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# DETERMINATION OF TRACE METALS BY ION-CHROMATOGRAPHY WITH CHEMILUMINESCENCE DETECTION

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A thesis entitled

**DETERMINATION OF TRACE METALS BY ION-CHROMATOGRAPHY WITH  
CHEMILUMINESCENCE DETECTION**

presented by

**TIMOTHY PAUL WILLIAMS, B.Sc., GRSC**

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requirements for the degree of

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DETERMINATION OF TRACE METALS BY ION-CHROMATOGRAPHY WITH  
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ABSTRACT

It is generally considered that the detection step for the determination of trace metals by liquid-chromatographic systems is the weakest area of the analytical procedure. Various post-column reagent systems, for trace metal analysis, based on the highly sensitive luminol-peroxide chemiluminescence (CL) reaction have been developed and are described.

A simple cation-exchange chromatographic system was developed and used to separate cobalt from other metal ions. These cobalt ions were detected using an optimised luminol-peroxide CL post-column reagent. A detection limit of  $0.5 \text{ ng l}^{-1}$  was achieved without the need for preconcentration, for a  $200 \mu\text{l}$  sample. The cobalt content of a rice flour certified reference material was determined using this system and gave good agreement with the certificate value.

A similar system, based on ion-exchange chromatography with CL detection was developed to simultaneously determine Cr(III) and Cr(VI) without any sample pretreatment. The separation system involved the employment of both anion and cation exchange columns connected in parallel and used a 10 port injection valve to introduce the sample. The determination of Cr(VI) required the investigation of various reducing agents which are discussed. Very high sensitivity detection for Cr(III) and Cr(VI) was achieved with a detection limit of  $0.3 \mu\text{g l}^{-1}$  for both species. The chromium content of a simulated fresh water certified reference material was determined and the results are discussed.

Finally, an ion-chromatographic system was developed for trace, multi-element analysis with CL detection. The system is highly sensitive and non-selective and is based on the displacement and determination of cobalt from a Co-EDTA post-column reagent by eluting metal species. The analyte metals originate from all areas of the Periodic Table and detection limits range between 2 and  $100 \mu\text{g l}^{-1}$  depending on the analyte. Some of the experimental data has been compared with a theoretical computer model and the results are discussed. Using this system the zinc and aluminium content of a certified sample was determined and gave good agreement with the certificate value.

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## CHAPTER 1

### INTRODUCTION

#### 1.1 The Requirement for Trace Metal Analysis

In recent decades, the importance of trace metal determinations has become recognised. Nowadays, the term 'trace analysis' no longer conveys a novel idea even though this branch of analysis was not distinguished until about 35 years ago with the determinations of trace elements in plants in order to elucidate their role in plant physiology.

The determination of trace concentrations of metals is of particular interest in health protection, as trace quantities can have a far reaching impact regarding health and diseases. Trace metal analysis is also of interest to public health institutions which are now able to establish threshold limit values for toxic substances contained within household articles, foodstuffs and industrial and laboratory atmospheres.

Some of the most important fields in which trace metal analysis is now used routinely include, for example, production technology of high purity materials such as those used in nuclear reactor design and in the production of semiconductors and fibre optics, where the presence of metal contaminants would adversely affect the performance of the product.

Another example in which trace metal analysis is important is in plant and animal systems where the trace metal ions play a vital role in a vast number of widely differing biological processes. Some of these processes are quite specific in their metal ion requirements in that only certain metal ions, in specified oxidation states, can fulfil the necessary catalytic or structural requirement. For example, the metal ions magnesium, manganese, iron, cobalt, copper, molybdenum and zinc are all important catalysts in a variety of enzyme reactions. Sodium, potassium and calcium, however, are heavily involved in certain physiological control and trigger mechanisms, while potassium, calcium and magnesium ions are all important in controlling the structure and function of cell walls.

In addition to those essential metal ions already mentioned, some metal ions are of environmental concern because of their toxic affect towards organisms. Even essential ions can, under certain conditions, reach toxic levels. For example, an over-exposure to iron can cause the condition siderosis.

Many non-essential ions can act as toxins and these are usually the soft, heavy metals and metalloids, which have minimal biological roles, and cause many problems when they appear in biological systems as a result of their very strong binding to biological macromolecules. They include compounds of arsenic, cadmium, mercury and lead.

There is also much controversial evidence of the carcinogenic effects of a wide range of metals and their compounds including chromium, nickel, arsenic, cadmium and beryllium, all of which are associated with cancer in humans (1).

The monitoring of trace metal pollutants from industrial processes has also been looked at more carefully as continually updated government legislation increases the pressure for industries to dispose of their waste products more responsibly. The coal combustion industry, amongst others, at present releases many metals as air pollutants. Some of these metals include lead, vanadium, manganese, arsenic, nickel, cadmium, mercury and beryllium. Water pollution is also of growing concern as almost every type of industrial chemical process involves the release of trace quantities of a ubiquitous cocktail of many metals in one form or another.

One specific industrial problem involves the monitoring and determination of corrosion products in coolant feeds of pressurized water reactors. A problem of radioactive build up occurs out-of-core resulting from the presence of the metal atoms nickel and cobalt in the material of construction of the primary circuit. The inactive metal ions are released into the coolant by aqueous corrosion and erosion processes, activated in the core, and subsequently deposited on out-of-core surfaces. Nickel and cobalt are the respective precursors of the high

energy, long lived  $^{58}\text{Co}$  and  $^{60}\text{Co}$  radionuclides and radioactive build up leads to increasingly high operational radiation exposure during shutdown in areas where maintenance and repair work are necessary. An accurate record of the exposure dose of radioactivity is required when assessing the possible health risk to workers in these potentially hazardous areas.

One would assume, from all the above examples of the presence and consequences of trace metals in industrial processes, biological systems and the environment, that there is an obvious requirement to monitor and determine such metals especially at the trace level where subtle differences in metal concentration can have a far reaching impact on the surrounding environment, be it industrial, biological or ecological.

### 1.2 The Importance of Trace Metal Speciation

To the scientist concerned with environmental problems, information on speciation, or chemical form, of a particular element can be of the utmost importance. This consequence arises from the fact that the form of the species which is present can have a dramatic affect on its toxicity and availability to biological systems.

Perhaps one of the simplest examples is given by the chemistry of chromium. Chromium exists in the environment predominantly in two oxidation states; Cr(III) and Cr(VI). The trivalent state is required by animals

for the maintenance of the normal glucose tolerance factor. The hexavalent state, however, has been shown to be much more toxic than the trivalent state in animal experiments (2, 3) and is considered to be particularly dangerous, primarily because of the associated risk of allergic reaction and cancer. As a consequence of this risk, there is an obvious need for the analyst to be able to distinguish between the two oxidation states.

Another, more recent example, is shown by the growing concern towards aluminium toxicity, initiated mainly by the "acid rain" problem. Studies have led to the growing realisation that the release of  $Al^{3+}$  ions from the soil has led to toxicity problems to fish (4). A number of different species of aluminium are present in normal natural waters, and these are summarized in Table 1.1.

The most toxic aluminium species towards organisms are the so-called labile monomeric species. In the other aluminium forms, the metal is chemically bound within an organic complex or polymeric systems and is less able to exhibit its toxic effect. The labile monomeric species are causing health concern, but at present, there are few analytical procedures to distinguish between these toxic and non-toxic forms.

Organometallic species can have many times the toxicity of the purely ionic species. Methylation greatly influences the absorption, distribution and toxicity of a number of

**Table 1.1** The aluminium species present in natural water

<u>Aluminium Form</u>	<u>Examples</u>
Labile monomeric species	$\text{Al}^{3+}$ , $\text{Al}(\text{OH})^{2+}$ , $\text{Al}(\text{OH})_2^+$ , $\text{Al}(\text{OH})_3$
Non-labile monomeric species	Al-humate, Al-fulvate etc
Polymeric species	alumina silicates and aluminium colloidal systems.

metals and metalloids. Bio-methylation has been demonstrated for mercury, arsenic, lead, thallium, chromium, selenium, platinum and palladium.

Analytical determinations must take into account the form in which the metal is present and without such information few meaningful results can be inferred.

### 1.3 Quantitative Inorganic Analysis

The main techniques employed in quantitative inorganic analysis are based upon (a) the quantitative performance of a suitable chemical reaction, (b) electrical measurements, or (c) the measurement of optical properties (absorption and emission techniques).

The use of chemical reactions for quantitative inorganic determinations are the basis of the traditional or classical methods of chemical analysis. These classical methods include gravimetry, titrimetry and volumetry. In gravimetric analysis the substance being determined is converted into an insoluble precipitate which is collected and weighed. Titrimetric, or volumetric, analysis involves the substance being allowed to react with an appropriate reagent added as a standard solution, and the volume of solution needed for complete reaction is determined. Volumetry measures the volume of gas evolved or absorbed in a chemical reaction.

Electrical methods of analysis involve the measurement of

current, voltage or resistance in relation to the concentration of a certain species in solution. These methods include (i) voltammetry (measurement of current at a specified voltage), (ii) coulometry (measurement of current and time needed to complete an electrochemical reaction), (iii) potentiometry (measurement of the potential of an electrode in equilibrium with an ion to be determined) and (iv) conductimetry (measurement of the electrical conductivity of a solution).

Optical methods of analysis are based upon (i) the absorption of radiant energy at a particular wavelength or (ii) the emission of radiant energy at a particular wavelength. Absorption methods are classified according to the wavelength involved as (a) ultraviolet spectroscopy, (b) visible spectroscopy and (c) infrared spectroscopy.

Atomic absorption spectroscopy involves the aspiration of a solution of a sample into a flame and then studying the absorption of radiation from an electric lamp producing the spectrum of the element to be determined. Emission methods involve an excitation step to raise atoms to excited levels causing them to emit energy on their return to the lower energy ground state. Common techniques are: (i) emission spectroscopy, where the sample is subjected to an electric arc or spark and the light that is emitted examined, (ii) flame photometry, in which a solution of the sample is injected into a flame and the light emitted

is measured and (iii) fluorimetry, in which a suitable substance in solution is excited by irradiation with visible or uv radiation, and the emitted radiation is then examined.

In addition to the general methods mentioned, there are also certain specialized techniques for quantitative inorganic analysis and these include X-ray fluorescence and methods based on radioactivity.

The methods based on measurements of electrical properties, and the extent to which radiation is absorbed or emitted all require the use of a suitable instrument. Instrumental methods are usually much faster than purely chemical procedures, are normally applicable at concentrations far too small to be amenable to determination by chemical procedures and find wide applications in industry.

Despite widespread adoption of instrumental techniques, it has not rendered classical techniques obsolete and instrumental and classical methods must be regarded as supplementing each other.

There are many solution and solid techniques available for present instrumental analysis and the careful consideration and choice of method is dependent on several criteria.

- (a) The type of analysis required; elemental or molecular, routine or occasional.
- (b) Possible interferences within the sample.
- (c) The concentration range, accuracy and precision required.
- (d) Turnover time for analysis to be completed.
- (e) The cost of the analysis.
- (f) Problems arising from the nature of the material to be investigated e.g. substances affected by water, corrosive substances, radioactive substances etc.

Some of the information that is required in order to make the relevant choice of the appropriate analytical method is given in Table 1.2.

#### 1.4 The use of Liquid Chromatography Systems for Trace Metal Analysis

Chromatographic methods and organic analysis have become almost synonymous. An analytical procedure for the separation and determination of several organic compounds may be difficult to achieve without application of a chromatographic method. The analysis of inorganic cations, however, is essentially dominated by purely spectroscopic methods (e.g. atomic absorption, photometry) and electrochemical methods (e.g. voltammetry, polarography). Nevertheless, chromatographic methods are, nowadays, of great interest to the inorganic analyst and in a number of cases, chromatographic systems are sometimes superior to more conventional elemental analysis

**Table 1.2** Some of the details required when considering the relevant choice of instrumental analytical method

Method	Relative Cost	Accuracy	Speed	Concentration Range/ g
Coulometry	L - M	H	S - M	$10^{-1}$ - $10^{-4}$
Voltammetry	M	M	M	$10^{-3}$ - $10^{-10}$
Potentiometry	L - M	M	M - F	$10^{-1}$ - $10^{-7}$
Spectrophotometry	L - M	M	M - F	$10^{-3}$ - $10^{-6}$
Atomic Spectroscopy	M - H	M	F	$10^{-3}$ - $10^{-9}$
Emission/Plasma Spectroscopy	H	M	F	$10^{-5}$ - $10^{-9}$
Chromatography (GLC, HPLC)	M	M	F	$10^{-3}$ - $10^{-9}$
Neutron Activation	H	M	S	$10^{-5}$ - $10^{-12}$
X-ray Fluorescence	H	H	F	$10^{-3}$ - $10^{-6}$
ICP-MS	H	H	F	$10^{-10}$ - $10^{-11}$

Abbreviations: F, Fast; H, High; L, Low; M, Moderate; S, Slow

determinations such as atomic absorption spectroscopy or voltammetry.

There are several reasons why chromatographic methods may be particularly useful. These are when:

- (a) Several elements need to be jointly identified and determined in a single procedure (so called multi-element analysis).
- (b) Determination of elemental species is necessary to give information on toxic/non-toxic fractions (See Section 1.2).
- (c) Systems are required that are able to lend themselves to routine analysis because of their ease of automation, their ability for on-line monitoring for potentially hazardous samples, their simplicity in operation and cost effectiveness.
- (d) There is a need for trace enrichment or preconcentration since sensitivities of other available methods may not be sufficient.
- (e) There is a requirement for matrix elimination by use of chelation exchange, for example, in the analysis of trace metals in sea-water and concentrated brines.

Considerable advances have been made in all of the above areas in the last ten or twenty years, although they cannot always be generalised and often apply only to highly specific problems.

The review of the literature concerning chromatographic

systems for inorganic and organometallic separations up to 1970 is presented in a book by J. Michal (5) with well over 700 references, beginning with the first investigation in the field of inorganic adsorption analysis in 1937 by Schwab and his co-workers, who studied the sorption of anions and cations on alumina columns. Numerous practical applications were subsequently developed for adsorption chromatography as documented by Michal with some 70 references. A good proportion of his review is devoted to partition chromatography and, in particular, the use of paper chromatography for inorganic separations. Within this partition section there are well over 400 references of particular applications. The review contains a small section of some 50 references on ion exchange chromatography which almost exclusively concerns itself with the use of well-known 'classical' ion-exchange resins such as Dowex and Amberlite in large diameter glass columns to perform ionic separations, or with those resins impregnated within papers.

The literature between 1970 and 1979 for chromatographic inorganic analysis is surveyed in a book by G. Schwedt (6) who provides detailed information and a literature review in a number of areas including:

- (1) Adsorption chromatography of metal chelates by thin-layer chromatography (TLC) (60 references).
- (2) Partition chromatography of inorganic ions and of metal complexes by TLC (45 references).

- (3) Reversed phase chromatography of inorganic ions by TLC (60 references).
- (4) Selected examples of ion-exchange chromatography for inorganic ions by TLC (40 references):
- (5) Adsorption chromatography of metal complexes by HPLC (20 references).
- (6) Partition chromatography of inorganics by HPLC (8 references).
- (7) Liquid chromatography of inorganic species with reversed phases (16 references).
- (8) Separation of anions and cations, as well as inorganic complexes by means of ion-exchange materials (32 references).

.. as well as numerous examples of practical applications for chromatographic procedures for a variety of inorganic analytical problems.

Schwedt later reviewed the literature in 1979 (7) in which only HPLC methods that were used with sensitive detection devices were taken into account. The review covers the separation of organo-metallic compounds, as well as transition metal complexes by adsorption and reversed phase methods. He also reviews the chromatographic determination of extractable metal chelates and ion-exchange procedures with some 60 references in all.

Two more recent reviews covering the same areas as those by Schwedt have appeared in 1985 (8) and 1986 (9), both of which provide an excellent source of reference with a

combined number of 160 relevant publications. Another review, worthy of note, was published in 1985 by Nickless (10), in which the advances and developments for trace metal determinations by chromatography are discussed thoroughly with many useful references.

#### 1.4.1 The Proceses Involved in Inorganic Analysis by Liquid Chromatography

From the preceding section, it is clear that the three main processes involved in inorganic analysis by chromatographic means are adsorption, partition and ion-exchange chromatography, all of which shall be discussed in more detail.

##### 1.4.1.1 Adsorption Chromatography

Adsorption chromatography, introduced by Tsvett in 1906, for the separation of plant pigments, was the first chromatographic method to be investigated. A mixture of substances in solution is separated by a column of suitable adsorptive material which should have widely differing adsorptive powers for the different components. Polar adsorbents are more common than non-polar ones and the most usual materials employed are primarily silica gel and, to a lesser extent, alumina. Numerous organic solvents can be used and careful choice of suitable adsorbent and solvent will allow the required separation to be achieved.

Due to the highly polar nature of both silica and alumina, elution occurs using normal phase solvents such as those that have a medium to low polarity compared with the stationary phase. The metal ions themselves, will not usually be soluble in the mobile phase and must, therefore, be converted to more non-polar organic compounds by simple organic extraction processes. These solvent extraction processes may be undesirable in terms of the time required for sample pretreatment, but the process itself has the advantage of (a) matrix elimination and (b) preconcentration, which will reduce interferences and increase sensitivity respectively. Thorough reviews by O'Laughlin on the development and practical application of adsorption chromatography are detailed elsewhere (11, 12). The most popular chelating agents are, by far, dithiocarbamate and dithizone although other chelating agents such as xanthic acid and mercaptoquinoline have also been used (7).

Adsorption chromatography has not really been developed to perhaps its full potential and is not used as much as bonded-phase chromatography. This is presumably because of the uncertainty of the behaviour of the stationary phase which tends to vary, quite considerably from batch to batch.

#### 1.4.1.2 Partition Chromatography

In partition chromatography, adsorption and desorption occur between two liquid phases. Practical problems exist

when using partition chromatography in HPLC determinations due to the mutual miscibility of the two phases and physical disturbance of the stationary phase giving rise to unreliable retention times and so partition chromatography has tended not to be exploited for analytical purposes to any great extent.

Bonded-phase materials, where organic functional groups are chemically bonded to the backbone of the stationary phase, are much more stable and are employed a great deal in a large number of varying chromatographic applications. This type of system, although classed as partition chromatography, combines the versatility of partition with the high efficiency of adsorption. The most usual organic groups to be bonded onto the surface silanol (Si-OH) groups are non-polar aliphatic chains of varying carbon length from C<sub>2</sub> to C<sub>18</sub> although polar amino and cyano groups can also be used.

Aliphatic C<sub>18</sub> octadecylsilane (ODS) groups are the most popular bonded phase used today and the mobile phase must be more polar to enable the separation of metal-chelates to occur. This is known as reverse-phase chromatography and separation of metals can only be achieved if stable metal-chelate systems are formed. Several reviews on the HPLC separation of metal chelates by reverse-phase chromatography have been written and some of the more successful chelate systems that have been used are;

(a) the  $\beta$ -Diketones

- (b) the  $\beta$ -Ketoamines
- (c) the thiosemicarbazones, hydrazones and dithizone
- (d) the dithiocarbamates
- (e) 8-hydroxyquinoline
- (f) Bipyridine, 1,10-Phenanthroline
- (g) Crown ethers
- (h) Macrocyclic amines
- (i) Porphyrins

all of which are described more fully in the reviews (7-12).

The most popular chelating agents to be used for metal separations and determinations are the dithiocarbamates. For example, the separation and determination of Cd, Co, Cu, Hg, and Ni can be achieved in about 15 minutes with limits of detection in the order of 5 ng (13). Also Pb, Cd and Bi have been determined at low levels in urine with similar detection limits, also using the dithiocarbamate chelates (14). In 1985 Mueller et al. determined trace amounts of Pd in platinum powder, again using the preformed dithiocarbamate chelates on a reverse phase column (15). Other examples include the simultaneous determination of Cu, Ni, Co, Cr(VI), and Cr(III) with electrochemical detection (16) and the multielement determination of the heavy metals Pb, Hg(I), Hg(II) and Cd dithiocarbamate complexes (17).

Dithiocarbamate complexes have also been exploited because of their ease of trace enrichment (18), thus increasing

the sensitivity of such reverse-phase systems. Other chelating systems that have been cited use 8-hydroxyquinoline for the reverse phase determination of V, Mo, W, Co and Cr (19) and also for the determination of Al, Cu, Fe and Mn in biological and other samples (20).

Metal ions have also been extracted and separated by reverse phase chromatography using ethyl-xanthate and 1,10-phenanthroline as chelating agents, although detection limits are not given (21). Uranium has been determined by reverse-phase chromatography after the complex formation with 2,6,-diacetyl-pyridine bis(benzoylhydrazone) which is particularly stable for complexing the uranyl ion (22), giving an absolute detection limit of 5 ng.

The examples given above are by no means exhaustive and there are a great many other chelating systems that have been studied. Most of the reagents studied form strong neutral complexes with a large number of metal ions. The majority of the separations reported have been carried out on silica based supports, and typically 3 - 8 complexes have been separated. Primarily, chelates of transition-metal ions have been separated although a few papers have been concerned with chromatography of various lanthanides and actinides (23).

Organometallic determinations by reverse-phase chromatography have increased in popularity over the last

few years and this is borne out by the number of papers and reviews that have appeared in the literature (7, 24-29). Organomercury determinations are the most prolific area of study but also organolead, organotin and organoarsenic studies have been carried out with a good deal of success.

A similar method for trace metal analysis is based on reverse-phase/ion-pair chromatography in which the charged metal species form an ion-pair that has a hydrophobic, long chain aliphatic end. The ion-pair end of the molecule is usually a sulphate or sulphonate functional group whilst the long chain aliphatic end is strongly attracted to the non-polar stationary phase of the ODS material. Early work by Skelly (30), focussed on the ion-pairing of inorganic ions with long chain aliphatics. More recently, metal complexes of positively charged 1,10-phenanthroline complexes of Fe(II), Ni(II), Ru(II), Co(II), Zn(II), Cd(II) and Cu(II) have been separated and determined (31, 32). A more recent example shows the determination of thorium, chromium and the rare-earth elements with 2-[(2-Arsenophenyl)azo]-1,8-dihydroxy [(2,4,6-tribromophenyl)azo]naphthalene-3,6-sulphonic acid which acted as both the ion-pairing reagent and the spectrophotometric means of detection (33).

#### 1.4.1.3 Ion-Exchange Chromatography

Ion-exchange substrates are insoluble solid materials which contain exchangeable cations or anions. There are

many types of exchanger on the market, some of which have a silica gel backbone, whilst others are organic in nature (the so called resins). Early studies on ion-exchange behaviour were achieved using synthetic organic ion-exchangers which are cross-linked polymers consisting of a 3-dimensional network of hydrocarbon chains carrying ionic groups. A strong cation exchanger e.g. Dowex 50, Amberlite IR1 30, would carry a sulphonate ( $\text{SO}_3^-$ ) ionic group, whilst a weak cation exchanger e.g. Amberlite IRC 50 would carry a carboxylate ( $\text{COO}^-$ ) functional group. Conversely a strong anion exchanger e.g. Dowex 1, Amberlite IRA 400 has quaternary ammonium ions ( $\text{C-NR}_3^+$ ) present, and a weak anion exchanger e.g. Dowex 3, Amberlite IR45 would usually have amine groups as the functional species ( $\text{C-NH}_3^+$ ). Many of these ion-exchange resins were developed in the 1940's and, after a short while, methods had been developed for the separation of most of the elements (34).

These resins were used in classical ion-exchange chromatography and were generally of large diameter and not particularly efficient. Often, separations would take many hours as the only driving force for analyte elution was gravity. The development of ion-exchange materials for high pressure work required that the resin beads were produced much smaller to increase efficiency. With the smaller beads came increased back pressure on the resin, which often resulted in collapse of the resin above a critical pressure. Some of the most successful resins

presently on the market have been developed by the Dionex Corporation, which produces a pellicular type resin that has had only the surface of the bead treated. This has several advantages and means that the beads are much more resilient and can withstand more pressure than conventional resins, the surface exchange mechanisms are quicker and more efficient, and the materials, themselves, are of lower capacity which may be an advantage in terms of more sensitive detection systems or, may be a disadvantage if the sample has a high ionic strength.

Bonded silica gel can also be used as ion-exchangers using the same exchange groups employed in the resins e.g. Partisil 10 SCX ( $\text{SO}_3^-$ ), Partisil 10 SAX ( $\text{NR}_3^+$ ) or Neucleosil 5 ( $\text{NH}_3^+$ ). These stationary phases can be made much smaller than their resin counterparts and are much more resistant to collapse, thus improving the efficiency of separation. Silica gel materials, however, can only be operated at a pH of between 2-7 because of solubility problems in aqueous media compared to the more versatile resins (pH 1-13).

Within the field of ion-exchange chromatography the term 'ion-chromatography' is increasingly used. This term 'ion-chromatography' was coined by Small et al. (35) in 1975 and its development led to a rapid expansion of improvements and applications to a number of commercial systems. The use of the term 'ion-chromatography' for one particular system which was originally developed by Small

is rather restrictive and nowadays the term is usually used as a general name for a process in which ions are separated chromatographically, for the purpose of analysis and which some form of automatic detection method is used. Usually ion-chromatography refers to a separation that uses an ion-exchange column, although ion-pairing and ion-exclusion are also used and included in this term.

The elution of metal ions from classical open column resin systems was traditionally performed by using high strength mineral acids (1-5 M), whilst affinity differences of simple anions for an anion-exchange resin are often sufficient to permit chromatographic separation that uses a competing anion to move the sample ions along the column. The use of mineral acids for HPLC separation, however, gave corrosion problems from the stainless steel components, a particular problem when dealing with trace metal analysis. Things improved considerably when complexing organic acid eluents were used giving good separations on both anionic and cationic exchange materials, thus avoiding the use of corrosive mineral acids. This fact, combined with the use of much smaller (3 - 50  $\mu\text{m}$ ) diameter materials, better chromatographic components, smaller samples and automatic on-line detection, has resulted in faster, more convenient separations. The most common complexing acids used at present are polyfunctional carboxylic acids including such types as oxalic, malonic, tartaric, lactic,  $\alpha$ -hydroxyisobutyric and citric acids. These complexing

agents work by chelating with the metal species, thus reducing the charge on the simple metal ions and facilitating separation by producing complexes with varying conditional stability constants. A simple example of this can be given by tartaric acid. At pH 4.0 the log of the stability constants,  $\log K_{stab}$ , for various cations is given in Table 1.3. From Table 1.3 it is clear that although all those cations mentioned are divalent, they all have different  $\log K_{stab}$  and thus the elution order would be: Cu, Zn, Pb, Cd, Mg.

Numerous papers reveal that the combination of cationic columns and organic acid eluents are very useful for trace metal determinations. Examples include the use of citrate (36) and malonate (37) eluents for alkaline-earth metal separations and the use of acetate for cerium (III), lanthanum (III), indium (III) and inorganic mercury (II) determinations (38).

With the development of low capacity pellicular and other ion-exchange materials, the complexing agents which offer the optimum separations of metal ions are weak complexing organic acids. Acids such as lactic, tartaric, citric, oxalic and phthalic have been used as chelating agents in ion exchange separations. Jones et al. (39) have used the chelating tartaric and lactic acids to separate transition metals and Group (III) metals using inverse photometry as a means of detection, whilst Hwang et al. have managed to separate 14 lanthanide elements in less than 40 minutes by

**Table 1.3** Log  $K_{stab}$  of various divalent cations with tartaric acid at  
pH 4.0

<u>Cation</u>	<u>Log <math>K_{stab}</math></u>
Cd	3.8
Cu	5.4
Mg	1.4
Pb	4.5
Zn	4.9

elution from a low capacity cation exchange material with  $\alpha$ -hydroxyisobutyric acid (40). The performance of these weak organic chelating eluents has been further investigated on a low capacity strong cation exchange column, by comparing the retention times of the following metal ions: Bi, Cd, Co, Cu, Fe(II), Fe(III), Pb, Mn, Ni and Zn (41).

More recently, the largest number of metal species separated (apart from the lanthanide series) in one injection was 13, taking 40 minutes for complete resolution. The metal species separated were Ba, Ca, Cd, Co, Cu, Fe(II), Fe(III), Mg, Mn, Ni, Pb, Sn and Zn (42).

Although ion-chromatography was originally developed to solve process monitoring and process control applications, the unique features of the technique resulted in applications and technology being developed mainly in the analytical laboratory. Ion-chromatography capabilities enable faster sample throughput, improved sensitivity, improved accuracy, and the ability to determine ionic species previously difficult or impossible to determine by other procedures. Cation analysis was a routine instrument procedure before ion-chromatography was developed. Now, cation and anion analysis by ion-chromatography are routine procedures. The analysis of inorganic species by ion-chromatography and the environmental applications of ion-chromatography are discussed fully in two recent reviews which offer an

excellent source of reference (43, 44).

A new area of development in ion-chromatography is the "dynamic" modification of cheap silica gel ODS materials to stationary phases that have ion-exchange capabilities. The ODS material can be dynamically coated with long chain aliphatic sulphonate groups (or more recently chelating functional groups), thus converting the reverse phase column into an ion-exchange or chelating-exchange stationary phase. Good separations have been achieved using this method (45, 46) and the advantage of this approach is that relatively cheap, efficient stationary phases are widely available.

#### 1.4.1.4 Chelation Exchange for Trace Metal Analysis

Most publications dealing with the determination of trace metals by liquid chromatographic means have concentrated on ion-exchange techniques as already mentioned.

However, several problems are associated with ion-exchange separations of metal ions. High ionic strength samples and highly concentrated solutions (e.g. wet digestions using concentrated acids) can cause drastic changes in column capacity, destroying the separation capability. Chelating-exchange can be used as an alternative to ion-exchange approaches. The solid substrate contains chelating groups rather than ion-exchange groups. Conditional stability constants are relatively unaffected by ionic strength which allows separations to proceed

unhindered.

Chelating-exchange is well known in classical column chromatography where it has been principally used for matrix elimination. An increasing number of publications are appearing that describe metal separations on HPLC grade chelating substrates. Most chelating-exchange publications deal with substrates with attached chemically bonded chelating groups synthesised by the workers themselves. For example Leyden et al. (47) immobilized chelating functional groups by reacting silica gel with various silylating reagents. In this way immobilized ethylenediamine, its dithiocarbamate, and primary and secondary amines were prepared and used to preconcentrate metal ions from various matrices. The Dow Al chelating resin (Chelex 100) was the first chelating resin of commercial importance. The functional group, iminodiacetic acid, reacts with many metal ions, although pH can be adjusted to impart selectivity. It has been used to concentrate metal ions from aqueous solution and matrix removal prior to analysis. The resin swells and shrinks considerably when pH conditions are changed, making it difficult to use in a moderate to high-pressure column system.

In 1970, Hirsch et al. (48) prepared an iminodiacetic acid substrate of higher cross linking that underwent very little volume change and is well suited for column operation. Other workers including Fritz et al. (49) and

Mayers and Fritz (50) have described a variety of chelating functions on resins and silica gels that act as separating stationary phases. One way of achieving chelating exchange without resorting to lengthy and difficult syntheses of chemical bonding of chelating groups, is to coat neutral or ion-exchange resins with dyestuffs. Several recent publications (51, 52, 53, 54) show the possibility of this permanent coating procedure for the separation and determination of both anions and cations. For example, the triphenyl-methane type dyestuffs Bromothymol Blue and Chrome Azural S were coated on the neutral polystyrene resin Benson BP1-10. The chelating stationary phases produced were able to separate and preconcentrate divalent and trivalent metal species. Similarly, the neutral resins PRP-1 and Amberlite XAD-1 were coated with Xylenol Orange and were used to preconcentrate and separate various divalent and trivalent cations using pH control as a means of gradient elution.

#### 1.4.2 Chromatographic Parameters

To appreciate the influence that chromatographic parameters have, it is important to gain an understanding of the basic theory of the liquid chromatographic process.

##### 1.4.2.1 Migration rates and band broadening

Figure 1.1 shows concentration profiles of solutes A and B on the column, at time  $t^1$  and at a later time  $t^2$ . B is held more strongly than A and is retained longer on the column and the distance between the two increases as they

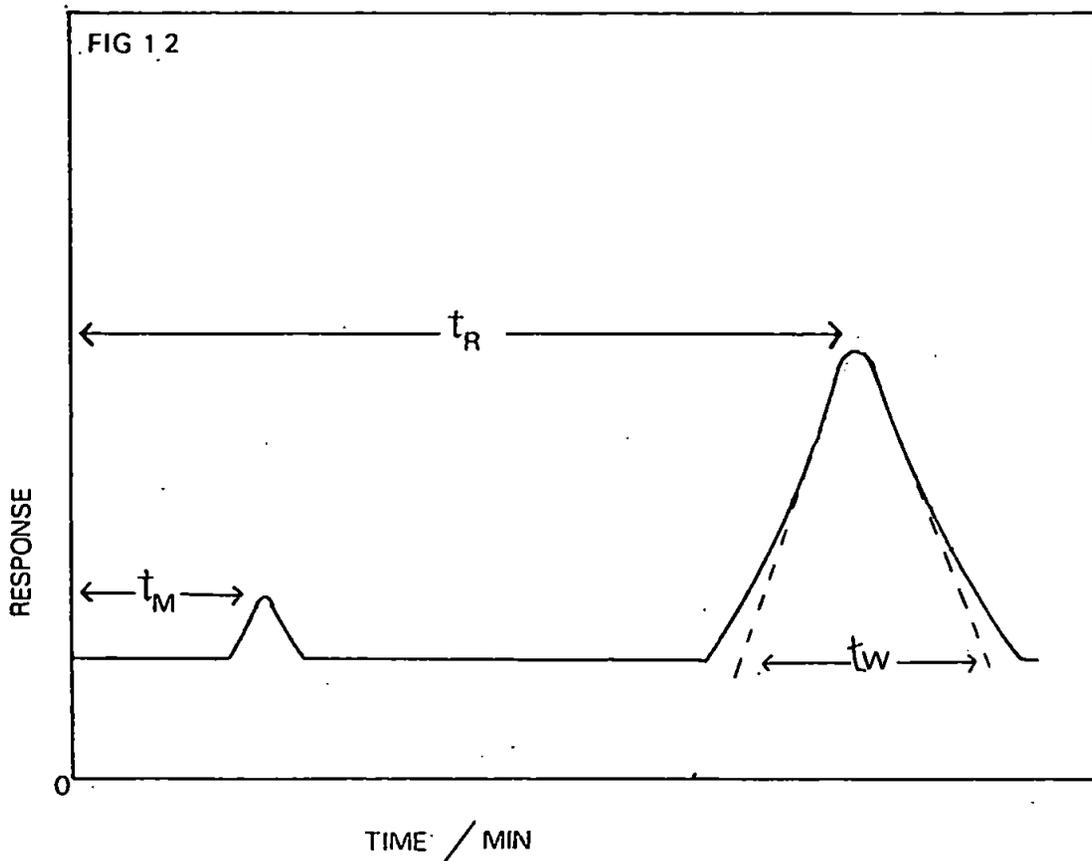
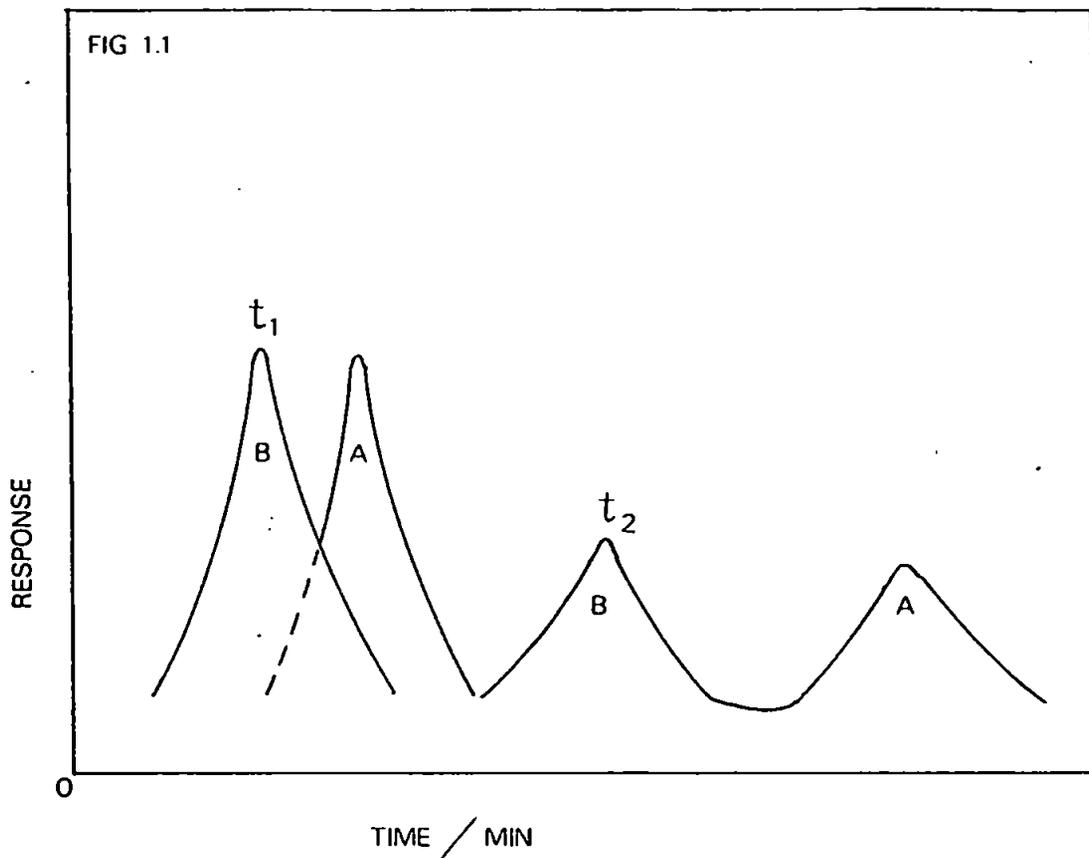


Figure 1.1 Concentration profiles for solute bands A and B at two different times in their migration down a column.

Figure 1.2 A theoretical chromatogram showing  $t_R$ , the retention time for a solute retained by the stationary phase and  $t_M$  is the retention time for one that is not.

both move down the column at different rates. At the same time broadening of both bands takes place which lowers the efficiency of the column as a separating device.

Several chemical and physical variables influence the rates of band separation and band broadening and improved separation can often be realised by the precise control of the variables that either increase the rate of peak separation or decrease the rate of band spreading.

#### 1.4.2.2 Partition ratios

All chromatographic separations are based upon differences in the extent to which solutes are partitioned between the mobile and stationary phases. For a solute species A the equilibrium involved is:

$$A_{\text{mobile}} = A_{\text{stationary}}$$

The equilibrium constant K (the partition co-efficient) is

$$K = \frac{C_s}{C_m}$$

where  $C_s$  is the concentration of the solute in the stationary phase and  $C_m$  is the concentration (M) of the solute in the mobile phase.

#### 1.4.2.3 Retention times

For the chromatogram shown in Figure 1.2, zero on the time

axis corresponds to the sample injection times.

The peak at  $t_m$  is known as the solvent pulse and species that are not retained by the column elute here. This gives an idea of the "dead volume" of the column. The retention time,  $t_R$ , for the second peak is the time required for that band to reach the detector at the end of the column. The average linear rate of solute migration  $V$  is

$$V = \frac{L}{t_R}$$

where  $L$  is the length of the column. The average linear rate of movement  $U$  of the molecules in the mobile phase is

$$U = \frac{L}{t_m}$$

1.4.2.4 The rate of migration of solutes. The capacity factor

The capacity factor  $k'$  is an important parameter that is widely used to describe the migration rates of solutes down a column. The  $k'$  of an ion  $A$ , is the amount of  $A$  in the resin phase of a column, divided by the amount in the solution phase:

$$k' = \frac{t_R - t_m}{t_m}$$

$t_R$  and  $t_m$  are easily obtained from a chromatogram. When

$k^1 < \text{unity}$ , elution occurs very rapidly and accurate determination of  $t_r$  is difficult. Conversely, when  $k^1$  is  $> 20$ , elution times become very long. Ideally, separations are performed under conditions in which the capacity factors for the solutes in a mixture lie in the range between 1 to 5. In liquid chromatography, the value of  $k^1$  can be manipulated by varying the competition for the mobile and stationary phase.

#### 1.4.2.5 The selectivity factor

The selectivity factor, or equilibrium constant,  $\alpha$ , of a column for the 2 species A and B is defined as

$$\alpha = \frac{k^1_B}{k^1_A}$$

where  $k^1_B$  and  $k^1_A$  are the capacity factors for B and A respectively. The value of  $\alpha$  can be determined from an experimental chromatogram

$$\alpha = \frac{(t_A) - t_m}{(t_B) - t_m} \cdot \frac{B}{A}$$

#### 1.4.2.6 Column efficiency

Two related parameters are widely used as measures of the efficiency of chromatographic columns: (1) number of theoretical plates,  $N$ , and (2) plate height  $H$ . The two are related by the equation

$$N = \frac{L}{H}$$

L = length of column packing (cm)

The efficiency of a column increases as the number of plates becomes greater and as the plate height becomes smaller.

Column efficiency describes the rate of band broadening as the analyte travels through the column. The quantitative measure of efficiency, N, can be calculated from the chromatogram by using .

$$N = 16 \left( \frac{t_r}{W} \right)^2$$

where  $t_w$  = peak width (same units as the retention time). The peak width is obtained from the intersection of the baseline with the tangents drawn through the inflection points on the sides of each peak (see Fig. 1.2).

#### 1.4.2.7 Column resolution

The resolution  $R_s$  of a column provides a quantitative measure of its ability to separate two analytes. A useful equation is readily derived that relates the resolution of a column to the number of plates it contains, as well as to the capacity and selectivity factors of a pair of solutes on the column i.e.

$$R_s = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{K'_B}{1 + K'_B} \right)$$

$K_B^1$  is the capacity factor of the slower-moving species and  $\alpha$  is the selectivity factor. This equation can be rearranged to give the number of plates needed to realise a given resolution:

$$N = 16 R_s^2 \left( \frac{\alpha}{\alpha - 1} \right)^2 \left( \frac{1 + K_B^1}{K_B^1} \right)^2$$

### 1.5 The Detection of Metals in Liquid Chromatography

Currently, the best and most reliable liquid chromatographic detectors are spectrophotometric, fluorescence and electro-chemical detectors. However, the mobile phase conditions necessary to ensure good chromatography do not always lend themselves to sensitive detection and the detection step is perhaps still the weakest part of the liquid chromatography system.

In order to improve the detection step, there have been tremendous advances in the field of post-column reaction detectors, sometimes referred to as chemical reaction detectors or post-column derivatizations. These methods provide a useful approach to increasing the range and sensitivity of such detectors. Three publications by some of the leaders in this area of chromatography report the advantages and disadvantages of such post-column systems, along with full reviews on applications, instrumentation

and general consideration when setting up such systems, with numerous references for the interested researcher (55, 56, 57).

The increase in the complexity of the instrumentation, with the use of an extra pump, and also the increase in peak width caused by increased dead volume and dilution of the sample are disadvantages to post-column reactor systems. These systems, however, are extremely versatile and, with control of the huge range of reagents available, can be used to make detectors both specific or universal depending on the requirements.

#### 1.5.1 Electrochemical Detectors for Trace Metal Determinations

Electrochemical detectors are usually divided into polarographic, amperometric, coulometric and conductometric detectors. Conductometric detectors differ from the others because (a) they measure cell resistance without including electrolysis and (b) they respond to all ions and are therefore universal detectors, whereas the other detectors will only respond to certain ions and are more specific in nature.

##### 1.5.1.1 Conductometric Detectors

These detectors can be extremely useful for a wide number of ions. The detectors do not respond to molecular substances such as water, ethanol and non-dissociated weak acid molecules. Conductometric cells have been used since

Small et al. (35) developed a technique that excluded the eluent ionic buffer by use of a suppressor column (1.4.1.3) to allow an increase in sensitivity. Suppressed ion-chromatography has been widely utilised for the determination of anions, but its application to metals has been limited. Moreover, there are several disadvantages to the use of a suppressor column, including increased band dispersion and the need to regenerate the suppressor column when the exchange capacity is exhausted. Alternatively, an eluent can be chosen that has a lower conducting ability, such as ethylene ammonium tartrate or EDTA, which have been used to determine metal cations without the need for a suppressor column (58, 59).

Numerous applications for conductometric detection have been reported and reviews by Schwedt (6), Fritz (49) and Nickless (10) outline the principles involved and provide an excellent source of reference.

#### 1.5.1.2 Voltammetric Detectors

These detectors may be sub-divided into amperometric and coulometric instruments. Both detectors are selective because they operate on the principles of oxidation and reduction of substances at an electrode. The ability of the substance to be oxidised or reduced is different for each substance and is measured by the potential required to induce the electrolysis.

Coulometric detectors are less popular than amperometric

instruments because they are less sensitive and more difficult to operate. Despite some of these problems, Takata and co-workers have shown the suitability of coulometry for a number of metal determinations (60). Also Girard has used coulometric detection for the analysis of heavy metals, alkaline-earth and rare earth elements (61).

Amperometric detectors are more widely used than coulometric instruments as they are more sensitive and have a wider scope for applications. Several papers by Bond and Wallace (62, 63, 64) and by White (65), all detail principles of operation and application of this type of detector.

#### 1.5.1.3 Potentiometric Detectors

This detector has not been used for very many metal determinations as difficulties exist that adversely affect the detector performance, mainly from incompatibility of the eluent with the detector. However, Haddad et al. (66) have developed a copper electrode for indirect potentiometric detection which has the capability to determine a range of metal species. The whole area of electrochemical detection was reviewed by Stulik et al. in 1985, but particular attention was paid to detector construction (67).

#### 1.5.2 Spectrophotometric Detectors for Trace Metal Determinations

Molecular absorption detectors are, by far, the most common detectors in the field of liquid chromatography. There are several reasons why this is so; (a) the detector is selective, but selectivity can easily be altered by changing the wavelength monitored by the detector, (b) versatility of the detector can be increased by adding a colour-forming reagent to the eluent or column effluent, the so-called post-column reaction and (c) considerable development of the detector has already been carried out by instrument manufacturers to make them as sensitive as possible for detection of organics by high performance liquid chromatography.

Spectrophotometric detectors are also employed for molecular emission (luminescence) techniques which are generally both more sensitive and more selective than absorption detectors.

#### 1.5.2.1 Molecular Absorption Detectors

Since most simple metal ions and compounds are not able to absorb radiation in the UV-Visible region, sensitivity is increased by the addition of an organic chelating agent either pre- or post-column.

##### (a) Pre-Column Reactions

The commonly used 254 nm UV detector responds poorly to most inorganic ions. However, many metal ions absorb light at lower wavelengths. Provided there is an absence of interfering organic material in the eluent, the

absorbance of metal chloride complexes in the UV region has been used extensively to detect metal ions in liquid chromatography i.e. 66 metal ions in a 6M HCl medium were detected using UV spectra (68).

Preformed metal chelates are often produced to facilitate their separation on reverse phase substrates (1.4.1) and often, the metal chelate will absorb strongly in the UV range (245-275 nm) and this can be used as a good means of detection. Dithiocarbamate metal chelates have been the most studied, but one problem arises from the fact that different metals produce metal chelates of varying maximum absorptivity. As the optimum wavelength for detection is not monitored, compromise conditions prevail (69).

#### (b) Post-Column Reactions

Post-column derivatisations of column fractions have been known for many years. The automatic addition of a colour forming reagent post-column, and analysis by flow-through cell detection is a more recent development.

The determination of metals by spectrophotometric means has also been known for a long time by classical chemists and there is a wealth of information concerning the determination of metal chelates with favourable detection characteristics. Only a few chelating agents have the right combination of properties that make them attractive for post-column reaction detection of metal ions. For favourable reaction detection the metal chelates should

exhibit strong absorbance in the UV or visible region that can be easily measured. The chelating agent, however, should be soluble in aqueous conditions, and formation of the metal-chelate bonds should be kinetically rapid. The maximum absorbance of the metal-chelate should be considerably different from the chelate itself to avoid wavelength overlap and thus allow sensitive detection.

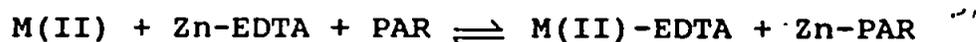
Besides the above criteria the colour forming reagent should also react with a large number of metals, be stable over a long period of time and responses should be linear and reproducible.

In one of the first applications of chelating reagents to the detection of metal ions, Kawazu and Fritz (70) examined 4-(2-pyridylazo) resorcinol, (PAR), for the detection of Cd(II), Zn(II), Fe(III), Pb(II), Cu(II), Co(II) and Mn(II) eluted from a low pressure cation exchange column. In a later study PAR, Arsenazo-I and Arsenazo-III were studied as PCR reagents for the detection of a number of metal species (71). PAR was found to be the most convenient and versatile reagent and generally the most sensitive, but Arsenazo-I was found to be preferable for Ca(II) and Mg(II) determinations.

Cassidy and Elchuck (72, 73) clearly showed the good quantitative performance and sensitivity of PAR. The metals that are best suited to PAR detection are Bi, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn and the lanthanides, with absolute detection limits ranging from 0.5 - 25 ng.

A number of colour-forming reagents have also been examined for the post-column reaction determination of Ca and Mg (74). The reagents examined were Arsenazo-I, Arsenazo-III, Sulfonazo-(III), Methylthymol blue, Titan yellow, Murexide, sodium rhodizoate, Eriochrome Black-T, Chlorophosphoazo, PAR, and PAR-Zn (EDTA). The most sensitive post-column reagent was found to be PAR-Zn (EDTA).

The use of a less selective system like the PAR-Zn (EDTA) overcomes the problem of wavelength selection for multi-metal analysis as the principle relies on the displacement of the Zn from the Zn-EDTA complex by eluting metal species. The Zn is then free to react with PAR and the detector wavelength can be set to monitor the Zn-PAR complex only. The reaction taking place in the post-column reaction coil on elution of a metal ion, M(II) is as follows:



(charges omitted for clarity)

This system was studied further (75) and it was found that both sensitivity and the range of metals that could be determined using Zn-EDTA were improved as, for example, the alkaline earth metals were detected which gave very poor sensitivity with PAR alone.

Xylenol orange has been used as a post-column colour forming agent for the determination of all of the rare-earth metals after their separation using  $\alpha$ -hydroxyisobutyric acid (76).

Another development that attempts to solve the problem of optimum wavelength detection is the application of inverse photometry for metal detection. The optimum absorbance of different metal chelates can vary quite considerably and the measurement of the decrease in absorbance of the chelating reagent gives a quantitative measure of the metal ion present. With initial studies, Eriochrome Black-T was used as the photometric reagent. The metals detected by Eriochrome Black-T are essentially the same as for PAR but with the addition of Ca and Mg. Absolute detection limits range from 1 - 10 ng but poorer responses are found for Pb, Cd and Ca (77, 78). The same author used a similar inverse photometric reagent, dithizone, to determine Cd, Co, Cu, Pb, Ni and Zn at nanogram levels, again avoiding the wavelength selection problem posed by other multi-element photometric detection systems (79).

#### 1.5.2.2 Molecular Emission Detectors

Emission techniques are generally more sensitive (by at least an order of magnitude) and more highly selective than absorption methods, but are often prone to quenching and other interfering effects.

### 1.5.2.2 (i) Fluorescence Detectors

Fluorescence methods have been widely applied in organic post-column reactions but there have only been a few publications for metal fluorescence determinations. This is as a result of the fact that many transition metals and heavy elements quench the fluorescence signal.

Selenium has been determined (80) some time ago, but only a few examples of fluorescence detection for metals using post-column reagents have been published.

One such example describes the separation of Zn, Cd, and Pb aniline EDTA complexes, followed by post-column derivatization with fluorescamine (81), with limits of detection reported at the low pg levels.

The reaction between trivalent metal cations with 8-hydroxyquinoline-5-sulphonic acid (8HQS) has been exploited by Jones et al. in two recent publications (82, 83). The detection limit for gallium was 0.1 ng absolute, and the system was used to determine trace gallium in a pure aluminium sample (82). The detection limit for aluminium was found to be 0.2 ng absolute and the aluminium content was successfully determined in certified reference material (Monel Alloy) and in tap water (83).

Soroka et al. (84) reported the use of 8HQS as a fluorimetric reagent for a range of metals and further demonstrated its potential as a post-column reagent for ion-chromatography.

### 1.5.2.2 (ii) Chemiluminescence Detectors

Chemiluminescence (CL) is observed when a chemical reaction yields an electronically excited product which either luminesces or transfers its energy to another molecule which then luminesces i.e.



where A and B are reactants and C\* is the excited state product.

Three conditions are generally required for a reaction to produce CL. These are; (i) sufficient energy to produce an excited state product, (ii) a favourable reaction pathway to produce this product and (iii) the excited product must be able to luminesce or transfer its energy to a potentially fluorescent molecule.

The term bioluminescence (BL) is generally used for light accompanying a reaction derived from nature (for example; fireflies, luminous bacteria and protozoa, the marine fireworms etc ...). Bioluminescence may be considered a special case of CL, although to the analytical chemist, the two processes may be considered to be the same.

CL methods can have significant advantages over other analytical methods. The limiting factor in the ultimate detectability of fluorescence techniques is the background

light that reaches the photomultiplier tube (PMT). This background light is a function of Rayleigh scattering, Raman scattering, second order interferences and the stray light characteristics of the wavelength selection devices employed. With the elimination of the light source, that comes with CL methods, a far reaching impact can be made in terms of the sensitivity for analyte detection.

The removal of any excitation source means that the instrumentation required for CL determinations does not need any complicated optics and detector design is much simplified and less expensive.

To use CL for chemical analysis, the reaction is performed under conditions so that the light intensity is a function of the level of analyte. This is frequently achieved by adjusting the component concentrations so that the analyte is the limiting reactant.

CL intensity will vary with time as the reactants are consumed. Therefore, it is important to initiate the CL reaction in some sort of controlled manner to achieve reproducible results. This can either be done by rapidly mixing the CL reactants and then measuring the resulting CL intensity as a function of time, the so-called batch method, or the CL reactants can be continuously mixed to measure a steady state light output. This is known as the continuous method. Continuous methods are generally more reproducible and lend themselves to automated analysis,

whilst batch methods are more intensive but use less reagents, but are not so reliable.

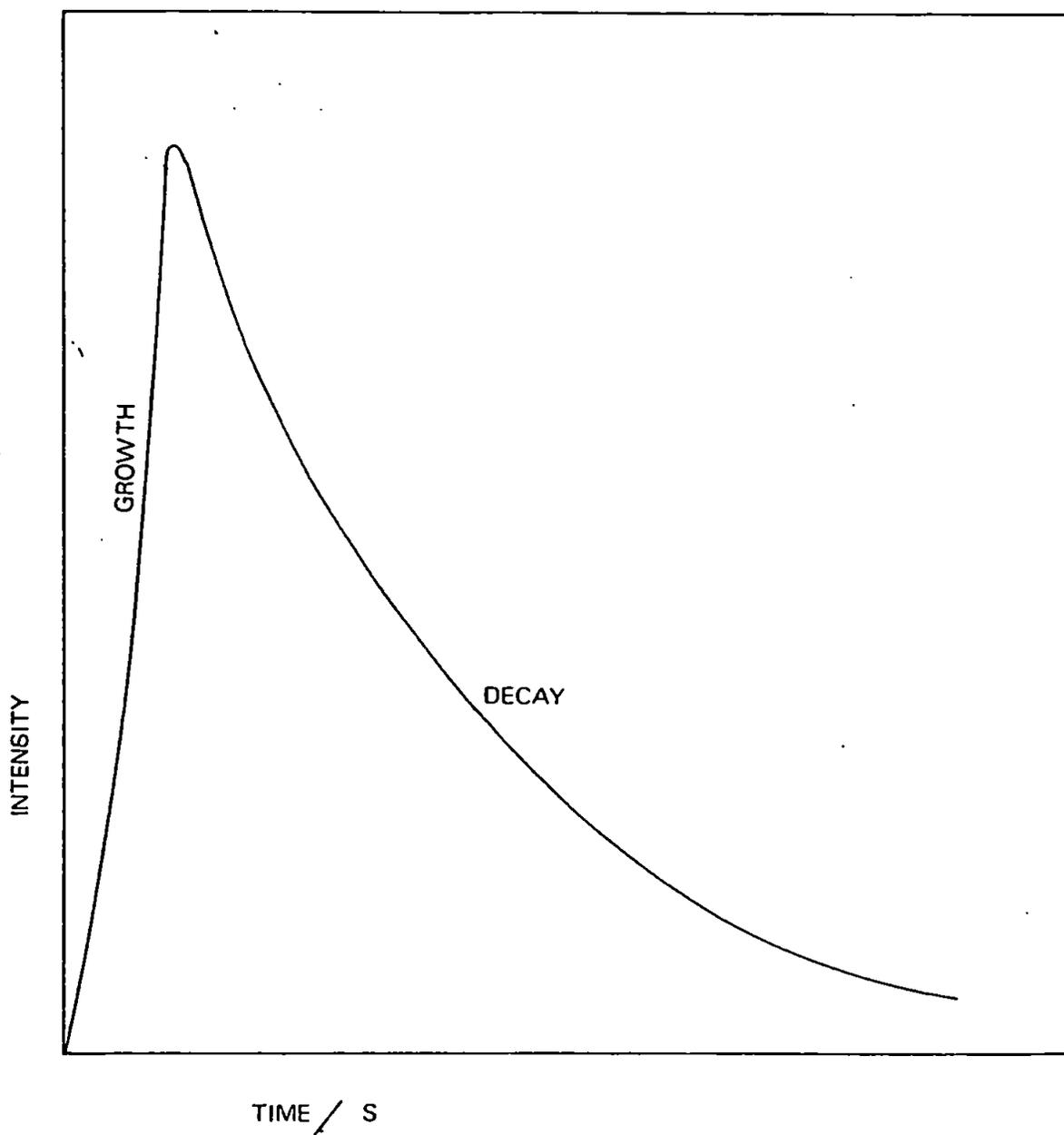
Figure 1.3 illustrates a hypothetical intensity/time curve. The shape of the curve depends on the kinetics of the reaction. The initial part of the intensity/time curve may also be affected by the mixing process.

This intensity/time curve can be analysed either by measuring intensity at a fixed time after mixing or by integrating intensity over time for part or all of the reaction.

The continuous method of analysis overcomes problems of intensity-time measurement as the reactants are mixed using some sort of flow cell. Provided the means of reagent delivery is reproducible, CL intensities will be the same for a given set of conditions. If the CL reaction is fast, relative to residence time in the flow cell, it is possible to observe all the light emitted. If the reaction is slow, however, much of the light may be produced after the sample has left the cell and so careful flow cell design may be required.

The batch method in CL studies, has been by far, the most popular way of applying CL reactions to analytical chemistry and there are many examples of the use of batch methods to determine a wide variety of organic, biomedical and pharmaceutical analytes. Two excellent reviews that

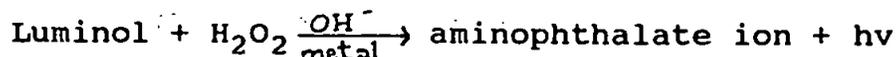
Figure 1.3 The intensity / time curve for a typical CL reaction.



give great detail to some of these reactions include a publication in 1974 by Isaacson and Wettermark (85) and a more recent example by Seitz (86) in 1981, which gives details on fundamentals, instrumentation and biomedical applications of both CL and BL. The most recent review by De Jong and Kwakman (87) details peroxyoxalate, luminol and lucigenin CL, with particular attention being paid to the detection of biomedical samples.

The use of CL detection for continuous post-column reagent work would seem a very attractive technique in terms of sensitivity and low capital cost investment, although it has yet to be fully exploited. Two publications that review CL as a means of analytical chromatographic detection by Imai (88) and Baeyens (89) both concentrate mainly on the most common peroxyoxalate type systems for biomedical applications, with little information on less popular inorganic determinations.

One of the first applications of CL detection for liquid chromatography by Neary et al. (90) used the well known luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) chemistry to determine certain transition metals separated by ion-exchange chromatography.



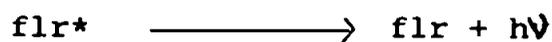
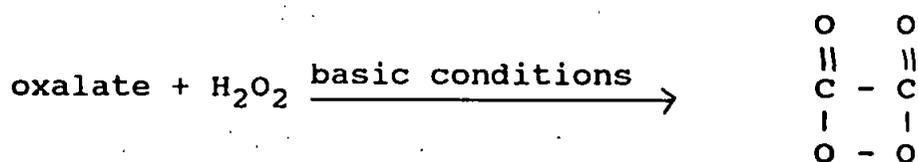
The aminophthalate ion has been shown to be the emitting species regardless of the metal or oxidant that catalyses the reaction. The use of luminol for the determination of

metals will be discussed in greater detail later.

Luminol has also been used for oxidant determinations, particularly hydrogen peroxide (91, 92) but also to measure oxidase enzyme reactions (93).

Lucigenin (bis-N-methylacridinium nitrate) can be used in a similar fashion to luminol, although it has different selectivities to transition metal catalysts. Veazy and Neiman (94) have used lucigenin to determine organic reductants such as vitamin C although it can also be used for hydrogen peroxide determinations (95).

Another type of CL uses energy transfer to excite fluorophores. Peroxyoxalate chemistry is almost exclusively used in this type of CL and refers to a large group of reactions summarised below:



flr = fluorescent acceptor.

The mechanism is thought to involve the cyclic  $C_2O_4$  intermediate shown above, but it has never been directly observed. Peroxyoxalate CL is the most efficient non-biological CL reaction known. The peroxyoxalate CL reaction was first adapted for LC detection by Kobayashi and Imai in 1980 (96). For dansylated amino-acids 10 fmol limits of detection were reported. The same author has determined dansylated oestrogens, extracts of shale oil and urinary porphyrins using this peroxyoxalate /oxidant/fluorescent acceptor system (97), with very impressive sensitivity and selectivity for those examples mentioned.

To function as a post-column reagent, an oxalate ester, usually bis-(2,4,6-trichlorophenyl) oxalate (TCPO) is mixed with hydrogen peroxide, followed by mixing with the mobile phase effluent. This system is applicable to detection of fluorophores as previously mentioned, but has found particular use in hydrogen peroxide determinations. This application is considered in a review of CL detection of peroxide (91). The lowest detection limit for  $H_2O_2$  was reported to be  $1 \times 10^{-9}$  M.

As already mentioned, fluorophores can be determined by peroxyoxalate reactions although not all fluorophores can be efficiently chemiexcited. Easily oxidized solutes are optimal CL energy acceptors. These include polycyclic aromatic hydrocarbons (PAH's) such as perylene or rubrene and certain long-wave emitters such as the rhodamines.

One area of post-column research that is rapidly growing is the development and employment of solid-state reactors as a cheaper and more efficient alternative to homogenous systems and CL solid state reactors have received a keen interest.

One group headed by Neiman, have immobilized luminol on silica or controlled pore glass by adsorption or by forming a glutaraldehyde linkage (98). Once immobilized, the material can be packed within a flow cell which can be housed in front of a PMT. This method has been used for the sensitive determination of peroxide at the  $10^{-7}$  M level and the immobilized luminol was found to last for over 500 peroxide assays (99, 100).

Other immobilization systems have been used, also for the determination of hydrogen peroxide at the sub picomole range, by the immobilisation of TCPO on glass beads (101, 102, 103), but no other applications have been reported. The performance of the immobilized system was compared with a conventional homogenous post-column reaction and was found to be greatly simplified and produced less band broadening. The system proved successful enough for it to be made into a field monitor with well over 40 sample injections per hour being made for sensitive peroxide determinations.

The use of CL for inorganic analysis has really not been exploited to any great extent. For most inorganic

determinations the usual method involves the use of some sort of flowing stream, either FIA or a chromatographic technique.

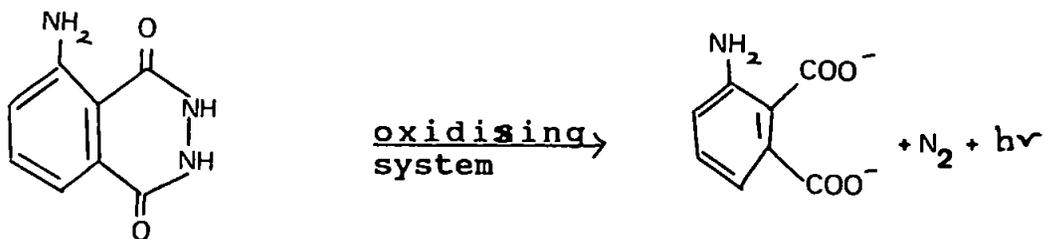
Luminol, once again, has been the main CL chemical that is employed for both anion and cation determinations, although other, less successful reagents have been tried.

In 1986 Cooper et al. (104) used a FIA system coupled with the luminol/peroxide reaction to determine several anions at low levels including chloride (LOD  $1.2 \times 10^{-6}$  M), nitrite (LOD  $1.5 \times 10^{-6}$  M), nitrate (LOD  $1.2 \times 10^{-6}$  M), bromide (LOD  $1.5 \times 10^{-5}$  M) and sulphate (LOD  $1.0 \times 10^{-6}$  M), with RSD's ranging from 1.9 to 5.0% for those anions mentioned. In this publication the author mentions the requirement for selectivity either by a separation or suppression technique although no such systems were tried.

FIA was once again used to determine nitrite at a lower LOD of  $1 \times 10^{-9}$  M (105) but the system used was rather complex.

Chromium (VI), Molybdenum (VI), and Vanadium (V) have all been determined by CL using a TCPO/H<sub>2</sub>O<sub>2</sub> acceptor type system (106). The anions were found to act as catalysts by facilitating the oxygen transfer from the H<sub>2</sub>O<sub>2</sub> to TCPO. The LOD's for the 3 anions were found to be between 5 and 10 µg.

The reaction of luminol with an oxidising agent is summarized below



There are a number of oxidising systems that react with luminol to yield light. Oxidising systems that involve hydrogen peroxide require a third component which serves as a catalyst and/or a co-oxidant.

Selected transition metals can act as catalysts for the luminol/peroxide reaction and this catalyst can be the analyte if the luminol and peroxide are kept in excess. As early as 1972, Rudolf Seitz (107) used a luminol/oxygen system for the determination of Fe(II) with a limit of detection of 0.005 µg. The system was applied to natural water and orchard leaf reference materials and gave good agreement with the certificate values.

As has already been reported, the determination of these transition metal catalysts by post-column reaction was achieved in 1974 when Neary *et al.* successfully determined cobalt and copper using the luminol/peroxide detection. Unfortunately, high-efficiency columns were not available at that time and so the chromatographic performance was relatively poor. In the same year a publication by Hartkopf *et al.* (108) revealed that many metals, including Al, Zn and Bi catalysed the luminol/peroxide reaction

although all the results were based on an FIA system which can often give misleading results.

Other examples of metal determinations almost invariably involve some sort of flowing stream in an attempt to ensure reproducible results. Many workers have opted to use FIA systems although interferences may be a problem and others have used simple ion-exchange chromatography which offers freedom from interferences and the possibility of rapid, sequential multi-element analysis.

From the literature survey, the 2 metals that give the most sensitive limits of detection are cobalt (II) and chromium (III) and consequently much work has been done looking at these 2 metals. Two recent publications involving the use of the luminol/peroxide CL system for Co determinations reveal tremendous sensitivity for the element. Boyle et al. (109) reports a Co(II) LOD of 20 pmol. l<sup>-1</sup> whereas Jalkian et al. (110) reports an absolute detection limit of 1.2 fmoles.

Cr(III) has been determined at low levels from biological samples (111) using the luminol system but detection limits for Cr(III) tend to be at least 3 orders of magnitude less sensitive than for Co analysis. Many workers e.g. Seitz et al. (112) have used EDTA to quench any interfering co-eluting CL species as the formation of Cr(III)-EDTA is extremely slow compared with most other metals. With this method he was able to determine Cr(III)

down to 25 picograms. Seitz's system was exploited by Chang et al. (113) in 1980 who also used EDTA to mask interfering species, and in doing so was able to successfully determine Cr(III) in seawater samples.

Other metals that have been determined by the luminol/peroxide reaction include silver (I) (114), iron (II) and titanium (III) (115) and copper (109), although the limits of detection for these metals is not as impressive as for Co(II) or Cr(III) determinations.

Other CL systems for the determination of selected metals, not including luminol, have not been studied to any great extent, presumably because detection performance is not so impressive. Gallic acid, however, has recently been used for the CL determination of picomolar levels of Co(II) from seawater samples using FIA (116) and Co(II) again was determined, this time after cation-exchange separation from the alkaline-earth metals with lophine CL detection (117). Lophine was previously used by MacDonald et al. (118) in conjunction with hydrogen peroxide for the determination of trace metal concentrations and other inorganic species. Detection limits were found to be:  $\text{OCl}^-$ ,  $1 \times 10^{-6}$  M; Co(II)  $8 \times 10^{-7}$ ; Cr(III)  $5 \times 10^{-6}$  M. All measurements were made with a stopped flow reagent delivery system.

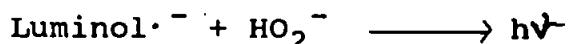
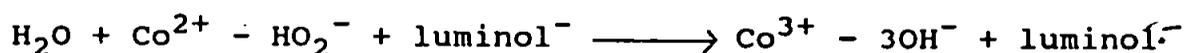
The use of CL detection systems coupled with chromatographic separation systems for multi-element

analysis have been achieved (90, 109) but only for 2 or 3 transition metal species. Sensitivity of detection is affected when looking at multielement determinations because each individual element requires specific detection conditions which vary considerably from metal to metal. Consequently, the multielement determinations that have been achieved so far, have used compromise detection conditions with an accompanying worsening of detection limits. This specific nature of CL reactions may be an advantage or disadvantage depending on the analytical problem.

A serious obstacle to further application of the luminol reaction to trace metal analysis, is the absence of knowledge as to how these metal ions function to induce CL. This knowledge is necessary to predict which metal ions will catalyse luminol CL. Elucidating the role of the metal catalyst would be an important step towards a more complete understanding of the luminol reaction which is still not fully explained. Possible explanations for the luminol CL reaction are described by Burdo and Seitz (119). They claim that  $H_2O_2$  and luminol will not react in basic solution in the absence of a catalyst, and so it can be assumed that the catalyst interacts with one of the reactants to produce a species that can then react with the other reactant. The metal ion can either form a complex with the luminol, or it can interact with the  $H_2O_2$  by complexing with the peroxide or by being oxidised to Co(III) with the production of an OH radical which can

then react with the luminol.

The metal-luminol complex is not likely to form as experiments show that the CL produced is not proportional to the [luminol], but is dependant on both  $[\text{Co}^{2+}]$  and  $[\text{H}_2\text{O}_2]$ . It is thought that the formation of a cobalt-peroxide complex is an important intermediate leading to luminescence. This intermediate is then able to react with luminol, in basic solution, to produce a luminol radical. This luminol radical can then react with  $\text{H}_2\text{O}_2$  and emit light. This proposed mechanism is summarised below:



The cobalt-peroxide complex is the limiting reagent in the rate determining step, and anything that affects its concentration will also reduce the intensity of CL. The cobalt-peroxide concentration is affected by complexation, pH, peroxide concentration and luminol concentration and experimental evidence (119) supports this proposed mechanism.

It is known that some of the other metal ions that can catalyse the CL reaction, which include  $\text{Cu(II)}$ ,  $\text{Ni(II)}$ ,

Cr(III), V(IV), Mn(II) and Fe(II) are all oxidizable by one electron from the  $M^{n+}$  to  $M^{(n+1)}$  state. This suggests that this one-electron oxidation step may be the requirement for the metal-ion catalysis of luminol CL.

### 1.6 Research Objectives

The detection step in liquid chromatography is generally considered to be the weakest part of analytical procedure. In an effort to strive towards lower and lower limits of detection, there is an obvious requirement for novel developments in both detector design and detection chemistry. With this in mind, the research objectives are to exploit new, sensitive detection procedures, based on post-column derivatisations, in particular with regard to CL reactions.

From the introductory chapter it is clear that CL determinations offer great potential for ultra sensitive detection of certain metals, particularly Co(II) and Cr(III) and these metals will be looked at in more detail to critically assess the detector performance and potential of selected CL determinations.

Although it is known that certain metals, particularly members of the first row of the 'd' block elements, can act as catalysts in the luminol CL reaction, it is hoped that a detection system can be designed to determine other metals, from all areas of the periodic table, that at present, exhibit little or no CL whatsoever. In effect

there will be an attempt to change the extremely selective nature of the luminol CL reaction for certain metals to create an almost universal detector to encompass a much wider range of analytes.

## CHAPTER 2

### INSTRUMENTATION AND EXPERIMENTAL

#### 2.1 Introduction

The instrumentation that was employed throughout this study varied, to some extent, but in general was the same conventional 'set up' used for normal post-column reaction techniques as shown in Fig. 2.1. Initial work, requiring one high pressure post column pump was carried out, but as the post-column systems became more complex, a greater number of pumps was required.

Wherever possible, all connections were achieved with 1/16th" O.D. PTFE tubing which had an inside diameter of between 0.020" and 0.043". PTFE unions and T-pieces were also used extensively. These measures combined with the use of plastic containers as reservoirs for both eluent and post-column reagent help to reduce contamination problems from leaching metal species and so reduce instrumental background noise.

The chromatography system consisted of a high pressure pump, an injector valve, an analytical column or columns, a detector and a chart recorder, later to be replaced by a chromatographic data handling station.

It is difficult to be specific about the chromatographic system used throughout the period of study as different combinations of pumps, columns, injector valves and

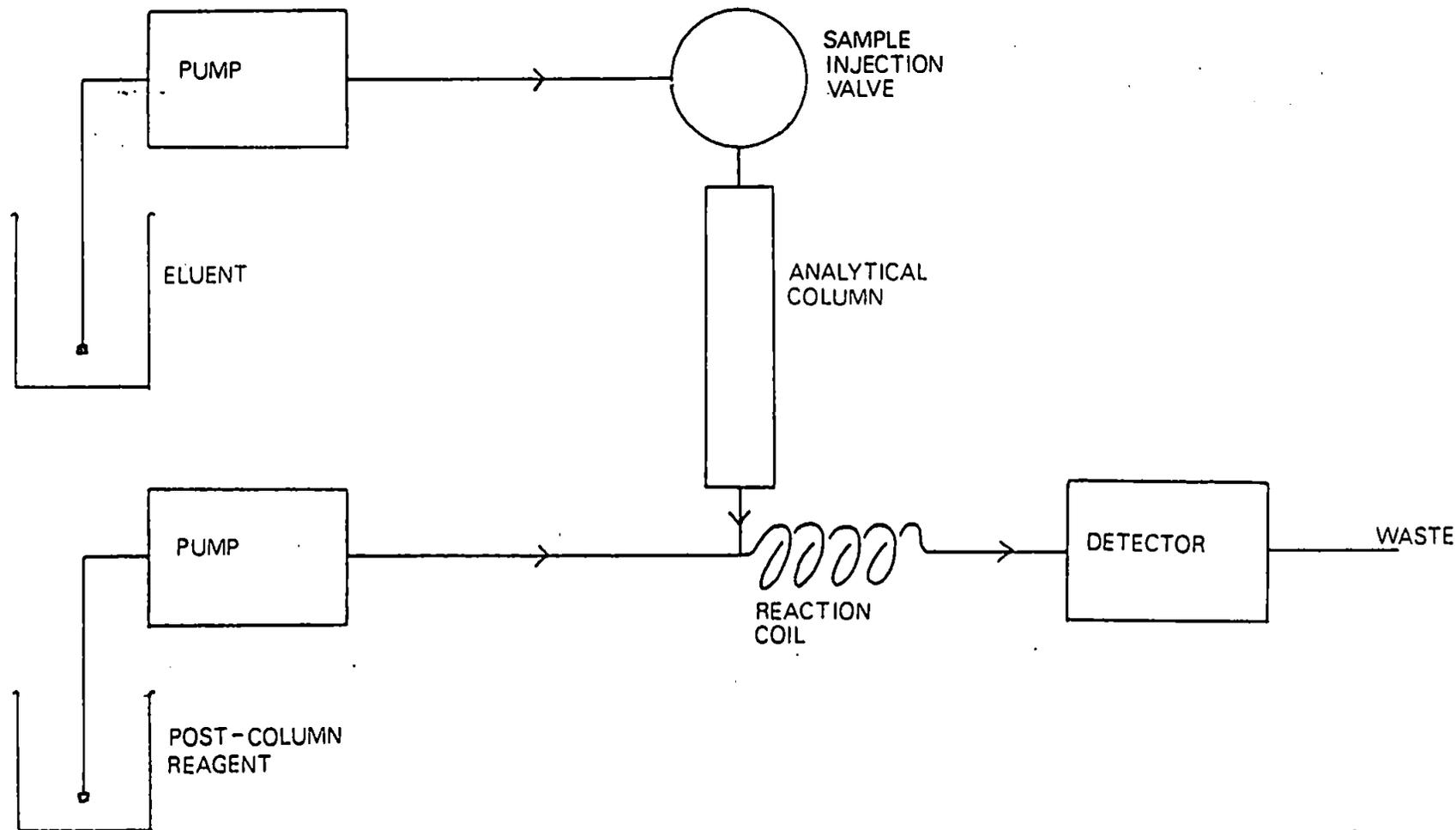


Figure 2.1 The instrumentaion required for post-column reagent studies.

reaction and detection systems were constantly being used. Consequently, a more detailed account of the instrumentation is given at the start of each relevant chapter.

## 2.2 The Development of the Chemiluminescence Detector

Perhaps, because of its relatively recent development, there are no commercial CL detectors that are suitable for trace metal detection presently on the market. It surely cannot be long before this fact is changed because of the extreme simplicity of both design and operation of such detectors. Until such a product is marketed, however, the researcher has to attempt to use other, perhaps less suitable systems to act as CL detectors. The most obvious way around this problem is to use a modified fluorescence detector with the excitation function made obsolete.

For the determination of cobalt, (Chapter 3) the fluorescence detector employed was a 950 Fluoromat from Kratos Instruments (Westwood, NJ, USA) which was operated with the excitation shutter closed and the emission filter removed. The post-column reagent and eluent were mixed directly before entering the quartz flow cell (30  $\mu$ l) and all the other optics remained unaltered.

With further developments, the flow cell was replaced with a PTFE coiled tube (volume 350  $\mu$ l, length 70 cm, I.D. 0.8 mm) as demonstrated by Townshend (120) although the detector electronics were left unaltered. The

introduction of this new flow cell now made the detector more versatile, easier to handle and improved detector performance. The photomultiplier tube (PMT) was removed from its housing and the coiled flow cell was mounted directly in front of the PMT quartz window with the whole assembly maintained in a "black box" as shown in Fig. 2.2.

This modified assembly was used for all chromium determinations (Chapter 4). With the breakdown and demise of the instrument, another fluorimeter was used and similarly modified (FD100 Spectrovision Inc, Chelmsford, MA, USA). This new detector was used successfully until the end of the research programme. Looking back over the research; it may well have proved fruitful to design and build a dedicated CL detector from scratch although this was not attempted.

### 2.3 Reagents and Standards

Analytical grade chemicals (BDH, Poole, Dorset, England), except luminol were used throughout. Solutions were prepared with high quality water from a Milli-Q system (Millipore, Bedford, MA, USA).

The preparation of both post-column reagent and eluent was carried out daily as and when required.

The preparation of the Co-EDTA solution (Chapter 5) was carried out by titration means and a more detailed account of the method used is given later.

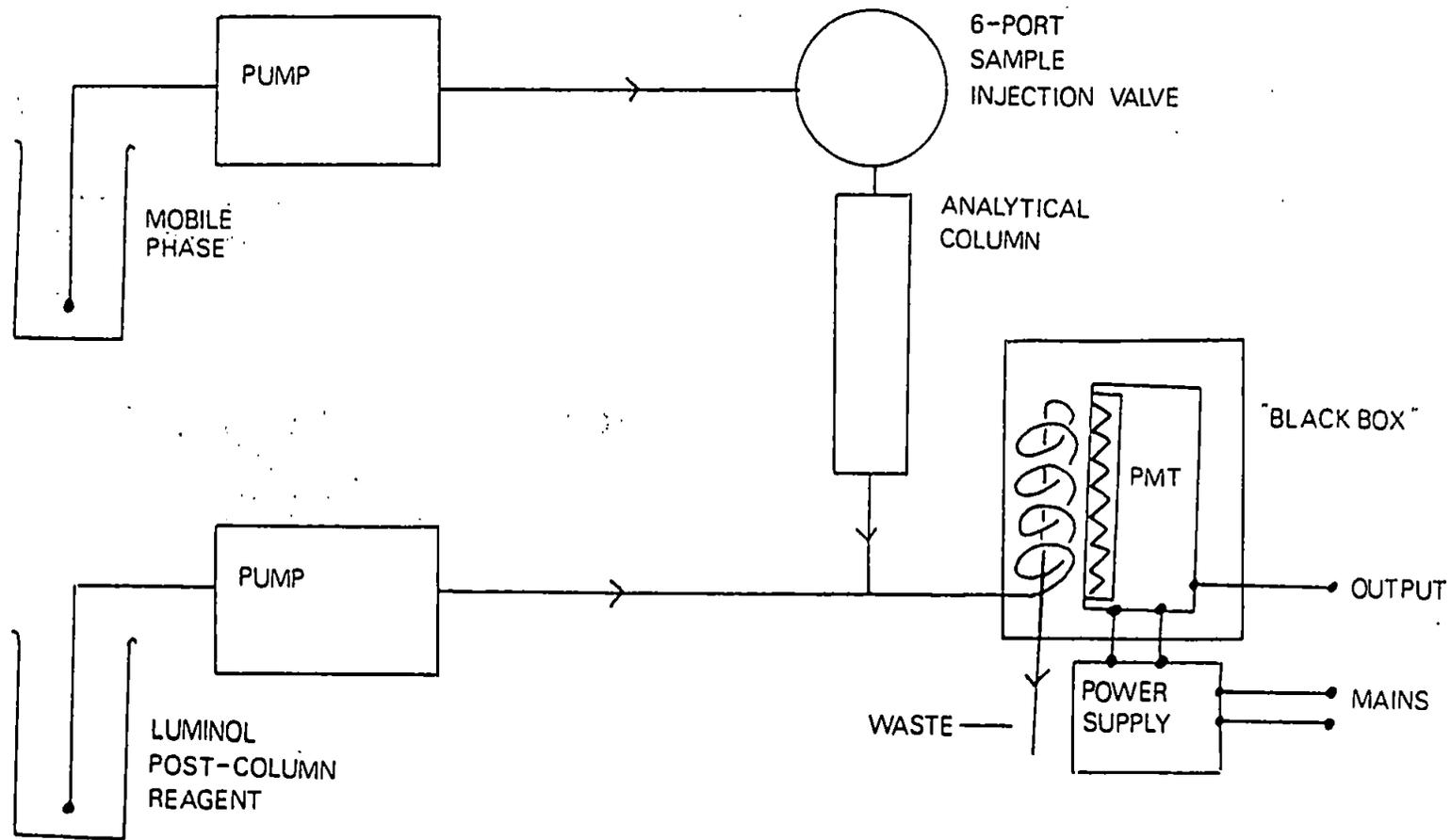


Figure 2.2 The instrumentation required for post-column CL detection showing the modified flow cell.

Metal standards were prepared by sequential dilution of 1000  $\mu\text{g ml}^{-1}$  Spectrosol stock solutions (BDH) except for iron(II) which was prepared from ammonium ferrous sulphate made up in helium degassed 0.1 M hydrochloric acid. Lower concentrations of these stock solutions were stored in their own polythene bottles in order to reduce cross contamination.

## CHAPTER 3

### THE DETERMINATION OF COBALT(II)

#### 3.1 Introduction

The determination of cobalt (II) at ultra trace levels is desirable for a number of reasons. Firstly, in environmental studies, the rapid and accurate analysis of many trace metals in natural waters is essential for the study of earth surface geochemical transfer processes. Very little information is available on the distribution of Co which is an essential element for every species in natural waters and this may be due to the rarity of the element ( $10^{-11}$  -  $10^{-9}$  mol kg<sup>-1</sup>). The low concentration levels at which Co occurs in natural waters leads to significant problems with its analysis, and very little reliable data is available on Co behaviour in fresh water systems.

Perhaps, as briefly mentioned in the introduction, in industrial processes, a more pressing area of need for Co determinations is in the coolant systems of pressurized water reactors (PWRs). The operators' radiation exposure is increased by accumulation and deposition of activated ionic products of corrosion from the inside surfaces of reactor pipes. To reduce the amount of corrosion products carried to the reactor, adequate anti-corrosion procedures are necessary which are based on the monitoring of plant-water quality. The most important element to be monitored for radioactivity reasons is Co, which undergoes

conversion to  $^{60}\text{Co}$ . This species is a gamma emitter and has a half life of 1924 days.

The main source of this  $^{60}\text{Co}$  comes from the alloy stellite (50 - 60% Co), used in heavy wear components, such as valves, control rod drive mechanisms etc. When new components are fitted, corrosion and transportation of species is of concern and requires intensive monitoring.

Since the concentration level of Co in the primary coolant ranges from parts per trillion to parts per billion, quantitative analysis will require a highly sensitive means of determination. Flame atomic absorption spectroscopy, inductively coupled plasma atomic emission spectroscopy and inductively coupled plasma - mass spectroscopy although sensitive techniques, are not anywhere sensitive enough for direct measurement. However even if they were, it would be undesirable to create an atomic vapour of radioactive isotopes in the laboratory and a safer, closed system, would be a better and safer choice.

The use of liquid chromatography would seem to lend itself to the monitoring of hazardous radioactive materials with the added potential for complete automation and on-line analysis. The problem, however, of using liquid chromatographic techniques, is that the detection step is not usually sensitive enough for direct analysis and so a pre-concentration step may be required.

A pre-concentration method was, in fact, developed and used by Jones et al., (121) for the determination of trace cobalt in simulated pressurized water reactor primary coolant. Typically 200 ml of sample was pre-concentrated using a short Aminex A9 pre-concentrator column and then eluted onto the analytical column by tartaric acid. The Co was separated from other species present and detection was achieved using inverse photometry (Eriochrome Black-T monitored at 610 nm). Using this method described by Jones, the limit of detection for Co after pre-concentration was  $0.01 \mu\text{g ml}^{-1}$  and the detector gave a linear response from 2.5 to 100 ng of Co absolute weight.

The liquid chromatography system that is presently employed for PWR monitoring is based on the more familiar Dionex system. Here, the lithium borate coolant is pre-concentrated (typically 50 ml) on a short low capacity cation exchange column (HPIC CG2) and eluted with a stronger complexing agent, 2,6-pyridinedicarboxylic acid (PDCA) onto the analytical column (HPIC CS2) and detection is achieved using pyridyl-azo resorcinol (PAR) monitored at 550 nm.

In order to reduce analysis time and so make each analysis more rapid and cost-effective, it would be desirable to negate the pre-concentration step altogether, but in order to do this, significantly better detection performance, in terms of sensitivity is required.

A significantly more sensitive detection system can be obtained using chemiluminescence systems, and this Chapter describes the development of such a system.

## 3.2 Experimental

### 3.2.1 Instrumentation

The liquid chromatographic system used was essentially the same as the one used by Jones et al., (121) but with some minor modifications as shown in Fig. 3.1. A high-pressure pump with titanium heads (Model 2150, LKB, Bromma, Sweden) was used to deliver the eluent and a high pressure stainless-steel pump (Knauer, Bad Homburg, FRG) was used for the post column reagent. All connections were achieved with either titanium (pre-column) or PTFE tubing. A 200  $\mu$ l sample was introduced using a six-port titanium switching valve (Valco, Schenkon, Switzerland). The separations were carried out using a Dionex HPIC CS2 cation-exchange column (250 mm x 4.6 mm i.d.), used in conjunction with a Dionex HPIC CG2 guard column (50 mm x 4.6 mm i.d.).

The eluent and post-column reagent (PCR) were mixed with the aid of a PTFE T-piece and the resulting solution was passed through a short flow cell (30  $\mu$ l).

Initial spectrophotometric determinations were carried out with a UV-Vis detector (SF 770 Spectroflow monitor, Kratos

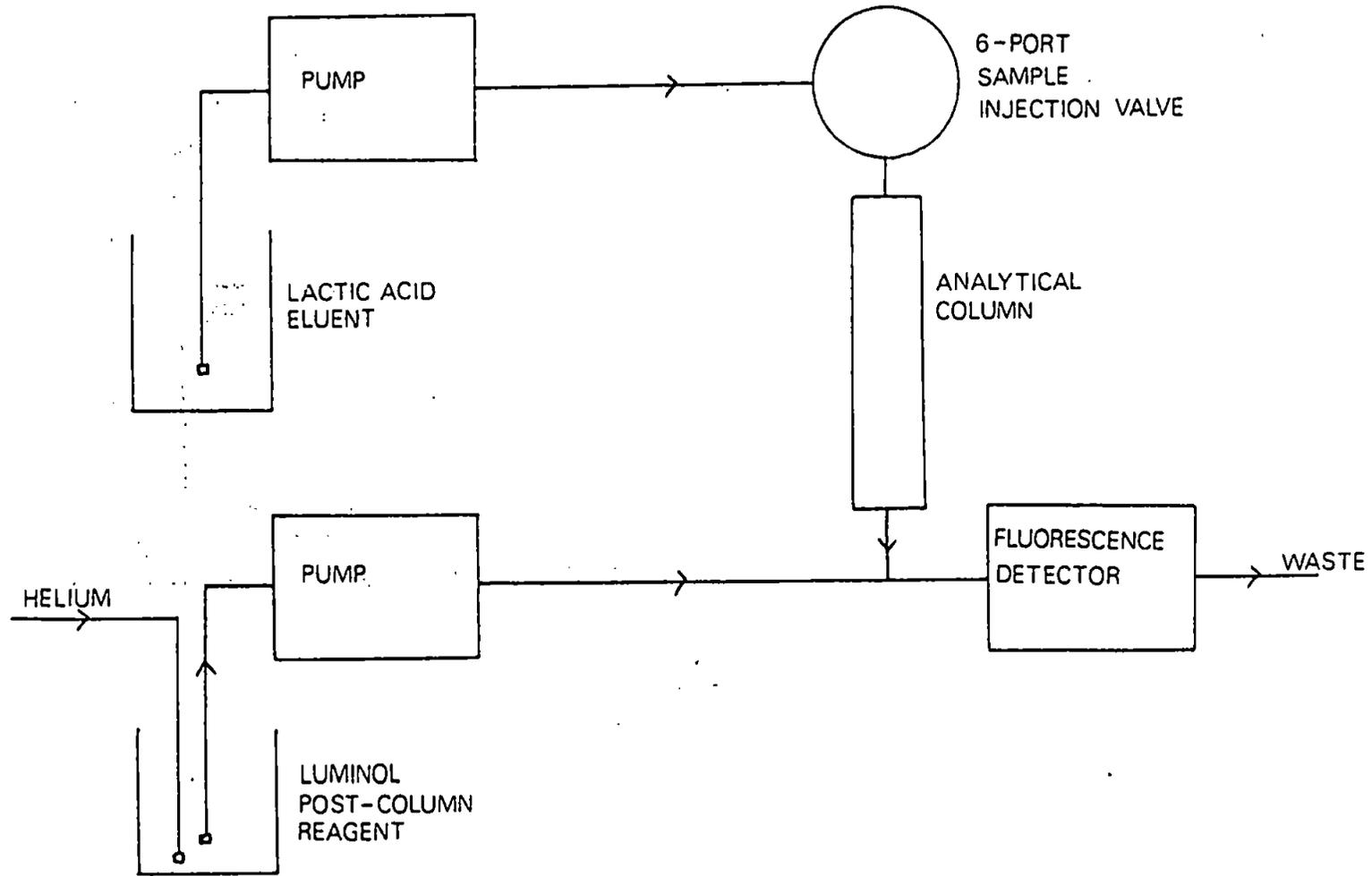


Figure 3.1 A schematic diagram of the apparatus required for Co determinations.

Inc., Schoeffel Instrument Division, Westwood, NJ, USA) but for CL measurements a fluorescence detector was employed (950 Fluoromat, Kratos, Westwood, NJ, USA) which, when in operation, had the lamp shutter in the closed position.

### 3.3 Results and Discussion

#### 3.3.1 Initial results

##### 3.3.1.1 The Separation System

Although for this particular part of research Co was the only metal under investigation, it was important to ensure that Co was isolated from any other metal that may interfere with the analysis. As has already been mentioned, one way to achieve this is to use ion exchange chromatography coupled to a less selective detector to ensure that no overlap of metal ions occurs.

Low capacity cation exchange Dionex columns were available for use (HPIC CG2, CS2) and the manufacturers recommend that they be used in conjunction with pyridine dicarboxylic acid (PDCA) as the eluent for the separation of selected members of the first row transition metals.

It was, however, decided to use a different eluent from PDCA. This decision was taken because PDCA forms a relatively strong complex with many transition metals and it was considered strong enough to adversely affect and possibly suppress any CL produced. It is well known that chelating materials such as EDTA can completely suppress

CL signals and indeed this fact has been exploited by several workers for the CL determination of Cr(III) in the presence of other metals by FIA (112). The EDTA reacts kinetically quickly with the majority of metals, thus masking them, and preventing them from producing any CL species. The slow formation of the [Cr(III)-EDTA] complex results in the Cr(III) being available to act as a catalyst in the luminol/peroxide CL reaction. Another reason why other elution reagents were chosen was because lactic acid and tartaric acid, both relatively much weaker than PDCA, have already been successfully employed for the separation of certain transition metals (77, 78).

Figures 3.2 and 3.3 show typical metal separations for both the lactate and tartrate separation systems, with detection being achieved by inverse photometry, with a Calmagite post-column reagent. From Figs. 3.2 and 3.3 the following elution order is produced with both lactate and tartrate eluents: Fe(III), Cu, Zn, Ni, Co, Fe(II), Mn and Cd. From studies at AEE Winfrith, this group contains most of the metals likely to be present with Co in the PWR coolant system.

The separation conditions for Figures 3.2 and 3.3 are described in Table 3.1

The detection system used a Calmagite solution in both cases. A stock solution of Calmagite reagent ( $4 \text{ g l}^{-1}$ ), in 2M ammonia was prepared from which the following daily

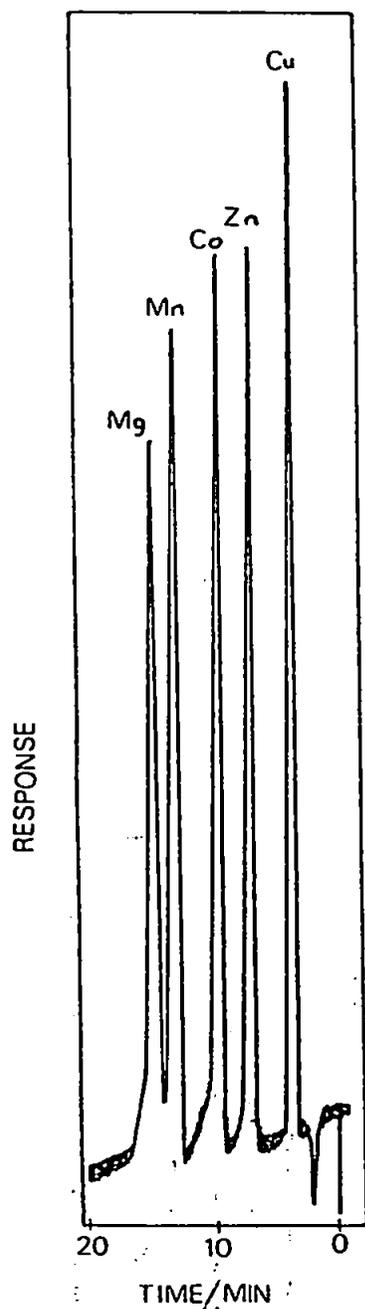


Figure 3.2 A typical transition metal separation using lactic acid as the mobile phase and a low capacity strong cation exchanger as the stationary phase. (2 ppm for each metal analyte).

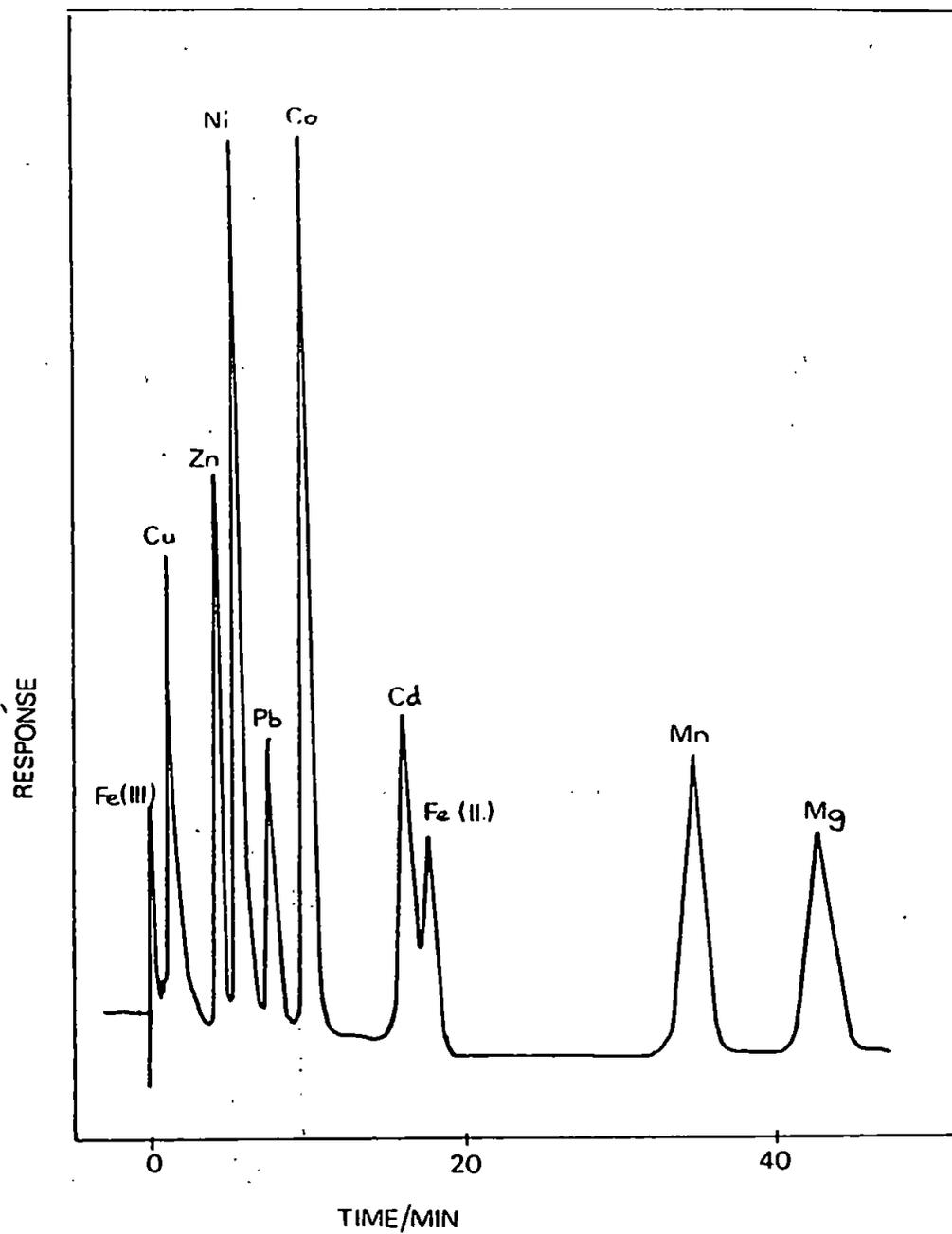


Figure 3.3 A typical transition metal separation using tartaric acid as the mobile phase and high capacity (Aminex A9) strong cation exchanger as the stationary phase. (5 ppm for each metal analyte).

**Table 3.1** The separation parameters required to produce the chromatograms in Figures 3.2 and 3.3

Mobile Phase	Concentration (M)	pH	Flow Rate (ml min <sup>-1</sup> )	Stationary Phase
Lactic acid	0.13	3.8	1.0	Dionex HPLC CS2 (25 cm x 4.6 cm id)
Tartaric acid	0.12	4.2	1.0	Aminex A9 (10 cm x 4.6 cm id)

working solution was made up; Calmagite : water : 2M ammonia (4:296:200).

As can be seen from Figures 3.2 and 3.3 separation conditions have been chosen so as to give clean, base-line resolution for most peaks and especially with regard to Co. Table 3.2 shows the retention times of various transition metals using the conditions stated in Table 3.1 for both lactate and tartrate systems.

The use of a detector, such as the one involving Calmagite, shows that the Co is not co-eluting with any of the metal species under study. Provided the separation conditions remain unaltered, the retention times of the metals will remain virtually identical. This is an important point when investigating a new detection system with unknown qualities.

#### 3.3.1.2 The CL Detection System

Previous work carried out at this Polytechnic looking at transition metal catalysis of CL reactions and the literature survey, have revealed that by far the most sensitive CL reaction for the determination of Co involves the catalytic oxidation of basic luminol with hydrogen peroxide (82, 83, 94).

Table 3.2 Lactate and Tartrate elution times for selected transition metals (see Table 3.1 for elution conditions).

<u>Lactate</u>		<u>Tartrate</u>	
Metal	Retention time / min	Metal	Retention time / min
Solvent front	3.8	Solvent front	2.3
Fe(III)	3.9	Fe(III)	2.4
Cu	3.9	Cu	2.6
Zn	6.8	Zn	4.3
Ni	9.1	Ni	6.2
Co	10.2	Co	16.0
Fe(II)	13.3	Fe(II)	18.0
Mn	14.1	Mn	36.2

The conditions of the post-column reagent that were required to give the most sensitive Co detection vary considerably depending on which publication you read, and it seemed that to get the correct detection parameters was rather a "hit and miss" affair. Initially, sensitivity was not of the utmost importance, and detection conditions were chosen that were known to give a reasonable CL signal. These conditions are described in Table 3.3.

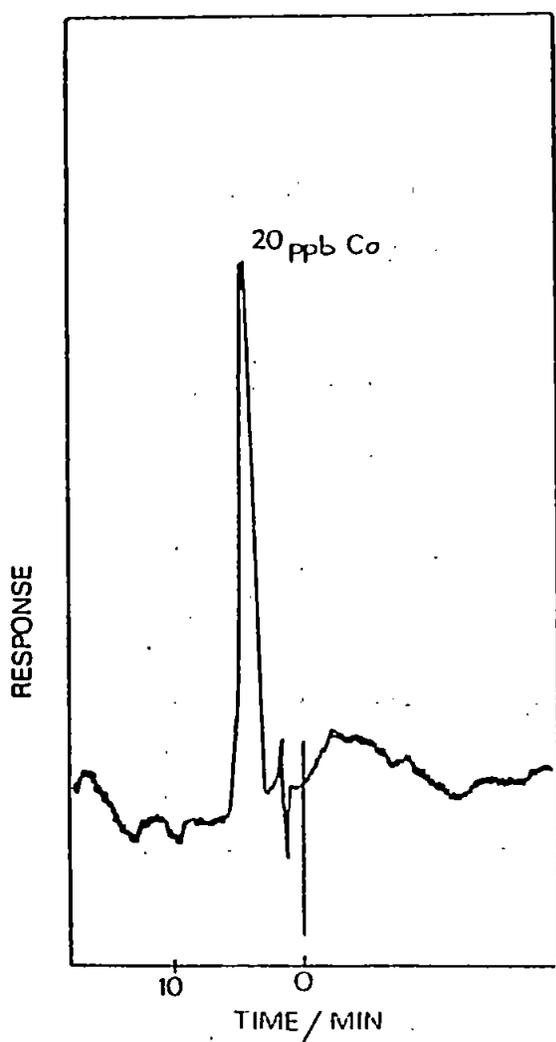
Using the lactate separation and detection parameters described a 20 ppb Co standard was injected and eluted after approximately 10 minutes and determined using CL detection as shown in Fig. 3.4. Interestingly, when using the tartrate elution system, although the base-line noise remained similar to that in Fig. 3.4, no CL signal was obtained for Co even at 100 ppb levels, although higher concentrations were not tried.

Fig. 3.4 also demonstrates the rather jagged and uneven baseline which is rather surprising as CL methods are reported to produce little or no background signal. Trace metal impurities in the analytical grade reagents are an obvious source of such background interference, although the background noise would not be so jagged if this were the only source of contamination. Leaching of cations e.g. chromium from the metal surfaces of the stainless steel pump may also contribute to the elevated background signal, although, again, a much smoother baseline would be

**Table 3.3** The Initial Detection Parameters used in the CL determination of  $\text{Co}^{2+}$

[Luminol] wt l <sup>-1</sup> g	[30% H <sub>2</sub> O <sub>2</sub> ] ml l <sup>-1</sup>	[Boric Acid] wt l <sup>-1</sup> g	pH adjusted with KOH	Flow rate ml min <sup>-1</sup>
0.044	0.60	6.20	13.0	1.0

**Figure 3.4** The CL response from an injection of a 20 ppb Co standard.



expected. Eventually it was decided that most of the noise was due to "micro" bubbles resulting from the decomposition of  $H_2O_2$ .

Improvement in the background condition was achieved by continual degassing of the luminol/peroxide PCR with a slow, steady stream of helium. This resulted in a far smoother baseline, presumably by delaying the decomposition of  $H_2O_2$  in the pump heads and connecting tubing.

#### 3.3.1.3 Discussion of Initial Results

The initial results showed that the use of CL for the determination of Co, even using a completely unoptimized detection system, indicated the potential for very sensitive detection.

A CL signal was only obtained using lactic acid as the mobile phase and the stronger the chelating ability of the mobile phase, the greater the likelihood that significant CL suppression may prevail. The logarithmic values of the conditional stability constants  $\log K_{stab}$  for Co with lactic acid and tartaric acid are 1.9 and 4.5 respectively, thus illustrating the significantly more stable metal-tartrate complex which compared with the metal-lactate complex, may explain why CL suppression occurs.

### 3.3.2 Detection Performance Optimisation of the CL Detection System

In order to fully explore and exploit the ultra sensitive detection possibilities of such a CL system the, detection parameters require full optimisation. Using the established lactic acid mobile phase as the elution system the following parameters were optimised: (a) luminol concentration, (b) hydrogen peroxide concentration and (c) the pH.

The optimisation procedure would seem ideally suited to using the simplex optimisation algorithm, which optimises interdependent variable parameters concurrently. This simplex optimisation was attempted but proved difficult to implement and was unsuccessful for a number of reasons. Perhaps the main stumbling block was the use of the signal-to-noise ratio as the figure of merit for the algorithm. It often proved impossible to obtain a reasonable noise level even at high instrument sensitivity and thus the signal-to-noise ratio was unobtainable. Another problem encountered was the lengthy time of preparation for each new set of detection parameters required and the subsequent lengthy instrument settling down period after the introduction of these new parameters.

In consideration of some of these problems, it was decided to carry out univariate searches for the three detection parameters. This proved much easier to perform. The

parameters which were optimised are listed in Table 3.4 with the possible ranges over which optimisation experiments could be conducted.

A univariate optimisation was performed for each parameter in turn, whilst holding the other parameters at the optimum established by their respective univariate search. The optimisation profiles for luminol concentration, hydrogen peroxide concentration and pH are shown in Figures 3.5, 3.6 and 3.7 respectively, and the optimised parameters are summarized in Table 3.5.

### 3.3.3 Quantitative Detector Performance

Having established the optimal detection parameters for the best Co signal in terms of sensitivity (3.2.3), the detector performance was then assessed in terms of linearity, calibration curves and detection limits.

Linear calibration curves were obtained covering approximately four orders of magnitude from  $5 \text{ ng l}^{-1}$  to  $10 \text{ } \mu\text{g l}^{-1}$  with excellent linearity as shown in Fig. 3.8. Although a wide dynamic range is expected from emission techniques, the linearity was excellent considering that detection was achieved by means of a complex and still incompletely understood catalytic reaction. Fig. 3.9 shows the chromatograms obtained for Co concentrations near the lower end of the calibration range ( $5 - 40 \text{ ng l}^{-1}$ ). A reproducibility trial was carried out to ascertain (a) the stability of the Co

**Table 3.4** Boundary limits of parameters studied during the univariate optimisation.

Parameter	Range
Luminol concentration	0.05 to 0.5 g l <sup>-1</sup>
H <sub>2</sub> O <sub>2</sub> (30%) volume	0.5 to 20.0 ml l <sup>-1</sup>
pH	8.0 to 13.0

**Table 3.5** The optimised parameters for sensitive Co(II) detection

Parameter	Optimised condition
luminol concentration	0.20 g l <sup>-1</sup>
hydrogen peroxide (30%) volume	10.0 ml l <sup>-1</sup>
pH	12.5

FIGURE 3.5 THE OPTIMISATION PROFILE OF [ LUMINOL ] FOR Co CL.

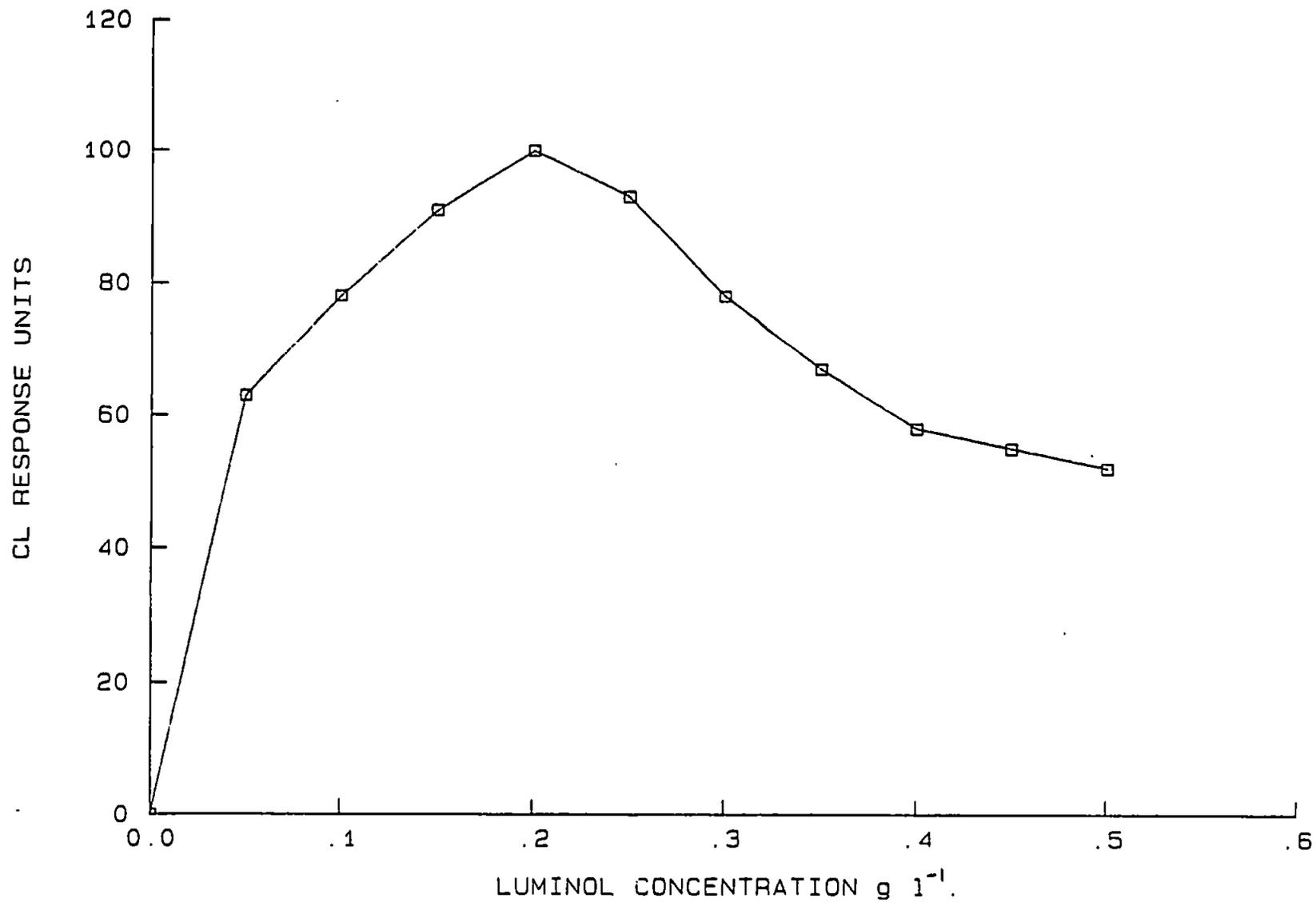


FIGURE 3.6 THE OPTIMISATION PROFILE OF [ H<sub>2</sub>O<sub>2</sub> ] FOR Co CL.

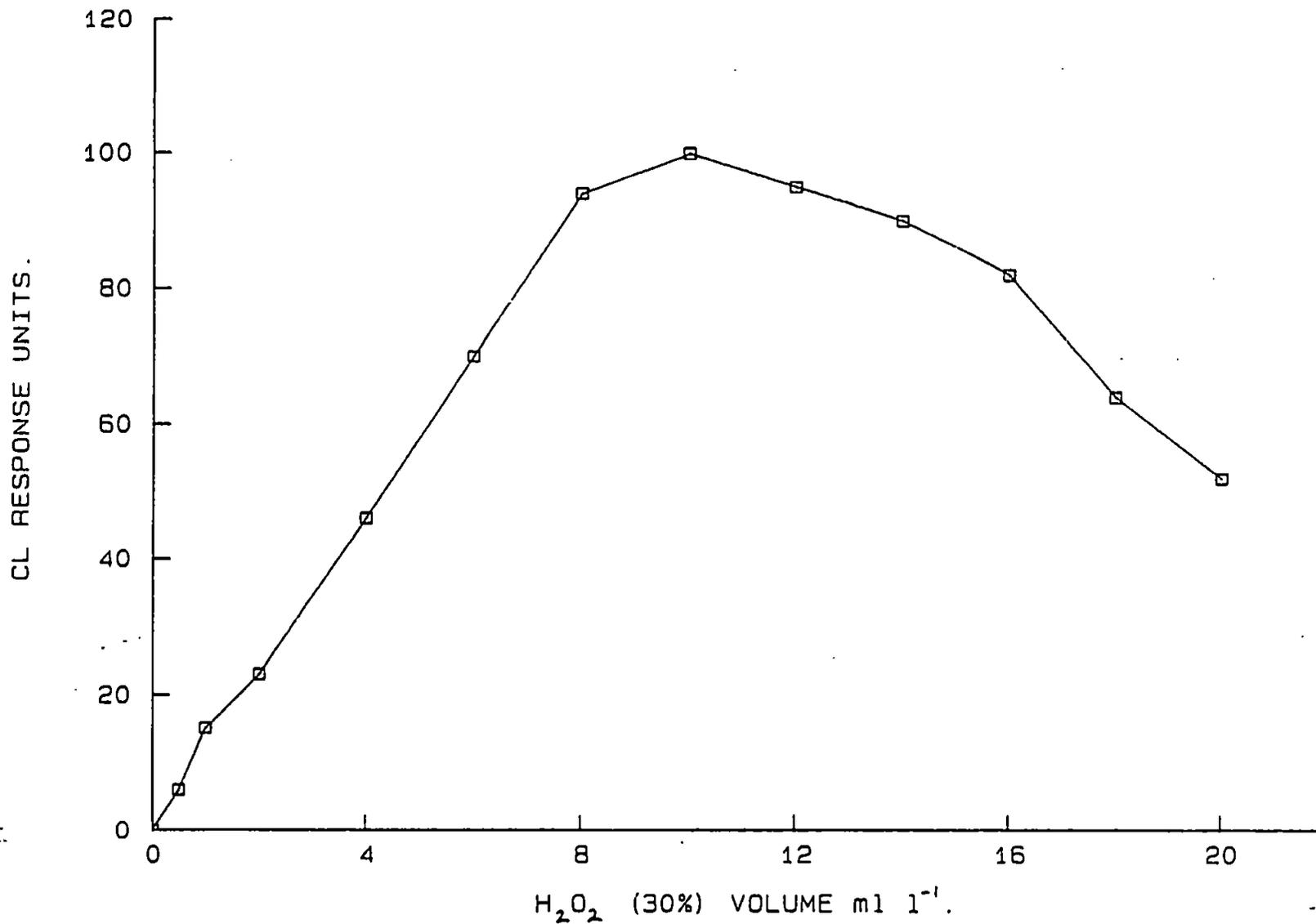


FIGURE 3.7 THE OPTIMISATION PROFILE OF pH FOR Co CL.

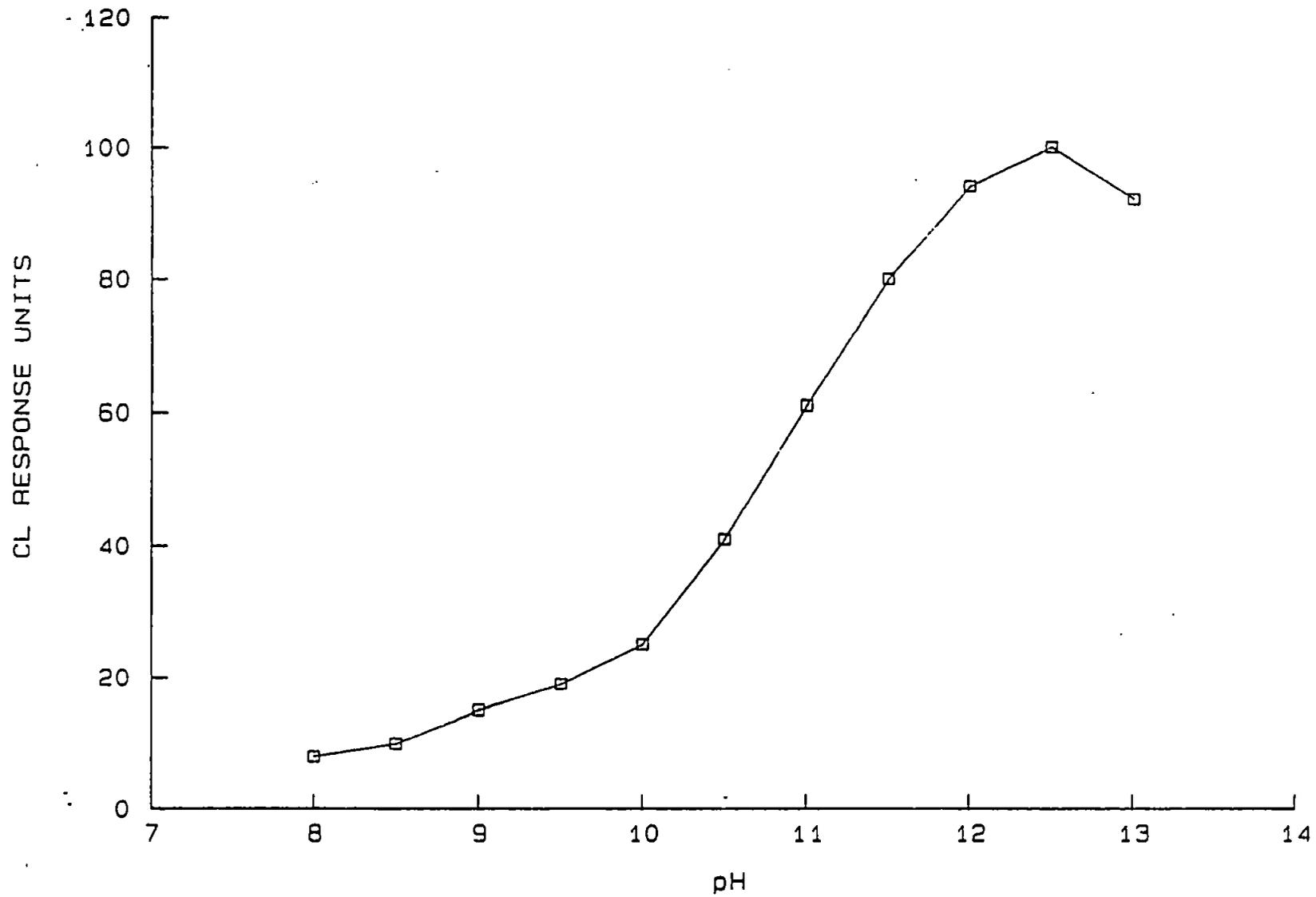
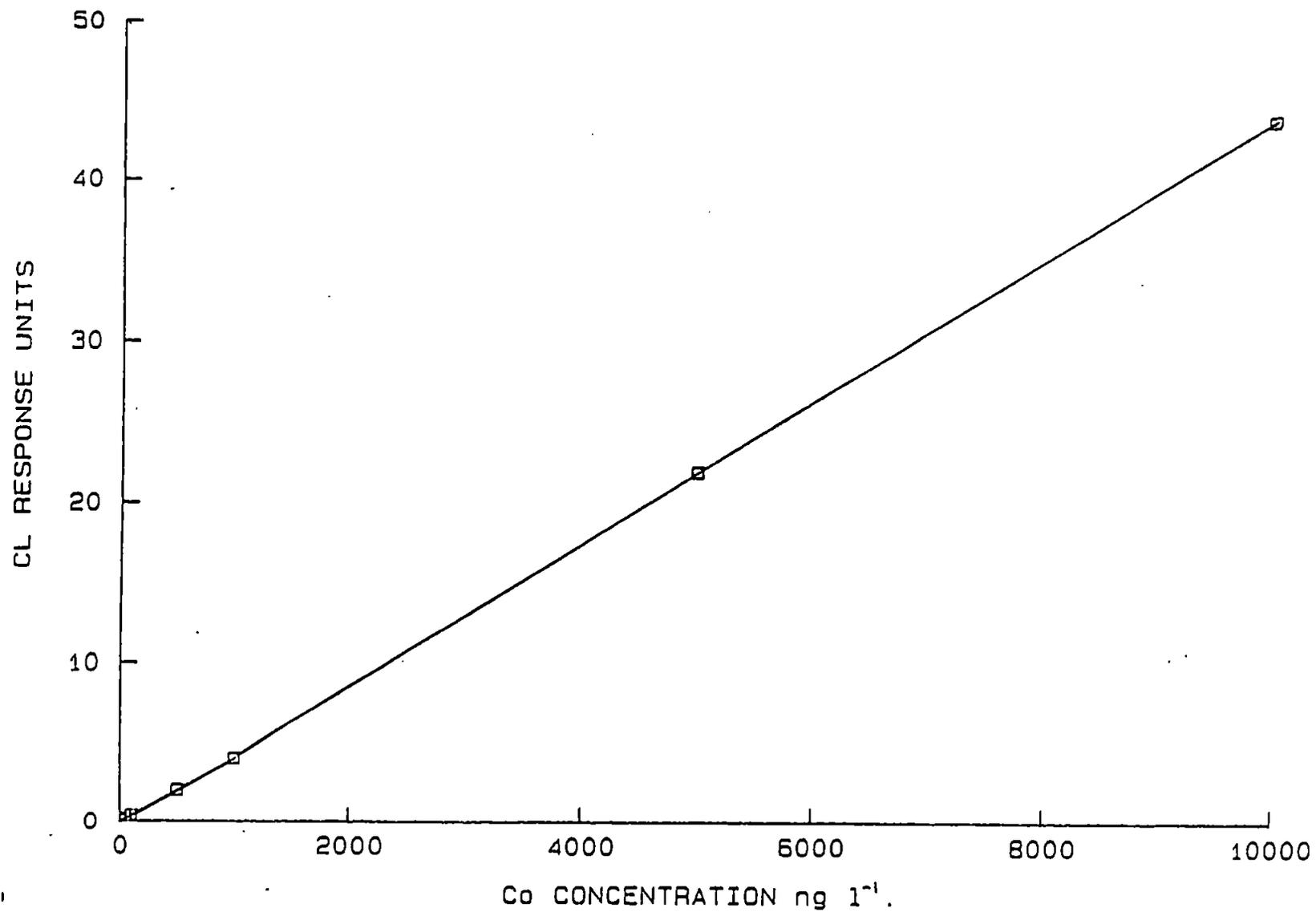


FIGURE 3.8

CALIBRATION CURVE FOR Co CL.



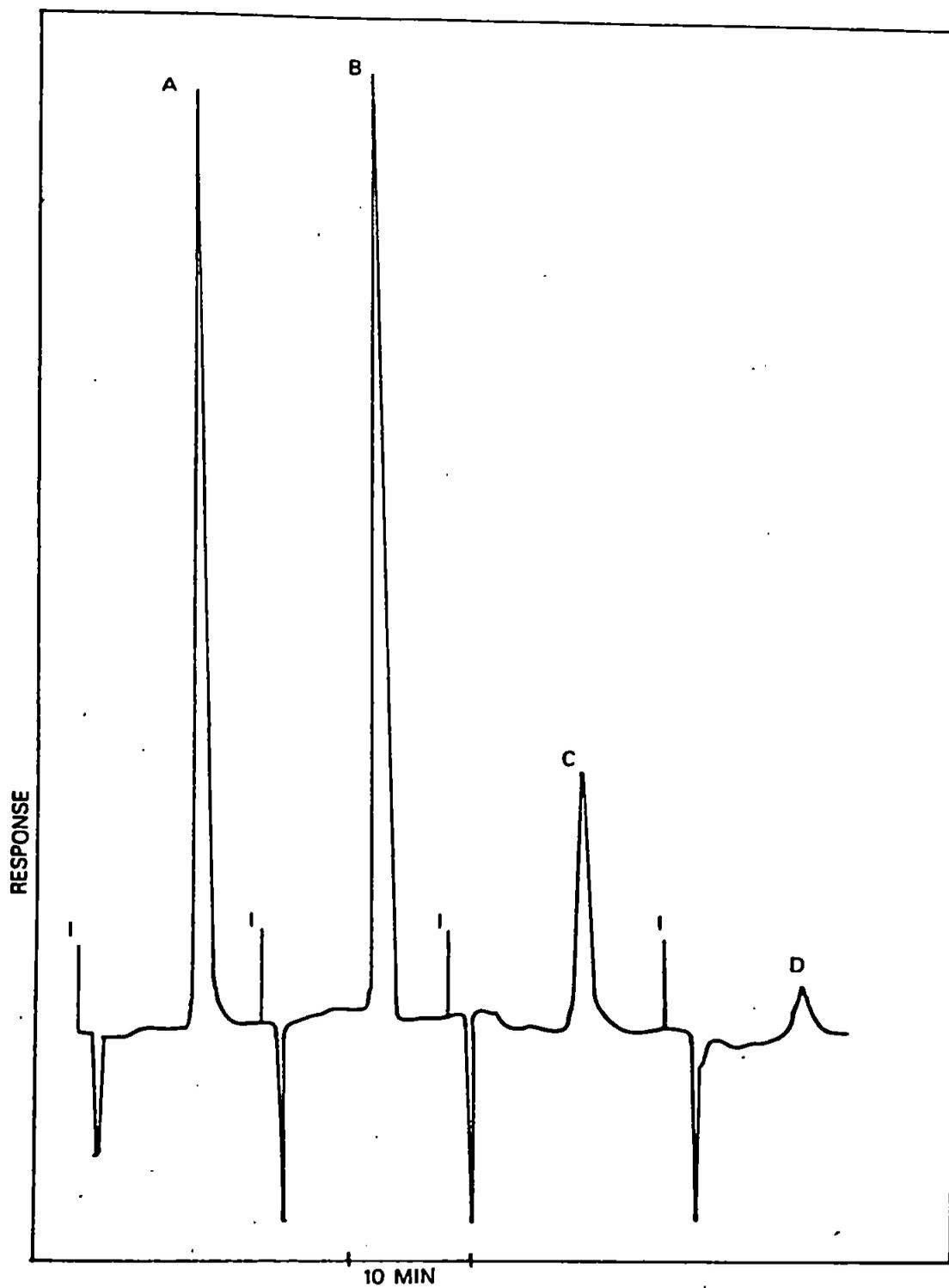


Figure 3.9 Chromatogram showing Co concentrations near to the detection limit; A.  $40 \text{ ng l}^{-1}$  (Scale x 0.5), B.  $20 \text{ ng l}^{-1}$ , C.  $5 \text{ ng l}^{-1}$ , D. blank.

standards and (b) the stability of the detection systems. Over a 2 hour period, 9 replicate injections were made onto the column from a  $40 \text{ ng l}^{-1}$  Co standard and the relative standard deviation was found to be 3.4% indicating that the low concentration standards, and the detection system, as a whole, were reasonably stable for at least 2 hours.

The detection limit, defined here as three times the peak to peak variation of the background noise, was  $0.5 \text{ ng l}^{-1}$ . This corresponds to 0.1 pg of Co absolute.

#### 3.3.3.1 Interference Studies

Of the suite of metals studied, the only metal, other than Co, that was found to give a CL response under the conditions employed was copper (II). The response due to Cu, however, was much lower than for Co and this point illustrates the selective nature of certain CL reactions as only weak responses were observed even for high Cu concentrations. Using the lactate system, Cu ions elute very quickly, just after the solvent front, and so do not cause any interference to the Co signal. Nevertheless, although no response was observed from the other elements tested, interference is still possible as any overlap with the Co peak could cause quenching. This is especially the case for nickel which elutes just before cobalt, and relatively high concentrations could produce an overlapping tail. For interference testing,  $10 \text{ } \mu\text{g ml}^{-1}$  solutions of Cu, Zn, Ni and Fe(II) were injected together

with a  $0.1 \text{ ng ml}^{-1}$  Co solution. The results of the interference study are presented in Table 3.6. No change in the Co peak height was observed for the mixed standards, compared with that produced by the pure  $0.1 \text{ ng ml}^{-1}$  Co standard.

### 3.3.3.2 Sample Analysis

To evaluate the sensitivity of the luminol CL detector, it was important to find a certified sample with a low Co concentration. A rice flour certified reference material (NBS SRM 1568) with a certified Co concentration of  $0.02 \pm 0.01 \text{ } \mu\text{g g}^{-1}$  had the lowest cobalt content available. Although this is well within the sensitivity range of the detector, the Co concentration, after sample preparation, was much lower.

Wet digestion techniques are inappropriate for sample preparation as high ionic strength solutions would saturate the low capacity analytical-column, producing variable retention times, thus making the analysis unsatisfactory. Dry ashing was satisfactory as Co compounds are relatively involatile.

Dry samples of 0.500 g of rice flour (SRM 1568, National Bureau of Standards, Washington D.C.) were dry ashed in a platinum crucible over a Meker burner. When cool, 1 ml of aristar hydrochloric acid was added to the mixture and evaporated to dryness with slow, careful heating. Hydrochloric acid (8.0 mls of 0.1 M) was added, then the

Table 3.6 The peak heights of  $0.1 \text{ ng ml}^{-1}$  Co standards co-injected with  $10 \text{ }\mu\text{g ml}^{-1}$  of possible interfering cations

Standard	$\bar{x}$ Peak ht mm 3 trials	normalised peak ht
$0.1 \text{ ng ml}^{-1}$ Co	96	1.00
$0.1 \text{ ng ml}^{-1}$ Co + $10 \text{ }\mu\text{g ml}^{-1}$ Cu	97	1.01
$0.1 \text{ ng ml}^{-1}$ Co + $10 \text{ }\mu\text{g ml}^{-1}$ Zn	98	1.02
$0.1 \text{ ng ml}^{-1}$ Co + $10 \text{ }\mu\text{g ml}^{-1}$ Ni	95	0.99
$0.1 \text{ ng ml}^{-1}$ Co + $10 \text{ }\mu\text{g ml}^{-1}$ Fe(II)	96	1.00

solution was warmed, cooled and transferred into a 10 ml volumetric flask with small volumes of water washings, finally diluting to the mark with water. The solution was transferred to a small polyethylene bottle to await analysis. Blanks were prepared in exactly the same manner, minus the rice sample, using the same containers.

The determination of the Co from the rice flour was carried out by both direct calibration methods and by standard addition injection and the results are presented in Table 3.7.

The results show acceptable agreement with the certified value of  $0.02 \pm 0.01 \mu\text{g g}^{-1}$ . Figure 3.10 shows chromatograms for one sample determination compared with deionized water standards and a standard addition injection.

The excellent sensitivity of the CL detector is clearly seen in Fig. 3.10 showing the noise free baseline suggesting that the detector was being operated well above the detection limit. The small step in the baseline may be due to a slight disturbance caused by the sample 'plug', with the relatively high salt content, disequilibrating the column for a short time.

#### 3.4 Concluding discussion

The detector performance in terms of sensitivity, linear working ranges, reproducibility and lack of interferences

**Table 3.7** The Determination of Co from a certified rice flour sample (NBS SRM 1568).

Method	Co content $\mu\text{g g}^{-1}$	$\bar{x}$
Certificate value	$0.02 \pm 0.01$	
Direct calibration	0.0162 0.0160 0.0158	0.016
Standard addition	0.0208 0.0214 0.0216	0.021

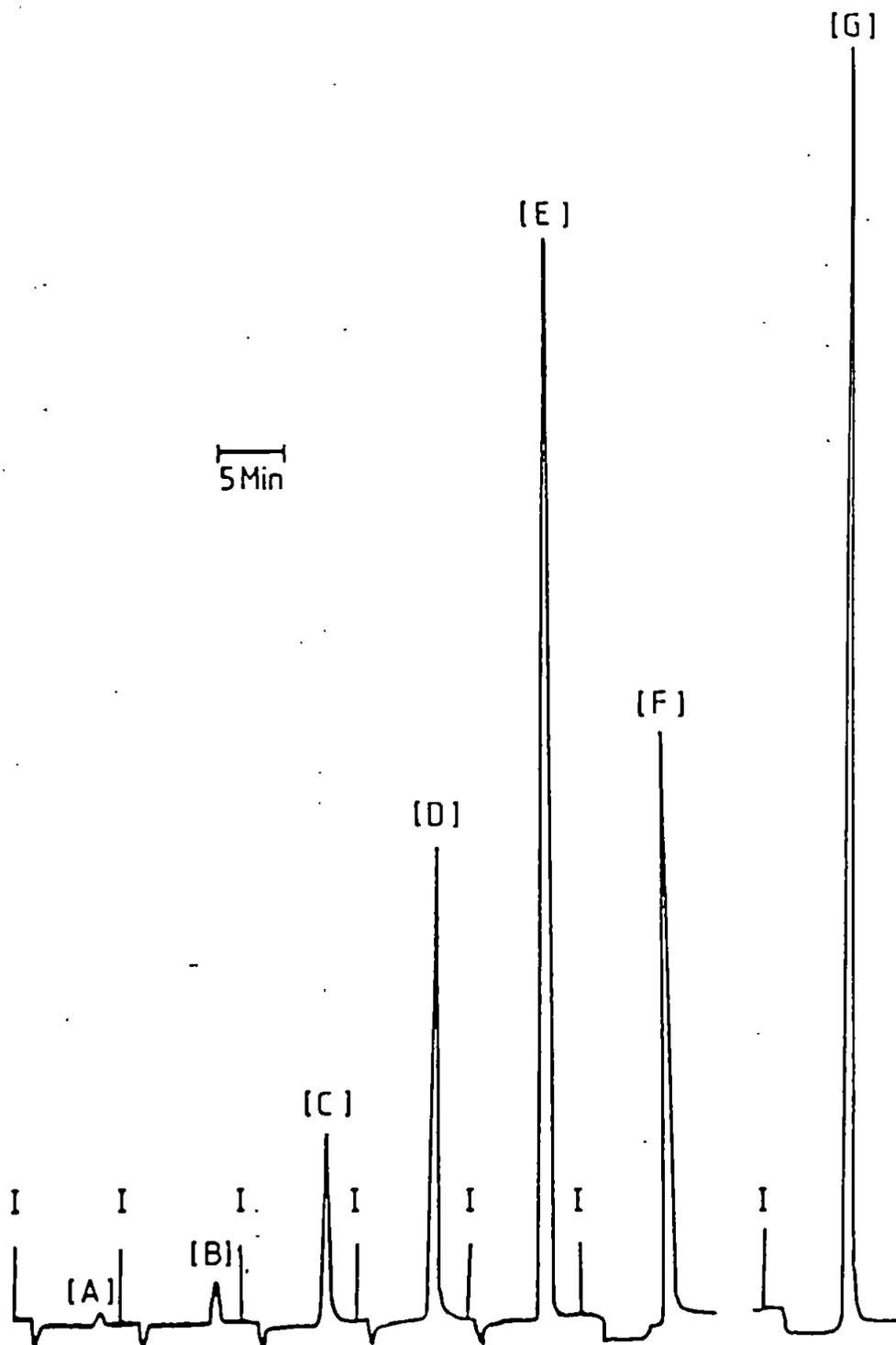


Fig. 3.10 Determination of cobalt in a rice flour sample. (A) Solvent blank; (B) procedural blank; (C), (D) and (E) 0.5, 1.0 and 2.0  $\text{ng ml}^{-1}$  cobalt standards, respectively; (F) sample; (G) sample + 1.0  $\text{ng ml}^{-1}$  of cobalt (standard addition).

is excellent, and the limits of detection better than any current analytical technique, including graphite furnace atomic absorption spectroscopy, and inductively coupled plasma mass spectroscopy. Simulated lithium borate coolant was manufactured by AEEW and was tested at Plymouth to see if similar sensitivities could be obtained for Co determinations. Co standards were made up in the lithium borate (1200 ppm boron, 3 ppm Li, pH 6.5) and injected into the system. No loss in CL signal or sensitivity was found. This system has proved successful enough to be employed at the Atomic Energy Establishment Winfrith (AEEW) for initial testing to ascertain the suitability of such a CL system for lithium borate coolant analysis.

The fluorescence detector used was obviously not optimised for CL determinations and even better sensitivities are possible if special flow cell/photomultiplier arrangements are constructed. The sensitivities available, however, using the commercial fluorescence detectors, are more than adequate for most samples. If, however, better sensitivities are required, one way to achieve this would be to remove the lactate from the mobile phase so as to reduce any possible suppression effects. If the lactate is removed from the mobile phase the analyte separation would have to be achieved by means other than ion-exchange chromatography. Such a procedure may involve chelation-exchange chromatography which requires no complexing agent in the mobile phase but relies on pH variation to control

separation mechanisms. No HPLC grade chelation-exchange stationary phases were commercially available at the time of this work and have only appeared in the last few months. It was found, however, that the sensitivity obtained with the lactate system was considered by the AEE Winfrith research team to be perfectly adequate for their use.

The determination of Co from rice flour shows the potential difficulty in using low capacity resins for trace metal analysis for real environmental samples which contain relatively high ionic strengths. The direct calibration data (Fig. 3.10) shows a retention time for the Co peak to be approximately 8 minutes, whereas, under the same elution conditions, the standard addition data (Fig. 3.10) shows a slight decrease in retention time for the spiked rice flour sample to approximately 7 minutes. The standard addition results show an apparent small bias compared with the pure standard and the possible cause may be the salt content of the ashed sample resulting in slightly shorter retention times. The use of an integrator to measure peak areas may solve this problem.

Having obtained such impressive results for one element by the CL technique, it would be desirable to develop a CL system to include a wider range of elements and thus to fully exploit the multielement capabilities of chromatographic systems, although to achieve this may require a novel approach to both the elution and detection

systems. Before the general detection problem was tackled, it was decided to study another metal with known high CL sensitivity namely, chromium, which unlike cobalt, has the added interest of being present as two major species under natural conditions. Environmental and medical interest, as well as a request from the Atomic Energy Establishment prompted the development of an HPLC/CL system for Cr(III) and Cr(VI) determinations described in the next chapter.

## CHAPTER 4

### THE DETERMINATION OF CHROMIUM

#### 4.1 Introduction

Occupational exposure to chromium (Cr) or its compounds frequently occurs in the working environment of industries handling chromium alloys or chromium compounds, such as the welding and grinding of stainless steel, chromium plating, tanning, wood preserving, painting and pigment production. The hexavalent state, Cr(VI), has been shown to be more toxic than the trivalent state, Cr(III) in animal experiments (2, 3), and is considered to be particularly dangerous because of the associated risk of allergic reaction and cancer. The trivalent state of Cr is a necessary trace element for the maintenance of the normal glucose tolerance factor and is non-toxic even at quite elevated levels. There is an obvious requirement for individual Cr species determination although there are very few analytical methods devoted to this task.

Atomic absorption, or plasma atomic emission techniques only provide information on the total amount of Cr in the test solution. Speciation measurement is possible with these methods, but the Cr(III) and Cr(VI) must be separated before they enter the absorption or emission instrument.

For the determination of water soluble Cr(VI), a photometric method based on 1,5-diphenylcarbazide (DPC) is

the most prevalent technique, although more recently a system based on lanthanide luminescence quenching by chromate has been developed (122). Measurements, however, are often unreliable because of significant interference from redox systems in the matrix of the actual samples. There are also no reliable methods for direct analysis of Cr(III). The calculation of the concentration of Cr(III) from the difference between total Cr and Cr(VI), as put forward by some workers, may involve some risk of uncertainty and one way to decrease such a risk would involve the simultaneous determination of both chromium species.

The use of chromatography would seem the most obvious way to separate these two chromium species and this has been achieved by several workers including Eijörvi et al. (3) who used reversed phase HPLC to separate Cr(III) and Cr(VI) chelates of 8-hydroxyquinoline, diethyldithiocarbamate and ammonium pyrrolidine-dithiocarbamate, using methanol-water and acetonitrile-water mobile phases with UV detection. Separation could not be achieved using 8-hydroxyquinoline as the Cr(VI) was reduced to Cr(III) in the chelating procedures. The other chelators used were successful for separations and average detection limits of 100 pg for both Cr species was found in an injection volume of 5  $\mu$ l. A method that has been developed for the determination of Cr(III) is based on the separation of a [Cr(III)-PAR] complex using reverse-phase chromatography with an impressive photometric detection

limit of  $0.5 \text{ ng ml}^{-1}$  (123).

Perhaps a more suitable chromatographic method for Cr species determination is the use of ion-exchange chromatography. This method was demonstrated by Suzuki et al. (124) in 1985, who used an anion exchange stationary phase to separate Cr(VI) and Cr(III) - EDTA anionic complexes and the species were detected by a UV monitor or an atomic absorption spectrophotometer. With this method, the authors were able to achieve an impressive detection limit for both Cr(III) and Cr (VI) of 5 ng absolute.

In all of the cited cases, a substantial amount of sample handling and pretreatment was required, which is undesirable from a trace analysis viewpoint, and also considerably lengthens the analysis time. For the improvement in limits of detection and less sample handling for quicker Cr analysis, an improvement in both the separation and the detection system is required.

One promising novel detection system that shows tremendous potential is the coupling of a CL post-column reagent to an ion-exchange separation system. As can be seen from Chapter 3, tremendous sensitivities are possible for the detection of Co(II) using CL and Cr(III) is known to act in a similar catalytic fashion to Co(II), although Cr(VI) exhibits no catalytic affect on the CL system. This fact has been exploited by a number of workers for the selective determination of Cr(III) from a sample which

contains both Cr(III) and Cr(VI). Trace Cr(III) can be determined by measuring Cr(III)-catalysed light emission from the luminol oxidation by peroxide. The light emission caused by other metals can be completely quenched by the addition of EDTA to form complexes that are not active as catalysts. [Cr(III)-EDTA] complexes are kinetically extremely slow to form and so the EDTA does not interfere with Cr(III) catalysis. Using this method, Seitz et al. (112) was able to determine 25 pg of Cr(III) as early as 1972. This method has also been used for Cr(III) determinations in sea water samples (113). EDTA was again used to eliminate the catalytic activity of other interfering metals present. Problems, however, were experienced with quenching effects caused by the presence of high levels of calcium and magnesium. Li et al. (111), have used a very similar technique for the determination of Cr(III) from a variety of biological materials, including orchard leaves and bovine liver. All these methods involving the CL determination of Cr(III) do not involve the use of chromatography systems which would, if used, negate the requirement for EDTA addition. However, no such system has been reported in the literature, presumably because of the difficulty in performing Cr(III) elution and separation from cation-exchange columns and hydrolysis problems. The choice of mobile phase is important because the Cr(III) has a very strong attraction for cation exchange resins and elution may prove to be difficult using conventional chelating mobile phases as complex formation is kinetically very slow, even at

elevated temperatures. The separation of Cr(III) from Cr(VI) by ion-exchange chromatography would seem the most obvious and easiest method for study, as Cr(III) exists in acid solution as cationic  $\text{Cr}^{3+}$  and Cr(VI) exists as anionic  $\text{CrO}_4^{2-}$  or the dichromate form  $\text{Cr}_2\text{O}_7^{2-}$ , depending on pH. The presence of these anionic and cationic forms makes separation easy, either by the use of mixed bed columns or anion-exchange and cation-exchange columns placed in series or in parallel.

CL methods have not been able to determine both Cr(III) and Cr(VI) simultaneously, although this is obviously a desirable aim and the following work describes the development of an HPLC system with CL detection for each individual species and then the simultaneous determination of Cr(III) and Cr(VI).

## 4.2 Experimental for Cr(III) Determinations

### 4.2.1 Instrumentation for Cr(III) Determinations

The instrumentation required for the determination of Cr(III) only, was essentially the same as that used for Co(II) determinations, but with some minor modifications as shown in Figure 4.1. A high-pressure plastic pump (Dionex 4000 gradient pump, Dionex, Sunnyvale, CA, USA) was used to deliver the eluent whilst a high-pressure stainless steel pump (Knauer, Bad Homburg, FRG) was used to deliver the luminol post-column reagent. All connections were achieved with either titanium or PTFE tubing. The sample was loaded into a 200  $\mu\text{l}$  titanium

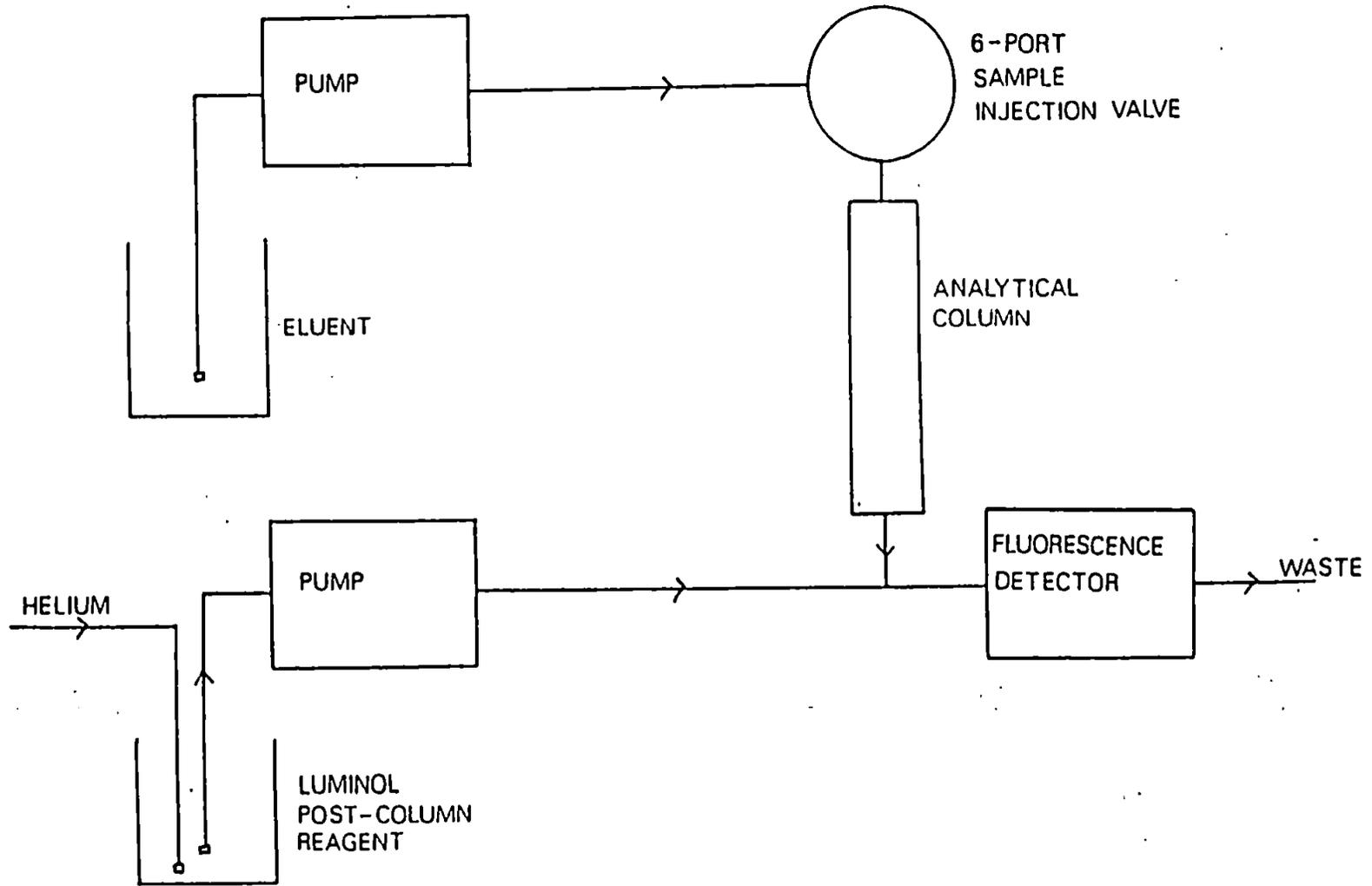


Figure 4.1 A schematic diagram of the apparatus required for Cr (III) determinations.

sample loop using a 6 port titanium valve (Valco, Schenkon, Switzerland) and injected onto a Dionex HPIC CG2 cation-exchange column (50 mm x 4.6 mm i.d.). The eluent and post-column reagent were mixed with a PTFE T-piece which was connected to a 350  $\mu$ l (70 cm x 0.8 mm i.d.) coiled PTFE flow cell which was mounted directly in front of the photomultiplier tube of a fluorescence detector (950 Fluoromat, Kratos, Westwood, NJ, USA) which had the lamp shutter closed.

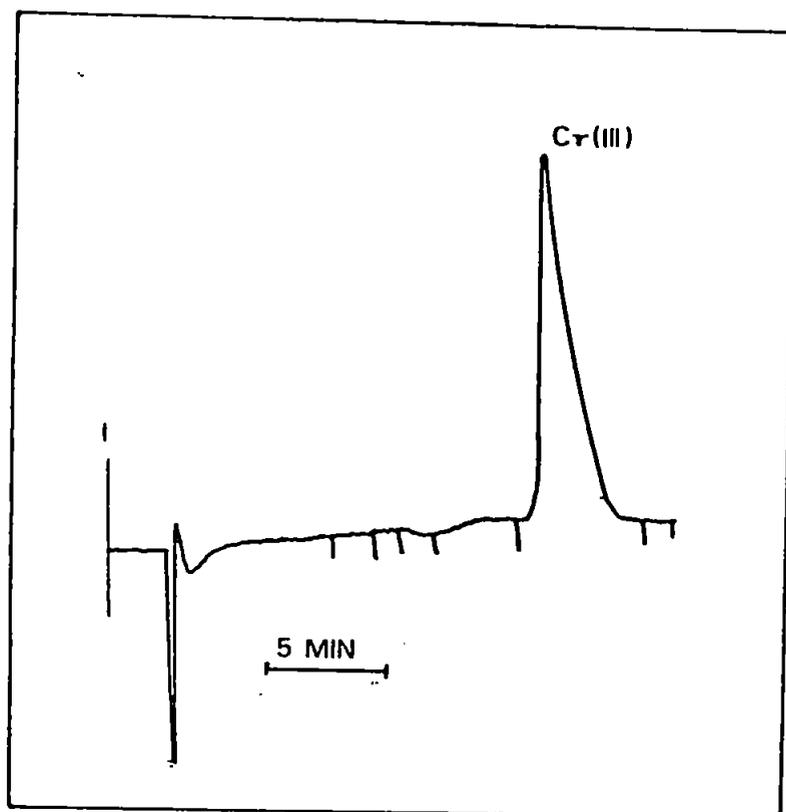
### 4.3 Results and Discussion for Cr(III)

#### 4.3.1 Preliminary Results for Cr(III)

The initial mobile phase that was chosen for Cr(III) elution was lactic acid which proved successful for Co(II) determinations and detection was achieved with the same Co CL post-column reagent conditions. Even at a relatively high lactate concentration, pH and flow rate, and with the use of a short column, the Cr(III) species took a rather long time to elute. Figure 4.2 shows a typical peak of a 5 ppm Cr(III) injection using 0.18M lactate, pH 3.8 and a flow rate of 1.8 ml min<sup>-1</sup>. The retention time was of the order of some 20 minutes. The peak shape was reasonably symmetrical although a little too broad to be ideal.

In order to decrease the retention time of the Cr(III) elution, it was envisaged to construct a column backflush facility. In this way, the Cr(III) standard could be injected in the usual manner, but after a relatively short time the direction of eluent flow could be reversed. The

Figure 4.2 The elution of Cr (III) using a lactic acid eluent with CL detection. (5 ppm metal analyte).



main advantage that is achieved by this is to produce faster elution times with the consequent increase in signal height and improved symmetry (although peak area should remain the same). This improvement in peak shape and signal arises because the ions do not have to travel the entire length of the column and, provided there is a long enough time allowed for any other metals present to elute from the system, overlap due to the presence of other metal ions should not pose a problem.

The backflushing mechanism is achieved by using a more complex switching valve system. Ideally, a 10 port valve would be employed, although, initially this was unavailable and so two 6 port valves were connected in series as shown in Figure 4.3. The conditions used for both the separation and detection conditions for the column backflushing are summarised in Table 4.1.

A typical chromatogram using this system is shown in Figure 4.4.

This backflushing system looked reasonably promising for further investigations. Problems soon arose, however, after a few working weeks of continuous column use. The baseline became very unstable and the background signal was significantly higher than had been previously experienced. The peak shape, due to Cr(III) ions was very uncharacteristic as can be seen from Figure 4.5. The explanation for these problems encountered is not clear,

**Table 4.1** Separation and detection parameters used for Cr(III) determinations using a column backflushing system.

Separation Parameter

lactic acid concentration	0.18 M
pH (adjusted conc. KOH)	3.8
flow rate	1.2 ml min <sup>-1</sup>
backflush time after injection	2.0 min
retention time Cr(III)	4.5 min

Detection Parameter

luminol concentration	0.2 g l <sup>-1</sup>
H <sub>2</sub> O <sub>2</sub> (30%) concentration	10.0 ml l <sup>-1</sup>
pH (adjusted conc. KOH)	12.0
boric acid concentration	6.0 g l <sup>-1</sup>
flow rate	1.0 ml min <sup>-1</sup>
outlet pH (after mixing with lactate)	10.8

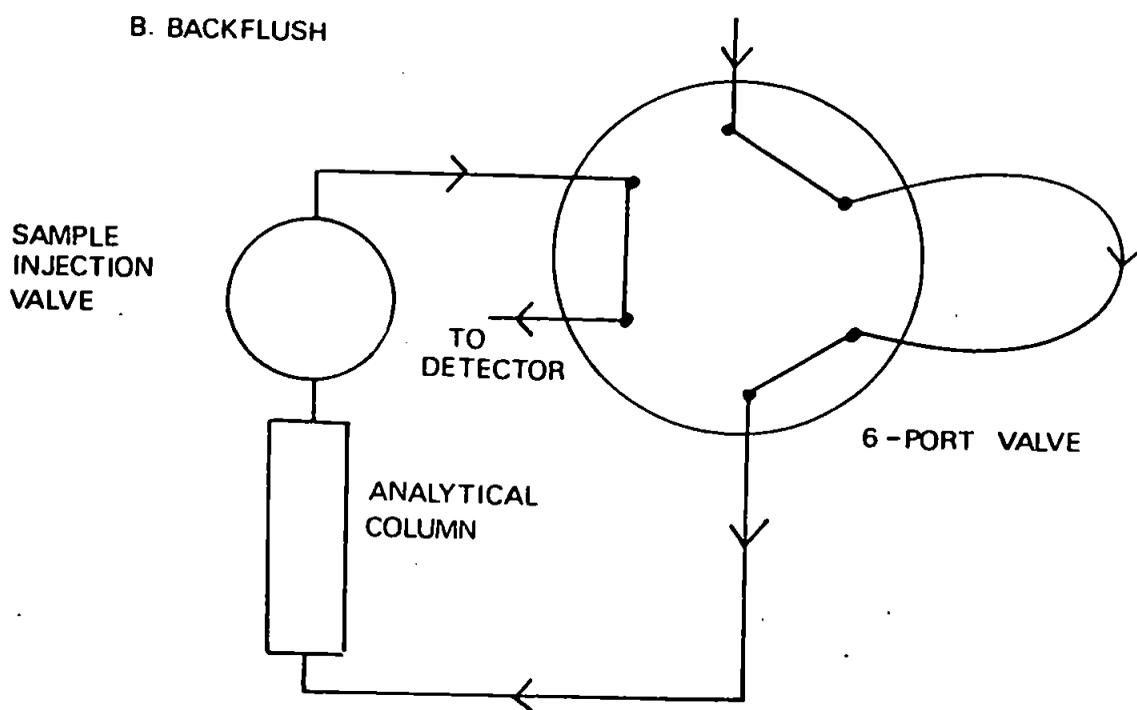
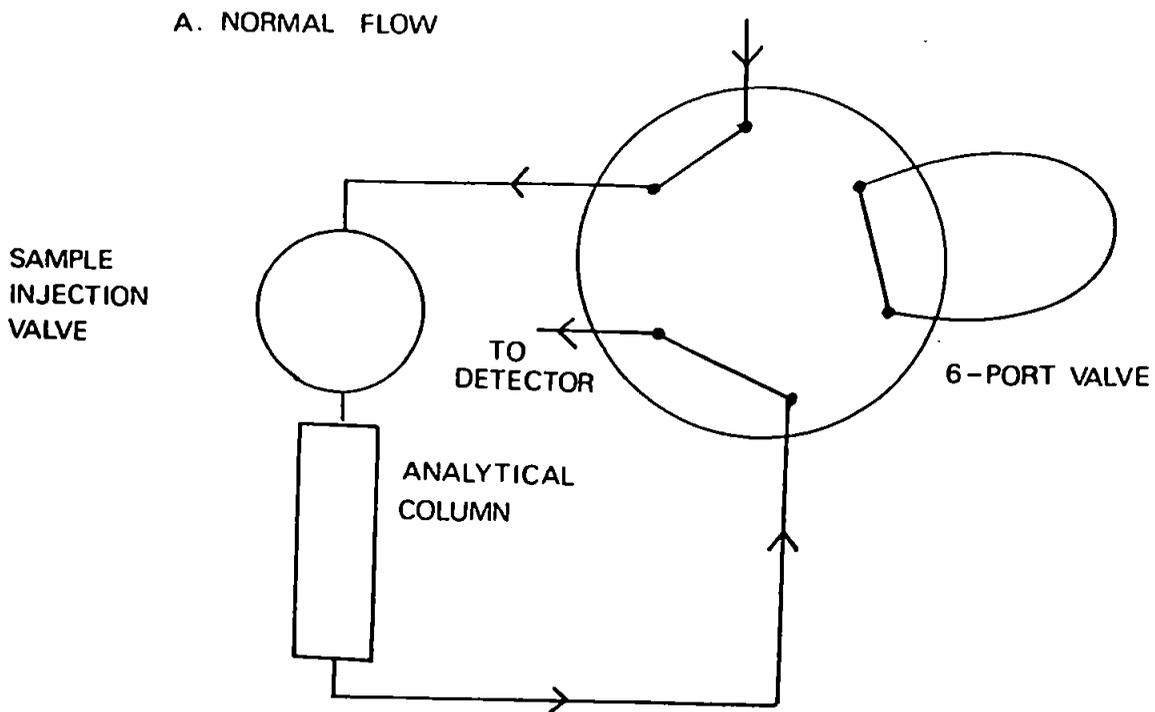


Figure 4.3 The use of 2, 6-port injection valves connected in series to produce; A. a normal flow and B. a backflush facility for Cr (III) elution.

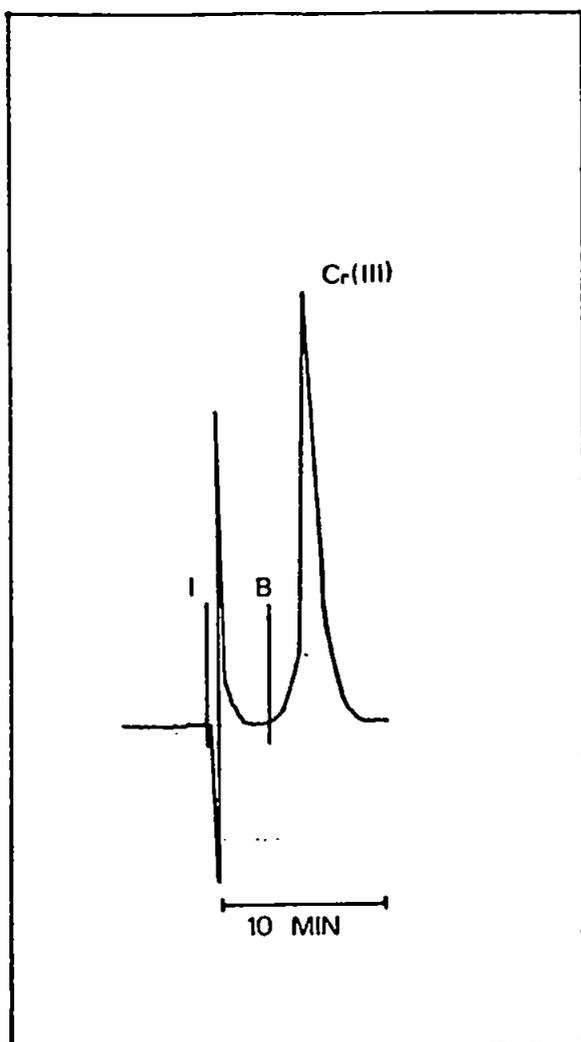


Figure 4.4 A typical Cr (III) peak obtained using the backflush facility with the lactic acid eluent. (3 ppm metal analyte).

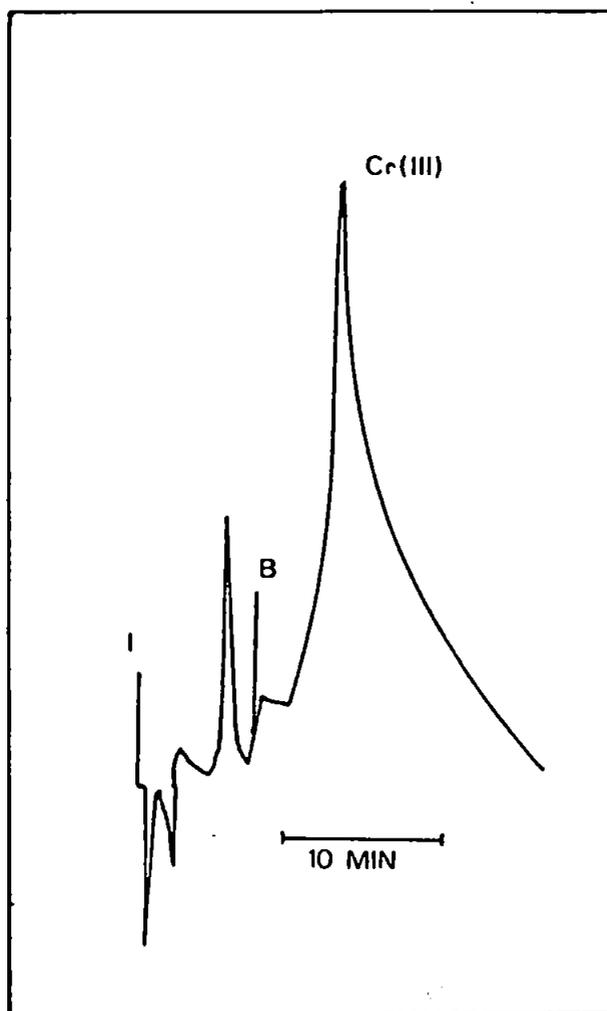


Figure 4.5 The uncharacteristic peak shape obtained for Cr (III) elutions after a few working weeks of continuous column use. (5 ppm metal analyte).

but it is presumed that with every injection of the Cr(III) standard, a small amount of it would not be eluted, due to its extremely strong affinity for the stationary phase, and slow, unfavourable complex formation with the mobile phase. Thus, over a period of time, the column would soon become saturated with Cr(III) ions and slow leaching of these Cr(III) ions from the column may well lead to the high background and an unsteady baseline observed. This presumption is further confirmed by the fact that when the column was cleaned with 1.0 M potassium nitrate, a huge CL emission was noted for some 8 minutes which was then followed by a rapid drop in signal to levels that were experienced in the initial stages of the Cr(III) study.

Previous work, looking at aluminium (Al(III)) determinations (82, 83) has required the use of a potassium sulphate eluent, which was used in place of a metal-chelate elution system. With the use of potassium sulphate as the eluent, the elution mechanism is based on ionic competition between the potassium ions and the analyte +2, +3 cations. This is one of the simplest types of elution system, although not widely adopted, as multielement analysis using this ionic competition approach is difficult to achieve. In general all the +1 cations will elute together, followed by the +2 metals and finally the +3's. It is relatively easy to separate the +2 from the +3 cations although more difficult to completely separate a suite of +2 or +3 metals from each

other. This is because separation is governed principally by ionic radius, which can be very similar when the hydration shells are taken into account, for metal species of the same charge. The potassium sulphate ionic strength can be chosen so that the +1 and +2 metals elute well before the Al(III) ions and do not, therefore, interfere. Since Cr(III) has a similar chemistry to Al(III), it was decided to use this same eluent as the mobile phase for Cr(III) elution. With the use of the potassium sulphate mobile phase, there is now no backflush step required, making the instrumentation a little simpler.

The new mobile phase conditions are described in Table 4.2 although the detection parameters remained the same as those described in Table 4.1.

Using the conditions described in Table 4.2, it is possible to obtain Cr(III) responses such as the one shown in Figure 4.6.

#### 4.3.2 Detector Performance for Cr(III) Determinations

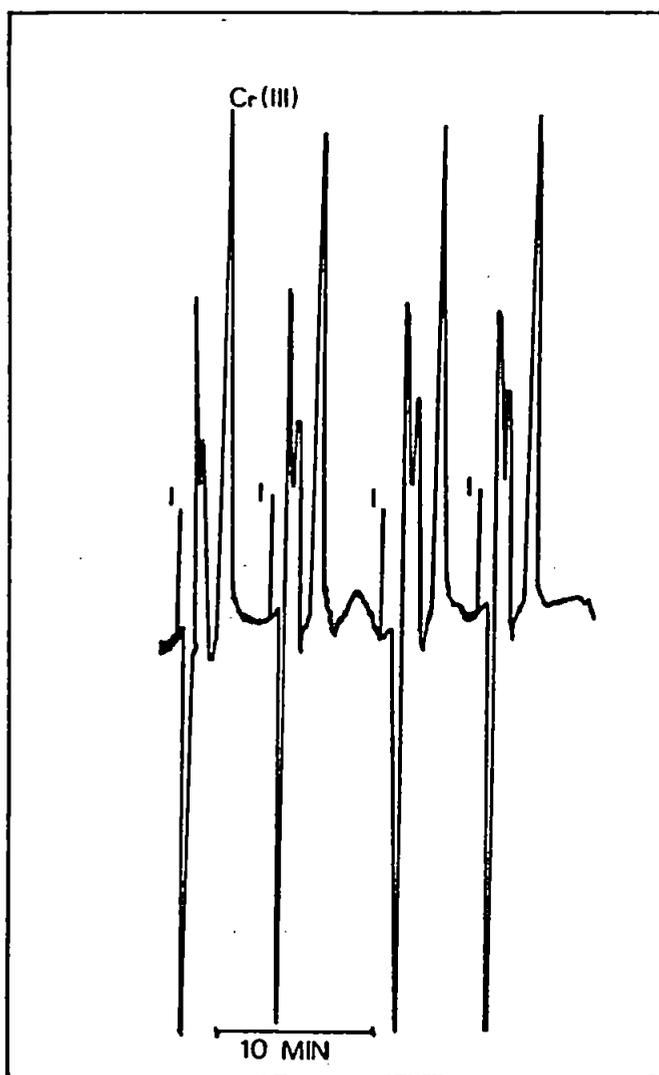
##### 4.3.2.1 Univariate Optimisation for CL Detection.

Until now, the conditions used for CL detection have been the same as those that were optimised for Co(II) determinations (Sec. 3.3). There will obviously be some variation in optimised conditions between Co(II) and Cr(III) and now that a suitable stable mobile phase is employed, univariate optimisation can be attempted. The parameters which were optimised are listed in Table 4.3

**Table 4.2** Separation conditions used for successful Cr(III) elutions.

<u>Parameter</u>	
K <sub>2</sub> SO <sub>4</sub> concentration	0.20 M
pH	3.0
flow rate	1.2 ml min <sup>-1</sup>
elution time Cr(III)	4.0 min
stationary phase	Dionex HPIC CG2 (50 mm x 4.6 mm)

Figure 4.6 Typical Cr (III) peaks obtained when using a potassium sulphate eluent. (200 ppb metal analyte).



**Table 4.3** Boundary limits of parameters studied for univariate optimisation for Cr(III) determinations.

<u>Parameter</u>	<u>Range</u>
Luminol concentration	0.05 to 0.5 g l <sup>-1</sup>
H <sub>2</sub> O <sub>2</sub> (30%) volume	0.5 to 5.0 ml l <sup>-1</sup>
pH	8.0 to 13.0

with the possible ranges over which optimisation experiments could be conducted.

Each parameter was optimised in turn whilst holding the other two variates at the optimum established for their respective searches. Figures 4.7, 4.8 and 4.9 show the optimisation profiles established for each respective parameter and the univariate optimised parameters are summarised in Table 4.4.

#### 4.3.2.2 Quantitative Detector Performance for Cr(III) Determinations

Having optimised the luminol CL system for Cr(III) detection, an interference study was carried out to ascertain whether there was overlap on the Cr(III) signal from the presence of other metals. As expected, using the potassium sulphate eluent none of the +2 metals, that were tested,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$ , were found to interfere even at relatively high levels ( $10 \mu\text{g ml}^{-1}$  metal<sup>2+</sup> co-injected with  $0.1 \mu\text{g ml}^{-1}$  Cr(III)).  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  were also tested for interference properties and were found only to have an interference effect at high levels. Table 4.5 summarises the affect of different concentrations of  $\text{M}^{3+}$  on a  $0.1 \mu\text{g ml}^{-1}$  Cr(III) signal.

As can be seen from Table 4.5 the Cr(III) signal is almost completely negated in the presence of  $40 \mu\text{g ml}^{-1}$  Al(III). A similar result is obtained when looking at injections of Fe(III) on the Cr(III) signal and this fact may limit the

FIGURE 4.7 THE OPTIMISATION PROFILE OF [ LUMINOL ] FOR Cr (III) CL.

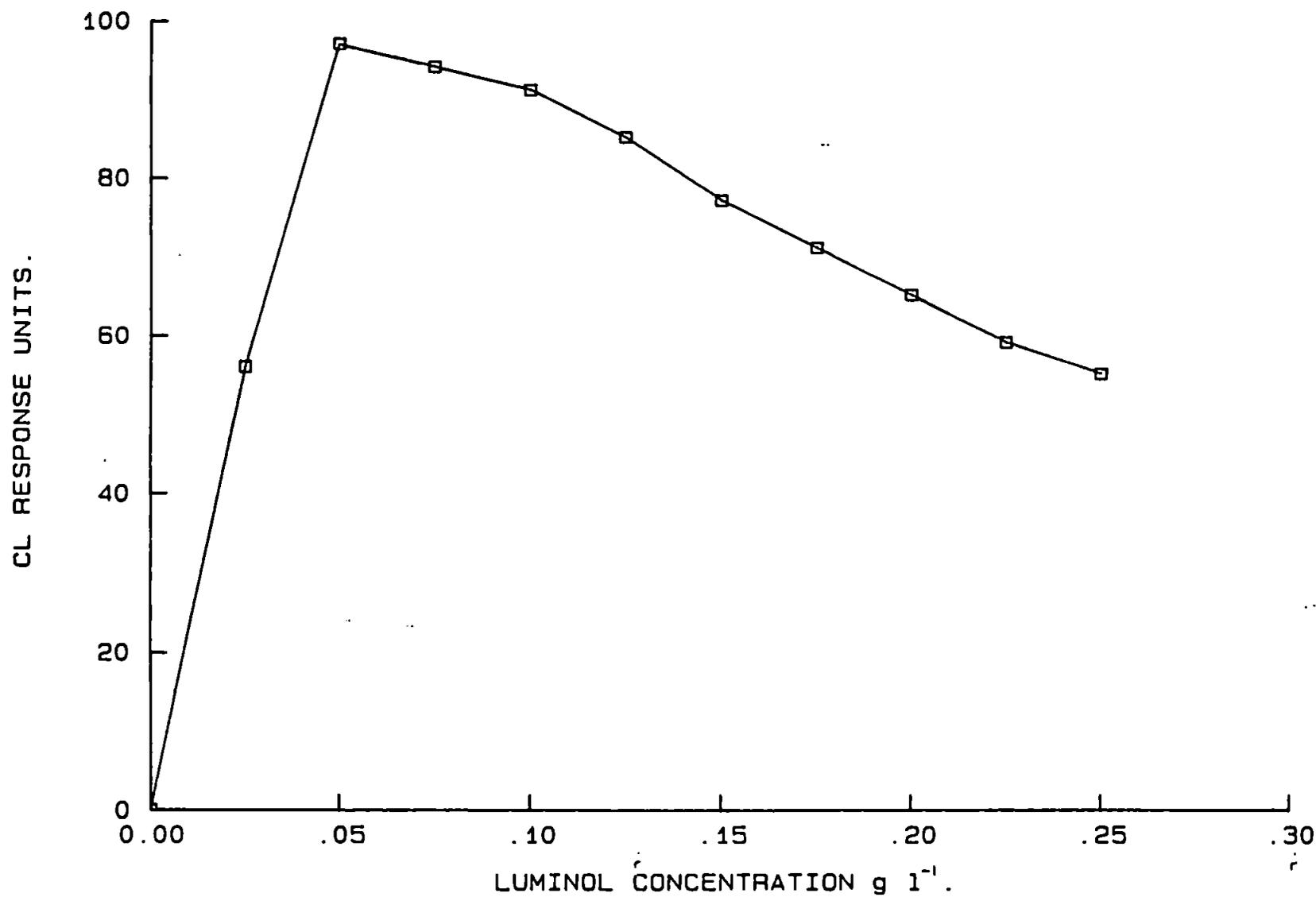


FIGURE 4.8 THE OPTIMISATION PROFILE OF  $[H_2O_2]$  FOR Cr (III) CL.

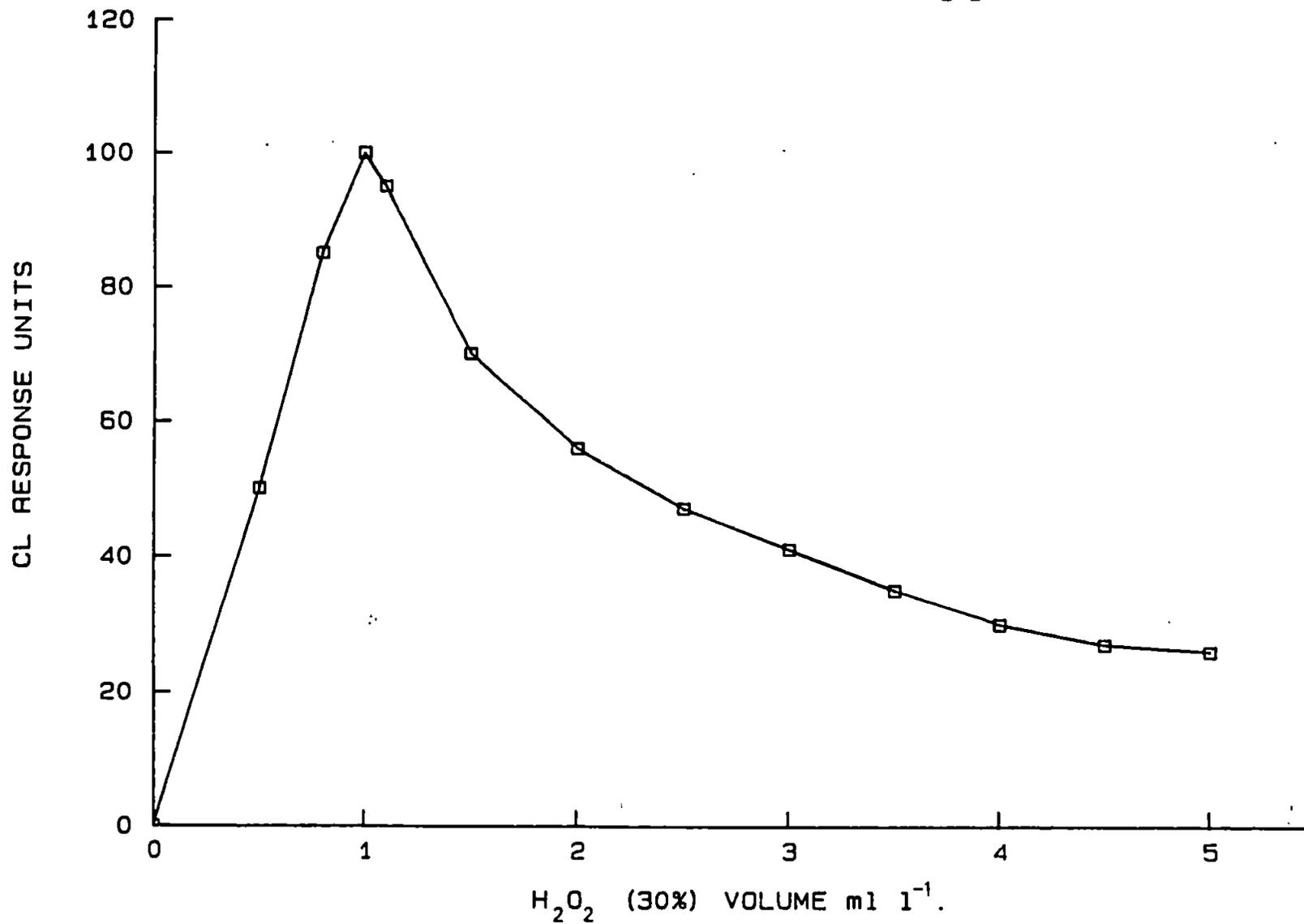
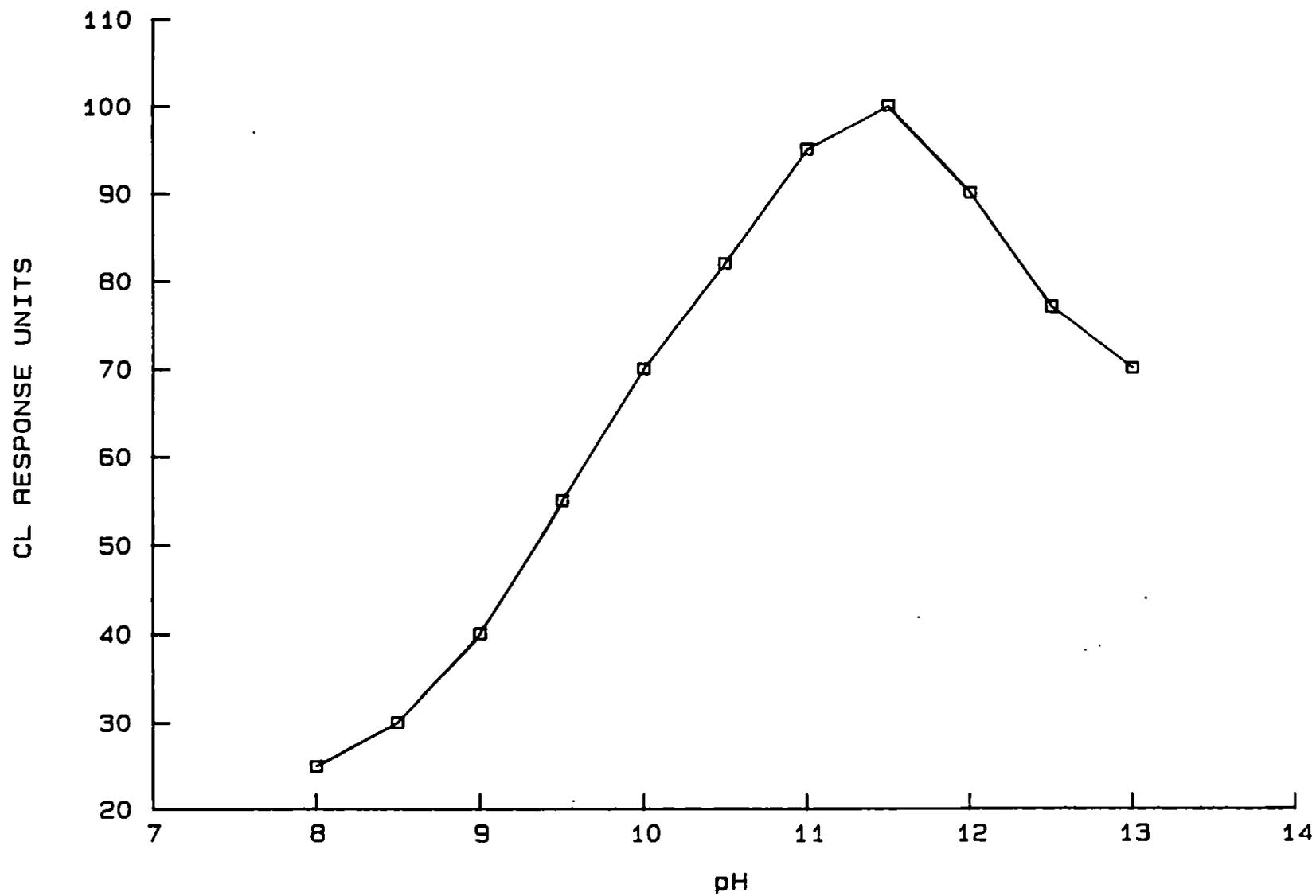


FIGURE 4.9 THE OPTIMISATION PROFILE OF pH FOR Cr (III) CL.



**Table 4.4** The optimised parameters for Cr(III) detection

<u>Parameter</u>	<u>Optimised conditions</u>
Luminol concentration	0.06 g l <sup>-1</sup>
H <sub>2</sub> O <sub>2</sub> (30%) volume	1.0 ml l <sup>-1</sup>
pH	11.5

**Table 4.5** Interference effect of Al(III) on a 0.1 µg ml<sup>-1</sup> Cr(III) signal

<u>0.1 µg ml<sup>-1</sup> Cr<sup>3+</sup> + x µg ml<sup>-1</sup> Al<sup>3+</sup></u>	<u>Peak height mm</u>
x = 0	95
x = 0.1	98
x = 1	97
x = 5	94
x = 10	73
x = 40	0

usefulness of the analytical system when looking at low concentrations of Cr(III) in the presence of high concentrations of Al(III) and Fe(III), both of which are particularly prevalent in many vegetation materials.

Linear calibrations were obtained covering approximately three orders of magnitude from  $0.5 \mu\text{g l}^{-1}$  to  $1.0 \text{mg l}^{-1}$ . Figure 4.10 shows a calibration plot with excellent linearity ( $r = 0.9999$ ). The reproducibility was reasonably good at the  $100 \mu\text{g l}^{-1}$  level where an RSD of 3.3% was found for 12 replicate injections and the detection limit, defined as three times the peak to peak variation of the base-line noise was  $0.1 \text{ng Cr(III)}$  absolute.

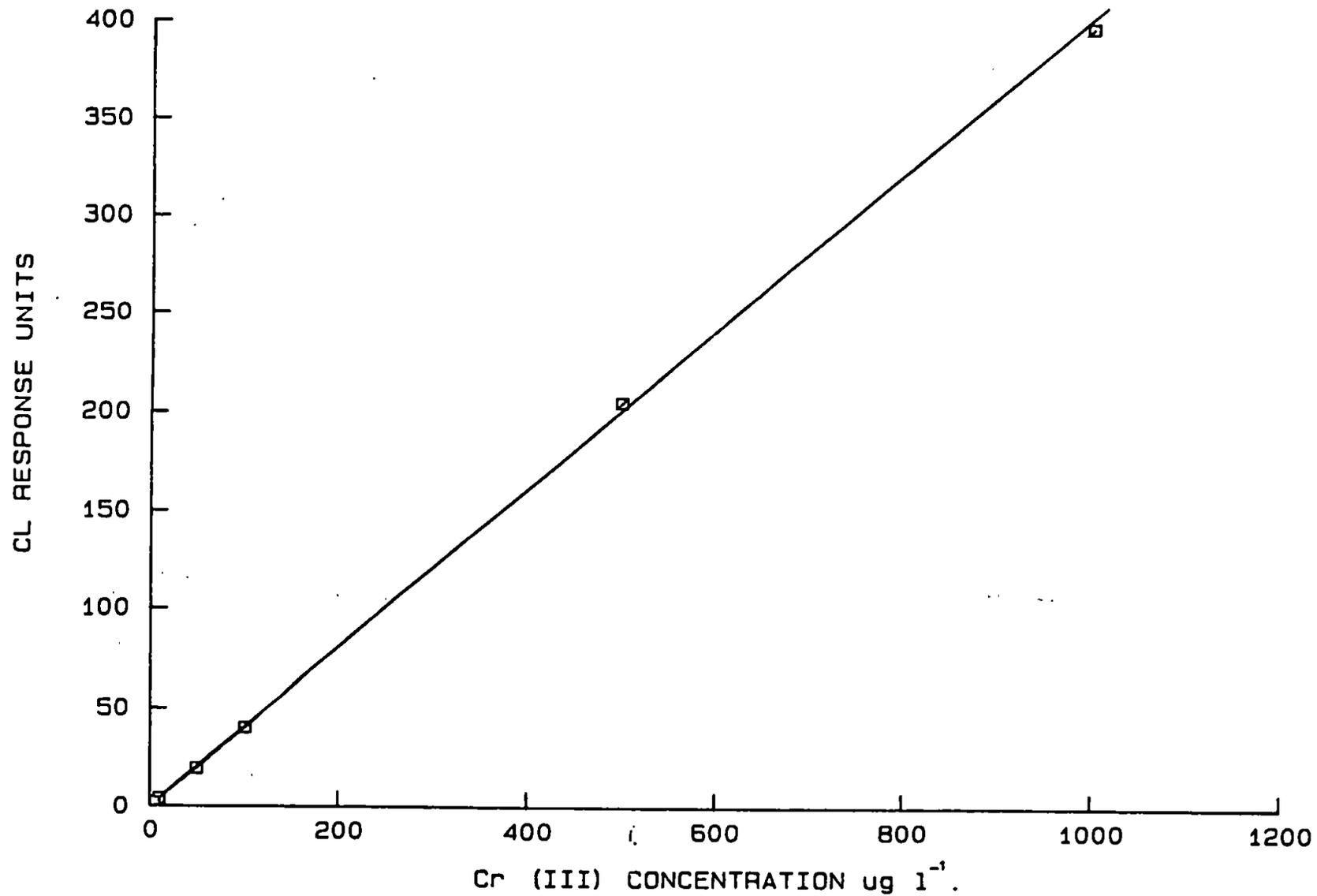
#### 4.4 Experimental for Cr(VI) Determinations

##### 4.4.1 Instrumentation for Cr(VI) Determinations

Once again, the instrumentation required for Cr(VI) determinations was essentially the same as had been previously used in Co(II) and Cr(III) determinations. One major difference, however, was that because Cr(VI) is known not to catalyse the luminol reaction, it must be chemically reduced post-column, to Cr(III), in order to produce any CL. This reduction step can be achieved either by using a solid phase reductor, such as a granular Cd, Zn or Sn metal packed column etc., or by using an homogenous reducing stream, both of which are discussed in more detail later on. A schematic diagram of the apparatus used is shown in Figure 4.11. A high pressure

FIGURE 4.10

CALIBRATION CURVE FOR Cr (III) CL.



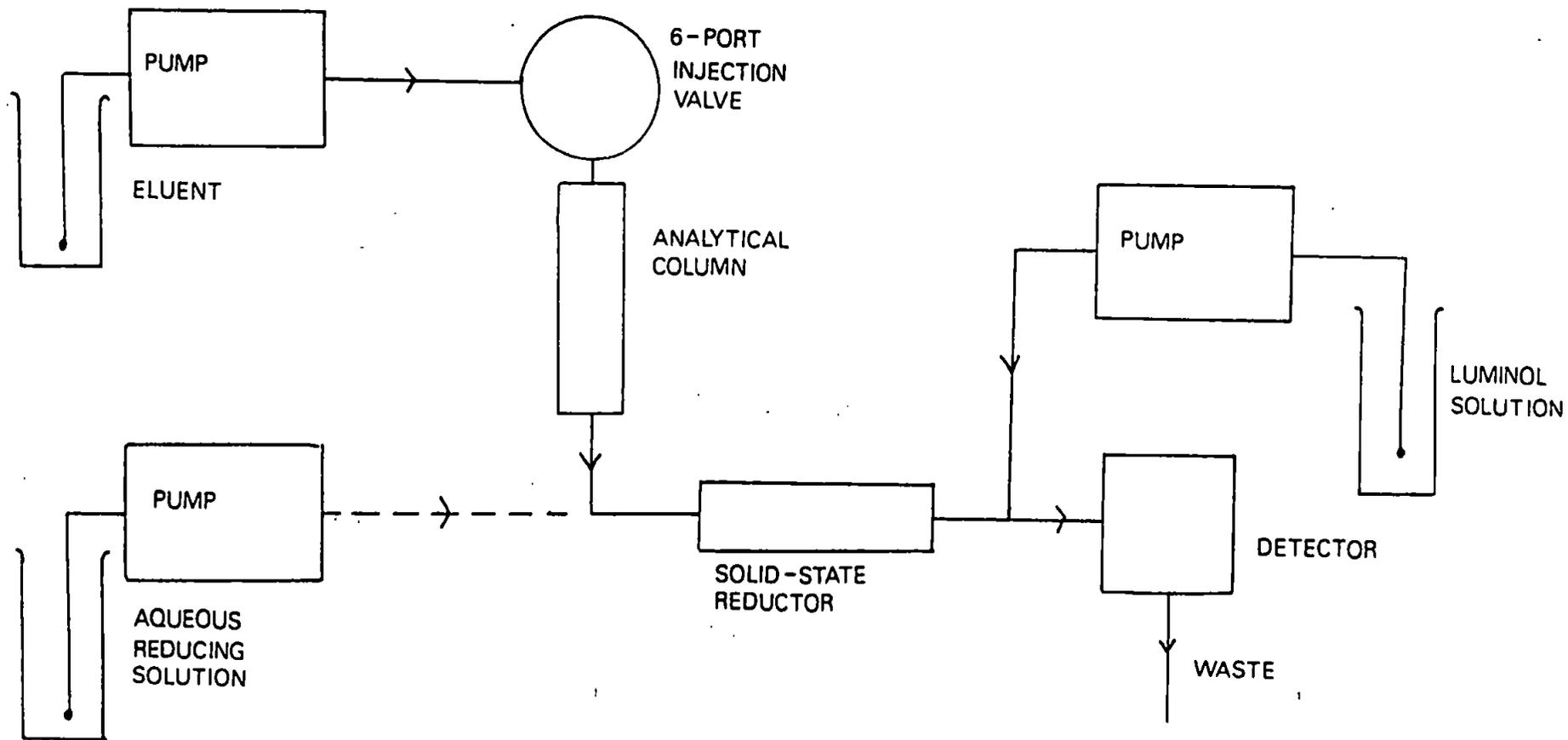


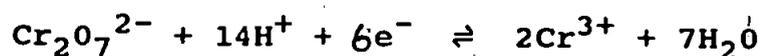
Figure 4.11 A schematic diagram of the apparatus required for sensitive Cr (VI) determinations.

plastic pump (Dionex 4000 gradient pump, Dionex, Sunnyvale, CA, USA) was used to deliver the eluent and a high pressure stainless steel pump (Knauer, Bad Homburg, FRG) was used to deliver the luminol post-column reagent. The Cr(VI) sample was loaded into a 200 µl titanium sample loop and injected onto a short (50 mm x 4.6 mm i.d.) Dionex AG4 column. After elution with aqueous potassium sulphate the sample was reduced by means of either a reduction column set up on-line, or by mixing with an aqueous reducing stream. When using the homogenous reducing stream, an extra pump was required (Model 600A, Waters Assoc., MA, USA). A PTFE open-coiled reaction tube was provided (750 µl 1.5 m x 0.8 mm i.d.) to ensure enough time for complete Cr(VI) reduction. The detection instrumentation was the same as for Cr(III) studies.

#### 4.5

##### 4.5.1 Preliminary Results for Cr(VI)

Having previously established the optimum detection parameters for the CL determination of Cr(III) ions, the main task faced was to develop an efficient means of reducing the Cr(VI) on-line to Cr(III), which could be easily and sensitively determined by the method already described (Sec. 4.2.3) i.e.



It was thought that a solid state reductor, such as the one containing granular cadmium and used to reduce nitrate to nitrite, would be useful for Cr(VI) reduction (125).

Three separate stainless steel columns (10.0 cm x 4.6 mm i.d.) were slurry packed with granular zinc, cadmium and tin metal respectively and each one tried out on-line, between the analytical column and the detector. All three metals successfully reduced the Cr(VI) to Cr(III) and a CL response was picked up by the detector. A typical response is shown in Figure 4.12, this particular example using granular tin metal as the reducing agent. The peak doublet shown in Figure 4.12 was experienced for all three columns tested and is presumably due to unsatisfactory nature of the metal packing materials. The dead volume produced by these reduction columns was relatively high (0.5 - 0.8 ml) and this was a direct result of the large metal particles present, typically 0.3 - 1.5 mm in diameter. The base-line noise was also rather high and unsatisfactory and this was presumably due to the increased contamination introduced by both the column packing material and the stainless steel columns and fittings that were used. These troublesome reduction columns were abandoned in favour of an homogenous aqueous reduction system which showed more promise.

After Cr(VI) elution from the analytical column, the effluent was mixed, with the aid of a PTFE T-piece with a variety of different homogenous reducing systems. Initial work with homogenous reducing solutions was carried out in the test tube to see the feasibility of each reducing agent. The reducing agents were mixed with a 0.1 M  $K_2Cr_2O_7$  solution and any colour change was noted (i.e. for

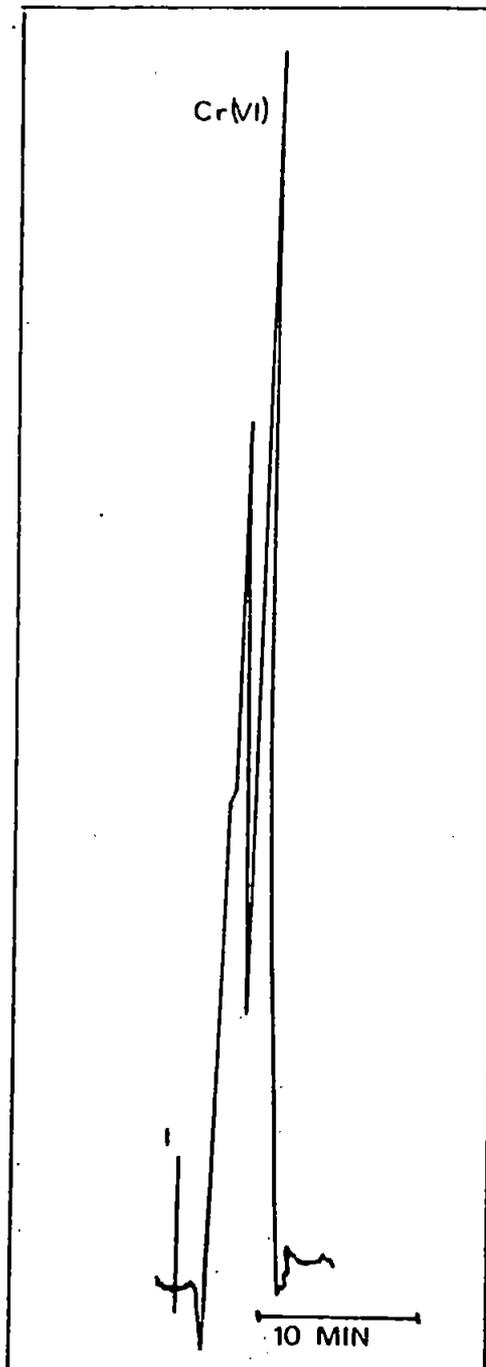


Figure 4.12 A typical peak for Cr (VI) obtained when using a solid state reducing column. (10 ppm analyte concentration).

successful reduction the orange Cr(VI) solution will change colour to a green Cr(III) solution.

A list of reducing agents and reducing performance is given in Table 4.6. This potassium sulphite solution was allowed to mix in a reaction coil with the Cr(VI) analyte where reduction occurred. Having been converted from Cr(VI) to Cr(III), the Cr(III) was able to catalyse the luminol/peroxide CL reaction as demonstrated previously (sec. 4.2.3).

#### 4.5.2 Detector Performance for Cr(VI) Determinations

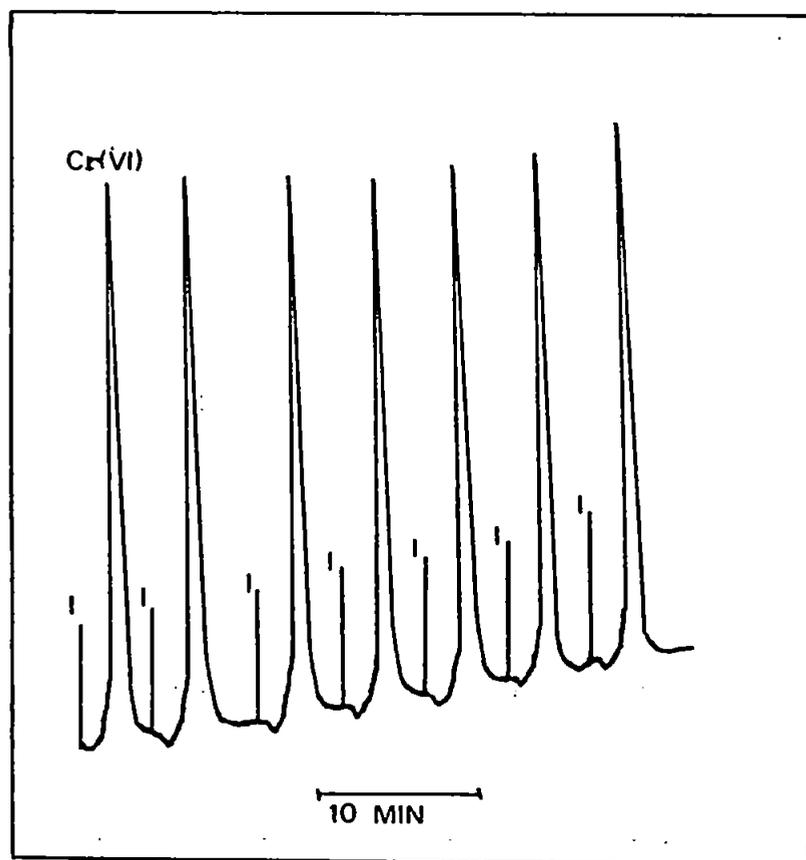
Figure 4.13 shows a typical response for the injection of 100 ppb Cr(VI) standard. The optimal conditions for both separation and detection for Cr(VI), are described in Table 4.7.

The most successful reducing agents, on the test-tube scale, were the arsenite and the sulphite solutions. The organic reagents were tried because very pure compounds could be obtained, thus decreasing contamination problems. These organic reagents, however, were found to be unsuitable. The potassium sulphite solution was eventually chosen as the homogenous reducing agent because (a) reduction of Cr(VI) to Cr(III) takes place rapidly (b) it avoids the use of toxic arsenite solutions and (c) the reducing solution is very compatible with the potassium sulphate mobile phase.

**Table 4.6** The reducing agent used to reduce Cr(VI) ions to Cr(III) ions.

Reducing agent	Successful Reduction (orange to green)	Comments
1) 0.1 M ethanol solution	✓	The colour change was not immediate and took several minutes to be complete
2) 0.1 M hydroxylamine	X	N <sub>2</sub> gas evolved but very little colour change
3) 0.1 M acetaldehyde	X	No reduction observed
4) 0.1 M potassium iodide	✓	The iodide reduce the Cr(VI) ions although I <sub>2</sub> was produced which resulted in a cloudy, dark solution
5) 0.1 M potassium arsenite	✓	Reduction took place rapidly. Only GPR grade available
6) 0.1 M potassium sulphite	✓	Reduction took place rapidly. Only GPR grade available

Figure 4.13 Typical responses for the repeat injection of 100 ppb Cr (VI) standards.



**Table 4.7** The conditions required for Cr(VI) determinations.

Separation

Stationary phase	Dionex HPIC AG4 (50 mm x 4.6 mm i.d.)
Mobile phase	potassium sulphate 0.085 M
pH	3.0
Flow rate	0.8 ml min <sup>-1</sup>
Retention time	8.0 min

Reduction of Cr(VI)

Reducing agent	potassium sulphite 0.015 M
pH	3.0
Flow rate	0.8 ml min <sup>-1</sup>

Detection

Luminol concentration	0.06 g l <sup>-1</sup>
H <sub>2</sub> O <sub>2</sub> (30%) volume	1.0 ml l <sup>-1</sup>
Boric acid concentration	6.0 g l <sup>-1</sup>
pH	11.5
Flow rate	0.8 ml min <sup>-1</sup>

N.B. Outlet pH of the mixture of potassium sulphate, potassium sulphite and luminol solution was 10.3

An interference trial was carried out on the Cr(VI) signal to see if there was any overlap from the presence of other anions which may lead to either suppression, or enhancement of the signal. Phosphate, vanadate and molybdate were all tested and found not to interfere even at relatively high concentrations (10  $\mu\text{g ml}^{-1}$  anion co-injected with 0.1  $\mu\text{g ml}^{-1}$  chromate).

#### 4.6 The Simultaneous Determination of Cr(III) and Cr(VI)

Two separate chromatographic systems have now been developed. One system is involved in Cr(III) determinations, whilst the other concerns itself with Cr(VI) determinations only. The obvious aim now, is to couple these two systems in an attempt to produce a single analytical procedure that would be capable of the simultaneous determination of both Cr(III) and Cr(VI).

##### 4.6.1 The Instrumentation Required for Simultaneous Cr(III) and Cr(VI) Determinations

Due to mobile phase incompatibilities anion and cation exchange columns could not be set up in series and so instead a system was designed so that the columns ran in parallel as shown in Figure 4.14.

The system now required the use of four high-pressure pumps, although later studies indicated that the potassium sulphite reducing solution and the luminol/peroxide post-column reagent could both be delivered using a single dual

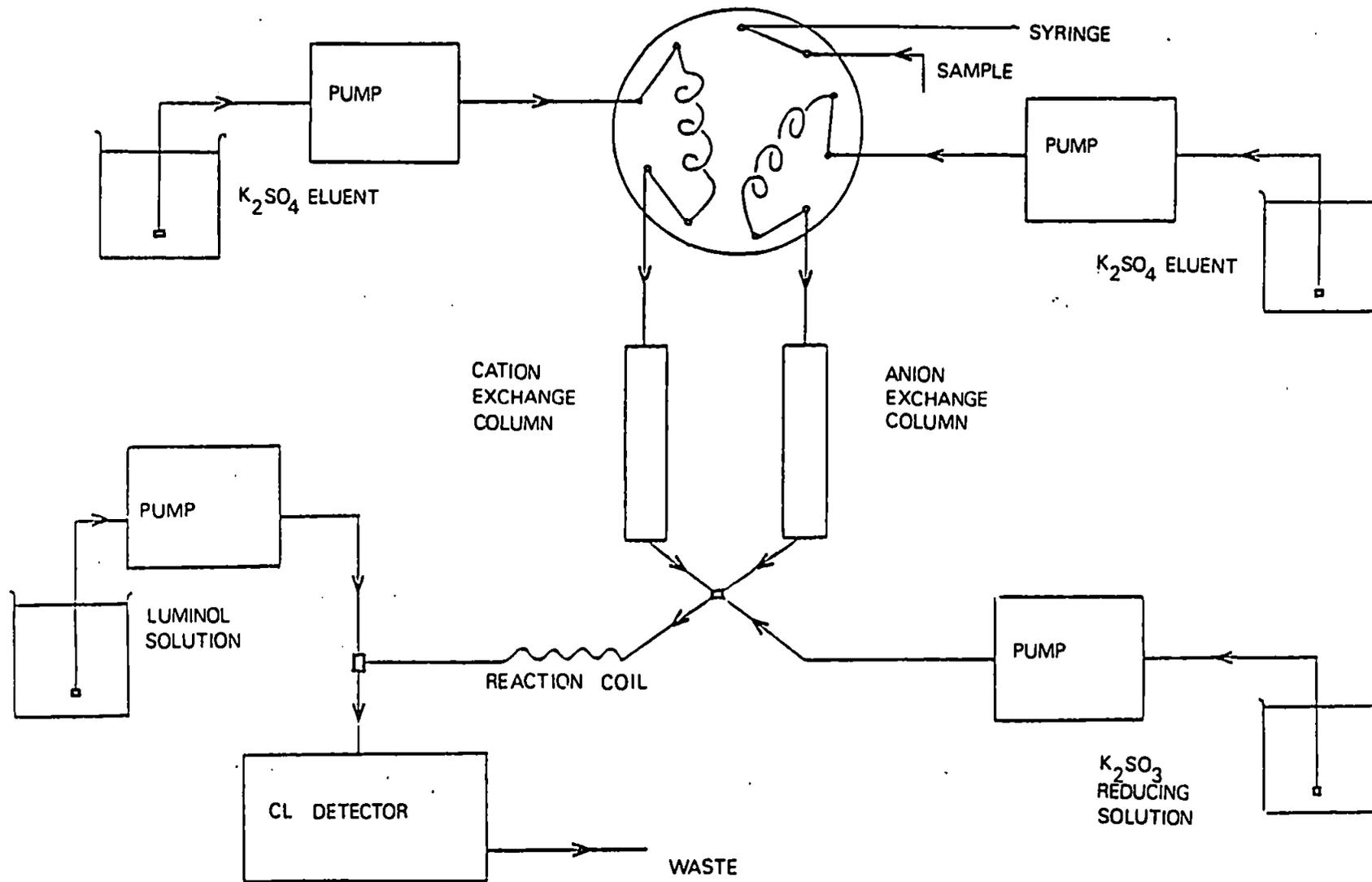


Figure 4.14 A schematic diagram of the apparatus required for the simultaneous determination of Cr (III) and Cr (VI) ions.

channel peristaltic pump, thus simplifying the instrumentation quite considerably. (Although worsening of limits of detection by an approximate factor of 2). All operating parameters remained the same, and a 10 port zirconium injection valve (Valco, Sweden) with two sample loops in the system was set up to load two samples from the same standard solution. Figure 4.15 shows the configuration of the 10 port injection valve in (a) the load and (b) the inject positions. When switched to inject, one sample was injected onto the cation-exchange column whilst the other was injected onto the anion-exchange column. Figure 4.16 shows a typical trace for this simultaneous determination, both analytes being  $150 \mu\text{g l}^{-1}$  in the standard. Sample injection volume on the cation-exchange was  $200 \mu\text{l}$  whilst for the anion exchange it was  $100 \mu\text{l}$ . For the sample that is injected onto the cation-exchange column any Cr(VI) that is present would have no attraction for the column and would consequently elute on the solvent front. Similarly, any Cr(III) that is present in the sample that was injected onto the anion-exchange column would also elute on the solvent front. It can be seen from Figure 4.16 that these two solvent fronts combine to produce a large response at the beginning of the chromatogram.

The conditions were optimised so that Cr(VI) eluted from the cation-exchange column after 5 minutes whilst the Cr(III) was retained on the cation exchange column for over 10 minutes ensuring complete separation of the two

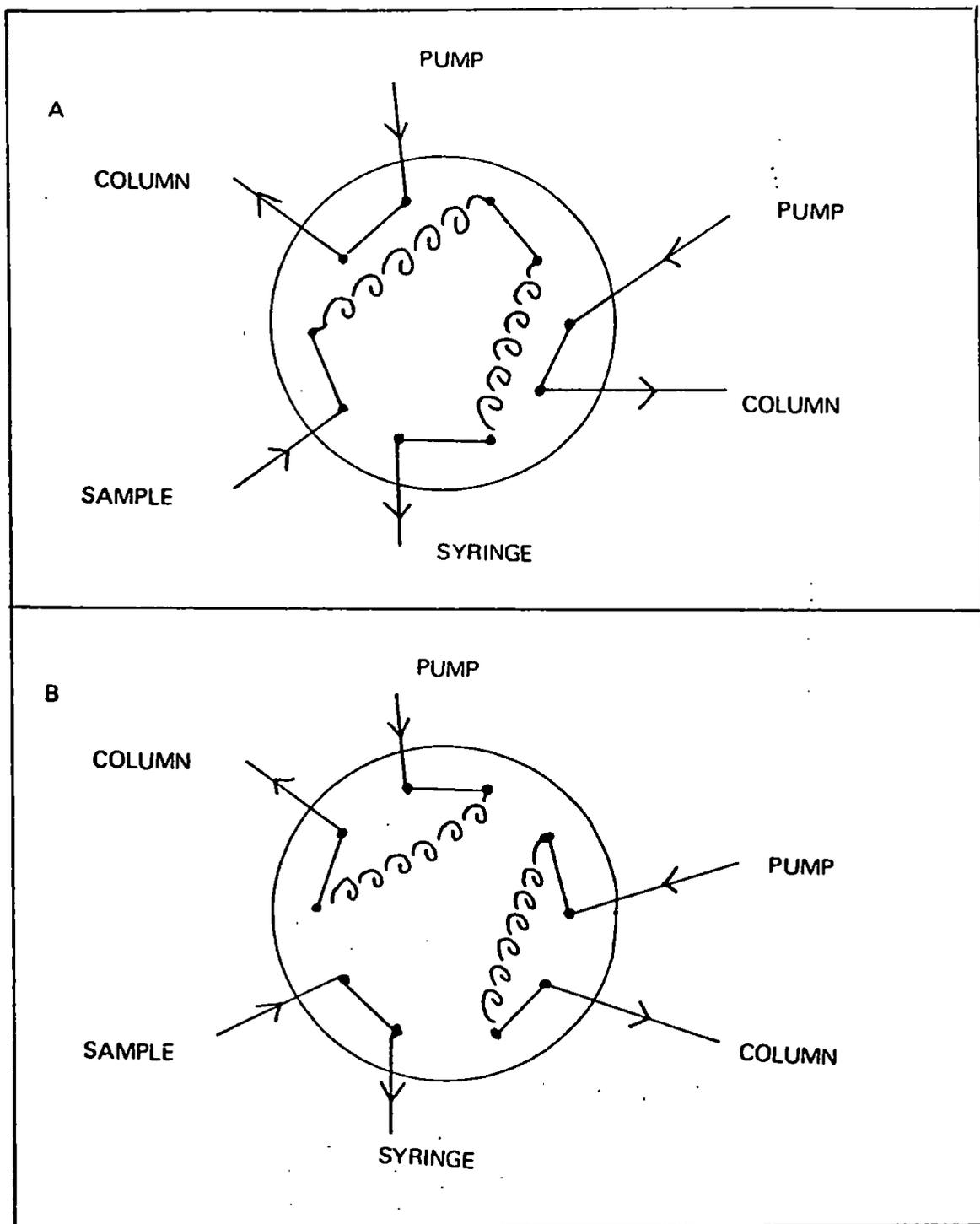


Figure 4.15 A schematic diagram of the operation of a 10-port injection valve; in A. the load and B. the inject positions.

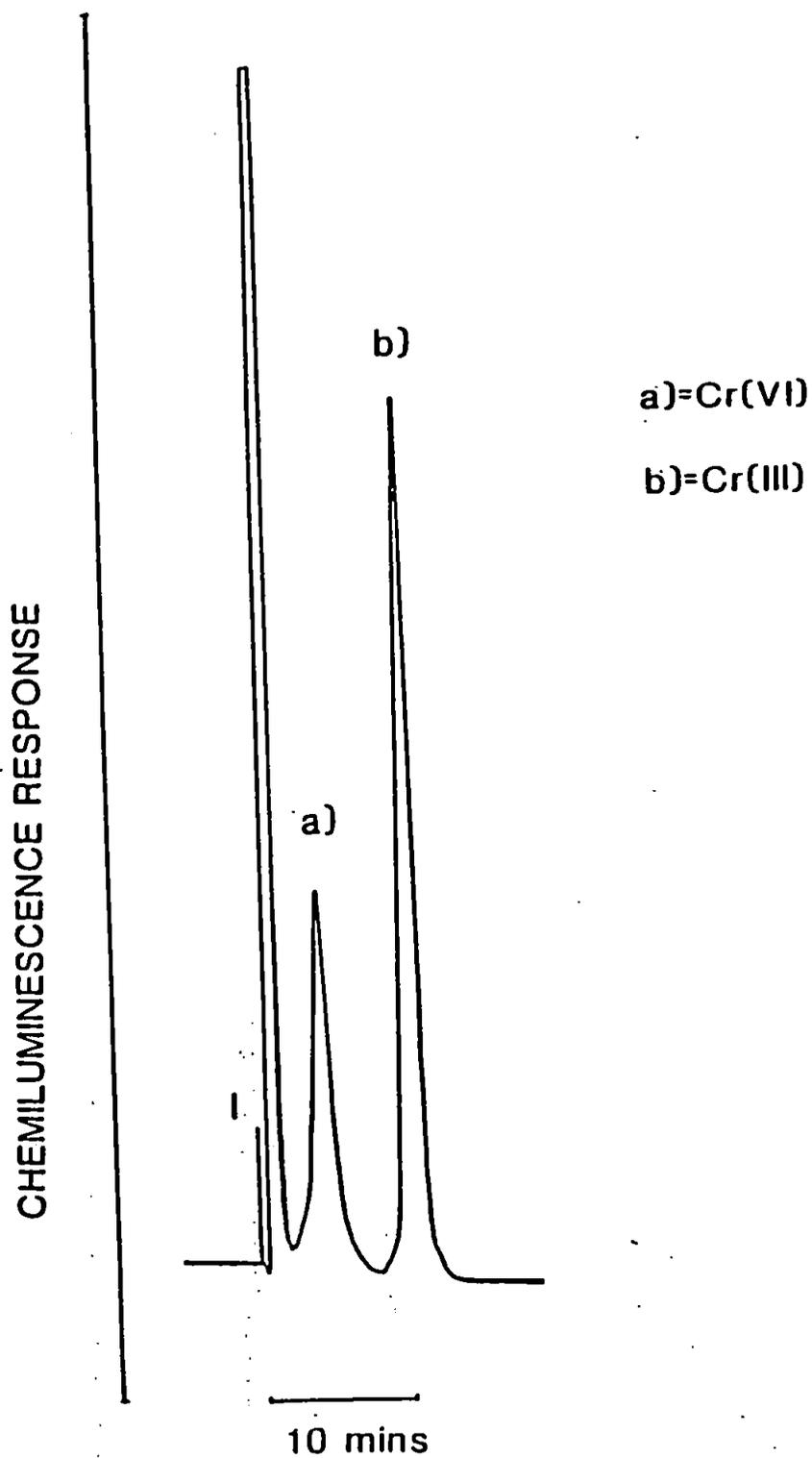


Figure 4.16 A typical trace for the simultaneous determination of Cr (III) (150 ppb) and Cr (VI) (150 ppb) using CL detection.

species. Separation conditions can easily be changed, if desired, for the Cr(III) ions to elute first, with Cr(VI) being the species retained longer on the column. Figure 4.17 shows a simultaneous calibration of the 2 species at the lower end of the linear range and the slight difference in the angle of the slope may be due to the measurement of peak height rather than area.

A linear range of at least three orders of magnitude was established ( $1 \mu\text{g l}^{-1}$  to  $1 \text{mg l}^{-1}$ ) although the absolute upper limit was not determined. The calibration plot for this data showed good linearity ( $r = 0.999$ ). The reproducibility was good at the  $150 \mu\text{g l}^{-1}$  level where a relative standard deviation of 2.1% was obtained for seven replicate trials.

The detection limit (three times the peak to peak variation of the baseline noise) was found to be 0.3 ng absolute for both chromium species.

#### 4.6.2 Sample Analysis

To evaluate the qualitative performance and accuracy of the system, a sample of low Cr(III) content was chosen. Unfortunately no certified reference material with both Cr(III) and Cr(VI) could be found, which perhaps illustrates the difficulty experienced in Cr(III)/Cr(VI) analysis. A simulated fresh water sample was chosen (IAEA/W4) with a Cr(III) content of  $9.9 \text{ ng ml}^{-1}$ . The results of the analysis are described in Table 4.8 and Fig. 4.18.

**Table 4.8** The determination of Cr(III) from a certified simulated fresh water IAEA/W4

Certificate value Cr(III)	9.9 ng l <sup>-1</sup>
Confidence level	9.0 - 10.5 ng l <sup>-1</sup>
Found value (x on 7 trials)	9.9 ng l <sup>-1</sup>
RSD on 7 trials (%)	3.3

See Figure 4.18

Figure 4.17 The simultaneous calibration of the 2 chromium species at the lower end of the linear range.

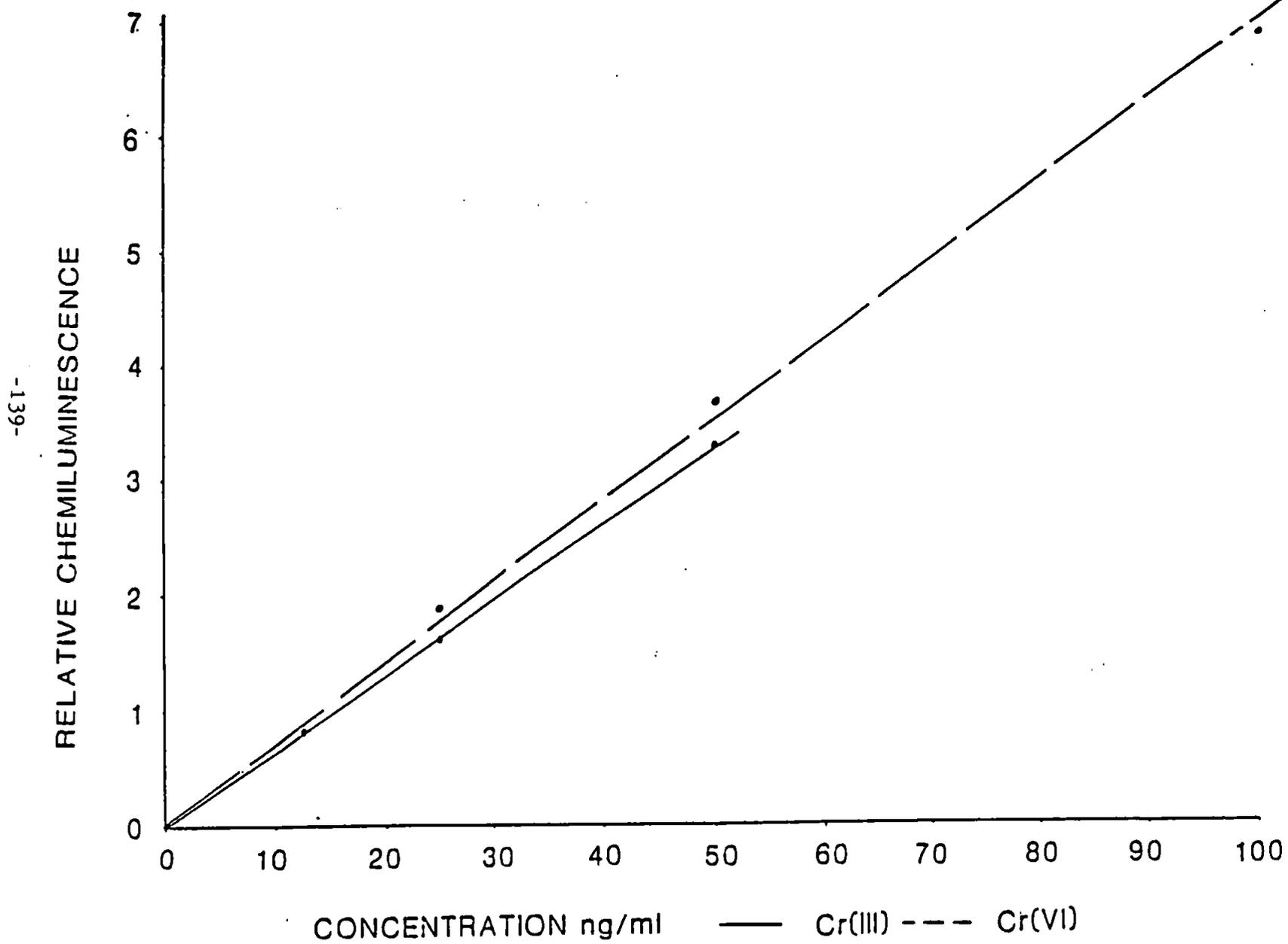
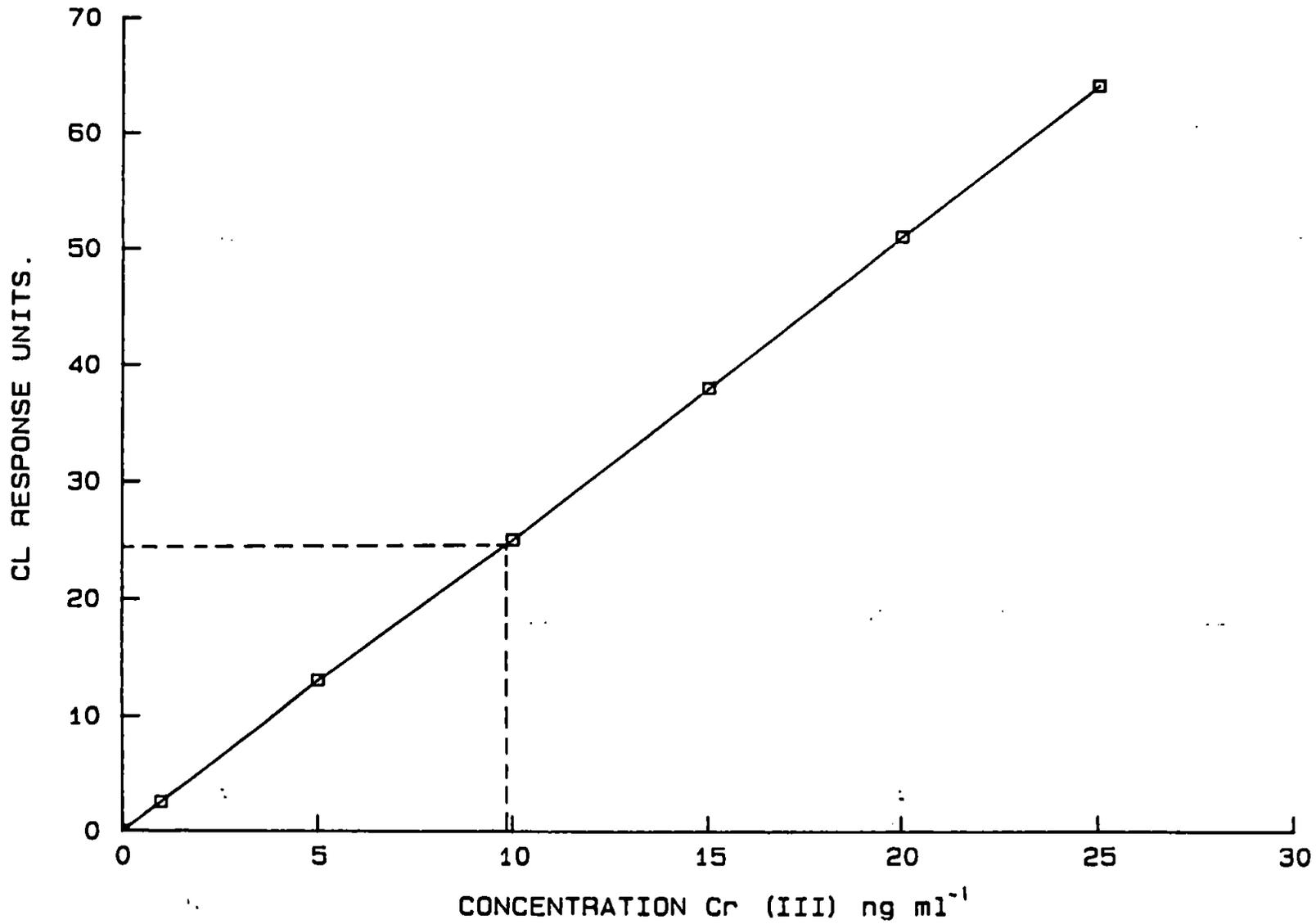


FIGURE 4.18 Cr (III) DETERMINATION FROM A CERTIFIED MATERIAL IAEA/W4.

-041-



#### 4.7 Overall Discussion and Conclusions

The sensitive, simultaneous determination of the two most abundant chromium species without any sample pretreatment has been achieved and outlined in this chapter. Once again, CL has been used as the means of detection which shows that very sensitive detection can be obtained for metal CL catalysts as has been described previously in Chapter 3 for Co(II) analysis.

The detection limits for Cr(III) and Cr(VI) are approximately the same as those achieved by graphite-furnace atomic emission spectroscopy and even better sensitivity would be experienced if an improved reduction step was developed.

The near absence of most interferences and the successful determination of Cr(III) in a certified reference material combined, with the excellent sensitivity and linearity of the system may possibly make it an attractive method for chromium analysis in real samples, although, as previously mentioned, high levels of iron(III) and aluminium (III) may cause some problems particularly from vegetation samples.

It is apparent that the detection conditions required for sensitive Co(II) detection vary, quite considerably, from those required for Cr(III) studies. This highly selective nature of such CL reactions may be advantageous for particular applications such as the monitoring of Co(II)

from the coolant of a PWR. For routine monitoring of industrial feeds, effluents and water samples, however, the ion-chromatographic procedure allows separation of several metal analytes but sensitive CL detection would only be possible for 1 or 2 of these. Thus, there is an obvious need for further development studies for multielement CL detection techniques and the following chapter addresses the problem of multielement analysis using CL as a means of detection

## CHAPTER 5

### MULTIELEMENT DETERMINATIONS WITH CL DETECTION

#### 5.1 Introduction

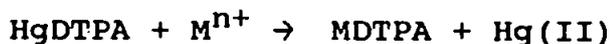
As has previously been mentioned, due to the selective nature of most CL reactions, multielement determinations are difficult to achieve as detection parameters can vary quite considerably from element to element. Multielement determinations have been achieved using CL detection, but only by using compromise conditions, thus adversely affecting the limits of detection (90, 109). One way to solve this selectivity problem is to use post-column reaction chemistry to produce a species that is more amenable to CL detection. A similar problem involving molecular absorption detectors has been solved to some extent using EDTA displacement reaction in the post-column system. Arguello and Fritz (74) used a PAR-Zn(II)/(EDTA) colorimetric reagent for determining metals that do not directly react with the PAR reagent, such as the alkaline-earths. Equal molar concentrations of PAR and Zn-EDTA were mixed together and buffered at pH 9.0. The reaction taking place in the post-column reagent on elution of a metal ion M(II) is as follows:



The Zn-PAR complex absorbs strongly in the visible region at 495 nm and the detector does not have to be set at a compromise wavelength to detect a number of metal species.

Improvements in sensitivity (2 - 10 fold) were obtained compared with other colorimetric techniques.

A similar post-column reagent system was described as early as 1973 by Takata and Arikawa (126). This paper described a system for electrochemical detection that was also based on a displacement type reaction, but this time, with coulombic detection, after the following exchange reaction;



DTPA = diethylenetriaminepentaacetate

The liberated Hg(II) ions were measured by their reduction in a coulombic detector. This system was applied to the following metal ions: Ag(I), Au(III), Bi(III), Cd(II), Co(II), Fe(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), Zn(II) and ions of alkaline-earth metals and rare earth metals, making it an almost universal type metal detection system.

It was decided to use a similar displacement approach to try and develop a universal detection system based on CL as a means of sensitive determination for a variety of metal species including those that are completely non CL active.

#### 5.1.2 The Displacement Reaction

Two main considerations involved in the decision to choose a suitable displacement reaction system are:

- (a) the correct choice of complexing agent.
- (b) the correct choice of metal suitable for displacement.

Both (a) and (b) are interdependent and the choice is vital if there is to be a successful development of the displacement system. The most obvious chelating agent to use was EDTA as it forms simple 1:1 complexes with most metals. It has been used in the past for displacement reactions and there is a wealth of information concerning metal-EDTA conditional stability constants reported in the literature. The choice of metal, however, was not so obvious.

The metal involved in the complex should have the following properties;

- (1) should be able to act as a catalyst in the luminol/peroxide CL reaction.
- (2) should have a favourable conditional stability constant to allow sufficient exchange to produce enough free metal to give a CL signal.
- (3) should not be easily oxidisable e.g. presence of air should not oxidise a  $M^{2+}$  to a  $M^{3+}$  complex which would be much more stable.
- (4) should be easily titratable against the EDTA to produce accurate 1:1 molar ratios.
- (5) should be able to rapidly exchange with another metal (i.e. not be constrained by kinetic factors).

Some of the metals that are catalysts in the luminol/peroxide reaction are Ag(I), Mn(II), Cu(II), Fe(II) and Co(II). Ag(I) and Fe(II), however, are unsuitable to act in a displacement reaction because the Ag(I) ions are not easily titrated against EDTA and Fe(II) ions will easily oxidise to the Fe(III) species, which is extremely stable and itself is non CL and, in any case, displacement by most other metal species, after formation of Fe(III)-EDTA, would be unlikely to occur.

Of those metals mentioned in Table 5.1, Cu(II), Mn(II) and Co(II) would seem the most appropriate metals for use in a CL displacement reaction as each potentially fulfils the necessary requirements.

Preliminary benchwork experiments were designed to determine the viability of either Cu(II), Mn(II) or Co(II) as the displacement metal and, whilst all three metals showed promising potential, as soon as lactic acid was introduced to the mixture, (as would be the case with the ion-chromatographic system) severe quenching resulted for the Cu(II) and Mn(II) CL. It is also true to say that the CL signal produced by Co(II), even in the presence of lactic acid, was very pronounced even to the naked eye. If the Mn(II) ions were not so suppressed by the presence of lactate, then they perhaps would have been the best choice to act in the metal-complex displacement reaction. This is because Mn-EDTA has a relatively low log stability constant,  $\log K_{stab.}$ , than many other metal EDTA

Table 5.1 Logarithmic conditional stability constants of metal-EDTA complexes.

pH	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Metal															
Ag(I)					0.7	1.7	2.8	3.9	4.9	5.9	6.8	7.1	6.8	5.2	2.5
Al(III)		0.2	3.0	5.4	7.5	9.6	10.4	8.5	6.6	4.5	2.4				
Ba(II)						1.3	3.0	4.4	5.5	6.4	7.3	7.1	7.8	7.6	7.0
Ca(II)				0.3	2.2	4.1	5.9	7.3	8.4	9.3	10.2	10.6	10.6	10.2	9.4
Cd(II)		0.1	3.3	6.0	7.9	9.9	11.7	13.1	14.1	15.1	15.5	14.5	11.9	8.4	4.5
Co(II)		0.1	3.7	5.9	7.8	9.7	11.5	12.9	13.9	14.6	14.7	14.1	12.1		
Cu(II)	0.4	3.4	6.1	8.3	10.2	12.2	14.0	15.4	16.2	16.4	16.3	15.8	15.4	15.3	15.3
Fe(II)			1.5	3.7	5.7	7.7	9.5	10.9	12.0	12.9	13.8	14.2	14.2	13.7	12.8
Fe(III)	5.1	8.2	11.5	13.9	14.7	14.8	14.6	14.1	13.7	13.7	14.0				
Hg(II)	3.5	6.5	9.2	11.1	11.5	11.5	11.3	10.7	9.8	9.0	8.6	7.2	7.0	6.0	5.0
La(III)			0.8	3.5	5.7	7.7	9.4	10.8	11.8	12.8	13.6	13.8	13.9	13.9	13.9
Mg(II)					0.4	2.1	3.9	5.3	6.4	7.3	8.2	8.5	8.0	7.1	
Mn(II)			1.4	3.6	5.5	7.4	9.2	10.6	11.7	12.6	13.4	13.4	12.6	11.6	10.6
Ni(II)	0.4	3.4	6.1	8.2	10.1	12.0	13.8	15.2	16.2	17.1	17.4	16.9			
Pb(II)		2.4	5.2	7.4	9.4	11.4	13.2	14.5	15.2	15.4	14.9	13.3	10.7	7.7	4.7
Sr(II)					0.3	2.0	3.8	5.2	6.3	7.2	8.1	8.5	8.6	8.4	7.7
Zn(II)			0.8	3.5	5.7	7.7	9.4	10.8	11.8	12.8	13.6	13.8	13.9	13.9	13.9

complexes. For example, as can be seen from Table 5.1, at a given pH e.g. 7.0, the conditional  $\log. K_{stab.}$  order for metal-EDTA is Ag < Ba < Sr, Mg < Ca < La, Mn, Hg, Fe(II) < Cd, Co, Zn < Pb < Cu, Ni.

From the above  $\log. K_{stabs}$  order, Co forms rather strong complexes with EDTA and therefore exchange with other metals of lower  $\log. K_{stab}$  would appear to be unfavourable. Nevertheless, because of the extreme sensitivity of the Co CL reaction it was considered worth a more detailed investigation.

#### 5.2.1 Instrumentation

A three pump system was now required to deliver (a) the eluent, (b) the metal-complex displacement solution and finally (c) the luminol post-column reagent. Throughout the course of the study, slight modifications were introduced in an attempt to improve the detector performance and these will be described as and when they occur. The instrumentation used was essentially the same as that used for Cr(VI) determinations and is shown in a schematic diagram in Figure 5.1. For the pump manufacturers and other instrumentational details, refer to Section 4.2.8. One modification that is worthy of note is that the flow cell used was taken from an absorbance instrument, and whilst it was useful for initial qualitative studies, linearity problems were found with quantitative results. The flow cell that was initially used had a very small internal volume of 20  $\mu$ l; and is

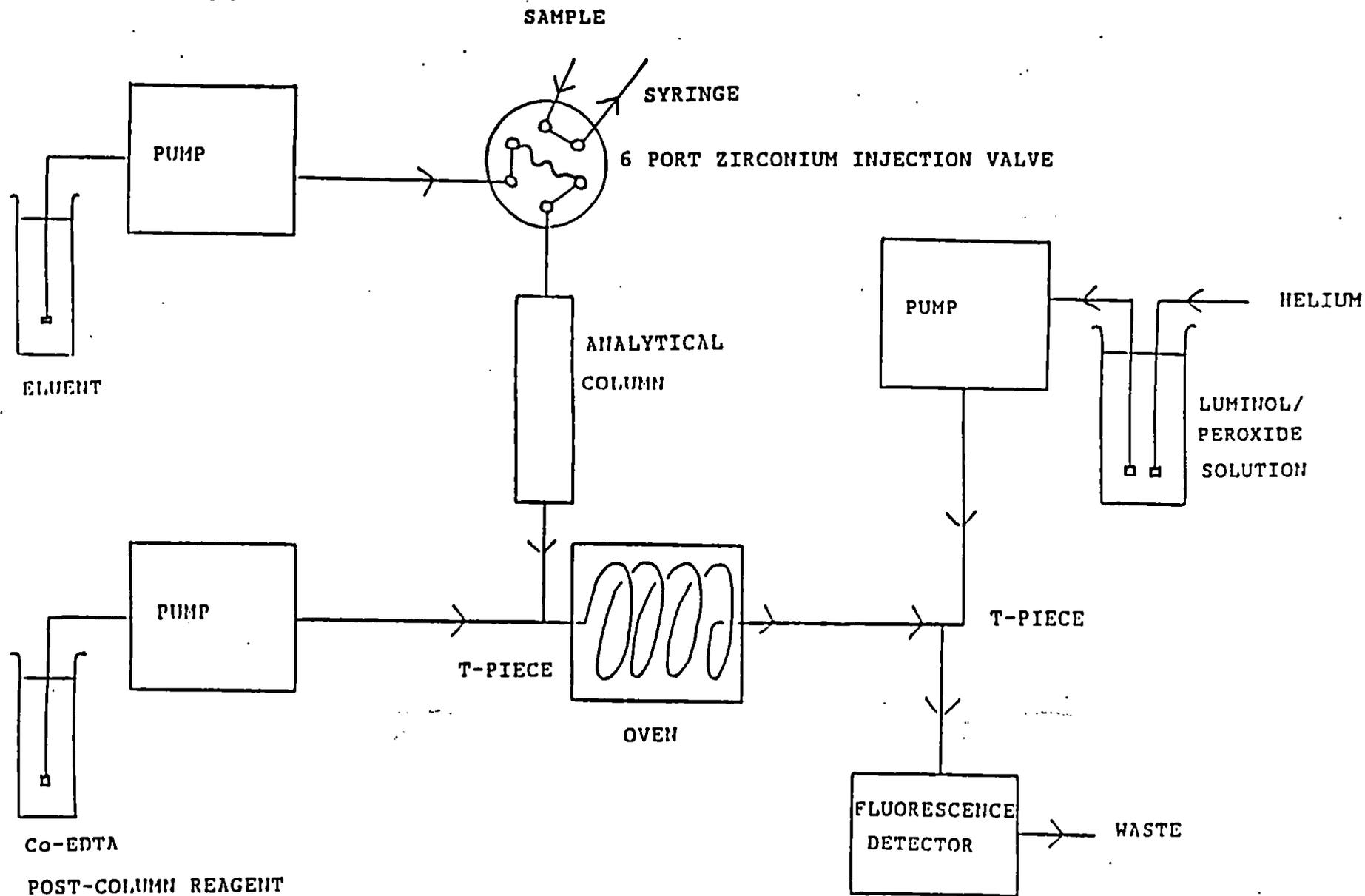
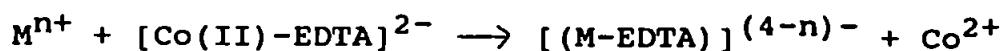


Figure 5.1 A schematic diagram of the apparatus required for multi-element analysis with CL detection.

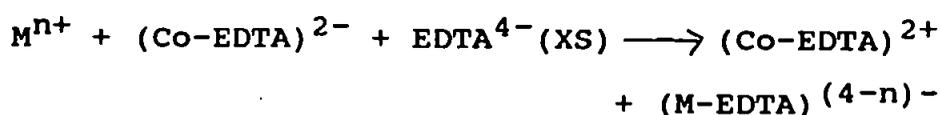
shown in Figure 5.2.

### 5.2.2 The Co-EDTA solution

The concept of the displacement reaction is a simple one:



It should be apparent that an accurate 1:1 molar ratio of Co:EDTA is required if the displacement system is to work successfully. If, for example, an excess of EDTA were to prevail, then there may not be any Co displacement i.e.



If, on the other hand, an excess of  $Co^{2+}$  was present then this would lead to extremely high background levels and overloading of the detector. (There only needs to be approximately 10 ppb free Co in the system for overload to occur). There is an obvious need, therefore, for an accurate method to produce this 1:1 molar ratio, and a titration method would seem the most appropriate.

A stock solution of  $2 \times 10^{-3}M$  cobalt was made up by dissolving 0.56 g of AnalaR cobalt (II) sulphate in a litre of distilled water. A similar strength solution  $2 \times 10^{-3}M$ , of EDTA was also made up by dissolving 0.744 g of the disodium salt also in a litre of water. When required, approximately 25 ml of  $2 \times 10^{-3}M$  EDTA solution

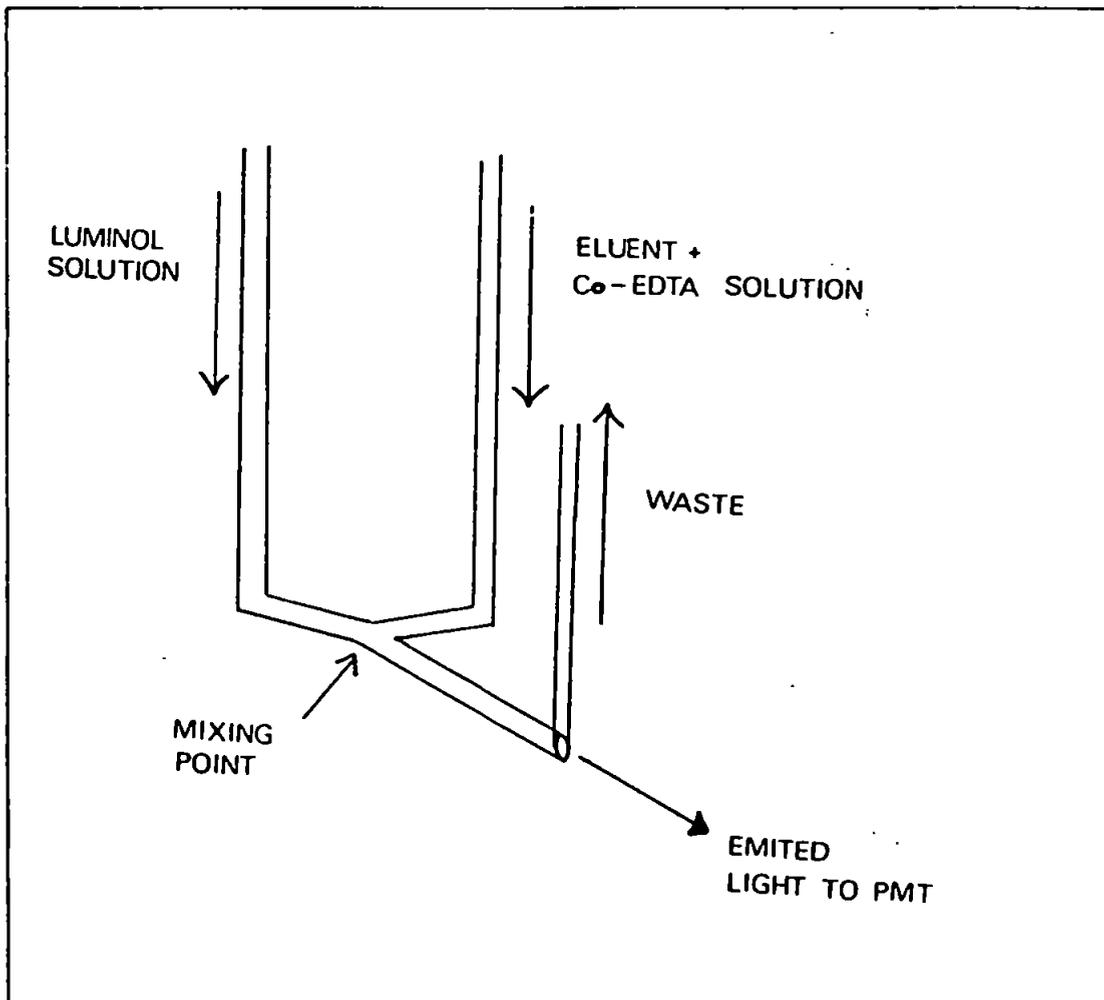


Figure 5.2 A schematic diagram of the original reaction coil and flow cell that was employed for preliminary studies, (internal volume 20  $\mu$ l).

was taken, a small amount of Murexide dye was added along with the minimum amount of dilute ammonium solution required to raise the pH to 9.0. This solution was then titrated against the standard  $2 \times 10^{-3} \text{M}$   $\text{CoSO}_4$  until the end-point was reached. (violet - orange/yellow). When the end point was reached, the addition of either one drop of EDTA, or one drop of  $\text{CoSO}_4$  would lead to a slight excess of EDTA, or  $\text{Co(II)}$  respectively. Approximately 50 ml of  $1 \times 10^{-3} \text{M}$  Co-EDTA was then stored in a tightly stoppered plastic bottle until it was ready for use.

### 5.3 Results and Discussion

#### 5.3.1 Initial Results

The instrumentation was set up as shown in Figure 5.1, but initially without the presence of an analytical column and so was in a so-called flow injection analysis (FIA) mode. The conditions used for some of these initial experiments are described in Table 5.2.

With the FIA system in operation, using the condition described in Table 5.2 several different metals were injected (200  $\mu\text{l}$ ) into the flow system. These metals included Co, Cu, Pb, Cd, Ni, Mn, Al(III) and Mg, and were all injected at the 10 ppm level. All the metals that were injected gave a response typically shown in Figure 5.3. When the Co-EDTA flowing stream was replaced by distilled water, the only metal to respond was, as expected, Co. FIA systems, however, can be rather misleading as large CL responses were also observed when

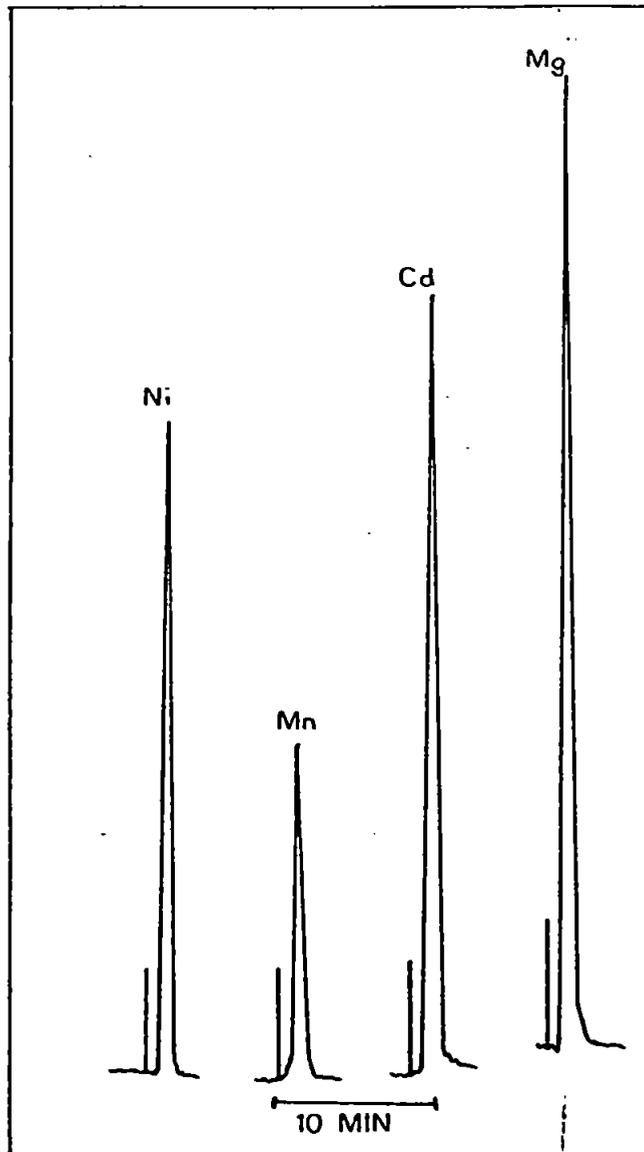


Figure 5.3 Typical responses observed for 10 ppm analyte metals injected into the Co-EDTA system using Flow-Injection Analysis.

acidic blanks were injected into the water : Co-EDTA : luminol system. These responses presumably result from the destabilizing affect of acidic pH on the metal-complex system. In order to be more certain that the response observed is a direct result of the presence of the analyte of interest, a separation technique must be used in order to separate and remove the analyte from the solvent front.

### 5.3.2 A Thorough Study of the Co-EDTA Displacement Reaction

The lactate eluent (0.15M, pH 3.5) was used in conjunction with the low capacity cation exchange Dionex columns (HPIC CG2 and CS2) to perform metal separations and ensuring that the analytes were removed from the solvent front. The other conditions that were used are described in Table 5.2. Many of the Co-EDTA parameters such as concentration and pH were not optimised but for the initial results a  $1 \times 10^{-3}$ M Co-EDTA solution was used at a pH of 9.0. At this concentration there should be plenty of Co-EDTA present for potential displacement and a pH value of 9.0 was chosen to ensure that the reaction:



is shifted to the right. This is an important point because at lower pH's there is a greater shift to the left hand side with a subsequent increase in the concentration of free  $\text{Co}^{2+}$  ions resulting in a higher background noise. At pH 9.0, assuming there exists an accurate 1:1 ratio of Co:EDTA the amount of free  $\text{Co}^{2+}$  can be approximately calculated i.e.

**Table 5.2** Initial experimental parameters used for the Co-EDTA displacement reaction

Mobile phase	Double distilled water (no column)
Flow rate	0.8 ml min <sup>-1</sup>
Co-EDTA concentration	1 x 10 <sup>-3</sup> M
Boric acid concentration	6 g l <sup>-1</sup>
pH	9.0
Flow rate	0.8 ml min <sup>-1</sup>
Luminol concentration	0.2 g l <sup>-1</sup>
Hydrogen peroxide concentration	10.0 ml l <sup>-1</sup>
Boric acid concentration	12 g l <sup>-1</sup>
pH	12.5
Flow rate	0.8 ml min <sup>-1</sup>
Reaction coil volume	50 µl
Reaction coil temperature	20°C

$$\log K_{(\text{conditional stab.})} = \frac{[(\text{Co-EDTA})^{2-}]}{[\text{Co}^{2+}][\text{EDTA}^{4-}]}$$

$$10^{14} = \frac{0.001}{[x][x]}$$

$$x^2 = \frac{0.001}{10^{14}} = 1 \times 10^{-18}$$

$$x = 1 \times 10^{-9} \text{ molar}$$

$1 \times 10^{-9}$  moles of Co = 0.06 ppb free  $\text{Co}^{2+}$  present.

On mixing with the lactic acid eluent, however, the pH of the lactate/Co-EDTA solution dropped from pH 9.0 to pH 4.0. The amount of free  $\text{Co}^{2+}$  ions will now increase considerably. i.e.

$$K_{(\text{conditional stab.})} = \frac{[(\text{Co-EDTA})^{2-}]}{[\text{Co}^{2+}][\text{EDTA}^{4-}]}$$

$$10^8 = \frac{0.001}{[x][x]}$$

$$x^2 = \frac{1 \times 10^{-3}}{10^7} = 1 \times 10^{-10}$$

$$x = 1 \times 10^{-5} \text{ molar}$$

$1 \times 10^{-5}$  moles of Co = 0.6 ppm free  $\text{Co}^{2+}$  present.

Although the background was considerable, the nature of the flow cell design (Fig. 5.2) desensitized the detection system and enabled the first displacement reactions to be

**Table 5.3** The original conditions used for preliminary multi-element determinations.

Mobile phase	Lactic acid
Concentration	0.15 M
pH	3.5
Flow rate	0.8 ml min <sup>-1</sup>
Column	Dionex HPIC CS5 (25 cm x 4.6 mm i.d.)
[Co-EDTA]	1 x 10 <sup>-3</sup> M
pH	9.0 (Boric acid 6 g l <sup>-1</sup> KOH)
Flow rate	0.8 ml min <sup>-1</sup>
Mixing pH (lactate and Co-EDTA)	4.0
[Luminol]	0.2 g l <sup>-1</sup>
H <sub>2</sub> O <sub>2</sub> /30%	10 ml l <sup>-1</sup>
[Boric acid]	12 g l <sup>-1</sup>
pH	12.5
Flow rate	0.8 ml min <sup>-1</sup>
Mixing pH (lactate, Co-EDTA, luminol)	10.1

monitored. Figure 5.4 shows the first results obtained for the time resolved simultaneous determination of Cd and Zn, neither of which do not act as catalysts in the luminol/peroxide CL reaction. This was indeed a very pleasing result, although it was clearly apparent that considerably more work was required to make the system much more sensitive and useful for analytical determinations.

The conditions employed for this Cd and Zn determination are described in Table 5.3.

With further optimization of the Co-EDTA system the optimum parameters were established for the determination of Cd using the best signal to background ratio as the figure of merit and are described in Table 5.4.

**Table 5.4** The initial optimised parameters for the Co-EDTA solution.

Parameter	Optimal Range	Optimum Condition Established
[Co-EDTA]	$1 \times 10^{-7} \text{M}$ to $1 \times 10^{-3} \text{M}$	$5 \times 10^{-5} \text{M}$
[Boric acid]	$3.0 \text{ g l}^{-1}$ to $10.0 \text{ g l}^{-1}$	6.0 g
pH (adjusted with KOH)	6.0 to 10.0	9.0

NB. The mixing pH of the lactate and Co-EDTA solution ranged from 4.3 to 5.5 depending on the concentration and pH of the Co-EDTA solution. In order to maintain the pH

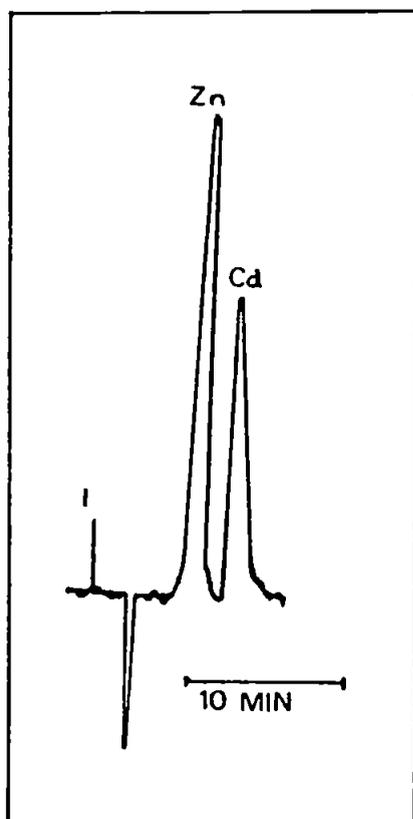


Figure 5.4 The time resolved determination of Cd and Zn (2 ppm both species) using the Co-EDTA displacement systems.

of the lactate/Co-EDTA/luminol mixture at 10.5, 18 g l<sup>-1</sup> of boric acid was required in the luminol post-column reagent, and adjusted to pH 12.0 with concentrated aqueous KOH.

The optimum pH of the lactate/Co-EDTA solution was found to be between 4.5 and 5.5. This result is rather surprising because it was presumed that a higher pH would be advantageous in order to reduce the background noise. This was true to some extent, as at higher pH levels the background was indeed reduced. The analyte signal, however, was severely affected as can be seen from Figure 5.5.

At mixing pH's of 6.0 and above the quality of the peak shape deteriorated. The peak shape has changed by what appears to be a chromatographic affect but this is obviously impossible as the chromatographic parameters remain unchanged. One possible explanation for this observation is that the Murexide dye, that is present in the Co-EDTA solution, is deposited on the inside of the PTFE post-column tubing. This phenomenon has been observed for other systems, e.g. when using Calmagite or Xylenol orange as post-column reagents. Due to the low concentration of the Murexide dye that is present it is difficult to observe this proposed coating effect. Assuming that there is a thin deposited layer of dye present then this could, provided the pH was favourable, chelate the metal ions passing over it. At higher pH's

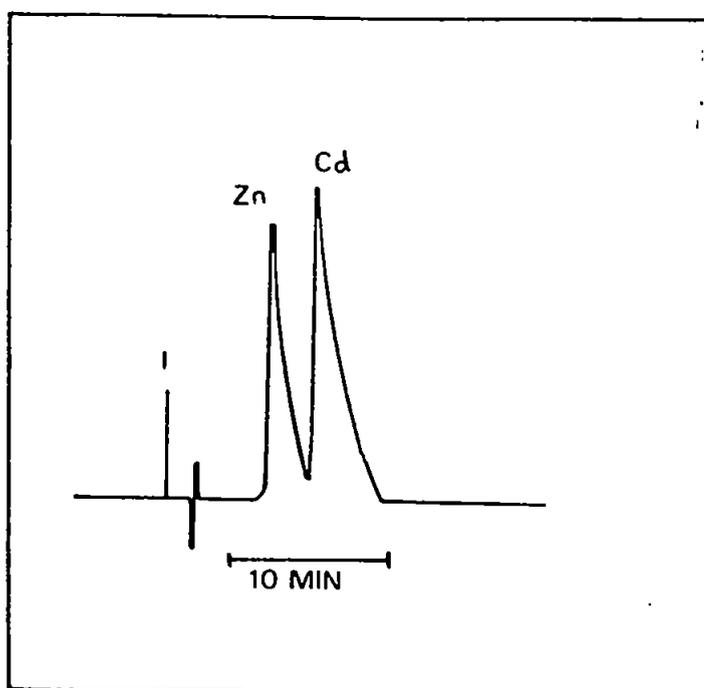


Figure 5.5 The affect of a rise in pH (from 3.5 to 6.5) on the analyte signal (both analytes 1 ppm).

the murexide dye will become ionised and be able to chelate the metal ions from solution causing the distorted peak shapes observed. This problem can not easily be overcome as the murexide dye must be present to establish an accurate 1:1 Co:EDTA ratio. Repeat titrations with the absence of the indicator dye were tried but proved very unreliable as accurate 1:1 ratios were very difficult to achieve. The system will improve greatly if this problem can be overcome as higher pH levels for the Co-EDTA/lactate mix are desirable to reduce the significant background noise levels and ultimately improve detection limits and linearity of the system.

### 5.3.3 Detector Performance

To summarise, the optimised chromatographic system parameters are described in Table 5.5.

With this system set up, various analyte metals were injected at the relatively high concentration of 1 ppm and the extent at which they produced any CL was measured. The results are described in Table 5.6.

To obtain more quantitative information Cd was initially chosen to discover more about this novel detection system, because (a) it is a completely non-active CL species and (b) it gave a medium response to the Co-EDTA displacement system.

**Table 5.5** The operating parameters used for the multi-element determination of selected metals by CL

[Lactic acid]	0.15 M
pH	3.5
Flow rate	0.8 ml min <sup>-1</sup>
Column	Dionex HPIC GS2 (250 mm x 0.46 mm i.d)
[Co-EDTA]	5 x 10 <sup>-5</sup> M
[Boric acid]	6.0 g l <sup>-1</sup>
pH	9.0
Flow rate	0.8 ml min <sup>-1</sup>
Mixing pH (lactate with Co-EDTA)	5.0
[Luminol]	0.2 g l <sup>-1</sup>
H <sub>2</sub> O <sub>2</sub> (30%)	10 ml l <sup>-1</sup>
[Boric acid]	18 g l <sup>-1</sup>
Flow rate	0.8 ml min <sup>-1</sup>
Outlet pH	10.5

**Table 5.6** Various metal injections (1 ppm) into the Co-EDTA displacement CL system.

Metal (1 ppm)	CL ✓ X	Normalized response
Co(II)	✓	100
Be(II)	X	-
Mg(II)	✓	20
Ca(II)	✓	40
Sr(II)	✓	20
Ba(II)	✓	5
Ti(III)	X	-
V(IV)	X	-
Mn(II)	X	-
Fe(II)	✓	50
Fe(III)	✓	80
Ni(II)	✓	20
Cu(II)	✓	80
Zn(II)	✓	85
Cd(II)	✓	55
La(III)	✓	40
Other Lanthanides	✓	40
Th	✓	30
U	X	-

### 5.3.3.1 The Calibration Curve for Cd

A calibration curve was constructed for Cd from 0.1 to 1.3  $\mu\text{g ml}^{-1}$  and is shown in Figure 5.6. As can be seen, there is marked non-linearity for the Cd calibration and is likely to be due to kinetic restraints in both the breakdown of the Co-EDTA complex and the formation of the new Cd-EDTA species. If this were the case, one would expect to see a significant improvement in signal response and linearity if (a) the time for the  $\text{M}^{n+}$  and Co-EDTA reaction was increased by employment of a reaction coil and (b) if the temperature for the  $\text{M}^{n+} + \text{Co-EDTA}$  reaction was increased.

Figures 5.7 and 5.8 show the results of increasing the reaction coil volume and increasing the reaction coil temperature. It is clear that there is a much improved linearity at higher temperatures for Cd, although there is still some degree of positive curvature even at the highest temperatures available ( $95^{\circ}\text{C}$ ). This phenomenon was experienced when other metals were tried including: Zn, Ni, Pb, In and Ga. Strangely enough, Co gave a pronounced negative curvature even at elevated temperatures. Figure 5.9 shows this positive curvature for Co analysis but its profile is not easily explained.

One consideration is that the nature of flow cell design (Fig. 5.2) was inappropriate for emission detection and, particularly at low analyte concentrations, a good deal of self-absorption may occur giving misleading information.

Figure 5.6 The calibration curve for Cd.

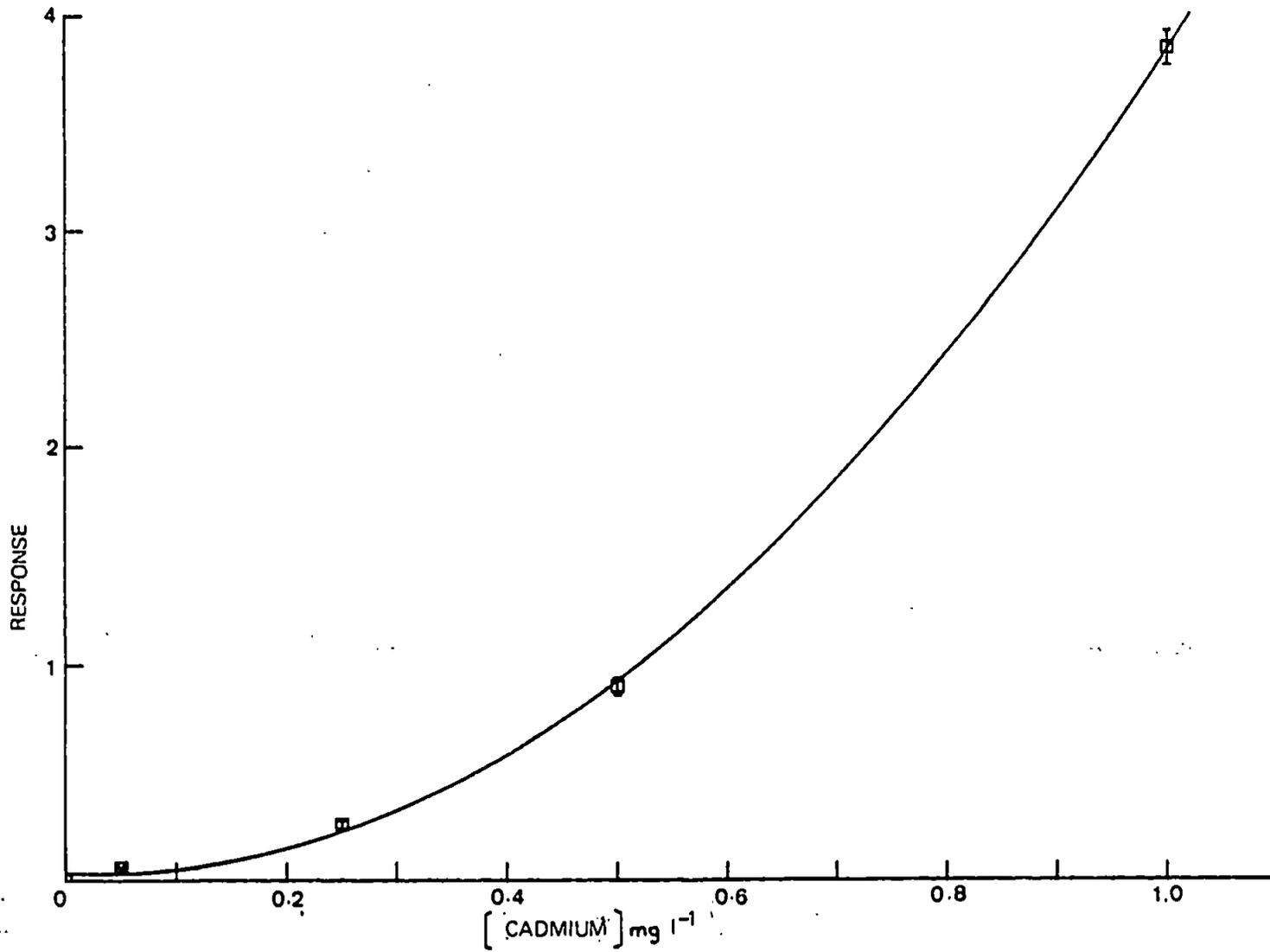


Figure 5.7 The affect on the Cd signal by an increase in the reaction coil volume.

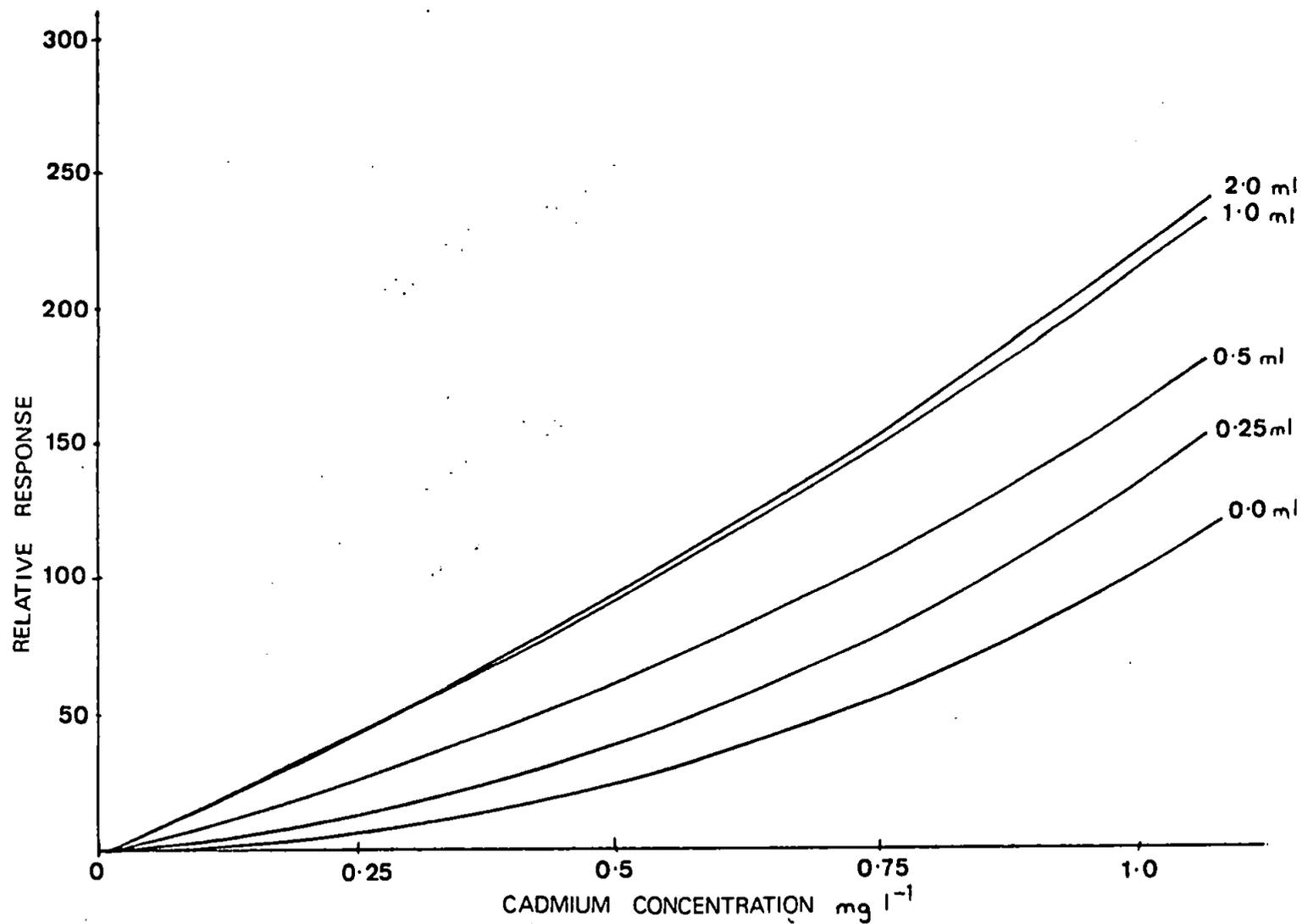


Figure 5.8 The affect on the Od signal by an increase in the reaction coil temperature.

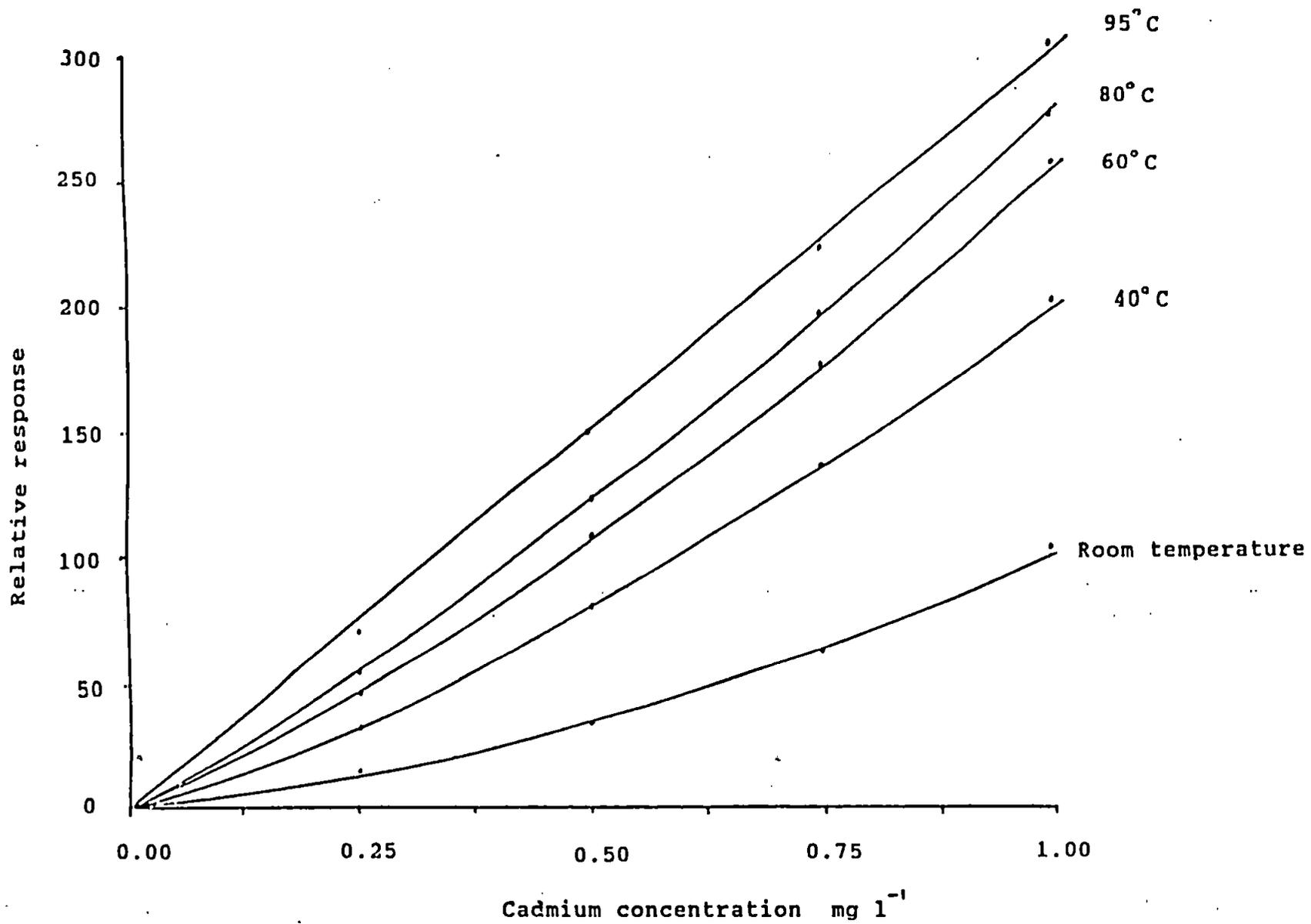
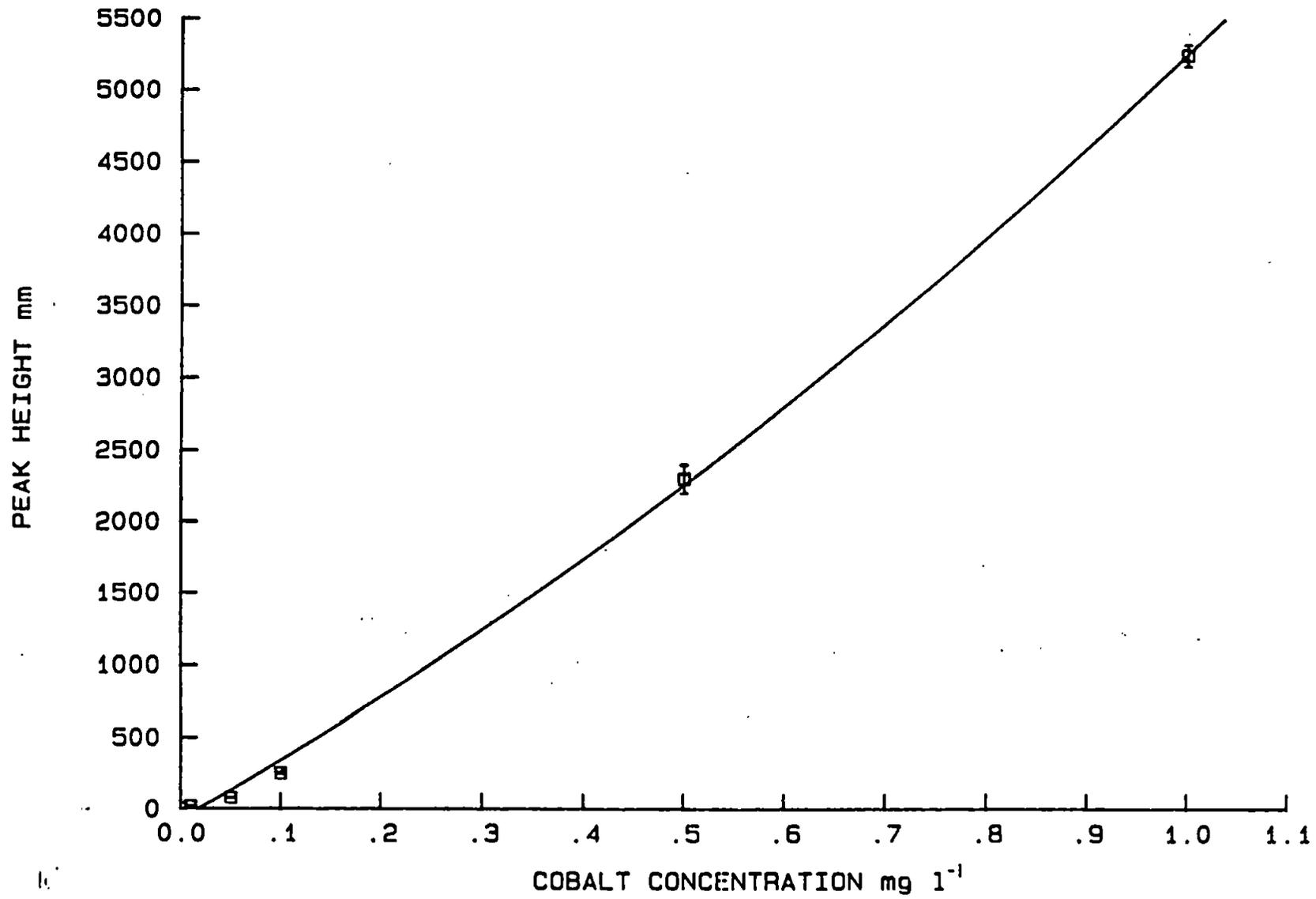


Figure 5.9 CALIBRATION CURVE FOR COBALT



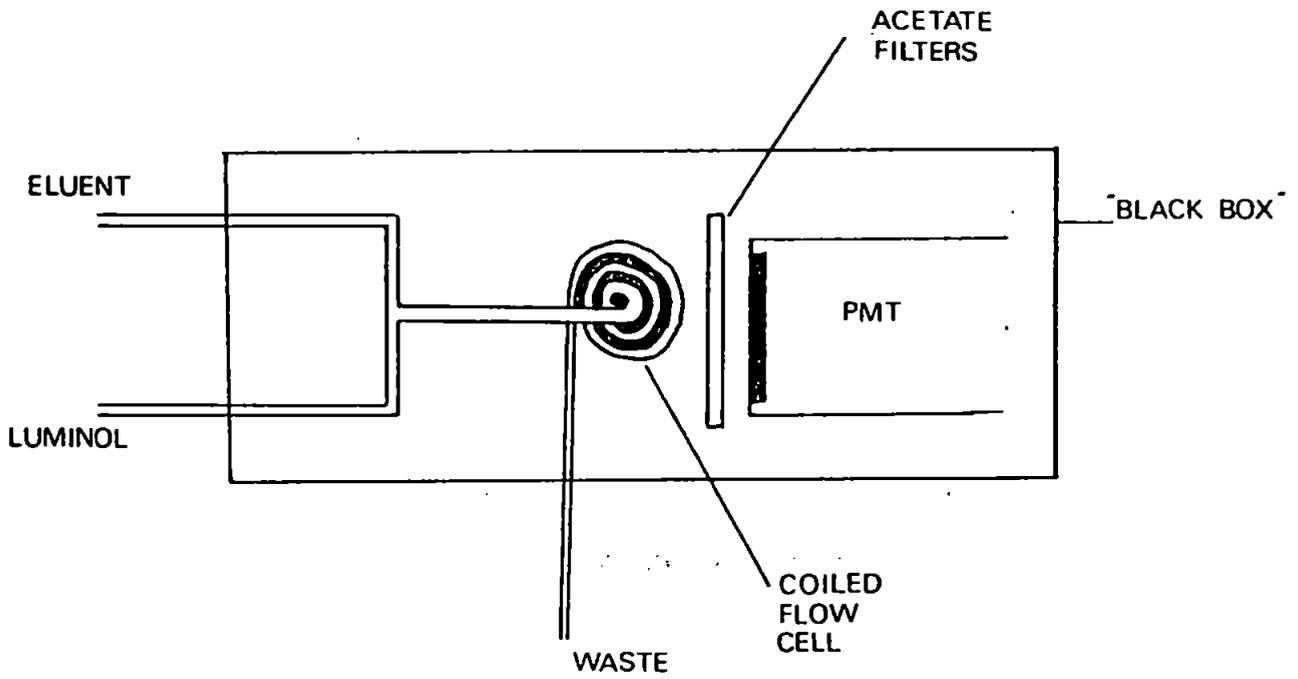
Another possibility is that there was inefficient mixing of the lactate/[Co-EDTA] with the luminol solution. For both of these reasons it was decided to alter the flow cell design to a more familiar one similar to the one used for chromium determinations (Chapter 4). The new flow cell is described in Figure 5.10.

#### 5.3.4 Quantitative Performance Using the New Design Flow Cell

With the employment of the new design flow cell, the background emission, reaching the photo-multiplier tube was very high, causing an overload and cut-out of the detector. In order to reduce this large emission a light filtering device was used. Dark, acetate sheets were placed between the flow cell and photo-multiplier tube. In all, 5 acetate sheets were required to reduce the background emission to a manageable level. NB. The 5 acetate sheets had an absorbance value of 3 units, thus filtering out 99.9% of the background emission.

In an attempt to reduce this large background, a buffer system was employed that used much lower concentrations of reagents, but maintaining the previously established pH's. In order to keep the pH of the lactate/Co-EDTA mixture at a pH of 5.5, the Co-EDTA solution was added to a 0.1 M solution of acetic acid adjusted to a pH of 6.5 with concentrated aqueous potassium hydroxide.

Figure 5.10 A schematic diagram of the new design flow cell.



With the inclusion of this new buffer system, less boric acid was required in the luminol solution to maintain the pH at 10.5. The new operating parameters are summarised in Table 5.7.

Although the parameters described are at an optimum for most of the analyte metals, it was discovered that the alkaline-earth metals and aluminium responded to a greater extent, in terms of signal to background ratio, if the Co-EDTA solution remained unbuffered at the pH of the mobile phase, typically pH 3.5. This is not what would be expected from purely thermodynamic data as all the alkaline-earth metals form very weak complexes with EDTA. For example at pH 3.5 the log  $K_{\text{conditional}}$  stability constants for metal-EDTA complexes are for:

$$\text{Mg} = < 2.1$$

$$\text{Ca} = < 2.2$$

$$\text{Sr} = < 2.0$$

$$\text{Ba} = < 1.3$$

$$\text{Co} = 6.9$$

From the above figures it would seem that the Co-EDTA complex is at least 100,000 times more stable than any of the alkaline-earth metal : EDTA complexes. The Co is also only weakly bound to the EDTA at this pH. Therefore, some displacement would not, perhaps seem unreasonable. With aluminium analysis, the potassium sulphate mobile phase eluent (69) has no chelating ability and it is not surprising that at elevated pH levels, there is a poorer response observed for Al, presumably because of hydroxide

**Table 5.7** Revised operating parameters requiring less buffering reagents.

Co-EDTA

[Acetic acid]	0.1M
pH (KOH)	6.5
Mixing pH lactate/Co-EDTA	5.5

Luminol

[Boric acid]	10.0 g l <sup>-1</sup>
pH (KOH)	12.0
Mixing pH lactate/Co-EDTA/luminol	10.5

formation and the Al precipitating out of solution.

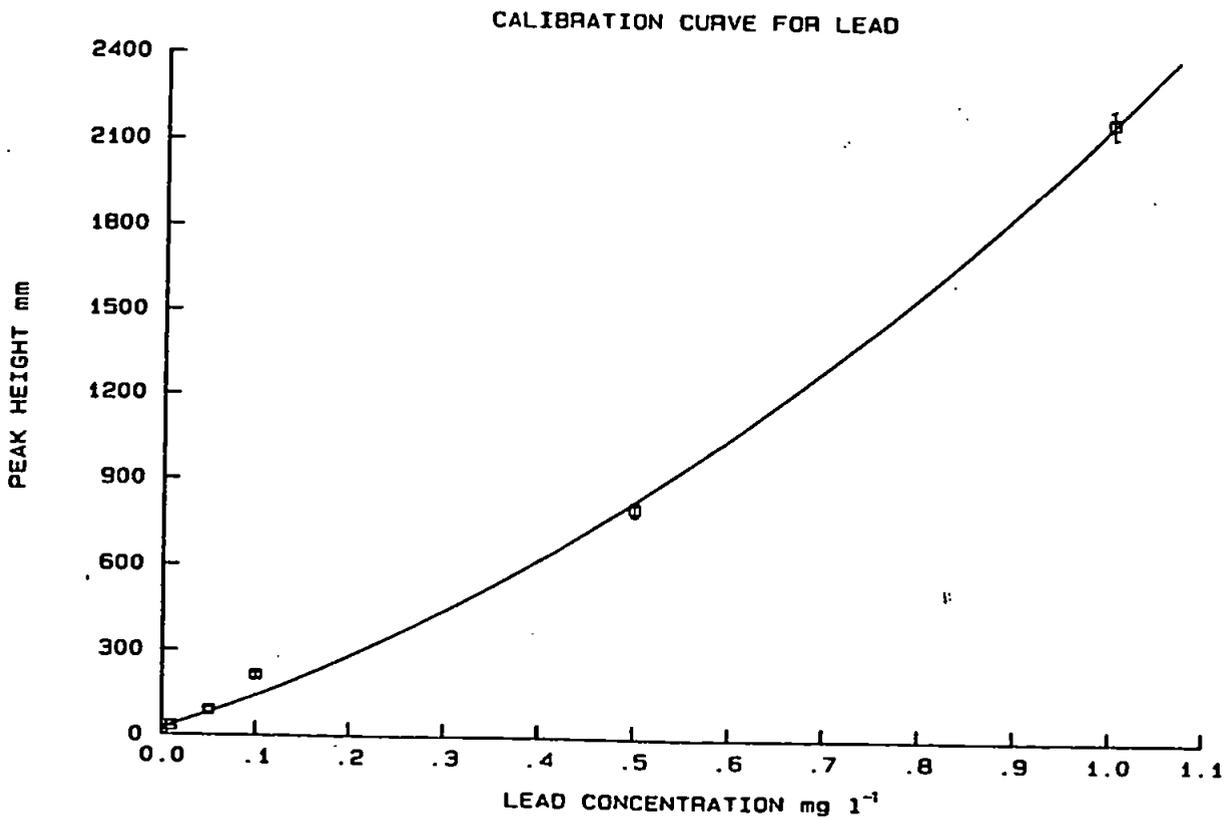
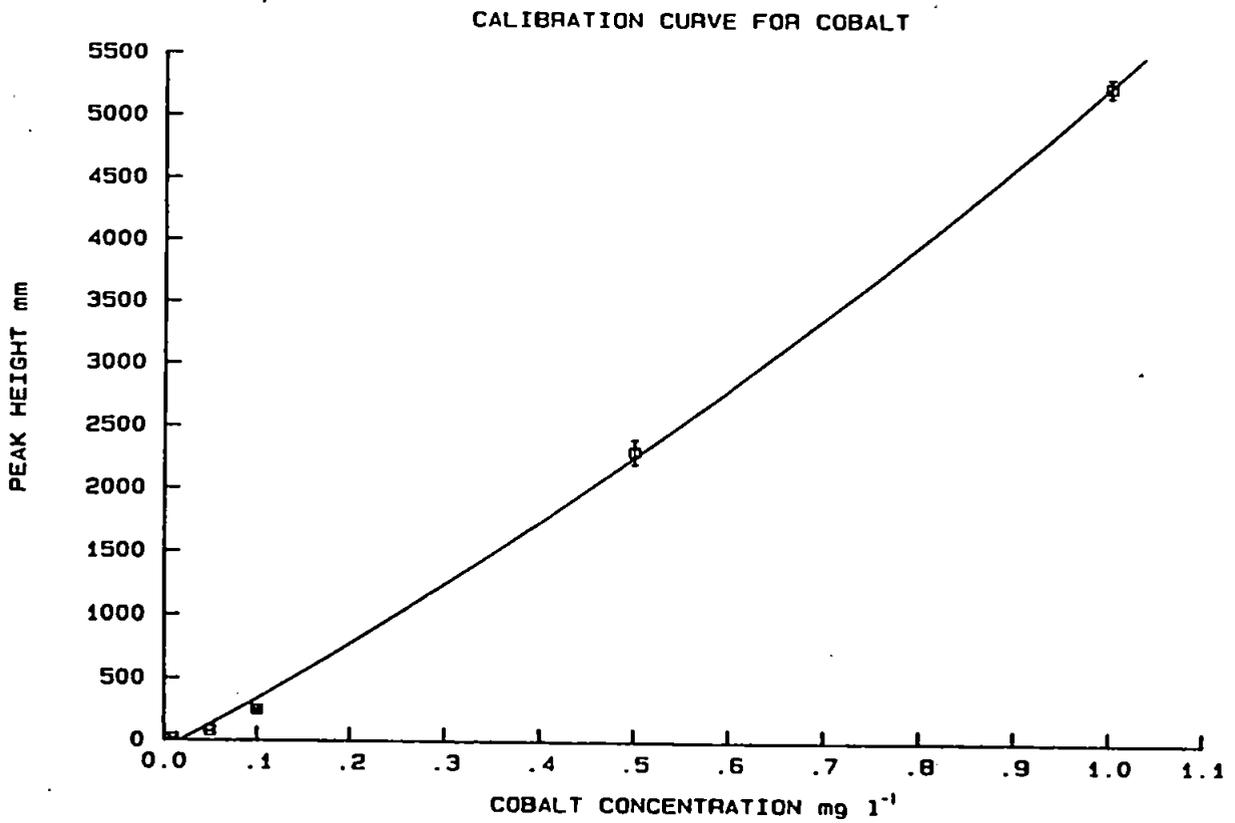
#### 5.3.4.1 Linearity and Detection Limits

Calibration curves were constructed for each of the following elements: Mg, Ca, Sr, Fe(III), Co, Ni, Cu, Zn, Cd, Pb, In, Ga, La, Ce, Pr and Al. The concentration range of the elements varied but was generally between  $10.0 \text{ ng ml}^{-1}$  to  $1.0 \text{ } \mu\text{g ml}^{-1}$ . Linear calibrations were obtained for Mg ( $r = 0.999$ ), Ca ( $r = 0.998$ ), Sr ( $r = 0.998$ ), Zn ( $r = 0.998$ ), and Al(III) ( $r = 0.998$ ).

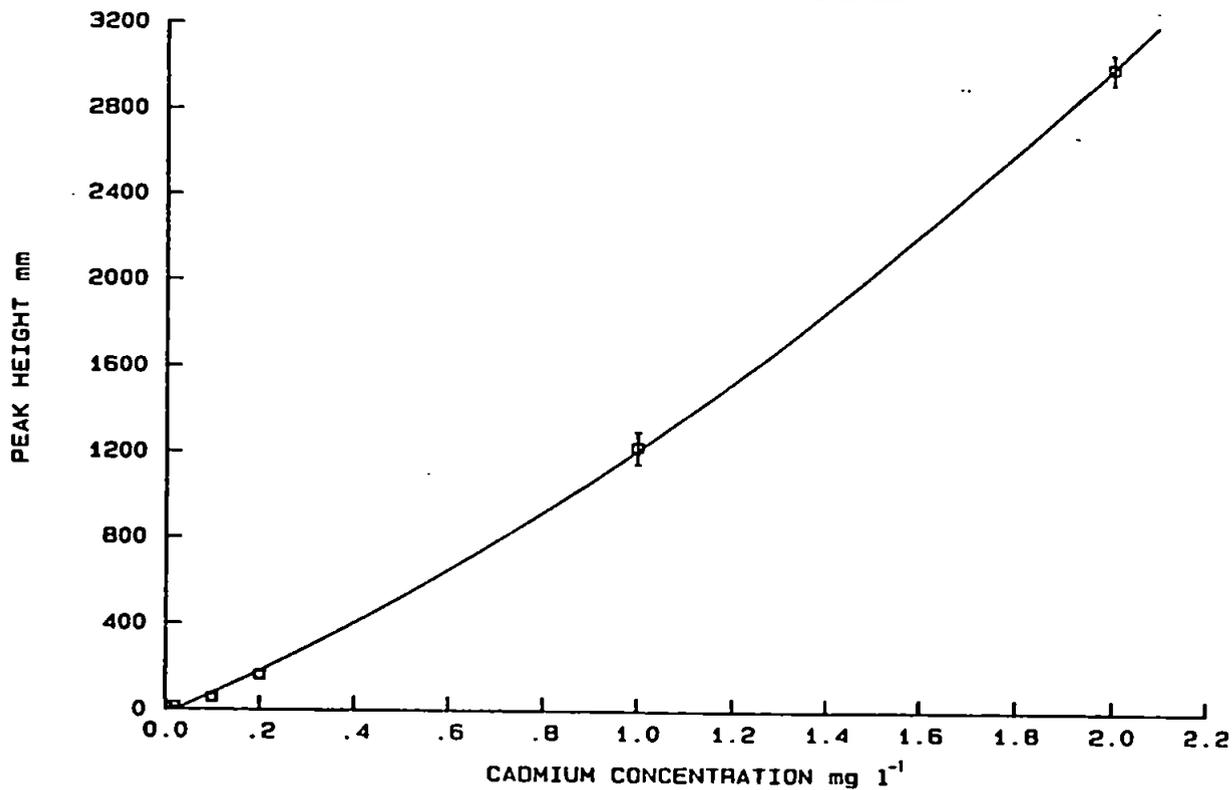
However, for the other elements: Cd, Pb, Co, Ni, In, Ga, Fe(III), La, Ce and Pr, a positive curvature is still observed, though still useful for quantitative analysis (Figure 5.11).

It is not clear why some elements produce linear calibrations, whilst others do not. Thermodynamic and kinetic factors for the Co/metal-ligand displacement reaction may contribute to the non-linearity of the system but this by no means explains why the calibration curve for Co is non-linear. There are, presumably, a number of factors involved in the production of these non-linear calibration curves. A very interesting recent development in the search for more information about the Co-EDTA displacement reaction has been to use a thermodynamic computer model. This model (127), given a specific set of conditions, is able to predict the different respective amounts of the Co species that are present in a mixture of metals and chelating

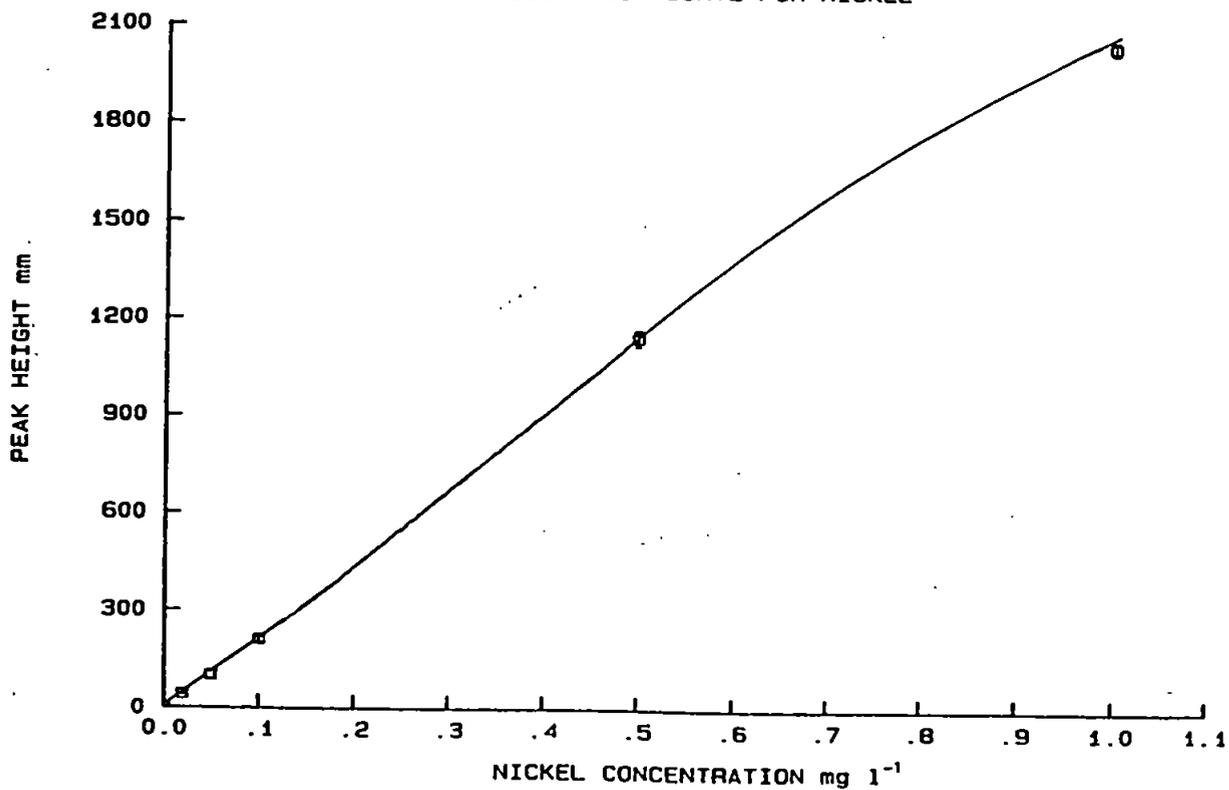
Figure 5.11 The calibration curves for the following analytes; Co, Pb, Cd, Ni, Fe (III), Ga, In, La, Ce and Pr.



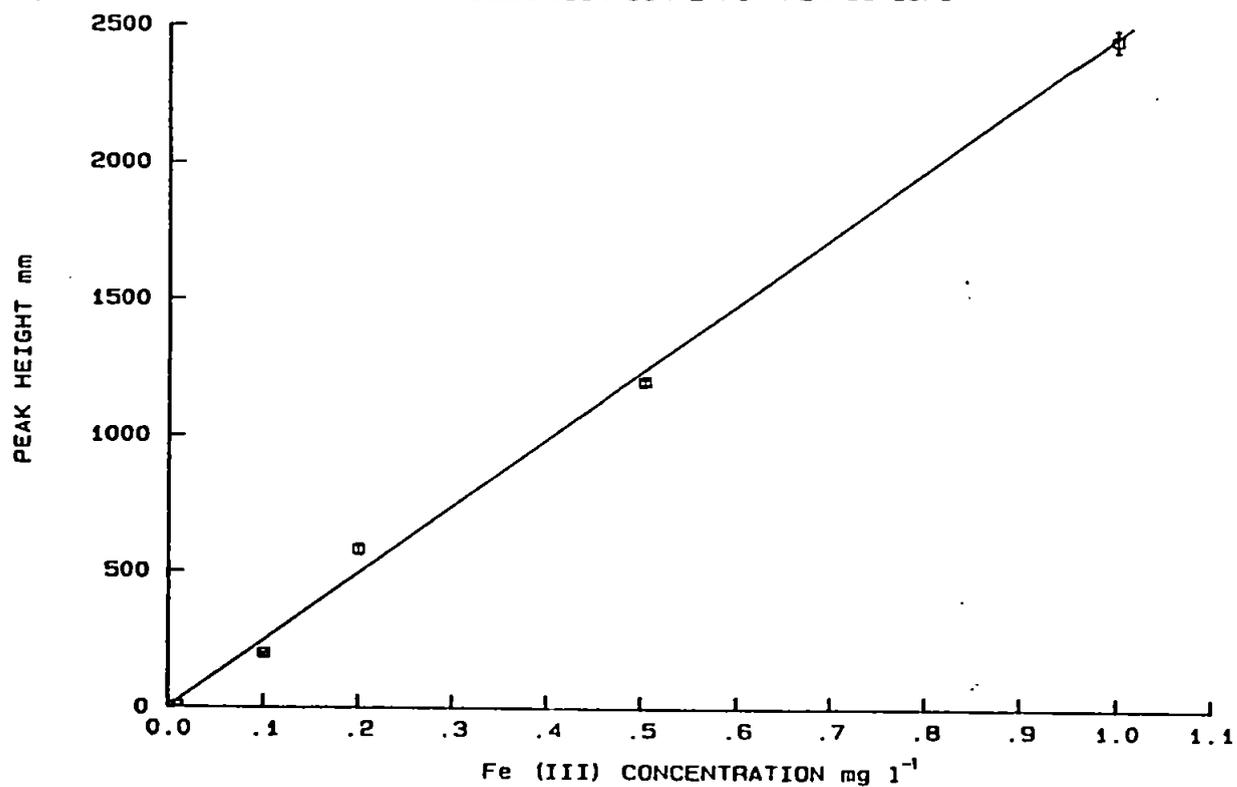
CALIBRATION CURVE FOR CADMIUM



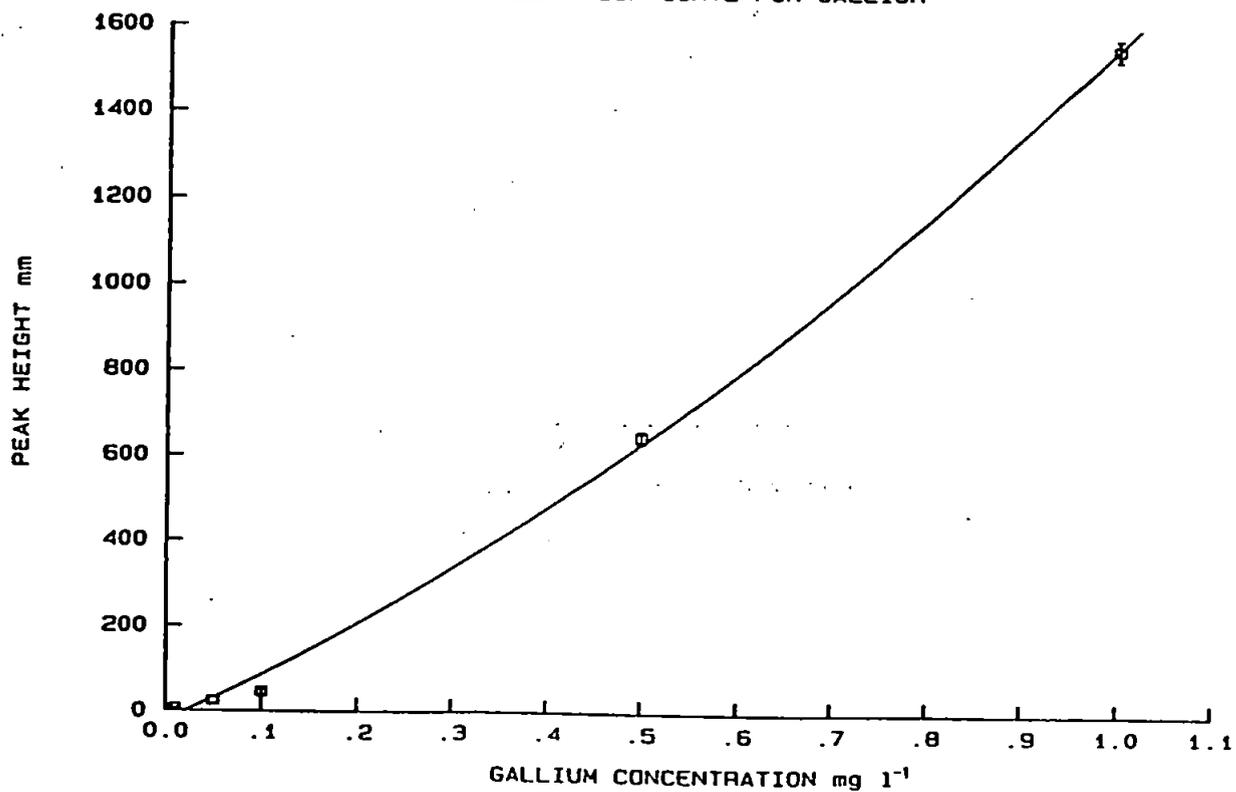
CALIBRATION CURVE FOR NICKEL



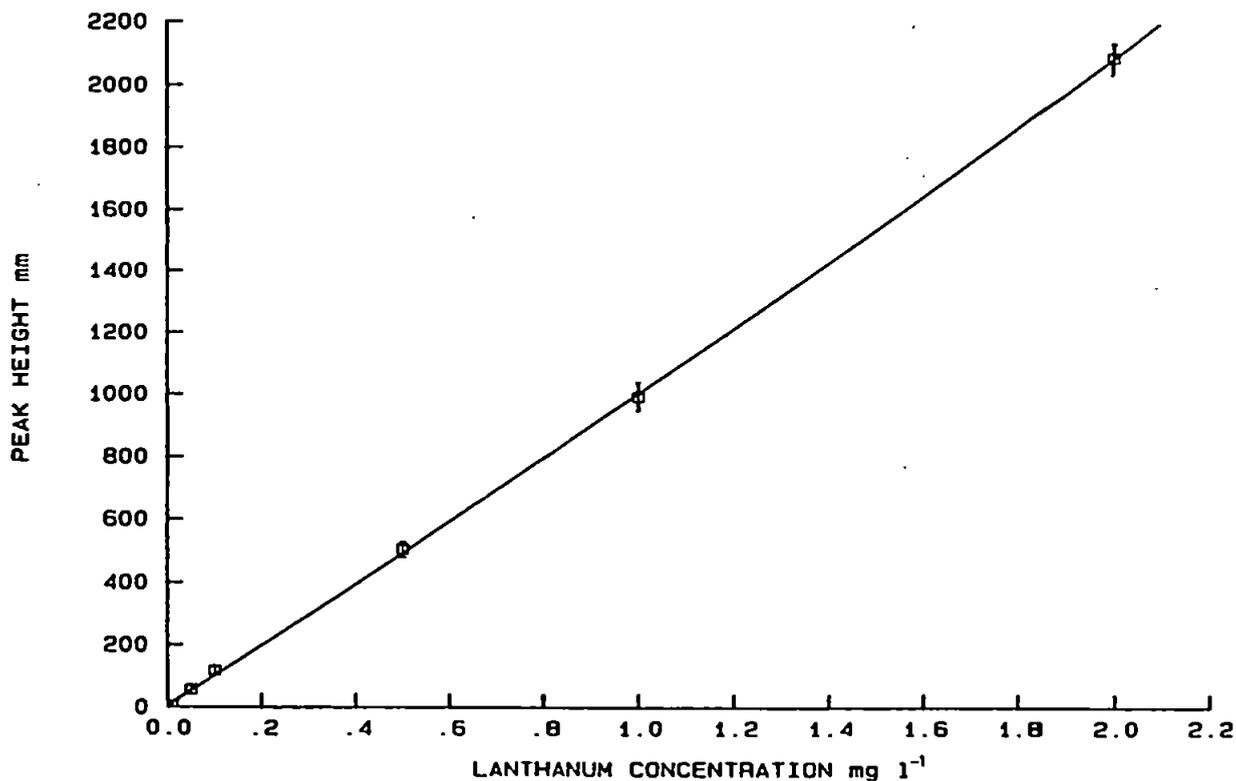
CALIBRATION CURVE FOR FERRIC IONS



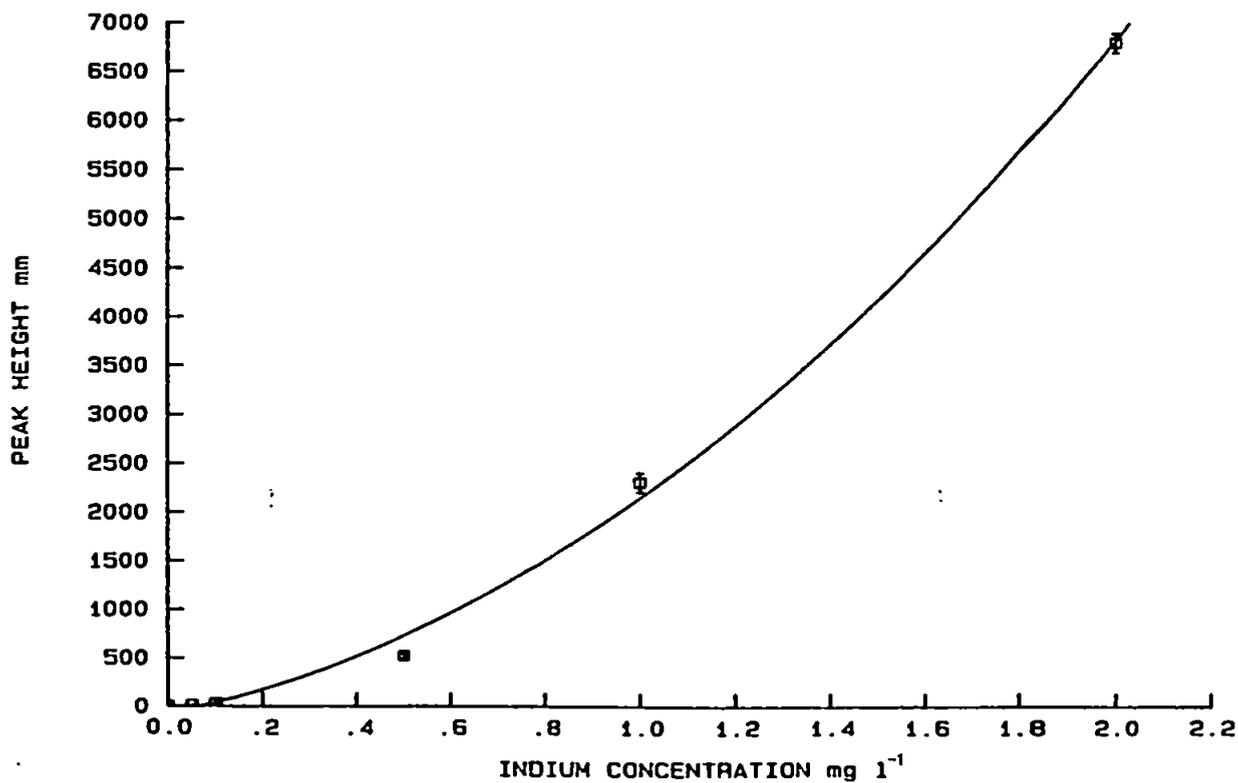
CALIBRATION CURVE FOR GALLIUM



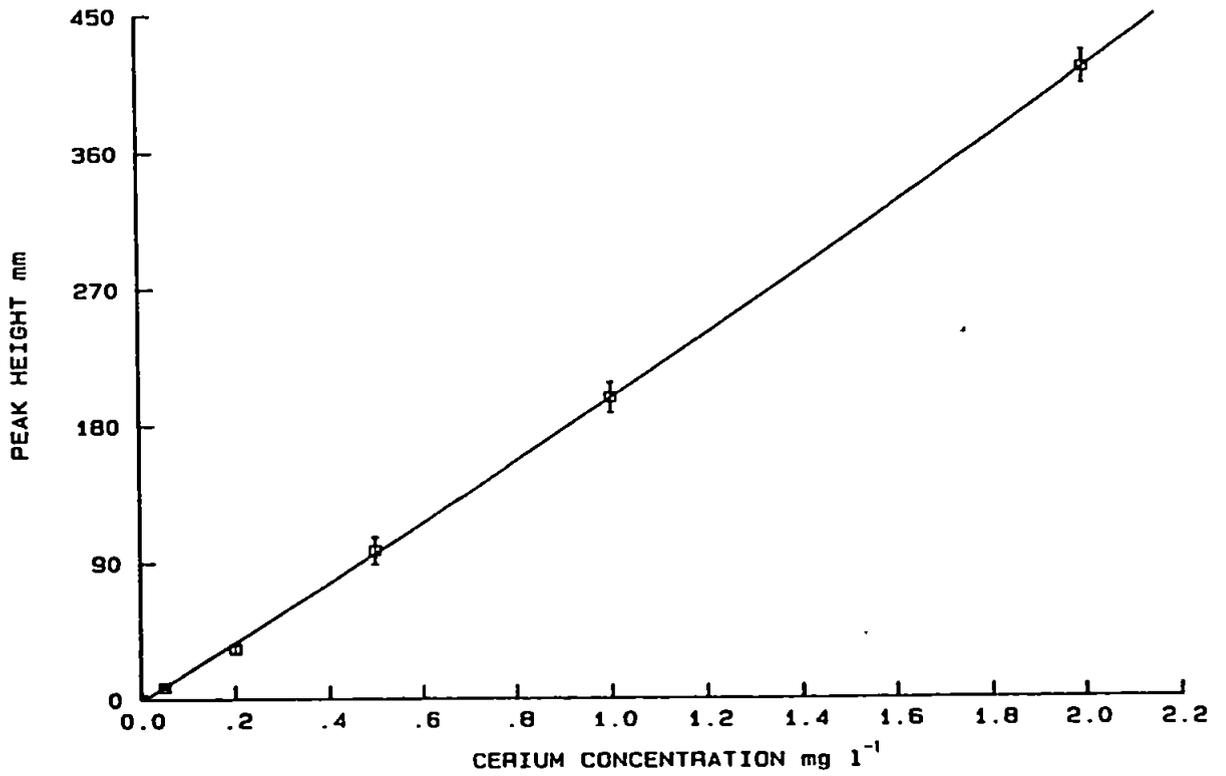
CALIBRATION CURVE FOR LANTHANUM



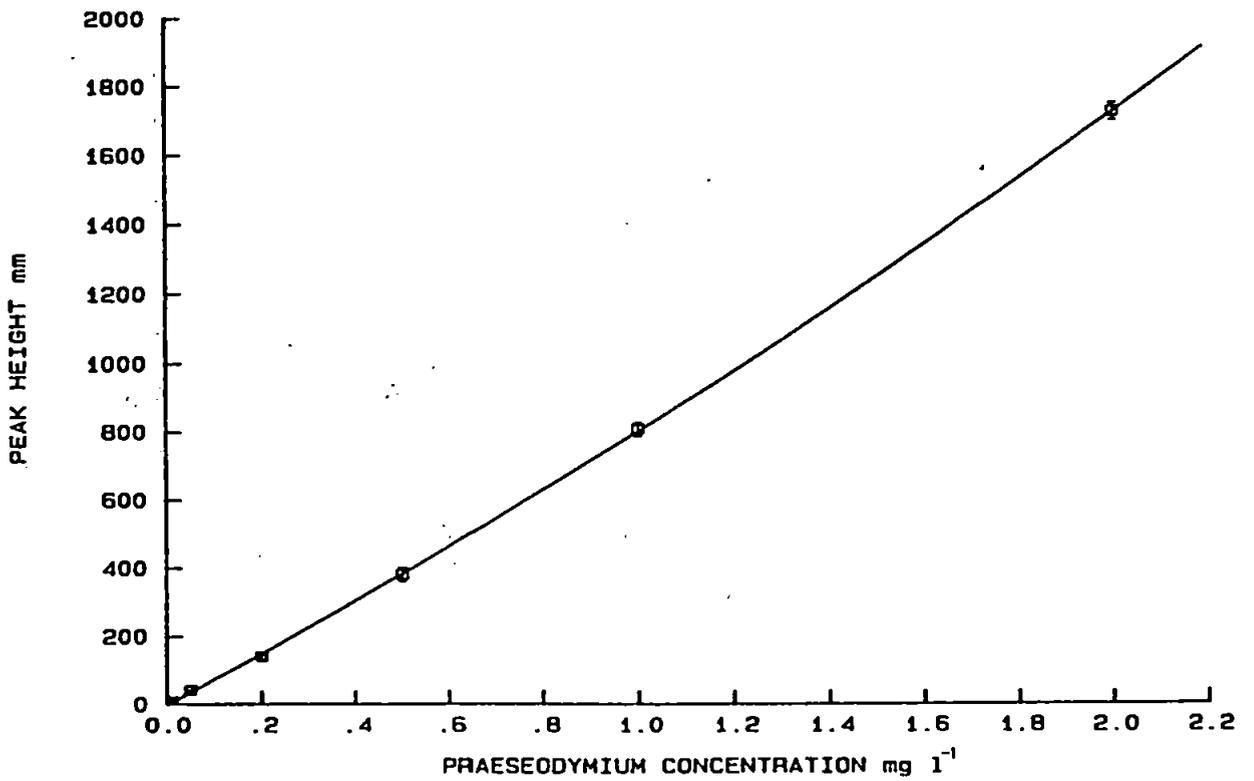
CALIBRATION CURVE FOR INDIUM



CALIBRATION CURVE FOR CERIUM



CALIBRATION CURVE FOR PRAESEODYMIUM



agents.

The species produced, that are of particular interest, are the ones that produce CL. All the Co-EDTA complexes are completely non-chemiluminescent and so can be ignored. The free  $\text{Co}^{2+}$  ions and the two Co-lactate complexes do produce CL and are included in the total CL emitted light. For the model to work successfully a column dilution factor must be applied to the [analyte metal] and also the ionic strength of the mixture must be taken into account.

The analyte dilution factor can be defined as:

$$\text{Dilution Factor} = \frac{\text{peak base width}}{\text{injection volume}} \quad \text{ml}$$

and for the majority of the analytes tested, the dilution factor was approximately 10.

The ionic strength for the eluent can be calculated:

$$\text{ionic strength } I = 1/2 \times (M_i Z_i^2 + M_{ii} Z_{ii}^2 \dots)$$

where M = concentration of i

and Z = charge on i

for 0.1 M lactic acid the ionic strength is (assuming complete dissociation)

$$\begin{aligned} I &= 1/2 \quad (0.1 \times 1^2) + (0.1 \times 1^2) \\ &= 0.1 \end{aligned}$$

for 0.1 M  $\text{K}_2\text{SO}_4$  the ionic strength is

$$\begin{aligned} I &= 1/2 \quad (0.2 \times 1^2) + (0.1 \times 2^2) \\ &= \quad \quad 0.3. \end{aligned}$$

Several calibration curves were constructed using the data produced by the thermodynamic model. Conditions and stability constant data that were used for the thermodynamic model to predict speciation are described in Table 5.8.

The calibration curves constructed from the thermodynamic model for the elements Co, Zn, Cd, Pb, Ca and Al are described in Figure 5.12.

It is clear from these theoretical calibration curves, that positive curvature exists for Co, Zn, Cd and Pb at the low ppb levels and this is a similar result to what has been found experimentally (Fig. 5.11). This curvature can be attributed to the fact that there is a very slight excess of EDTA present which is able to chelate a small proportion of the analyte species. A linear working range is difficult to achieve whilst there is more uncomplexed EDTA present than that produced by dissociation of an exact 1:1 molar ratio Co -EDTA complex

The calibration curve for Ca is linear in both the experimental result, and the theoretical data. This is presumably because, from thermodynamic considerations, a lot more of the analyte is required before there is any significant displacement Co from the Co-EDTA solution. The low concentration end of the Ca calibration curve is

**Table 5.8.** The conditions and data used in the thermodynamic model to predict speciation

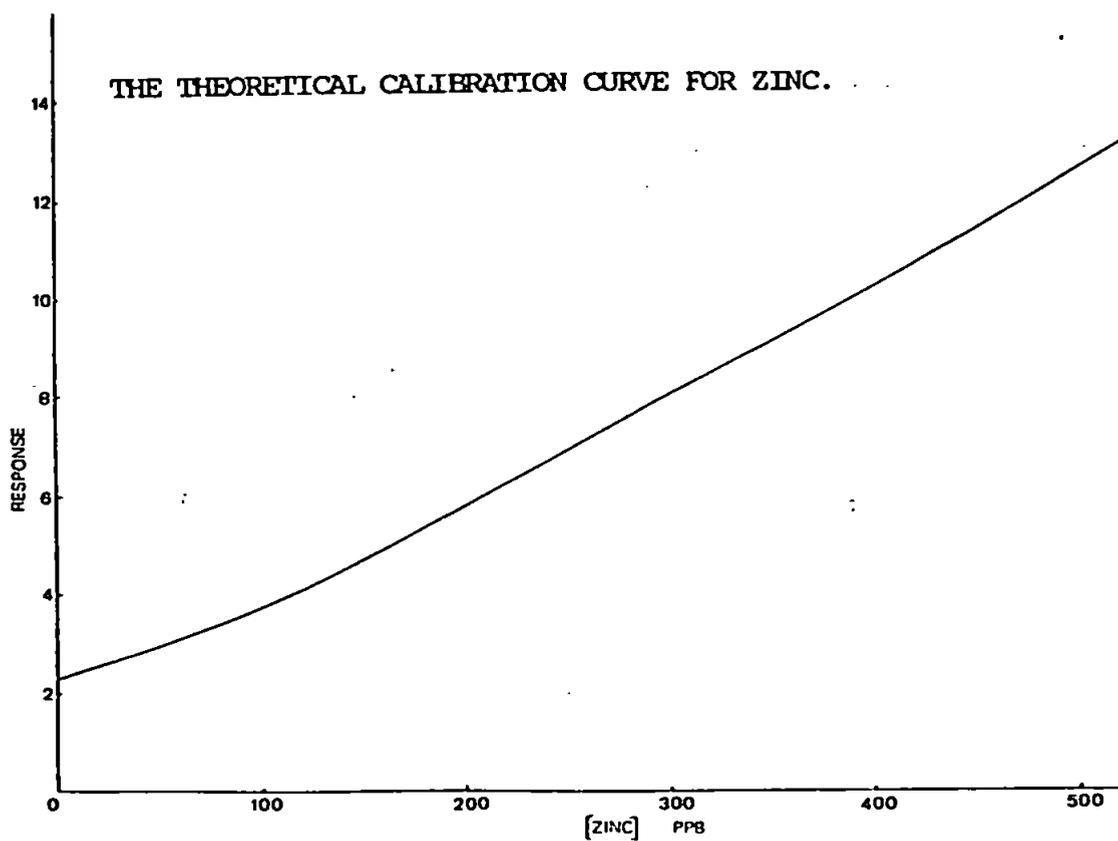
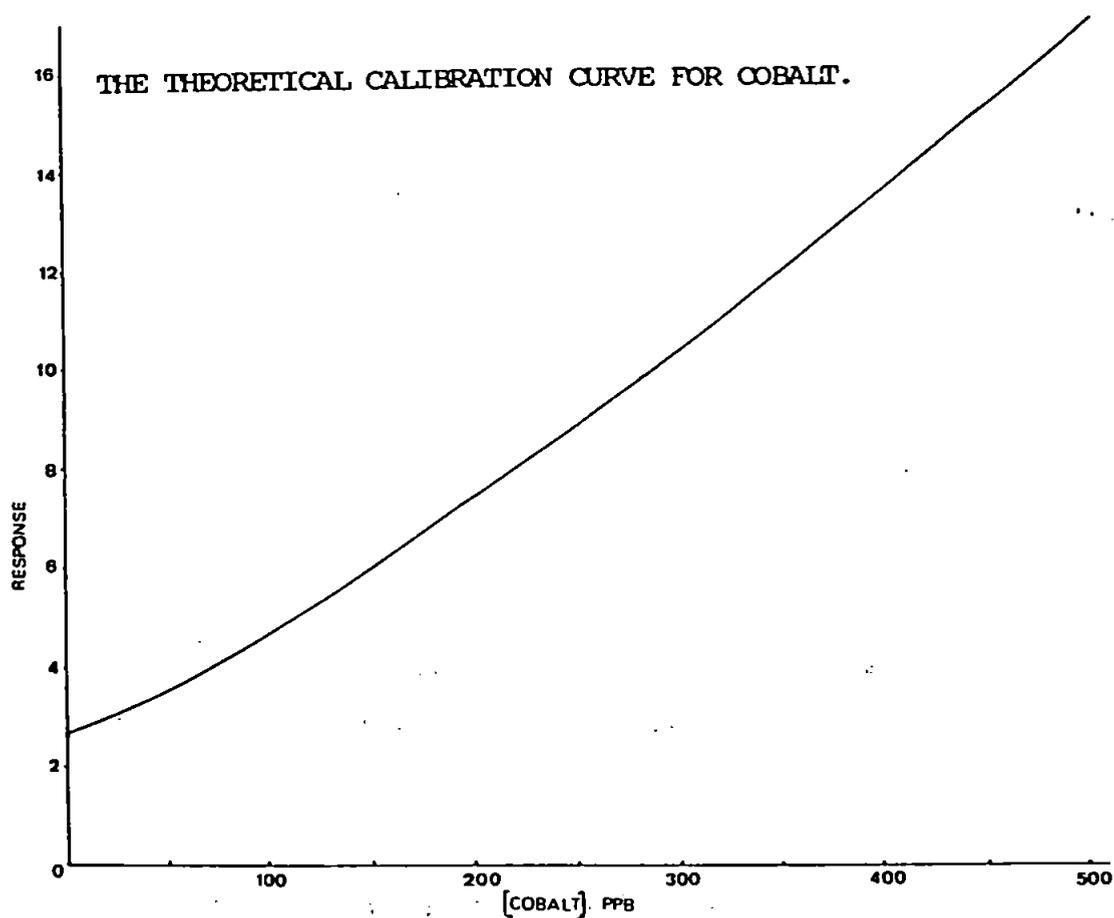
Analyte $M^{n+}$	[Co] moles	[EDTA] moles	$K_1(M-EDTA)^{4-n}$	$\beta_1(M-lactate)$
Co	$5 \times 10^{-6}$	$5.02 \times 10^{-6}$	16.3	2.52
Zn	"	"	16.5	3.2
Cd	"	"	16.5	2.28
Pb	"	"	18.0	3.50
Ca	$1 \times 10^{-5}$	$1.04 \times 10^{-5}$	10.7	1.44
Al	$5 \times 10^{-6}$	$5.02 \times 10^{-6}$	16.1	NA.

The calibration curve data for Co, Zn, Cd, Pb, Mg and Al are described in Table 5.9

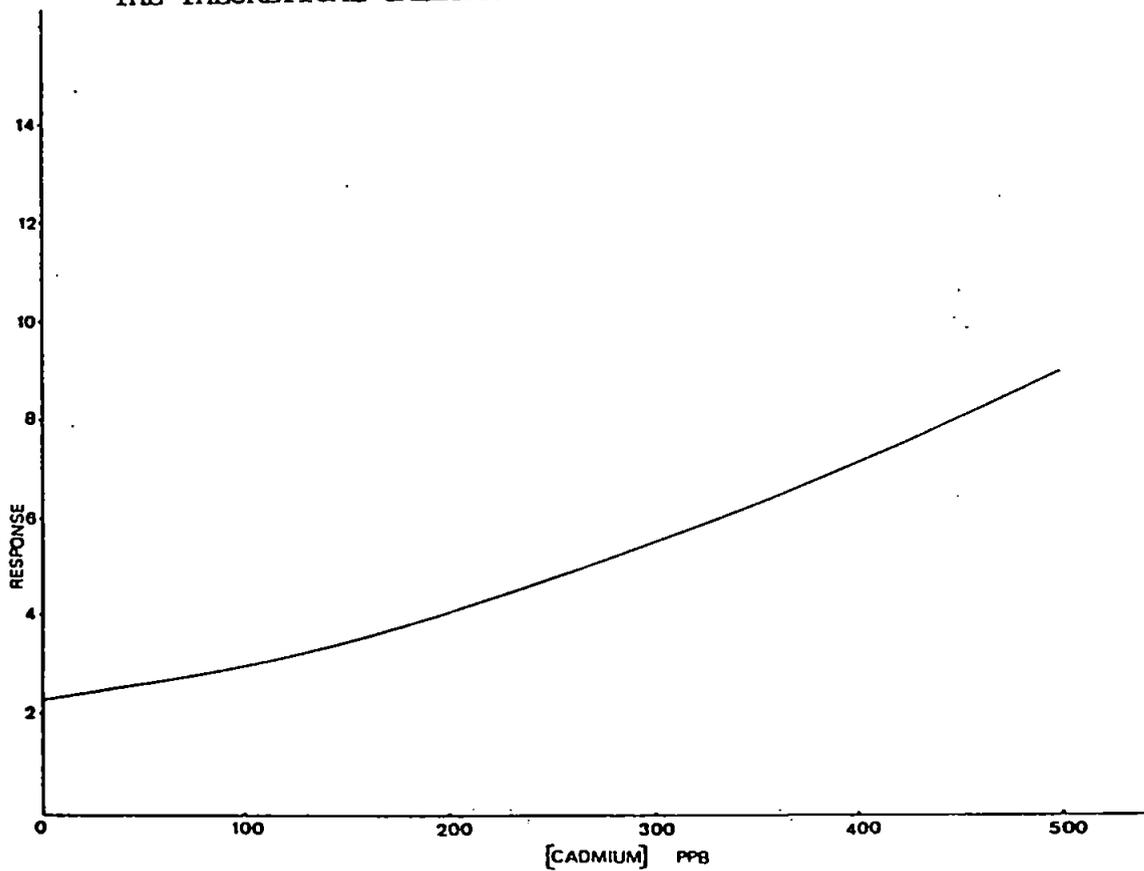
**Table 5.9** The CL species produced by the addition of metal analyte species to the eluent/Co-EDTA solution (pH between 3.0 and 4.5 depending on the analyte).

Metal	Concentration	Free Co <sup>2+</sup> %	Co-Lac( $\beta_1$ ) %	Total %
Co <sup>2+</sup>	0.0 ppb	0.62	2.0	2.62
	25.0	0.72	2.33	3.05
	50.0	0.84	2.70	3.54
	100.0	1.10	3.58	4.67
	250.0	2.10	6.78	8.88
	500.0	3.98	12.84	16.82
Zn <sup>2+</sup>	0.0 ppb	0.71	1.77	2.48
	25.0	0.83	1.79	2.62
	50.0	0.95	2.07	3.02
	100.0	1.25	2.71	3.96
	250.0	2.29	4.96	7.25
	500.0	4.00	8.65	12.65
Cd <sup>2+</sup>	0.0 ppb	0.68	1.57	2.25
	25.0	0.78	1.66	2.46
	50.0	0.85	1.83	2.68
	100.0	1.01	2.18	3.19
	250.0	1.61	3.48	5.09
	500.0	2.77	5.99	8.76
Pb <sup>2+</sup>	0.0 ppb	0.75	1.61	2.36
	25.0	0.75	1.61	2.36
	50.0	0.78	1.69	2.47
	100.0	0.86	1.87	2.73
	250.0	1.14	2.48	3.62
	500.0	1.72	3.72	5.44
Ca <sup>2+</sup>	0.0 ppm	1.82	4.54	6.36
	50.0	1.82	4.55	6.37
	100.0	1.82	4.56	6.38
	250.0	1.83	4.58	6.41
	500.0	1.84	4.61	6.45
	1000.0	1.86	4.68	6.54
Al <sup>3+</sup>	0.0 ppb	40.70	NA	40.70
	50.0	41.33	NA	41.33
	100.0	41.97	NA	41.97
	250.0	43.83	NA	43.83
	500.0	46.78	NA	46.78
	1000.0	50.07	NA	50.07

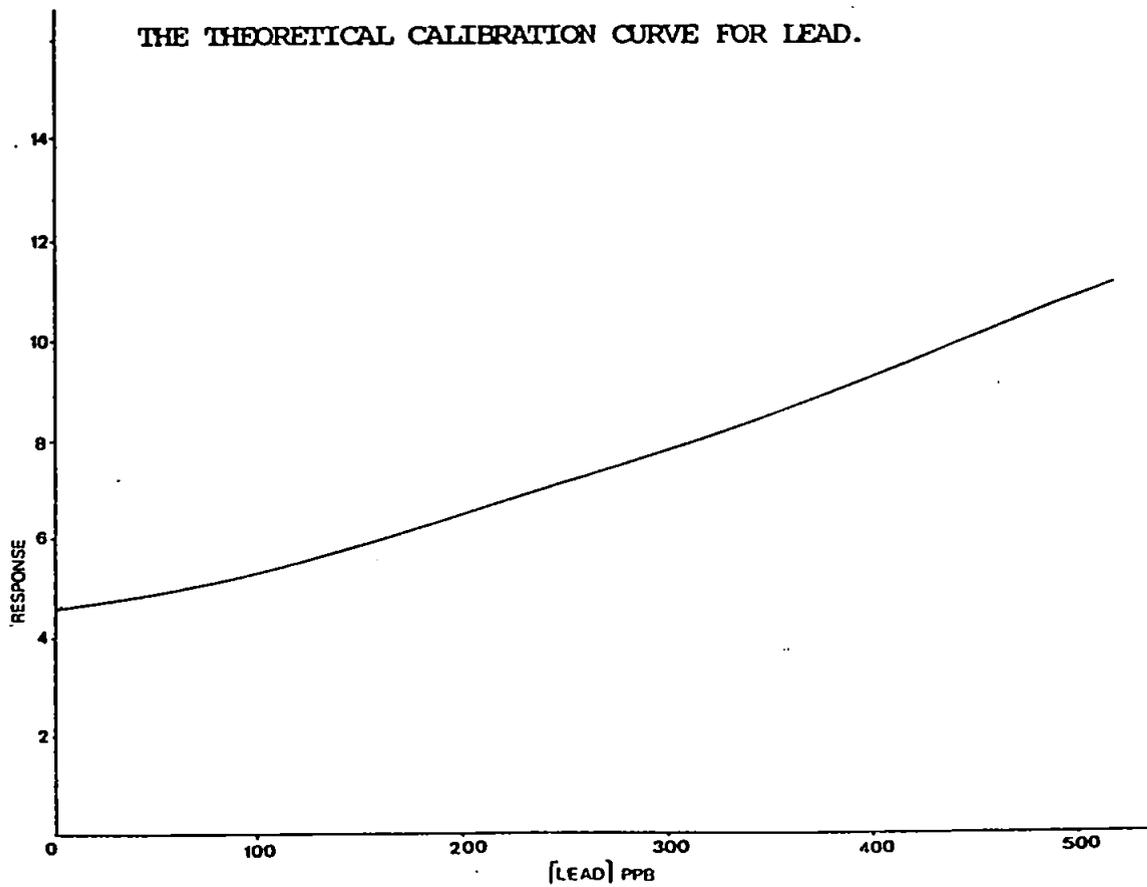
Figure 5.12 The theoretical calibration curves obtained by the thermodynamic model for the following analytes; Co, Zn, Cd, Pb, Ca and Al.



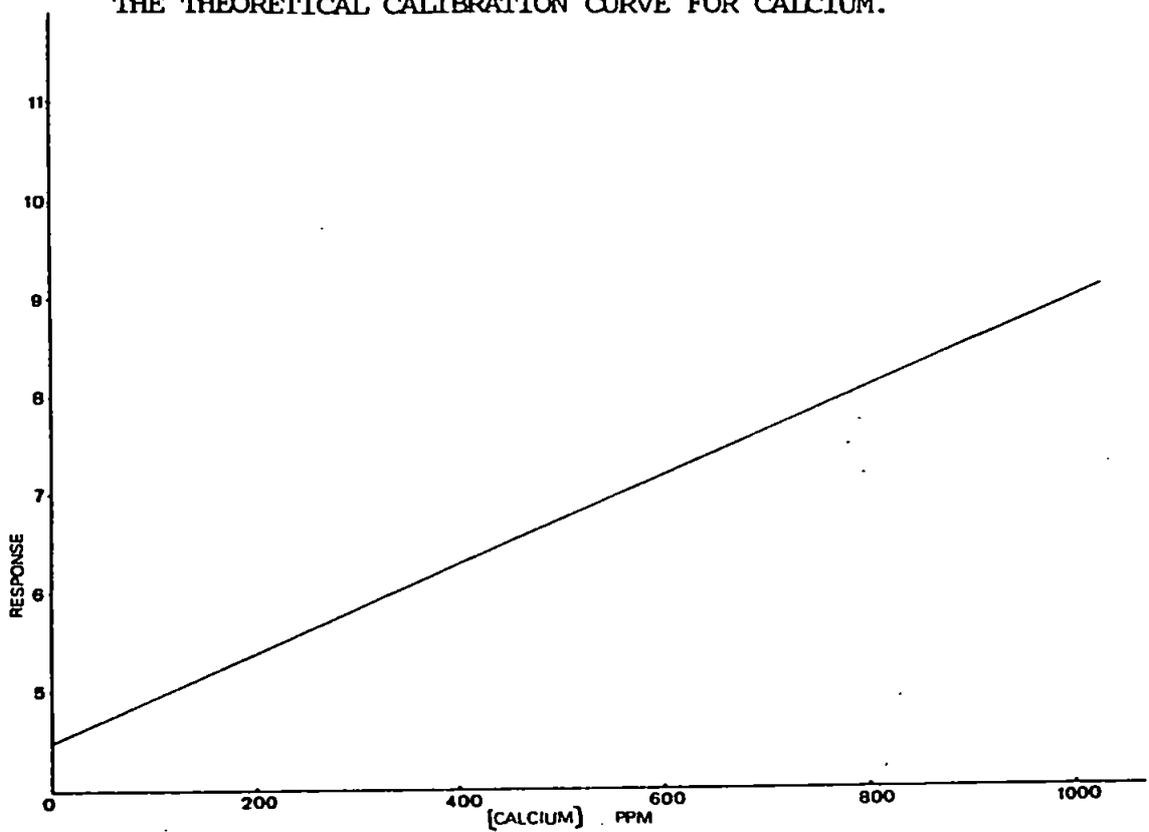
THE THEORETICAL CALIBRATION CURVE FOR CADMIUM.



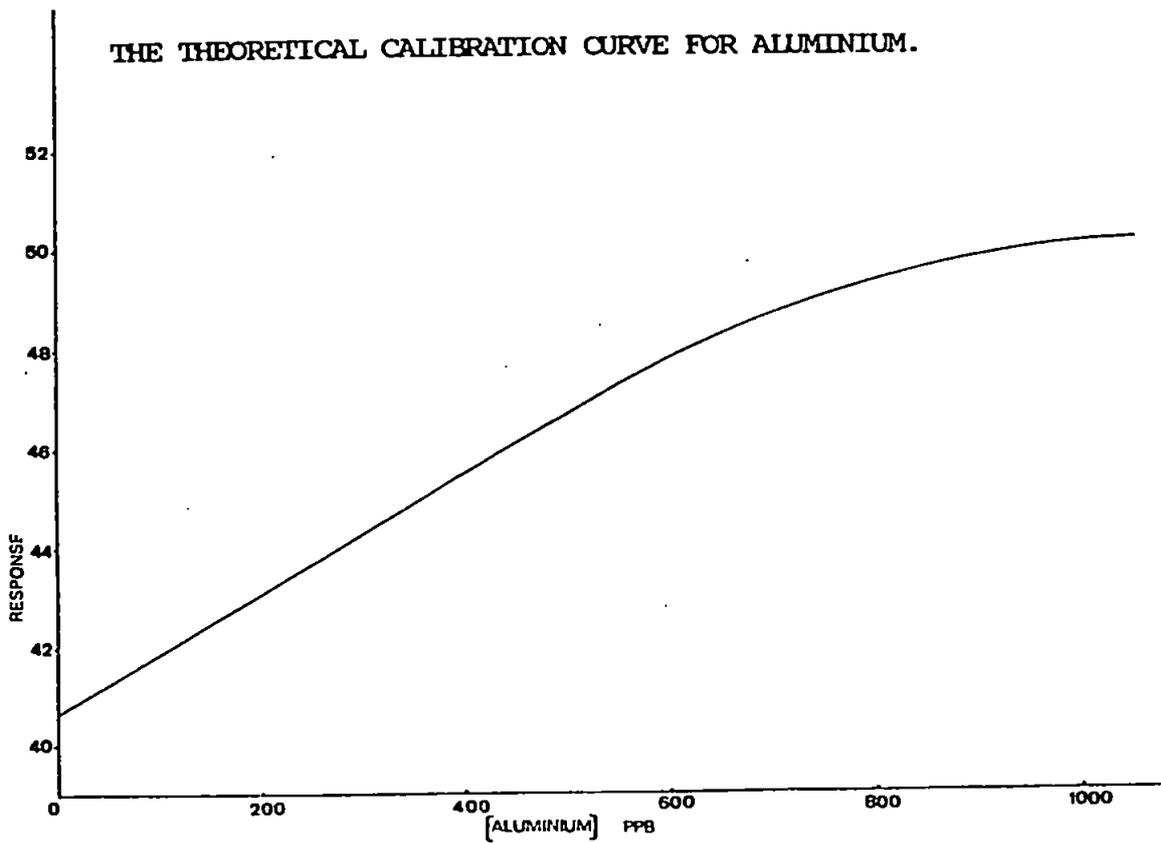
THE THEORETICAL CALIBRATION CURVE FOR LEAD.



THE THEORETICAL CALIBRATION CURVE FOR CALCIUM.



THE THEORETICAL CALIBRATION CURVE FOR ALUMINIUM.



also obscured by the very high background noise levels that are produced and thus, low detection limits are impossible to achieve. The positive - curvature that is experienced for other analyte metals possibly occurs for the alkaline-earths below this noise threshold and cannot be seen.

The presence of the lactic acid is thought to have a significant affect on the linearity of the system. The calibration curve for Al goes a long way to support this proposal as, both in theory and in practice, a linear calibration curve for Al is obtained when using a potassium sulphate eluent. The reason for this may be that the lactic acid is acting as a competitive chelating agent against the EDTA and complicating the simplistic metal/cobalt displacement reaction. Calibration curves for other metals using a potassium sulphate eluent were tried, but the sensitivity was badly affected and the separation of metals of similar charge was difficult to achieve.

Chelating eluting agents other than lactic acid were tried, including tartaric acid, malic acid, succinic acid and malonic acid. With the general increase in the  $\log K_1$  values for the metal complexes, the background noise levels were reduced but analyte sensitivity was adversely affected and no improvement in the linearity of the system was noted. The  $\log K_1$  values for succinic acid and malonic acid were sufficiently high to suppress the CL

signal completely.

With little or no improvement to the overall system when using different mobile phases, it was decided to continue with the lactic acid eluent for the remainder of the study.

Using lactic acid as the eluent, the detection limits for the various metal species, defined as three times the peak to peak variation of the background noise, are described in Table 5.10

#### 5.3.4.2 Multielement and Speciation Analysis

Figures 5.13, 5.14 and 5.15 show the separation and detection of various groups of cations including the alkaline-earths, members of the d block and also members of the p block. All the lanthanides respond and produce CL, although base-line resolution is difficult to achieve as shown in Figure 5.16.

Speciation studies are also possible to achieve as can be seen from Figure 5.13 which clearly shows the separation and detection of Fe(II) and Fe(III).

#### 5.3.4.3 Sample Analysis

To evaluate the quantitative performance and accuracy of this novel detector, it was decided to determine cations which have no direct catalytic effect on the luminol system. A simulated fresh water sample, IAEA/W4, was

**Table 5.10** Detection limits for various metal species using CL detection using experimental conditions described in Table 5.

Cation	Limit of Detection $\mu\text{g l}^{-1}$
Mg	100
Ca	50
Sr	100
Fe(II)	25
Fe(III)	5
Co	2
Ni	2
Zn	2
Cd	5
Pb	4
La	5
Ce	5
Pr	6
Ga	3
In	20
Al	5

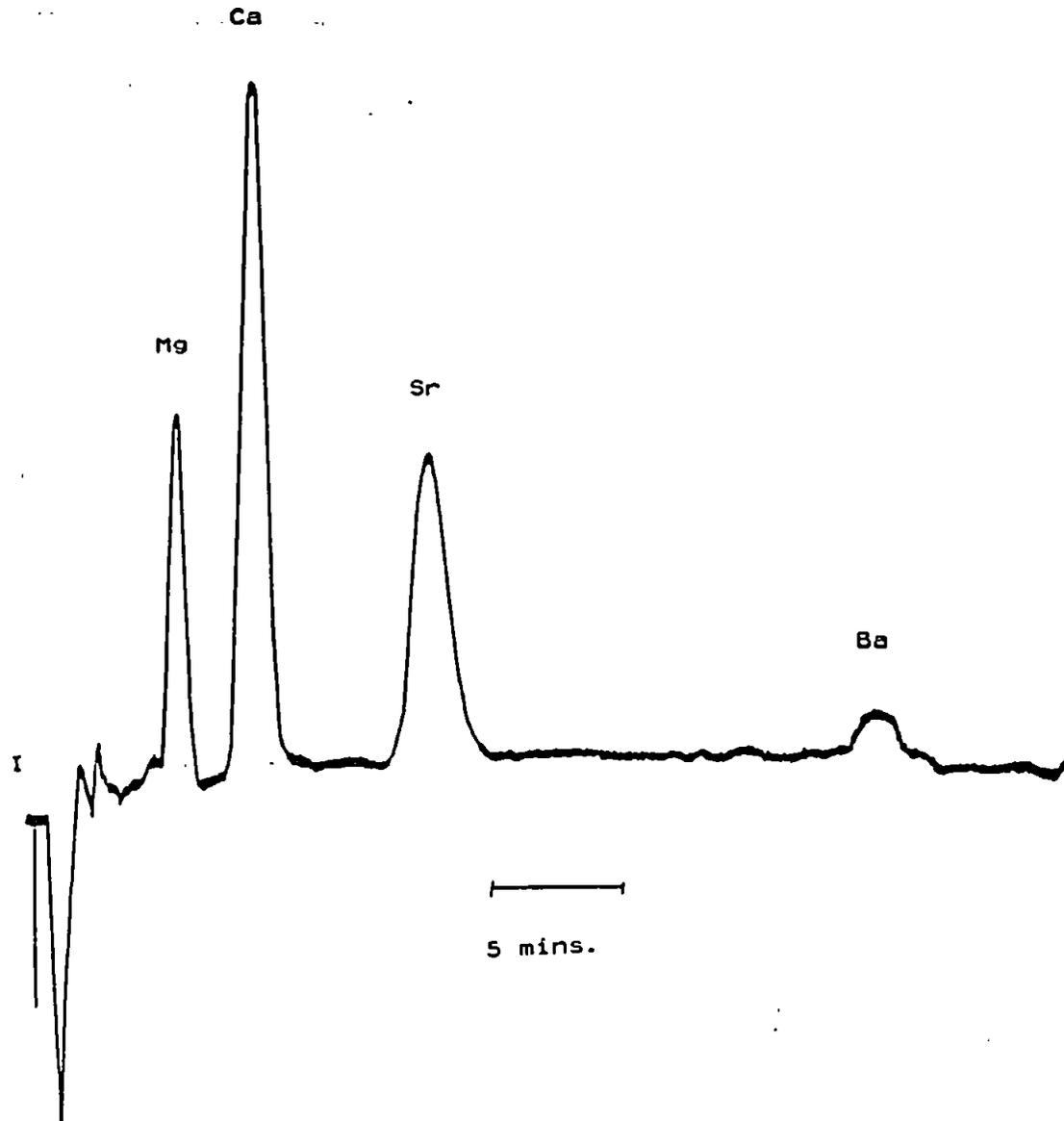


Figure 5.13 The multi-element determination of the alkali-earth metals.

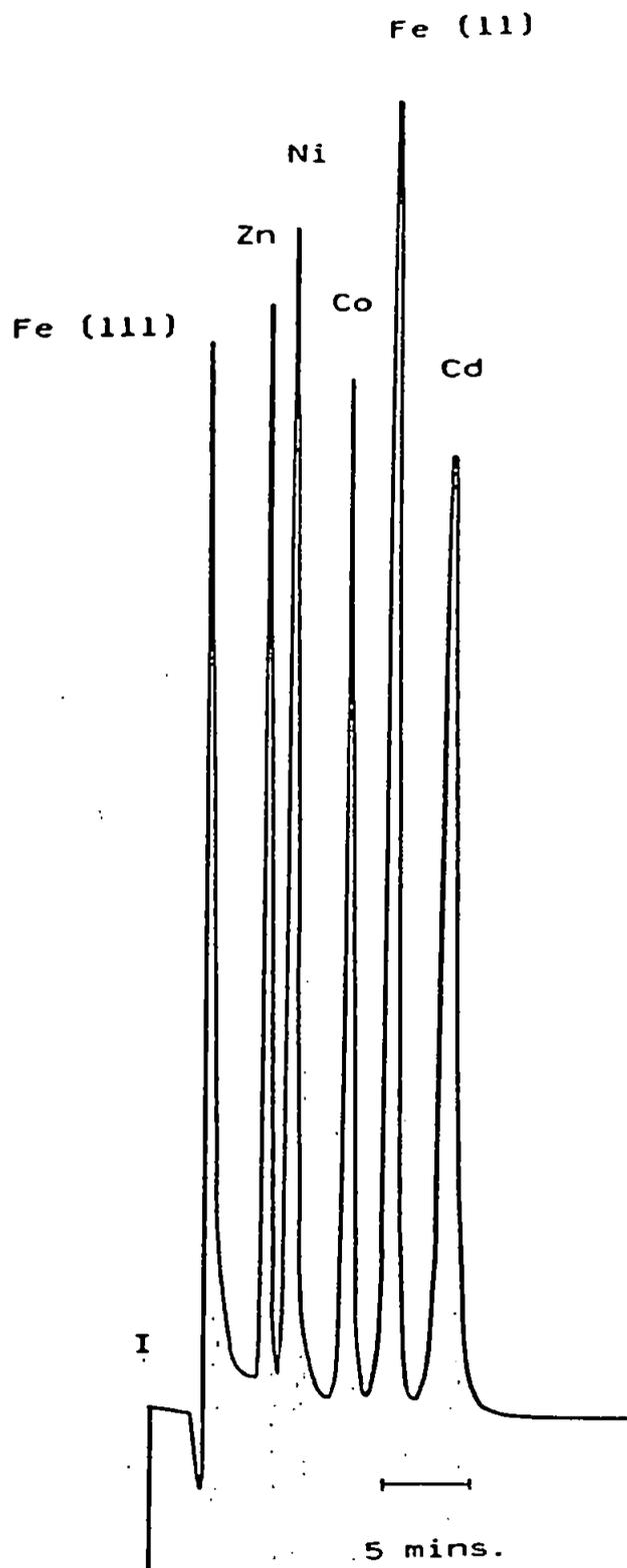


Figure 5.14 The multi-element determination of selected members of the d-block metals.

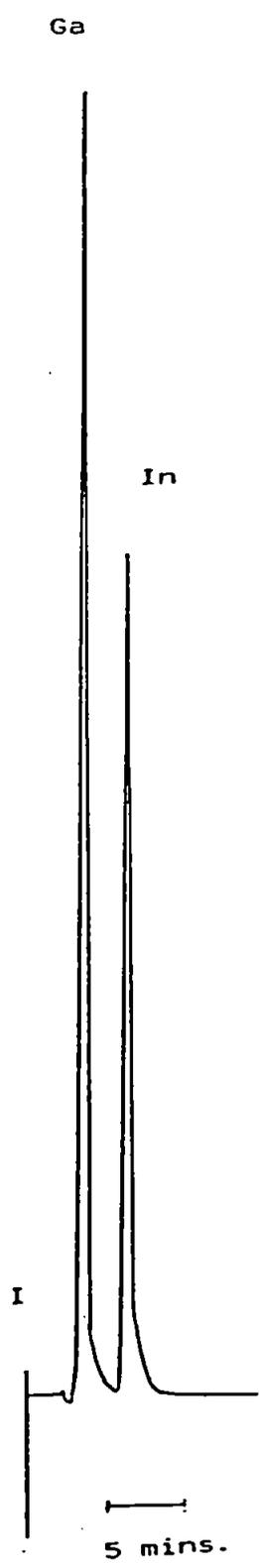


Figure 5.15 The multi-element determination of selected members of the p-block metals.

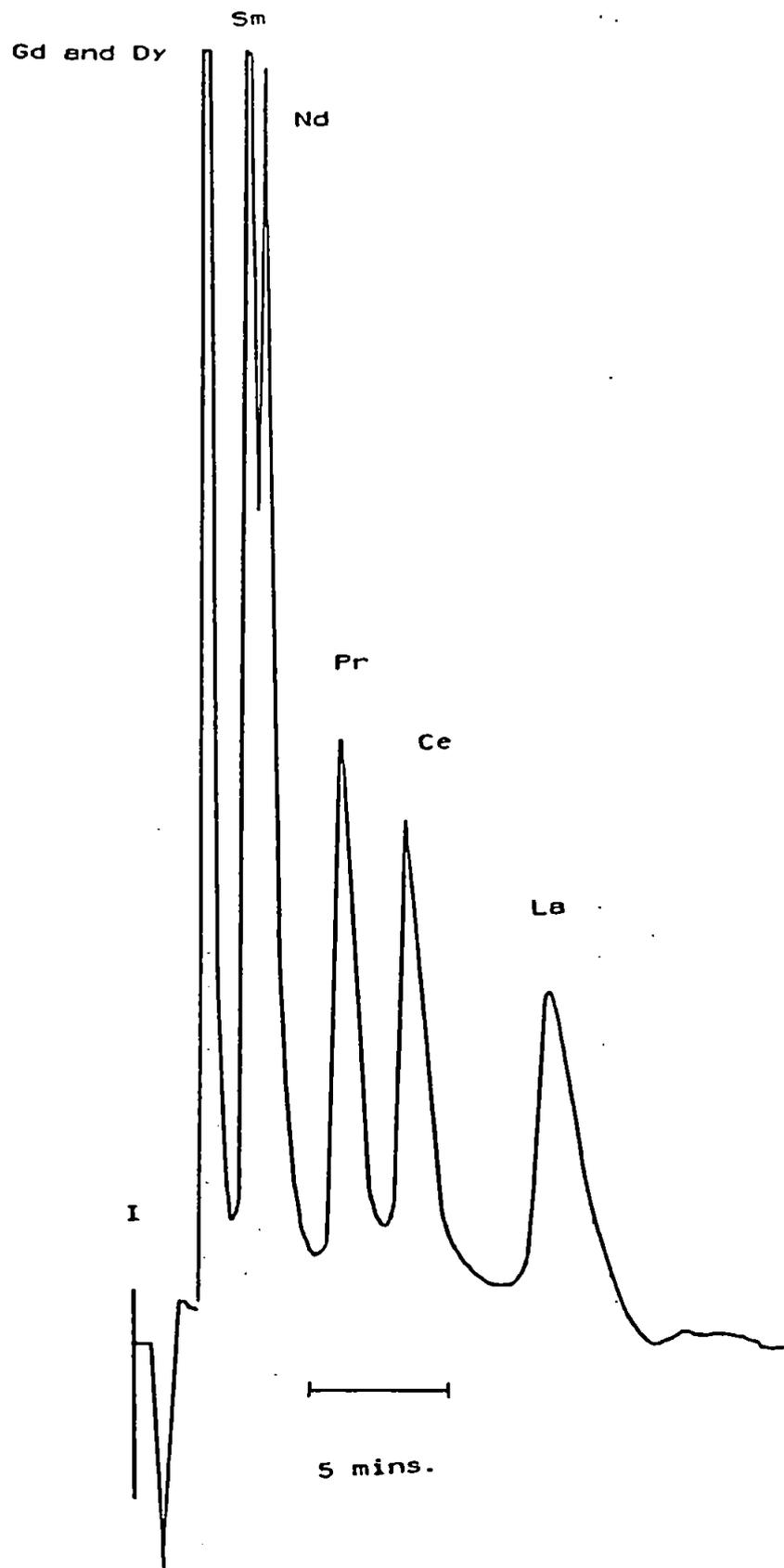


Figure 5.16 The multi-element determination of selected members of the lanthanide series.

chosen with a zinc content of  $48 \text{ ng ml}^{-1}$ , and an aluminium content of  $48 \text{ ng ml}^{-1}$  also.

The analysis was performed by simply injecting a 200  $\mu\text{l}$  aliquot of the sample followed by the standards and blank solutions. Figures 5.17 and 5.18 shows the standard calibration curves for zinc and aluminium respectively and Table 5.11 describes the certificate values and expected results.

#### 5.4 Concluding Discussion

The flexibility of rapid, sequential, multi-element determinations of selected metals using ion-chromatography coupled to a novel CL detector has been demonstrated. The employment of a displacement system that can detect weak or completely non-CL cations (e.g. Zn, Al, Cd, Pb, Ca, La etc) shows the universal and non-selective nature of such a detector and overcomes the restrictions imposed by the other highly selective CL systems such as those seen in Chapters 3 and 4.

The non-linearity of some calibration curves is a little disappointing and there is obviously quite a large scope for improvement of the detection system. One encouraging result is that the data obtained from the computer thermodynamic model also predicts this non-linear aspect of the system. Perhaps one way around this problem is to have the  $\text{Co}^{2+}$  ions in slight excess instead of the EDTA although, with the present system, an unacceptably high

Figure 5.17 The determination of zinc from a certified reference material IAEA / W4.

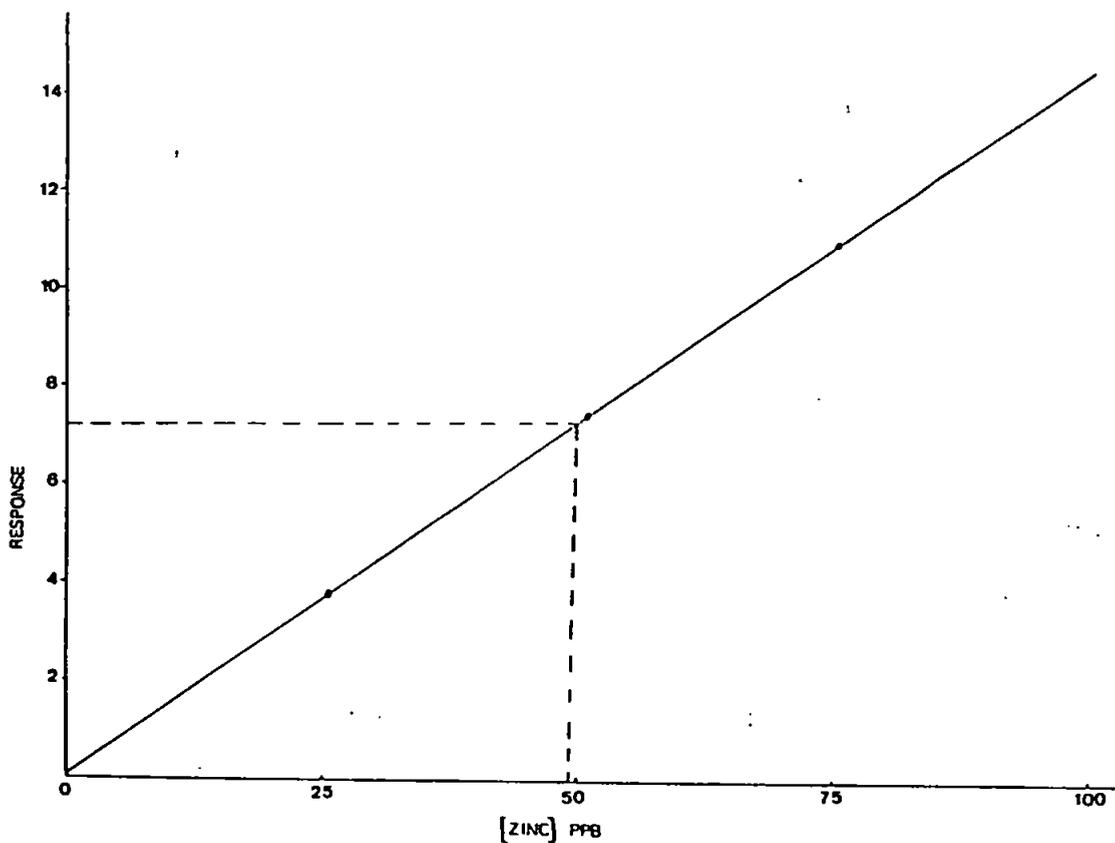
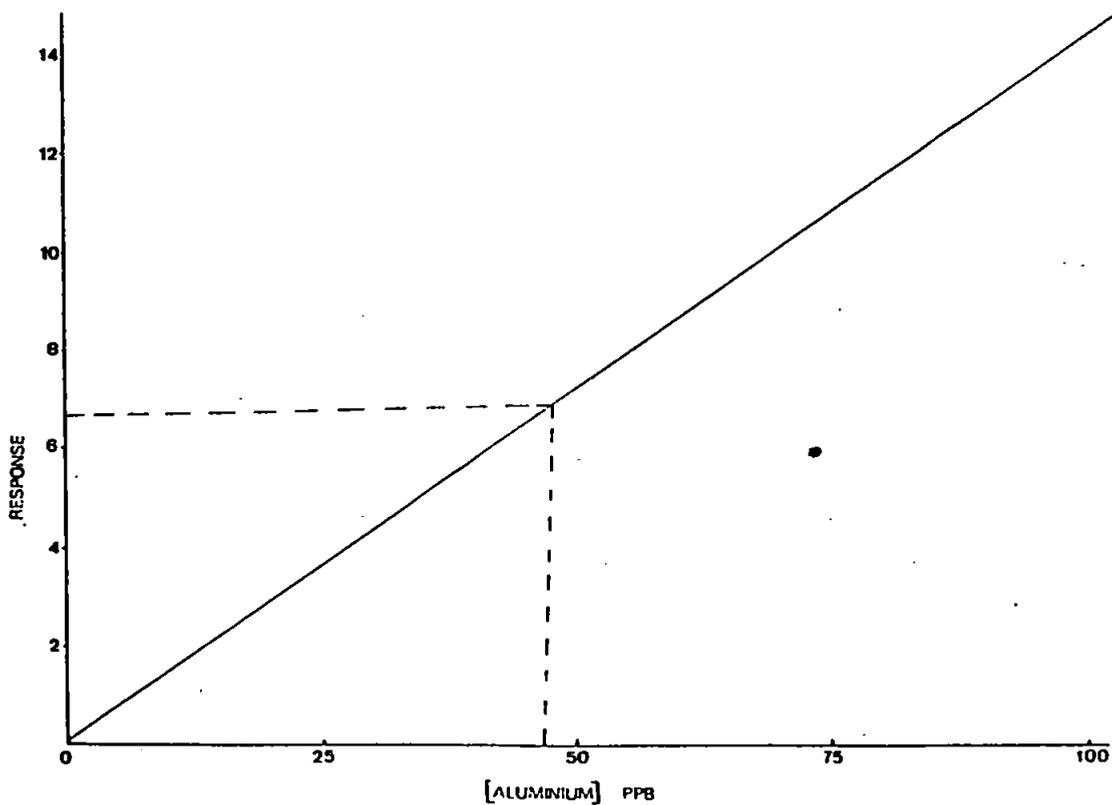


Figure 5.18 The determination of aluminium from a certified reference material IAEA / W4.



**Table 5.11** The determination of zinc and aluminium from a Certified Reference Material IAEA/W4

**Zinc**

Certificate value	48 $\mu\text{g l}^{-1}$
Confidence interval (signif. level 0.05)	43-54 $\mu\text{g l}^{-1}$
Found value $\bar{x}$ on 3 trials (this method)	48 $\mu\text{g l}^{-1}$
RSD on 3 trials	3.1%

**Aluminium**

Certificate value	48 $\mu\text{g l}^{-1}$
Confidence interval (signif. level 0.05)	40-53 $\mu\text{g l}^{-1}$
Found value $\bar{x}$ on 5 trials (this method)	44 $\mu\text{g l}^{-1}$
RSD on 5 trials	3.0%

background noise prevails. There is enormous potential for such a system if improvements in the background emission can be achieved because, at present, detection is based on the determination of a small signal on top of a large background level and this is obviously undesirable.

Even with the present drawbacks the limits of detection using this novel system are comparable with or better than most colorimetric post-column reagents, although again, it must be stressed that much better detection limits are possible with the elimination of the background emission.

This detection approach shows particular potential for the monitoring of natural and waste waters for elevated levels of trace metals. The detector can be made small and compact and the complexity of the pump system can be reduced by replacing the Co-EDTA and luminol post-column reagent with a dual channel peristaltic pump. Studies, however, indicate a worsening of detection limits by approximately a factor of two, due to increased baseline noise.

## CHAPTER 6

### CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

The research project was successful in meeting its original objectives and useful liquid chromatographic detection systems, based on CL, have been developed.

The determination of the ultra-trace levels of cobalt indicate how sensitive CL systems can be and the ultra-sensitive nature of such systems are of the utmost importance for particular applications. The use of the CL system described for ultra-trace Co determinations is presently being adopted and developed by A.E.E. Winfrith in an attempt to significantly improve the analysis of their P.W.R. coolant.

The simultaneous determination of trace levels of Cr(III) and Cr(VI) using CL detection, has been successfully achieved. No other system, for Cr determinations, reported in the literature has been able to simultaneously determine both Cr(III) and Cr(VI) without any sample pretreatment. The method developed and described in this research programme does not require sample pretreatment and, consequently, is less time consuming, not subject to pretreatment errors and is more amenable to automated analysis. For successful Cr determinations, many of the problems of both separation and detection have been solved with the consequent development of a reasonably cheap and reliable Cr detector showing, particular potential for the

continual monitoring of Cr in surface waters. Improvements can be made to this system with particular reference to changing the reduction system from an homogenous to a solid-state one. With this alteration the instrumentation would become simpler to use and less expensive to operate. With a further view to simpler instrumentation, the separation system could be altered to use mixed bed anion and cation-exchange columns or ion-exchange columns in series. With this alteration, a more conventional 6-port injection valve could be employed again making the instrumentational design more attractive.

The specific nature of CL reactions can limit their usefulness in terms of multielement analysis. One of the main research objectives was to create a more non-specific type post-column reagent system that was able to respond to a number of metal species. This problem of high specificity was successfully tackled using a displacement type system although there are still problems that need to be overcome. With the displacement system described in Chapter 5, metals from the s, p and d blocks were detected at levels approaching, or in some cases, better than current colorimetric IC detection techniques. For CL techniques to be adopted for routine trace-metal determinations, significant improvements in sensitivity, linearity and linear working ranges must be made.

The major problem that was found, when using the system described for multi-element analysis, was the high

background noise levels that were experienced. This background noise is a direct result of the relatively low pH level used for the Co-EDTA solution. At higher pH's it is known that the noise level, as expected, drops considerably thus enabling the possibility of much lower detection limits. In practice, however, higher pH's could not be used because of the problems experienced with peak distortion. It is thought that this distortion may be due to a thin coating of dye present on the post-column tubing. In order to eliminate this dye, a new method of producing the accurate 1:1 Co:EDTA molar ratio is required. It is not easy to see how a 1:1 ratio can be achieved, although careful weighing of pure Co and EDTA salts may prove to be accurate enough.

The linearity problem of the Co-EDTA system is thought also to be mainly due to the low pH level of the Co-EDTA solution. At this low pH level, the Co-EDTA is significantly uncomplexed giving rise to a lot of free  $\text{Co}^{2+}$  ions and consequently a high background. The background noise is so high, there needs to be a very slight excess of EDTA present in order to maintain noise levels at manageable levels (even with the use of light filters). At the low end of the calibration curves, for most of the elements studied, there was a notable non-linear curve produced. One pleasing aspect about this result is that the theoretical thermodynamic computer model predicts the non-linearity observed. The non-linearity is presumably due to the excess EDTA mopping up

a small amount of the analyte species. Some degree of linearity is seen as analyte concentration increases which supports this idea, as the calibration linearity can only occur when the excess EDTA is exhausted. This problem needs to be looked at in more detail, and the computer model could prove to be extremely useful in the design of new experiments.

Another factor that has been shown to influence the linearity of the Co-EDTA system is the presence of a competing complexing agent in the form of the mobile phase. One way to reduce this problem is to move away from ion-exchange chromatography and to use chelation-exchange which requires no complexing agent present, as separation is achieved merely by pH control. As the mobile phase is potentially very simple for chelation exchange, even lower detection limits should be possible and the pursuit of this type of system may prove to be fruitful in the future.

Finally, the employment of the Co-EDTA displacement system has been shown to work reasonably well. Other systems, based on solid-state reactors, may prove to be extremely useful, not only in terms of better detector performance but also would produce simpler instrumentation. Cobalt ions, or other CL species, could perhaps be immobilized onto chelating exchange materials. The presence of analyte ions would displace the CL active species and produce an analyte signal. This type of displacement

system, be it homogenous or solid-state, offers attractive possibilities as a great number of various CL species could be exploited including; the catalyst, the oxidant, the co-oxidant or the luminol itself, in an attempt to produce better liquid chromatographic detection systems.

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## MEETINGS ATTENDED, PRESENTATIONS AND PUBLICATIONS

### Meetings Attended

- (i) North East Region, Atomic Spectroscopy and Molecular Spectroscopy Groups jointly with the UV Spectroscopy Group and Atomic Spectrometry Group of the RSC, meeting on "Recent Advances in Atomic and Molecular Spectroscopy", 29th and 30th March, 1988, Hull.
- (ii) Analytical Division of the RSC, meeting on "25th Anniversary of the Research and Development Topics in Analytical Chemistry", 18th and 19th July, 1988, Plymouth.
- (iii) The International Association of Environmental Analytical Chemistry, meeting on "6th Symposium on Ion-Chromatography", 9th-13th April 1989, Sils-Maria, Switzerland.
- (iv) The International Association of Environmental Analytical Chemistry, meeting on "1st International Symposium on Detection in Liquid Chromatography and flow Injection Analysis", 19th -21st September, 1989, Cordoba, Spain.
- (v) The Department of Trade and Industry, meeting on "The Analysis by Emitted Light", 5th-7th March, 1990, Plymouth.
- (vi) Analytical Division of the RSC, meeting on "Research and Development Topics in Analytical Chemistry" ICI Runcorn, 18th and 19th July, 1990, Runcorn.

(vii) Austrian Society for Analytical Chemistry, European Conference on Analytical Chemistry "Euroanalysis VII" 26th - 31st August, 1990, Vienna, Austria.

### Presentations and Publications

Resulting from the work reported in this thesis, the following papers have been presented and published.

#### Presentations

- (i) "The ultra-sensitive determination of cobalt using chemiluminescence after separation by ion-chromatography". Poster presented at Research and Development Topics in Analytical Chemistry, 18th and 19th July, 1988, Plymouth.
- (ii) "The determination of trace metals by ion-chromatography with post-column reagent detection". Paper presented during a research visit to ICI Brixham 18th October, 1988, Brixham.
- (iii) "Simultaneous determination of Cr(III) and Cr(VI) at ultra trace levels using ion-chromatography with chemiluminescence detection". Poster presented at the 6th Symposium on Ion-Chromatography, 9th - 12th April, 1989, Sils-Maria, Switzerland.

- (iv) "The development of a novel, multi-element detection system for trace metal analysis based on chemiluminescence after separation by ion-chromatography". Poster presented at the 1st International Symposium on Detection in Liquid Chromatography and Flow Injection Analysis". 19th -21st September, 1989, Cordoba, Spain.
- (v) "Multi-element detection for trace metals based on chemiluminescence after liquid chromatographic separation". Poster presented to the Research and Development Topics in Analytical Chemistry meeting, 18th-19th July, 1990, Runcorn.
- (vi) "Multi-element trace metal detection using ion-chromatography coupled with chemiluminescence detection". Poster presented at Euroanalysis VII, 26th-31st August 1990, Vienna, Austria.

#### Publications

- (i) Jones, P., Williams, T. and Ebdon, L.,  
"Determination of Cobalt at Picogram Levels by HPLC with Chemiluminescent Detection".  
Anal. Chim. Acta., 1989, 217, 157.
- (ii) Williams, T., Jones, P. and Ebdon, L.,  
"Simultaneous Determination of Cr(III) and Cr(VI) at Ultra Trace Levels using Ion-Chromatography with Chemiluminescence Detection".  
J. Chrom., 1989, 482, 361.

- (iii) Jones, P., Williams, T. and Ebdon, L.,  
"Development of a Novel Multi-Element Detection  
System for Trace Metal Determination", Based on  
Chemiluminescence after Separation by Ion-  
Chromatography.  
Anal. Chim. Acta, 1990, 237, 291.