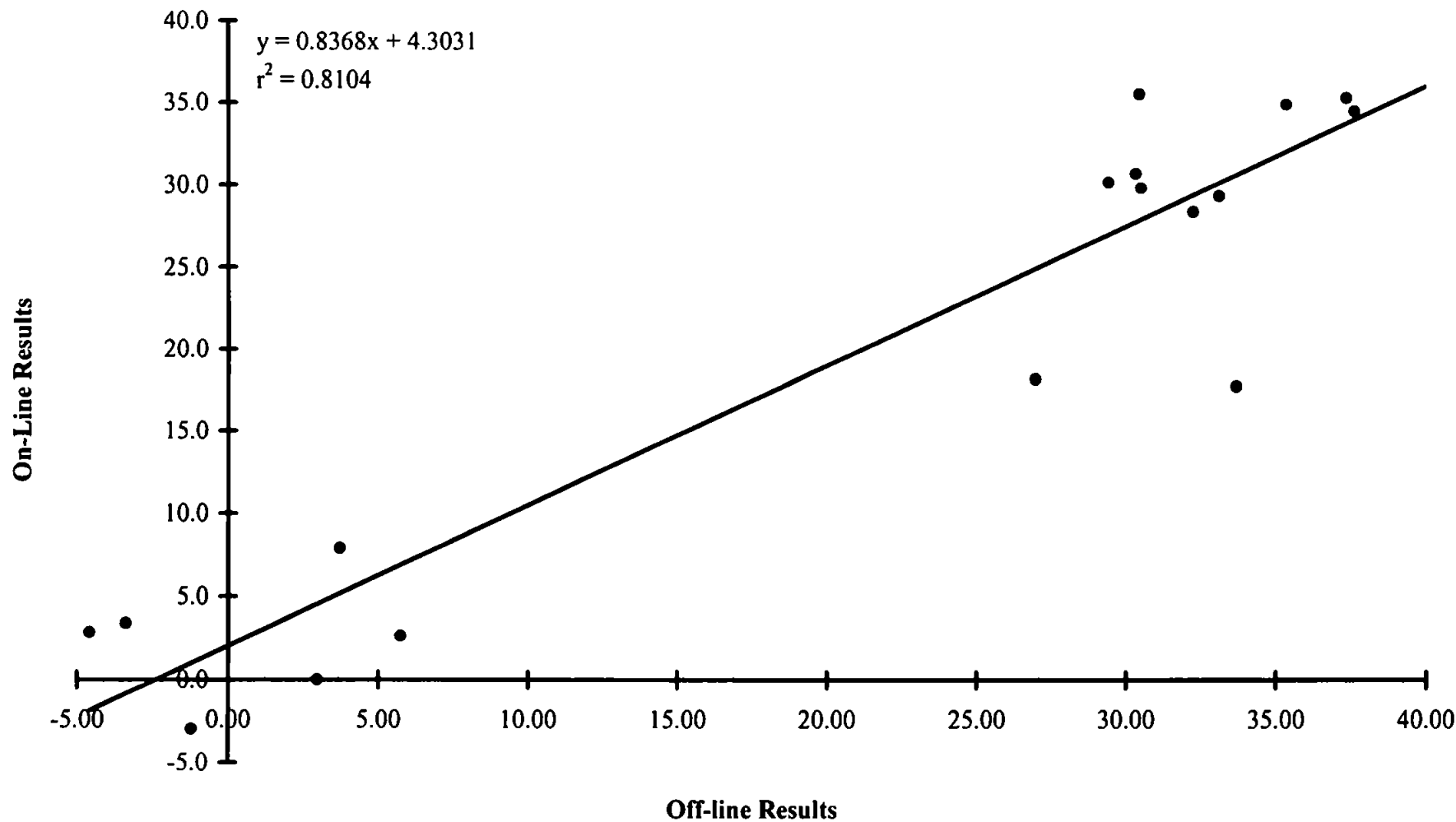


Figure 4.31: Linear regression plot of results obtained from on-line and off-line SPE.

higher for the off-line samples except for the spiked Hamburg samples and the fully formulated heptane sample. The on-line and off-line results were compared using a *t*-test (see Section 3.3.5). The corresponding *t* value for this number of degrees of freedom with 95 % confidence is 4.3. A table of the calculated *t*-test values for the on-line and off-line SPE results is shown in Table 4.5. If the results were significantly different, then the magnitude of the *t*-values would be greater than 4.3. All the calculated values were lower than this, showing that there is no significant difference between the on-line and off-line SPE results at the 95 % confidence level.

Table 4.5: *t*-test comparison of the on-line and off-line SPE results.

Fuel	Pooled estimate of the standard deviation (s) / mg L⁻¹	Calculate <i>t</i>
Heptane	0.83	3.54
Swedish	0.74	2.42
Stanlow	2.31	1.35
Hamburg	3.21	2.11
SNV	3.12	2.38
Brazilian	1.16	3.61
Fully formulated heptane	4.76	1.05
Fully formulated Swedish	3.87	0.55
Fully formulated Stanlow	3.29	0.22
Fully formulated Hamburg	2.39	0.29
Fully formulated SNV	9.18	1.74
Fully formulated Brazilian	5.00	0.79

A *t*-test comparison of the on-line SPE-FI-CL results for fully formulated fuels with those of the base fuels is shown in Table 4.6. All of the *t* values were greater than 4.3 except SNV, showing that statistically the results were significantly different for all of the base fuels than for the fully formulated fuel (with the exception of SNV). The reason SNV could not be differentiated was owing to the large error in the measurement of its fully

formulated sample. The reproducibility for this sample could possibly be improved by increasing the conditioning times to ensure the column is fully cleaned prior to analysis.

Table 4.6: *t*-test comparison of results obtained from the fully formulated fuels with those obtained from their respective base fuels.

Fuel	Pooled estimate of the mean (s)	
	/ mg L⁻¹	t-value
Fully formulated heptane	2.37	14.96
Fully formulated Swedish	1.04	36.68
Fully formulated Stanlow	0.67	40.54
Fully formulated Hamburg	1.58	16.94
Fully formulate SNV	5.95	2.50
Fully formulate Brazilian	3.00	6.80

4.4 CONCLUSIONS

The specific conclusions of the work carried out in this chapter are as follows:

1. The manual on-line solid phase extraction FI-CL system gave a good calibration for dodecylamine standards in heptane over the range 0 - 50 mg L⁻¹, ($R^2=0.9936$) with good reproducibility (8.5 % for a 40 mg L⁻¹ DDA sample($n=3$)). Analysis times were approximately 7.5 minutes, allowing up to 8 samples to be analysed per hour.
2. LabVIEW is an excellent software environment for instrument control and data processing and provides flexibility for further development and refinement of the SPE instrumentation.
3. SPE can be incorporated on-line into FI-CL detection to provide a fully automated system. The automated on-line SPE-FI-CL manifold gave comparable results to the manual system ($R^2=0.9927$) with acceptable reproducibility (13 % for a 40 mg L⁻¹

DDA sample). Analysis times were shorter than for the manual system allowing 13 samples per hour to be analysed.

4. Results using on-line solid phase extraction showed no significant difference from those obtained using off-line SPE (at the 95 % confidence level).
5. Results obtained using the on-line SPE-FI-CL system showed that all results were significantly different (at the 95 % confidence level) for fully formulated fuels when compared with results for the base fuels (except in the case of SNV diesel fuel due to the poor reproducibility for this sample).

Chapter 5

Determination Of Glycols Using FI-CL

5 DETERMINATION OF GLYCOLS USING FI-CL

5.1 INTRODUCTION

Additive formulations with poly-alcohol functionalities such as Paramin 655 (P655) shown in Fig 5.1, are commonly used as lubricity agents in diesel fuels. P655 consists of mono-, di- and tri-esters of linoleic acid and glycerol in a carrier oil and the mixture is typically dosed in diesel fuel at a concentration of 150 mg L^{-1} .

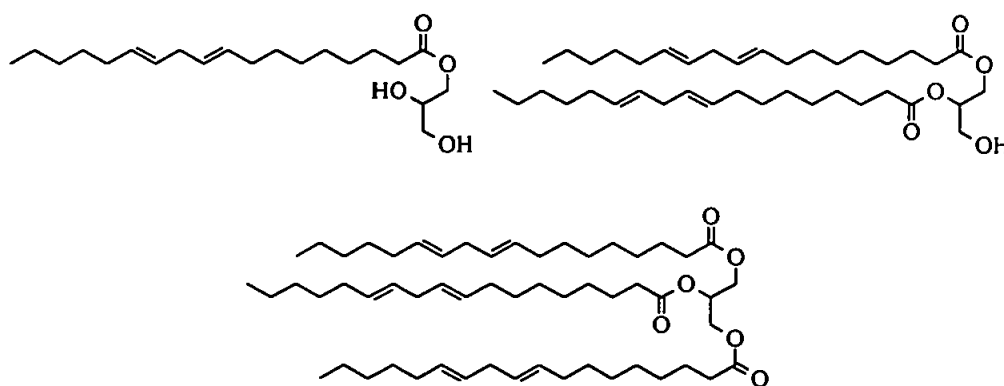


Figure 5.1: Structures of mono-, di- and tri-esters of glycerol and linoleic acid which comprise the additive Paramin 655 (P655).

As with the detection of detergents in diesel fuels (see Chapter 2) the determination of lubricity agents in fuels using portable field instrumentation is desirable. Poly-alcohols in simple matrices can be determined using many different analytical methodologies. High performance liquid chromatography with pulsed electrochemical detection can be used to detect and separate alcohols (Johnson and LaCourse, 1990), but HPLC requires a high pressure pump and therefore is not appropriate for fully portable instrumentation. Gas chromatography with flame ionisation detection can also be used for the separation and detection of alcohols (Leary, 1983), but again GC is not readily adapted to portable instrumentation, as gas bottles are needed which are heavy and GC instrumentation is not

very robust. Another method for the determination of alcohols is based on the conversion to alkyl nitrite using NaNO_2 , which can then be detected by near UV spectrophotometry (Leenson, 1997). This has the advantage of being readily portable, however, UV spectrophotometric instrumentation requires a light source, whereas chemiluminescence detectors do not require this additional component. Various flow injection chemiluminescence methods are available for the detection of alcohols and a summary of these is shown in Table 5.1.

Table 5.1: Chemiluminescence reactions that have been used for the determination of alcohols.

Alcohol	L.O.D.	Reaction	Reference
Ethanol	4 % (v/v)	Oxidation of ethanol by hypochlorite / H_2O_2	(Lu et al., 1993)
Ethanol	1 % (v/v)	Oxidation of ethanol by acidified potassium permanganate using Fe(II) as catalyst.	(Montalvo and Ingle, 1993)
Polyphenols, monophenols and sugars	-	Peroxyoxalate / perylene detection of H_2O_2 produced from the reaction of poly-alcohols with imidazole at 80°C .	(Nozaki et al., 1995)
Ethanol	0.01 % (v/v)	Luminol detection of H_2O_2 produced by the oxidation of ethanol by alcohol oxidase.	(Danet et al., 1997)
Ethylene glycol	0.5 μM	Non CL reaction of ethylene glycol with periodate competing with the CL reaction of periodate in the presence of pyrogallol and hydroxylamine.	(Evmiridis, 1989)

Chemiluminescence can be obtained from alcohols by oxidising them with hypochlorite and hydrogen peroxide (Lu et al., 1993), although this method is only useful at high concentrations and has a detection limit of 4 % (v/v). The oxidation reaction of alcohols using acidified permanganate (Montalvo and Ingle, 1993) has the same problem, with a detection limit of 1 % (v/v). This reaction also requires reagents to be prepared in 95 % nitric acid, which is undesirable in a flow system. Another method (Nozaki et al., 1995)

uses the peroxyoxalate CL reaction to detect hydrogen peroxide produced from the mixing of alcohols with imidazole. Production of hydrogen peroxide requires mixing to take place at 80°C, which is a disadvantage for portable instrumentation. A further method for the detection of ethanol using FI-CL is by detecting the hydrogen peroxide produced when ethanol reacts with alcohol oxidase (Danet et al., 1997). This is an enzymatic reaction that is specific to ethanol, and is therefore not suitable for poly-alcohols such as those comprising P655.

A simple flow injection system utilising a chemiluminescence reaction has been applied to the determination of poly-alcohols in a laboratory environment (Evmiridis, 1989). Potassium periodate reacts with pyrogallol to produce visible light. Periodate also reacts with alcohols, and so the two reactions are in competition with one another, resulting in less chemiluminescence when alcohol is present. This reaction has good sensitivity (an L.O.D. of 0.5 µM for ethylene glycol) and should therefore be suitable for the purposes of determining poly-alcohols using simple portable instrumentation. In order to analyse petroleum based fuel samples using the aqueous CL reaction and to remove all matrix interferences, solid phase extraction is also needed.

This chapter describes the optimisation of the pyrogallol / periodate FI-CL method as applied to the determination of a simple poly-alcohol (ethylene glycol) and the adaptation of this method for the detection of P655 in its carrier oil. A solid phase extraction procedure to extract P655 from a simple non-polar matrix (*i.e.* heptane), used as a model system to demonstrate the feasibility of applying the method to diesel fuel, is also described.

5.2 EXPERIMENTAL

5.2.1 REAGENTS

AnalaR grade butan-1-ol, butan-2-ol, propan-2-ol, acetone, potassium periodate and potassium dihydrogen phosphate were obtained from Merck (Poole, Dorset, U.K.). Dichloromethane (Hipersolve grade), ethanol (Hipersolve grade), ethyl acetate (Hipersolve), sodium hydroxide (reagent grade) and hydroxylammonium chloride (Spectrosol grade) were also obtained from Merck. Ethylene glycol (Puriss grade) was purchased from Fluka (Gillingham, Dorset, U.K.), pyrogallol (2,6-dihydroxyphenol analytical grade) was obtained from Riedel-de Haen, and HPLC grade acetonitrile and methyl t-butyl ether were obtained from Rathburns (Walkerburn, U.K.). Water used was analytical reagent grade ($18.2 \text{ M}\Omega \text{ cm}^{-1}$) deionised water from an Elgastat UHQ II system (Elga Ltd., High Wycombe, Bucks., U.K.). Solid phase extraction columns were Isolute® 200 mg aminopropyl obtained from International Sorbent Technology (Mid Glamorgan, U.K.).

A 1 M NaOH solution was prepared by dissolving 40 g of NaOH in 1 L of water. Buffer solutions were prepared by dissolving 13.6 g of potassium dihydrogen phosphate (KH_2PO_4) in 900 mL of water and adjusting the pH with addition of 1 M NaOH solution until the pH reached the desired value. The solutions were then made up to 1 L with water. 1×10^{-2} M potassium periodate (KIO_4) stock solutions were prepared by dissolving 23 g in 1 L of phosphate buffer with the required pH. Stock solutions of 1×10^{-2} M pyrogallol were prepared by dissolving 1.261 g in 1 L of the phosphate buffer of the required pH. Stock solutions of 1×10^{-2} M hydroxylammonium chloride (HONH_3Cl) were prepared by dissolving 0.695 g of hydroxylamine in 1 L of the buffer solution. All other solutions were prepared by serial dilutions of the above stock solutions. All solutions containing

pyrogallol were prepared daily and stored in amber glassware. All other reagents were found to be stable for a number of weeks (periodate stock solutions were stored in the dark). 0.5 M stock solutions of ethylene glycol were prepared by dissolving 15.52 g in 500 mL of phosphate buffer at the required pH.

5.2.2 INSTRUMENTATION

Minipuls 3 (Gilson, Villiers-le-Bel, France) peristaltic pumps were used for both the sample and reagent streams. 1.02 mm i.d. Ismaprene pump tubing (Ismatec, Weston-super-Mare, U.K.) was used to propel all reagent and carrier streams through 0.75 mm i.d. (PTFE) manifold tubing and T-pieces (Omnifit, Cambridge, U.K.). The sample injection valve (6 port, 2 position [C22-3716EH] cheminert microactuated valve) was obtained from Valco Instruments (Switzerland). Chemiluminescence emission was measured using a CamSpec CL-2 detector (Cambridge Instruments, Cambridge, U.K.). Reagent streams were mixed using an internal T-piece fitted in the CamSpec detector immediately in front of the flow cell as shown in Fig. 5.2.

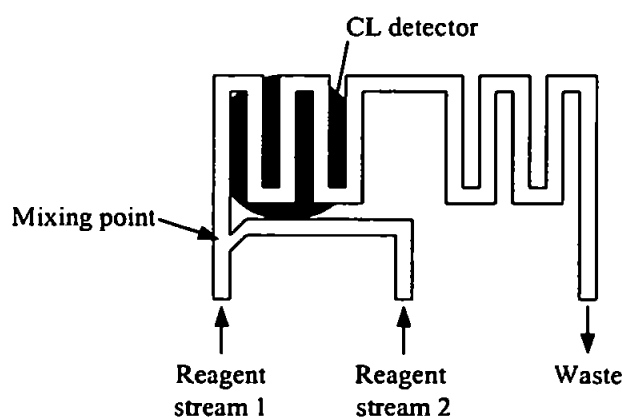


Figure 5.2: Diagram of the flow cell.

The signal from the detector was acquired using a multifunctional DAQ-700 data acquisition / digital I/O card (National Instruments, Berkshire, U.K.) attached to a laptop

computer. LabVIEW® software (see Section 4.3.2) was used to acquire the signal and to operate the switching valve. A charge coupled device (CCD) detector was used with a liquid nitrogen cooled (256 x 1024 pixels) chip and a 270M imaging spectrograph (Instruments SA) to record CL emission spectra (see Section 2.2 for details). A Carlo-Erba high resolution gas chromatograph (HRGC) with an on-column injector and fitted with a flame ionisation detector was used for the analysis of solid phase extracts. A J&W Scientific DB-5 column was used and 1 µL sample volumes were injected. A Hewlett Packard GC-MSD fitted with a mass spectrometric detector was also used to analyse solid phase extracts. This was fitted with a DB-5 column and 1 µL sample volumes were injected using a septum injector.

5.2.3 PROCEDURES

5.2.3.1 *Optimisation of a CL manifold for the detection of periodate*

The manifold described in the literature (Evmiridis, 1989), which is shown in Fig. 5.3, was optimised to achieve the greatest sensitivity for the detection of a 1×10^{-2} M solution of KIO_4 in buffer solution. The parameters optimised were pyrogallol concentration, hydroxylamine concentration, pH and flow rate. When the pH was optimised the buffer was systematically varied for all solutions including the periodate sample matrix. A 40 µL sample injection loop was used throughout.

5.2.3.2 *Recording of the emission profile*

A continuous flow system was used to record the emission profile for this reaction. The background signal was recorded with buffer solution as the carrier stream, and 1×10^{-2} M potassium periodate solution replacing the carrier stream when the emission profile was

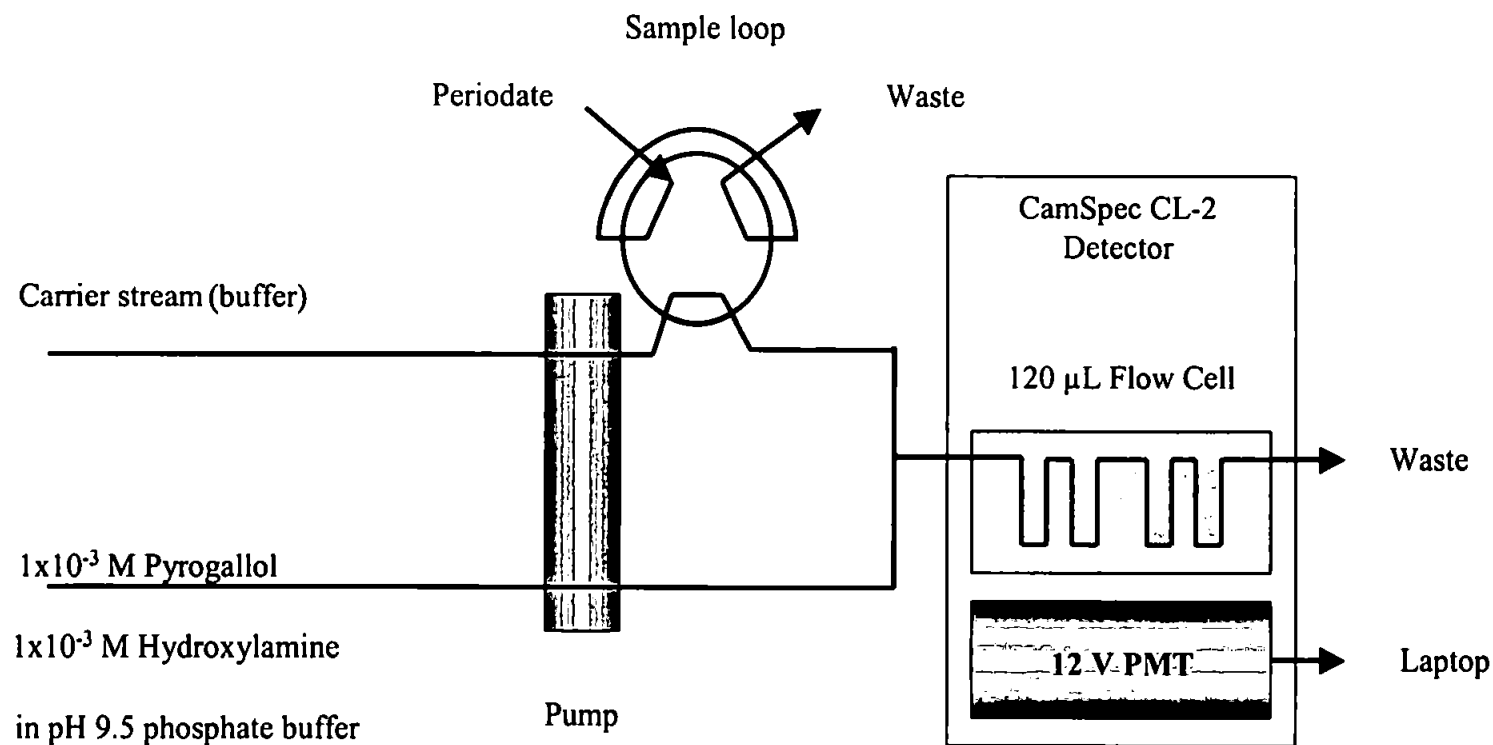


Figure 5.3: FI-CL manifold for the determination of periodate in buffer solution (Evmiridis, 1989).

being recorded. The emission was recorded for 500 s and the resulting output was exponentially smoothed using Microsoft Excel 2000.

5.2.3.3 *Determination of alcohols using the FI-CL manifold*

Calibration graphs for ethanol and ethylene glycol were recorded using the optimised flow injection manifold. The manifold was altered to allow a constant stream of periodate as shown in Fig 5.4. A potassium periodate solution with a concentration of 1×10^{-3} M in buffer was used as the carrier stream. Samples of ethanol and ethylene glycol were prepared in buffer solution and sample injection volumes of 40 μ L and 100 μ L were both evaluated.

5.2.3.4 *Solvent effects*

40 μ L of various solvents (methanol, ethanol, propan-2-ol, butan-1-ol, butan-2-ol, acetone, acetonitrile, dichloromethane and methyl-t-butyl ether) were injected into the manifold shown in Fig 5.4 with 1×10^{-3} M KIO_4 in buffer acting as the carrier stream, in order to determine their effects on the CL response.

5.2.3.5 *Gas chromatographic validation of solid phase extraction*

GC-MS

A Hewlett Packard GC-MSD was used with the following temperature program: Injection at 50°C with no temperature hold, 10°C min⁻¹ temperature ramp to 200°C, temperature ramp of 5°C min⁻¹ from 200 - 300°C and a hold at 300°C for 10 minutes. Masses were recorded in the range 1-850 D.

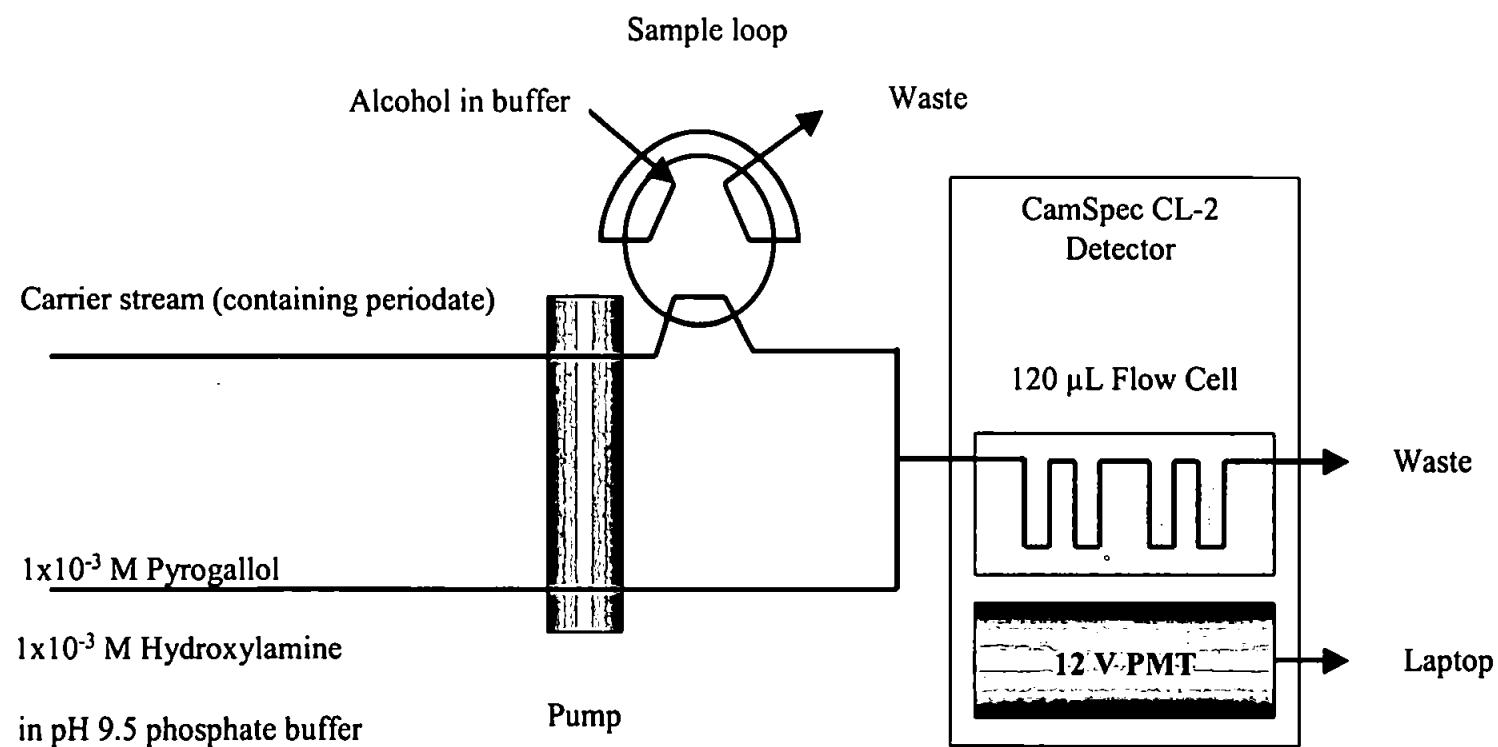


Figure 5.4: FI-CL manifold for the determination of alcohols in buffer solution.

GC-FID

Gas chromatographic conditions for measurement of P655 using flame ionisation detection were as follows; temperature programme: 250°C held for 5 minutes, 10°C / min up to 340°C with 10 minute hold. 1 µL sample volumes were injected directly onto the column. Recoveries were measured against standards prepared in the same solvents.

5.2.3.6 *Optimisation of the solid phase extraction of P655 in heptane*

Elution solvent selection

In order to find a suitable elution solvent for P655 in heptane, a solid phase extraction cartridge was conditioned and loaded as shown in Table 5.2. 4 mL aliquots of heptane, toluene, dichloromethane, acetonitrile and methanol were subsequently passed through the column to determine which solvent would be the best to elute P655 from heptane. The resulting aliquots were analysed using GC-FID to measure the recovery in each elution.

Table 5.2: Solid phase extraction procedure to determine a suitable elution solvent for P655 in heptane.

	Step	Volume / mL	Solvent
Conditioning	1	4	Acetonitrile
	2	4	Toluene
	3	4	Heptane
Loading	4	5	1000 mg L ⁻¹ P655 in heptane
Eluting	5	4	Heptane
	6	4	Toluene
	7	4	Dichloromethane
	8	4	Acetonitrile
	9	4	Methanol

Acetonitrile elution profile

To optimise the volume needed to elute P655 into acetonitrile, the solid phase extraction procedure shown above was repeated, however this time 0.5 mL aliquots of acetonitrile were used as the elution solvent and, once again, the eluents were analysed by GC-FID. The solid phase extraction procedure used is shown in Table 5.3.

Table 5.3: Solid phase extraction procedure to determine the elution volume needed to elute P655 with acetonitrile.

	Step	Volume / mL	Solvent
Conditioning	1	4	Acetonitrile
	2	4	Toluene
	3	4	Heptane
Loading	4	2	1000 mg L ⁻¹ P655 in heptane
Washing	5	2	Heptane
	6	2	Toluene
Eluting	7	0.5	Successive 0.5 mL aliquots of acetonitrile

5.2.3.7 *Choice of elution solvent compatible with FI-CL detection*

The manifold shown in Fig 5.4 was modified by the addition of a solvent line in order to allow samples of ethylene glycol in various solvents to be injected into the system. 1% ethylene glycol samples in pH 8.5 buffer, ethyl acetate and methanol were injected into a carrier stream containing the same solvent using the manifold shown in Fig. 5.5. The aim here was to evaluate whether any useful signal could be obtained for poly-alcohols in each particular solvent using the FI-CL system.

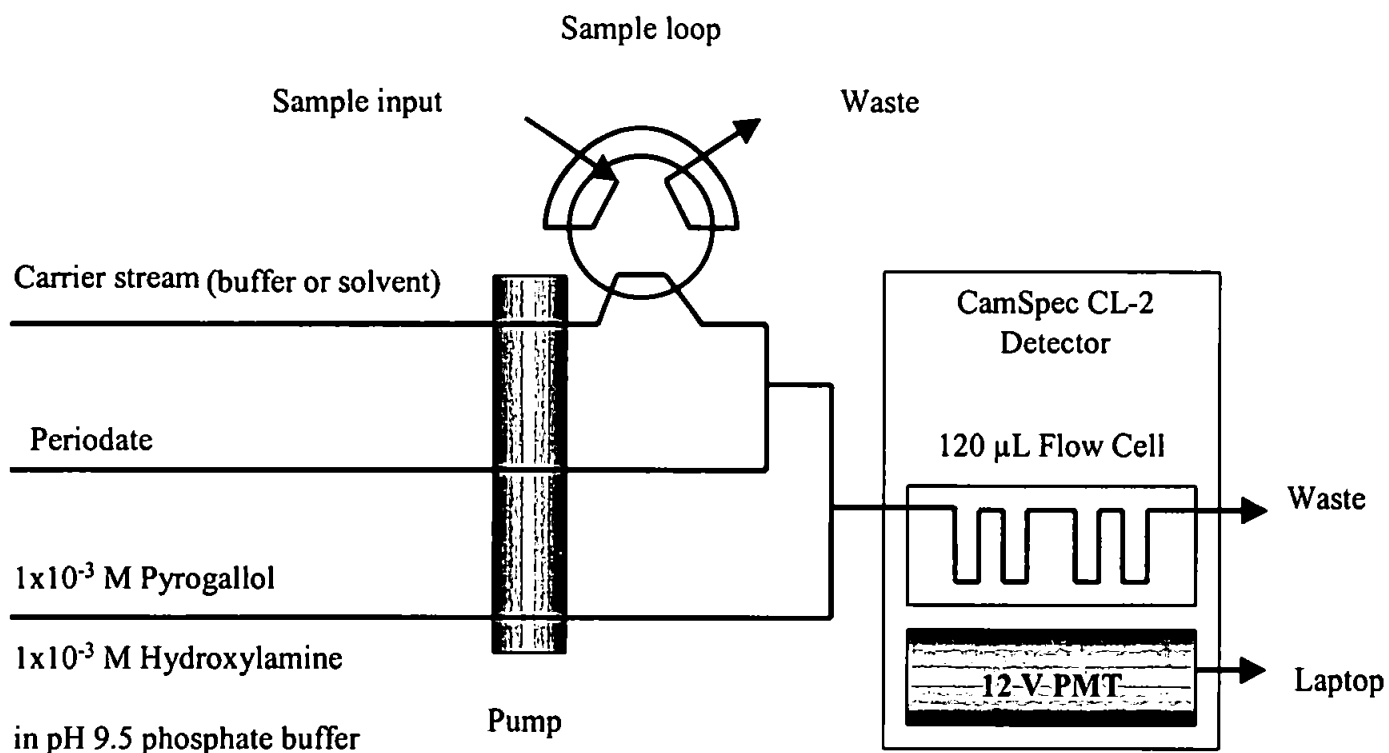


Figure 5.5: Modified FI-CL manifold for the determination of alcohols in various solvents.

5.2.3.8 *Optimisation of the modified FI-CL manifold for the detection of ethylene glycol in acetonitrile*

The modified manifold (Fig. 5.5) was optimised for the determination of ethylene glycol in acetonitrile. Acetonitrile was used as the carrier solvent, and the parameters optimised were pH, flow rate, pyrogallol concentration and hydroxylamine concentration. 0.1 % (v/v) solutions of ethylene glycol were injected into the system in all cases.

5.2.3.9 *Analysis of P655 extracts*

Solid phase extracts of P655 were prepared using the procedure shown in Table 5.3 (conditioning: 4 mL acetonitrile / toluene / heptane, washing: 2 mL heptane / toluene) with the exception that 4 mL samples of P655 in heptane in the range 2500 – 100000 mg L⁻¹ (0.25-10%) were loaded, and samples were eluted using 4 mL of acetonitrile. The same loading and elution volumes were used so that no preconcentration occurred.

5.3 RESULTS AND DISCUSSION

5.3.1 CHEMILUMINESCENCE EMISSION FROM THE OXIDATION OF PYROGALLOL

The oxidation reaction of polyhydroxy phenols (such as gallic acid) with hydrogen peroxide and formaldehyde was discovered in 1905 (Trautz and Schorigin, 1905) and was shown to cause the emission of red light. The emission was thought to be either directly from excited singlet oxygen molecules or indirectly from excited organic molecules (F^{*}) generated from an energy transfer reaction with excited singlet oxygen molecules (as shown in Equations 5.1 and 5.2)



A proposed method for the formation of singlet oxygen molecules ($^1\text{O}_2^*$) is shown in Fig. 5.6. In step (1) pyrogallol is oxidised by hydrogen peroxide / oxygen to a hydroxybenzoquinone, which in step (2) polymerises in the presence of oxygen to produce a polymer of unknown structure and an excited singlet oxygen molecule. Step (3) is in competition with step (2) and also results in the formation of a singlet oxygen molecule. Periodate has been shown to react in the same way as hydrogen peroxide with pyrogallol (Evmiridis, 1987). The oxidation of 2,6-dimethoxypyrogallol by periodate (Sklarz, 1967) has been shown to generate a variety of products which are shown in Fig. 5.7. It has been proposed that the oxidation of pyrogallol by periodate produces similar conjugated organic species (Evmiridis, 1987) which can act as fluorophores for chemiluminescence emission.

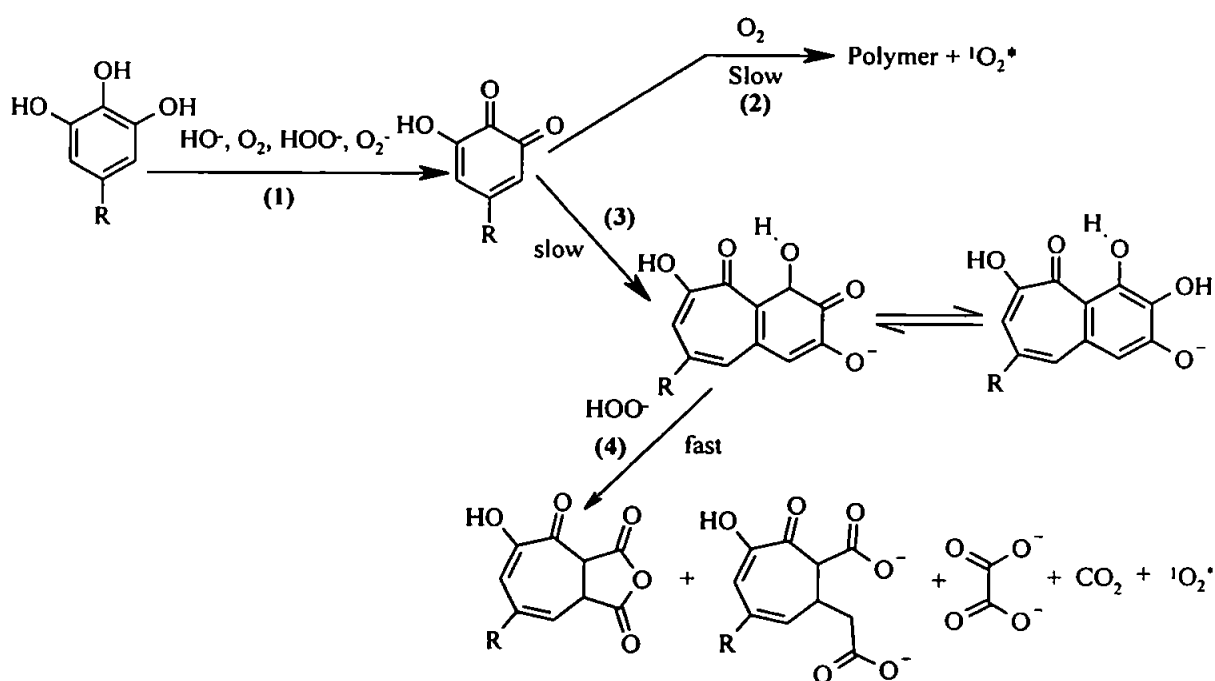


Figure 5.6: Proposed mechanism for the Trautz-Schorigin reaction, where R = H for pyrogallol and COOH for gallic acid (Evmiridis, 1987).

The pyrogallol oxidation reaction has a variety of analytical applications including the detection of glucose, fructose (Evmiridis et al., 1999), cobalt(II) (Cannizzaro et al., 1999), chromium(III) (Nakano et al., 1993), permanganate, cerium(IV), periodate, hypochlorite, peroxide (Evmiridis et al., 1998) and polyhydroxyl compounds (Evmiridis, 1987).

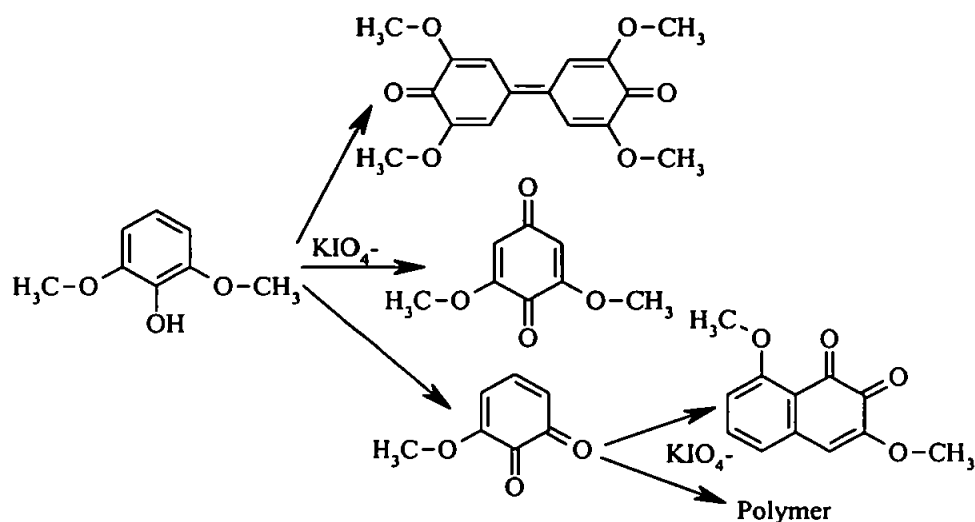


Figure 5.7: Products identified from the reaction of periodate with 2,6-dimethoxyphenol (Evmiridis, 1987). (Sklarz, 1967)

The detection of poly-alcohols using the pyrogallol CL reaction can be achieved using its competing reaction with periodate, which lowers the concentration of the oxidant. The removal of periodate from the pyrogallol CL reaction produces a suppression of the chemiluminescence response, which is directly proportional to the amount of poly-alcohol present. Dihydroxy compounds and hydroxy-ketones will react with periodate as shown in Fig. 5.8

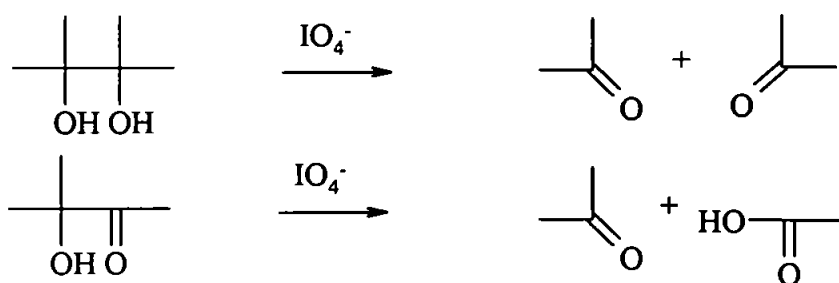


Figure 5.8: Reaction of hydroxy compounds with periodate (Sklarz, 1967).

Periodate has been also been shown to react with secondary mono-alcohols to produce aldehydes (Evmiridis, 1989). Both the mono- and di- esters components of P655 (shown in Fig. 5.1) should therefore cause a suppression of the pyrogallol CL reaction. In order to detect small changes in periodate concentration (brought about by the competing reaction with alcohols) by chemiluminescence, the FI manifold and reaction conditions were optimised to maximise the sensitivity for periodate detection.

5.3.2 OPTIMISATION OF A CL MANIFOLD FOR THE DETERMINATION OF PERIODATE

The pyrogallol CL reaction (Evmiridis, 1987) was univariately optimised (using the manifold shown in Fig. 5.3) for the determination of periodate (A 1×10^{-2} M solution was used for the optimisation). The ranges used and the optimum conditions for each parameter are shown in Table 5.4, and optimisation graphs for each parameter are shown in Fig. 5.9. The reaction has negligible background chemical noise, and therefore peak height (and not signal/noise ratio) has been reported.

Table 5.4: Optimum conditions for the determination of periodate.

Parameter	Condition used whilst optimising other parameters	Lower Limit	Upper Limit	Optimum Condition
Pyrogallol concentration / M	1×10^{-3}	1×10^{-4}	1×10^{-2}	1×10^{-3}
Hydroxylamine concentration / M	1×10^{-3}	1×10^{-4}	1×10^{-2}	1×10^{-3}
pH	8.0	7	10.5	9.5
Flow rate / mL min ⁻¹	1.5	0.5	2.25	1.25

The rate of the pyrogallol oxidation reaction is relatively fast and therefore the greatest sensitivity was found when the reagents were mixed as close to the point of detection as

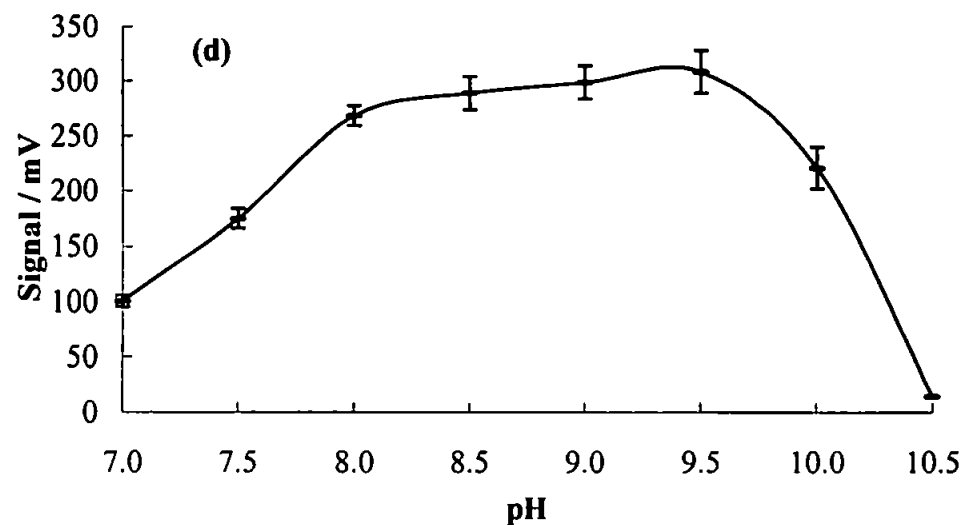
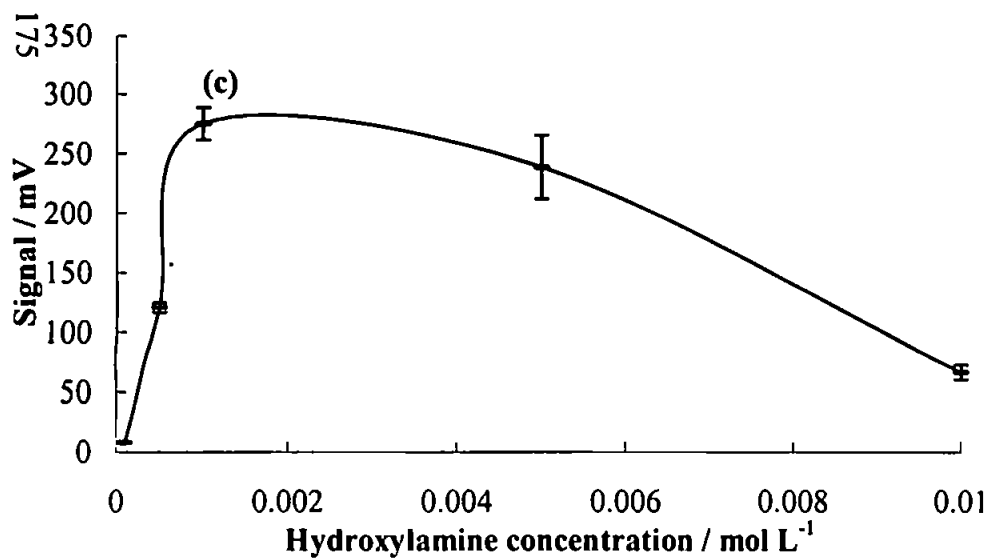
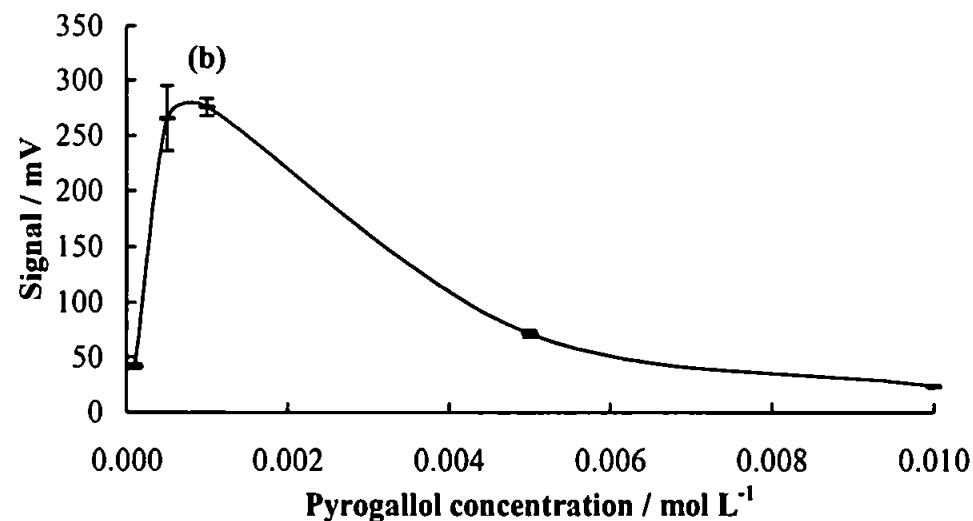
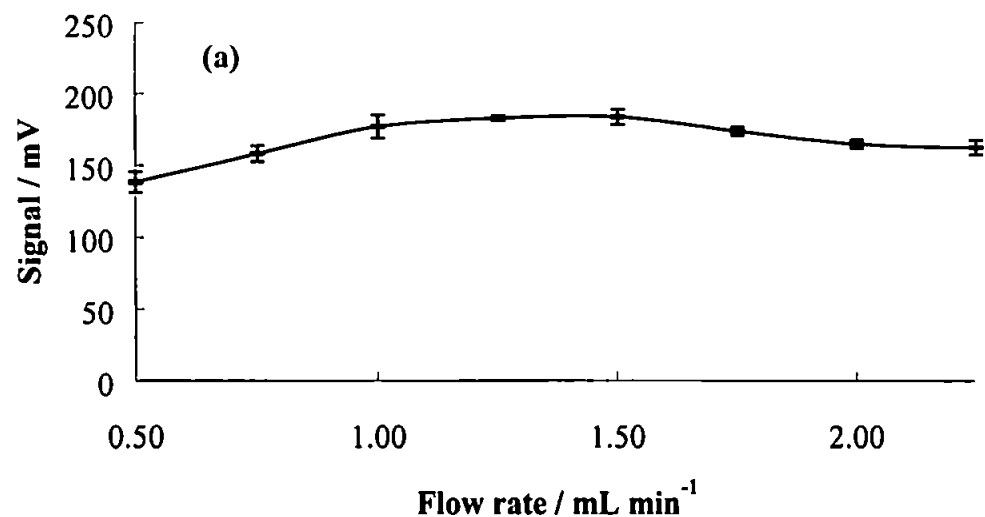


Figure 5.9: Optimisation of the FI-CL manifold for the determination of KIO_4 : a) Flow rate, b) pyrogallol concentration, c) hydroxylamine concentration and d) pH of buffer solution. Error bars represent 3s.

possible (*i.e.* with no reaction coil prior to detection). With the mixing point so close to the point of detection (as shown in Fig. 5.2), changing the overall flow rate of the reagents would not be expected to have a significant influence on the sensitivity. Maximum CL emission was found at 1.25 ml min^{-1} and although the effect of the flow rate was relatively small, this was selected as the optimum flow rate.

Increasing the pyrogallol concentration increased the CL emission up to a maximum value of $1 \times 10^{-3} \text{ M}$. With concentrations greater than this the emission sharply decreased due to quenching (self absorption) by the pyrogallol or one of the oxidation products.

Hydroxylamine concentration also reached a maximum at $1 \times 10^{-3} \text{ M}$. It has been proposed that enhanced CL produced by hydroxylamine is due to the product of the reaction between pyrogallol and hydroxylamine acting as a sensitiser (Evmiridis, 1987). This explains why an equimolar amount of hydroxylamine and pyrogallol is needed to produce maximum CL. When the concentration of hydroxylamine is greater than the concentration of pyrogallol there will be excess hydroxylamine present that has not reacted with pyrogallol. When this happens the free hydroxylamine reacts immediately with periodate to produce N_2O and iodine, explaining why a reduction in the sensitivity was seen when the concentration of hydroxylamine was greater than that of the pyrogallol ($1 \times 10^{-3} \text{ M}$). The pH was found to have little effect in the range 8.0 to 9.5 (when all reagents were buffered at the same pH).

The CL emission profile for the pyrogallol reaction was measured using a continuous flow system (see Fig.5.10). Maximum emission was observed at 560 nm, which is significantly different to the reported emission maximum of 630 nm for pyrogallol in the presence of hydrogen peroxide and formaldehyde (Biswas and Dhar, 1931).

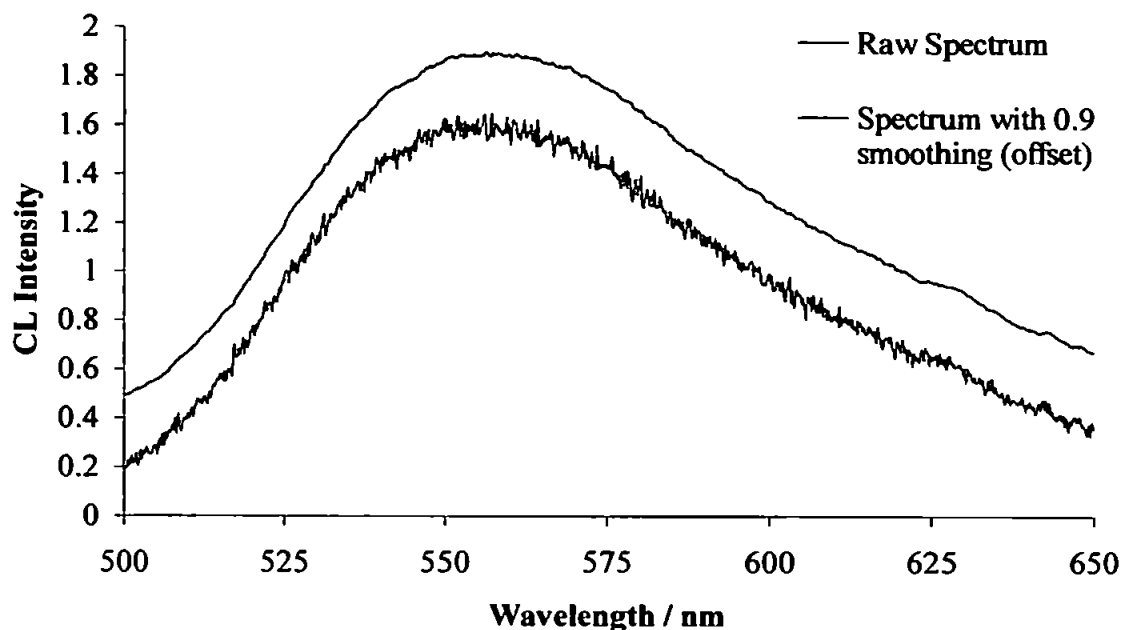


Figure 5.10: CL emission spectrum from the oxidation of pyrogallol.

This suggests that there is a different reaction mechanism and that emission is far more likely to be coming indirectly from reaction products rather than from direct emission from excited singlet oxygen.

Examples of periodate peaks are shown in Fig. 5.11, which demonstrate good reproducibility and excellent baseline stability. A calibration graph for potassium periodate is shown in Fig. 5.12.

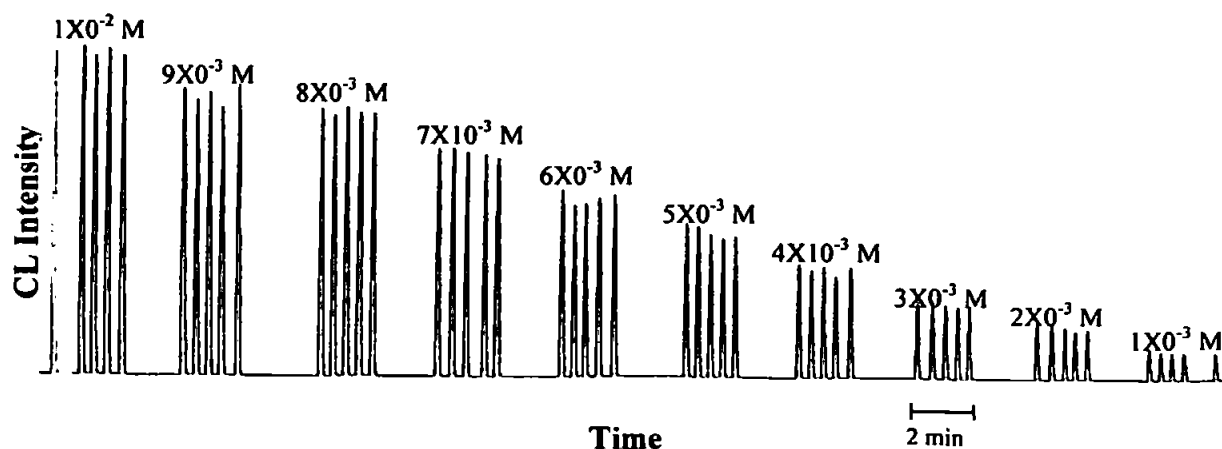


Figure 5.11: Periodate peaks from the optimised FI-CL manifold (1×10^{-3} – 1×10^{-2} M)

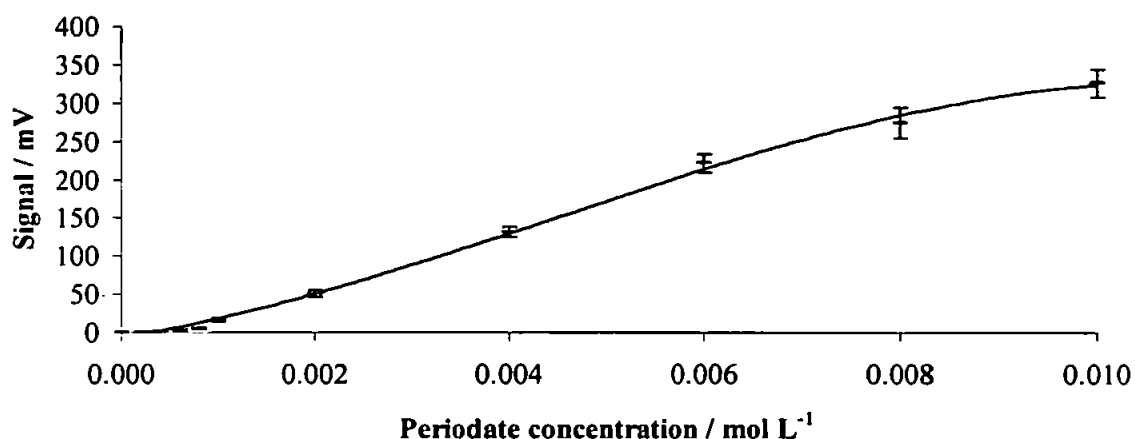


Figure 5.12: Calibration graph for potassium periodate using the optimised FI-CL manifold.

The calibration graph for potassium periodate was linear in the range 8×10^{-4} - 6×10^{-3} M ($R^2 = 0.9983$). At higher concentrations the graph levelled out due to reagent consumption. By expanding the baseline, the limit of detection for periodate was found to be 8×10^{-4} M ($S/N = 3$).

5.3.3 DETERMINATION OF ALCOHOLS USING THE FI-CL MANIFOLD

In order to determine alcohols using suppression of the pyrogallol CL reaction, periodate (the oxidant) was added to the carrier stream (which had previously been pH 9.5 phosphate buffer only) and samples (in pH 9.5 phosphate buffer) were injected into this (see Fig 5.4). Examples of suppressed CL peaks are shown in Fig. 5.13. Calibration graphs for ethylene glycol and ethanol are shown in Fig. 5.14.

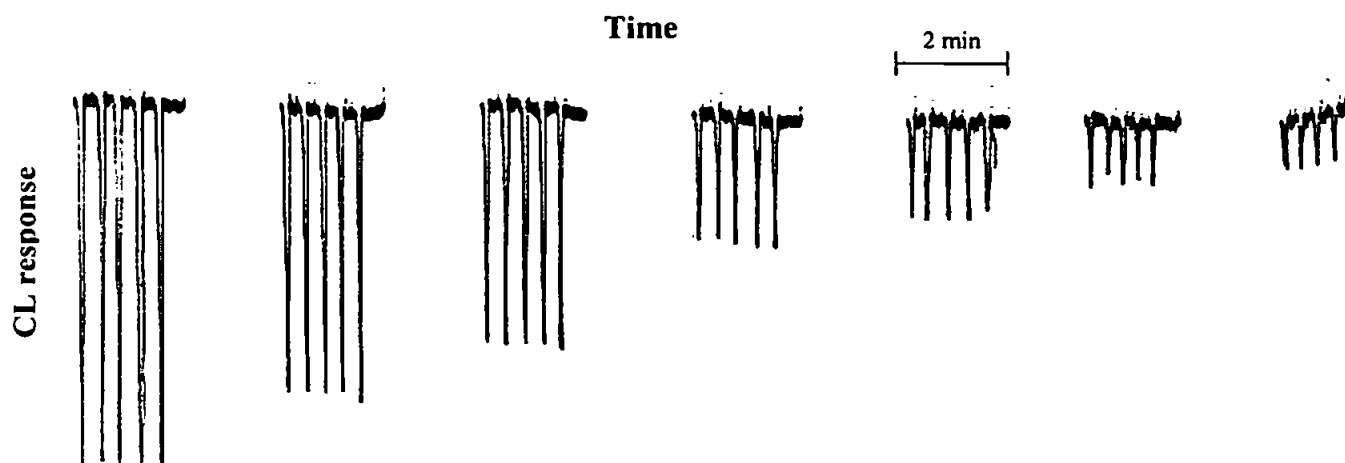


Figure 5.13: Ethylene glycol suppressed CL peaks.

The baseline noise level is much greater than previously shown. Previously, there was negligible background signal, so pulses in the reagent pumping (slightly altering the reagent concentrations in the reaction mixture) would have little effect. With this suppressed system there is a large background signal, and therefore pulses from the pumps cause variations in the chemiluminescence signal.

Limits of detection for ethylene glycol in pH 9.5 phosphate buffer (3 times the baseline noise) were 5×10^{-4} M for a 100 μL sample injection and 2×10^{-4} M for a 40 μL injection. The 100 μL sample loop gave a better calibration ($R^2 = 0.9909$), however RSDs were much larger than for the 40 μL sample loop (3 % and 0.2 % respectively for a 5×10^{-3} M ethylene glycol sample), most likely due to poorer mixing of the periodate stream. Limits of detection for ethanol were 1.3×10^{-3} M and 8×10^{-4} M (for 40 and 100 μL sample loops respectively). RSDs were again generally higher for the larger sample loop (6 % and 9 % for 40 and 100 μL sample loops respectively). For greater sensitivity, (which was more important than really low RSDs) a 100 μL sample loop was used in all further experiments.

Limits of detection were lower for ethylene glycol than for ethanol, which was as expected, due to the dual alcohol functionality present in ethylene glycol.

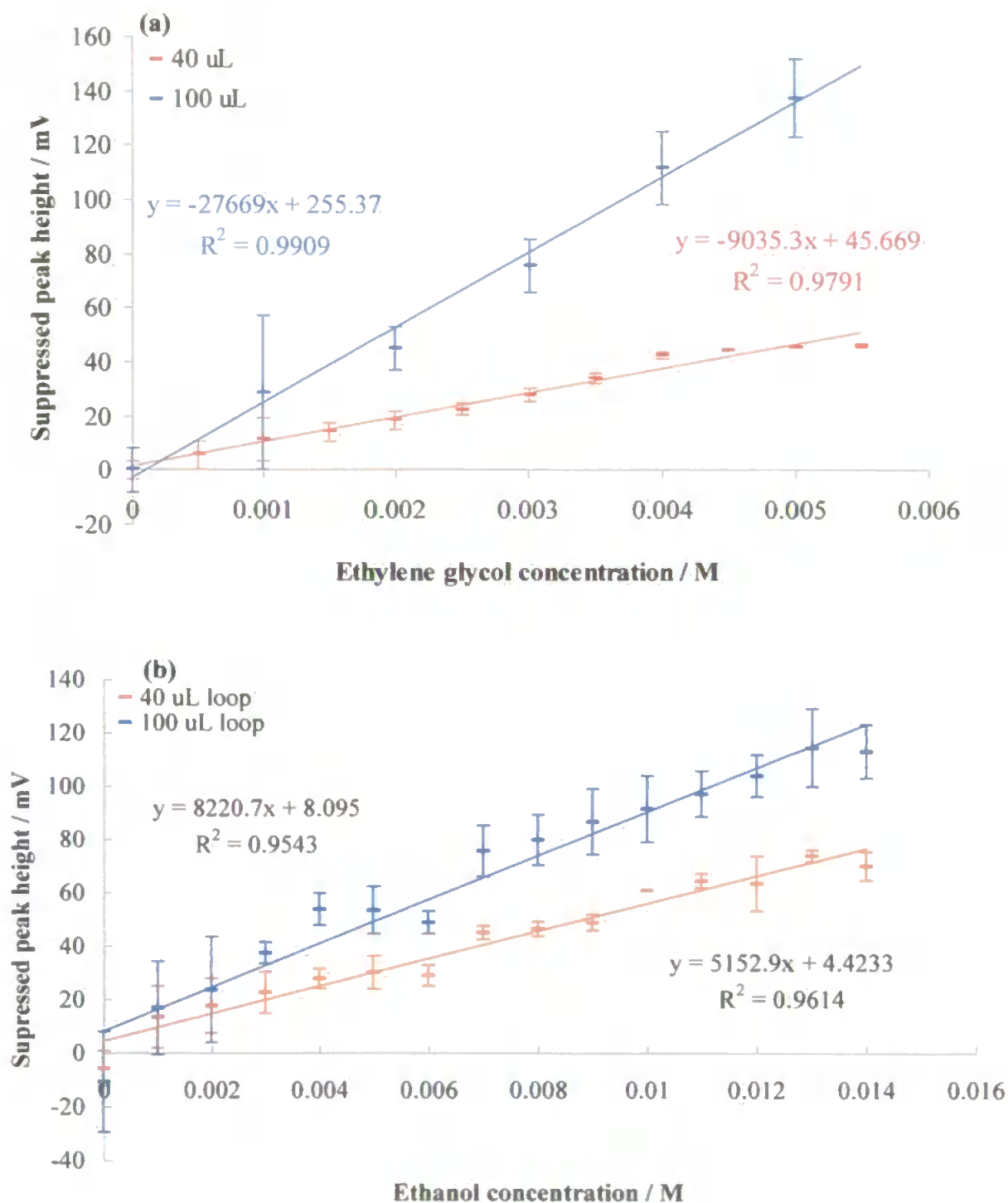


Figure 5.14: Calibration graphs for (a) ethylene glycol and (b) ethanol (error bars = 3s).

5.3.4 SOLVENT EFFECTS

The additive P655 is present in a carrier oil which is immiscible with water. In order to analyse it using FI-CL, a solid phase extraction procedure is again required. However, any solvent that is used as an extraction solvent in SPE must be compatible with the FI-CL reaction. If the extraction solvent suppresses all of the chemiluminescence then it is not possible to measure anything in the extract. For this reason the effect of various solvents was determined by directly injecting solvents into the FI-CL manifold. The effects of these solvents on the CL signal are shown in Fig. 5.15.

As can be seen above, all of the alcohols suppressed the CL reaction (as expected). They are therefore of no use as extraction solvents in solid phase extraction. Acetonitrile had the least effect on the CL signal and was therefore the most promising solvent for the extraction.

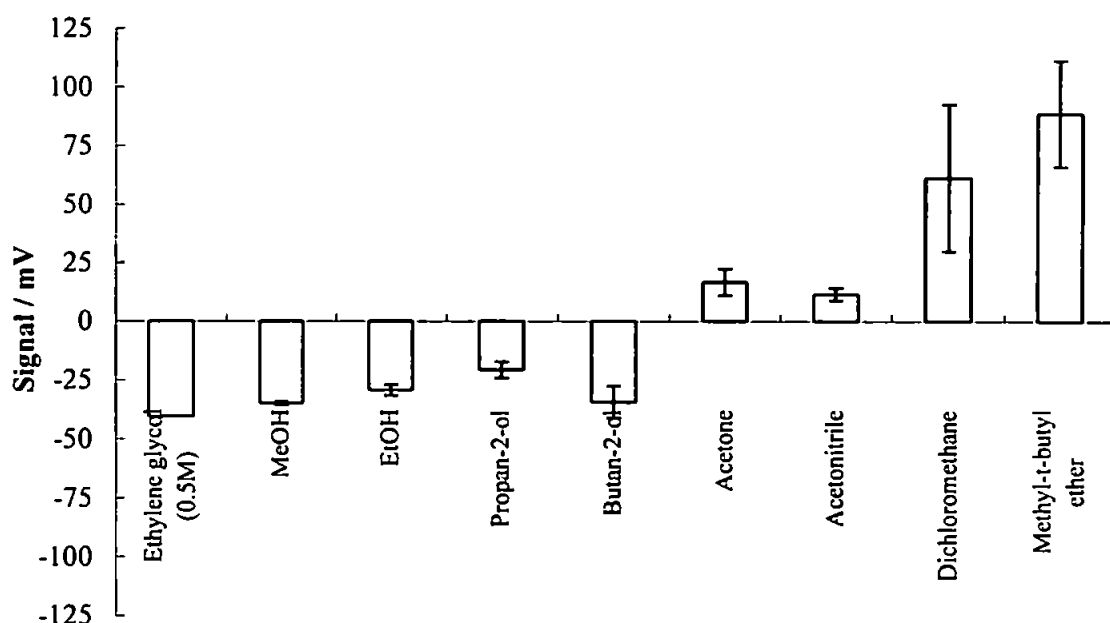


Figure 5.15: Effect on baseline of various solvents using the manifold shown in Fig 5.4 with 1×10^{-3} M KIO_4 as carrier.

5.3.5 GC VALIDATION OF SPE

Analysis of P655 can be performed using GC-MS (Forbes, 2000) as shown in Fig. 5.16. The temperature limit for this GC analysis was 380°C, however an expensive (and non-standard) high temperature GC column is needed to elute the di- and tri- ester components. When using a standard GC-MS fitted with a typical column (DB-5) that has an upper temperature limit of 340°C only the mono-ester component can be detected (see Fig. 5.17). The GC-MS is however able to resolve 2 distinct mono-esters, with the structures shown in Fig. 5.18.

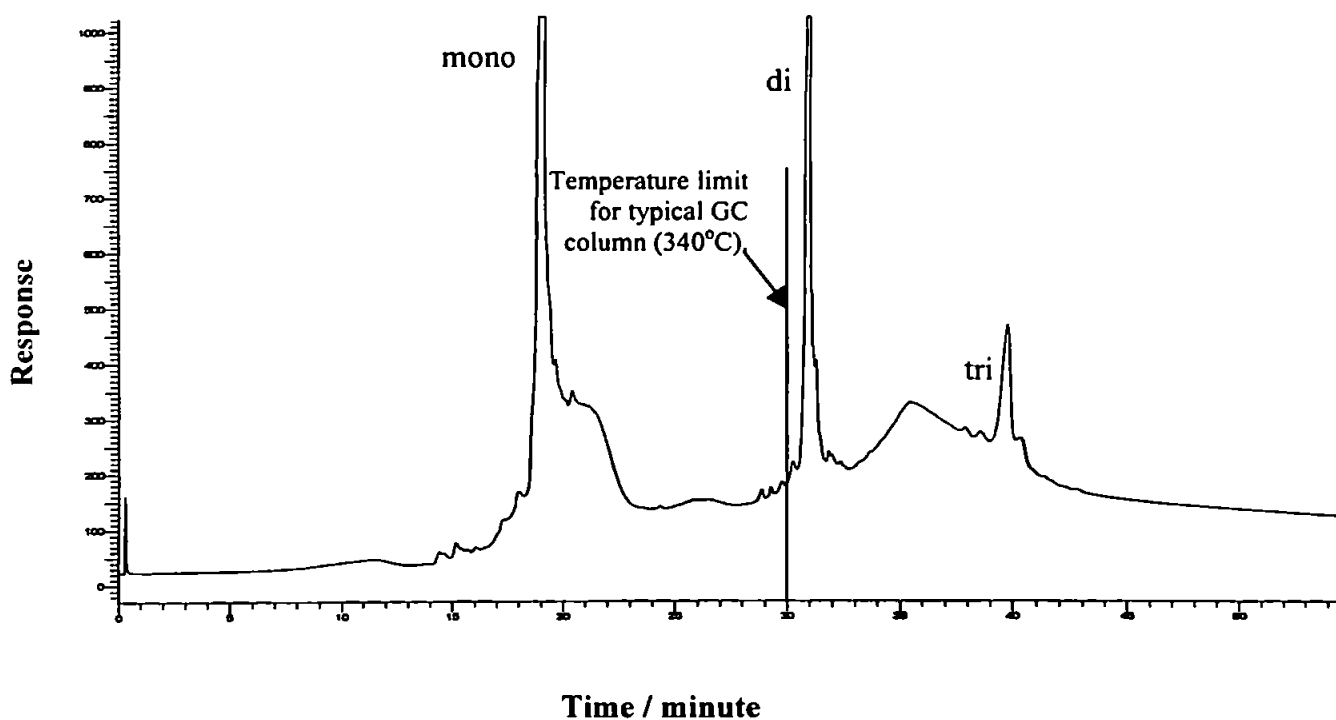


Figure 5.16: GC-MS of P655 diesel additive in carrier oil. Temperature program: 100°C held for 0 minutes, 8°C / minutes up to 380°C held for 20 minutes, detection using an FID (Forbes, 2000).

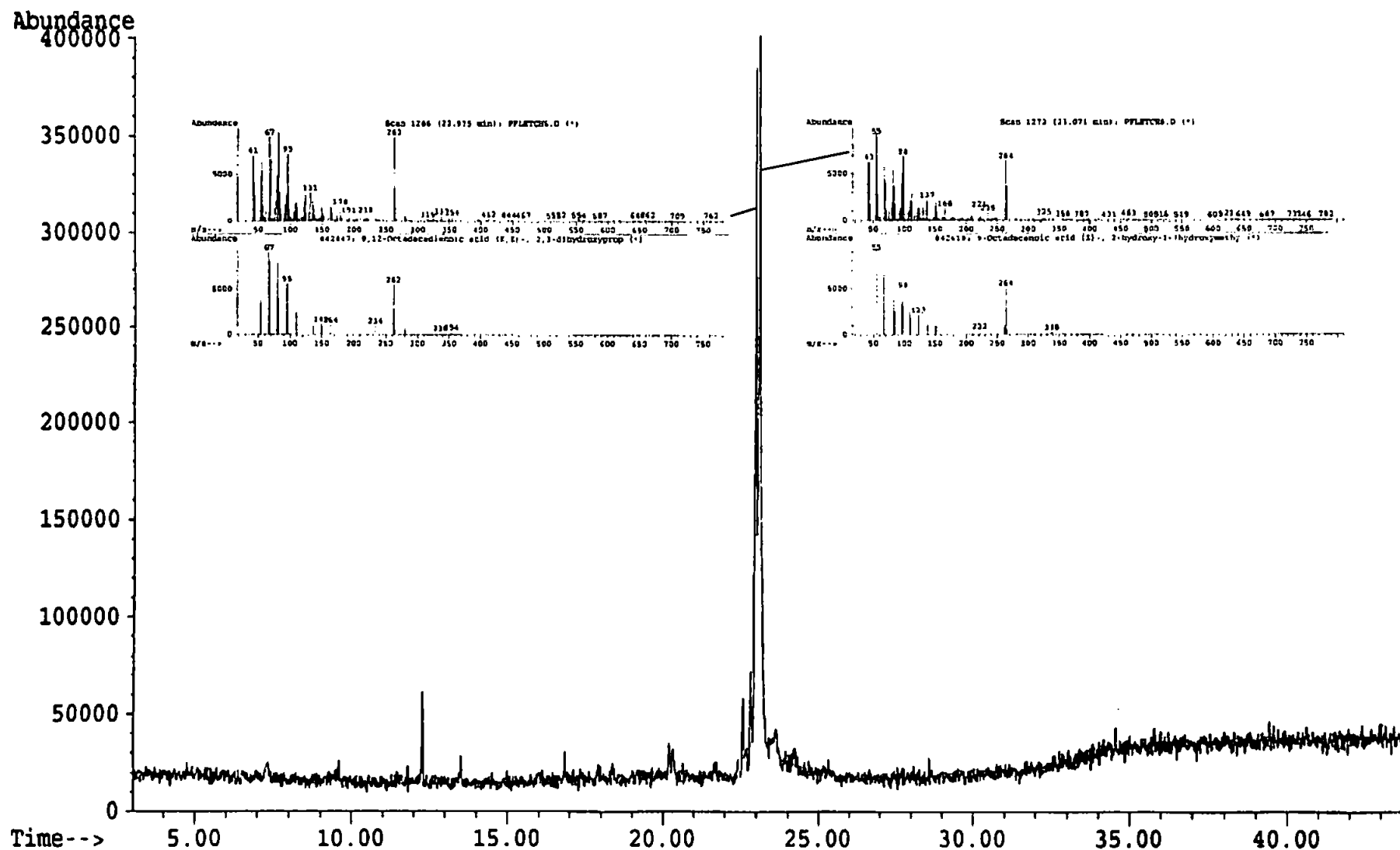


Figure 5.17: GC-MS Chromatogram of P655 using DB-5 column with temperature program: Injection at 50°C, 10°C / minute to 200°C, 5°C / minute to 300°C held for 10 minutes.

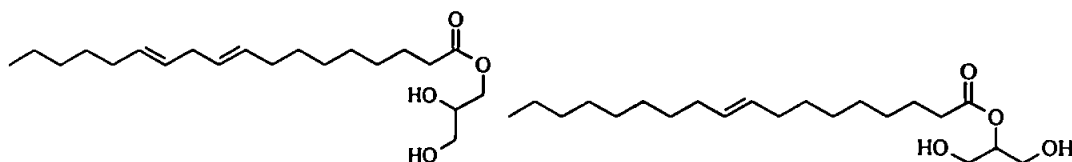


Figure 5.18: Left structure of peak at 22.975 mins (9,12-octadecadienoic acid (Z, Z)-, 2,3-dihydroxypropyl ester), right structure of peak at 23.071 mins (9-octadecenoic acid (Z)-, 2-hydroxy-1- (hydroxymethyl) ethyl ester).

GC-FID was used for the development of a solid phase extraction procedure. A typical GC-FID chromatogram for P655 in heptane solution is shown in Fig. 5.19. The column used also had a temperature limit of 340°C, and so (as with the GC-MS) only the mono-ester could be seen.

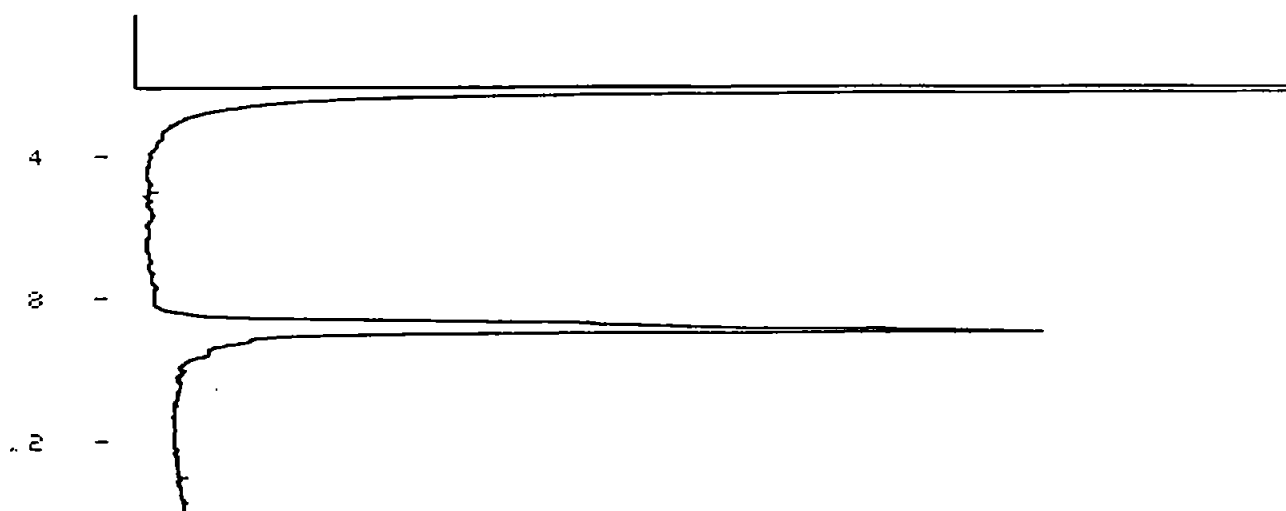


Figure 5.19: GC-FID chromatogram of 1000 mg L⁻¹ P655 in heptane (temperature program 200°C for 5 mins, 10°C / min up to 300°C).

5.3.6 OPTIMISATION OF THE SOLID PHASE EXTRACTION OF P655 IN HEPTANE

Using sequential 4 mL elutions of solvents with increasing polarity, an elution profile can be constructed. Fig. 5.20 shows elutions (measured by GC-FID) from an SPE column loaded with 4 mL of P655 solution (1000 mg L⁻¹ in heptane). Toluene with a solvent polarity of 0.29 was not strong enough to elute any of the P655, while dichloromethane

(polarity of 0.39) was strong enough to elute some of the P655 (76 % in 4 mL). Using a greater volume of solvent would have eluted more P655, however, a robust SPE method should be able to elute the desired component in a small volume to allow preconcentration, therefore a stronger elution solvent was needed. Acetonitrile (polarity of 0.65) eluted 95 % of the P655, which is an acceptable level of recovery, and no more P655 was recovered in the methanol elution. Acetonitrile was therefore shown to be a suitable solvent for this procedure.

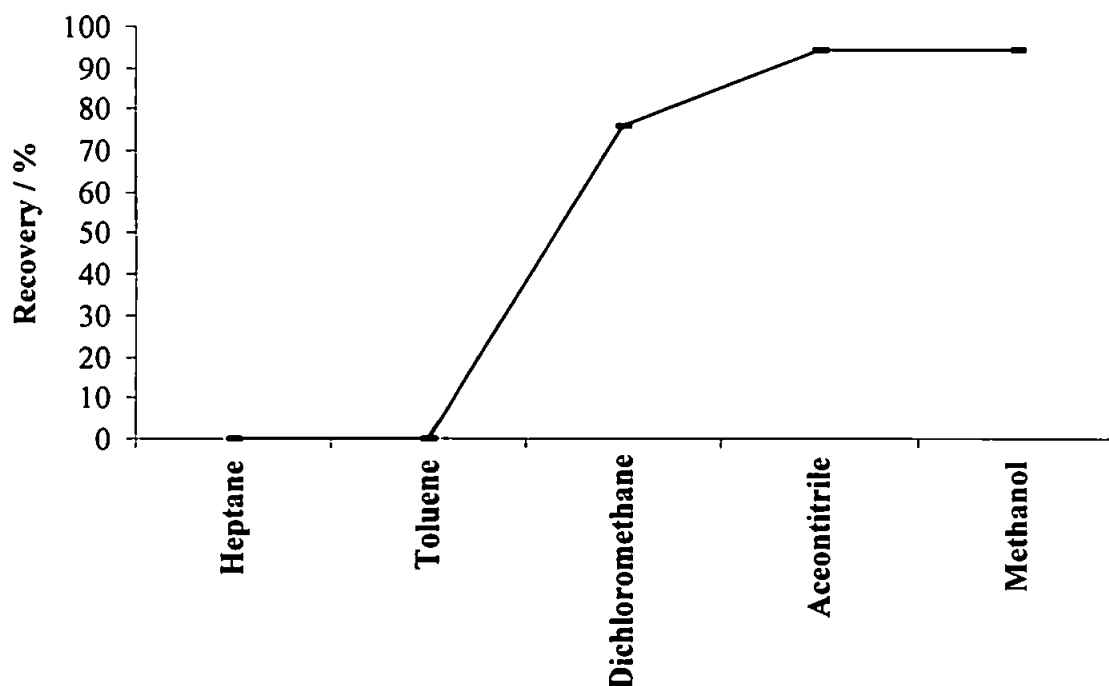


Figure 5.20: Sequential 4 mL solid phase extraction eluents (sample loaded was 100 mg L⁻¹ P655 in heptane).

4mL of ethyl acetate, which has a similar polarity to that of acetonitrile (0.62 and 0.65 respectively) was also used to extract P655 using the same conditioning and loading conditions as above. 93 % recovery was obtained, showing that ethyl acetate was also suitable as an extraction solvent for P655.

In order to optimise the elution volume required to extract P655 into acetonitrile, sequential elutions of 0.5 mL acetonitrile were analysed by GC-FID, the results of which are shown in Fig. 5.21. 94 % of P655 was extracted into 1 mL of acetonitrile, and no further significant improvement in recovery level was produced by using larger volumes.

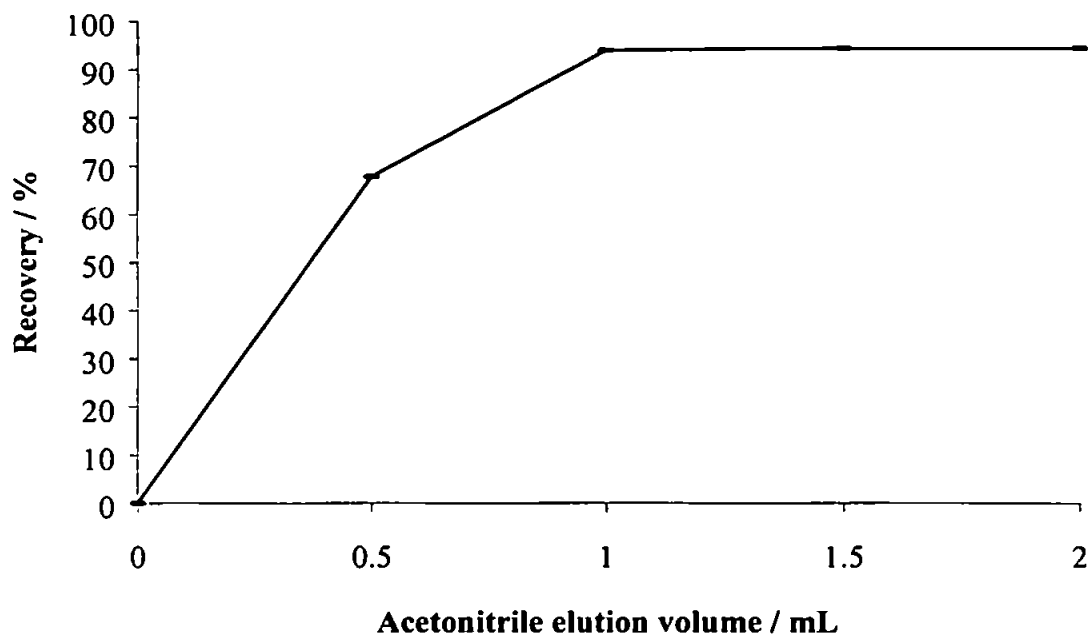


Figure 5.21: Elution profile for P655 with acetonitrile as elution solvent.

5.3.7 CHOICE OF ELUTION SOLVENT COMPATIBLE WITH FI-CL DETECTION

Ethyl acetate, acetonitrile and methanol all have the solvent strength to extract P655 using solid phase extraction. However if extracts are to be analysed using the FI manifold, the extraction solvent must be compatible with flow injection. For this reason, samples of the model poly-alcohol (ethylene glycol) were made up in each of the extraction solvents and these were injected into a carrier stream consisting of the same solvent (see Fig 5.5) to determine their effect on the response. Ethyl acetate in pH 8.2 buffer was injected in a carrier stream of the same buffer for comparison. The effects are shown in Fig. 5.22.

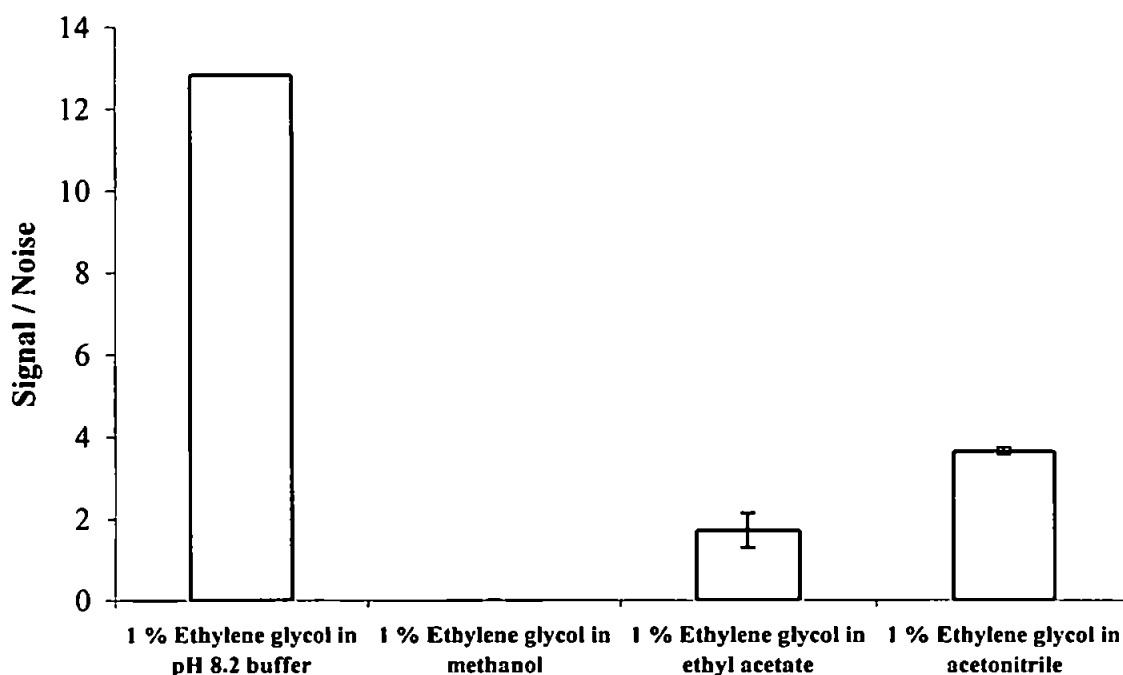


Figure 5.22: Measured suppression of a 1 % solution of ethylene glycol in various solvents using matched solvents as the carrier stream (errors bars = 3s).

When pH 8.2 buffer was used as the sample matrix / carrier stream a S/N ratio of 13 was measured (for 1 % ethylene glycol). No signal at all was observed when methanol was used as the carrier. This is expected, as methanol, having by its very nature alcohol functionality, reacts with all of the periodate present resulting in no CL reaction. Methanol was therefore not suitable as a carrier solvent for this reaction. A suppression of the CL response was obtained when ethyl acetate was used as the sample matrix and carrier (S/N = 1.6). The reproducibility was however not particularly good in this case (RSD of 8 %, n = 5). A greater sensitivity was obtained for acetonitrile than for ethyl acetate (S/N = 3.6) and the reproducibility was also much better (RSD of 0.8 %, n = 5). Acetonitrile was therefore determined to be the most applicable solvent for solid phase extracts that are to be analysed using this FI-CL manifold.

5.3.8 OPTIMISATION OF THE MODIFIED FI-CL MANIFOLD

The modified manifold shown in Fig. 5.5 was optimised for the detection of the poly-alcohol ethylene glycol in acetonitrile, with acetonitrile as the carrier. The optimisation graphs shown in Fig. 5.23 display the same trends that were seen with the unmodified manifold. The pH, however, was found to be more critical and the maximum sensitivity was obtained at pH 8.5.

When the solvents were being used as the carrier the signals measured contained a high degree of noise. Air bubbles were produced at the point where the solvent (carrier) stream mixed with the periodate stream. An example of this is shown in Fig. 5.25. The bubbles were dissolved oxygen in acetonitrile, which has been reported to come out of solution when acetonitrile is mixed with water (Freeman, 1999). The air bubbles caused positive spikes in the CL signal. Ultrasonication for 30 minutes reduced the bubbles, however, to fully eliminate the problem, helium sparging or fitting a debubbling device inline would be required. Time on this project, however, was limited and did not permit these modifications. A temporary solution was to use a larger sample loop (100 μ L) so that the samples had relatively long lifetimes compared with air bubble spikes.

5.3.9 ANALYSIS OF P655 EXTRACTS

The solid phase extraction procedure described in Section 5.2.3.9 (conditioning: 4 mL acetonitrile / toluene / heptane, loading 4 mL P655 in heptane, washing: 2 mL heptane / toluene, elution 4 mL acetonitrile) was used to extract samples containing P655 from heptane in the range 2500 – 100000 mg L⁻¹ (0.25-10% m/v). The extracted acetonitrile solutions were analysed using FI-CL, and example flow injection peaks are shown in Figs. 5.24 and 5.25.

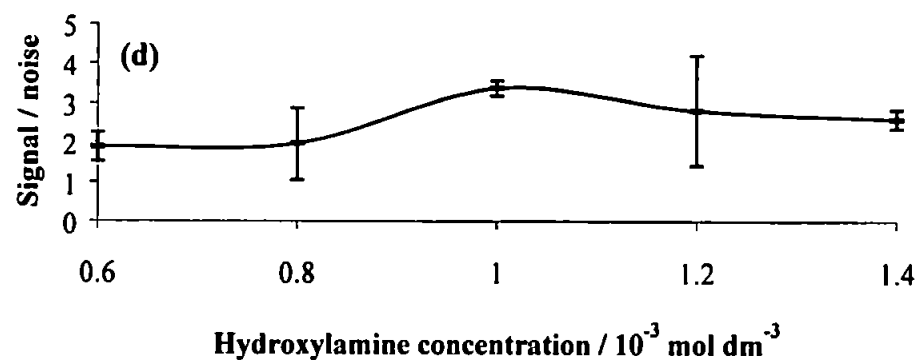
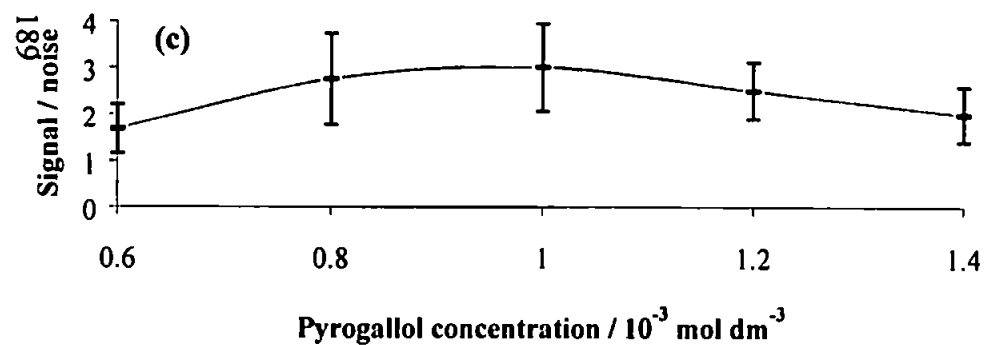
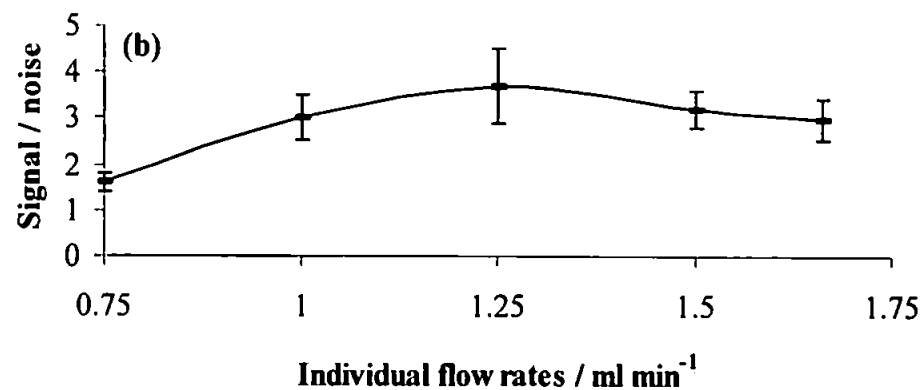
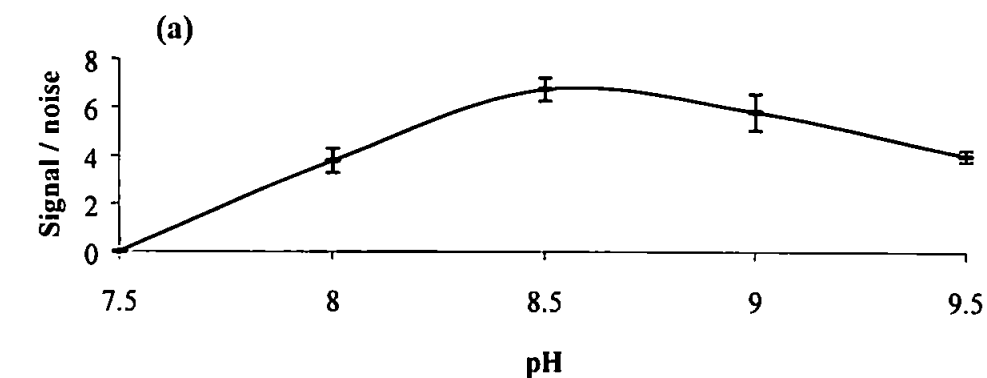


Figure 5.23: Optimisation charts for modified pyrogallol FI-CL manifold with acetonitrile as carrier solvent: a) pH optimisation, b) overall flow rate, c) pyrogallol concentration, and d) hydroxylamine concentration. Sample injected was 0.1 % (v/v) ethylene glycol in acetonitrile (error bars = 3s).

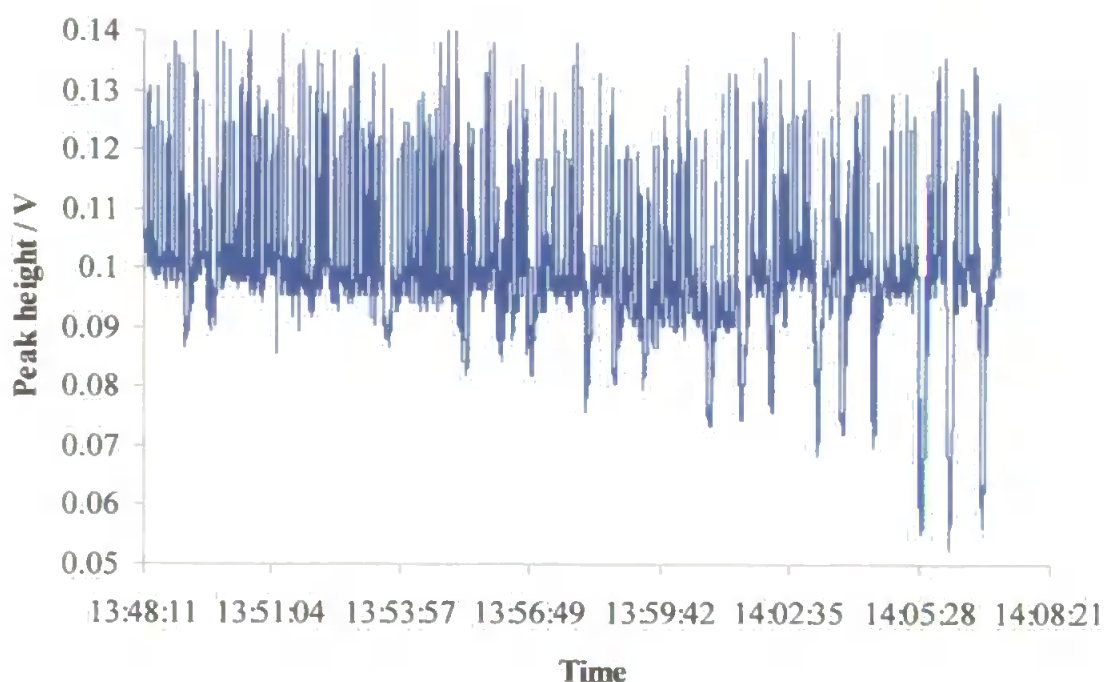


Figure 5.24: Example FI-CL peaks of P655 acetonitrile extracts (samples loaded in heptane in the range $0 - 15000 \text{ mg L}^{-1} = 0 - 1.5\% \text{ m/v}$).

As can be seen in Fig 5.24, the peaks appear to be very noisy but a major advantage of acquiring signals digitally on a computer is the ability to 'zoom in' on a particular peak. An enlargement of 3 replicate injections of a sample are shown in Fig 5.25. When the peaks are enlarged a clear difference can be seen between the baseline and the peaks. This was sufficient to allow useful quantitative information to be obtained and demonstrates the enhanced information recovery available with automated digital data collection.

An excellent calibration was obtained from P655 extracts over the range $0-150 \text{ mg} / 10 \text{ mL}$ extract, in spite of the noisy signal and is shown in Fig. 5.26. The limit of detection (measured by taking 3 times the predicted error in blank (Miller and Miller, 1993)) for P655 extracts in acetonitrile was $8.6 \text{ mg in } 10 \text{ mL} = 860 \text{ mg L}^{-1} = 0.086\% \text{ (m/v)}$. This L.O.D. is not particularly sensitive, however, no preconcentration was used for these samples. The linear range for P655 was $0 \text{ to } 150 \text{ mg in } 10 \text{ mL}$ ($0 - 1.5\%$). At higher

concentrations the calibration levels off due to all of the KIO_4 being used up (see Fig. 5.27).

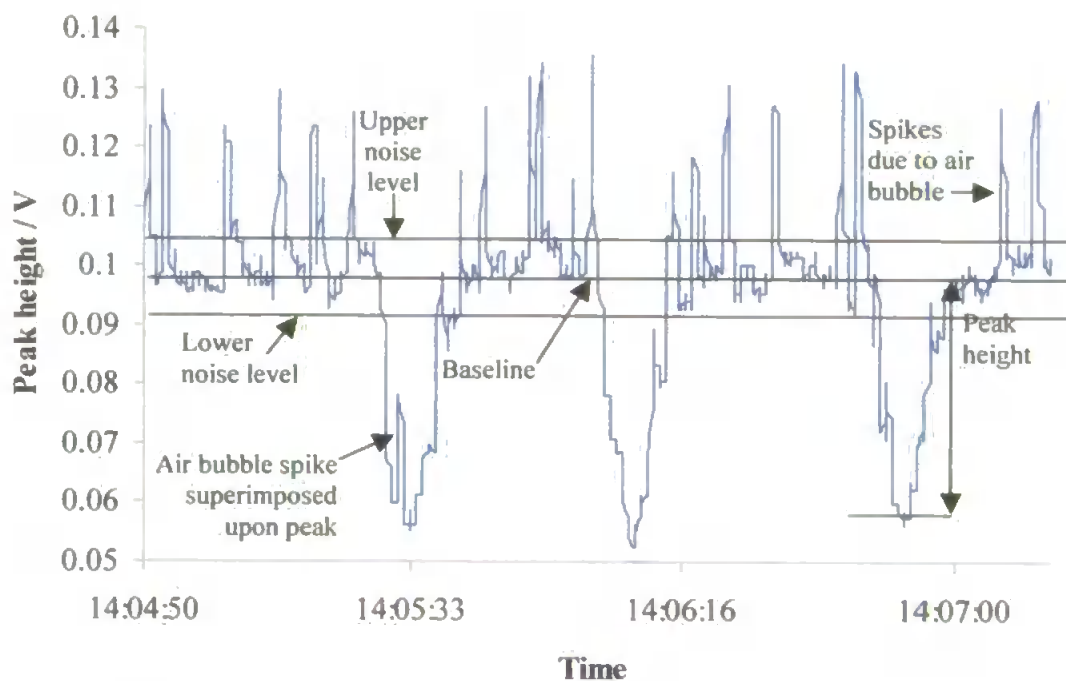


Figure 5.25: Peak height measurement for P655 extracts using FI-CL (a 1.5% m/v sample is shown).

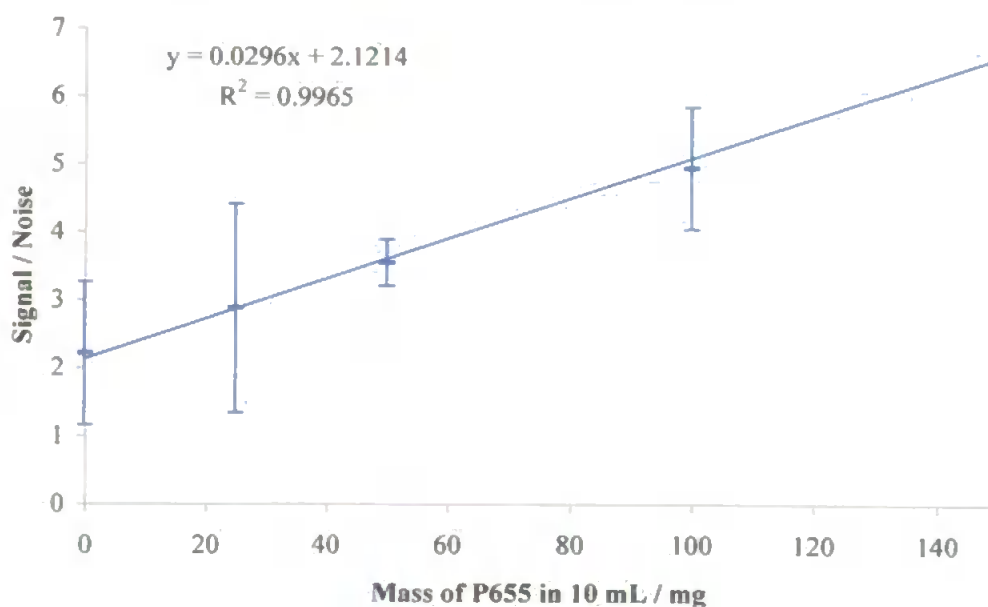


Figure 5.26: Acetonitrile extracts of P655 measured using FI-CL in the range 0-1.5 % m/v (error bars = 3s).

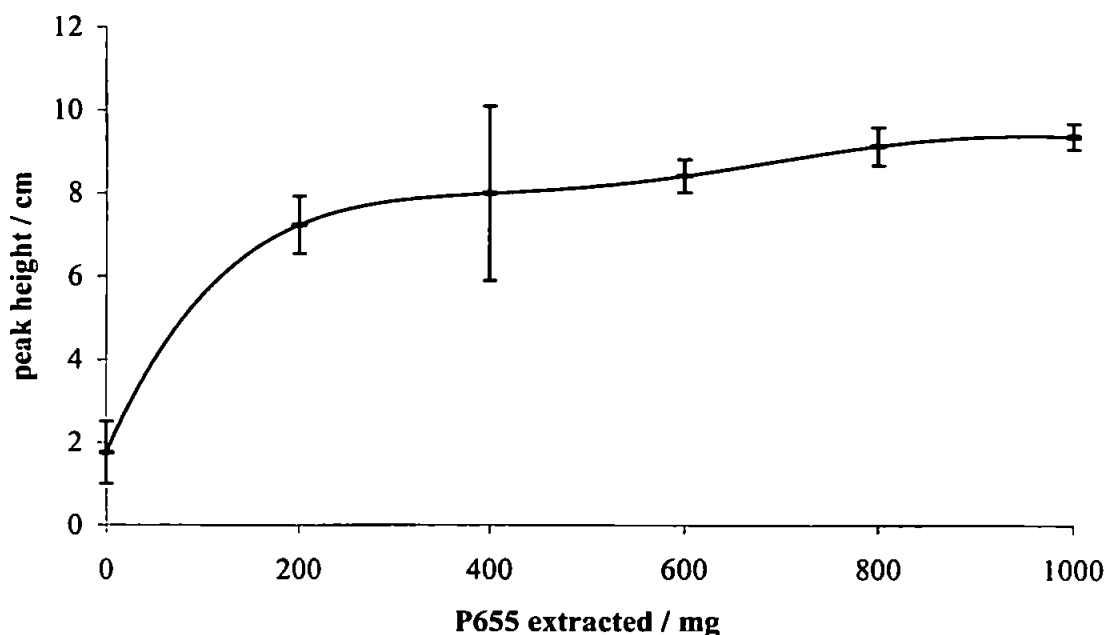


Figure 5.27: Extraction of higher concentrations of P655 in heptane (in the range 2-10 % m/v), error bars = 3s.

The limit of detection of 860 mg L^{-1} is significantly higher than the desired detection limit of 150 mg L^{-1} , however, these results were obtained using no preconcentration (4 mL was loaded and the extraction volume was 4 mL). With a larger sample volume being loaded, and using a smaller elution volume (1.5 mL), this reaction should be able to achieve detection limits of the order needed to detect P655 in diesel fuel. Further improvement could be also achieved by additional optimisation of the manifold design. Nonetheless the manifold shows that P655 can be quantitatively determined in a non-aqueous matrix using its suppressive effect on the CL emission from the periodate / pyrogallol oxidation reaction.

5.4 CONCLUSIONS

The specific conclusions from the research discussed in this chapter are:

1. Flow injection incorporating the pyrogallol oxidation chemiluminescence reaction with periodate can be used to determine potassium periodate in pH 9.5 buffer over the range 8×10^{-4} - 6×10^{-3} M ($R^2 = 0.9983$) with a limit of detection of 8×10^{-4} M (3 times the baseline noise).
2. Using the adapted FI-CL manifold described in this chapter, ethylene glycol and ethanol can be determined with limits of detection of 2×10^{-4} M and 8×10^{-4} M respectively.
3. The solid phase extraction procedure described in this chapter (conditioning: 4 mL of acetonitrile / toluene / heptane, loading: P655 in heptane, washing: 2 mL heptane / toluene, elution: 1.0 mL acetonitrile) can extract P655 from an organic matrix (heptane) with recovery rates of 94 %.
4. Acetonitrile extracts of P655 in heptane can be detected using FI incorporating the suppressed pyrogallol oxidation CL reaction with a limit of detection of 0.086 % (m/v) = 860 mg L^{-1} . Preconcentration would however be required to achieve detection limits below 150 mg L^{-1} which would be necessary for the determination of P655 in diesel fuels.

Chapter 6

Conclusions And Suggestions For Further Work

6 GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

6.1 GENERAL CONCLUSIONS

In addition to the specific conclusions listed at the end of each chapter, the following general conclusions can be made from the work described in this thesis.

1. Flow injection with peroxyoxalate / sulphorhodamine 101 chemiluminescence detection is a very sensitive, rapid and reproducible method for the determination of dodecylamine in acetonitrile / water mixtures. Up to 80 samples per hour could be analysed and DDA was measured with a detection limit of $190 \mu\text{g L}^{-1}$. The response was linear over the range $0.1 - 50 \text{ mg L}^{-1}$ ($R^2 = 0.9979$) with RSDs typically $< 4\%$. This reaction is therefore suitable for determining the additive in diesel fuel. The following indigenous diesel components have been shown to interfere strongly with this reaction: aniline, *p*-cresol, hexanoic acid, and indole (>1 , >300 , >100 and $>1 \text{ mg L}^{-1}$ caused a suppression equal to that of 5 mg L^{-1} DDA). Solid phase extraction is therefore needed prior to detection.
2. Solid phase extraction allows dodecylamine to be extracted into acetonitrile / water mixtures that are compatible with FI-CL using aminopropyl SPE columns. The SPE procedure used involves conditioning with 5 mL of each of methanol, toluene and heptane, loading with 25 % sample in heptane, washing with 2 mL of each of heptane, toluene and dichloromethane and elution with 2.5 mL of 90 / 10

acetonitrile / water. Greater than 92 % recoveries can be obtained from dodecylamine samples in heptane, and at least 75 % recoveries for all samples of diesel fuel spiked with an additive package containing dodecylamine at 40 mg L⁻¹. Gas chromatography can be used as a reference method to validate SPE procedures.

3. LabVIEW is an ideal software platform for graphical programming that can be used to control instrumentation such as pumps and valves and can also be used to acquire data from an analogue output. Constructing an on-line solid phase extraction FI-CL system offers the advantages of reducing analysis time (compared with off-line SPE with FI-CL detection), reducing the chance of entailing operator errors and simplifying the whole procedure. Throughput was 8 samples per hour for the manual on-line SPE manifold and 13 samples per hour for the automated dual column on-line SPE manifold. A reasonable correlation was obtained between off-line and on-line solid phase extraction with FI-CL detection ($R^2 = 0.8104$) for heptane and four base fuels (Swedish, SNV, Stanlow and Brazilian) that were unspiked, spiked with 40 mg L⁻¹ DDA or with the fully formulated additive package.
4. The pyrogallol oxidation chemiluminescence reaction can be used for the detection of periodate. Poly alcohols such as ethylene glycol and the lubricity additive P655 can also be determined using their competing reaction with periodate and its suppression of the CL emission. P655 can be extracted from heptane into acetonitrile with a recovery of 94 %. The limit of detection for P655 using solid phase extraction without preconcentration and FI-CL detection is 860 mg L⁻¹.

6.2 SUGGESTIONS FOR FUTURE WORK

1. The prototype field monitor has demonstrated that FI-CL with on-line SPE can be fully automated and applied to the determination of specific additives in fully formulated diesel fuels. However in order for this automated system to be useful as a routine field instrument for use in the petrochemical industry (*e.g.* at the filling station forecourt) it would need to be re-engineered to meet the demands of industrial safety requirements and the absence of mains power. This would need to be followed by extensive field trials and used by non-experts to ensure reliable long term operation.
2. In order to develop an SPE-FI-CL system capable of determining P655 at levels typically present in diesel fuels (*i.e.* 150 mg L⁻¹) the solid phase extraction procedure would need to be adapted to incorporate preconcentration to significantly lower the detection limit. This could be achieved by increasing sample loading and decreasing the elution volume and further optimisation of the elution protocol. The use of other solid phases such as cyano, alumina, fluorosil. and diol could also be investigated. The problem of air bubbles discussed in Section 5.3.8 could be address by introducing an in-line de-bubbler to the FI-CL manifold. The manifold could also be further adapted to allow on-line solid phase extraction as with the DDA system. Diesel samples spiked with P655 would then analysed using the optimised on-line SPE-FI-CL system.

extraction, as with the DDA system. Diesel samples spiked with P655 would then analysed using the optimised on-line SPE-FI-CL system and validated in a similar manner to the dodecylamine method.

3. The potential of FI-CL has been demonstrated for the determination of specific additives (DDA and P655) in diesel fuels. However for more generic applications, other additives would need to be targeted. For example, the reduction of nitrates to nitrite followed by the derivatisation reaction by hydrogen peroxide with luminol detection (Mikuska et al., 1995), as described in Section 1.4.1.2, could potentially be adapted for quantifying the cetane improver ethyl hexyl nitrate.

4. In order to better understand the nature of the amine catalysed peroxyoxalate / sulphorhodamine 101 reaction, fundamental studies on the reaction mechanism and kinetics could be undertaken. This would help to improve understanding of the specific roles of interfering species such as indoles, cresols, organic acids and anilines within this reaction, and may enable a further refinement of the SPE procedure in order to remove the most significant sources of interference from the eluted sample.

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Published Papers

Analytical applications of flow injection with chemiluminescence detection—a review

Philip Fletcher,¹ Kevin N. Andrew,¹ Anthony C. Calokerinos,² Stuart Forbes³ and Paul J. Worsfold^{1*}

¹Department of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK

²Department of Chemistry, University of Athens, Laboratory of Analytical Chemistry, Panepistimiopolis, 157 71 Athens, Greece

³Analytical Technology Business Group, Shell Global Solutions, Cheshire Business Park, PO Box 1, Chester CH1 3SH, UK

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ABSTRACT: This paper reviews the literature on analytical applications of flow injection (FI) techniques with chemiluminescence (CL) detection from 1995–1999. The focus is on the application of FI–CL to the quantitative determination of specific analytes in real sample matrices. Therefore, entries have been tabulated under the most appropriate application area, i.e. pharmaceutical, environmental, foods and beverages and biomedical, as defined by the matrix that has been analysed. Each table lists analytes alphabetically and gives details of the exact sample matrix, the limit of detection (as reported in the original paper) and comments on the CL reaction used. Copyright © 2001 John Wiley & Sons, Ltd.

KEYWORDS: flow injection; chemiluminescence; pharmaceutical; environmental; foods and beverages; biomedical

INTRODUCTION

Flow injection (FI) is now well established as an excellent technique for rapid, automated, quantitative analysis that combines on-line chemical and physical sample treatment with a range of flow-through detection systems in an enclosed, continuous flow environment. It is particularly well-suited to monitoring transient light emission from liquid phase chemiluminescence (CL) reactions due to the rapid and reproducible mixing of sample and reagent in close proximity to the detector.

FI has been used to investigate the fundamental chemistry of CL reactions, to optimize post column reaction conditions for liquid chromatography and to quantify analytes in relatively clean or synthetic matrices. In the last 5 years (1995–1999, the period covered by this review) however, there has been a notable increase in the application of FI–CL to the analysis of real sample matrices (1–227). This has been achieved by a combination of more sophisticated on-line sample treatment, e.g. the use of solid phase reagents to preconcentrate selected analytes and/or to remove the sample matrix, and the use of more inherently selective CL reactions. For an historical perspective on the development of FI–CL the

reader is referred to two previous reviews (228, 229) that cover the periods late 1970s–mid-1992 and 1991–mid-1995, respectively.

In view of this development, and to make the review more directly useful for analytical problem solving, papers have been classified by generic application area and analytes have been listed alphabetically within each category. The application areas are pharmaceutical (Table 1), environmental (Table 2), foods and beverages (Table 3) and biomedical (Table 4) and a group of papers in which the matrix has not been specified (Table 5). All limits of detection (LODs) are quoted as reported in the original paper.

CHEMILUMINESCENCE REACTIONS

If one looks at the range of chemistries used in FI–CL systems it is clear that variants of the luminol reaction are the most popular, but there are also a significant number of papers that utilize the oxidizing power of permanganate and cerium(IV). There are also several references to 1,10-phenanthroline, gallic acid/pyrogallol, lucigenin and peroxyoxalate. Other reagents that have been used include ruthenium(II), periodate, TCNQ, fluorescein and quercetin. The relatively small number of reactions involving peroxyoxalate is in marked contrast to the situation in liquid chromatography (LC) and is related to the fact that the majority of FI–CL applications involve aqueous matrices.

This review does not cover the use of CL detection

*Correspondence to: P. J. Worsfold, Department of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK.

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Table 1. Pharmaceutical applications

Analyte	Sample matrix	Reaction	LOD	Reference number
Amidopyrine	Injection solutions	Formaldehyde-acidified potassium permanganate CL	3×10^{-5} mol/dm ³	(1, 2)
Analgin	Pharmaceutical preparations	Auto-oxidation of analgin in the presence of Tween 80 with rhodamine 6G as a sensitizer immobilized on a cation-exchange column	0.15 mg/L	(3)
Analgin	Tablets	Ce(IV)-sulphuric acid CL with rhodamine 6G as a sensitizer	0.02 µg/mL	(4)
Ascorbic acid	Vitamin C tablets and multivitamin capsules	Luminol-Fe(II)-Na ₂ B ₄ O ₇ -potassium hydroxide CL	0.2 ng/mL	(5)
Ascorbic acid	Pharmaceutical samples and tablets	Luminol-K ₇ [Cu(IO ₆) ₂]-potassium hydroxide CL	1.5×10^{-8} mol/dm ³	(6)
Ascorbic acid	Pharmaceutical samples	Lucigenin CL with ascorbic acid in a basic medium, enhanced with iron(III) and Brij 35	2×10^{-9} mol/dm ³	(7)
Ascorbic acid	Tablets	Luminol-sodium hydroxide-hydrogen peroxide CL	8.6×10^{-9} mol/dm ³	(8)
Ascorbic acid	Pharmaceutical preparations	Luminol-potassium periodate-ascorbic acid CL	0.8 ng/mL	(9)
Ascorbic acid	Pharmaceuticals and blood	Potassium hexacyanoferrate(III)-luminol CL	4×10^{-8} mol/dm ³	(10)
Ascorbic acid	Tablets	Inhibition of luminol-hexacyanoferrate(III) CL (immobilized on an anion-exchange resin column, elution with sodium phosphate)	5.5×10^{-3} µg/mL	(11)
Aztreonam, penicillin G, cephalothin, 6-amino-penicillanic acid, 7-aminocephalosporanic acid, panipenem, latamoxef and faropenem	Aqueous	Luminol-hydrogen peroxide CL with hexacyanoferrate(III) and hexacyanoferrate(II) as catalysts	100, 60, 40, 20, 4, 2, 1 and 1 ng respectively (5 µL injection)	(12)
Benzocaine, butacaine, butoform, procaine, tetracaine	Pharmaceutical preparations	Acidic permanganate CL	30 ng/mL, 20 ng/mL, 30 ng/mL, 40 ng/mL and 3 ng/mL, respectively	(13)
Captopril	Pharmaceutical preparations	Cerium(IV)-sulphuric acid CL	2×10^{-7} mol/dm ³	(14)
Cefadroxil monohydrate	Pharmaceutical preparations and biological fluids	Potassium permanganate-sulphuric acid CL with quinine as sensitizer	0.05 µg/mL	(15)
Ciprofloxacin hydrochloride	Tablets and capsules	Cerium sulphate-sulphuric acid-Na ₂ SO ₃ CL	0.27 mg/L	(16)
Co(II)	Eye lotions	Lophine-Co(II)-hydrogen peroxide CL enhanced with hydroxylammonium chloride	4.5×10^{-8} mol/dm ³	(17)
Codeine	Pharmaceutical samples	Permanganate-polyphosphoric acid CL	2×10^{-7} mol/dm ³	(18)
Dopamine	Aqueous	Inhibition of the lucigenin-Fe(II)-Brij 35 CL reaction	2×10^{-9} mol/dm ³	(19)
Dopamine, adrenaline and isoprenaline	Injection solutions	Treatment with EDTA-Reineche's salt. Detection using luminol-hydrogen peroxide CL	4 ng/mL, 20 ng/mL and 16 ng/mL, respectively	(20)
Ergonovine maleate	Pharmaceutical preparations	Potassium hexacyanoferrate(III)-sodium hydroxide CL enhanced using hexadecylpyridinium chloride	0.07 µg/L	(21)
Furosemide	Tablets	Ce(IV)-sulphuric acid chemiluminescence, sensitized by rhodamine 6G	2.2×10^{-7} mol/dm ³	(22)
Glutathione and cysteine	Aqueous	Ce(IV)-hydrocortisone CL	2×10^{-7} mol/dm ³ and 1.4×10^{-6} mol/dm ³ respectively	(23)

Table 1 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
Hydrochlorothiazide	Pharmaceutical preparations and tablets	Ce(IV)–sulphuric acid CL, sensitized by rhodamine 6G	1.5×10^{-7} mol/dm ³	(24)
Hydrogen peroxide, glucose and ascorbic acid	Pharmaceuticals	Luminol–hydrogen peroxide–potassium periodate CL (indirect CL using glucose oxidase for the detection of glucose)	3×10^{-8} mol/dm ³ , 0.08 µg/mL and 6×10^{-8} mol/dm ³ respectively	(25)
Imipramine	Tablets	Imipramine–glyoxal–potassium permanganate CL	12 ng/mL	(26)
Imipramine and chlorpromazine	Urine	Acidified permanganate CL	5×10^{-5} mol/dm ³ and 2×10^{-5} mol/dm ³ respectively	(27)
Isoniazid	Pharmaceutical formulations	Inhibition of the luminol–hydrogen peroxide–potassium hexacyanoferrate(III) reaction	5 mg/L	(28)
Isoniazid	Pharmaceuticals	Mn(II)–luminol–potassium periodate CL	30 ng/mL	(29)
Levodopa	Tablets	Acidified permanganate CL	62 µg/L	(30)
Medazepam	Drug formulations	Potassium permanganate–sulphuric acid CL	1.85×10^{-5} mol/dm ³	(31)
Menadione sodium bisulphite	Injection solutions	Ce(IV)–menadione sodium bisulphite CL	2×10^{-3} µg/mL	(32)
Methotrexate	Injection solutions and tablets	Permanganate–H ₂ SO ₄ –formaldehyde CL	3.4×10^{-9} mol/dm ³	(33)
Morphine, sinomenine and codeine	Pharmaceutical preparations and tablets	Treated with EDTA–Reinecke's salt. Detected with luminol–hydrogen peroxide CL	60 ng/mL, 70 mg/mL and 600 ng/mL respectively	(34)
Naltrexone	Pharmaceutical preparations	Acidified permanganate CL	2.5 ng/mL	(35)
Naproxen	Pharmaceutical preparations	Ce(IV)–sulphuric acid CL	15 ng/mL	(36)
Paracetamol	Pharmaceutical formulations	Inhibition of the luminol–hydrogen peroxide–potassium hexacyanoferrate(III) reaction	2.5 µg/mL	(37)
Penicillamine	Pharmaceutical preparations	Cerium(IV)–sulphuric acid CL with quinine as sensitizer	15 pmol (50 µL injection)	(38, 39)
Perphenazine	Drug formulations	Acidified permanganate CL	50 mg/L	(40)
Persantin	Pharmaceutical preparations and tablets	Sodium hypochlorite CL with Triton X-100 as enhancer	11 ng/mL	(41)
Phenothiazines	Pharmaceutical preparations and biological fluids	Cerium(IV)–acid CL with rhodamine B as a sensitizer	0.01–0.1 µg/mL	(42)
Prednisone acetate	Tablets	Na ₂ SO ₃ –ammonium ceric sulphate–sulphuric acid CL	31 µg/L	(43)
Progesterone and hydrocortisones	Aqueous	Cerium(IV)–cysteine–sulphuric acid CL sensitized by the analyte	0.10 µg/mL	(44)
Promethazine hydrochloride	Tablets	Potassium permanganate–oxalic acid CL	3.5×10^{-8} g/mL	(45)
Pyridoxine hydrochloride	Tablets	Luminol–hydrogen peroxide CL	6 µg/mL	(46)
Reserpine	Injection solutions	Permanganate–hydrogen peroxide–H ₆ P ₄ O ₁₃ CL	0.3 µg/mL	(47)
Riboflavine	Injection solutions and tablets	Acidified potassium permanganate–sodium dithionite CL	62 ng/mL	(48)
Rutin	Sophora japonica L (traditional Chinese medicine)	Luminol–potassium hexacyanoferrate CL	6.7×10^{-9} g/mL	(49)
Rutin	Traditional Chinese medicines	Sodium hypochlorite–rutin–semicarbazide hydrochloride CL	13 pg/L	(50)
Salicylamide	Human urine and pharmaceutical formulations	Acidified potassium permanganate CL	30 ng/mL	(51)
Sodium nitroprusside	Pharmaceuticals	Luminol–hydrogen peroxide CL	9×10^{-9} mol/dm ³	(52)

Table 1 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
Tannic acid	Chinese gall (traditional medicine)	Inhibition of luminol–hydrogen peroxide–Cu(II) CL	9×10^{-9} mol/dm ³	(53)
Tetracyclines	Commercial formulations	Acidic permanganate CL with octylphenyl polyglycol ether as sensitizer	0.4–0.6 µg/mL	(54)
Tetracyclines	Pharmaceutical preparations	Cerium(IV)–sulphuric acid CL with quinine as sensitizer	0.025–0.25 nmol (50 µL injection)	(55)
Tetrahydropalmatine	Pharmaceutical preparations and tablets	Acidified potassium permanganate–sodium dithionite CL	3.2 ng/mL	(56)
Thiamine nitrate	Tablets	Potassium hexacyanoferrate(III)–sodium hydroxide–uranine CL	2.0×10^{-3} mol/dm ³	(57)
Tiopronin	Pharmaceuticals	Ce(IV)–sulphuric acid CL using rhodamine 6G and quinine as fluorophores	1×10^{-7} mol/dm ³	(58)
Tiopronin	Pharmaceuticals	Cerium(IV)–sulphuric acid CL with quinine as a sensitizer	3.4×10^{-7} mol/dm ³	(59)
Vitamin B ₆	Injection solutions and tablets	Na ₂ S ₂ O ₃ –potassium permanganate–sodium polyphosphate CL	58 ng/mL	(60)

Table 2. Environmental applications

Analyte	Sample matrix	Reaction	LOD	Reference number
Acetaldehyde	River/waste water	Gallic acid–hydrogen peroxide–sodium hydroxide CL	0.31 ng/mL	(61)
As	Ores and rocks	Dissolution with HCl. Reduction of As(V) to As(III) using potassium iodide–thiourea followed by luminol–hydrogen peroxide–Cr(III) CL detection	3.4×10^{-5} mol/dm ³	(62)
As(III)	Geochemical samples	Sample mixed with K ₂ Cr ₂ O ₇ –H ₂ SO ₄ followed by luminol–hydrogen peroxide CL	1×10^{-10} mol/dm ³	(63)
<i>Chattonella antiqua</i> (red tide phytoplankton)	Aqueous	2-Methyl-6-(<i>p</i> -methoxyphenyl)-3,7-dihydroimidazo[1,2- α]-pyrazin-3-one–superoxide CL	2×10^3 cells/mL	(64)
Chlorine	Tap water	Luminol is immobilized on an anion exchange resin column. Sodium hydroxide is passed through the column to elute luminol, which is mixed with a sample stream to produce CL	8×10^{-9} g/mL	(65)
Co(II)	Seawater	Preconcentration using 8-quinolinol immobilized on 8HQ-MAF and CL detection using gallic acid–hydrogen peroxide	0.62 ng/L	(66)
Co(II)	River, sea and tap water	Luminol chemiluminescence enhanced with CO _{2(g)}	5×10^{-13} mol/dm ³	(67)
Co(II)	Sea water and river water	Quercetin–hydrogen peroxide–potassium hydroxide reversed flow injection CL	0.1 ng/mL	(68)
Co(II) and Cr(III)	Mineral waters	Cetyltrimethylammonium bromide–hydrogen peroxide–luminol CL	10 pg/mL and 12 pg/mL respectively	(69)
Co(II) and Ni(II)	Hair	Microwave digestion in HNO ₃ and hydrogen peroxide. Detection using alizarin purple–ethanol cetyltrimethylammonium bromide–potassium hydroxide	0.1 µg/mL	(70)
Cr(III)	Tap water	Hydrogen peroxide–luminol CL	0.5 µg/mL	(71)

Table 2 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
Cr(III) and Cr(VI)	Waste water	Reduction to Cr(III) using hydrogen peroxide followed by detection with luminol–hydrogen peroxide CL	$<10^{-9}$ mol/dm ³	(72)
Cr(III) and Cr(VI)	Natural waters	On-line oxidation of Cr(III) to Cr(VI) on a PbO ₂ column followed by quercetin–hydrogen peroxide–potassium hydroxide CL	1 ng/mL	(73)
Cr(III) and Cr(VI)	Waste water	Reduction using copper-coated zinc followed by luminol–hydrogen peroxide CL detection	2.3×10^{-8} mol/dm ³	(74)
Cr(VI)	Waste water	CL produced by luminol and hexacyanoferrate(II) (immobilized on an anion-exchange resin column, eluted with sodium phosphate)	0.014 µg/mL	(75)
Cu (complexed) Cu(II)	Sea water	1,10-Phenanthroline–hydrogen peroxide CL	1×10^{-10} mol/dm ³	(76)
	Hair	Luminol–potassium permanganate immobilized onto D201*7 anion-exchange resin. Elution with sodium hydroxide to react with Cu(II) to produce CL	0.2 ng/mL	(77)
Cu(II)	Natural waters	Immobilized luminol–cyanide are eluted with Na ₃ PO ₄ and mixed with the sample–sodium hydroxide for CL detection	1.3×10^{-9} mol/dm ³	(78)
Cyanide	River water	Sulphite generated by the reaction of cyanide and sodium thiosulphate catalysed by immobilized rhodanese reacts with immobilized sulphite oxidase and produces sulphate and hydrogen peroxide, which is detected with luminol and peroxidase	1.2×10^{-8} mol/dm ³	(79)
Cyanide	Tap and waste water	Luminol immobilized on Amberlyst resin with copper immobilized on D151 resin. CL is produced with cyanide	2×10^{-9} g/mL	(80)
Fe (dissolved)	Sea water	Preconcentration with TSK-8HQ, followed by detection with luminol–hydrogen peroxide CL	2.2×10^{-11} mol/dm ³	(81)
Fe and Mn (dissolved)	Underground water	Luminol–potassium periodate CL	3×10^{-6} µg/mL and 5×10^{-6} µg/mL respectively	(82)
Fe(II)	Natural waters	Luminol–hydrogen peroxide CL	2×10^{-9} mol/dm ³	(83)
Fe(II)	Treated waters	O-phenanthroline–luminol–potassium periodate CL (reversed flow-injection)	3 ng/L	(84)
Fe(II)	Hair	Lucigenin–sodium hydroxide CL with cetyltrimethylammonium bromide as sensitizer	2 pg/mL	(85)
Fe(II) and Fe(III)	Sea water	Preconcentration with 8-HQ, followed by detection with luminol CL	4×10^{-11} mol/dm ³	(86)
Fe(II) and Fe(III)	Natural waters	Fe(III) reduced to Fe(II) with Cu-coated Zn. Luminol immobilized on an anion-exchange resin. Eluted with sodium hydroxide for CL detection	0.4 ng/L	(87)
Fe(II) and hydrogen peroxide	Rain water	Oxygen–peroxyoxalate CL	$<1 \times 10^{-7}$ mol/dm ³	(88)
Fe(II) and total Fe	River and sea water	Preconcentration on Amberlite XAD-4 functionalized by N-hydroxyethylethyl-ethylenediamine groups followed by brilliant sulphoflavine–hydrogen peroxide CL detection	0.80 nmol/L and 0.36 nmol/L, respectively	(89)
Fe(II) and total Fe	Human hair and natural waters	Luminol–hydrogen peroxide CL enhanced with cationic surfactant tetradecyltrimethylammonium bromide	2×10^{-9} mol/dm ³ and 1×10^{-9} mol/dm ³ , respectively	(90)

Table 2 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
<i>Heterosigma carterae</i> (red tide phytoplankton)	Aqueous	2-Methyl-6-(<i>p</i> -methoxyphenyl)-3,7-dihydroimidazo[1,2- α]-pyrazin-3-one (MCLA)-superoxide CL	1×10^2 cells/mL	(91)
Hg(II)	Environmental waters	Cu(II)-luminol-sodium hydroxide-potassium hexacyanoferrate(II) CL	0.33 $\mu\text{g/mL}$	(92)
Hydrazine	Drinking water	CL produced from N-bromosuccinimide in alkaline medium with dichloro-fluorescein as sensitizer	5×10^{-7} mol/dm ³	(93)
Hydrazine	Air	Absorption in sulphuric acid followed by luminol-potassium periodate-sodium hydroxide CL	2 $\mu\text{g/m}^3$	(94)
Hydrogen peroxide	Rain water	Octylphenyl polyglycol ether-acidic potassium permanganate CL	6.0×10^{-9} mol/dm ³	(95)
Hydrogen peroxide	Snow water	Potassium periodate-potassium carbonate CL	5×10^{-9} mol/dm ³	(96)
Hydrogen peroxide	Sea water	Co(II)-luminol CL	1.06×10^{-8} mol/dm ³	(97)
Hydrogen peroxide	Rain water	Immobilized Co(II) and luminol are eluted with NaSO ₄ . CL is produced in the presence of hydrogen peroxide	3.5×10^{-8} mol/dm ³	(98)
Hydrogen peroxide	Rain water	Luminol and Co(II) are immobilized on a strongly basic anion-exchange resin and a weakly acid cation-exchange resin, respectively. Reagents are eluted by hydrolysis for CL detection.	1.2×10^{-8} mol/dm ³	(99)
Hydrogen peroxide	Air	Hydrogen peroxide diffusion scrubbed. Detection with 1,1'-oxalyldi-imidazole-peroxyoxalate CL	3.4 ppbv	(100)
Hydrogen peroxide	Sea water	Co(II)-hydrogen peroxide-luminol CL	4.2×10^{-10} mol/dm ³	(101)
Hypochlorite	Tap water	Oxidation of indole in propan-2-ol with hydrogen peroxide	5 $\mu\text{g/mL}$	(102)
Ir(IV)	Ores and rocks	Potassium hydroxide-hydrogen peroxide-Tween-80 CL	11 pg/L	(103)
Mn	Human hair	Sample ashed and dissolved in acid. Detected using I ₂ -luminol CL	0.1 ng/mL	(104)
Mn(II)	Potable water	7,7,8,8-Tetracyanoquinodimethane oxidation with Eosin Y as sensitizer	4.5 $\mu\text{g/mL}$	(105)
Mn(II)	Natural waters	Immobilized luminol and IO ₄ ⁻ eluted with Na ₃ PO ₄ for CL with Mn(II)	1×10^{-9} g/mL	(106)
Mn(II)	Sea water	Luminol-hydrogen peroxide CL	2.9×10^{-11} mol/dm ³	(107)
Nitrite	Natural waters	Nitrite reacts with hydrogen peroxide to form peroxynitrite, which produces CL with luminol	1×10^{-9} mol/dm ³	(108)
Nitrite	Drinking water and food	Luminol-I ₂ (produced from potassium iodide in acid) CL	1.6 ng/mL	(109)
Oxygen (dissolved)	River and tap water	Reaction with MnSO ₄ -iodine-potassium iodide-ammonia to form a precipitate that is dissolved in H ₂ SO ₄ -H ₃ PO ₄ . Detection with luminol-sodium hydroxide	0.412 mg/L	(110)
Ozone	Treated waters	Luminol-ozone CL using gas diffusion FIA	8 $\mu\text{g O}_3 \text{ L}$	(111)
Pb(II)	Natural waters	Lucigenin-hydrogen peroxide-sodium hydroxide CL enhanced with ethanol	0.1 $\mu\text{g/mL}$	(112)
Pb(II)	Waste water	Pb(II) replaces Fe(II) from an EDTA complex. Fe(II) reacts with luminol-sodium hydroxide to produce CL	20 ng/L	(113)
Phosphate	Natural waters	Hydrogen peroxide produced from the reaction of immobilized pyruvate oxidase with phosphate is detected using luminol-horseradish peroxidase CL	7.4×10^{-8} mol/dm ³	(114, 115)

Table 2 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
Phosphate	Drinking water	Phosphate ion-dependent pyruvate oxidase reaction produces hydrogen peroxide which is detected using luminol CL catalysed by <i>Arthromyces ramosus</i> peroxidase	1.6×10^{-7} mol/dm ³	(116)
Phosphate	River water	Immobilized pyruvate oxidase G producing hydrogen peroxide for luminol CL detection	9.6×10^{-8} mol/dm ³	(117)
Phosphate	River water	Maltose phosphorylase, mutarotase, and glucose oxidase immobilized on N-hydroxysuccinimide beads with <i>Arthromyces ramosus</i> peroxidase–luminol CL detection	1×10^{-6} mol/dm ³	(118)
Phosphate	Natural waters	Purine nucleoside phosphorylase and xanthine oxidase immobilized on amino-propyl-controlled pore glass beads convert phosphate to hydrogen peroxide for peroxyoxalate–rhodamine B CL detection	3.9×10^{-8} mol/dm ³	(119)
Phosphorus	Natural waters	Conversion to phosphomolybdic acid using HClO ₄ –ammonium molybdate with luminol–sodium hydroxide CL detection	36 µg/mL	(120)
Polyaromatic hydrocarbons	Organic (hexane or acetonitrile)	Peroxyoxalate–hydrogen peroxide CL	0.6–79 µg/mL	(121)
Sb(III)	Ores and rocks	K ₂ Cr ₂ O ₇ –luminol–sodium hydroxide CL	0.1 ng/mL	(122)
Sulphite	Tap water	Auto-oxidation sensitized by rhodamine 6G (immobilized on cation exchange resin) enhanced with Tween 80	0.01 mg/L	(123)
Sulphite and sulphur dioxide	Water and air	Luminol immobilized on an anion exchange column. Luminol is eluted by hydrolysis for CL detection	1×10^{-7} mol/dm ³	(124)
Sulphur dioxide	Air	Adsorption using triethanolamine followed by Ru(2,2'-bipyridyl) ₃ ²⁺ –SO ₃ ²⁻ –K ₂ S ₂ O ₈ CL	4.1×10^{-8} mol/dm ³	(125)
Sulphur dioxide	Air	Absorption on triethanolamine followed by Tris(1,10-phenanthroline)ruthenium–potassium periodate CL	7×10^{-10} mol/dm ³	(126)
Sulphur dioxide	Air	Adsorption using triethanolamine followed by Ru(2,2'-bipyridyl) ₃ ²⁺ –SO ₃ ²⁻ –potassium permanganate CL	2.5×10^{-8} mol/dm ³	(127)
V(IV)	Tap water	Potassium dichromate–potassium iodide–sodium hydroxide–luminol CL	7×10^{-10} mol/dm ³	(128)
V(V)	Geochemical and hair samples	Luminol and hexacyanoferrate(II), are both immobilized on an anion-exchange resin column, and are eluted with phosphoric acid to produce CL	5.4×10^{-3} µg/cm ³	(129)
Volatile phenols	Polluted waters	Quenched <i>p</i> -chlorobenzenediazonium fluoroborate–hydrogen peroxide CL	0.015 – 0.03 µg/mL	(130)
Zr(IV)	Rocks and ores	Luminol–hydrogen peroxide CL	30 pg/L	(131)

with other flow systems, eg LC, CL immunoassay labels or electrogenerated CL. There are also a limited number of FI–CL papers (not tabulated) in which unstable oxidants have been used by electrochemical generation of the reagent within the FI manifold (230–236).

PHARMACEUTICAL APPLICATIONS

The high sensitivity of FI–CL has attracted considerable interest for the analysis of pharmaceutical samples (Table 1) using a variety of oxidants. The luminol–hydrogen peroxide reaction, with and without K₇Cu(10₆)₂, has been used for the measurement of ascorbic acid in tablets (6, 8), morphine, sinomenine and codeine in tablets and

Table 3. Food and beverage applications

Analyte	Sample matrix	Reaction	LOD	Reference number
3,4-Dihydroxybenzoic acid	Wines	Inhibition of the luminol–hydrogen peroxide–Co(II) CL	2.7×10^{-7} mol/dm ³	(132)
Acetaldehyde	Alcoholic beverages	Gallic acid–hydrogen peroxide–sodium hydroxide CL	0.31 ng/mL	(61)
Amino acids	Food	Cu(II) amino acid complex formation, catalysis of the luminol–hydrogen peroxide and <i>o</i> -phenanthroline–hydrogen peroxide CL systems	pmol	(133)
Ascorbic acid	Beverages	Oxidation with permanganate–acid	5×10^{-7} mol/dm ³	(134)
Ascorbic acid	Mung bean sprouts, tomato and cucumber skins	Luminol–Fe(II)–Na ₂ B ₄ O ₇ –potassium hydroxide CL	0.2 ng/mL	(5)
Ascorbic acid	Fruit juices	Luminol and potassium permanganate immobilized on resins in a glass column. Eluent mixed with sodium hydroxide to produce CL	5 µg/L	(135)
Ascorbic acid	Vegetables	Acidified permanganate–luminol CL	0.1 µg/mL	(136)
Ascorbic acid	Vegetables	Inhibition of the CL produced from luminol and ferricyanide (immobilized on an anion-exchange resin column, eluted with sodium phosphate)	5.5×10^{-3} µg/mL	(11)
Carbohydrates	Aqueous	Acidified permanganate/Mn(II) CL	1×10^{-4} mol/dm ³	(137)
Choline	Cabbage	Luminol–Co(II) detection of hydrogen peroxide produced from an immobilized choline oxidase column	1×10^{-7} mol/dm ³	(138)
Citric acid	Non-alcoholic beverages	Reduction of Fe(III) to Fe(II) with citric acid followed by luminol CL detection	0.1 µg/mL	(139)
Ethanol	Beer	Production of hydrogen peroxide using alcohol oxidase followed by luminol CL	0.01% (v/v)	(140)
Free L-malate	Wines	Malate dehydrogenase/reduced nicotinate adenine dinucleotide oxidase co-immobilized on polymer beads to produce hydrogen peroxide for detection using luminol–hexacyanoferrate(III) CL	8×10^{-8} mol/dm ³	(141)
Glucose and fructose	Aqueous	Pyrogallol–hydroxylamine hydrochloride–periodate CL	Not reported	(142)
Glycerol	Wines	Glycerol dehydrogenase and NADH oxidase are co-immobilized on poly(vinyl alcohol) beads to produce hydrogen peroxide, which was detected using luminol–hexacyanoferrate(III) CL	7×10^{-8} mol/dm ³	(143)
Hydrogen peroxide	Fermented liquors	Peroxyoxalate CL in an emulsion of ethyl acetate, non-ionic surfactant polyoxyethylene(20) sorbitane monolaurate (Tween 20) and water	1×10^{-6} mol/dm ³	(144)
L-lactate	Food samples	Immobilized L-lactate oxidase to produce hydrogen peroxide for luminol–horse-radish peroxidase–luminol CL detection	1×10^{-7} mol/dm ³	(145)
Nitrite	Water and food	Luminol–I ₂ (produced from potassium iodide in acid) CL	1.6 ng/mL	(109)
Sulphite	Sugar	Ru(2,2'-bipyridyl) ₃ ²⁺ –SO ₃ ²⁻ –K ₂ S ₂ O ₈ CL	4.1×10^{-8} mol/dm ³	(125)
Sulphite	Beers and wines	Auto-oxidation sensitized by rhodamine 6G (immobilized on cation exchange resin) enhanced with Tween 80	0.03 mg/L	(146)
Sulphite	Wines	Na ₂ CO ₃ –NaHCO ₃ –Cu(II) CL with a gas diffusion module	5×10^{-7} mol/dm ³	(147)
Sulphite	Sugar	Ru(2,2'-bipyridyl) ₃ ²⁺ –SO ₃ ²⁻ –potassium peroxide CL	2.5×10^{-8} mol/dm ³	(127)
Tannic acid	Hop pellet samples	Inhibition of luminol–hydrogen peroxide–Cu(II) CL	9×10^{-9} mol/dm ³	(53)

Table 4. Biomedical applications

Analyte	Sample matrix	Reaction	LOD	Reference number
Acetylcholine and choline	Rat brain tissue	Following sample preparation, injection into stream, passed through two reactors containing acetylcholinesterase and choline oxidase immobilized on glass beads. Detection using Co(II)–luminol CL	600 and 500 fmol, respectively	(148)
Acetylcholine and choline	Culture media	Production of hydrogen peroxide using an immobilized enzyme reactor followed by detection with luminol–Co(II) CL enhanced using sodium dodecylsulphate	1×10^{-6} mol/dm ³	(149)
Adenosine-5'-triphosphate	Aqueous	Alkaline phosphatase from <i>Escherichia coli</i> (immobilized) is used to produce hydrogen peroxide for detection with luminol–heteropoly acid	1×10^{-8} mol/dm ³	(150)
α -Chymotrypsin, trypsin and a commercial protease	Aqueous	Immobilized tripeptide and isoluminol–Co(II)–hydrogen peroxide CL	2.7×10^{-4} mg/L, 4×10^{-2} mg/L and 2×10^{-3} mg/L respectively	(151)
Amino acids	Aqueous	Inhibition of the lucigenin–Co(II) reaction	1×10^{-9} – 2×10^{-7} mol/dm ³	(152)
Amino acids	Aqueous	Ninhydrin–hydrogen peroxide–Cu(II) or Co(II) CL	4.2×10^{-2} – 9.2×10^{-6} mol/dm ³	(153)
Amino acids, polyamines, and salicylic acids	Aqueous	Unsaturated complex of Cu(II) and organic ligands enhanced 1,10-phenanthroline–hydrogen peroxide–CTMAB CL	2.7–90 pmol	(154)
Bilirubin	Aqueous	N-bromosuccinimide or sodium hypochlorite CL	1.75 μ g/mL	(155)
Branched-chain L-amino acids	Plasma	Leucine dehydrogenase and NADH oxidase are co-immobilized on aminated poly(vinyl alcohol) beads to produce hydrogen peroxide which is detected using luminol–hexacyanoferrate(III) CL	3×10^{-7} mol/dm ³	(156)
Catecholamines	Plasma	Imidazole-catalysed decomposition by catecholamines producing hydrogen peroxide which is detected using peroxyoxalate CL	Not reported	(157)
Chlorotetracycline	Urine	Copper sulphate–hydrogen peroxide–ammonium carbonate–cetyltrimethylammonium bromide CL	4×10^{-8} mol/dm ³	(158)
Cholesterol	Human serum	Cholesterol oxidase immobilized on amine-modified silica gel in a column is used to produce hydrogen peroxide. Luminol and ferricyanide are co-immobilized on an anion-exchange column for CL detection	5×10^{-6} g/mL	(159)
Cholesterol	Blood serum	Hydrogen peroxide produced from an immobilized cholesterol oxidase column is detected using luminol–Co(II) CL	0.1 mg/L	(160)
Cr(III)	Urine	Luminol–hydrogen peroxide CL	0.01 μ g/L	(161)
Cu(II)	Serum	Hydroxylamine–fluorescein–hydroxide CL	0.5 μ g/L	(162)
D-amino acids	Human plasma	Immobilized enzyme column reactor with peroxyoxalate CL detection	0.4–30 pmol (10 μ L injection)	(163)
D-glucose and 3-hydroxybutyrate	Serum	Two enzyme reactors, one containing glucose dehydrogenase and NADH oxidase, the other containing 3-hydroxybutyrate dehydrogenase and NADH oxidase, co-immobilized on beads to produce hydrogen peroxide for detection using luminol–hexacyanoferrate(III) CL	1×10^{-5} mol/dm ³ and 1×10^{-6} mol/dm ³ , respectively	(164)
Dopamine	Biochemical samples	Imidazole–peroxyoxalate CL	10 nmol (20 μ L injection)	(165)

Table 4 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
Fe(III)	Blood	Luminol and potassium hexacyanoferrate(II) are immobilized on a D201 anion exchange column. Elution with Na ₂ SO ₄ . Detection with sodium hydroxide	7 µg/L	(166)
Glucose	Rabbit fluid and blood	Microdialysis followed by reaction in an immobilized glucose oxidase reactor to produce hydrogen peroxide, detected with luminol-hexacyanoferrate CL	1×10^{-5} mol/dm ³	(167)
Glucose	Biochemical samples	K ₇ [Cu(IO ₆) ₂]-luminol-sodium hydroxide CL	0.18 ng/mL	(168)
Glucose	Plasma	Immobilized pyranose oxidase produced hydrogen peroxide for reaction with luminol in a flow cell containing immobilized peroxidase	3×10^{-9} mol/dm ³	(169)
Glucose, acetylcholine and choline	Urine	Production of hydrogen peroxide using immobilized glucose oxidase or acetylcholine esterase-choline oxidase followed by peroxyoxalate CL	3×10^{-9} mol/dm ³ and 5×10^{-8} mol/dm ³ , respectively	(170)
Glycerol-3-phosphate	Aqueous	Glycerol-3-phosphate oxidase immobilized on controlled pore glass. Detection with luminol-Co(II) CL	5×10^{-7} mol/dm ³	(171)
Hydrogen peroxide	Human serum	3-Propyl-7,8-dihydropyridazino[4,5-g]quinoxaline-2,6,9(1H)-trione	1.3 pmol (100 µL injection)	(172)
Iodide	Urine	Conversion to iodine by potassium dichromate followed by detection with luminol-Co(II) CL	10 µg/L	(173)
L-alanine, 2-oxoglutarate and L-glutamate	Cell cultivation media of mammalian cells	Sample passed through reactor containing alanine aminotransferase and glutamate oxidase immobilized on sieved porous glass beads before passing into a flow cell containing NaHCO ₃ -luminol-Co(II) immobilized peroxidase from <i>Arthromyces ramosus</i> for CL detection	2×10^{-6} mol/dm ³ , 5×10^{-6} mol/dm ³ and 1×10^{-6} mol/dm ³ , respectively	(174)
L-valine, L-leucine and L-isoleucine	Plasma	Leucine dehydrogenase, NADH oxidase and peroxidase are co-immobilized covalently on tresylate-hydrophilic vinyl polymer beads in a flow cell. NAD ⁺ and luminol used to produce CL	1×10^{-8} mol/dm ³	(175)
Lysine and glucose	Aqueous	Immobilized lysine oxidase and glucose oxidase producing hydrogen peroxide with luminol-Co(II) CL detection	4×10^{-8} and 7×10^{-7} mol/dm ³ , respectively	(176)
Lysine and ornithine	Aqueous	Immobilized lysine oxidase producing hydrogen peroxide with luminol-Co(II) CL detection	4×10^{-7} mol/dm ³	(177)
Polyphenols, monophenols and sugars	Not reported	Imidazole-peroxyoxalate CL	Not reported	(178)
Porphyrins	Aqueous	Peroxyoxalate-hydrogen peroxide CL	0.1 µg/L	(179)
Proteins	Bovine serum albumin, human serum albumin, human γ-globulin, and egg albumin	Cu(II)-proteins complexes catalyse luminol-hydrogen peroxide	0.03–0.05 µg/mL	(180)
Proteins	Bovine serum albumin, human serum albumin, γ-globulin, and egg albumin	1,10-Phenanthroline-hydrogen peroxide-Cu(II) cetyltrimethylammonium bromide CL	0.02 µg/mL	(181)
Pyruvate	Blood serum	Acidified permanganate-quinine CL	0.8 µg/mL	(182)
Serotonin and related indoles	Aqueous	Potassium permanganate-sulphuric acid CL	2×10^{-9} – 1.5×10^{-8} mol/dm ³	(183)

Table 4 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
Serum 3-hydroxybutyrate	Serum	Two immobilized enzymes, 3-hydroxybutyrate dehydrogenase and NADH oxidase producing hydrogen peroxide, detection with luminol-hexacyanoferrate CL	Not reported	(184)
Sulphated bile acids	Urine	Production of hydrogen peroxide using multistep enzymatic reactions with detection using luminol CL	1×10^{-7} mol/dm ³	(185)
Tryptophan	Tissue	Cerium(IV)-sulphuric acid CL	0.1 µg/mL	(186)
Uric acid	Urine	Luminol-K ₂ [Cu(IO ₆) ₂]-potassium hydroxide CL enhanced with polyhydroxy compounds	7.2×10^{-9} mol/dm ³	(187)
Uric acid	Urine	Acidic permanganate CL with octylphenyl polyglycol ether as a sensitizer	0.055 µg/mL	(188)
Uric acid	Aqueous	Inhibition of luminol-potassium periodate-Mn(II) CL	1.8×10^{-9} g/mL	(189)
Vanilmandelic acid	Urine	Luminol-hexacyanoferrate(III) CL	2×10^{-8} mol/dm ³	(190)

injection solutions (34), pyridoxine in dietary sources, eg peanuts, yeast, lemons, tomatoes and apples (46), nitroprusside in injection solutions (52) and rutin in traditional Chinese medicines (49). Some catecholamines (dopamine, adrenaline, isoprenaline) have been determined by the same reaction after treatment with Reinecke's salt (20). Addition of potassium periodate to the luminol stream has been proposed for the determination of ascorbic acid (9) and isoniazid (29) in pharmaceutical preparations and glucose (after treatment with glucose oxidase) (25). Ascorbic acid has also been determined by the luminol-hydrogen peroxide reaction in the presence of iron(II) in vitamin C tablets, multivitamin capsules, mung bean sprouts, tomatoes and cucumber skin, with recoveries of 96–105% (5). The presence of hexacyanoferrate in the reaction mixture allowed measurement of β -lactam antibiotics via generation of hydroxy and superoxide radicals (12). Ascorbic acid has also been determined using hexacyanoferrate(III) instead of hydrogen peroxide (10). Inhibition of luminol CL has been applied to the determination of ascorbic acid in vitamin pills and vegetable extract tablets (with good agreement with iodimetry) (11), isoniazid (28) and paracetamol (37). Tannic acid in Chinese gall has been determined by its inhibition of the copper(II)-catalysed luminol-hydrogen peroxide reaction (53).

A variety of other oxidants in acidic and alkaline media have been investigated for drug analysis using FI-CL. Potassium permanganate in acidic media, with or without CL enhancers or promoters, has been used for the determination of amidopyrine (1, 2), benzocaine, procaine and other local anaesthetics (13), cefadroxil (15), codeine (18), imipramine (26, 27), levodopa (30), medazepam (31), methotrexate (33), naltrexone (35), perphenazine (40), promethazine (45), reserpine (47),

salicylamide (51), tetracyclines (54) and tetrahydropalmitate (56) in pharmaceutical preparations. All gave good recoveries and agreement with official or standard analytical methods. Initiation of CL reactions in acidic media has also been achieved using cerium(IV) for the determination of analgin (4), captopril (14), furosemide (22), hydrochlorothiazide (24), naproxen (36), penicillamine (38, 39), phenothiazines (42), tetracyclines (55) and tiopronin (58, 59).

The CL reaction of cerium(IV) with sulphite has been used for excitation by energy transfer to ciprofloxacin (16) and prednisone acetate (43). CL also occurs if sulphite is substituted by a mercapto-compound, such as glutathione or cysteine, and the reaction is sensitized by hydrocortisone (23), while the reaction with glutathione and energy transfer has been used to determine progesterone and hydrocortisone in human serum by standard additions (44). Menadione sodium bisulphite has been determined in injection solutions by release of sulphite and reaction with cerium(IV) and the results were in good agreement with a standard spectrophotometric method (32). Similarly the reaction of permanganate with dithionite has been used to determine riboflavin (48) and with thiosulphate to determine vitamin B₆ (60).

Potassium hexacyanoferrate(III) in alkaline solutions has been used for the determination of ergonovine maleate in synthetic pharmaceutical preparations (with good agreement with the official method) (21) and thiamine (57). Alternatively, sodium hypochlorite can be used for initiation of CL reactions in alkaline media for the assay of persatin in injection solutions and tablets (41). Rutin has been measured in Chinese traditional medicines by the hypochlorite-semicarbazide CL system (50).

Table 5. General/unspecified applications

Analyte	Sample matrix	Reaction	LOD	Reference number
2,4-Dinitrophenylhydrazine	Aqueous/propan-2-ol	Potassium permanganate–formic acid CL with rhodamine B as sensitizer	5×10^{-10} mol/dm ³	(191, 192, 193)
4-(5',6'-Dimethoxybenzothiazolyl) phthalhydrazide	Dimethyl sulphoxide	4-(5',6'-Dimethoxybenzothiazolyl) phthalhydrazide–hydrogen peroxide–potassium hexacyanoferrate(III) CL	1.3×10^{-11} mol/dm ³	(194)
Al(III), Zn(II), Cd(II), and In(III)	1,4-Dioxane	Derivatisation with γ -hydroxyquinoline followed by peroxyoxalate–hydrogen peroxide CL detection	20–70 μ g/L	(195)
Benzaldehyde	Benzyl alcohol	Gallic acid–hydrogen peroxide–sodium hydroxide CL	4.4×10^{-6} mol/dm ³	(196)
Cl ⁻ , Br ⁻ , NO ₂ ⁻ , NO ₃ ⁻ , SO ₄ ²⁻	Aqueous	N-(β -carboxypropionyl) isoluminol immobilized on a resin is replaced by anions releasing isoluminol which reacts with hydrogen peroxide and potassium hexacyanoferrate(III) producing CL	8.0×10^{-7} – 1.4×10^{-6} mol/dm ³	(197)
Co(II)	Aqueous	1,10-Phenanthroline–hydrogen peroxide–Co(II) CL with the cationic surfactant cetyltrimethylammonium bromide	5 μ g/L	(198)
Cu(II)	Aqueous	Peroxyoxalate chemiluminescence with 3-aminofluoranthene as fluorophore and imidazole as catalyst	Not reported	(199)
Fe(II) and Fe(III)	Aqueous	On-line liquid–liquid extraction into chloroform followed by Fe(III) complex formation with 8-quinolinol and detection with luminol–hydrogen peroxide–cetyltrimethylammonium chloride	5 ng/mL	(200)
Fe(II) and Fe(III)	Aqueous	Reduction of Fe(III) to Fe(II) using Cu-coated zinc followed by luminol CL detection	2.7×10^{-10} mol/dm ³ and 3.5×10^{-10} mol/dm ³ , respectively	(201)
Fe(III)	Aqueous	1,10-Phenanthroline–hydrogen peroxide–sodium hydroxide CL with the cationic surfactant zephiramine	0.1 μ g/L	(202)
Heterocyclic compounds	Acetonitrile	Peroxyoxalate–hydrogen peroxide–3-2 aminofluoranthrene CL	Not reported	(203)
Horseradish peroxidase	Aqueous	Luminol–hydrogen peroxide CL enhanced with sodium tetraphenyl borate	6×10^{-14} mol/dm ³	(204)
Hydrogen peroxide	Aqueous	Flow-cell reactor containing immobilized horseradish peroxidase is used to mix the sample with luminol to produce CL	10 pmol	(205)
Hydrogen peroxide	Aqueous	Luminol–potassium periodate CL	3×10^{-8} mol/dm ³	(206)
Hydrogen peroxide	Aqueous	Immobilized luminol and Co(II) are removed from a column by hydrolysis and CL is detected	4×10^{-8} mol/dm ³	(207)
Hydrogen peroxide	Aqueous	Horseradish peroxidase–sol–gel immobilized on a glass plate. Luminol CL detection	8×10^{-6} mol/dm ³	(208)
Hydrogen peroxide	Not reported	Peroxyoxalate CL with perylene as a fluorophore	1×10^{-9} mol/dm ³	(209)
Hydrogen peroxide	Aqueous	Peroxyoxalate CL with immobilized 3-aminoperylene or 3-aminofluoranthene as fluorophore	3×10^{-10} mol/dm ³	(210)
Hydrogen peroxide	Aqueous	Peroxyoxalate CL with immobilized 2-(4-hydrazinocarbonylphenyl)-4,5-diphenylimidazole as fluorophore	1×10^{-9} mol/dm ³	(211)
Hydrogen peroxide, pyrogallol, and α -thioglycerol	Aqueous	Periodate–polyhydroxyl compounds in acid medium producing CL, enhanced with carbonate	5×10^{-9} mol/dm ³ , 5×10^{-9} mol/dm ³ and 1×10^{-8} mol/dm ³ , respectively	(212)

Table 5 continued.

Analyte	Sample matrix	Reaction	LOD	Reference number
Hypoxanthine	Aqueous	Production of hydrogen peroxide from a xanthine oxidase bioreactor followed by <i>Artheromyces ramosus</i> peroxidase-luminol CL	1×10^{-8} mol/dm ³	(213)
Indole, indole-3-acetic acid and tryptophan	Aqueous	Hydrogen peroxide-Fe(III)-sodium hydroxide CL	5.5×10^{-7} mol/dm ³ , 6.1×10^{-9} mol/dm ³ , and 5×10^{-7} mol/dm ³ , respectively	(214)
Lophine	Dimethylformamide/methanol	Lophine-Co(II)-hydrogen peroxide CL enhanced with hydroxylammonium chloride	72 fmol (20 μ L injection)	(215)
Luminol and lucigenin	Aqueous	CL produced by adding luminol and lucigenin solutions to magnesium oxide and barium oxide powders, respectively	3×10^{-11} mol/dm ³ and 3×10^{-12} mol/dm ³ , respectively	(216)
Oxovanadium(IV) acetylacetonate	Chloroform	Luminol-cetyltrimethylammonium chloride (in chloroform/cyclohexane)	0.1 ng/mL	(217)
Potassium permanganate, Ce(IV), potassium periodate, NaOCl and hydrogen peroxide	Aqueous	Pyrogallol CL	3×10^{-4} mol/dm ³ , 3.5×10^{-4} mol/dm ³ , 2.5×10^{-4} mol/dm ³ , 6.1×10^{-4} mol/dm ³ and 4.5×10^{-4} mol/dm ³ , respectively	(218)
Pyrogallol	Aqueous	Lucigenin-hydroxylamine hydrochloride-sodium hydroxide CL	4.5×10^{-8} g/mL	(219)
Pyrogallol	Aqueous	Oxidation of pyrogallol by N-bromo-succinimide in alkaline media, enhanced with hydroxylammonium chloride and cetyltrimethylammonium bromide	2×10^{-7} mol/dm ³	(220)
Sulphite	Aqueous	Cerium(IV)-acidified permanganate CL with cyclooctylamine as sensitizer	5.4×10^{-7} mol/dm ³	(221, 222)
Uric acid, phenacyl alcohol, cortisone, ascorbic acid, corticosterone, glutathione, cysteine, fructose, glucose and creatinine	20% Acetonitrile/80% H ₂ O	Luminol CL with hexacyanoferrate(III) and hexacyanoferrate(II) as catalysts	1.7 pmol, 3.0 pmol, 4.0 pmol, 6.0 pmol, 16.0 pmol, 55.0 pmol, 62.0 pmol, 0.6 nmol, 1.5 nmol and 15.0 nmol, respectively (10 μ L injection)	(223)
V(IV)	Aqueous	On-line liquid-liquid extraction into chloroform followed by cetyltrimethylammonium-luminol CL	50 ng/mL	(224)
V(IV) and Fe(II)	Aqueous	Lucigenin adsorbed on non-functionalized silica gel used a solid phase CL reagent with potassium hydroxide	0.7 μ g/L and 0.8 μ g/L, respectively	(225)
Yb(III)	Chemical reagents	Quenching of the Cr(II)-luminol-hydrogen peroxide CL	3×10^{-8} mol/dm ³	(226)
Zn(II)	Aqueous	1,10-Phenanthroline-hydrogen peroxide-sodium hydroxide CL with the cationic surfactant trimethylstearyl ammonium chloride	2.3×10^{-8} mol/dm ³	(227)

The auto-oxidation of analgin in Tween 80, in the presence of rhodamine 6G as sensitizer, to generate CL emission has been used for its determination in pharmaceutical preparations (3). Lucigenin CL has been used to determine ascorbic acid (7) and inhibition of the iron(II)-catalysed reaction can be used to measure dopamine (19). Cobalt(II) has been determined in eye lotions by the hydroxylammonium chloride-sensitized reaction with lophine and hydrogen peroxide (17).

ENVIRONMENTAL APPLICATIONS

FI-CL methodologies have been applied to wide ranging analyses of environmental samples, as detailed in Table 2. Here these are categorized according to the type of sample matrix analysed, ie aquatic (natural waters, drinking water and wastewaters), geological, atmospheric and biological (human hair).

Natural, potable and waste waters

Metal ion determinations comprise the majority of published FI-CL applications for aqueous environmental samples. Many of these are based on the luminol reaction, which is catalysed by certain metal ions. Examples include the determination of cobalt(II) at sub-picomolar levels in river, sea and tap water (67) and of copper(II) at nanomolar levels in natural water samples (78). Cobalt(II) and chromium(III) in admixtures have been monitored via their catalytic effect on the reaction of luminol-hydrogen peroxide-cetyltrimethylammonium bromide. Initially, both ions are determined and then cobalt is masked with EDTA and chromium is measured. The procedure has been used in the analysis of mineral water, with recoveries of 94–105% (69). Luminol CL has been used to detect iron(II) and iron(III) at nanomolar or subnanomolar levels in seawater (81, 86), natural freshwaters (83, 87, 90), groundwater (82) and treated waters (84). Similar methods have measured chromium(III) at part-per-billion levels in tap water (71), and both chromium(III) and chromium(VI) at nanomolar levels in wastewaters (72, 74–75). Modified luminol-based methods have also been used to determine mercury(II) at part-per-billion levels in natural waters (92), manganese(II) at sub-nanomolar levels in seawater (107), lead(II) at part-per-trillion levels in wastewaters (113), and vanadium(IV) at nanomolar levels in tap water (128). The chemiluminescence reaction between 1,10-phenanthroline and hydrogen peroxide has been applied to the determination of copper complexation in seawater (76), while cobalt(II) in seawater has been determined using methods involving both gallic acid-hydrogen peroxide (66) and quercetin-hydrogen peroxide-potassium hydroxide (68). The latter reaction has also been applied to determinations of chromium(III) and chromium(VI) in

natural waters (73). Iron(II) in rainwater has been determined using peroxyoxalate CL (88), and iron(II)/total iron have been measured in river water and seawater using brilliant sulphoflavine-hydrogen peroxide CL (89). A CL method based on the oxidation of 7,7,8,8-tetracyanoquinodimethane has been used to measure manganese(II) in drinking water (105), and the reaction between lucigenin, hydrogen peroxide and sodium hydroxide has been applied to the determination of lead(II) in natural waters (112).

As shown previously, a number of CL reactions are based on the oxidative properties of hydrogen peroxide. FI-CL methods have therefore proved highly suitable for determining low levels of dissolved hydrogen peroxide in natural waters. Nanomolar levels have been detected in rainwater using methods based on either the octylphenyl polyglycol ether-potassium permanganate (95) or cobalt(II)-luminol (98, 99) reactions. The latter method has also been applied to nanomolar and sub-nanomolar determinations of hydrogen peroxide in seawater (97, 101), while a periodate-potassium carbonate CL method has been used to measure nanomolar levels in melted snow (96).

FI-CL methods have been used to determine a range of dissolved inorganic molecular and anionic species in aqueous samples. Free chlorine has been measured at part-per-billion levels in tap water using a luminol CL method (65), and modified luminol methods have been used to determine cyanide at nanomolar levels in river (79), tap and wastewaters (80). Sub-micromolar levels of hydrazine in drinking water have been determined with a CL method based on *n*-bromosuccinimide (93). Hypochlorite ions have been measured in tap water using CL generated by the oxidation of indole by hydrogen peroxide (102). Variants of the luminol reaction have been applied to the determination of nanomolar levels of nitrite in natural (108) and potable (109) waters, and to the determination of phosphorus and phosphates at micro-nanomolar levels in natural freshwaters (114–115, 117–120) and drinking water (116). Sulphite in tap water has been measured using an auto-oxidative CL method, sensitized with rhodamine 6G (123). Dissolved oxygen has been quantified at part-per-million levels in river and tap waters using a luminol-sodium hydroxide CL method (110), while a gas diffusion method based on luminol-ozone CL has been applied to the measurement of part-per-billion levels of dissolved ozone in treated waters (111).

Determinations of a small number of organic/biological parameters using FI-CL methods have been reported. Acetaldehyde has been measured at part-per-billion levels in both natural and wastewaters using a gallic acid-hydrogen peroxide CL method (61). Similar levels of volatile phenols present in polluted waters have been determined using a hydrogen peroxide-*p*-chlorobenzene-diazonium fluoroborate CL reaction (130). FI-CL

methods have also been used to identify the presence of two strains of red tide phytoplankton in natural waters (64, 91), with a CL reaction based on 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo(1,2- α)-pyrazin-3-one and superoxide used in each case.

Geological samples

FI-CL methods have been reported for the determinations of five elements in a variety of geological/geochemical samples. Trace levels of arsenic(III) in geochemical solutions have been determined using a luminol-hydrogen peroxide CL reaction (63), while a similar method incorporating prior reduction of arsenic(V) to arsenic(III) has been applied to the measurement of total arsenic in acidic extracts of rocks and ores (62). Picogram levels of iridium(IV) have been determined using a hydrogen peroxide-potassium hydroxide CL reaction (103), and a luminol method incorporating potassium dichromate and sodium hydroxide has been applied to the measurement of part-per-billion levels of antimony(III) in mineral extracts (122). Variants of the luminol reaction have also been applied to the determinations of vanadium(V) and zirconium(IV) in geochemical samples. The former uses luminol and hexacyanoferrate(II) as immobilized reagents on an anion-exchange column (129), while the latter uses the frequently applied luminol-hydrogen peroxide reaction (131).

Atmospheric samples

Four FI-CL applications have been reported for the determination of micromolar to sub-nanomolar levels of sulphur dioxide in air and sulphite in aqueous solution. Three of these methods used triethanolamine for initial adsorption, followed by CL reactions involving either ruthenium (2,2'-bipyridyl)₃/potassium persulphate (125), Tris(1,10-phenanthroline) ruthenium-potassium periodate (126) or ruthenium (2,2'-bipyridyl)₃-potassium permanganate (127). The fourth method was based on luminol CL, with luminol initially immobilized on an anion exchange column, then eluted by hydrolysis (124). Trace levels of hydrazine in air have been determined using a CL method based on the luminol-potassium periodate-sodium hydroxide reaction, following initial absorption of hydrazine in sulphuric acid solution (94). Part-per-billion levels of gaseous hydrogen peroxide have been measured with a method based on 1,1'-oxalyldi-imidazole-peroxyoxalate CL and incorporating a diffusion scrubber (100). The peroxyoxalate-hydrogen peroxide CL reaction has also been used in the determination of a range of polycyclic aromatic hydrocarbons (PAHs) at part-per-billion levels in synthetic hexane and acetonitrile solutions (121). This method of detection, in combination with chromatographic separation, could also prove suitable for the determination of

PAHs in solvent-extracted atmospheric particulate samples.

Human hair

Analysis of human hair samples can often provide useful indications of the body's intake of a range of chemical species, either through diet or by environmental exposure. The determination of trace metals in human hair samples using FI-CL has been reported in several papers. Part-per-billion levels of cobalt and nickel have been measured in microwave-digested samples by utilizing their catalytic effect in the CL reaction of alizarin purple-ethanol cetyltrimethylammonium bromide-potassium hydroxide (70). Similar levels of copper(II) have been determined using the luminol-potassium permanganate reaction, with these reagents initially immobilized on an anion-exchange resin, then eluted with sodium hydroxide (77). Iron(II) in hair has been determined using both the lucigenin-sodium hydroxide (85) and the luminol-hydrogen peroxide (90) CL reactions, either of which are capable of nanomolar/part-per-trillion detection limits when sensitized with cationic surfactants. Manganese has been measured at part-per-billion levels using an iodine-luminol CL method (104), and part-per-million levels of vanadium(V) have been determined with a CL method based on the luminol-hexacyanoferrate(II) reaction (129).

FOOD AND BEVERAGE APPLICATIONS

This has been an area of considerable development in recent years. The bioluminescence reaction involving firefly luciferase has been used to monitor bacterial contamination of foods but CL reactions have also been used to quantify species such as ascorbic acid, sulphite and carbohydrates in alcoholic beverages and a variety of foods (Table 3).

Ascorbic acid

Various FI-CL systems have been used to determine ascorbic acid in vegetables and non-alcoholic beverages, with detection limits in the range 0.2 $\mu\text{g/L}$ –5.5 mg/L . The oxidation of ascorbic acid by permanganate-produced CL (134) and results compared well with spectrofluorimetric and titrimetric methods, but transition metals, sorbitol and mannitol interfered. The inhibition of the CL reaction between hexacyanoferrate(III) and luminol can also be used for the detection of ascorbic acid. This reaction can be performed with (11) or without (5) the reagent being immobilized on an anion exchange column. The CL reaction between luminol, permanganate and sodium hydroxide has also been used for the determination of ascorbic acid and, as with the previous reaction, the

reagent can be used with (135) or without (136) reagent immobilization on an anion exchange column. Manganese(II) and copper(II) interfered when present at five-fold excess.

Sulphite

A variety of CL reactions have been used for the determination of sulphite in diverse matrices, including sugar, beers and wines with sub-micromolar limits of detection. Chemiluminescence produced from tris(2,2'-bipyridyl)ruthenium(II) in the presence of either $K_2S_2O_8$ (125) or $KMnO_4$ (127) was used to quantify sulphite in sugar and sulphur dioxide in air (absorbed in triethanolamine) with interference from copper(II) and EDTA. Sulphite (detection limit 30 $\mu\text{g/mL}$) has also been determined in beers and wines using the auto-oxidation of sulphite sensitized by rhodamine 6G in the presence of Tween 80 surfactant micelles (146), with interference from sulphite, nitrite and ascorbic acid. This is a good example of the increasing trend to add surfactants to enhance CL emission by providing a protective environment for the reaction. FI-CL has been used to determine sulfite in wines using the NaHCO_3 -sodium carbonate-copper(II) CL reaction (147). The manifold incorporated a gas diffusion module to enhance selectivity and remove physical interferences but iodide, cobalt(II), nickel(II), and sulphite interfered at two-fold excess.

Carbohydrates

Chemiluminescence is produced when carbohydrates are oxidized by acidified potassium permanganate (137). Glucose, galactose, fructose, arabinose, xylose, lactose and sucrose have all been detected over the linear range 10^{-4} – 10^{-1} mol/dm³. The oxidation of pyrogallol by periodate has been used for the detection of carbohydrates (142) with a detection limit for hexose of 20 μg . An interesting aspect of this method was the use of differential CL reaction kinetics to allow the resolution of binary mixtures of glucose and fructose.

Other analytes

Luminol chemiluminescence has been used for a variety of other analytes in food and beverages, including 3,4-dihydroxybenzoic acid (132), choline (138) citric acid (139), ethanol (140), L-malate (141), glycerol (143), L-lactate (145), nitrite (109) and tannic acid (53). 3,4-Dihydroxybenzoic acid (protocatechuric acid) (132) has been measured in wines using CL produced by the oxidation of luminol with hydrogen peroxide, with cobalt(II) as a catalyst. Citric acid in orange drinks (139) has also been determined using the luminol reaction. Iron(III) is reduced by citric acid to iron(II), which can then be detected with luminol, but ascorbic

acid interfered. Ethanol in beer can be indirectly determined by the enzymatic generation of hydrogen peroxide using alcohol oxidase (140). L-Cysteine interference was removed by prior complexation with copper(II). L-Malate (141), glycerol (143) and L-lactate (145) have all been indirectly detected using immobilized enzyme reactors to produce hydrogen peroxide, followed by luminol detection. Nitrite can be detected in foods by reaction with KI (109) to produce I_2 , which then reacts with luminol. Iron(II), copper(II), AsO_4^{3-} and SbO_4^{3-} interfere. Tannic acid (53) has been determined in hop pellets, using the lumino-peroxide reaction with a copper(II) catalyst, and hydrogen peroxide has been measured in fermented liquors (144) using bis-(2,4,6-trichlorophenyl)oxalate and perylene chemiluminescence. The latter method has been applied to the determination of L-glutamic acid in culture media. Acetaldehyde has been determined using gallic acid-hydrogen peroxide-sodium hydroxide chemiluminescence (61), but formaldehyde, cobalt(II), manganese(II), silver(I), cadmium(II), lead(II) and permanganate all interfered. Amino acids have been detected in food (lysine) and serum (phenylalanine) using both the luminol-hydrogen peroxide and *o*-phenanthroline/hydrogen peroxide reactions (133).

BIOMEDICAL APPLICATIONS

A particularly wide variety of analytes have been determined by enzymatic conversion to produce hydrogen peroxide, which is then quantitatively detected by the luminol or peroxyoxalate reactions (Table 4).

The luminol reaction has been used for the measurement of hydrogen peroxide generated from acetylcholine and choline after passing the sample through two consecutive columns containing immobilized acetylcholinesterase and choline oxidase (148, 149). Similarly, ATP has been determined with immobilized alkaline phosphatase (150) and glycerol-3-phosphate with immobilized glycerol-3-phosphate oxidase (171). Branched chain L-amino-acids have been determined in human plasma, with recoveries in the range 98–102%, by passing the analyte solution through an enzyme reactor column containing leucine dehydrogenase and NADH oxidase (156) or by introducing the solution, mixed with luminol and NAD^+ , into a spiral flow cell onto which leucine hydrogenase, NADH oxidase and peroxidase have been immobilized (175). Cholesterol has been monitored by passing the sample through immobilized cholesterol oxidase (159, 160) and L-alanine, α -ketoglutarate and L-glutamate determined with immobilized alanine aminotransferase and glutamate oxidase (174). Glucose has been monitored in subcutaneous tissue fluid and blood of rabbits after passing through immobilized glucose oxidase (167), in plasma by flowing through

immobilized pyranose oxidase (169), in mixtures with 3-hydroxybutyrate by passing through two NADH oxidase enzyme reactors containing glucose dehydrogenase and 3-hydroxybutyrate dehydrogenase (164), and in mixtures with lysine by passing through immobilized glucose oxidase to measure glucose and through lysine oxidase to measure lysine (176). The same principle has been applied to the measurement of lysine and ornithine (177). On-line co-immobilized 3-hydroxybutyrate dehydrogenase and NADH oxidase has been used for the measurement of 3-hydroxybutyrate in serum, with very good agreement with other accepted analytical methods (184). Hydrogen peroxide can also be produced from sulphated bile acids by passing through a column of bile acid sulphate sulfatase and 3 β -hydroxysteroid dehydrogenase (185). α -Chymotrypsin, trypsin and a commercial protease have been determined by passing through a mini-column containing immobilized isoluminol (151).

Catecholamines can be measured in plasma by imidazole conversion to hydrogen peroxide prior to the peroxyoxalate CL reaction (157). D-Amino acids in plasma have been determined with good agreement with a colorimetric method by flowing through a specific enzyme reactor containing D-amino acid oxidase before peroxyoxalate CL detection of hydrogen peroxide (163). Incubation of dopamine with imidazole at 60°C for 30 min in the dark generated hydrogen peroxide, which was then introduced into a FI analyser for peroxyoxalate CL detection (165). Glucose or choline and acetylcholine, separated by a cation exchange column, have been determined in urine by conversion to hydrogen peroxide via a glucose oxidase reactor or a choline oxidase-acetylcholine esterase reactor, respectively (170). Compounds containing an alcoholic or phenolic hydroxyl group (polyphenols, monophenols and sugars) have been determined by mixing with imidazole and heating to 80°C prior to peroxyoxalate detection of hydrogen peroxide (178).

Several other reactions have also been used for biomedical applications of FI-CL. Amino acids have been determined by complexation with cobalt(II) and inhibition of the lucigenin CL reaction (152), by the inhibiting effect of cobalt(II) complexes or enhancing effect of copper(II) complexes on the ninhydrin-hydrogen peroxide CL reaction (153) and by their effect on the 1,10-phenanthroline/copper(II)-hydrogen peroxide CL reaction (154). Chlorotetracycline has been assayed in urine, with recoveries of 98.8–101.1% by its effect on the copper(II)-ammonium carbonate-cetyltrimethylammonium bromide-hydrogen peroxide CL reaction (158). Proteins have been monitored via the catalytic effect of copper(II) complexes on the luminol (180) or phenanthroline (181) CL reaction with hydrogen peroxide and porphyrins have been measured in urine via the peroxyoxalate-hydrogen peroxide reaction (179).

Chromium(III) has been determined in urine, blood

serum and hair by its catalytic effect on the luminol-hydrogen peroxide CL reaction. The method was validated by analysing a certified reference material and recoveries were within the range 89–115% (161). Copper(II) in serum has been determined with recoveries of 94–97% by its effect on the hydroxylamine-fluorescein CL reaction (162) and iron(III) in blood has been monitored by its effect on the luminol reaction and the results showed good agreement with those obtained by flame AAS (166). Iodide in urine can be monitored after oxidation to iodine with dichromate and measurement by the cobalt(II)-catalysed luminol reaction (173). The luminol reaction with $K_7[Cu(IO_6)]_2$ has been used for determining glucose (168) and uric acid (187), while vanilmandelic acid has been determined by its enhancement effect on the luminol-hexacyanoferrate(III) CL reaction, with an excellent detection limit (190), and uric acid has been measured in urine by its inhibiting effect on the luminol-periodate reaction, with results comparable with those obtained by spectrophotometry (189).

Serum glucose has been assayed by the CL reaction of hydrogen peroxide with 3-propyl-7,8-dihydropyridazino-[4,5-g]quinoxaline-2,6,9(1H)-trione, which is a luminol-related compound (172). Acidic permanganate has been used as a chemiluminogenic reagent for the determination of pyruvate in serum (182), serotonin and related indoles (183) and uric acid (188). Tryptophan has been found to exhibit CL by the action of cerium(IV) (186), while bilirubin generates CL by reaction with N-bromosuccinimide or sodium hypochlorite (155).

GENERAL/UNSPECIFIED APPLICATIONS

A number of published FI-CL methodologies have involved the analysis of synthetic aqueous or organic solutions, but either no real sample application has been specified or the application is not one that easily fits into the categories described above. However, in most cases the analytes of interest and the CL chemistries concerned are similar to those described in the preceding sections, so with little or no adaptation these methods should be equally applicable to analyses of such real sample matrices. Details of these methods are summarized in Table 5.

The majority of these methods have been applied to determinations of metal ions or hydrogen peroxide. Aluminium(III), zinc(II), cadmium(II) and indium(II), for example, have all been measured at part-per-billion levels in a 1,4-dioxane matrix using peroxyoxalate-hydrogen peroxide CL (195), while ytterbium(III) has been measured at nanomolar levels in chemical reagents, owing to its quenching effect on the chromium(III)/luminol-hydrogen peroxide CL reaction (226). Luminol-hydrogen peroxide CL methods incorporating immobilized horseradish peroxidase have been used to determine

both hydrogen peroxide (205) and horseradish peroxidase (204) at picomolar and femtomolar levels, respectively. A number of organic analytes have also been measured, in both organic and aqueous media. Two examples are 2,4-dinitrophenylhydrazine and benzaldehyde. The former has been measured at sub-nanomolar levels in aqueous-propan-2-ol solution, using a CL method based on the potassium permanganate-formic acid reaction sensitized with rhodamine B (191–193), while the latter has been determined in a benzyl alcohol medium with a gallic acid-hydrogen peroxide-sodium hydroxide CL method (196). Other reported methods worthy of mention here include a luminol CL reaction catalysed with hexacyanoferrate, which has proved suitable for the measurement of nanomolar levels of fructose, glucose and creatinine, and picomolar levels of uric acid, phenacyl alcohol, cortisone, ascorbic acid, corticosterone, glutathione and cysteine (223), and a method based on pyrogallol CL that has been applied to sub-millimolar determinations of potassium permanganate, cerium(IV), potassium periodate, sodium hypochlorite and hydrogen peroxide (218).

CONCLUSIONS

The major trend in the last 5 years has been the significant increase in the application of FI–CL reactions to real sample matrices. A survey of all FI literature has shown that CL is now the fourth most commonly used detector for FI applications (after UV/visible, fluorescence and amperometry). This popularity is due to the attractions of rapid and reproducible mixing of sample and reagents and the ability to perform on-line chemical and physical sample treatment. With regard to CL chemistries, the recent trend has been to modify well known CL reactions to suit particular applications rather than to develop novel CL reactions.

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High temporal resolution field monitoring of phosphate in the River Frome using flow injection with diode array detection

Grady Hanrahan^{a,b}, Martha Gledhill^a, Philip J. Fletcher^a, Paul J. Worsfold^{a,*}

^a Department of Environmental Sciences, Plymouth Environmental Research Centre, University of Plymouth, Plymouth PL4 8AA, UK

^b Centre for Ecology and Hydrology (CEH), Winfrith Technology Centre, Dorset DT2 8ZD, UK

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Abstract

The design and deployment of an in situ flow injection (FI) monitor for high temporal resolution monitoring of phosphate in the River Frome, Dorset, UK, is described. The monitor incorporates solenoid, self-priming micropumps for propulsion, solenoid-operated switching valves for controlling the fluidics and a miniature CCD spectrometer for full spectrum (200–1000 nm) acquisition and operates in a graphical programming environment. A tangential filtration unit is attached to the sample inlet line to remove suspended particulate matter and prevent blockage of the micropumps and valves. Detection (at 710 nm) is based on molybdenum blue chemistry with tin(II) chloride reduction. The detection limit is 0.67 μM PO_4 and the linear range can be adjusted by using different wavelengths for detection. Pump noise is eliminated by subtraction of the signal at a non-absorbing wavelength (447 nm). Data from an intensive (sample every 30 min) field trial on the River Frome performed in October 2000 are presented, and the implications of the data for refining an export coefficient model for phosphorus from the catchment are discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Flow injection; Eutrophication; Solenoid

1. Introduction

Phosphorus export (loading) into catchments from both point and diffuse sources can result in eutrophication, i.e. an enrichment of nutrients, which can in turn lead to an increase in biomass and primary productivity within aquatic communities [1,2]. Eutrophication of natural waters has become a subject of increasing public concern which, in the European Union, has been recognised by legislation setting 0.1 mg l^{-1} PO_4 as an indicator level for possible problematic algal growth in rivers [3]. An assessment of water quality

is an essential prerequisite for the implementation of successful management strategies for minimising the likelihood of eutrophication. Phosphorus loading is an important factor in this assessment and is often the best indicator of the nutrient status of receiving waters. Models have been implemented to predict nutrient loads and their sources and sinks [4–7] and for this study a simple export coefficient model for the River Frome, Dorset, UK, was constructed. The annual and seasonal total phosphorus (TP) loads were predicted as the sum of the export of phosphorus from each nutrient source [8]. However, the transfer of phosphorus from land and its concentration in catchments such as the River Frome are strongly influenced by rainfall.

There are extensive data on instantaneous (grab sample) phosphorus concentrations in rivers but very

* Corresponding author. Tel.: +44-1752-233006;

fax: +44-1752-233009.

E-mail address: p.worsfold@plymouth.ac.uk (P.J. Worsfold).

little information that shows short-term changes in phosphorus concentration occurring, for example, immediately before, during and after a rain event. Consequently, field instrumentation capable of acquiring high quality analytical data with good temporal resolution, i.e. every 15–30 min, would be a useful development in its own right [9,10]. In addition such data are essential for building improved models for predicting the transport and biogeochemical behaviour of phosphorus in catchments.

This paper describes the design and deployment of an in situ flow injection (FI) monitor for high temporal resolution monitoring of phosphorus in the River Frome. The monitor incorporated a miniature diode array detector, self-priming micropumps and electronic switching valves for controlling the fluidics. Analytical performance, in the laboratory and in the field, is presented and the influence of the data on an export coefficient model for phosphorus in the Frome catchment considered.

2. Experimental

2.1. Reagents

All solutions were prepared in ultra-pure water (Elga Maxima[®], 18.2 M Ω) and all reagents were of AnalaR (or equivalent) grade and purchased from BDH Merck, unless otherwise stated. All containers, bottles and glassware used during this study for manipulating and storing reagents, samples and standards

were first cleaned overnight with nutrient free detergent (Neutracon[®], Decon Laboratories), rinsed three times with ultra-pure water and soaked in 10% (v/v) HCl for at least 24 h. All were then rinsed three times with ultra-pure water and dried at room temperature.

2.1.1. Flow-injection manifold

The optimum concentrations for the two phosphorus reagent streams were: ammonium molybdate solution (10 g l⁻¹ ammonium molybdate in 35 ml l⁻¹ sulphuric acid), and tin(II) chloride solution (0.2 g l⁻¹ tin(II) chloride and 2 g l⁻¹ hydrazinium sulphate in 28 ml l⁻¹ sulphuric acid). Working standard solutions in the range of 0.8–8.0 μ M PO₄ were prepared by dilution from a 3 mM stock solution (0.4393 g of oven dried potassium dihydrogenorthophosphate diluted to 1000 ml with water). The carrier stream was ultra-pure water.

2.1.2. Batch method

The three reagents used for batch determination were: ammonium molybdate (15 g diluted to 500 ml with water), ascorbic acid (5.4 g diluted to 100 ml) and potassium antimonyl tartrate (0.34 g diluted to 500 ml). Working standard solutions in the range 0.3–12.0 μ M PO₄ were prepared by dilution from a 3 mM stock solution (0.4393 g of oven dried potassium dihydrogenorthophosphate diluted to 1000 ml).

2.2. Flow injection (FI) manifold

The manifold used is shown in Fig. 1. It consisted of four solenoid-operated self-priming micropumps

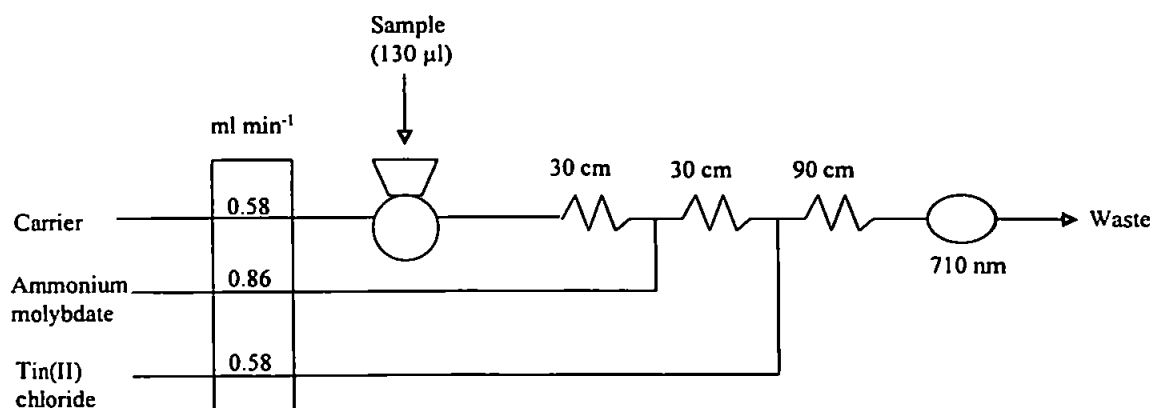


Fig. 1. FI manifold for the determination of PO₄ in freshwaters.

(Bio-Chem Valve series 120SP12-25, PD Marketing, Chichester, UK) connected to solenoid-operated PTFE switching valves (Bio-Chem Valve series 075T12-32, PD Marketing) with PTFE tubing (0.8 mm i.d.). The fluidics and the operation of the system are discussed in detail in Section 3.1. An Ocean Optics PSD-1000 miniature fibre-optic spectrometer (Anglia Instruments Ltd., Cambridge, UK) consisting of two 1024 element linear CCD arrays was used for detection. The master channel (UV–VIS range), with 600 lines mm^{-1} blazed at 300 nm, enabled a spectral range of 200–700 nm. The slave channel (VIS–NIR range), with 600 lines mm^{-1} blazed at 750 nm, enabled a spectral range of 500–1000 nm. The light source was a miniature halogen lamp (LS-1 Ocean

Optics Inc.). Data communication was performed using a type II PCMCIA DAQ-DIO-24 card (National Instruments Corp., Berks, UK) and a Toshiba Satellite 4030 CDS notebook PC (Toshiba Information Systems Ltd., Surrey, UK). Automation of the manifold and data acquisition was controlled using an in-house graphical program (LabView™ 5.0, National Instruments Corp.). A schematic diagram of the complete system is shown in Fig. 2.

2.3. Field study site

The River Frome rises on the North Dorset Downs near Evershot and flows into Poole Harbour in the Southwest of the UK. The most important geological

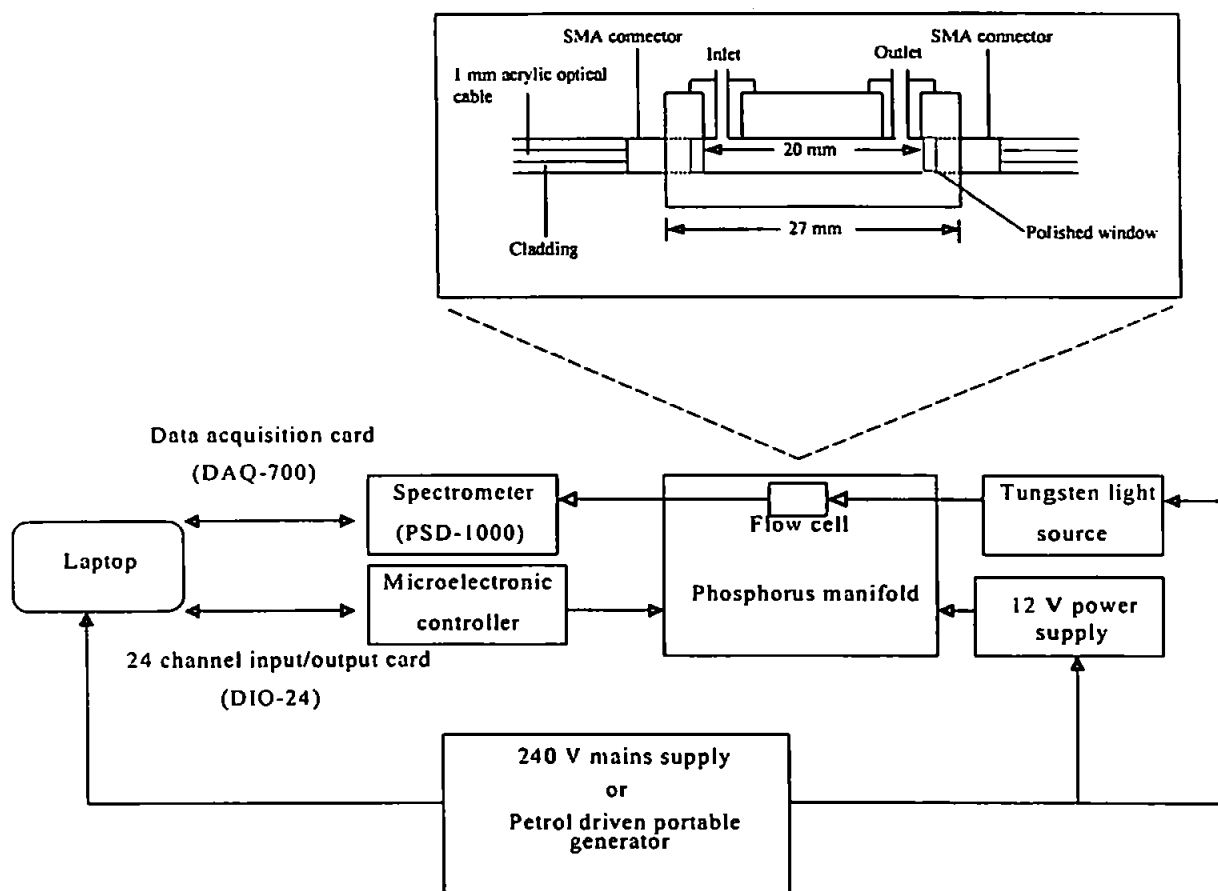


Fig. 2. Schematic diagram of the complete FI system for PO_4 determination with an insert showing the PVC flow cell incorporating 1 mm acrylic optical fibre cables.

formation is chalk, which comprises nearly 50% of the 414 km² catchment [11]. Land use in the catchment is predominately permanent grassland, dairying or stock rearing, cereals and natural wetlands. The hydrology of the Frome catchment is dominated by a highly reactive aquifer passing beneath the lower catchment and Poole Harbour. River flow is dependent on upper reach streams and groundwater levels and drains into Poole Harbour, UK.

2.4. Procedures for field trial

A 3 day high temporal resolution campaign was undertaken on the River Frome at the Environment Agency gauging station, East Stoke (grid reference SY866867). River water was pumped up a vertical height of 2 m into the filtration unit, incorporating a 0.45 µm cellulose acetate filter (0.47 mm o.d.), using a rotary pump (Cole-Parmer 7532-02). Calibration was performed each morning and evening using 0.8, 1.5, 4.0, 6.0 and 8.0 µM PO₄ standards, with a 4.0 µM standard injected after every fourth or fifth river sample as a quality control (QC) sample. Calibration was then adjusted according to the QC result. The sampling cycle was configured to sample every 30 min. Samples for batch analysis were collected in 125 ml HDPE sample bottles (Nalgene®) at the same time as sample was introduced into the FI monitor. Bottles were first rinsed twice with filtrate then filled with approximately 50 ml aliquots of sample. Samples were then preserved with 0.1% (v/v) chloroform, labelled, placed in resealable plastic bags and stored at 4°C in the dark. Upon return to the laboratory (within 6–12 h), samples were allowed to warm to room temperature and then analysed immediately for PO₄ using a Beckman DU-8 spectrophotometer according to the method of Eisenreich et al. [12].

3. Results and discussion

3.1. Instrument design

Previous field FI systems have typically incorporated solid-state detection based on light-emitting diodes (LEDs) and photodiodes, peristaltic pumps and text-based software. Such solid-state detection is limited in that it can only provide an integrated

response over the spectral bandwidth of the LED (typically 20–30 nm). In addition, peristaltic pumps require frequent recalibration and maintenance as well as regular replacement of pump tubing. The use of text-based software allows instrument control, but is difficult to use and has limited data acquisition and processing capabilities. The instrumentation reported here therefore provides major advantages in terms of spectral acquisition and signal processing, component reliability and ease of operation.

A prototype instrument, used to determine nitrate in the River Frome, has been previously reported [13]. The current instrument has several design modifications to improve reliability for field use. Firstly, the conventional quartz flow cell was replaced by a rigid, in-house 20 mm bore PVC flow cell incorporating 1 mm acrylic fibre optical cables (see Fig. 2 insert). Secondly, an on-board tangential flow filtration unit [14] was incorporated to prevent blockage of the micropumps (a limitation of the original design) and thereby significantly extend operating lifetime and reliability in the field. In addition, the system was housed in a secure, impact resistant IP67 rated polycarbonate box (FIBOX, Finland) for adequate protection during field use and the software was modified to allow for replicate analyses and in-field adjustment of the calibration.

The self-priming micro pumps and miniature solenoid valves provided a viable alternative to peristaltic pumps. Fig. 3 shows the fluidic layout of the FI automation injection sequence. Switching valves were set to normally open — common (NO/COMM) when de-energised and to normally closed — common (NC/COMM) when power was supplied to the valves. The advantages of the system are low maintenance (and therefore potentially longer deployments) and electronic control of the flow rates. The two drawbacks are the additional pulsing of the micropumps compared with peristaltic pumps and their susceptibility to blockage from particulate matter. The first problem was overcome by using the spectral capability of the detector to subtract the signal at a non-absorbing wavelength and the second problem by incorporation of the tangential flow filter.

The phosphorus FI manifold was based on a published design [15] but the limit of detection was improved to meet the needs of the study site (annual variability of 1–4 µM PO₄) [9,15]. This was achieved

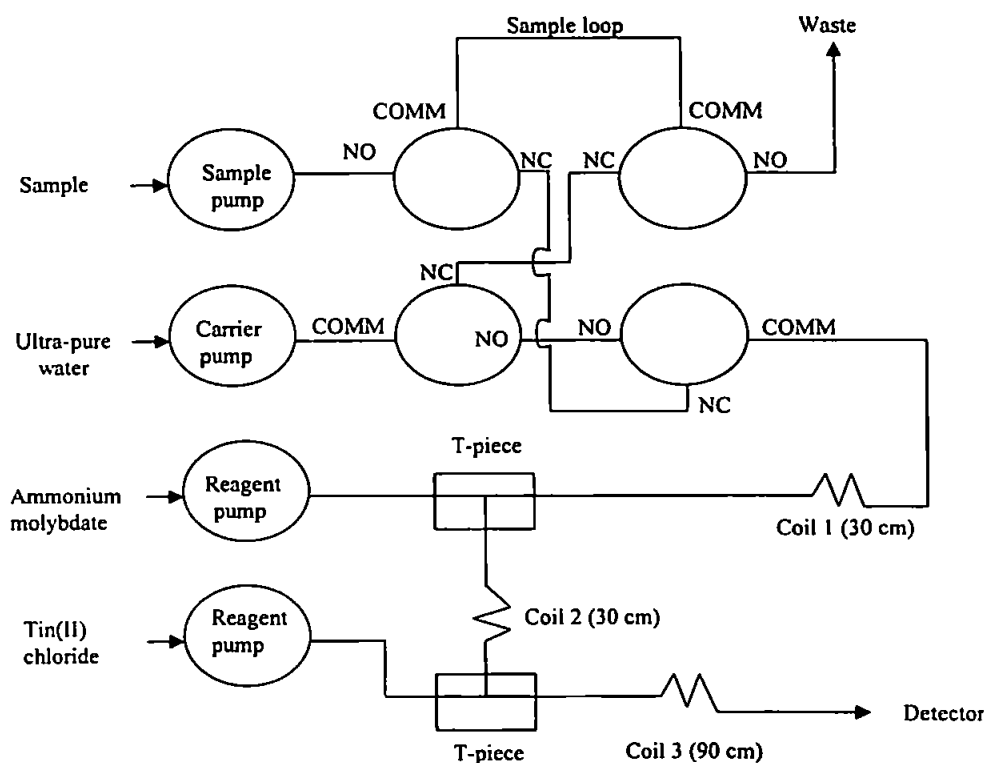


Fig. 3. Fluidics of the automated FI monitor for PO_4 determination. Switching valves were set to normally open — common (NO/COMM) when de-energised and to normally closed — common (NC/COMM) when power was supplied to the valves.

by increasing the sample volume from 40 to 130 μl and monitoring the phosphomolybdenum blue species produced at 710 nm (instead of 690 nm).

3.2. Laboratory FI calibration

The FI determination of PO_4 was based on the standard reaction with acidic molybdate to form 12-molybdophosphate, which was then reduced to form an intensely coloured molybdophosphate blue species [16]. Five phosphate standards covering the range 0.8–8.0 μM PO_4 gave linear calibration graphs ($r^2 > 0.998$), with a gradient of 0.0043 absorbance μM^{-1} and an intercept of -0.009 absorbance. The relative standard deviations (R.S.D.) for these standards were typically in the range of 0.0–1.3% ($n = 3$) and the limit of detection (calculated from the mean of the blank plus three times the standard deviation of the blank) was 0.67 μM PO_4 . All measurements were made at 710 nm (λ_{max}) and processed by sub-

tracting the absorbance at a non-absorbing reference wavelength (447 nm). Fig. 4a and 4b compare the single (710 nm) and dual wavelength (710–447 nm) FI responses for the standards and clearly demonstrates the success of using the non-absorbing wavelength to remove the effect of pulsations caused by the micropumps.

The linear calibration range was extended to 0.8–50 μM PO_4 by reducing the sample volume injected from 130 to 90 μl and monitoring at 650 nm (selected as the wavelength giving 50% of maximum absorbance at 710 nm). This gave a linear calibration graph ($r^2 = 0.994$, $n = 6$) with a gradient of 0.0034 absorbance μM^{-1} and an intercept of 0.009 absorbance. Good reproducibility was also observed throughout the range, with R.S.D.s typically $< 3.0\%$ ($n = 7$). This shows that the linear range can easily be adjusted in the field to suit local conditions and changing circumstances, e.g. storm events. For this deployment, 710–447 nm with a sample loop volume

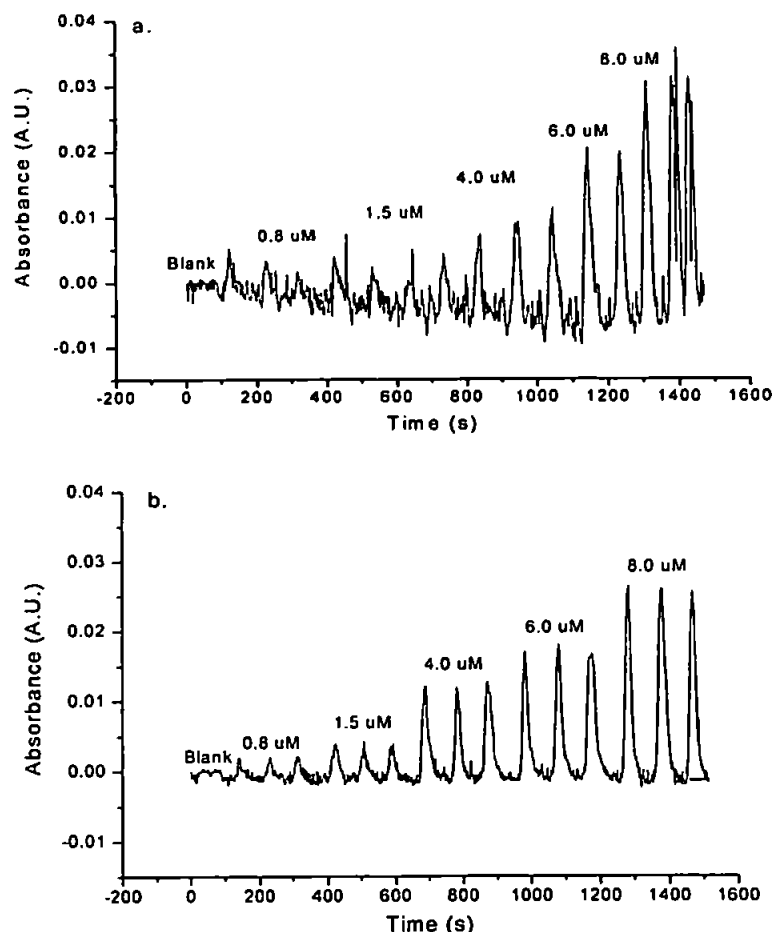


Fig. 4. (a) Single wavelength (710 nm) and (b) dual wavelength (710–447 nm) scan of a PO_4 calibration series (0.8–8.0 μM).

of 130 μl was considered the best measurement protocol for the levels typically found in the River Frome in October. The addition of the QC (4.0 μM PO_4) after every fourth or fifth sample allowed for correction of drift arising from, e.g. changes in air temperature.

3.3. River Frome field study (October 2000)

A comparison of the data for the three field calibrations (1–3 October 2000) is given in Table 1. Reproducibility for replicate injections of standards was typically <4.0% R.S.D. ($n = 3$) and the pooled data (between batch) also showed R.S.D. of <4.0%, with a linear correlation coefficient (r^2) of 0.997. The QC sample was analysed 15 times over the 3 day

period and the results were all within 3.7% (range 3.81–3.95 μM PO_4), showing that external factors did not significantly affect the response. Fig. 5 shows the diurnal PO_4 concentration profile over the course of the 3 day monitoring campaign, during which the monitor ran continuously after initial installation except for deliberate stoppages for the calibrations and subsequent changes of filter in the tangential flow filtration unit. The environmental significance of the data is discussed in Section 3.5.

3.4. Intercomparison study

An intercomparison study between the FI instrument and the batch method on 30 samples was

Table 1

Table of field calibration data for PO₄ obtained during the field deployment on the River Frome.

[PO ₄] (μM) (n = 3)	Day 1		Day 2		Day 3		Pooled Data	
	Mean absorbance	R.S.D. (%) (n = 3)	Mean absorbance	R.S.D. (%) (n = 3)	Mean absorbance	R.S.D. (%) (n = 3)	Mean absorbance	R.S.D. (%) (n = 3)
0.8	0.0027	2.7	0.0021	3.7	0.0019	1.5	0.0020	2.6
1.5	0.0040	6.0	0.0049	2.3	0.0040	2.7	0.0043	3.7
4.0	0.0121	0.9	0.0135	4.3	0.0121	0.8	0.0126	2.0
6.0	0.0168	1.0	0.0181	0.7	0.0166	2.3	0.0172	1.3
8.0	0.0260	1.6	0.0276	2.0	0.0257	1.9	0.0263	1.8
r ²	0.993	0.993	0.998	0.998	0.995	0.995	0.997	0.997
Gradient (absorbance, μM ⁻¹)	0.0038	0.0038	0.0043	0.0043	0.0038	0.0038	0.0038	0.0038
Intercept (absorbance)	0.0003	0.0003	-0.009	-0.009	0.0004	0.0004	0.0007	0.0007

undertaken. Samples were analysed immediately using the FI monitor (range 4.74–5.13 μM PO₄) and also collected manually, stored and analysed later (within 8 h) using the batch method (range 4.75–5.34 μM PO₄). A paired *t*-test showed no significant difference at *P* = 0.05 (the critical value of *l*_l was 1.96 and the calculated value of *l*_l was 0.88). This means that data acquired using the field instrumentation can

be directly compared with historical data acquired using the batch method and can therefore also be incorporated into the export coefficient model.

3.5. Effect of FI data on the export coefficient model

The export coefficient model for the Frome catchment [8] predicted a TP load of 26,100 kg per year,

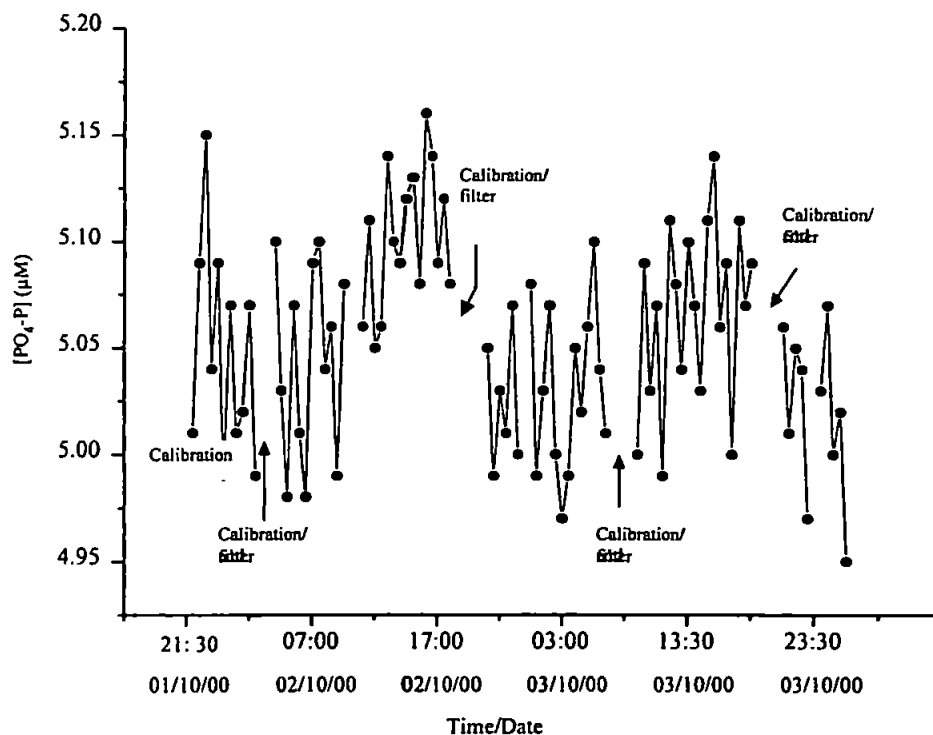


Fig. 5. Time series plot of PO₄ concentration obtained over a 3 day sampling campaign on the River Frome in October 2000.

compared with an observed (measured) load of 23,400 kg per year for 1998. Data for the observed load were obtained from manually collected samples (four per month) analysed in the laboratory using the batch method. The concentration data were integrated with flow data from the same sampling point and the observed annual load determined. Due to the variation in number of days between measurements, the loads were calculated on a time interval basis and aggregated to obtain the annual load. The observed load for 1998 was 11% less than the predicted load, due in part to the lack of resolution of the manual sampling programme, which meant that insufficient samples were collected during periods of peak river flow (when phosphorus loads are at their highest).

During the 3 day deployment, and for 10 days prior to that, it remained dry and therefore it is not surprising that Fig. 5 does not show major changes in PO_4 concentration. Nonetheless the difference between the highest and lowest concentrations of PO_4 observed would have resulted in an annual difference of 770 kg in an aggregated calculation which, assuming a constant ratio of TP to PO_4 of 1.6:1.0 [8], is equivalent to 1230 kg TP. It is reasonable to assume that over the lifetime of a rain event the PO_4 concentration will fluctuate much more rapidly and that this data will have significant leverage on the aggregated observed annual load. The above hypothesis will be tested during future deployments but this paper demonstrates the feasibility of acquiring the necessary high temporal resolution data using FI based field instrumentation.

4. Conclusions

The FI field monitor described is effective and reliable for measuring PO_4 in freshwaters, with a detection limit of $0.67 \mu\text{M}$ and the ability to respond to changes in environmental conditions by adjusting the wavelength used for detection. The addition of the tangential flow filtration unit prevented blockage of the micropumps seen in earlier deployments, which allowed extended and uninterrupted introduction of samples. The monitor can sample with high temporal resolution (every 30 min) which is necessary to measure short-term changes in PO_4 concentration. In

addition, the acquired data can be used to refine the export coefficient model for phosphorus loading in the Frome catchment.

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