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STUDY OF TRACE ELEMENT DETERMINATION BY ION CHROMATOGRAPHY WITH CHEMILUMINESCENCE DETECTION

BEERE, HUGH GRAHAM

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STUDY OF TRACE ELEMENT DETERMINATION

BY ION CHROMATOGRAPHY

WITH CHEMILUMINESCENCE DETECTION.

by

HUGH GRAHAM BEERE

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AUTHOR'S DECLARATION

At no time during the registration for degree of Doctor of Philosophy has the author been registered for any other University award.

Author:

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STUDY OF TRACE ELEMENT DETERMINATION BY ION CHROMATOGRAPHY WITH CHEMILUMINESCENCE DETECTION.

Hugh Graham Beere

ABSTRACT

This thesis details the development of several highly sensitive liquid chromatography post-column reaction detectors based on chemiluminescence (CL) for the ion chromatography (IC) determination of metal species in a range of sample types.

The first chapter describes a non-selective multi-element CL detector based on metal-ligand reactions involving cobalt (II) and EDTA. A complexation reaction was designed so that eluting metal species displaced or produced equivalent amount of free cobalt ions which then catalysed the CL oxidation of luminol. The effect of pH, temperature and reaction time was investigated and optimised. It was found that the detector responded to a wide range of metals capable of forming EDTA complexes, even the relatively weak complexes such as those of magnesium and calcium. However, problems with the high back-ground signal limited sensitivity to the low $\mu g \ l^{-1}$ range.

The next chapter deals with the development of a highly sensitive IC method for the determination of two environmentally important chromium species, namely chromium (III) and chromium (VI). A rapid ion exchange separation was achieved using a single column with potassium chloride eluent. This was incorporated into a luminol-H_2O_2 CL detector, specific for chromium (III). On-line reduction was required in order to visualise the chromium (VI). Detection limits for chromium (III) and chromium (VI) wer 0.05 $\mu g \ l^{-1}$ and 0.1 $\mu g \ l^{-1}$ respectively. Good results were obtained with a freshwater standard reference material and lyophilised samples as part of the author's participation in a Bureau Communtaire de reference (BCR) certification exercise. Sample pH was found to have considerable influence on the stability of the species and this is described and discussed.

The third and largest part of the study involved the development of an IC system for the ultra-trace determination of silver in pressurised water reactor (PWR) primary coolant, of particular concern to the nuclear power industry. A novel ion exchange separation was achieved on hydrophillic resins giving excellent separation from divalent cations. A CL post column reaction detector was designed based on the oxidation of luminol with persulphate. Good quantitative performance was accomplished based on the analysis of a certified reference material and simulated PWR coolant with detection limit for silver of 0.05 $\mu g \ l^{-1}$.

Finally, a CL detection system was developed for determining gold (III) after IC separation. A novel aspect was that no added co-oxidant was required for the luminol reaction. Results for a standard reference metal alloy sample was in good agreement with the certified value. Again, high sensitivity was achieved with a detection limit of 0.25 $\mu g \ l^{-1}$.

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CHAPTER ONE.

1.0   INTRODUCTION

1.1   Trace Elemental Analysis

Trace analysis has now come to mean the low level determination of analytes, although the term was first used in the 1950's to describe determination of analytes in plant material at the limits of detection of the techniques then available. Detection limits were generally 0.1 mg l^{-1} to 10 mg l^{-1}. The methods available at present far exceed the limits of detection shown by earlier methods, thus detection below 0.1 mg l^{-1} is often referred to as ultra-trace analysis.

Grasselli [1] describes how the detection limits, analysis time and sample size has decreased over the last forty years since the introduction of instrumental techniques into analytical chemistry. This is illustrated with a presentation of lead determination over this period, in which the detection limits of the techniques used up until 1970's would be incapable of detecting lead at present Federal guideline concentrations (American) [1].

Lead is not the only element of concern in the environment, its non-essential nature has made it easier to determine toxic effects. However, essential metals can also exhibit toxicity if present at elevated concentrations, for example the presence of iron (III) in high concentrations gives rise to siderosis whilst decreased concentrations can lead to anaemia. The effects of essential trace metals in biological systems is more complex

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than that of non-essential metals as there are upper and lower limits on the acceptable levels which vary from metal to metal. The role of metals in biological systems and metabolism is slowly being understood. Apart from biological considerations there is tremendous interest in trace element determination connected with many industrial processes, not only the process itself but also in waste management. Two diverse examples illustrate this point from the semiconductor and nuclear industries. However, there is a need in industry and waste management to determine trace element levels in a wide variety of complex matrices.

The electronics industry based upon silicon semiconductor technology uses very high purity compounds which are doped with various elements to modify characteristics. The presence of contaminants such as iron, copper and nickel at sub ng g^{-1} level may alter semiconductor performance (2). High purity quality control is required in related industries eg. fibre optic, synthetic gem stone and pharmaceutical industries.

The nuclear industry is also concerned about trace element contamination. Secondary coolant circuits in Pressurized Water Reactors (PWRs) contain trace impurities which can concentrate to form corrosive brine, corroding the secondary circuit especially in the steam generator. The steam generator is housed in the reactor building where it interfaces, out-of-core, with the primary coolant circuit, Figure 1 (3). Replacement of corroded parts of the secondary circuit in this area is expensive as it requires the shut-down of the reactor and working time is limited because of the radioactive environment (4, 5). Radioactivity in this out-of-core area is affected mainly by the radiation emitted by trace impurities in the primary coolant (4). The primary coolant circuit of PWRs is constructed from specialist materials which are chosen for their resistance to corrosion.
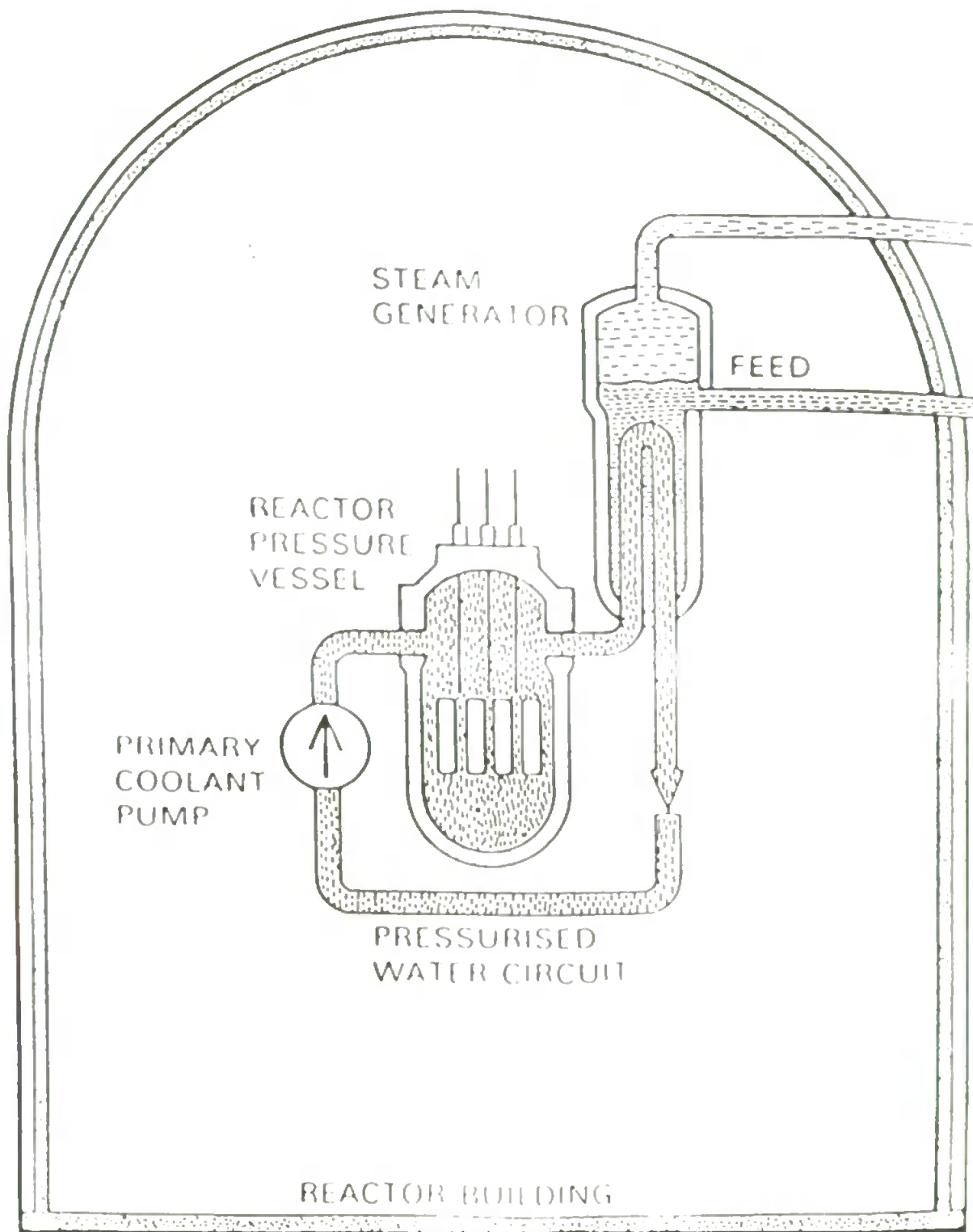
Figure 1   Diagram showing the relationship between the primary and secondary coolant circuits in a pressurized water reactor. Vertical dashed lines = primary coolant curcuit, horizontal dashed lines = secondary coolant curcuit.

However, trace amounts of metals are released from the surface of these construction materials or are introduced from the high purity chemicals and water used. These can then be activated in the core and deposited in out-of-core areas. The radionuclides particularly of cobalt (^{58}Co and ^{60}Co), but also other cations, are of concern due to the effect power cycling has on their mobility and out-of-core activity (5, 6, 7, 8).

There is little doubt that with improvements in detection has come an understanding of various processes that occur in the environment, biological/medical fields and industry. However, it has also become apparent that very low detection limits are only part of the answer. Many authors report that there is presently interest not just in detection limits but also in determining the speciation or chemical form of an element. As Behne (9) points out "However, with increasing knowledge of the metabolism and biological effects of trace elements, it has become increasingly apparent there is a need not only to determine the total levels of an element in tissues and body fluids but also to measure its different chemical forms quantitatively." The speciation of an element describes the form in which the element is present and this may differentiate between different phases. Speciation also refers to the oxidation state of an element or the incorporation of an element in different molecular types. This can have a dramatic effect on the toxicity of an element, or its fate in metabolism. The focus of much attention regarding speciation is directed towards the environmental aspect and toxic effects of speciation. This is particularly so for metallic compounds because once a metal is in the environment it is not biodegradable, its toxicity is controlled by its chemical form (10).

There are many examples where the change in speciation of a metal can effect the toxicity, in general the formation of organo-metallic compounds increases the toxic effect of metals, (9, 11) and has been related to easier transport across the blood-brain barrier of the organo-metallic species, for example methylmercury, triethyltin and tetraethyllead (9).

1.2   Trace Analytical Techniques.

Elements can be determined quantitatively by chemical or instrumental methods. Chemical methods of analysis eg. titrimetry and gravimetry are still used to determine concentrations of analytes where the need for trace analysis is not required. Chemical methods take longer, are in general not as sensitive as instrumental methods and therefore have tended to be superseded by instrumental methods when there is a requirement for sensitive or rapid determination.

Each of the techniques have their merits and are complementary to each other, the choice of technique to be used is often based upon a number of factors, not always analytical, these include: limit of detection, reproducibility, reliability, capital cost, running costs, skilled personnel, ability to determine species and place of use. (1, 10, 12, 13). These are shown for a number of chosen instrumental methods in Table 1.

Table 1   Some aspects that may be relevant in choosing an instrumental technique.

H = high, L = low, M = moderate, F = fast, Y = yes, N = no.

Method	Accuracy	Typical concentration range (moles l^{-1})	Speed	Robust	Cost
Atomic Spectrometry	M	10^{-3} to 10^{-9}	F	N	M-H
Plasma-OES	M	10^{-5} to 10^{-9}	F	N	H
Neutron Activation	M	10^{-5} to 10^{-12}	S	N	H
ICP-MS	H	10^{-2} to 10^{-11}	F	N	H
Spectrophotometry	M	10^{-3} to 10^{-6}	M-F	Y	L-M
Fluorescence	M	10^{-3} to 10^{-9}	M-F	N	M-H
Voltammetry	M	10^{-3} to 10^{-10}	M	N	M
Potentiometry	M	10^{-1} to 10^{-7}	M-F	Y	L-M
Chromatography	M	10^{-3} to 10^{-9}	F	Y	M

Chromatography, in particular high performance liquid chromatography (HPLC) offers

several advantages over other instrumental techniques, which include the following:

1) The initial outlay required is not great, from Table 1 it can be seen that a
   moderately priced instrument is capable of detection as sensitive as other
   more expensive instrumentation.

2) The time for the analysis for a sample is not high if a number of analytes
   are to be determined in a single injection.

3) The instrumentation is simple to operate on a routine basis, and may be
   easily automated. Chromatographs can be easily moved and may be
   used as self contained units in industrial environments.

4) Different species of a compound may be separated, this is useful in
   environmental applications where the toxic fraction of a compound or
   element has to be determined.

5) Preconcentration methods can be used, where the detection method is not
   sufficiently sensitive to determine the analyte directly.

6) Separation of the sample matrix from the analyte is a feature of most
   chromatographic applications, and can lead to improved detection of the
   analyte.

The above list is general and parts may be more applicable in some applications than in

others. However, two of the advantages should be highlighted namely the ability to

investigate speciation and the ease of adaption to monitoring industrial processes. Thus

further developments or chromatographic techniques will produce an important

complementary or alternative approach to atomic spectroscopic techniques. In fact the

linking of chromatography and spectroscopy in hybrid systems in now an essential part of present trace element investigations.

1.3   Chromatography.

The main types of chromatography used in trace analysis are gas chromatography (GC) and high performance liquid chromatography (HPLC). Although other forms of chromatography are used eg. planar chromatography, the faster separations, higher resolution and lower detection limits of GC and HPLC have led the use of these to far exceed other types of chromatography in trace element analysis. Although GC can be used in the analysis of metals, HPLC, in particular ion chromatography, is the most important chromatographic technique used for the analysis of metal ions.

1.4   High Performance Liquid Chromatography.

1.4.1   Development.

In the 1950's classical column chromatography had become more efficient. The improved efficiency, by reduction of the particle size, had led to unacceptably slow elution with a liquid mobile phase. Two possibilities existed for further development, either reduce the viscosity of the mobile phase or develop a forced flow system. In practical terms the use of a lower viscosity fluid, gas, was easier and led to the development of gas chromatography (14). The development of a forced flow system

with a liquid eluent took longer. However one advantage that forced flow chromatography has over gas chromatography is the ability to chromatograph thermally labile, non-volatile and ionic compounds.

Forced flow chromatography led to the development of HPLC during the 1960's. HPLC uses small diameter packing on which to separate the solutes. Particle size is typically 5 to 50 μm diameter, packed into columns between 5 cm and 25 cm long and 3 to 5 mm in diameter. The use of small particles, 3-10 μm diameter gives very efficient chromatography columns, though the lower the particle diameter the higher the back-pressure.

The chromatography of metals paralleled the development of HPLC in the 1960's and 1970's towards separation and detection in organic compound analysis, though at much lower levels of investigation. Organic molecules have been successfully chromatographed using adsorption or partition chromatography. However, as metals and some non-metals exist in solution as the hydrated ions or charged complexes, cations and anions are more amenable to straightforward separation by ion exchange chromatography than by adsorption or partition chromatography. The development of high performance ion exchange liquid chromatography in the 1970's revolutionized the detection of non-metallic anions and the amenability of HPLC to metal ions. Modern high efficiency ion exchange separations form the largest part of HPLC techniques for the separation of ions now called ion chromatography (IC).

A HPLC system consists of three distinct interdependent parts: the solvent delivery system, the separation system and the detection system. The solvent delivery system

is usually a twin headed reciprocating pump. Development of inert pump heads, either

titanium, poly ethyl ether ketone (PEEK) or poly tetrafluoro ethylene (PTFE) and

electronic feedback to suppress pump noise have been the most important advances in

recent years. The separation system and detection system are of most interest and shall

be treated separately. It is important to remember that changes in separation can

influence detection eg. complexes used in the separation step in partition and absorbtion

chromatography are also chosen for their ability to be detected. Conversely,

improvement in detection systems has led to the ability to develop ion exchange

chromatography elution systems.

1.4.2   Separation of solutes by high performance liquid chromatography.

Elution of a solute (or analyte) occurs by a series of repetitive interactions of the solute

between the mobile and stationary phases. Differences in these interactions for

different solutes leads to different elution times and so separation occurs. HPLC is

classified according to the type of interaction and the manner with which these

interactions occur.

There are three important modes of HPLC in inorganic analysis. These are adsorption,

partition and ion exchange. Both adsorption and partition chromatography require that

hydrated metal ions are first derivatized into very stable neutral complexes with organic

molecules. The inclusion of the metal ions in complexes can effect the ability of the

ions to be detected by post column reaction detection. The work presented here

exploits the catalysis of chemiluminescence reactions by metal ions, the efficiency of

which can be seriously affected by complexation. It is therefore unlikely that these two forms of chromatography would be suitable for use with chemiluminescence detection. In any event the pre-column formation of complexes could seriously disturb the integrity of the sample and make speciation virtually impossible. Thus, ion exchange is the preferred separation mode. This is both because it is likely that this separation mode will have the least detrimental effect on the detection system, and because it is the most useful way to analyze metal ions as the metal ions are soluble in the aqueous eluents used, and can be injected with minimal sample pretreatment. Whilst complexes are frequently used in IC eluents to separate multivalent cations, these complexes are intentionally not as stable as the complexes used in adsorption or partition chromatography and hence have less effect on chemiluminescence detection systems.

The different types of stationary phases that may be used in IC and the processes involved in the retention and subsequent elution of an analyte on a column by IC are described below. Methods for experimentally determining the chromatographic parameters of column efficiency and resolution are also given. Resolution of two or more solutes (or analytes) is dependent on column efficiency, capacity and the separation coefficient of the solutes to be separated. The effect that selectivity, column efficiency and capacity has on the resolution of two solutes is also described.

1.5   Ion Chromatography.

1.5.1   Stationary phases in ion chromatography.

Classical column ion exchange mainly involved resins of polystyrene cross-linked with divinyl benzene (PS-DVB resins). They are formed by polymerising monomers which contained suitable moieties to give functionalisation. With low cross linking these resins provided a packing of quite large particle size that proved satisfactory at the low pressure used, generally gravity fed. However, at the higher pressures required in HPLC large, low cross-linked resins collapse.

Silica had been widely used as a support for HPLC due to its ability to withstand the high pressures, the small particles with narrow particle size distribution gave high efficiency. However, silica is soluble in the aqueous eluents used in IC especially at high pH, with an operating range between pH 2 to 8. Pellicular silica is used in IC but careful pH control and stability in aqueous eluents has limited its use.

Increasing the cross linking and decreasing the particle size made the PS-DVB resin much more resistant to collapse. PS-DVB resin is now the preferred choice of substrate in IC. However, PS-DVB resins contain benzene rings which exhibit hydrophobicity (15), and have been involved in causing non-specific effects on column, such as denaturation of proteins and effecting the chromatography of easily polarizable ions, such as lead (16). Other resin types which do not contain π bonding systems are being developed but are only recently appearing on the market and are, at present, relatively expensive.

Choice of stationary phase is a compromise between different elution characteristics, interferences caused by non-specific interaction, stability and cost.

1.5.2 Principles of ion exchange in ion chromatography.

The principles that govern IC are discussed below. Initially the process that leads to the exchange between two solutes is discussed. This exchange reaction is often used to enable the elution of univalent ions by salt solutions containing other ions. Elution of multivalent cations by simple salt solutions, as above, is not generally satisfactory. First, the concentration of the salt solution has to be greater to overcome the greater affinity of the multivalent cations for the stationary phase. Secondly, similarly sized and charged metal species, eg transition metals or the lanthanides will co-elute. This problem is overcome by the addition of complexing agents to the eluent, reducing the charge on the cation. The retention times for the different cations is then more related to the stability of the complex formed. In order to determine individual analytes by IC they must be separated or *resolved* from each other, this is best achieved on a high efficiency column. High efficiency columns elute solutes as narrow bands rather than more diffuse band(s) when compared to similar but less efficient columns, giving better resolution and greater sensitivity. Parameters that can be measured to determine efficiency and resolution in HPLC and the effect efficiency has on resolution are also discussed below.

1.5.3   Retention.

IC requires an ion exchanger which is composed of an insoluble stationary matrix on which there are chemically bound ions at ion exchange sites known as the *fixed* ions. Also at these ion exchange sites are oppositely charged *counter-ions*. The counter-ions are mobile and may be displaced by other charged ions present. It therefore follows that cation exchange material has fixed ions that are negatively charged and anion exchange material has fixed ions that are positively charged.

The counter-ions are generally the same as those in the eluent and are used to elute the other solute ions, including analytes. When the stationary phase has been conditioned with a counter-ion and the counter-ions occupy all the ion exchange sites, then the column is said to be in the *form* of that counter-ion. For example, cation exchange columns may be in the potassium or hydrogen form and anion exchange columns may be in the chloride or sulphate form.

1.5.4   Elution.

The most simple elution of an ion from the column is dependent upon equilibria of the solute ion and the counter-ion between the stationary and mobile phases. These equilibria control the ion exchange of the ions either into the mobile phase from the stationary phase or vice versa. Equation 1.1 shows the general case for exchange between two cations, x moles of the counter-ion (E^+) and y moles of the solute ion (A^{x+}). Subscripts refer to whether the ions are either in the mobile phase ($_m$) or on the

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stationary phase (r) (often a resin). The exchange is stoichiometric to maintain the electroneutrality of the solution and stationary phase.

$$\text{Equation 1.1} \qquad y\,A^{X+}_m + x\,E^{Y+}_r \rightleftharpoons y\,A^{X+}_r + x\,E^{Y+}_m$$
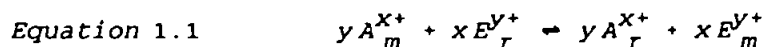
The equilibrium constant of the above reaction ($K_{A,E}$) also known as the *selectivity coefficient* is shown in Equation 1.2. Equation 1.2 is often shown simplified without the activity coefficients (γ), as in Equation 1.3. This simplified form infers that the activity coefficients are unity, although this is not always the case. In fact the activity coefficients for either E^+ or A^+ on the stationary phase cannot be measured. The selectivity coefficient ($K_{A,E}$) therefore is not a thermodynamically defined equilibrium constant but determined experimentally.

$$\text{Equation 1.2} \qquad K_{A,E} = \frac{[A^{X+}_r]^y\,[E^{Y+}_m]^x}{[A^{X+}_m]^y\,[E^{Y+}_r]^x}\; \frac{\gamma_{A^{X+}_r}^{y}\,\gamma_{E^{Y+}_m}^{x}}{\gamma_{A^{X+}_m}^{y}\,\gamma_{E^{Y+}_r}^{x}}$$

$$\text{Equation 1.3} \qquad K_{A,E} = \frac{[A^{X+}_r]^y\,[E^{Y+}_m]^x}{[A^{X+}_m]^y\,[E^{Y+}_r]^x}$$

The magnitude of $K_{A,E}$ represents the degree of take up of the solute ion by the stationary phase. If $K_{A,E}$ is unity then the affinity of either ion to the stationary phase

is the same. A value of $K_{A,E}$ greater than one describes a solute that has a higher affinity for the stationary phase than the counter-ion and $K_{A,E}$ less than one describes a solute that has a lower affinity for the stationary phase than the counter-ion. Selectivity coefficients are measured between two ions under specific conditions however, a general order for selectivity coefficients has been determined for certain conditions. Selectivity coefficients may be used as a predictive tool to determine the elution order of different solutes. They are also useful in predicting the changes in elution when using different counter-ions. For example, the ions that have the highest selectivity coefficient are stronger eluents, ie. elute ions more rapidly than weaker counter-ions, having lower selectivity coefficients. The general selectivity coefficient order for various cations on strong acid cation exchange resin and anions on strong base anion exchangers are given below.

General order of selectivity coefficients for cations taken up on strong acid cation exchange resin.

$Pu^{4+} >>$

$La^{3+} > Ce^{3+} > Pr^{3+} > Eu^{3+} > Y^{3+} > Sc^{3+} > Al^{3+} >>$

$Ba^{2+} > Ca^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+} > Mg^{2+} >>$

$Tl^+ > Ag^+ > Cs^+ > Rb^+ > K^+ > Na^+ > H^+ > Li^+$
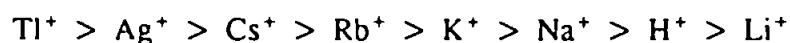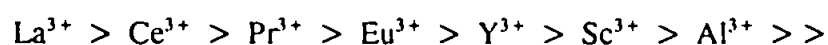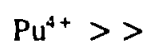
General order of selectivity coefficients for anions taken up on strong base anion exchanger.

citrate > salicylate > $ClO_4^- > SCN^- > I^- > S_2O_3^{2-} > WO_4^{2-} > MoO_4^{2-} > Cr_2O_4^{2-} >$

$C_2O_4^{2-} > SO_4^{2-} > SO_3^{2-} > HPO_4^{2-} > NO_3^- > Br^- > NO_2^- > CN^- > Cl^- > HCO_3^- >$

$H_2PO_4^- > CH_3COO^- > IO_3^- > HCOO^- > BrO_3^- > ClO_3^- > F^- > OH^-$

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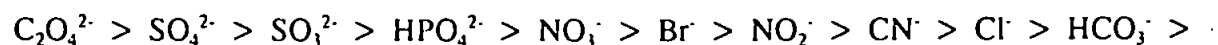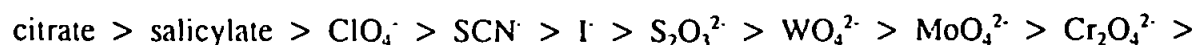
It should be borne in mind that the selectivity coefficients do not describe the separation of two different solutes, as information concerning the capacity of the column for the solutes and the distribution of the solutes between the two phases is required. These are determined experimentally for given conditions and are given as the distribution coefficient, often given the symbol k_D or for a specific solute D_A. Alternatively the capacity factor k_A' is given. The distribution of a solute, A, between the two phases is given by its distribution coefficient, D_A, in Equation 1.4, where $[A^{x+}]$ refers to all forms of A whether in simple hydrated form or in complexes.

$$Equation\ 1.4 \qquad D_A = \frac{[A^{x-}_r]}{[A^{x+}_m]}$$

The distribution coefficient is related to the capacity of the column for that solute by the capacity factor (k_A') in Equation 1.5. Information on the weight of the stationary phase (w) and the volume of eluent (V_m) is required.

$$Equation\ 1.5 \qquad k_A' = D_A \frac{w}{V_m}$$

The separation of two solutes A^{x+} and B^{z+} on a column is given by the separation factor α_{AB} which is given in Equation 1.6 in terms of the relative capacity factors for the two solutes.

$$Equation\ 1.6 \qquad \alpha_{AB} = \frac{k_A'}{k_B'}$$

1.5.5 Complexing eluents.

The above equilibria involving a counter ion describes what is generally known as the *pushing effect* used to move ions along a column. With cations, another effect may be introduced which modifies the elution of the cation. This can be described as the *pulling effect*. Cations may complex with an anionic ligand or chelate in the mobile phase so effectively reducing the positive charge on the cation. Equations 1.7-1.10 show some possible reactions between a ligand (L) and a metal (M). The ligands are often weak organic acids that can complex only when deprotonated. Equations 1.7 and 1.8 show the equilibrium between acid and conjugate base, this emphasizes that changes in pH can drastically effect the concentration of the ligand and therefore elution of cations by complexing eluents.

$$Equation\ 1.7 \qquad H_2L \rightleftharpoons HL^-_m + H^+_m$$

$$Equation\ 1.8 \qquad HL^-_m \rightleftharpoons L^{2-}_m + H^+_m$$

$$Equation\ 1.9 \qquad M^{x+}_m + L^{2-}_m \rightleftharpoons ML^{x-2}_m$$

$$Equation\ 1.10 \qquad ML^{x-2}_m + L^{x-2}_m \rightleftharpoons ML^{x-4}_2$$
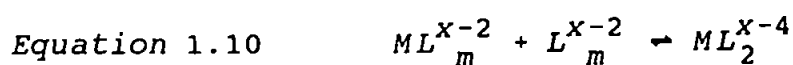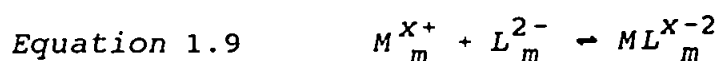
The complexation of the metal decreases the concentration of the free metal cation in solution. The free metal in solution (M^{x+}_m) can be written as a fraction (α_M) of the total

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metal in solution (C_M), Equation 1.11.

Equation 1.11
$$\alpha_M = \frac{[M^{X+}_m]}{[M^{X+}_m] + [ML^{X-2}] + [ML_2^{X-4}]} = \frac{[M^{X+}_m]}{C_M}$$

Assuming that only the free metal is bound to the cation exchange stationary phase then the distribution coefficient for the metal is given below in equation 1.12.

Equation 1.12
$$D_M = \frac{[M^{X+}_r]}{C_M}$$

By using the terms for all the metal species present in the system and not only those that refer to the free metal species D_M will be less when metal complexes are formed than when they are not. The lower the value of D_M the less the metal is retained on the column. Partitioning of a free cation between the two phases is not altered, but the proportion of free cation is reduced by complex formation. Thus when complexes of the metal ions are formed, those that form significantly stronger complexes will have a smaller proportion of free metal ions present than those metals that form weak complexes. Therefore when D_M is measured for a cation with a complexing eluent D_M decreases most for those metals that form the stronger complexes. Since for a series of metals stability constants increase with decreasing ionic radius the elution order remains more or less the same as for simple elution using a counter ion. The formation of these chelates has proved extremely useful in the separation of divalent cations where only small differences in D_M are obtained using the simple elution of a ionic species by

a counter-ion, and was a major breakthrough in the separation of the trivalent lanthanides and actinides nearly fifty years ago.

1.5.6   Separation of cations on an anion exchange column.

Some separations of cations are carried out on anion exchange stationary phases. This has been achieved by the formation of anionic complexes with the metal cation and in a similar manner to the above example the separation is dependent upon the difference in the stability constants of the complex. However, an increase in the stability constant leads to a greater degree of complex formation, therefore a greater proportion of negative charge leading to increased retention times. Equations 1.13 and 1.14 show the complex formation reaction of an anionic complex between the metal M and a ligand L. The distribution coefficient for the metal D_M is given in Equation 1.15, it may be noted that as M^{2+} is not retained on the anion exchange stationary phase that D_M is given in terms of $[ML_{2r}^{2-}]$ the retained anionic complex.

$$Equation\ 1.13 \qquad M_m^{2+} + L_m^{2-} \rightleftharpoons ML_m$$

$$Equation\ 1.14 \qquad ML_m + L_m^{2-} \rightleftharpoons ML_2^{2-}$$

$$Equation\ 1.15 \qquad D_M = \frac{[ML_{2\,r}^{2-}]}{C_M}$$

The above expressions show how it is possible to separate different ionic components of a sample by three commonly used IC processes. It is also possible to describe the efficiency of the chromatography column and the degree of separation of components in the sample.

1.5.7 Chromatographic efficiency and resolution.

As the solutes pass along the chromatography column small differences in the retention behaviour of the same type of molecule or the different path lengths that these molecules take lead to broadening of the elution band. In chromatographic systems the solutes enter the chromatographic column as a single complex band, the aim of chromatographic separation is to separate this band into its constituent parts. The sections above showed that the distribution coefficient (K_D or D_M) is different for different cations (and anions). Exploitation of this difference enables one solute to pass along the chromatographic column faster than another solute, when driven along by exchange with a counter-ion. Quantifying the separation of the different solutes is one measure of chromatographic efficiency. Another equally important measure is the rate at which elution bands broaden as they pass down the column. This can be described in terms of plate theory.

The measure of chromatographic efficiency is a measure of the number of theoretical plates in a column or the length (height) of the column equivalent to a theoretical plate (HETP), (usually expressed in mm). This can be calculated from the width of the peak, at a number of positions. Equation 1.16 shows how HETP is related to the

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length of the column (L) and the number of theoretical plates (N). Equation 1.17 shows the relationship between N, the retention time of the peak and the standard deviation (σ), defined from the peak width at that retention time. Figure 2 shows how the standard deviation is related to various widths (W_{50}, W_T and $W_{4.4}$) that may be measured for an idealized peak, normally the widths at tangents dissecting the baseline are measured to determine efficiency. Equation 1.18 shows how these measurements may be used to calculate the standard deviation. Using these various measured widths substituted in Equation 1.19 the number of theoretical plates may be calculated from the retention times and the peak widths.

$$Equation\ 1.16 \qquad HETP = \frac{L}{N}$$

$$Equation\ 1.17 \qquad N = (\frac{t_R}{\sigma})^2$$

$$Equation\ 1.18 \qquad \sigma = \frac{W_{50}}{2.345} = \frac{W_T}{4} = \frac{W_{4.4}}{5}$$

$$Equation\ 1.19 \qquad N = 5.54(\frac{t_R}{W_{50}})^2 = 16(\frac{t_R}{W_T})^2 = 25(\frac{t_R}{W_{4.4}})^2$$

In can be seen from these equations that the more efficient columns are those that have the greatest number of theoretical peaks for a given length. The degree of separation of the peaks obtained for two or more different solutes is termed the resolution. This
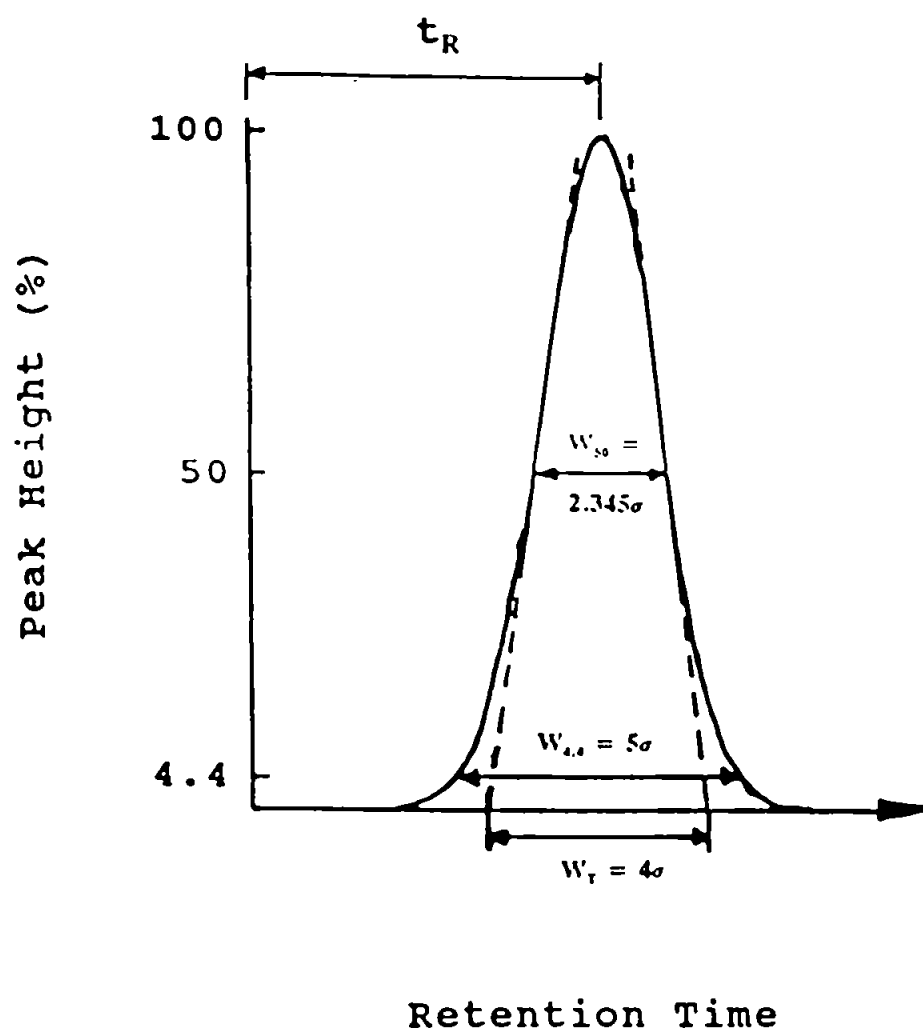
Figure 2   Idealized chromatogram showing the elution of a solute.

Retention time (t_R) and peak widths (W_{50}, $W_{4.4}$ and W_T) shown

can be used to calculate column efficiency.

can be calculated from the retention time and standard deviation of the two peaks. In Equation 1.20 the resolution (R$_s$) is given between solute one at retention time t_{R1} and solute two at retention time t_{R2}, shown in Figure 3. The standard deviations can be calculated as above. Normally an R$_s$ value of 1.5 is considered as just complete resolution of two peaks.

$$Equation\ 1.20 \qquad R_s = \frac{t_{R2} - t_{R1}}{2(\sigma_1 + \sigma_2)}$$

Equation 1.20 describes the resolution for idealized peaks such as those shown in Figure 3. However, it is often used to describe non-symmetrical peaks. Peak shape can deviate from Gaussian when K$_D$ alters with the concentration of the ion on the column, leading to either peak tailing or peak fronting.

The main factors that affect the resolution are the separation factor, the capacity of the column and the number of theoretical plates. This relationship can be seen in Equation 1.21 in terms of the second component in a pair of solutes (hence subscript 2).

$$Equation\ 1.21 \qquad R_S = \left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{1+k_2'}\right)\left(N_2\right)^{\frac{1}{2}}$$

The separation factor (α) for the pair of solutes "1" and "2" ($\alpha_{1,2}$) can be obtained from Equation 1.6 and is dependent on selectivity coefficients of solutes "1" and "2". The greater the difference in selectivity coefficients the greater the separation factor, and so
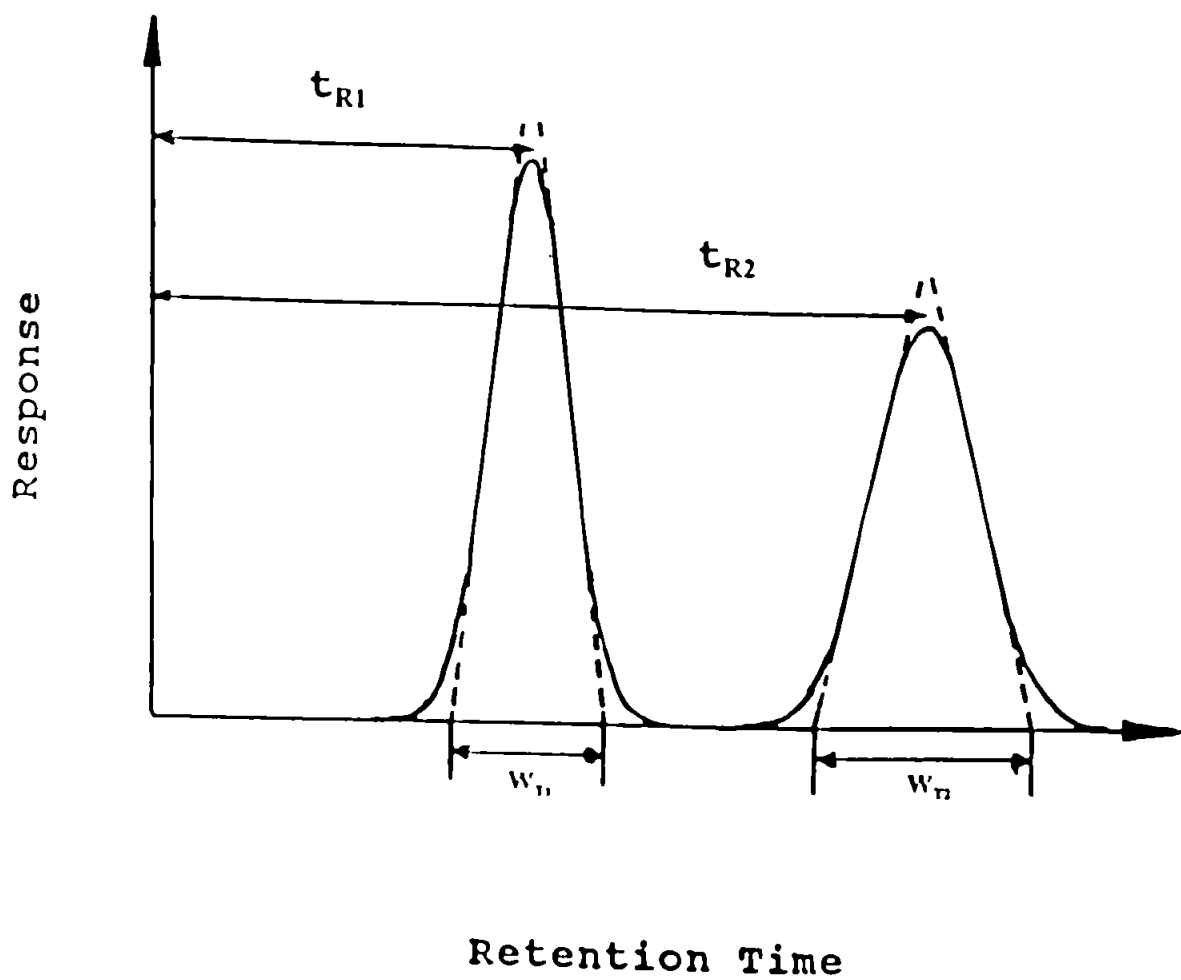
Figure 3   Idealized chromatogram showing the elution of two solutes

showing the retention times and peak widths at peak tangents used ·

to calculate the resolution of the two peaks.

the greater the resolution. The capacity factor, which can be calculated from Equation 1.5 for component "2" has little effect on the resolution once $K'_2/(1+K'_2)$ tends to unity. The number of theoretical plates (N) may be calculated from Equation 1.19. An increase in N only leads to a square root increase in resolution. However, when the increase in N is significant, for example by a decrease in packing size then the resolution of two solutes is also significantly increased.

1.6   The Current State of Ion Chromatography.

Ion chromatography (IC) is a term which covers the high efficiency chromatography techniques used to separate ionic species, including ion pairing and ion interaction chromatography; chelation ion chromatography and ion exchange chromatography. The majority of separations performed by IC use ion exchange.

1.6.1   Ion chromatography using ion exchange.

The first forced flow ion exchange chromatography for metal ions was the separation of the iron (III) chloro complex from other transition metal chloro complexes by anion exchange chromatography, presented by Seymour et al. in 1971 (17). The iron was detected as its chloro complex by UV detection. Formation of chelates post column to aid detection by colourimetry was used early in the development of IC, for example in 1973 Kawazu (18) determined various transition metals as their 4-(2-pyridylazo)resorcinol chelates. In 1975 Small et al. presented the use of suppressed

conductivity detection for the detection of anions and cations in IC (19). Since the development of these detection strategies in IC for the determination of ionic species between twenty and twenty-five years ago IC has developed to become a technique widely used in routine analysis of trace element ions (20, 21, 22, 23).

Recent reviews on IC (20, 22) report that IC has developed into a mature technique and that the principles are well understood. As a result recent work has focused on developing IC for applications rather than new methods of separation (20), although this is still reported as new ion exchangers are developed.

A review by Frankenberger (23) reports the widespread use of IC for the determination of anions and cations in environmental samples. The determination of anions in environmental samples exceed those reported for cations and is a reflection in the lack of other suitable methods for the determination of anions. This interest in both anions and cations has led research into developing strategies for simultaneous single column detection of anions and cations (24). This has been achieved by using chromatographs equipped with either dual columns (25, 26) or a mixed bed column containing both anion and cation exchange sites (27) or by the formation of anionic complexes with metal cations present and separation on an anion exchange column (28, 29).

Passel (30) and Bostic (31) both report the use of IC for the routine monitoring of anions and univalent cations in secondary coolant in nuclear power plants, in relation to corrosion control. Buck *et al.* report the use of IC for the determination of anions, cations and alkaline earth cations in snow and ice cores (32).

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Anions and univalent cations can be separated with simple salt eluents. However, IC of transition metals, p-block metals and the lanthanides usually involves the use of mildly chelating eluents in order to obtain suitable retention times and separation. This was discussed above in Section 1.5.5. The addition of chelating agents to the eluent was used in classical column ion exchange chromatography and was used early in the development of IC during the 1970's. One of the first separations using complexing eluents in IC was by Takata and Fugita in 1975. Takata and Fugita used 0.4 M tartaric acid with 0.03 M sodium chloride at pH 3.63 as the eluent. They achieved the separation of six transition metal cations on a cation exchange column in 7 minutes (33). The separation of the lanthanides has also been important in showing the ability of complexing eluents to separate metals with similar charge. In 1979 fourteen lanthanides were separated by Elchuk and Cassidy in 26 minutes on an Aminex styrene-divinylbenzene resin column (34), using an α-hydroxyisobutyric acid (HIBA) eluent and a gradient elution programme.

Chelating eluents were used throughout the 1980's and enabled the simultaneous separation of even more metals. For example in 1983 Sevenich and Fritz used isocratic elution with tartaric acid or HIBA eluents for the separation of eight lanthanides, seven transition metals and some alkaline earth metal cations (35). The complete resolution of 15 lanthanides in 28 minutes was presented by Wang et al. in 1984 on a bonded phase column, Nucleosil SA using 2-methyllactic acid, 0.01 M to 0.04 M in a 30 minute gradient elution programme (36). Also, the separation of 13 transition metals in 40 minutes on a Nucleosil SA-10 column with 0.5 M tartaric acid eluent at pH 2.76 was presented by Yan and Schwedt in 1985 (37).

The most popular chelating agents have been the polyfunctional carboxylic acids, some mentioned above. Other polyfunctional carboxylic acids such as lactic acid (38), oxalic acid and malonic acid (39, 40) and other chelating agents such as EDTA (28) have been used. The use of polyfunctional carboxylic acids have been favoured because of practical considerations, for example, stability or solubility of the chelate, the effect on metal components in the chromatographic system and the effect on detection, (34, 36, 39). Some chelating agents such as oxalic acid and EDTA are rather strong and could interfere with certain detection systems. This resulted in Jones *et al.* using lactic acid in the eluent rather than tartaric acid (38). The complex formed between cobalt (II) and lactic acid is weaker and interfered less with the chemiluminescence detection used. Another example is by Legras who used EDTA as a complexing agent for elution purposes and to enable UV detection (28). Legras used a lower pH than the neutral pH commonly used, that is, pH 4.8 instead of pH 6 to 8. This led to reduced degradation of stainless steel parts but was done mainly to improve detection by increasing the UV absorbance of the metal-EDTA chelate, relative to the uncomplexed EDTA background signal.

Where differences in charge allow metals may be separated by simple salt eluents rather than using complexing agents, such as the separation of different chromium species by Williams *et al.* (25). However, the use of a weak complexing agent in the eluent has enabled IC to be used effectively when a large number of metals is involved as in the study of transition metals in the presence of p-block and alkaline earth metals. Recent reviews (21, 22, 23, 41, 42) give an idea of the scope of IC in environmental samples including the analysis of trace metals in soils, natural and waste waters, as well as industrial applications.

Preconcentration of analytes onto ion exchange columns has enabled detection of the analyte even when present in samples in concentrations lower than the limit of detection by direct injection. In this way Jones *et al.* were able to preconcentrate 200 cm^3 of solution containing cobalt (II) and determine it at sub μg l^{-1} levels. However, if the ionic strength of the solution is too great either elution of the analyte will be caused, or the column will become swamped. The development of chelation ion chromatography has enabled preconcentration of trace metal ions even in high ionic strength samples.

1.6.2   Chelation ion chromatography.

Stationary phases have been prepared that contain chelating groups. Thus, retention of solutes is governed by the strength of complexes formed with the stationary phase rather than with ligands in the mobile phase. This can give rise to very different selectivity effects compared to ion exchange phases. The use of chelation ion chromatography has enabled the analysis of concentrated solutions, such as saturated brine. Alkali and alkaline earth cations form only weak complexes with imino-diacetic moieties, transition metal complexes are considerably stronger and are little affected by ionic strength. Columns containing imino-diacetic moieties will form stable complexes with transition metals and weaker complexes with alkali or alkaline earth cations. By careful control of pH and therefore the conditional stability constants, chelation chromatography columns unlike conventional ion exchange columns do not become swamped when preconcentrating trace metal ions from concentrated solutions. By using stepped gradients, preconcentration and separation can be achieved on one column. This has enabled analysis of industrial brine (43), sea-water (44, 45) and milk

(46). The final application shows another interesting feature of these columns, viz the normal ion exchange elution order is reversed. Thus, Jones *et al.* (46) were able to elute strontium prior to the much larger concentration of calcium.

1.7   Detection in Ion Chromatography.

In order to take advantage of the high resolution and the small quantities of analyte HPLC requires a detector that is sensitive and can be fitted on-line without giving rise to significant loss of resolution.

HPLC was developed in the 1960's for the analysis of relatively large, thermally labile or involatile organic molecules. The presence of many organic molecules in the eluent can be determined by changes in the UV-Vis absorption or refractive index of the column effluent and detection systems for these compounds were developed along these lines. By contrast, the UV-Vis absorption or refractive index of an ionic eluent is not significantly altered by the presence of simple hydrated metal ions. Therefore these detection strategies were inappropriate for IC and the development of a suitable detector hampered IC's development. This has led to a variety of different approaches for the detection of metal species each with its own advantages and disadvantages. The principal detection methods at the moment are conductivity, hyphenated systems and post column reactions, the latter being the most versatile.

1.7.1 Conductometric detection.

Although IC is an attractive means of separating ionic species the development of a suitable continuous on-line detector remained a problem. The development of conductance detection in 1975 provided a detector for IC that measured a parameter characteristic for all ionic species. Sensitivity was greatly improved by using eluent suppression where a solid state reactor was incorporated post-column to remove eluent ions oppositely charged to analyte ions, thus lowering the background conductance of the effluent. The solid state reactor was a column of much greater capacity than the low capacity analyte column but a relatively weak eluent had to be used so as not to exceed the capacity of the suppression column. Even so frequent regeneration of the suppression column was required.

Recently membrane suppressor technology has replaced these columns which has enabled much greater eluent volumes to be passed before regeneration is required. Low conductance eluents which do not need eluent suppression led to the development of non-suppressed conductance detection, but is little used for trace analysis due to poor sensitivity. Suppressed conductance is still principally used for the detection of anions. The need for low ionic strength eluents and lack of selectivity has resulted in limited application for metal ions which require more complex elution conditions.

1.7.2 Hyphenated techniques.

Hyphenated systems essentially involve the coupling of two independent analytical

techniques. One of the most important is the coupling of liquid chromatographs to atomic spectrometers and mass spectrometers.

A major advantage of this type of approach is the exploitation of the sensitive and selective detection offered by atomic absorption/emission spectrometers and mass spectrometers combined with the ability of chromatography to separate the analyte from the sample matrix, often a cause of interference in instrumental analysis. Equally important, it allows the determination of different species of the element (47).

Hyphenated techniques were originally used with classical column chromatography, where the analyte was concentrated on the column and subsequently eluted as discrete aliquots. These were then analyzed by a suitable spectroscopic technique. However, the application of on-line hyphenated techniques coupled to HPLC presented a number of interface problems. In particular the rapid broadening of sharp peaks in the large dead volume of the interface. These problems are now being overcome and hyphenated techniques can provide important and useful detection systems for HPLC.

Van Loon and Barefoot give examples of the different coupling strategies of HPLC to flame atomic absorption spectroscopic instruments (FAAS) (48). Hill *et al.* review the more recent coupling of chromatography (HPLC and GC) to inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectroscopy (ICP-MS) (41). The higher sensitivity and multi-element capability of ICP-MS has been utilized to determine low concentrations of different species and elements simultaneously (41). Coupled IC-FAAS has been used to determine the speciation of single elements, such as the alkyl-tins, inorganic and organo-mercurial

compounds. An example of a hyphenated technique which also uses a post column reaction, discussed below, to overcome coupling problems in the detection of arsenic species. Ricci *et al.* (47) describe the determination of arsenite, arsenate, monomethylarsenate, dimethylarsenate and *p*-aminophenylarsenate. The arsenic species are converted to arsine in a continuous arsine generator (post column reaction) after separation by IC. The arsine can then be determined by FAAS.

One major drawback of using such systems is the added complexity of using two instrumental techniques. Some instruments such as ICP-MS or ICP-OES can determine a number of different elements simultaneously. Coupled systems using these can be used to determine different species of different elements in one injection. However, other less complex instruments such as FAAS can only determine single elements in an injection, multi-element speciation studies using these instruments would require greater complexity. Also, as the analyte is spread out in a peak, detection limits in coupled techniques are generally higher than by direct nebulization into atomic absorption/emission spectrometers or mass spectrometers. Band spreading due to poor coupling leads to greater dispersion of the analyte and therefore even higher detection limits. Thus the spectroscopic instruments must be inherently sensitive and these instruments are often expensive thereby drastically increasing the cost of the IC system. Perhaps the biggest disadvantage of hyphenated techniques is the difficulty and impracticability of using them in a monitoring and/or automated application, for example in process control or waste management. Some applications are not possible such as on oil rigs, petrochemical plants or nuclear power stations where flames and plasmas are excluded. Therefore, self contained HPLC systems are preferred and this has led to detection based on post column reactions.

1.7.3.1   Post column reactions.

Post column reactions (PCRs) have been used with liquid chromatography since 1958 when Spackman *et al.* reacted eluting amino acids post column with ninhydrin (49). In 1961 Lundgren and Loeb used PCR detection to determine inorganic phosphates (50). A decade later forced flow systems used for the trace metal ions were being developed (17). Early in the development of IC PCRs were used to enable the detection of trace metals, for example in 1973 Kawazu added 4-(2-pyridylazo)resorcinol (PAR) to the eluent post-column and determined several metal ions (18). PCR detection has continued to increase in popularity and has resulted in the application of many reaction chemistries in order to obtain highly sensitive detection systems for analytes.

PCR detection includes all those techniques that incorporate a reaction post column. Strictly speaking this includes suppressed conductance detection. However, suppressed conductance detection is generally classified along with non-suppressed conductance detection as conductance detection and therefore is not discussed here.

The major disadvantage of the PCR detection systems is the added complexity where an additional pump (sometimes more) is needed to add post column reagents. Additional pumps also increase the contribution to the base-line noise. The other possible disadvantage is the dilution of the analyte and band broadening if an analyte has to be kept in a long reactor for a slow reaction to occur. However, the use of narrow bore tubing in either knitted or tightly coiled reactors prevents dispersion and development of PCRs using rapid reactions means dispersion is generally not a major problem when . PCR detection is used with IC. However, PCR reaction systems have many advantages

that outweigh these disadvantages.

One main advantage of PCR detection systems used with IC is they have enabled the use of relatively concentrated, complexing eluents. Therefore, transition metal ions may be eluted and sensitively determined using PCR detection systems. PCRs are the most common detection method for transition metal ions separated by IC. A review by Dasgupta (52) is a good source of different PCRs used with IC and a book by Krull is a good source of reference for many aspects of PCR detection (53).

The use of PCRs has enabled exploitation of a range of the most sensitive colourimetric reactions for metal ions to enable detection by spectrophotometric or luminescent detection. As the reaction occurs post-column the complexes formed do not have to be chromatographed, unlike the complexes formed pre-column for adsorption or partition chromatography. Also, PCRs may be altered without affecting the chromatography. One alteration that can be achieved using PCRs is the measurement of the disappearance of a ligand as a chelate forms rather than the appearance of the analyte chelate. It is also possible to measure an analyte by an exchange reaction. This approach has been used to increase the range and sensitivity of determination for some analytes. PCRs can also be used for highly sensitive determinations of selected analytes by the application of chemiluminescent reactions.

1.7.3.2 Detection using post column reactions involving spectrophotometry.

The most common method of detecting metals is to form a UV-Vis absorbing complex with the cations as they elute from the column. (34, 36, 54, 40). 4-(2-pyridylazo)resorcinol (PAR) has been used to detect various transition metal cations and rare earth metal cations (36, 54). However, the absorbance maxima of different metal chelates can differ and thus the detection wavelength is a compromise if more than one metal is to be detected. The use of diode and spectral array detectors have alleviated this as simultaneous multiple wavelengths can be used for detection. Detection is limited by the absorption at the detection wavelength by the free ligand, especially for metal ions that form weak complexes with the ligand. However, other PCR strategies such as inverse photometry have been developed that have alleviated this problem.

Detection of fluorescent complexes that are formed post column can be measured in a similar "direct" manner but offer greater sensitivity. For example, the determination of aluminium by Jones *et al.* (55, 56) after PCR derivitization to its fluorescent 8-hydroxy quinoline sulphonate (8-HQS) complex. However, as with UV-Vis detection, fluorescence detection for different elements also have different wavelength emission maxima for different complexes, as well as different excitation maxima. Also, fluorescence detection can be quenched by spin-orbit coupling of transition and heavy elements in the effluent, limiting the range of detectable elements by direct fluorescence mostly to the lighter representative elements.

1.7.3.3   Inverse photometry.

Two problems associated with detection of the formation of complexes, above, are the different wavelengths of absorbance maxima and the fact that only certain metals react with the reagent. The first has been overcome by the determination of the disappearance of a ligand, rather than the appearance of a complex. This measurement therefore is not effected by the different wavelengths of absorbance maxima and extinction coefficient (ϵ_{max}) values of the different metal complexes. Such systems have been based upon ligands that absorb strongly at a given wavelength when uncomplexed but absorb weakly at those wavelengths when complexed. Ligands such as dithizone, Eriochrome Black T (EBT) (53, 57) and its analogues, for example, Calmagite (58) have been used extensively. The peaks observed result from a decrease in the absorbance signal as the free ligand is depleted when it complexes with the eluting metal ions. A major disadvantage is that pump noise is accentuated because the baseline is at relatively high absorbance. Thus, the pump noise is generally greater using inverse photometric PCR detection, affecting the limit of detection.

1.7.3.4   Metal-ligand exchange reactions.

The second problem mentioned above where certain analytes form no complexes or perhaps very weak complexes with ligands used in PCR detection can be solved by involving the eluting metal ions in an exchange reaction. When using an exchange reaction the increase in absorbance is normally measured, like in "direct" PCR detection methods. This means that one is measuring against low background noise.

Two such ion displacement reactions have been cited the most often, namely, zinc-EDTA-PAR and magnesium-EDTA-EBT. A similar displacement reaction based on the magnesium-EDTA-EBT reaction but using 8-HQS instead of EBT has been used with fluorescence detection.

The zinc-EDTA-PAR post-column ion displacement reaction was introduced by Arguello and Fritz in 1977 (59). The main reason this approach was adopted was because EDTA is able to form stable complexes with far more cations than PAR. Zinc was chosen because the zinc-PAR complex is intensely coloured and the zinc-EDTA complex is more stable than the zinc-PAR and as such low backgrounds can be achieved.

The zinc-EDTA complex is considerably more stable than the EDTA complexes of some other metal-EDTA complexes. However, the conditional stability constant of the zinc-EDTA complex can be controlled by the addition of ammonia to the PCR. Addition of ammonia to the PCR lowers the conditional stability constant of the zinc-EDTA complex, allowing detection of metal ions that form only weak EDTA complexes but which do not form ammonia complexes, such as calcium and magnesium. Altering the ammonia concentration in the PCR alters the effect on the zinc-EDTA conditional stability constant and so can be used to alter selectivity in the PCR detection system.

In the zinc-EDTA-PAR PCR system, the zinc-EDTA complex is added with PAR as a post column reagent. In the exchange reaction, Equation 1.22, the eluting analyte cation displaces zinc from the zinc-EDTA complex. The displaced zinc can then form

a complex with PAR and be detected at 510 nm (53). Sensitivity of detection is controlled by the relative stability constants of analyte-EDTA to zinc-EDTA.

Equation 1.22 $$M^{2+} + Zn\text{-}EDTA + PAR \rightarrow M\text{-}EDTA + Zn\text{-}PAR$$

The magnesium-EDTA-EBT ion displacement as described by Bowles is similar to the above but uses the displacement of magnesium from an EDTA complex (60). Magnesium forms one of the weakest EDTA complexes and should be easily displaced without the need for an additional ligand, ammonia. The magnesium-EBT complex formed by the displaced magnesium is determined at 520 nm.

Williams and Barnett (61) describe a magnesium-EDTA-8-HQS system in which magnesium displaced from its EDTA complex forms a fluorescent complex with 8-HQS. The magnesium-8-HQS complex can be determined by highly sensitive fluorescence. Detection limits quoted are around 10^{-7} M for a range of metal cations.

Extremely sensitive detection systems for use with HPLC have been provided by PCRs based upon chemiluminescence (CL). Sub ng l^{-1} (10^{-11} M) detection limits for trace metal ions separated by IC (38) and detection limits below 10^{-10} M for other inorganic compounds have been reported (62). Thus, development of CL PCR detection systems for use with IC are of interest in order to determine very low concentrations of trace elements.

1.8.1   Chemiluminescence.

The luminescence called chemiluminescence (CL) is the light released as a result of a chemical reaction rather than by excitation with electromagnetic radiation. The quantum yield of chemiluminescence (Φ_{CL}) is given as the product of the quantum yield of the reaction (Φ_R) and the quantum yield of luminescence (Φ_L), given in Equation 1.23.

Equation 1.23 $\Phi_{CL} = \Phi_R \cdot \Phi_L$

Quantum yields of CL reactions (Φ_{CL}) used analytically are typically 0.001 to 0.1 (62). The oxidation of luminol in aqueous alkali has a quantum yield of 0.01 (62), whereas some of the most efficient CL reaction systems used in analytical chemistry, the peroxyoxalate systems have quantum yields in the range 0.07 to 0.5 (62).

CL and fluorescent emission spectra have been found to be the same for the same compound. Thus the photophysical properties of the two phenomena are thought to be similar. Some of the photophysical properties that are involved in fluorescence are given below (63).

1.8.2 Photophysical properties of fluorescence.

1.8.2.1 Singlet, doublet and triplet states.

Molecules may occur in singlet, doublet or triplet states. This is a reference to the quantum spin of the atom or molecule. When electrons have filled the orbitals, according to Hund's rule (64) and Pauli's principle (64) one of three possible situations occur: all electrons are paired; there remains one unpaired electron or there are two unpaired electrons of similar spin. This leads to the quantum spin number, S, of 0, ½ or 1 respectively. The state to which a molecule is assigned, is given by the equation $(2S+1)$. When $S=0$ then $(2S+1)=1$, the singlet state, when $S=½$ then $(2S+1)=2$, the doublet state and when $S=1$ $(2s+1)=3$, the triplet state.

Organic molecules in the ground state invariably have their electrons paired and so occupy the lowest singlet state S_0 or ground state (S_0 refers to the singlet state and not the quantum spin number). Excitation of this state can lead to promotion to an energetically excited singlet state $S_1, S_2, ... S_n$. This promotion can be due to absorbance of photons, which may result in fluorescence, or as a result of a chemical reaction, which may result in CL. Promotion from the singlet state to a doublet or triplet state is called inter-system crossing (ISC) and is "forbidden" under the quantum selection rule $\Delta S=0$. The occurrence of ISC is discussed in a following section, energy levels are assigned D_n for doublet and T_n for a triplet states.

1.8.2.2 Energetically excited states.

Figure 4 shows an idealized energy profile for a theoretical excited fluorescent organic molecule. Absorption of light energy promotes the molecule from the singlet ground state S_0 to an excited state S_1. At this point a number of events may happen to this molecule, one eventuality is fluorescence but other possibilities are discussed below.

1.8.2.3 Vibrational relaxation (VR).

In the liquid phase nuclear motion occurs on a shorter time scale than fluorescence or CL, approximately every 10^{-13} to 10^{-12} seconds. Interaction results in the deactivation of vibrational modes of a molecule. Molecules therefore tend to occupy the lowest vibrational mode, ie. $V=0$. This being the case luminescent emission occurs from the lowest vibrational mode of the excited singlet state.

1.8.2.4 Internal conversion (IC).

Promotion of a molecule from the ground state to an excited state above the first excited state is possible, that is, promotion from S_0 to $S_2...S_n$ but the fluorescence spectrum is generally the same as that observed if promotion is to the first excited state (S_1). This is because rapid conversion from S_n to S_1 occurs in the order of $0 \cdot 1$ to 10 picoseconds. A conversion such as this is called internal conversion.

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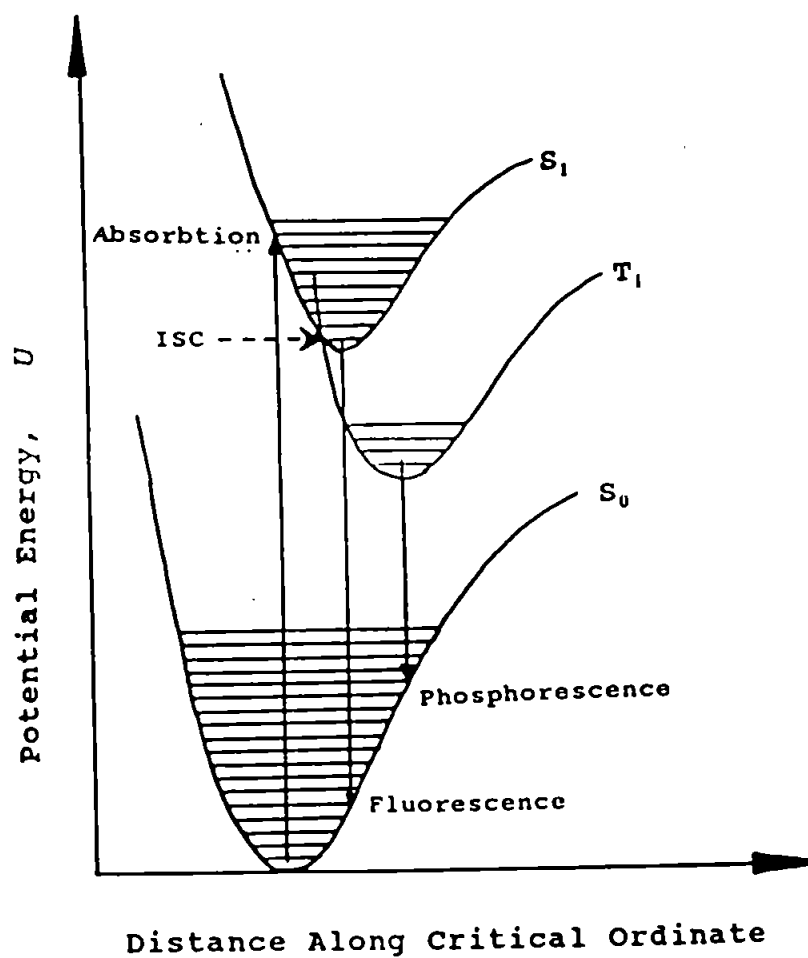
Figure 4   Idealized potential energy curves for the S_0, S_1 and S_2 energy

levels. Quantized vibrational energy levels are shown by

horizontal lines in each energy level. The energy transfer

processes of absorption, phosphorescence and fluorescence are

also shown.

The transfer from an excited state to a lower energy level of the same spin with the release of energy in a radiationless form is called internal conversion. The rate to which internal conversion occurs is related to the difference in the overlap of the vibrational wave functions of the two states. Overlap is more likely to occur where energy levels are closer. S_1 is generally energetically closer to higher excited singlet states than the ground state and so internal conversion is more rapid between S_n and S_1 than between S_1 and S_0. Furthermore the large energy difference between the ground and first excited state results in a metastable S_1 state.

1.8.2.5   Inter system crossing (ISC).

The transition from a singlet to a triplet is strictly forbidden by the quantum mechanical selection rule $\Delta S = 0$. However, if spin-orbit coupling is present and the vibrational wave functions overlap ISC can occur.

The transition from the excited singlet state to a lower lying triplet state is a form of ISC. Energetically excited triplet states may decay to ground singlet states either by non-radiative ISC or radiative ISC which is called phosphorescence. Phosphorescence which is spin forbidden occurs more slowly and with lower intensity than fluorescence, normally in a period of seconds or minutes. Heavy atoms promote spin-orbit coupling and if present in a molecule or solution can increase ISC. This has been used to increase phosphorescence.

1.8.2.6 Fluorescence.

A molecule that has been raised to an excited state by the absorption of light has a number of paths that it may go back down apart from that of re-emitting the light in what is known as fluorescence. It may undergo a chemical reaction, it may transfer energy to another molecule or it may undergo one of the transitions above to return to the ground state. The quantum yield (ϕ_F) for the fluorescent reaction is therefore dependent upon the rate constants of fluorescence and the other processes, as shown in Equation 1.24.

Equation 1.24
$$\phi_F = \frac{k_F}{k_F + k_{IC} + k_{ISC} + k_{ET} + k_R}$$

Where

k_F = Rate constant for fluorescence.

k_{IC} = Rate constant for internal conversion.

k_{ISC} = Rate constant for inter system crossing.

k_{ET} = Rate constant for energy transfer.

k_R = Rate constant for reaction.

The radiative lifetime (τ_F) of fluorescence has been calculated for a number of molecules and is in the region of 10^{-6} to 10^{-8} seconds.

Fluorescence can be affected by structural modifications. The more rigid structured

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fluoroscein for example is highly fluorescent whereas a very similar molecule phenolphthalein is non-fluorescent. Substituents can also affect fluorescence, anthroquinone is non fluorescent due to rapid ISC. However, dihydroxyanthracene is highly fluorescent.

Clearly, this study exploits the chemical excitation of luminescence, which introduces a further complication. The mechanisms involved in the chemical excitation of luminescence are often complex, multi-step processes. With relatively low quantum yields mechanisms are generally poorly understood or controversial. The next section details the main CL systems exploited for PCR detection.

1.9   Detection Systems using Chemiluminescence.

1.9.1   General aspects of chemiluminescence detection.

Numerous CL reactions and reaction strategies have been used with a number of different CL reactions. The light output of the luminescent reaction in all cases is the measured response, but unlike fluorescence there is no need for a light source. This has several advantages in terms of detector simplicity. The lack of an external light source means there is generally no need to provide wavelength selection and the associated complicated optics. In many circumstances all that is required to construct a detection cell is to form a coil of PTFE chromatography tubing flat against the window of a photo-multiplier tube.

Fluorescence detection is often limited by the scatter of stray light in the detection cell and by fluctuation in the light source. However, neither of these problems are encountered with CL. As the background CL signal is often extremely low the signal to noise ratio of the luminescent response is very high and leads to the extreme, sub nM, sensitivity found for some determinations. In many CL reactions the CL reagent is the measured species. However, with some CL reactions, such as the peroxyoxalate reaction, the CL measured is from a fluorophore excited by a separate reaction. The background CL is from the CL reagents which emit weak CL or from fluorescent contaminants. Kwakman discusses the use of filters to cut-out background CL in the peroxyoxalate system at wavelengths below 550 nm whilst using fluorophores that emit at longer wavelengths (65).

In flow systems it is the intensity of CL that is measured, either as peak intensity or the signal integrated over the period of time the analyte is in the detector. Equation 1.25 shows how the intensity (I) is dependent upon the quantum yield of the CL reaction and the rate of reaction (dC/dt).

Equation 1.25 $\qquad\qquad I = \Phi_{CL}\ (dC/dt)$

Increase in reaction rate leads to an increase in intensity, so increasing the ability to be detected. Also, the detector must be able to detect maximum intensity if it is to reach maximum sensitivity. The maximum intensity is reached at different times for different reactions and different catalysts. The time interval between the column effluent (and analyte) mixing with the CL reagent before entering the detection cell and residence time in the detection cell is important. Therefore the time window of the mixed

solution in the detector is optimized to give the greatest response for all analytes. Allowance for shorter or longer rise time can be made simply by adjusting the length of tubing prior to the flow cell. Mixing of the eluent with the CL reagent can lead to background CL emission. If the rise and fall time of this CL is different from the rise and fall time of the analyte, careful selection of the time window can be used to obtain the greatest signal to noise ratio for the analyte.

The rapid rise and fall times of many CL reagents means that CL occurs in the first part of the detector, the rest of the detector remaining dark, this is known as "chemical band narrowing". The chemical band narrowing effect means that relatively large flow cells may be used without significantly increasing the background CL whilst optimising the collection of light when analytes are present. The use of large flow cells also collect light from different phases of reciprocating pump motion and can lead to a more even background pump noise, a limiting factor in many CL detection systems.

1.9.2 Utilization of chemiluminescence reactions.

The same CL reaction may be utilized in a manner of different ways by using different detection strategies. An idealized CL reaction is shown in Equation 1.26.

Equation 1.26 $\qquad A + B \xrightarrow{C} D^* + E$

The reaction between A and B is catalysed by C. The products D^* and E are formed, D^* is in an excited state and may decay back to the ground state releasing a photon

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giving rise to CL, as in Equation 1.27.

Equation 1.27 $$D^* \rightarrow D + h\nu$$

Alternatively D^* may decay back to the ground state via internal conversion, inter system crossing or by transferring the energy to another molecule F, promoting F to an excited state, F^*, as in Equation 1.28.

Equation 1.28 $$D^* + F \rightarrow D + F^*$$

F^* may then decay back to the ground state by emitting a photon, in which case F is a fluorophore. F^* produced may also return to the ground state via radiationless decay. If this leads to a decrease in CL then F may be determined by quenching.

In the above reaction scheme any of the reagents A, B, C and F may be determined. Equation 1.28 shows that F may be determined by acting as a fluorophore, a strategy that has been used extensively with peroxyoxalate CL reactions.

1.10.1   Peroxyoxalate.

Fluorescence detection has provided sensitive detection systems for a number of analytes in HPLC. However, stray light in the flow cell is often a limiting factor. Using a chemical excitation source instead of a photo-excitation source has solved this problem and resulted in detection limits 10 to 100 times less than the respective

fluorescence detection technique (62, 65).

Peroxyoxalate CL was discovered by Chandcross in 1963 (66). Chandcross reported the appearance of a bluish-white emission on mixing oxalylchloride with hydrogen peroxide in solution of dioxane or benzene. Fluorescent compounds such as 9,10-diphenylanthracene (DPA), anthracene and N-methyl acridone were shown to undergo fluorescence if present (67).

The oxalylchloride CL reaction was further studied with other fluorescent compounds by Rauhut (68). Rauhut proposed that the excitation energy that promotes the fluorescent compounds to an excited state comes from electron exchange from a high energy intermediate in the peroxyoxalate reaction. Hence the CL is known as chemically initiated electron exchange luminescence (CIEEL).

The luminescence from fluorescent compounds via CIEEL rather than CL direct from the aryl oxalates is much more intense and weak bases are often included to catalyse the reaction and provide larger peak intensities. Bases such as triethylamine, tris(hydroxymethyl)aminomethane or imidazole have been used. However, stability problems with some post column reaction mixtures have been found.

1.10.2   Stability.

Peroxyoxalate esters such as bis-2,4-dinitrophenyl)oxalate (DNPO) and bis(2,4,6-trichlorophenyl)oxalte (TCPO) are the most popular oxalates used (62). However,

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DNPO and TCPO and their products have limited solubility and stability in water, thus requiring the use of an organic solvent (62, 65, 69). Water soluble oxalates have been developed but are less sensitive (62, 65).

Many peroxyoxalate detection systems have been used with reversed phase HPLC (RP-HPLC), which uses a higher content of organic solvent than IC. However, polar solvents can seriously affect the response. The effect is thought to be due to the nucleophilic solvents such as water reacting with the oxalate esters in non-CL side-reactions (65). Also, reagent solutions containing oxalate and hydrogen peroxide degrade as the oxidative reaction proceeds in the reagent container. The effect different solvents have on this process has also been studied (69).

Peroxyoxalate detection systems are widely used and two detection strategies have been used, the first is to include a PCR mixture that contains the aryl oxalate and a fluorophore in excess for the determination of hydrogen peroxide. The second is to use a PCR mixture that contains aryl oxalate and hydrogen peroxide in excess for the determination of fluorescent compounds. This is widely used for the determination of organic compounds by CIEEL because of superior detection limits to fluorescence. However, the problems associated with the need for organic solvent has meant that peroxyoxalate CL has been little studied for the determination of metal ions separated by IC.

1.10.3   Determination of hydrogen peroxide.

Hydrogen peroxide detection is a common feature of many CL reaction strategies, as reagent B in Equation 1.26. Many CL reactions are oxidation reactions and hydrogen peroxide is often a suitable oxidizing agent. When limiting the reaction, hydrogen peroxide can be sensitively detected. However, certain aspects of peroxyoxalate CL make it particularly suitable for the determination of hydrogen peroxide.

Hydrogen peroxide can be generated as a result of many enzyme reactions which occur in buffered solutions around neutral pH, and so become amenable to detection at a similar pH. The pH required for peroxyoxalate reactions is much lower than for other CL reactions. The peroxyoxalate reaction pH depends upon the aryl oxalate used and can be as low as pH 4 although more rapid at higher pH 8. This pH is still much lower than for other CL reaction systems that can be used to detect hydrogen peroxide for example the luminol CL system discussed later generally requires a pH >9.

Kwakman in his review (65) cites work by a number of authors including Rigin and Van Zoonen who indirectly determined biologically important molecules from the hydrogen peroxide they generated from post column enzymic reactions. The hydrogen peroxide generated was determined by peroxyoxalate CL.

The photo-generation of hydrogen peroxide has also been used. In the presence of oxygen quinones act as photocatalysts for the production of hydrogen peroxide. Aichinger applied the above reaction to the detection of alcohols and sugars in the low   . nanogram range, which is much lower than that achieved by UV-Vis detection (70).

1.10.4   Detection of fluorophores.

As mentioned above, fluorescence detection is often limited by stray light in the detection cell. CIEEL offers a means of exciting some fluorescent molecules without a photo-excitation source. Most of the compounds determined by peroxyoxalate CL have been organic and it has only been used to a very limited extent for trace metal determination although some potential has been shown by Fujimaki who describes the FIA determination of organo-tin species after the formation of various fluorescent quinoline derivatives (71). Exploitation of CIEEL to excite fluorescent metal compounds formed post-column after IC separation may provide a more sensitive detection system for these compounds than by radiative excitation, but would require oxalate esters that are more compatible with the aqueous eluents used in IC.

Chemiluminescence using the peroxyoxalate system is from the fluorophore rather than the oxalate ester. However, in most CL reactions the emitter is the species that undergoes the chemical reaction. Whilst most CL reactions involve the oxidation of organic compounds, a metal complex tris(2,2'-bipyridine)ruthenium can undergo either oxidation or reduction reactions from two different oxidation states to yield CL.

1.11.1   Tris(2,2'-bipyridine)ruthenium (II).

Hercules and Lytle first reported orange light upon reaction of tris(2,2'-bipyridine)ruthenium (III) with either hydrazine or hydroxide ion in 1966 (72). Tris(2,2'-bipyridine)ruthenium (II) $Ru(bpy)_3^{2+}$ is the stable species of this ruthenium

complex in aqueous solution although it may be electrochemically oxidized to the ruthenium (III) complex at $+1.3$ V or reduced to the ruthenium (I) complex at -1.3 V. The oxidized or reduced ruthenium complexes may then be reduced or oxidized back to the $Ru(bpy)_3^{2+}$ complex, for example by oxalic acid or potassium persulphate respectively (73, 74), as shown in Equations 1.29 and 1.30 (74). In both cases the $Ru(bpy)_3^{2+}$ is excited and becomes the emitting species.

1.11.2 Reduction of Tris(2,2'-bipyridine)ruthenium (II) by oxalate.

Equation 1.29
$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + e^-$$

$$Ru(bpy)_3^{3+} + C_2O_4^{2-} \rightarrow Ru(bpy)_3^{2+} + C_2O_4^-$$

$$C_2O_4^- \rightarrow CO_2 + CO_2^- \cdot$$

$$Ru(bpy)_3^{2+} + CO_2^- \cdot \rightarrow Ru(bpy)_3^+ + CO_2$$

$$Ru(bpy)_3^{3+} + Ru(bpy)_3^+ \rightarrow Ru(bpy)_3^{2+} + Ru(bpy)_3^{2+\cdot}$$

$$Ru(bpy)_3^{2+\cdot} \rightarrow Ru(bpy)_3^{2+} + h\upsilon$$
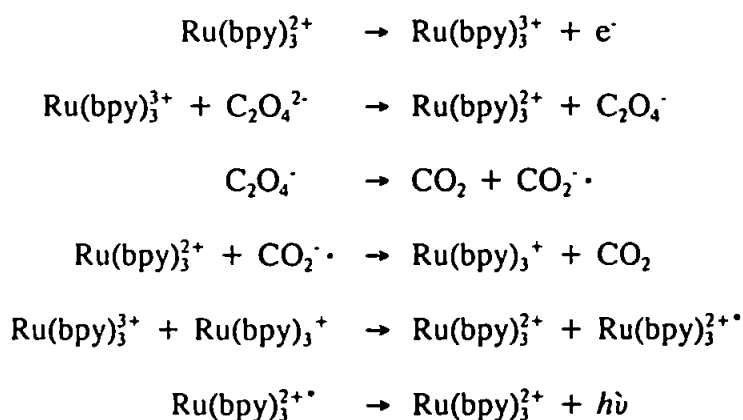
1.11.3   Oxidation of Tris(2,2'-bipyridine)ruthenium (II) by potassium persulphate.

Equation 1.30

$$Ru(bpy)_3^{2+} + e^- \rightarrow Ru(bpy)_3^+$$

$$Ru(bpy)_3^+ + S_2O_8^{2-} \rightarrow Ru(bpy)_3^{2+} + S_2O_8^{3-}$$

$$S_2O_8^{3-} \rightarrow SO_4^{2-} + SO_4^- \cdot$$

$$Ru(bpy)_3^{2+} + SO_4^- \cdot \rightarrow Ru(bpy)_3^{3+} + SO_4^{2-}$$

$$Ru(bpy)_3^{3+} + Ru(bpy)_3^+ \rightarrow Ru(bpy)_3^{2+} + Ru(bpy)_3^{2+*}$$

$$Ru(bpy)_3^{2+*} \rightarrow Ru(bpy)_3^{2+} + h\upsilon$$
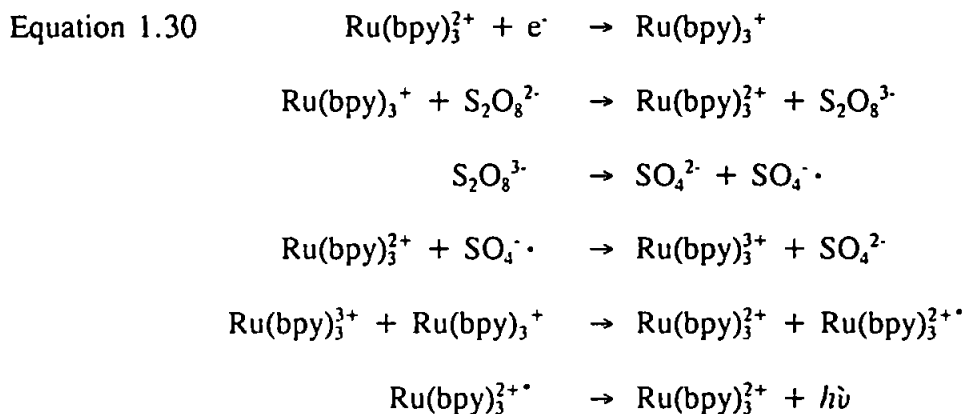
The reduction of $Ru(bpy)_3^{3+}$ by aliphatic amines was used by Noffsinger to detect primary secondary and tertiary aliphatic amines (75). Noffsinger found this detection system particularly sensitive to tertiary amines, which unlike the primary and secondary amines cannot be tagged with dansyl chlorides and sensitively determined by other CL detection systems ie. peroxyoxalate (76).

Downey used a nafion (perfluorinated ion exchanger) film in the detection cell which contained $Ru(bpy)_3^{2+}$ which could then, by electrochemical oxidation, be converted to $Ru(bpy)_3^{3+}$ (77). The CL from the $Ru(bpy)_3^{3+}$ reaction could then be used to determine various analytes, such as, oxalate, amines and NADH. Jackson used a similar system for the rapid sensitive determination of amino acids (78).

1.12.1   Luminol.

The above CL reactions have shown the use of CL in the determination of organic or occasionally organo-metallic molecules. In general, the determination of metal species has been achieved using other CL systems, such as the luminol CL system. Although there are a few reports of metal ions determined by CL detection systems used in conjunction with HPLC, CL metal detection systems have been used predominantly in batch and FIA systems. In such systems the analyte ion may co-exist with interfering ions in the detection cell. CL reagents such as lophine (2,4,5-triphenylimidazole) or gallic acid (3,4,5-trihydroxybenzoic acid), with greater specificity than luminol, can be used in such systems with fewer interferences. However, lophine is only slightly soluble in water and requires an organic solvent. Lucigenin (10,10'-dimethyl9,9'-bisacridium nitrate), has been used for the determination of metal ions by the catalytic effect these ions have on the CL reaction, but the product of the lucigenin CL reaction, N-methyl-acridone, is insoluble and tends to coat the detection cell decreasing the analytical response over time, and requiring regular removal. Klopf added low concentrations of the surfactant sodium dodecyl sulphate to the lucigenin reagent and found that this reduced the build up of N-methyl-acridone (79). Luminol CL has an advantage over these other systems for the detection of metal ions in aqueous solutions as it is water soluble. Thus, the majority of publications describing determination of metal species quote the use of luminol. Table 2 shows the range of metals that have been determined by different CL systems.

Luminol (5-amino-2,3-dihydropthalahydrazine-1,4-dione or 5-aminophthalahydrazide) CL was first reported in 1928 (62, 107, 108, 109) by H. O. Albrecht (110).   The

Table 2   Range of metals detectable using CL reaction systems.

Applications

Chemiluminescence System	Metal Ions Determined	Author Reference
Peroxyoxalate	Sn	(71)
Peroxyoxalate	Cr(VI), Mo(VI), V(VI)	(80)
Tartaric Acid	Co(II)	(81)
Gallic Acid	Co(II)	(82)
Gallic Acid	Co(II), Ag(I)	(83)
Luminol	Ag(I), Co(II), Fe(II), Cu(II), Mn(II), Cr(III), Au(III), Ti(III), Cr(VI and two multi-element detection systems	(25), (38), (84), (85), (86), (87), (88), (89), (90), (91), (92), (93), (94) (95), (96), (97, (98)

Reviews

Chemiluminescence System	Number of CL active metals	Author Reference
Lucigenin	9 metals	(99)
Lophine	10 metals	(102)
Lophine	9 metals	(106)
Gallic acid	14 metals	(101)
Gallic acid	5 metals	(62)
Aequorin	Ca (II)	(103)
Luminol	9 metals	(62)
Luminol	6 metals	(100)
Luminol	10 metals	(104)
Luminol	20 metals	(105)
Luminol	6 metals	(103)

oxidation of luminol to yield CL occurs in alkaline solutions, although the use of

ordered media has allowed the use of lower pH (84). The overall reaction scheme for

the oxidation of luminol in alkali solution in the presence of a catalyst is given in

Equations 1.31 and 1.32. A common oxidant is hydrogen peroxide and one of the

most studied catalysts is cobalt (II). The emitting species is the 3-aminophthalate

anion.

Equation 1.31

Luminol + oxidant + catalyst $\xrightarrow{OH^-}$ 3-aminophthalate + N_2

Equation 1.32
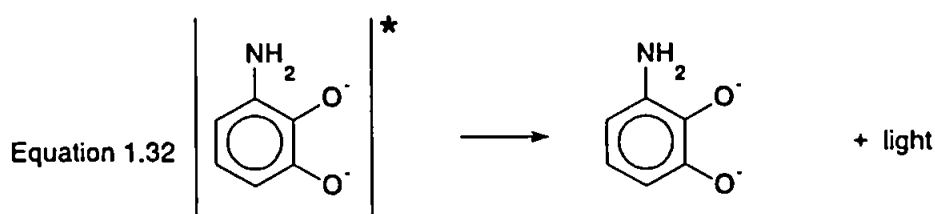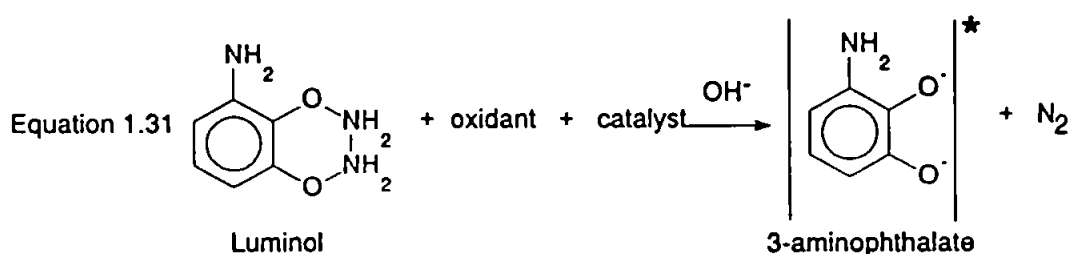
The CL reaction mechanism for oxidation of luminol in alkaline peroxide in the

presence of catalyst is poorly understood (108, 109, 111). The understanding of CL

reactions is made more difficult as multi-step processes and the low quantum yields,

(luminol Φ_{CL} = 0.01) serve to hide reaction pathways. Catalysis of this system by

cobalt (II) was studied by Burdo *et al.* and a cobalt (II) hydrogen peroxide complex is

believed to be involved (109). Later Lind *et al.* investigated the role of luminol in this

reaction (108).

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When using hydrogen peroxide as the oxidant the CL reaction may be catalysed by a range of transition metal cations. The use of the term catalysis is often not correct as many metal ions do not remain unchanged, but are oxidised permanently to a higher oxidation state. However, the term catalyst has been used historically and has become the accepted term. Interestingly nickel (II) has been reported to be a true catalyst for this reaction (104).

A number of metals can catalyse the CL reaction in the absence of added oxidant. The most widely reported of these is iron (II) which uses the dissolved oxygen in the reaction solution. Catalysis of the CL reaction in the absence of added co-oxidant has also been reported for solutions containing gold (III). Other oxidizing systems have been reported for the CL oxidation of luminol. One such is potassium persulphate which can oxidise luminol in a reaction catalysed by silver (I) (86).

Equations 1.26-1.28 show that CL reactions can be used in detection systems in a number of ways, for example from Equation 1.26 reagents A, B, and C can be determined. By ensuring an excess of other reagents, either luminol (or derivatives), (A); oxidant, (B) or catalyst, (C) can be determined. It is also possible to determine other reagents that interact with one of the reagents affecting the CL signal. However, most typically the catalyst is the target reagent.

1.12.2   Determination of analyte via Luminol (Pre-column Labelling).

Luminol and its derivatives such as iso-luminol can be used in pre-column

derivitization. Analytes are most often attached to the amine moiety on the benzene ring. This procedure has been applied to the determination of organic compounds rather than metal ions.

Aminobutylethylisoluminol (ABEI), an analogue of iso-luminol, has an alkyl chain which distances the substitution point and the CL site. This distance decreases the effect the analyte has on CL (73). Niemann and Kawasaki describes the use of ABEI for pre-column derivatization of primary and secondary amines and carboxylic acids before separation by reversed phase HPLC (73). Ishida presents 4,5-diaminophthalhydrazide for the specific pre-column derivitization of α-dicarbonyl compounds prior to separation and CL determination (112).

1.12.3   Determination of analyte via oxidant.

The most common oxidant in luminol reactions is hydrogen peroxide. Hydrogen peroxide may be the actual analyte or may be generated in the PCR, such as the enzymatic PCRs described with peroxyoxalate CL determination of hydrogen peroxide, Section 1.10.3.

Another variation is the suppression of the action of hydrogen peroxide on luminol CL which was used by Huang to determine sulphite in wine by FIA (113). The limit of detection for sulphite by this method was $10\mu M$.

Ci *et al.* (114) describes the PCR detection of amino-acids by the inhibitory effect they

have on the oxidation of luminol by mimetic peroxidases. The amino-acids complex with the mimetic peroxidases, which are metallo-porphyrins, reducing CL.

1.12.4   Determination of analyte via the catalyst.

The main species that catalyse the oxidation of luminol by hydrogen peroxide are transition metals, including cobalt (II), copper (II) iron (III), iron (II) (with or without peroxide), and chromium (III). Hartkopf (105) and Seitz (104, 103) give more examples of cations that catalyse the CL reaction, with detection limits. Only a few non-metals catalyse the luminol CL, including iodine and hypochlorite (104). The analyte may either be the catalyst or another species that causes the CL intensity to alter by interacting with the catalyst.

1.12.5   Analyte as the catalyst.

Sensitive determination of the metal catalysts by CL has been reported for some cations. These cations tend to be those to which the CL reaction is very sensitive, for example cobalt (II) or suffer few interferences in the absence of hydrogen peroxide, such as iron (II).

The light emission spectrum of the luminol CL system is not catalyst dependent, the only difference between different catalysts is the difference in magnitude of the signal and the difference in signal rise and fall profiles. However, the time window is fixed

and a detector cannot differentiate between different catalysts based on different rise and fall times in a flow system. In this case catalysts have either to be introduced to the luminol reagent singly or interfering species have to be masked to give quantitative analysis of the catalyst.

Flow injection techniques and batch systems have been used but suffer due to the number of interferents. Seitz determined iron (II) in seawater by luminol CL without peroxide (85). The number of interfering ions is low by this method and iron (II) could be determined at 0.005 μg l^{-1}, Seitz also determined chromium (III) in water at 0.025 μg l^{-1} by a similar method but needed hydrogen peroxide (87). The number of interfering ions is greatly increased by using a hydrogen peroxide system. However, Seitz masked these ions by adding EDTA to the eluent, which rapidly forms complexes with interfering cations, but not with chromium (III) in the time taken to reach the detector. Reports of other catalysts that are sensitively determined are few, for example, gold (III) (84, 115), silver (I) (86, 115), copper (II) and vanadium (88), though less sensitive than cobalt (II), chromium (III) or iron (II).

1.12.6   The use of chemiluminescence detection in ion chromatography.

The emission spectrum of luminol is identical irrespective of catalyst used. Hence, separation of the analyte from interfering species was proposed. Seitz and other authors proposed the separation of the analytes by ion exchange chromatography followed by CL detection in the 1970's (104, 100, 116). In 1973 Seitz (104) showed the separation of nickel (II), copper (II) and cobalt (II) as their anionic chloride

complexes followed by CL detection.

The very high sensitivity of CL detection has more recently been used with IC to detect cobalt (II) at sub ng l^{-1} levels. For example, Jones *et al.* could detect 0.5 ng l^{-1} cobalt (II) (38). Detection of cobalt (II) at the ng l^{-1} level was achieved by Sakai *et al.* (1 ng l^{-1}) (89) and Boyle *et al.* (2.4 ng l^{-1}) (88). Boyle *et al.* also determined vanadium, copper (II), iron (II) at low ng l^{-1} levels (88). Detection limits of 0.1 μg l^{-1} chromium (III) and 0.3 μg l^{-1} chromium (VI) were reported by Williams *et al.*, after simultaneous separation on a twin column IC system (25). Gammelgard *et al.* reported detection limits of 0.5 μg l^{-1} for both chromium species after separation on a single column IC system (90). Obata *et al.* reports a detection limit 2.8 ng l^{-1} iron (III) in sea-water using chelation IC and preconcentrating 18 cm^3 sample (45). However, IC with CL detection has not been developed for other species that cannot be determined as sensitively.

IC of transition metals has mostly relied upon the addition of weakly complexing organic acids to acidic eluents. The use of even weak complexing agents often reduces the ability of a catalyst to catalyse the CL reaction. Burdo proposes a reaction pathway that involves a catalyst-peroxide complex (109). Cations already in a complex with another chelate might not be able to form this peroxide complex or catalyse the reaction. Such reduction in sensitivity was reported by Burdo (109) and also by Jones (38) when using complexing agents for IC. The decrease in CL when a catalyst is complexed has been exploited in PCR systems where the analyte is determined by the effect it has on the catalyst and therefore CL.

Also, the luminol CL reaction occurs in strongly alkaline solution and therefore a post-

column pH change is required. This can be simply done by adding a suitable buffer to the luminescent reagent. However, increasing salt concentration can decrease CL response and increase possible contamination.

The IC systems developed so far have been for the metal ions that most sensitively catalyse the CL reaction. Exploitation of Cl detection systems to enable sensitive detection of metals ions and other species by involving them in a reaction with catalyst are described below.

1.12.7   Determination of analyte via post column reaction with the catalyst.

Post column reactions have been developed that either increase or decrease the amount of catalyst that is available to catalyse the CL reaction. Niemann describes examples where the metal catalyst is complexed with organic compounds leading to a decrease in CL intensity (73).

The use of exchange reactions between an analyte metal and the metal catalyst contained in a complex have been investigated. The aim of these investigations was to develop a sensitive system for the determination of non-CL active or weakly CL active metals based on CL. The most sensitively determined metal ion catalysts for the luminol reaction, cobalt (II) and copper (II), were used in these investigations.

Yan *et al.* (92) described a FIA system in which copper (II) was displaced from a strong cation exchanger in the copper form by monovalent, divalent and trivalent

cations. The high number of sites on the copper form column enabled detection of cations with significantly lower affinity. The same system was used to determine weak acids after separation by liquid chromatography. However, the ionic strength of the mobile phase had to be very low to a prevent high background signal by displacement of copper (II) ions.

Jones *et al.* (93) described a novel ion displacement reaction in which cobalt (II) was displaced from a cobalt-EDTA complex by divalent cations. The displaced cobalt (II) was then available to catalyse the CL reaction. This system was used to determine various divalent transition metal cations, calcium and magnesium separated by IC. However, the system required the use of long reaction coils heated in a water bath to 90°C and there was a very large background signal.

1.13   Conclusion on the use of chemiluminescence detection.

The very high signal to noise ratio of CL detection makes CL detection very sensitive to low levels of analyte. This has enabled the use of CL detection systems to provide highly sensitive detection for both metals and non-metals, for a range of chromatographic, flow injection and static methods of analysis. The most used CL system for the determination of transition metal ions in aqueous solutions is the luminol system.

Separation of transition metal ions is most conveniently carried out by IC. By separating metal ions by IC they elute either as simple metal ions or in weak

complexes. Both of these situations have enabled the sensitive detection of some of these ions by CL.

The choice of CL detection system used in conjunction with liquid chromatography systems is governed by compatibility with the mobile phase of the eluent. These include solubility and stability of the CL reagents in different organic and aqueous phases, the effect solvents used in the eluent have on the intensity of the CL reaction and the effect that the eluent has on the ability of catalysts or analytes to react in the CL reaction or PCR. Hence, the luminol CL system has also been the CL system most used for the determination of metal ions separated by IC.

1.14   Aims.

Briefly, the aim of this work is to investigate and develop CL PCR detection systems for trace metals after separation by IC. From previous studies the most suitable CL reagent to use with aqueous eluents is luminol and so luminol will be used throughout as the CL reagent.

CL detection has been used successfully with IC only in a limited number of cases to provide sensitive but highly selective detection for some transition metal cations, for example cobalt (II), copper (II) and chromium (III). The aim of the first part of this work is to investigate the use of complexation reactions between cobalt (II) and EDTA in order to develop a sensitive CL detection system for a wider range of metal ions than presently available, that is, for cations that cannot catalyse the CL reaction

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directly.

The second section of work will be devoted to the development of a simultaneous single column separation of chromium (III) and chromium (VI) in order to develop an IC system with CL detection for the study of chromium speciation in natural waters. The developed system will be evaluated using a certified reference simulated fresh water sample and by involvement in a Bureau Communtaire de Reference (BCR) programme to determine chromium speciation in lyophilised fresh water samples.

There is concern in the nuclear industry about trace metal contaminants in the primary coolant of PWR installations. Silver has been detected by neutron activation analysis in some VVER designed PWR plants and there is a need to study the deposition and release using chemical detection methods. The third section and largest part of the programme deals with the development of an IC system with CL detection for the determination of silver (I) in simulated VVER (PWR) primary coolant at the sub μg l^{-1} level. The IC method needs to be developed alongside the CL detection system to ensure compatibility.

The fourth and final section deals with the determination of gold. Gold (III) in water and mineral samples is of interest to those in the gold prospecting and processing industries. However, there are few sensitive methods for the determination of gold at the μg l^{-1} level. Gold (III) can be used to give CL with luminol in alkaline solution in the absence of co-oxidant. A method for the IC separation of gold (III) as its chloroauric complex has been presented by Rocklin. This final section presents the development of a CL detection system for gold (III) to be used with this IC method.

CHAPTER TWO.

2.0 MULTI-ELEMENT DETECTION SYSTEM FOR ION CHROMATOGRAPHY BASED ON CHEMILUMINESCENCE.

2.1 Introduction.

Detection in liquid chromatography has often been considered to be the weak link in the HPLC system. The use of hyphenated techniques has often provided a means of obtaining sensitive detection of elements. However, the coupling of instruments not designed to work with each other has been problematic. Non-hyphenated detection systems have relied on conductivity measurement of ions, spectrophotometry or inverse photometry, often using post column reactions (PCRs). These techniques, whilst more widespread, lack sensitivity. However, PCRs based on chemiluminescence (CL) are an exception. These have provided highly sensitive detection for a limited number of elements, that is those that most effectively catalyse the CL reaction eg. cobalt (II), copper (II), iron (II) and chromium (III). It would be useful therefore to extend the range of metals determinable by CL after separation by IC.

One promising approach is to use metal-ligand exchange reactions in the PCR system. One of the best known of these systems is the zinc-EDTA-PAR exchange reaction developed by Aguello and Fritz (59), discussed in Section 1.7.3.4 and shown in Equation 2.1.

Equation 2.1 $\qquad M^{2+} + Zn\text{-}EDTA^{2-} + PAR \rightarrow M\text{-}EDTA^{2-} + Zn\text{-}PAR$

Using the same principle Yan *et al.* (92) and Jones *et al.* (38) have both developed PCR systems based on exchange reactions in order to develop a more universal CL detector based on luminol CL catalysis by copper (II) or cobalt (II) respectively. The method developed by Yan *et al.* was limited for use with IC as it could only be used with very dilute eluents. Jones *et al.* successfully used their system as a PCR detection system with IC for a range of metals eluted from a cation exchange column. The reaction used by Jones *et al.* relied on the release of cobalt (II) from an EDTA complex by eluting cations, as shown in Equation 2.2. The free cobalt (II) could then catalyse the CL reaction.

Equation 2.2 $\qquad M^{n+} + Co\text{-}EDTA^{2-} \rightarrow M\text{-}EDTA^{(4-n)-} + Co^{2+}$

It is well known that a relatively small EDTA complex stability constant is needed in order to enable significant exchange to take place. However, the cobalt-EDTA complex does not undergo rapid exchange, requiring a long reaction coil heated to 90°C in a water bath to speed up the reaction rate and achieve significant exchange. Jones *et al.* also found it necessary to decrease the pH of the reaction in order to promote acid catalysed exchange and get significant exchange with cations that formed significantly weaker EDTA complexes than cobalt, for example calcium and magnesium.

In general therefore the exchange reaction of an EDTA complex is slow, (118) and it is

difficult to see how direct exchange reactions involving the strong cobalt-EDTA complex would be kinetically fast enough to be analytically useful without the long reaction times and high temperatures described above. In contrast the direct formation of a metal-EDTA complex is fast. Therefore, it was considered that by using two direct formation reactions shown in Equations 2.3 and 2.4, the long, heated reaction coils used in the direct exchange reaction would not be required.

Equation 2.3 $\qquad y\,M^{n+} + x\,EDTA^{4-} \rightarrow y\,M\text{-}EDTA^{(4-n)-} + (x-y)\,EDTA^{4-}$

Equation 2.4 $\qquad (x-y)\,EDTA^{4-} + x\,Co^{2+} \rightarrow (x-y)\,Co\text{-}EDTA^{2-} + y\,Co^{2+}$
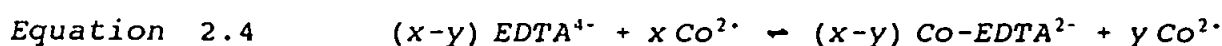
The procedure required the use of another pump and reaction coil so that equivalent amounts of EDTA and cobalt (II) could be added sequentially. In the proposed system the eluting metal ion, M^{n+}, would react with EDTA in the first reaction coil according to Equation 2.3. The remaining uncomplexed EDTA reacts with cobalt (II) in the second reaction coil according to Equation 2.4. The result is an increase in uncomplexed cobalt (II) as analyte metals elute. The combined eluent would then mix with the luminol solution directly before the detector. Cobalt (II) remaining uncomplexed will then be available to catalyse the CL reaction.

To put it another way, in the absence of any analyte cation the amounts of cobalt (II) and EDTA have to be balanced as close to a 1:1 ratio as possible, so that there is no excess cobalt (II) or EDTA. The amount of free cobalt (II) able to catalyse the CL reaction is dependent on the amount of analyte present. From Equations 2.3 and 2.4,

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when there is no analyte present then $y=0$, therefore $(x-y)=x$ and ideally no cobalt (II) is left uncomplexed to catalyze the CL reaction. When analyte is present then $y>0$ and there will be insufficient EDTA to complex all of the added cobalt (II). The remaining uncomplexed cobalt (II) can catalyse the CL reaction.

2.2 Experimental.

The chromatograph, shown in Figure 5, allowed sequential addition of the EDTA and cobalt (II) reagents. The two reaction coils allowed independent control of some reaction conditions for the formation reactions of the analyte-EDTA and the cobalt-EDTA complexes. Reaction conditions investigated included reaction time and reaction temperature. Reaction pH was also examined. However, changes in reaction pH affected the reactions in both reaction coils.

Analyte cations were separated and detected using the conditions reported by Jones *et al.* (38). This included a dilute complexing eluent containing 0.1 M lactic acid at pH 3.7 to elute the analyte cations from a Dionex CG2 IC column and a luminol CL detection system responsive to free cobalt (II). The flow rates reported by Jones *et al.* were used where possible. However, the use of separate EDTA and cobalt (II) reagent solutions required that the flow rate for addition of each was half the flow rate Jones *et al.* had used to deliver the single cobalt-EDTA complex reagent. The equipment and reagents used are detailed below.

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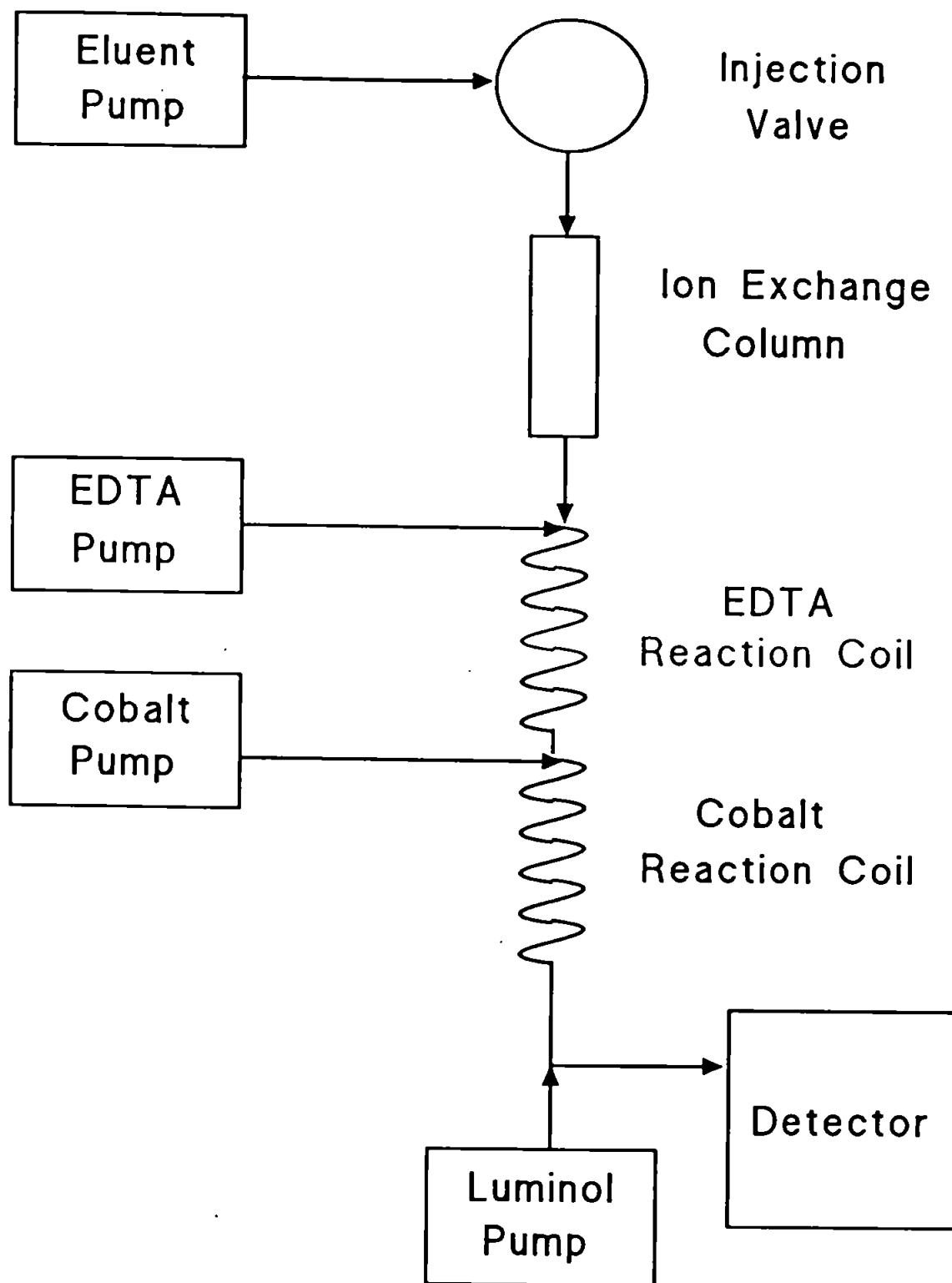
Figure 5   Schematic diagram of the four pump chromatographic system employed.

2.2.1   Equipment.

A high-pressure inert plastic pump (Dionex Gradient pump, Dionex, Sunnyvale, CA, U.S.A.) delivered the column eluent to a Dionex CG2 IC column. The EDTA solution was delivered by a post-column stainless-steel pump (model 64, Knauer, Bad Homburg, Germany). A high-pressure titanium pump (Model 2150, LKB Bromma, Sweden) was used to deliver the cobalt (II) solution. The luminol solution was delivered by a high-pressure inert plastic pump (Dionex 2000i).

Titanium tubing connected the gradient pump to the injection valve, a ten port zirconium injection valve (Valco, Schenkon, Switzerland). All other tubing was 0.3 mm i.d. PTFE. The detector was made by coiling the tubing (volume $33\mu l$) flat against the face of a photo-multiplier tube from a fluorescence detector (FD-100, Spectrovision, Chelmsford, MA, U.S.A.).

2.2.2   Reagents.

Milli Q deionized water (Millipore Corp., Bedford, MA, U.S.A.) was used throughout. All reagents were AnalaR (Merck, Poole, U.K.) except luminol (Fluka, Poole, U.K.). Spectrosol (Merck) 1000 mg l^{-1} or 10,000 mg l^{-1} solutions of the analyte metals: magnesium, calcium, manganese, zinc and nickel were diluted to make working standards.

2.2.3   Eluent.

The dilute complexing eluent of 0.1 M lactic acid was adjusted to pH 3.7 by the addition of potassium hydroxide solution.

2.2.4   EDTA solution.

Ethylenediaminetetraacetic acid disodium salt was used. The pH of this solution was buffered by the addition of disodium tetraborate (0.05 M) if the pH of the reaction coils was to be maintained above that of the lactic acid eluent. Solutions containing 5×10^{-5} M, 10^{-5} M and 5×10^{-6} M EDTA were investigated initially. 5×10^{-5} M EDTA was used subsequently.

2.2.5   Cobalt (II) solution.

A solution of the cobalt (II) chloride hexahexahydrate corresponding to the molar concentration of EDTA solution was used. The pH of this solution was slightly acid and was not adjusted.

2.2.6   Luminol solution.

A solution containing 0.001 M luminol, 0.16 M orthoboric acid and 0.1 M hydrogen

peroxide was prepared and adjusted to pH 12.0 by the addition of potassium hydroxide.

2.2.7   Flow rates.

The flow rates for the eluent and luminol solutions were the same as those used by

Jones *et al*. The flow rate Jones *et al*. used for the cobalt-EDTA solution was split

equally between the cobalt (II) and EDTA solutions. The flow rates used are shown in

Table 3, the cobalt flow rate was adjusted by 0.01 ml min^{-1} increments as required to

balance the cobalt (II) and EDTA in the system.

2.3   <u>Results and Discussion.</u>

2.3.1   Previous work.

The metal/ligand exchange reaction used by Jones *et al*. (38) had a number of

problems, which are listed below:

1)   Cations that form considerably weaker complexes with EDTA than cobalt (II),

such as magnesium or calcium, required that the pH of the reaction coil was

reduced to pH 3 in order to achieve sufficient exchange for detection.

2)   The metal/ligand exchange reaction used is acid catalysed. This required the

reaction pH to be less than pH 7, otherwise the exchange reaction was too slow.

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Table 3   Flow rates of the reagents used.

Solution	Flow Rate ml min^{-1}
Lactic Acid	1.0
EDTA	0.3
Cobalt (II)	0.3
Luminol	0.8

.

3) As the pH is lowered, to satisfy 1) and 2), the conditional stability constant of the cobalt-EDTA complex, shown in Figure 6, is lowered. As a result the proportion of uncomplexed cobalt (II) is increased, raising the background CL.

4) Even in slightly acidic solution the system required that there was a long reaction coil to increase the reaction time, which inevitably lead to peak broadening.

5) The reaction coil was heated up to 90°C to increase the rate of metal/ligand exchange as much as possible.

2.3.2 Speed of reaction.

The metal/ligand exchange reaction used by Jones *et al.* was slow whereas the direct formation of a metal-EDTA complex is known to be fast (118). Therefore, by using a system that avoided the exchange reaction, but formed the metal-EDTA complex from the uncomplexed metal and EDTA, the high temperatures and long reaction coils may be avoided.

2.3.3 Important variables.

In this, the four pump system, a number of variables were decided to be important and these may differ, in terms of importance and effect, from those variables investigated in
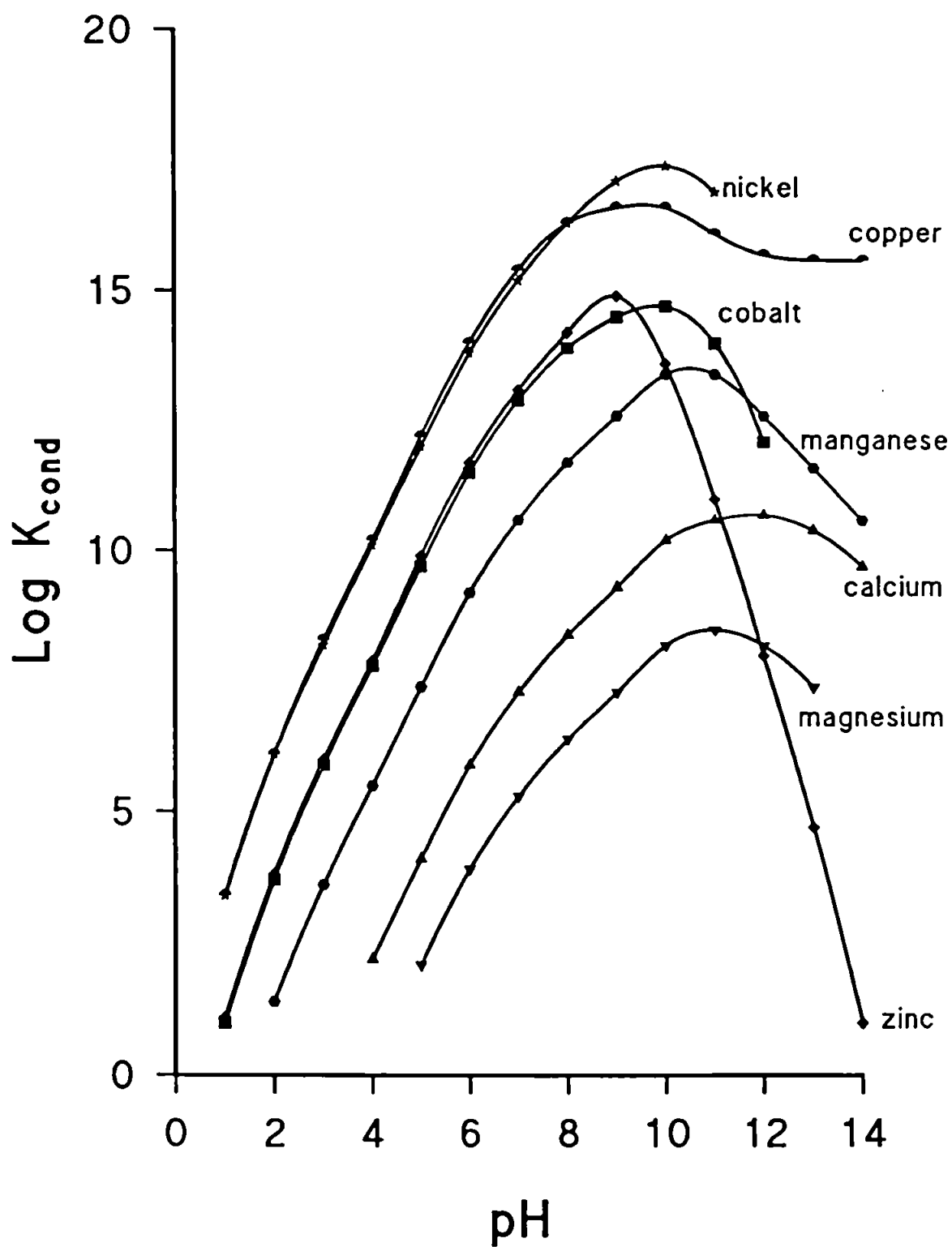
Figure 6   Variation of conditional stability constants with pH for selected

metal-EDTA complexes (117).

the previous system. The important variables chosen were temperature of reaction coils, length of reaction coils, pH of the solution in the reaction coils and the balancing of the separate cobalt (II) and EDTA addition to the flow system.

2.3.4   Addition of cobalt (II) and EDTA to the flow system.

In the system developed by Jones *et al.* the cobalt-EDTA solution was prepared prior to use by titration. In the system used in this work the complex was prepared *in situ* in the reaction coils. The need for matching these reagents is critical in this system to prevent overloading of the detection system by the presence of too much free cobalt (II). It is also important that there is not an excess of EDTA, this would cause the formation of metal-EDTA complex with eluting analytes without leading to the "release" of cobalt (II) and hence free cobalt (II) in the detector. This could lead to curved calibrations or poor detection limits.

2.3.5   Balancing of cobalt (II) and EDTA addition.

The flow rate of the EDTA solution was kept constant at 0.3 ml min^{-1} whilst the delivery of the cobalt (II) was increased or decreased by 0.01 ml min^{-1} according to the amount required. The addition of too much cobalt (II) led to high background signal which obscured low levels of analyte, whereas too little cobalt (II) addition led to lower background but loss of signal at low levels of analyte.

The conditional stability constant of the EDTA complex (K_{cond}) given in Equation 2.5 is pH dependent and increases with increasing pH.

$$Equation\ 2.5 \qquad K_{cond} = \frac{[Co\text{-}EDTA^{2-}]}{\sum [Co^{2+}]\ \sum [EDTA]}$$

The K_{cond} for the cobalt-EDTA complex is given in Table 4 at selected pHs, it can be seen that as the pH increases (and K_{cond} increases) the amount of free cobalt (II) decreases. The amount of free cobalt (II) and EDTA is given as X, which can be calculated approximately from the dissociation of the cobalt-EDTA complex, at a known concentration, according to Equations 2.6 and 2.7, assuming that X is small compared to the concentration of cobalt-EDTA.

$$Equation\ 2.6 \qquad K_{cond} = \frac{5 \times 10^{-5}\ M}{X^2}$$

$$Equation\ 2.7 \qquad X^2 = \frac{5 \times 10^{-5}\ M}{K_{cond}}$$

However, in the system used the complex is formed by the mixing of two separate streams of cobalt (II) and EDTA, therefore the free concentrations of cobalt (II) and EDTA may differ. If they differ greatly then the high background or low sensitivity mentioned above would occur.

Table 4   Calculated amount of free cobalt arising from the dissociation of 5×10^5 M cobalt-EDTA complex at different pHs, using Equation 2.7. The conditional stability constant ($K_{(cond)}$) is defined in Equation 2.5.

pH	4	5	6	7	8
$K_{(cond)}$	6.3×10^{-7}	5.0×10^{-9}	3.2×10^{-11}	7.9×10^{-12}	7.9×10^{-13}
X^2	6.3×10^{-13}	9.9×10^{-15}	1.6×10^{-16}	6.3×10^{-18}	6.3×10^{-19}
Free Cobalt M (X)	9×10^{-7}	1×10^{-7}	1×10^{-8}	2.5×10^{-9}	8×10^{-10}
Free Cobalt $\mu g\ l^{-1}$	55	5.6	0.74	0.16	0.05

The need for a low background especially at the lower pH tends to favour a slight excess of EDTA, however, as the pH increases the free cobalt (II) decreases and the slight excess of EDTA becomes too great requiring the addition of more cobalt (II). This effect can be seen in Table 5 which shows the result of adding borax (sodium borate) to the EDTA solution, thereby increasing the pH of the reaction in the coils and the conditional stability constant. At two points it was necessary to increase the addition of cobalt (II) to again obtain a signal given on injection of a 50 μg l^{-1} zinc. This was necessary because the increase in the conditional stability constants of the cobalt (II)-EDTA complex led to a large excess of EDTA, capable of complexing the injected zinc without causing any cobalt (II) to be "released".

The exact balancing of EDTA and cobalt (II) addition was problematic due to the increments with which increase or decrease of addition could be made. Using 5 x 10^{5} M solutions a change in 0.01 ml min^{-1} cobalt (II) led to a change equivalent to 12 μg l^{-1} cobalt (II) in the system. The use of more dilute solutions would obviously lead to a smaller change in cobalt (II) concentration with change in flow rate. However, the use of more dilute solutions did not lead to lower background CL and gave poorer response. 5 x 10^{5} M EDTA and cobalt (II) solutions gave the best results and so were used throughout.

2.3.6   Effect of temperature.

The heating of the reaction coil in the system described by Jones *et al.* was used to increase the reaction rate and therefore the exchange between analyte and cobalt (II) in

Table 5   Addition of Borax to 500 ml EDTA solution. Effect on the peak height of 50 μg l^{-1} cobalt and zinc, the pH of the reaction coils and the amount of cobalt required.

Borax added (g l^{-1})	Cobalt Pump Flow (ml min^{-1})	pH of -		Peak Height (mm)	
		Reaction Coil	Final Solution	Cobalt	Zinc
0	3.0	3.6	10.3	15.5	22
5		4.1	10.1	3	10
6		4.3	10.0	2	5.5
7		4.6	10.0	N/D	N/D
7	3.1	4.6	10.0	4.5	10
8		4.8	10.0	5	8.5
9		7.1	9.9	N/D	N/D
9	3.2	7.1	9.9	12	15

the EDTA complex. In order to find out whether increasing the temperature of the direct formations reactions would be advantageous, the reaction coil(s) were heated in the following manner. The first reaction coil was immersed in a water bath at the desired temperature. The effluent was either cooled prior to entering the second reaction coil, or was allowed to pass directly into the second reaction coil uncooled in order to heat the second reaction coil. Cooling was achieved by immersing a small section at the end of the first reaction coil in an ice bath. The results of heating the coil(s) in such a manner is shown in Tables 6 and 7, Figures 7, 8 and 9 and is discussed below. The reaction coils were maintained at pH 5.0.

As the reaction coil(s) was heated the background noise increased, this being most pronounced at the highest temperatures. Cooling of the eluent prior to the second reaction coil did not decrease the noise significantly and therefore cannot be due in total to an increase in exchange of cobalt (II) or increase in CL reaction in the detector.

Whilst a significant increase in signal to noise ratio (S:N) was obtained by heating the first reaction coil to 45°C and then cooling the end of the reaction coil in ice prior to the second reaction coil, this added even greater complexity and the requirement that ice had to be continuously replaced as it melted. The length of the EDTA reaction coil had yet to be determined and therefore the amount of tubing which could be cooled or heated.

Allowing the effluent to pass into the second reaction coil uncooled thereby heating the second reaction coil, as expected, increased the displacement of analyte from its EDTA complex by cobalt (II). This reaction is comparable to the back reaction of Equation

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Table 6   Effect of increasing the temperature of the EDTA reaction coil on the background noise and the CL response for 50 μg l^{-1} nickel. Noise measured as the peak to peak variation in the background signal. Peak height measured from baseline mid-point to the top of peak.

Temperature (°C)	Cooling (Yes/No)	Peak Height (mm)	Baseline Noise (mm)	S:N Ratio
20	Yes	31	3	10
45	No	40	4	10
45	Yes	42	3	14
60	Yes	75	15	5
90	No	146	25	6
90	Yes	86	20	4

Table 7   Effect of increasing the temperature of the EDTA reaction coil on the background noise and the CL response for 50 μg l^{-1} zinc. Noise measured as the peak to peak variation in the background signal. Peak height measured from baseline mid-point to the top of peak.

Temperature ($^\circ$C)	Cooling (Yes/No)	Peak Height (mm)	Baseline Noise (mm)	S:N Ratio
20	No	74	6	9
20	Yes	63	4	16
45	No	62	4	16
45	Yes	85	3	28
60	No	76	7	11
60	Yes	119	9	13
90	No	121	25	5
90	Yes	136	20	7

50 μg l⁻¹ Zinc   50 μg l⁻¹ Zinc   50 μg l⁻¹ Nickel

No Ice   Ice   Ice
Bath   Bath   Bath
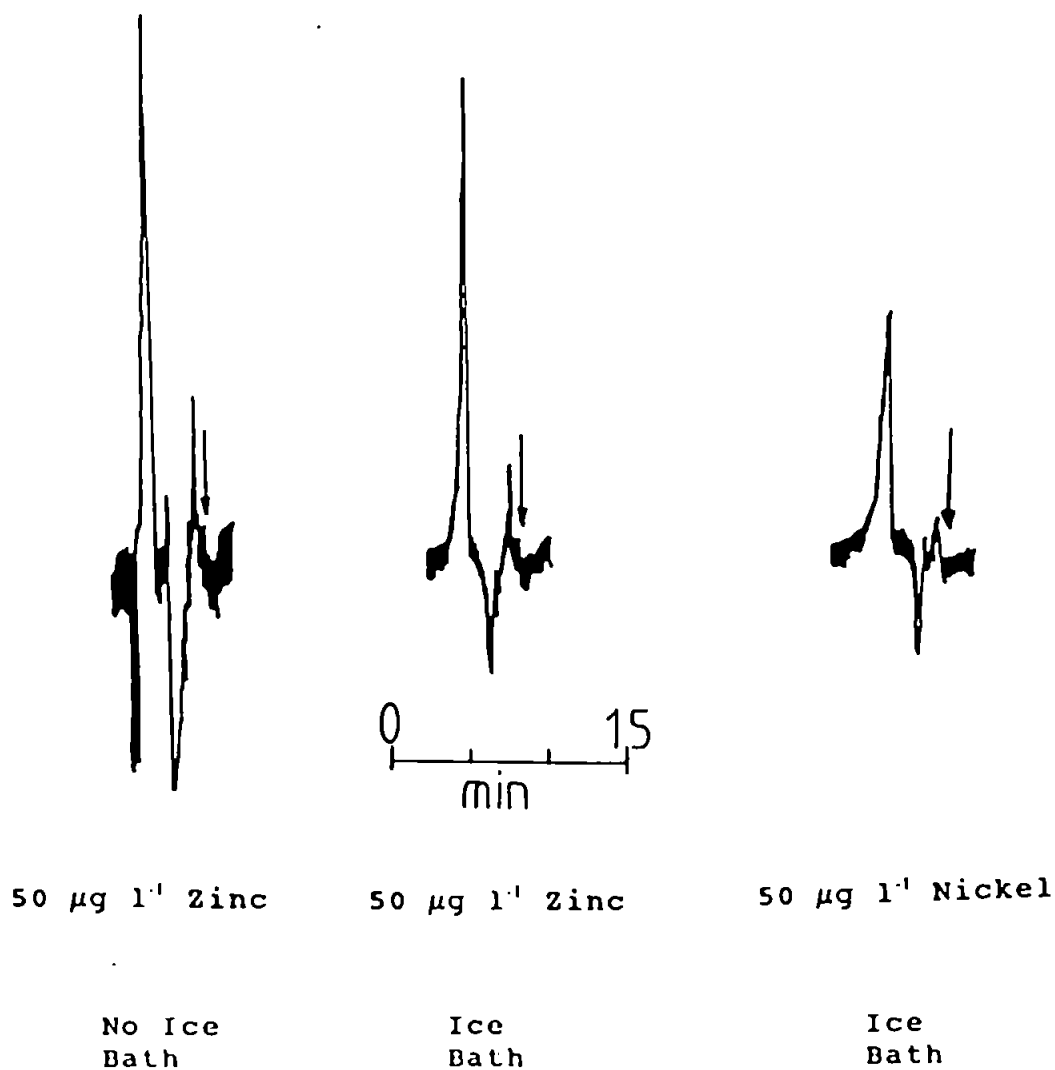
Figure 7   Effect of heating the reactions in the reaction coils on CL signal.
                  The column effluent was heated in the EDTA reaction coil by
                  immersion of the coil in the appropriate water bath. The effluent
                  was then cooled before leaving the EDTA reaction coil or left
                  heated to pass through the cobalt reaction coil. Cooling was
                  achieved by immersion of the final 50 cm of the EDTA reaction
                  coil in an ice bath.
                  The following Figures, 7, 8 and 9 show the CL response to either
                  50 μg l⁻¹ zinc or nickel on heating the coil(s).

45°C

50 μg l⁻¹ Zinc   50 μg l⁻¹ Nickel

No Ice   Ice   No Ice   Ice
Bath   Bath   Bath   Bath
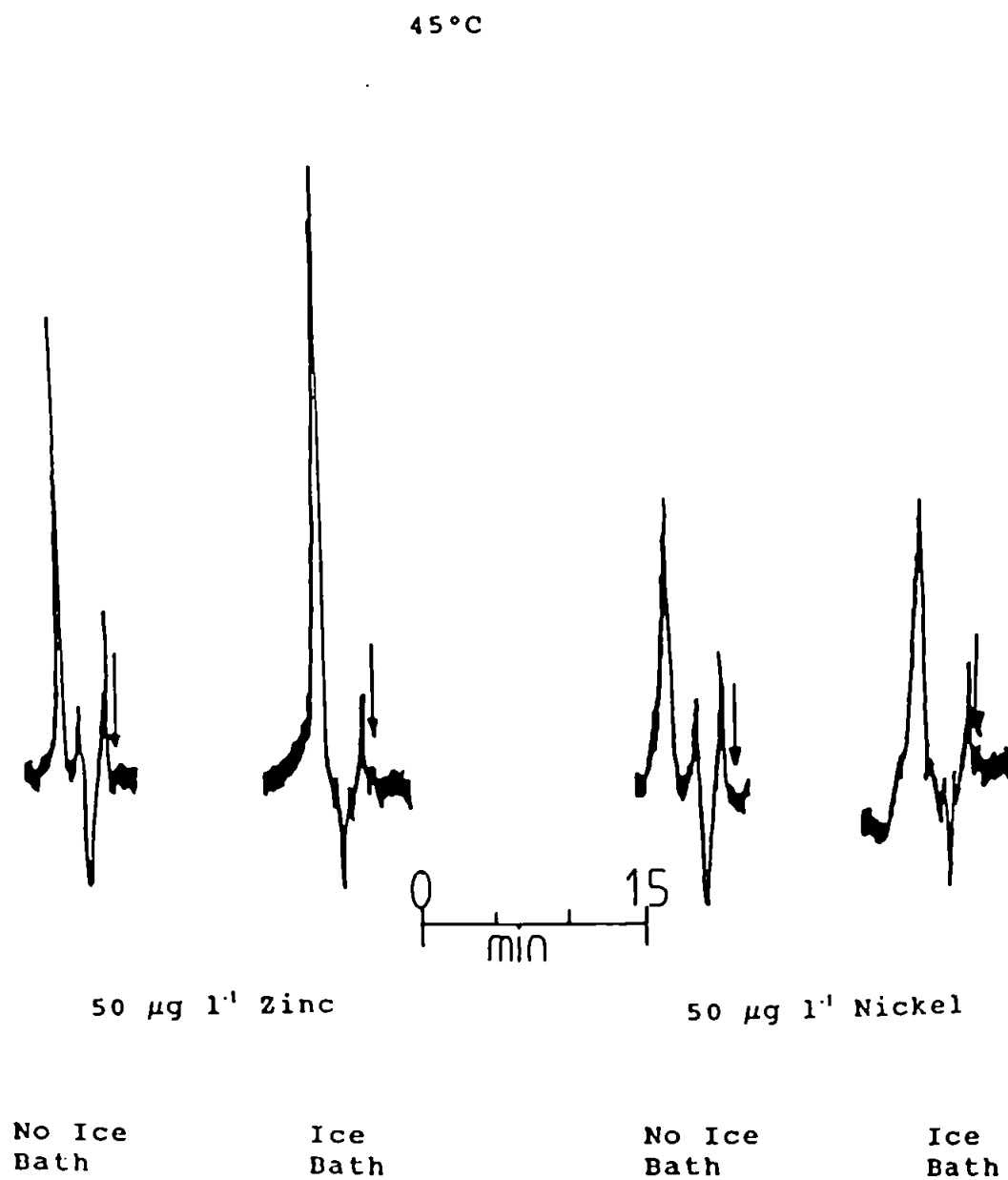
Figure 8   Heating of the coil(s) to 45°C.

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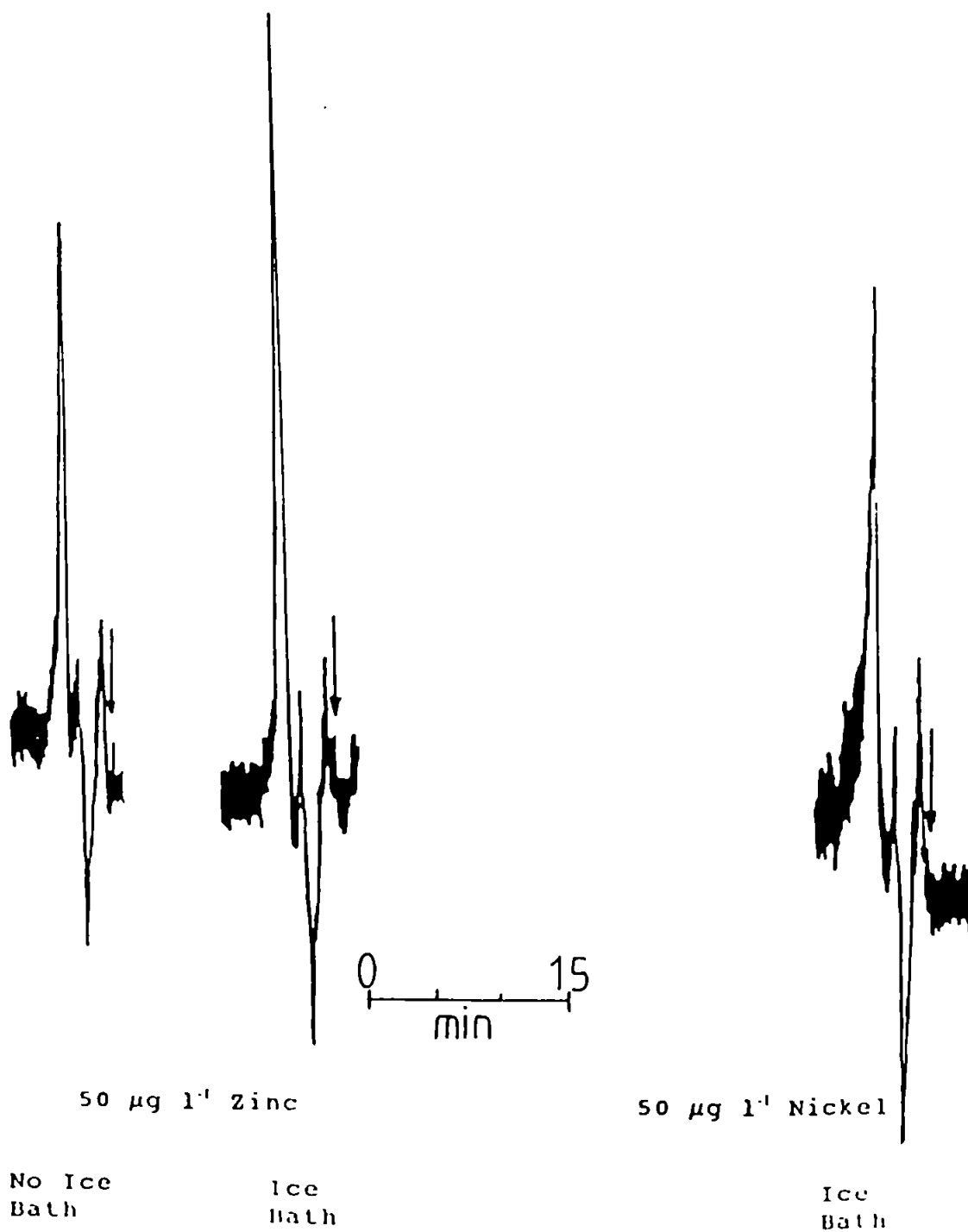
Figure 9   Heating of the coil(s) to 60°C.

2.2 and led to a decrease in the S:N ratio for both nickel and zinc at most temperatures. This effect was more pronounced for zinc than nickel. The nickel-EDTA complex has a higher conditional stability constant than the cobalt-EDTA, whereas the zinc-EDTA complex has a similar conditional stability constant. Therefore it is possible that heating the second reaction coil increases the displacement of analytes from their EDTA complexes, particularly if they form weaker EDTA complexes than cobalt (II).

2.3.7 Length of EDTA reaction coil.

The EDTA reaction coil was lengthened by the addition of sections of 0.05 cm i.d. tubing, increasing the residence time of the effluent in the reaction coil. The length of these sections and the respective residence time and effect on peak height for 50 μg l^{-1} zinc are given in Table 8. It can be seen from Table 8 and in Figure 10, which shows the effect of tube length on peak height, that the EDTA complex is rapidly formed and that increasing the EDTA reaction coil length led to a decrease in peak height, due to peak broadening. Hence the first reaction coil was essentially disposed of and was replaced by 25 cm of 0.03 cm i.d. tubing which was sufficient to connect the cobalt and EDTA "T" pieces. At a flow rate of 1.3 ml min^{-1} this gave a residence time of 1 second, enough time for the reaction to occur, the narrower tube should limit band broadening.

Table 8   Effect of EDTA reaction coil length on the CL response of 50 μg l^{-1}

zinc. EDTA reaction coil 0.05 cm i.d. (except, * 0.015 cm i.d.).

Coil length (cm)	Retention Time (seconds)	Peak Height (mm)
20*	0.34	93
25	3.0	159
50	5.7	113
75	8.9	59
100	11.0	30
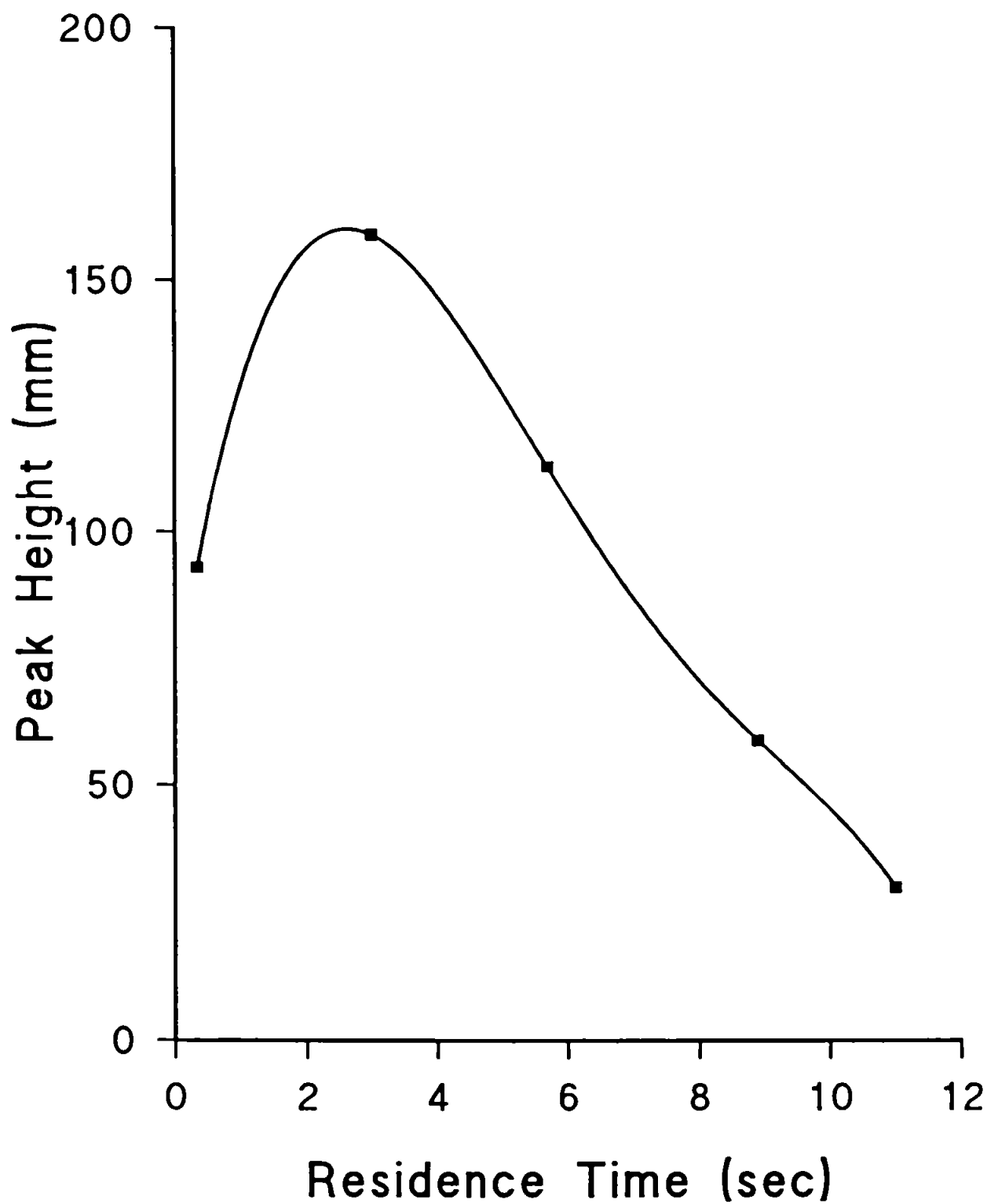
Figure 10   Effect on the CL reponse to zinc on change in length of the

EDTA reaction coil.

2.3.8   Length of the cobalt reaction coil.

Increasing the cobalt (second) reaction coil length increases the amount of time cobalt (II) has to form its EDTA complex, as well as increasing the time for the displacement reaction between the analyte and cobalt (II) ie. the back-reaction of Equation 2.2. A further complication is that the displacement reaction is also governed by pH and so it was important that the length of the cobalt reaction coil was investigated at different pHs. Table 9 shows the effect of increasing the reaction coil length at three different pHs for two different cations, zinc and manganese. The conditional stability constant of the zinc-EDTA complex is slightly less than that of the cobalt-EDTA complex, whereas that of the manganese-EDTA complex is considerably less. The variation of conditional stability constants with pH are shown in Figure 6.

At the lowest two pHs investigated lengthening the cobalt reaction coil has little effect on the zinc response. However, the manganese response rapidly decreases as it is displaced from its EDTA complex and this will occur to a greater extent in longer reaction coils. At the highest pH both the zinc and the manganese peak responses decrease with increasing reaction coil length. The peak widths at half peak height when using the 200 cm long reaction coil increase from 1.5 mm to 2.5 mm for manganese as the pH was increased. An increase in peak widths were observed for zinc, however, zinc elutes much closer to the solvent front and has inherently much sharper peaks. Measurement of peak width in a similar fashion showed that at the lowest pHs the widths were slightly less than 1 mm whereas at the higher pH the peaks were just over 1 mm wide. The increase in peak widths with increasing pH in the reaction coils was believed to be due to some effect occurring on the walls of the

Table 9   Effect of the cobalt reaction coil length on the response of 0.2 mg l^{-1} zinc (Zn) and 1 mg l^{-1} manganese (Mn) at different reaction coil pH. Peak width was measured at half peak height. O/S = off scale.

Reaction Coil length (cm)	Peak Height (mm) pH = 5.0		Peak Height (mm) pH = 5.7		Peak Height (mm) pH = 6.8	
	Zn	Mn	Zn	Mn	Zn	Mn
0			80	O/S		
25	92	O/S	92	177	62	O/S
50	105	142	93	156	75	144
100	117	124	78	132	67	155
150	117	102	87	154	47	120
200	109	60	83	105	43	103
Peak Width	1.5		1.5		2.5	

reaction coil tubing used and hence cause a decrease in peak height. The decrease in peak height with lengthening reaction coil length was similar for both analytes pointing to a decrease in displacement reaction at the higher pH.

It would seem therefore that the smaller reaction coils gave the optimum signal, especially if the conditional stability constants for the analytes' EDTA complexes where considerably less than cobalt's. However, the pump noise from three different pumps was considerable when the shortest coils were used and therefore a 100 cm reaction coil was found to give the most reasonable compromise between absolute peak heights and pump noise.

2.3.9 Effect of pH and calibrations.

The effect of pH in the PCR system on the response by various analytes is dependent upon the conditional stability constants of the analyte, the ease at which the analytes may be displaced from their EDTA complexes by cobalt (II) and on an effect believed to be occurring on the surface of the tubing, apparent at higher pHs and discussed above. Figures 11 to 15 show the effect pH has on the determination of five cations. Two of the cations, namely magnesium and calcium form quite weak EDTA complexes, the remaining three form more stable EDTA complexes, similar in strength to cobalt's.

As the pH of the reaction coil is increased, the conditional stability constants of the analyte EDTA complexes increase. Therefore, the proportion of EDTA complexed
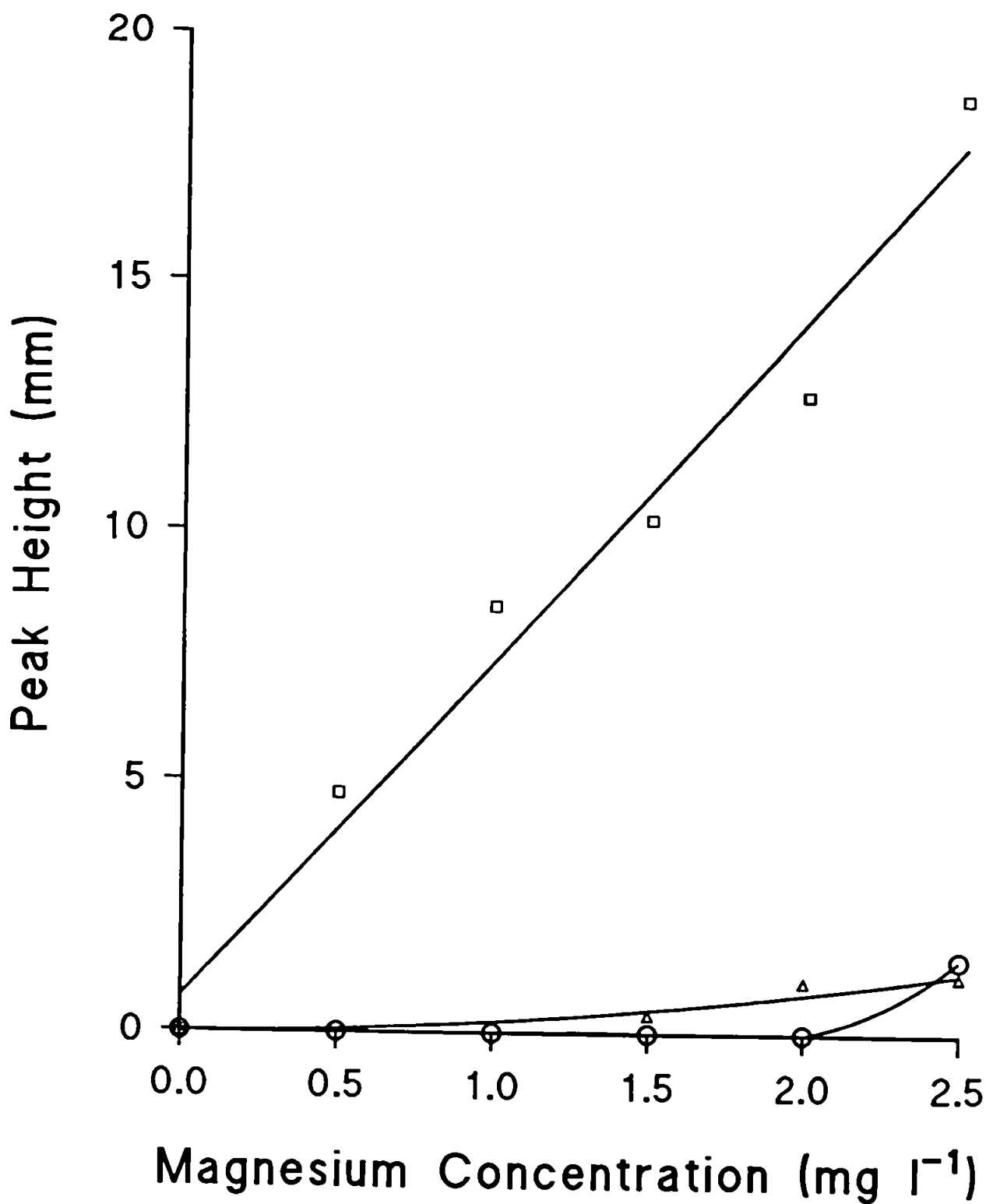
Figure 11   Effect of cobalt reaction coil pH on the CL response observed for

magnesium calibrations at three pHs. Cobalt reaction coil pH 5.3
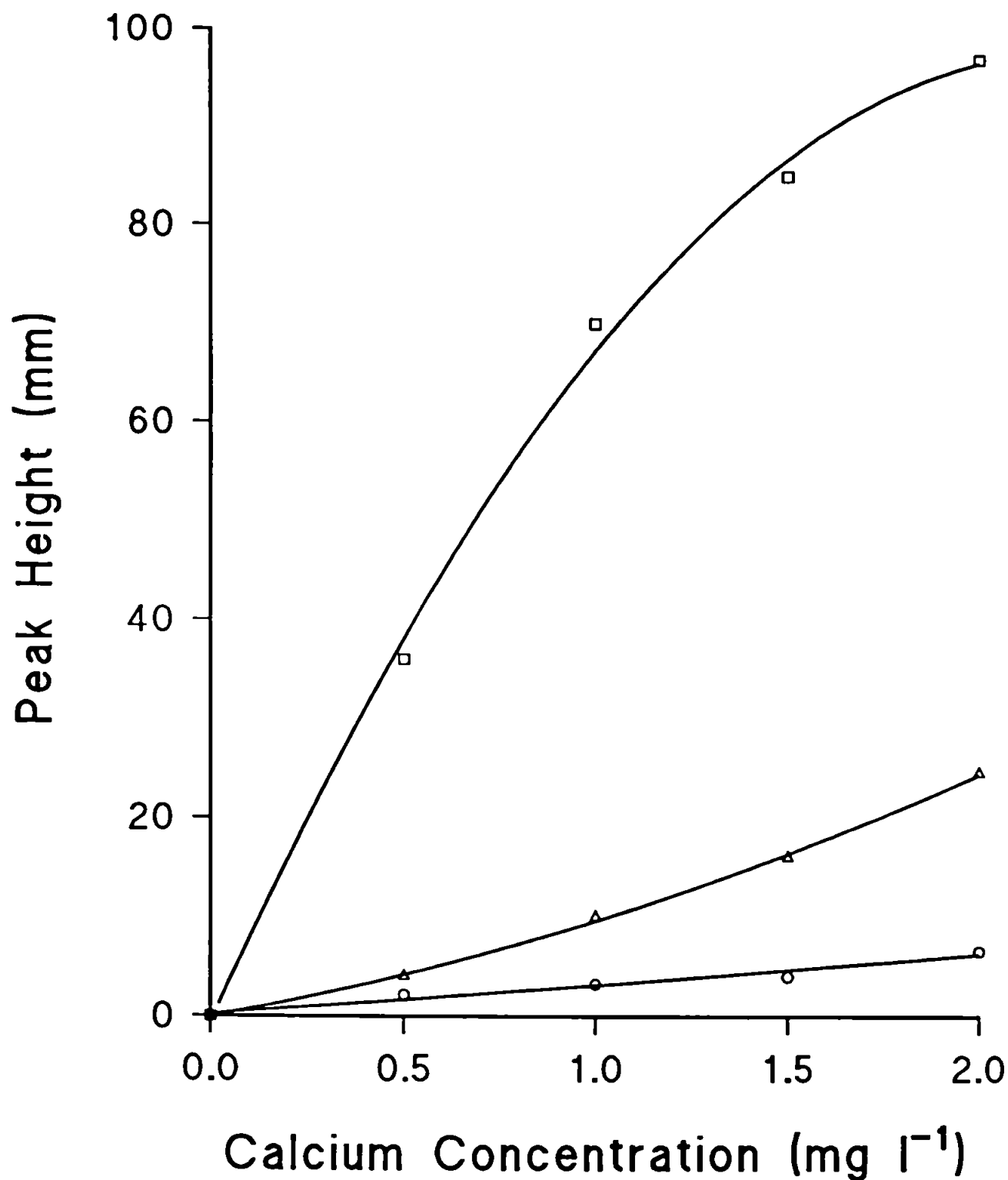
= o, pH 7.0 = Δ, pH 8.0 = □.

Figure 12   Effect of cobalt reaction coil pH on the CL response observed for

calcium calibrations at three pHs. Cobalt reaction coil pH 5.3 =   .
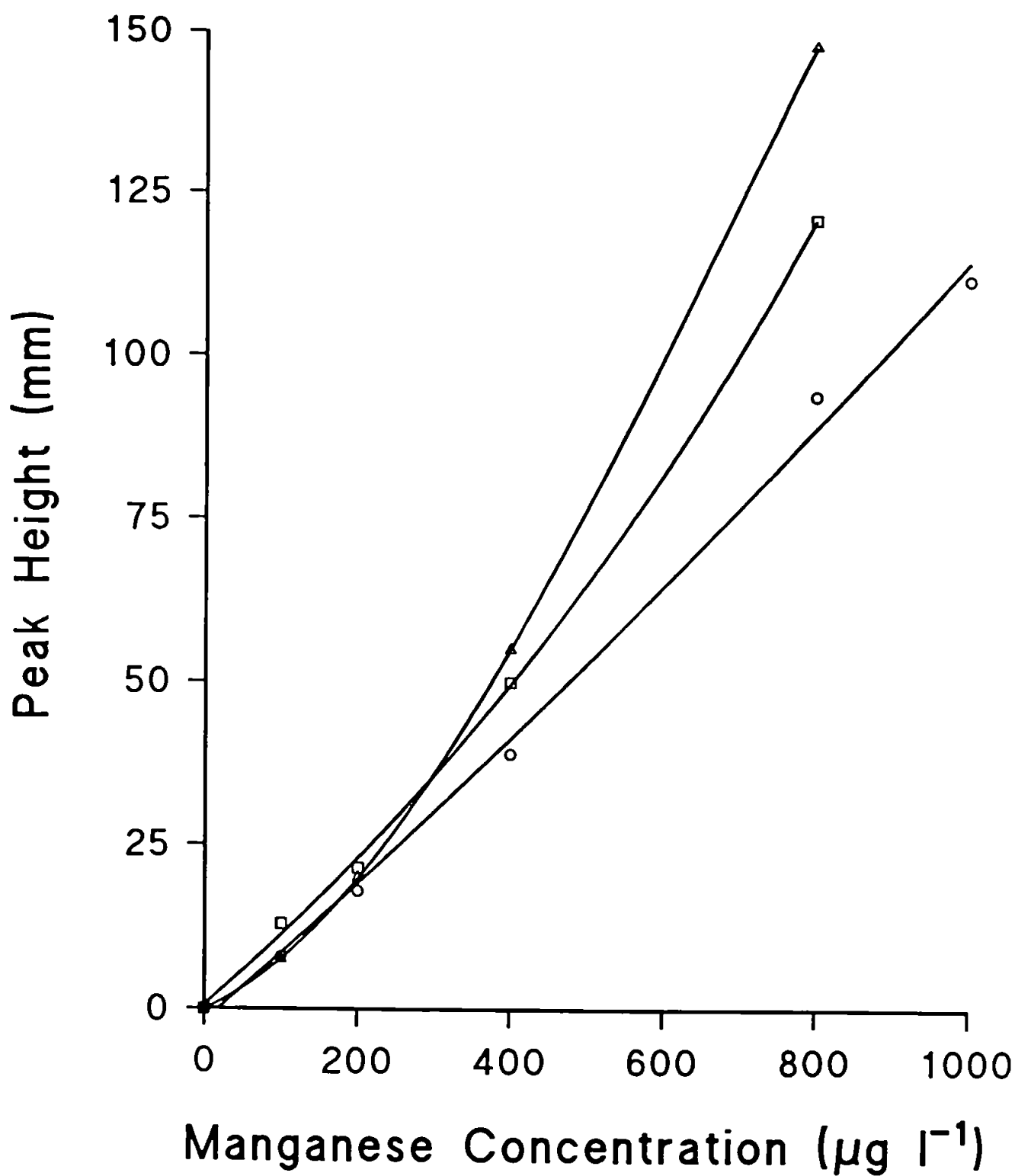
○, pH 7.0 = ∆, pH 8.0 = ●.

Figure 13   Effect of cobalt reaction coil pH on the CL response observed for

manganese calibrations at three pHs. Cobalt reaction coil pH 5.3
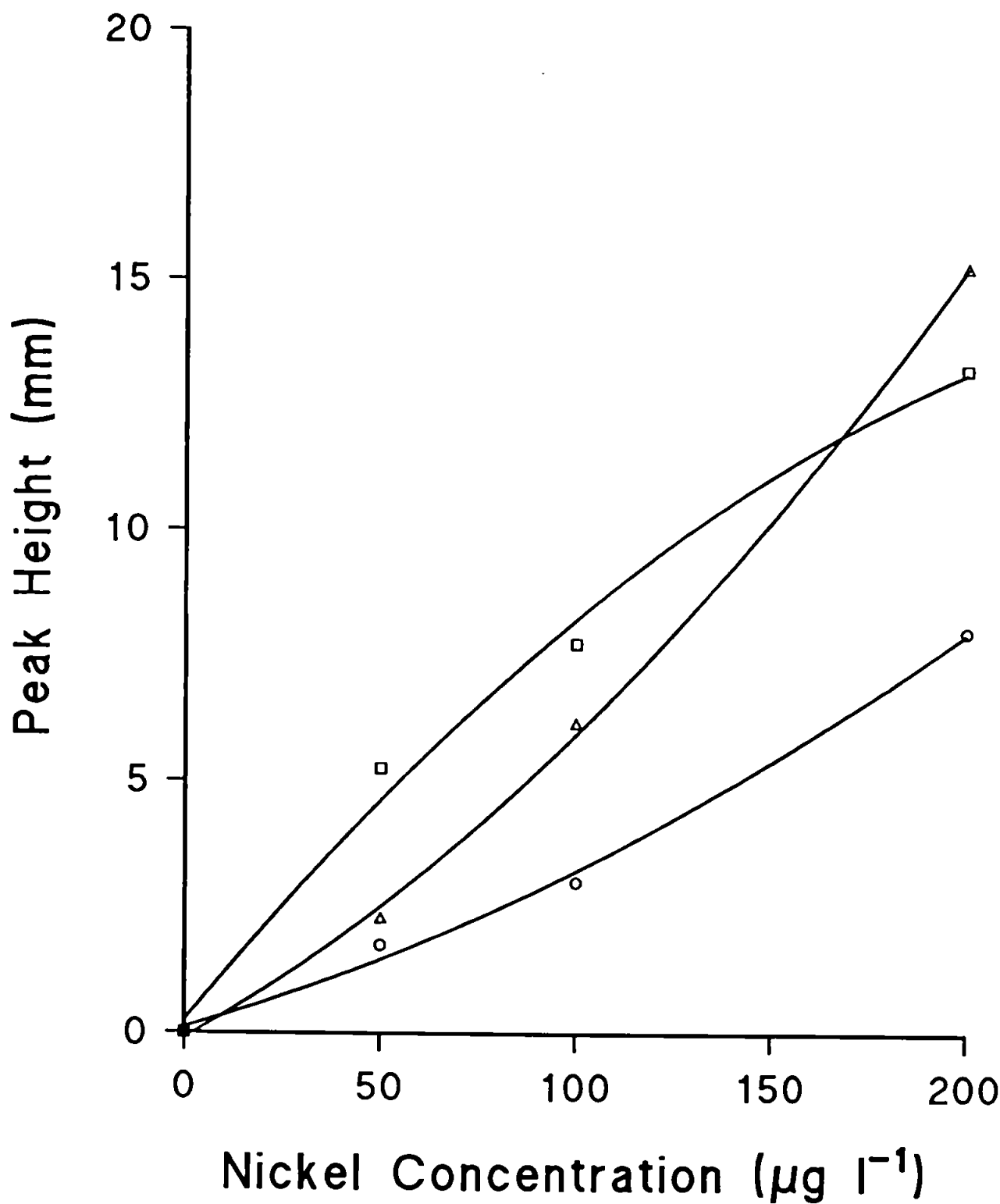
= o, pH 7.0 = ▲, pH 8.0 = □.

Figure 14   Effect of cobalt reaction coil pH on the CL response observed for

nickel calibrations at three pHs.   Cobalt reaction coil pH 5.3 =
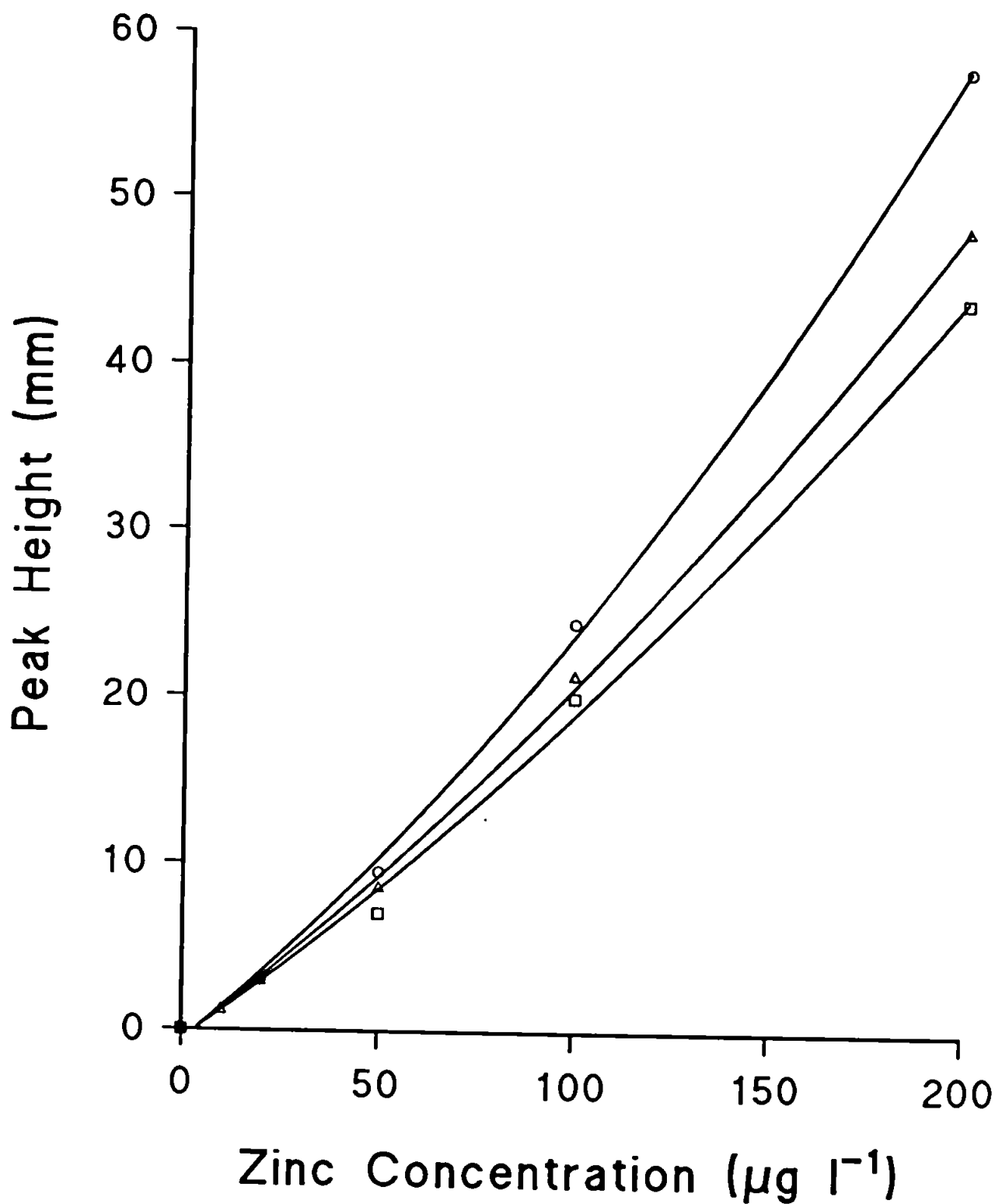
○, pH 7.0 = ▲, pH 8.0 = □.

Figure 15   Effect of cobalt reaction coil pH on the CL response observed for

zinc calibrations at three pHs. Cobalt reaction coil pH 5.3 = o,

pH 7.0 = △, pH 8.0 = □.

with the analyte increases. The second effect is that the acid catalysed exchange reaction decreases, thus cobalt displaces less analyte and so more free cobalt is present in the detector. Displacement of analyte from its EDTA complex by cobalt was found, as expected, to have greatest effect on those analytes that form the weakest EDTA complex and therefore have complexed less EDTA initially. The final effect on response with increasing pH was that of band broadening due to an effect believed to be occurring on the tubing surface.

Change in peak heights with increasing pH for the different cations is related to the relative magnitude of these effects. Thus, for magnesium and calcium which form relatively weak EDTA complexes, the increase in the conditional stability constants and the decrease in the exchange reaction with increasing pH is more significant in increasing sensitivity than the effect that causes peak broadening. Conversely, with zinc the most apparent effect is that of peak broadening with increasing pH, leading to a decrease in sensitivity. Change in response for nickel and manganese seems to be more complex as sensitivity increases then decreases. Also, the nickel-EDTA complex is more stable than the zinc-EDTA complex at a given pH and as the formation of the metal-EDTA complexes is rapid, it is difficult to explain why the cation that forms the more stable EDTA complex gives a lower response.

2.4   Conclusion.

The system developed here involving two fast forward reactions was not as sensitive as other CL systems used for single element determination, although it did extend the range of metals that can be determined. However, it was reasonably sensitive to a number of cations that form EDTA complexes of similar stability to that formed by cobalt (II). The approach used here differs considerably from that used by Jones *et al.* (38) and as a result shows different effects with changes in reaction temperature, time and pH.

Jones *et al.* increased the temperature of the reaction coil to increase the rate of reaction to obtain significant exchange in the reaction used (Equation 2.2). Heating improved linearity in Jones *et al.*'s system but in the system described here heating the reactions was detrimental. This was shown by an increase of the pump noise generated from four pumps and by an overall decrease in signal response for those cations that form weak EDTA complexes. Moderate heating followed by cooling in the first reaction coil did improve detection but was not feasible due to the length of the reaction coil.

The previous system made use of a long reaction coil to increase the reaction time ensuring significant exchange occurred. Increasing the length of the first reaction coil led to band broadening, also since the reaction was rapid long reaction coils were not required. Increasing the length of the second reaction coil led to an increase in displacement of those cations that formed weak EDTA complexes. Also, in conjunction with the surface effect on the tubing increasing coil length led to

broadening of the peaks. The length of the second reaction coil was a compromise between the damping effect it had on the considerable pump noise and the possibility for significant displacement reactions and peak broadening to occur.

In this system the sensitivity increases for cations that form weak EDTA complexes as the pH of the reaction coils increases, which is the reverse of that found in Jones *et al.*'s system. As the pH increases the conditional stability constant of the metal-EDTA complex increases and there is a decrease in the displacement of metal from its EDTA complex by cobalt (II). This is most pronounced for those cations that form weak EDTA complexes, such as magnesium and calcium. Unfortunately the increase in sensitivity also makes the system more prone to background contamination by magnesium (II) and calcium (II) in the reagents. Furthermore a surface effect on the walls of the PTFE tubing, which increased with pH led to peak broadening, limiting the extent of the pH increase.

The direct formation reactions made the HPLC system quite complicated and the exact balancing of EDTA and cobalt (II) addition was problematic. However, it did show the more favourable kinetics of this type of approach and thus enabled the use of short reaction coils at ambient temperature. This was an important step in developing a multi-element detection system based upon CL. Further developments may make use of more selective chelating agents which would be less affected by contaminating cations, this is discussed in Chapter 6.

CHAPTER THREE.

3.0   INVESTIGATION OF CHROMIUM SPECIATION.

3.1   Introduction.

There is continuing interest in the simultaneous determination of chromium species as the two predominant oxidation states of chromium in the environment (119), chromium (III) and chromium (VI), are known to have very different toxicities. For example chromium (VI) is a known carcinogen (120) whereas chromium (III) is essential in mammals to maintain the glucose, lipid and glucose metabolism (121). The increasing interest in chromium speciation in environmental samples has led to a variety of approaches for differentiating and determining chromium (III) and chromium (VI) species. A number of studies have focused on just one species of particular interest, such as chromium (III) in water and foodstuffs by Escobar *et al.* (94) or chromium (VI) in water by Elleouet *et al.* (121). Other investigators have measured one species and obtained the other by difference after total chromium measurement. Mugo *et al.* (122) and Beceiro-Gonzalez *et al.* (123) measured chromium (III) and total chromium, whereas Chakraborty *et al.* (124), Gao *et al.* (125) and Sperling *et al.* (126) determined chromium (VI) and total chromium. The aim of other studies has been to determine both species simultaneously. Essentially, this involves the determination of chromium (III) and chromium (VI) as separate species after elution from a chromatography column. Several workers have reported the use of hyphenated systems linking the chromatography column to atomic spectrometric instruments, such as flame atomic absorption (127, 128) or inductively coupled plasmas (129).

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Another approach, involves the use of self-contained ion chromatography systems with standard on-line detectors. However, the chromatography is not straightforward as the two chromium species are of opposite charge. This can be simplified by converting chromium (III) to a negatively charged complex ion such as an EDTA complex and then separating it from chromium (VI) using an anion exchange column (119, 120, 130, 131). However, this usually requires a major disturbance of the sample such as boiling for a short period (132). Clearly this is not satisfactory, since to maintain species integrity, particularly as chromium (VI) is not a very stable species, sample treatment should be kept to a minimum. In an attempt to solve this problem Williams *et al.* developed an ion chromatography method capable of separating chromium (III) and chromium (VI) as their simple ions in an aqueous sample (25). Very high sensitivities were achieved using a chemiluminescence (CL) detector optimised for chromium (III). The chromium (VI) species, which is not chemiluminescence active, was reduced to chromium (III) post-column using sulphite. Although good separations were obtained the chromatography was very complex, involving two eluents, two parallel columns and four pumps. Gammelgaard *et al.*, using the same CL detection system with sulphite reduction, reported a simpler separation system based on a single column (90). However, the chromium (VI) eluted very close to the solvent front, which makes it vulnerable to distortion or disturbance depending on the sample composition.

The aim of this study was to improve and simplify the chromatography system used and provide resolution of both chromium species from each other and the solvent front. An effective means of simplifying the system would be to separate the two species on a . single column or on columns joined in series so that only a single eluent would be

required. This would result in removal of one of the eluent pumps and a greatly simplified injection port assembly.

Once optimized the IC system will be used to study chromium speciation in aqueous media. In particular the effect of pH on stability of reference standards. Finally, the IC method will be used to analyze samples containing chromium, as part of a calibration exercise for the Bureau Communtaire de Reference (BCR) programme.

3.2 Experimental.

The system developed by Williams *et al.* (25) involved a complex chromatographic set-up. Several approaches were possible to simplify the chromatography. The two areas chosen for study were investigations into various column configurations, giving different anionic and cationic column capacities and examination of different counter ions in the eluent. Thus obtaining a single eluent capable of separating both species on a single column or columns in sequence. The CL detection system reported by Williams *et al.* was used and the on-line reduction system was modified to 0.015 M sulphur dioxide solution instead of a 0.015 M potassium sulphite solution. The chromatographic system and reagents used are detailed below.

3.2.1 Equipment.

As shown in Figure 16 the chromatograph consisted of a high-pressure inert plastic

INJECTION VALVE

ELUENT PUMP

ION EXCHANGE COLUMN

REACTION COIL

REDUCING AGENT PUMP

DETECTOR

LUMINOL PUMP
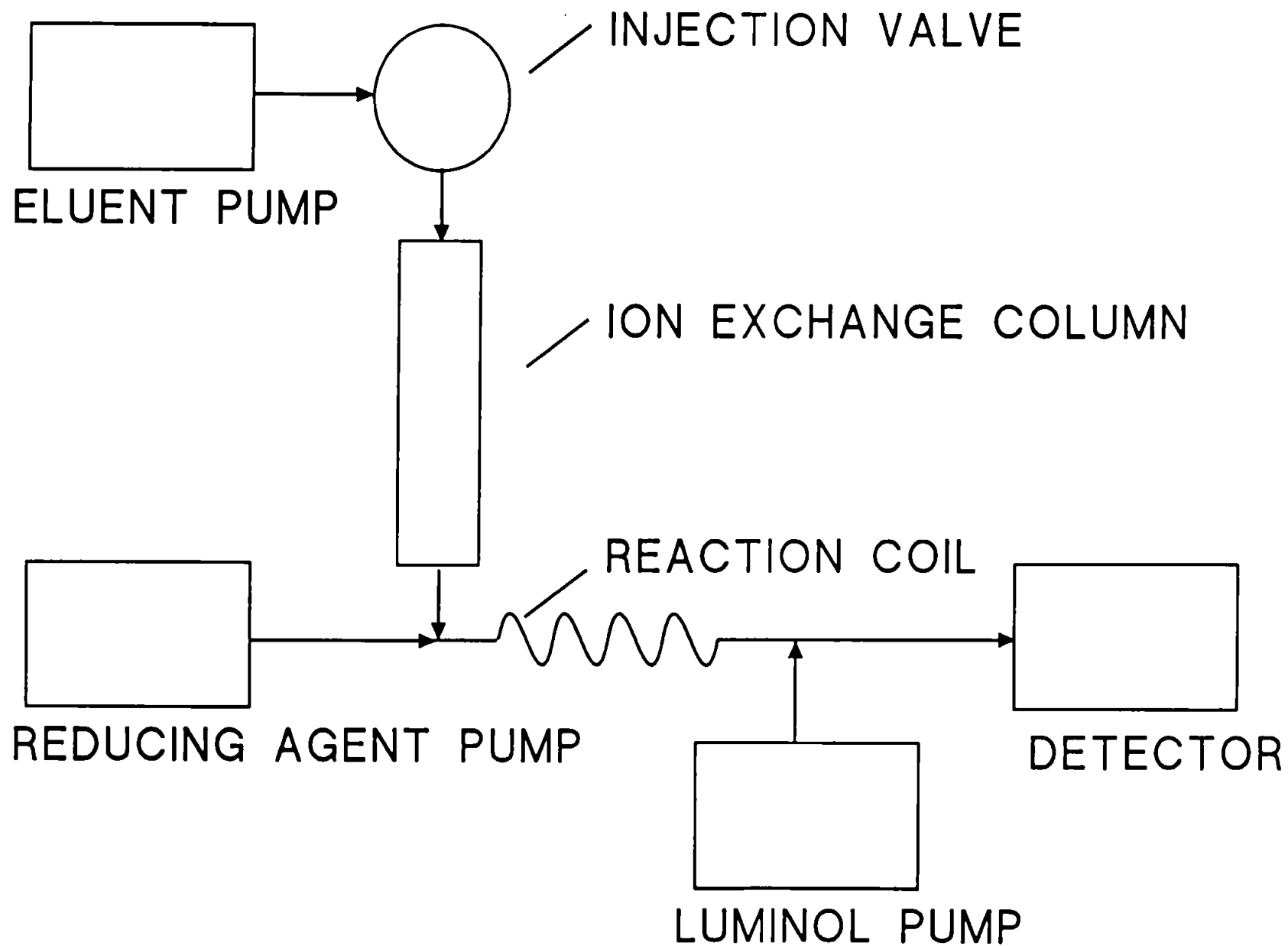
Figure 16   Schematic diagram of chromatograph employed.

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pump (Dionex Gradient pump, Dionex, Sunnyvale, CA, U.S.A.) which was used to deliver the eluent to the column at 1 ml min^{-1}. The solution used to reduce the chromium (VI) to chromium (III) was mixed with the column eluent post-column and was delivered by a stainless-steel pump (model 64, Knauer, Bad Homburg, Germany) at a flow rate of 0.06 ml min^{-1}. The CL reagent was added at 1 ml min^{-1} by a high-pressure inert plastic pump (Dionex 2000i), directly prior to the detector. The detector consisted of PTFE tubing coiled flat against the face of the photo-multiplier tube from a fluorescence detector (FD-100, Spectrovision, Chelmsford, MA, U.S.A.).

PTFE tubing (0.3 mm i.d.) was used throughout. An inert PEEK Rheodyne (Model 9010, Cotati, CA, U.S.A.) injection valve with a PEEK 100μl sample loop was used for sample injection. All eluents were degassed with helium prior to use and the luminol solution was constantly purged with nitrogen.

3.2.2 Columns.

A number of Dionex anion and cation exchange columns shown in Table 10 were used in various combinations.

3.2.3 Reagents.

Milli Q deionized water (Millipore Corp., Bedford, MA, U.S.A.) was used throughout. All reagents were AnalaR (Merck, Poole, U.K.) except luminol (Fluka, Poole, U.K.)

Table 10   Exchange Capacities of Some Dionex Columns (133).

Dionex column	Column Length (cm)	Anion Exchange Capacity (μeq)	Cation Exchange Capacity (μeq)
AS4A	25	20	/
CG3	5	/	20
CG5	5	30	14
CS5	25	150	70

and Aristar potassium hydroxide (Merck). Spectrosol (Merck) 1000 mg l^{-1} or 10,000

mg l^{-1} solutions were diluted to make standards as required. Standards were prepared

in 0.01 M nitric acid.

3.2.4   Eluent.

Initially potassium sulphate concentrations of between 0.0085 M and 0.03 M at pH 2.5,

were used. After the preliminary experiments 0.3 M, later 0.28 M, potassium chloride

was used as the eluent. 0.001 M EDTA was added to the eluent solution to keep the

background CL low.

3.2.5   Reducing solution.

Initially 0.015 M sodium sulphite ($Na_2SO_3.7H_2O$) or potassium sulphite ($K_2SO_3.7H_2O$)

was used to reduce the chromium (VI) to chromium (III). Later a 0.015 M sulphur

dioxide solution was used.

3.2.6   Luminol solution.

A solution containing 3.4 x 10^{-3} M luminol, 0.1 M orthoboric acid and 0.01 M

hydrogen peroxide at pH 11.5 was used as the CL reagent.

3.3   Results and Discussion.

3.3.1   Chromatography.

Williams *et al.* used potassium sulphate as the eluent which was passed through two columns in parallel but the concentrations required for each column differed considerably, namely 0.085 M potassium sulphate to elute chromium (III) and 0.003 M potassium sulphate to elute chromium (VI) (25). With such differences in concentration it was not possible to use the two columns in series with potassium sulphate as the eluent. It was shown by Gammelgaard *et al.* (90) that if the higher concentration of potassium sulphate is used with the cation exchange column (Dionex CG2) chromium (VI) elutes on the solvent front. If the lower potassium sulphate concentration was used chromium (III) would be retained indefinitely on the cation exchange column.

In order to delay the elution of chromium (VI) and elute the chromium (III) in a reasonable time, there were a number of possibilities.

1) A high capacity anion exchange column could be used with the 0.085 M potassium sulphate.

2) 0.003 M potassium sulphate used with a lower capacity cation exchange column.

3) Potassium sulphate be replaced by an eluent that contains :-

a) weaker counter anion.

b) stronger counter cation.

Options 2 and 3b would require very weak eluents. This may lead to a situation where the ionic strength of the sample is much greater than that of the eluent and this would be undesirable. Also the Dionex CG2 column is already a low capacity column (12μeq per column) and an even lower capacity column may easily become swamped.

A number of different columns were tried in an attempt to increase the anion exchange capacity (option 1) but this proved to be unsatisfactory.

Option 3a therefore seemed to be the most promising to investigate further, particularly as the selectivity coefficient of the sulphate anion is greater than many other counter anions. The problem with the previous system was the great disparity between the concentration of potassium and sulphate required for the elution of the anion and cation. By replacing sulphate with a weaker counter anion, such as chloride, higher concentrations of counter anion would be needed in the eluent. In this way the concentration of anion and cation counter ions required in the eluent may be brought closer, eluting chromium (VI) away from the solvent front whilst eluting chromium (III) in a reasonable time.

Initial trials were with a Dionex CG5 column and 0.2 M potassium chloride eluent, the results are summarized in Table 11. Chromium (III) was retained for 16 min whilst chromium (VI) eluted on the solvent front. Introducing a Dionex CS5 column in

Table 11   Retention of chromium (VI) and chromium (III) on various

Dionex HPIC columns with potassium chloride eluent, pH 2.5.

SF = Solvent Front. N/I = Not Injected.

Column Type/Combination	Concentration Potassium Chloride (M)	Retention Time (min)	
		Cr (VI)	Cr (III)
CG5	0.2	SF	16
CS5	0.2	10	N/I
CG5 + AS4A	0.2	6	>30
CG5 + AS4A	0.3	5.5	12.5
AS4A	0.3	4	7
AS4A	0.28	4.5	8.5

addition to the CG5 column led to retention of the chromium (VI) for 10 minutes. However, the retention of chromium (III) was not determined due to the expected very long retention time.

In order to increase the anion exchange capacity of the system to prevent the rapid elution of chromium (VI) on the solvent front when using the Dionex CG5 column a Dionex AS4A, anion exchange separating, column was placed in series. The retention of chromium (VI) was increased to 6 minutes, but with the 0.2 M potassium chloride solution chromium (III) was not detected even after 30 minutes. Increasing the potassium chloride solution to 0.3 M, chromium (VI) eluted off the solvent front at 5.5 minutes and eluted chromium (III) in 12.5 minutes. The retention of chromium (III) was still considered to be too great. Also the use of the Dionex AS4A column in conjunction with the Dionex CG5 column had led to an increase in chromium (III) retention from 16 min to >30 min, when using 0.2 M potassium sulphate eluent, therefore it seemed that the Dionex AS4A anion exchange column had some cation exchange capacity. A further increase in the potassium chloride concentration to significantly decrease the retention time of chromium (III) would lead to elution of chromium (VI) on the solvent front. Therefore the use of the Dionex AS4A column alone was investigated, but with the 0.3 M potassium sulphate eluent this led to the co-elution of the tail of the chromium (VI) peak and the front of the chromium (III) peak. However, by decreasing the concentration of potassium chloride to 0.28 M separation of the two chromium species from each other and the solvent front was achieved.

Since the AS4A column is an anion exchange column it seems surprising that significant hold up of chromium (III) should occur. However, Dionex AS4A anion

exchange columns are formed by electrostatic agglomeration of smaller anion exchange particles on larger cation exchange particles. This leads to a pellicular layer of excess anion exchange particles upon the cation exchange resin particles. Pellicular layers such as these show improved mass transfer but are composed essentially of both anion and cation exchange resins. The anion exchange pellicle should repel the similarly charged cations. However, there must remain some cation exchange capability and it is assumed that the trivalent chromium (III) undergoes cation exchange on the internal cation exchange matrix.

3.3.2   Calibration.

In the early work the calibration curves for both chromium species were non-linear, the response increasing faster than the concentration. This was found to be due to oxygen dissolving in the degassed luminol solution over a period of time, increasing the sensitivity of the CL reaction to chromium (III). By purging the luminol solution constantly with nitrogen a consistent response was obtained. Figure 17 shows the calibrations obtained for this system from mixed chromium standards. The correlation coefficient of 0.999 was found between the peak area and chromium concentration in both cases. The relative standard deviation (RSD) for six replicate injections of 10 μg l^{-1} chromium (III) was 4.3% and the RSD for six replicate injections of 10 μg l^{-1} chromium (VI) was 2.9 %. Figure 18 shows a typical chromatogram of 10 μg l^{-1} chromium (III) and 10 μg l^{-1} chromium (VI). A chromatogram near the detection limit is shown in Figure 19. The limits of detection given as a peak height twice that of the peak to peak noise of the baseline were 50 ng l^{-1} for chromium (III) and 100 ng l^{-1} for

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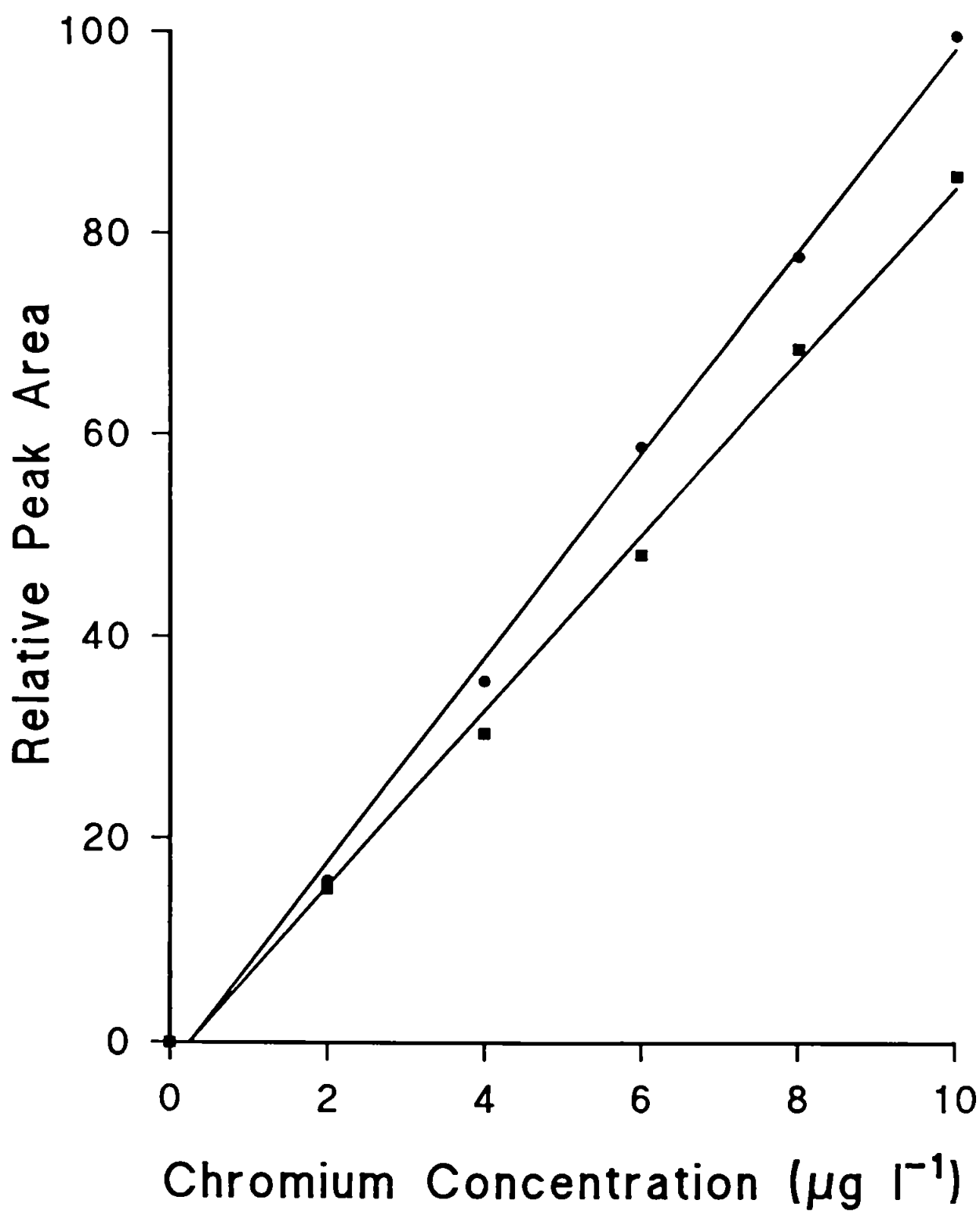
Figure 17   Calibration curves for chromium (III) and chromium (VI)

chromium (III) ● and chromium (VI) ■.
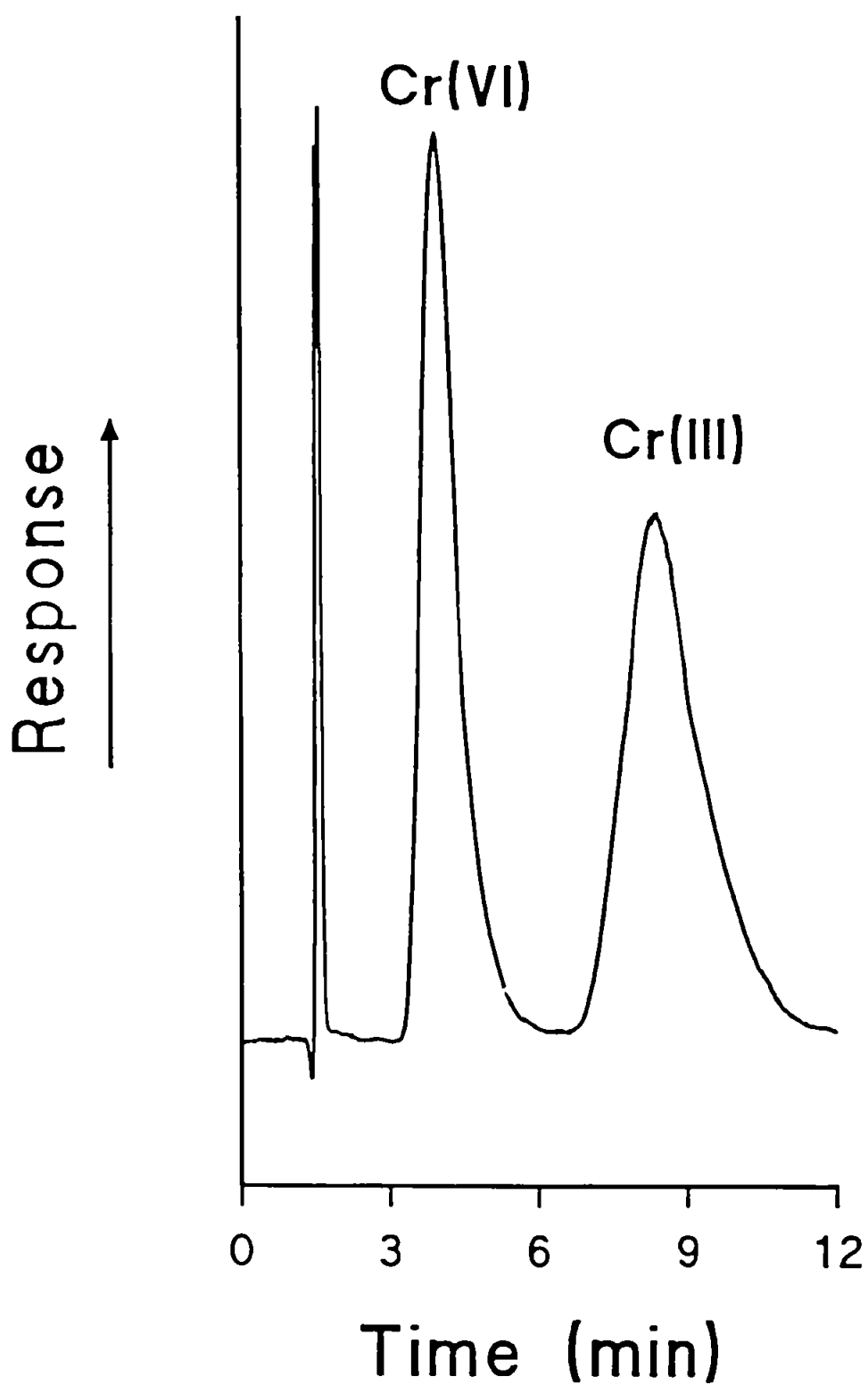
Figure 18   Typical chromatogram for 10 μg l^{-1} chromium (III) and chromium (VI).
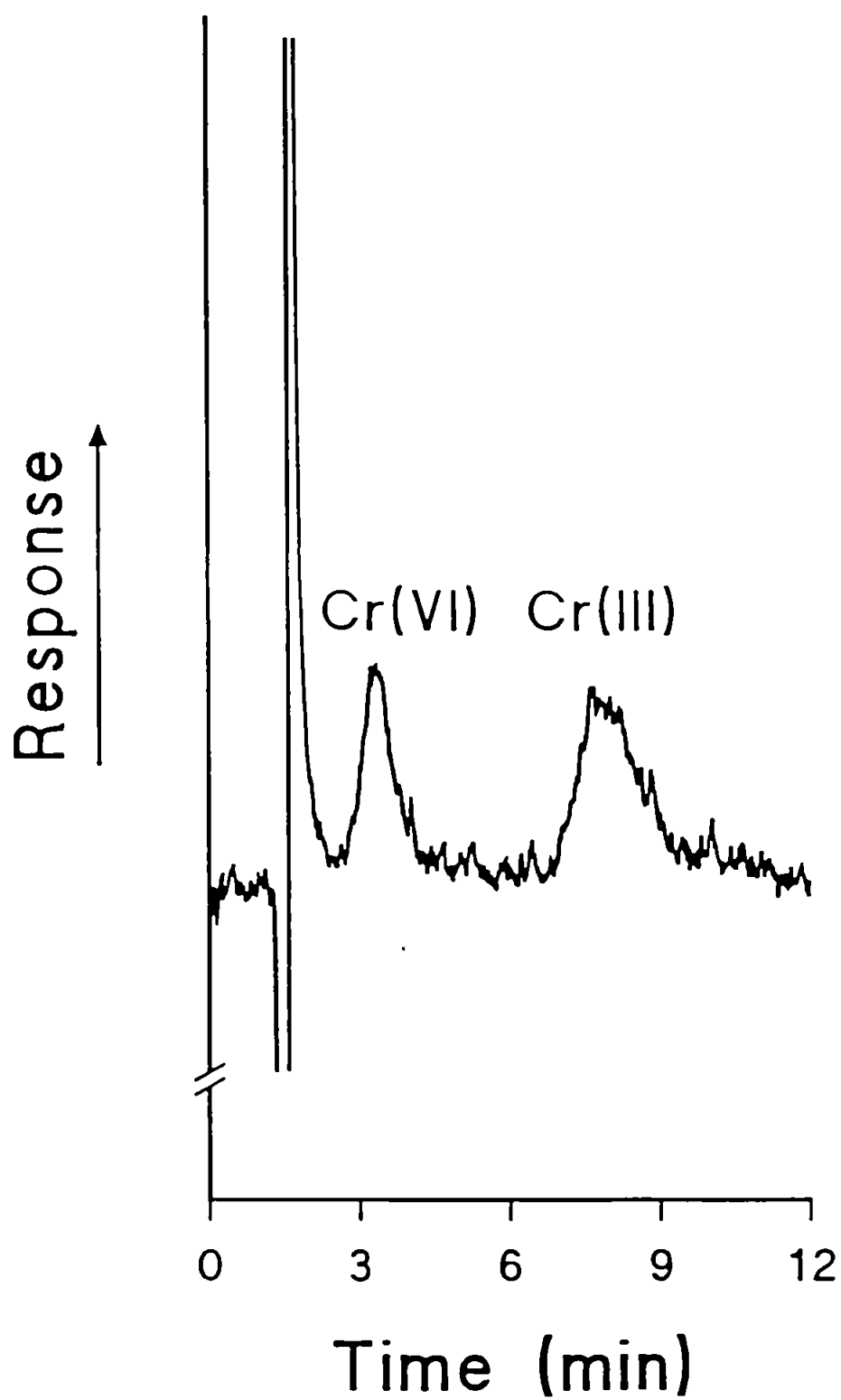
Figure 19   Typical chromatogram near the detection limit. 0.25 µg l⁻¹

chromium (III) and chromium (VI).

chromium (VI).

The area of the chromium (VI) peaks were found to be less then that of the chromium (III) peaks at a given concentration. This is difficult to explain as it is chromium (III) that catalyses the CL reaction in both cases. This may be explained by the fact that although chromium (III) forms a strong complex with EDTA it occurs extremely slowly (118). However, when chromium (VI) is reduced to chromium (III) the hydration layer takes a finite time to reform and a proportion of the chromium may become complexed with the EDTA.

3.3.3   Interferences.

10 μg l^{-1} injections of chromium (III) or (VI) showed little interference from a range of co-injected cations, including: cobalt (II), zinc (II), nickel (II), manganese (II), magnesium (II), calcium (II), aluminium (III) and iron (III), even in concentrations of up to 10 mg l^{-1}. This is as expected as they all should be eluted on the solvent front away from the chromium peaks.

<u>3.4.1   Certified Reference Material.</u>

To test the IC system, chromium speciation in an International Atomic Energy Agency, Certified Reference Material, Simulated Fresh Water (SFW), IAEA/W-4, was investigated. As the SFW contained only chromium (III), chromium (VI) was added as

a spike. After the first couple of experiments it became apparent that chromium (VI) was not stable in the SFW. There are two probable reasons for this. First the speciation of chromium (VI) can alter with pH (134). The SFW was kept in 1.0 M nitric acid and under acid conditions, dilute solutions of the chromate anion are singly protonated, below pH 2 the chromate anion may be doubly protonated and therefore uncharged. However, on injection into the eluent this protonation should rapidly reverse and no effect should be observed. It is therefore likely that a different effect is occurring. It can be seen in the Pourbaix diagram Figure 20, that as the pH decreases the reduction potential required to reduce chromium (VI) becomes more positive, ie chromium (VI) is more readily reduced. It was therefore expected that at the low pH of 1.0 M nitric acid chromium (VI) would be reduced rapidly to the simple chromium (III) by certain species in the water sample. However, if this did occur then an increase in the chromium (III) peak would be expected. Figure 21 shows that although an increase was observed for chromium (III) a significant signal also occurred on the solvent front indicating the presence of low or uncharged chromium species. It may be that some chromium (VI) in the spiked sample is reduced to chromium (III) and as discussed earlier some of this chromium (III) could form a non-labile complex before the hydration layer forms around it. Thus, in order to remove this problem the pH of the SFW was raised to pH 6.15 before being spiked with chromium (VI). However, as can be seen in Figure 22, at this pH chromium (VI) is stable, and as expected chromium (III) is now unstable as it forms hydroxide species above pH 3.5, as shown in the Pourbaix diagram, Figures 20 (135). In the Pourbaix diagram solubility limits are for a 10^{-4} M chromium solution. However, the concentration of chromium (III) in the SFW was 10 μg l^{-1}, approximately 2 x 10^{-7} M. A more detailed solubility and species profile is given in Figure 23 displaying the solubility of different chromium
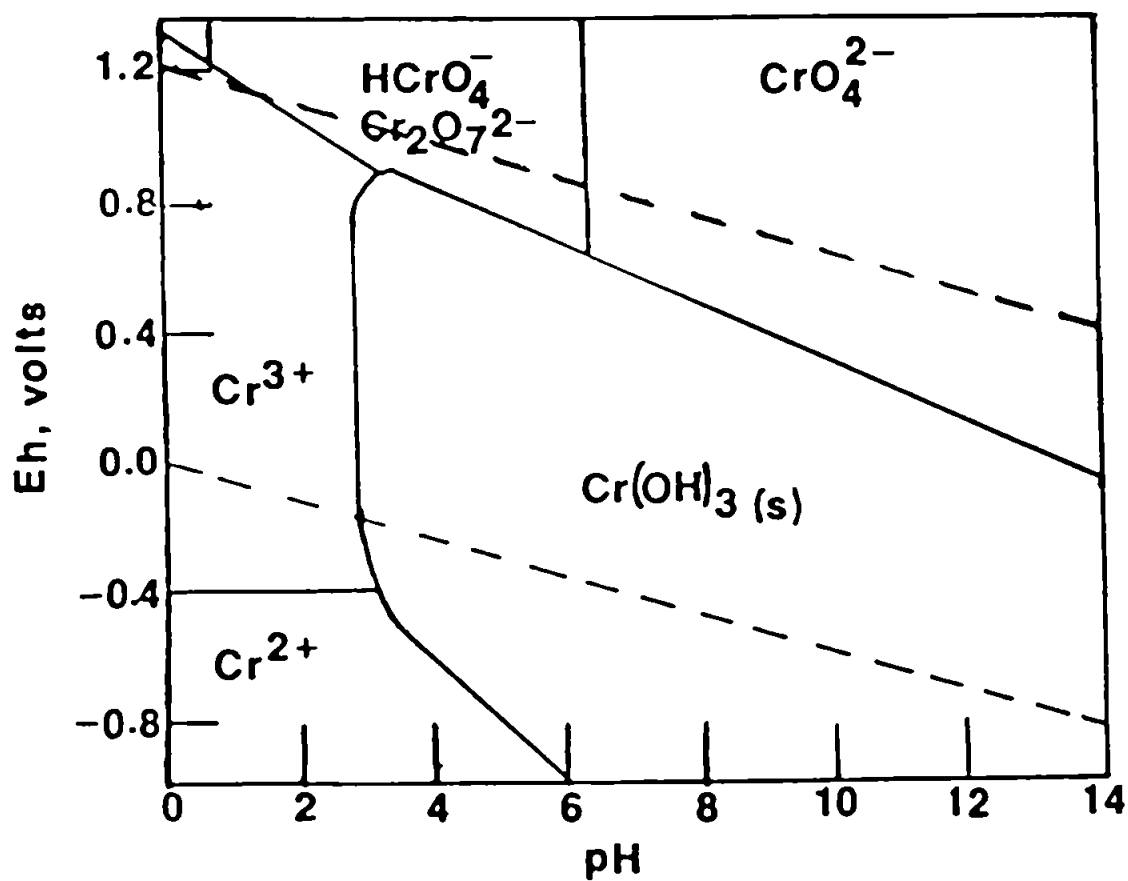
Figure 20   Eh-Ph diagram (Pourbaix diagram) for the chromium-water system at 25°C. Chromium concentration 10^{-4} M.
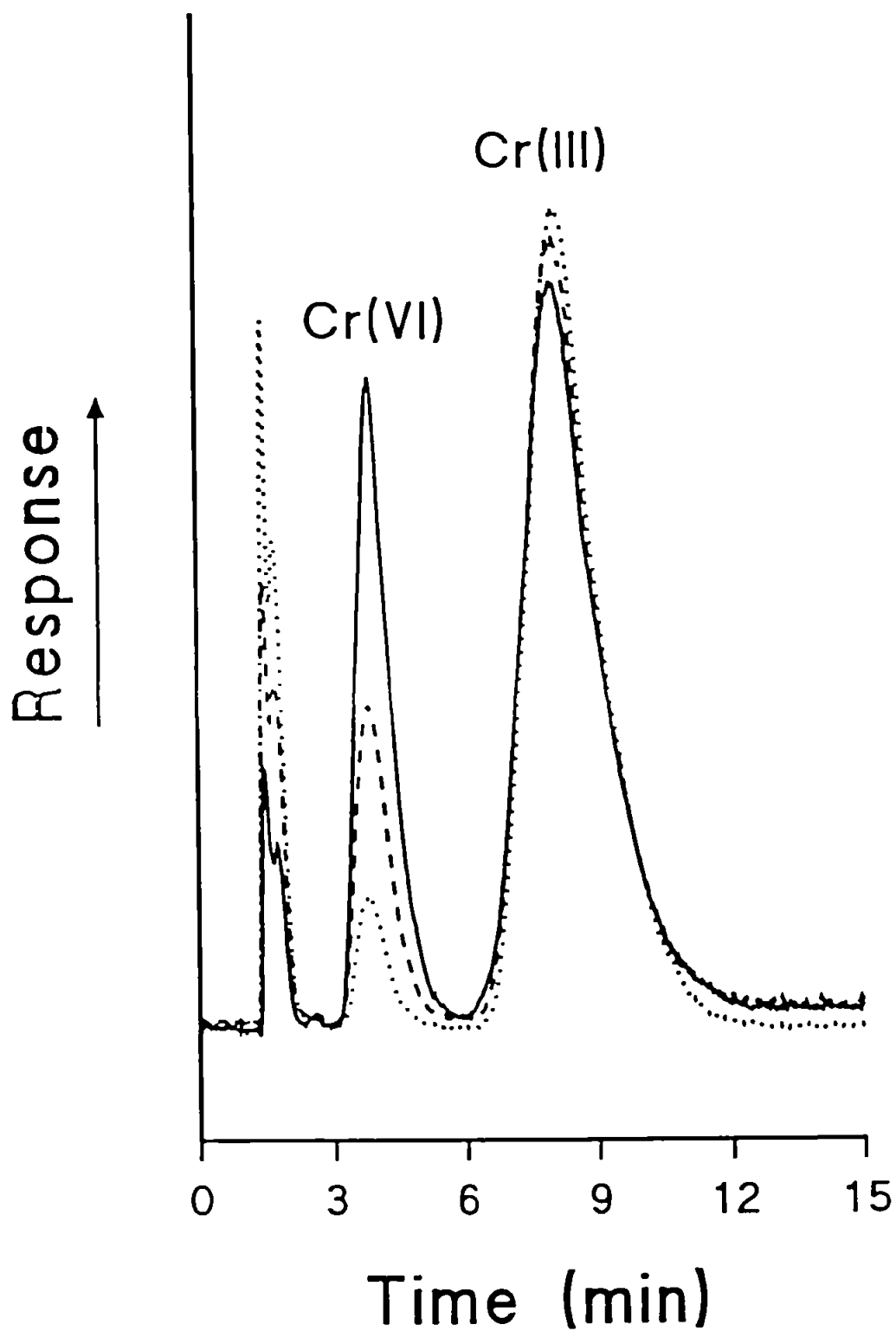
Figure 21   Decrease in response over time shown by chromium (VI) in

acidified SFW. Sample, SFW adjusted to pH 2.98 and spiked

with 10 μg l^{-1} chromium (VI). Time: 0 min (————), 60 min (-- .

-) and 120 min ($\cdots$).
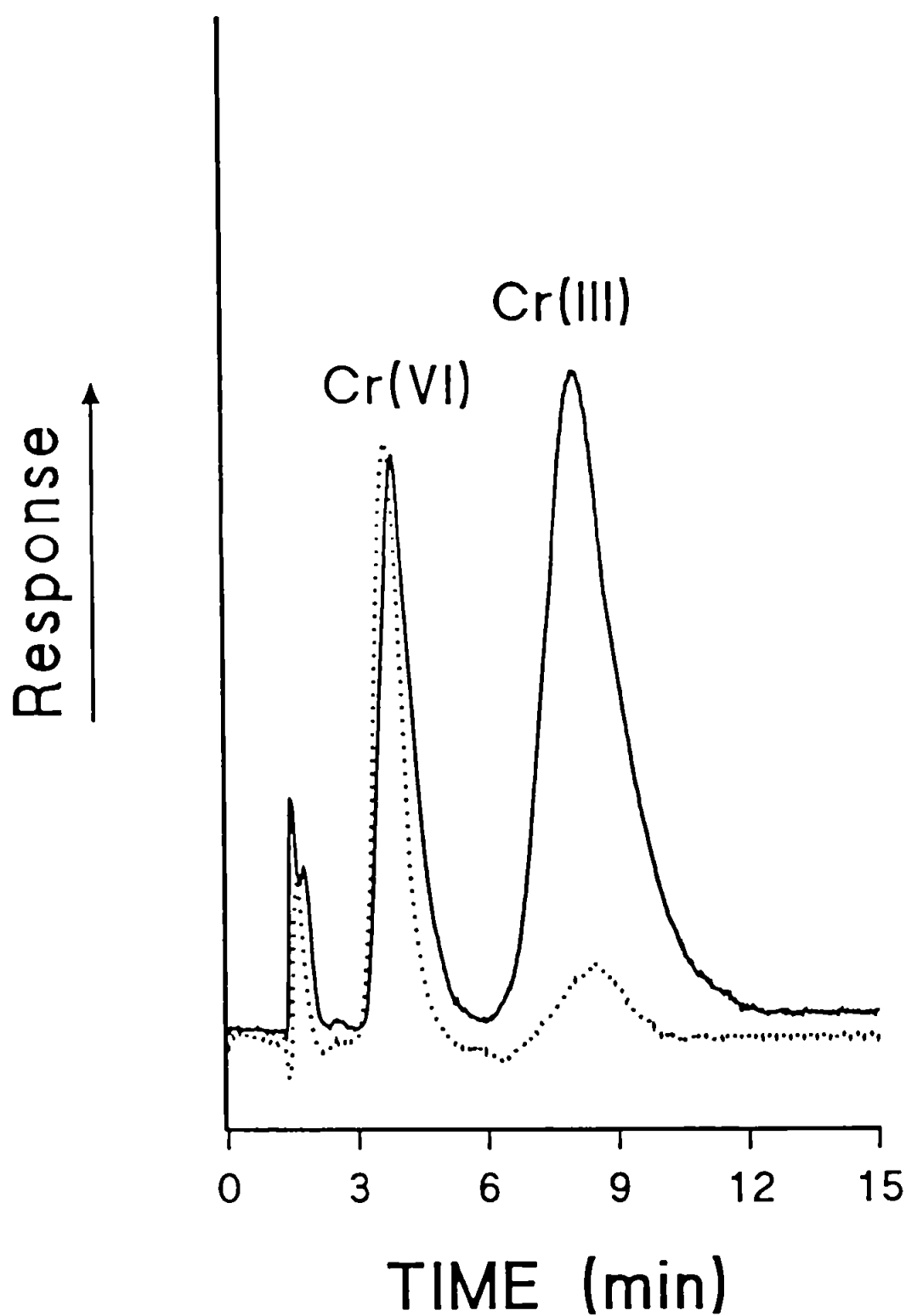
Figure 22   Effect of pH on the stability of chromium (III) in SFW. Samples,

SFW spiked with 10 μg l^{-1} chromium (VI) at pH 2.98 (————)

and 6.15 ($\cdots$).
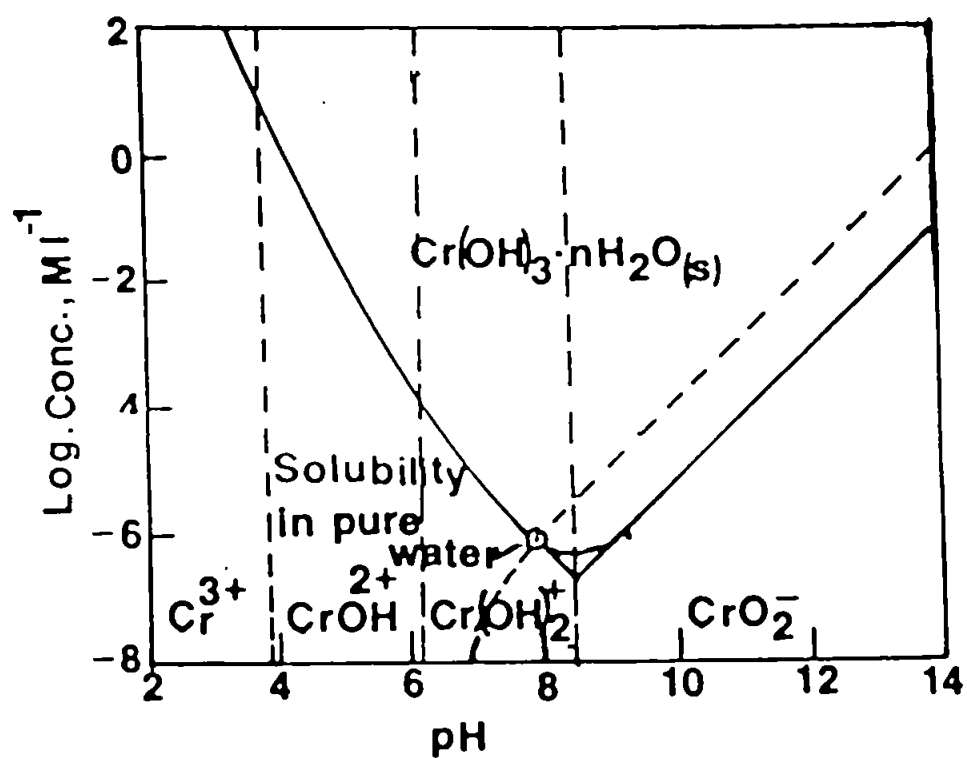
Figure 23   The effect of pH on the solubility of chromium hydroxide species

Cr(OH)₃.nH₂O (s) in solutions containing chlorides.

(III) species in solutions containing chloride and pure water (135). This indicates that soluble chromium (III) hydroxy species may be formed. Change in speciation with pH is rapid, hence any soluble chromium hydroxide species injected into the eluent (pH 2.5) would be expected to rapidly change speciation to chromium (III) aqua ion and elute accordingly. However, as can be seen from Figure 22 there is a large decrease in the chromium (III) response, presumably as insoluble chromium (III) species form as the pH of the sample is increased. Having noted that chromium (III) was unstable an intermediate pH was tried (pH 3.58) where chromium (III) is stable, however, chromium (VI) was still unstable. These findings have an important bearing on future studies of chromium speciation by IC. Simultaneous determination of both species is certainly possible at low pH, but only in the absence of reducing agents. Real samples, are unlikely to be free of low levels of reducing agents such as iodide or even species such as dissolved nitrogen dioxide formed during acid digestion. Thus, in practise chromium (III) and chromium (VI) may not be determined simultaneously in one sample injection. Separate aliquots will be required, adjusted to different pHs, a low pH for chromium (III) and a high pH for chromium (VI).

Chromium (III) was determined when the pH of the SFW was not adjusted i.e. in 1 M nitric acid and chromium (VI) was determined in SFW that had first had its pH adjusted. The amount of chromium (III) determined, namely 10.0 μg l^{-1} (average of six replicates), compared favourably with the certified amount of 9.9 μg l^{-1} chromium (III) in the simulated fresh water (confidence interval 9.0-10.5 μg l^{-1}) The reproducibility was good with a relative standard deviation (RSD) of 4.3% for the six replicates. Chromium (VI) determined in the SFW spiked at 10 μg l^{-1} giving a six replicate average of 10.5 μg l^{-1} with an RSD of 2.7%.

3.5.1   Study of Lyophilised Chromium Samples.

The Bureau Communtaire de Reference (BCR) prepared two lyophilised water sample types for independent analysis to ascertain the chromium content in each. The author was one of twenty laboratories that took part in a round robin analysis exercise. Ten lyophilised water samples were supplied, five samples of each type, type A and type B. They contained between 10 μg l^{-1} to 40 μg l^{-1} chromium (III) and chromium (VI) for sample type A. Sample type B contained only chromium (VI), at a concentration of 5 mg l^{-1} to 10 mg l^{-1}.

The instructions given required that the lyophilised samples were reconstituted by the addition of 20 ml, 0.05 M sodium carbonate buffer, at pH 6.5. It was found that if a solution of the buffer, spiked with either chromium (III) or (VI) was injected onto the column chromium eluted on the solvent front as a result of the ionic strength of the buffer. Dilution of the buffer solution by a factor of 100 prevented this. The large concentration of chromium (VI) present in sample B meant it was possible to simply dilute reconstituted lyophilised sample B a hundred fold prior to injection. However, the lower concentration of chromium in the A sample meant that dilution was not an option. The only option was to acidify the sample to remove the carbonate.

Carbonate buffer was acidified by the addition of 6 ml 0.1 M hydrochloric acid which gave a solution with a pH of 2.2 and did not cause chromium to elute on the solvent front. Sample A was reconstituted as instructed, then added to 6 ml 0.01 M hydrochloric acid in a polypropylene container, mixed and then returned to the original bottle directly before injection onto the column. The results are summarized in Table

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12.

The results obtained from this method are compared in Figures 24 and 25 to those

obtained by other laboratories using different methods. The BCR programme requires

that the chromium species are detected directly and not inferred by the determination of

one chromium species and total chromium. Overall more laboratories participated in

the determination of chromium (VI) than chromium (III), which is a result of the

greater difficulty in determining chromium (III) as a separate species.

The amount of chromium (III) determined by this method was less than many other

methods, although within the proposed confidence limits. This may reflect the

expectation that chromium (III) was present as the simple relatively inert, CL active

aqua ion. Instead, if chromium (III) was present as a complex with a different

retention time or impaired CL activity, chromium (III) determination would be

affected. However, no evidence such as spurious peaks were observed to indicate other

CL active species of chromium (III) were formed. Other laboratories that determined

chromium (III) as the aqua ion also expressed problems in determination, the overall

mean was less than the amount of chromium (III) added prior to lyophilization and may

indicate the possibility that the composition of chromium (III) may alter from the

chromium (III) aqua ion in the sample.

Chromium (VI) was determined in both samples. In both instances the determination

of chromium (VI) by this method was lower than the mean of all methods. However,

for both samples, the mean results by this method were within the proposed confidence

limits of the combined results. It was seen in the SFW sample that chromium (VI) was

Table 12   Summary of the results of chromium determination for the lyophilized water samples. * Five repeat injections, ⁸ six repeat injections.

Sample Type	Chromium (VI) Concentration	Chromium (III) Concentration	Reproducibility (%RSD)
A	10.5 μg l^{-1}	20.0 μg l^{-1}	Cr (VI) 4.3 *
			Cr (III) 8.8 *
B	5.0 mg l^{-1}		Cr (VI) 4.6 ⁸

A 16.000   20.000   24.0   28.0 μg l⁻¹ chromium
method
ETAAS
ETAAS
ICPAES
⎯ CHEMI
ICPMSa
ICPMSb
IDMS

|MEANS|

B 5.000   15.000   25.0 μg l⁻¹ chromium
method
COLOR
COLOR
COLOR
· COLOR
SPEC
ETAAS
ETAAS
ETAAS
ETAAS
ETAAS
ETAAS
ETAAS
ETAAS
ETAAS
ICPAES
ICPAES
⎯ CHEMI
DPP
ACSV
ICPMSa
ICPMSb
IDMS

|MEANS|
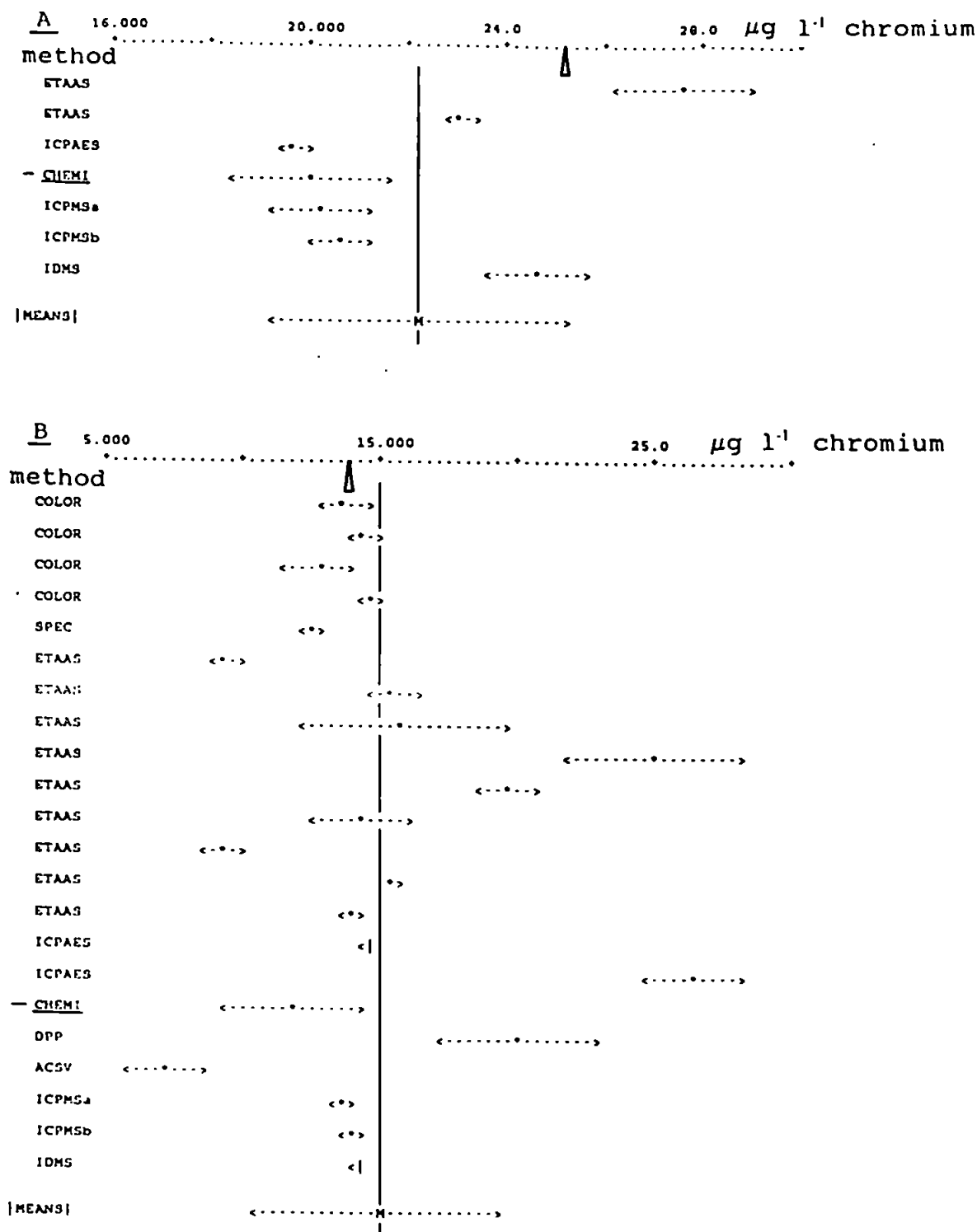
Figure 24   Comparison of results obtained by this method to those obtained
by other laboratories for the determination of A) chromium (III)
and B) chromium (VI) in **lyophilized water sample type A.**
Arrow indicates concentration of chromium in sample.
Abbreviations: ACSV, adsorptive cathodic stripping voltammetry; CHEMI,
chemiluminescence; COLOR, colorimetry; DPP, differential pulse polarography;
ETAAS, electrothermal atomic absorption spectrometry; ICPAES, inductively
coupled plasma atomic emission spectrometry; ICPMS, inductively coupled plasma
mass spectrometry; SPEC, UV-vis light spectrometry; IDMS, isotope dilution
mass spectrometry.

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method

4.000   5.000   6.000   7.000   8.000   mg l^{-1} chromium

COLOR

COLOR

COLOR

COLOR

COLOR

SPEC

SPEC

ETAAS

ETAAS

ETAAS

ETAAS

ETAAS

ETAAS

FAAS

FAAS

FAAS

ICPAES

ICPAES

CHEMI

DPP

ACSV

ICPMSa

ICPMSb

IDMS

|MEANS|
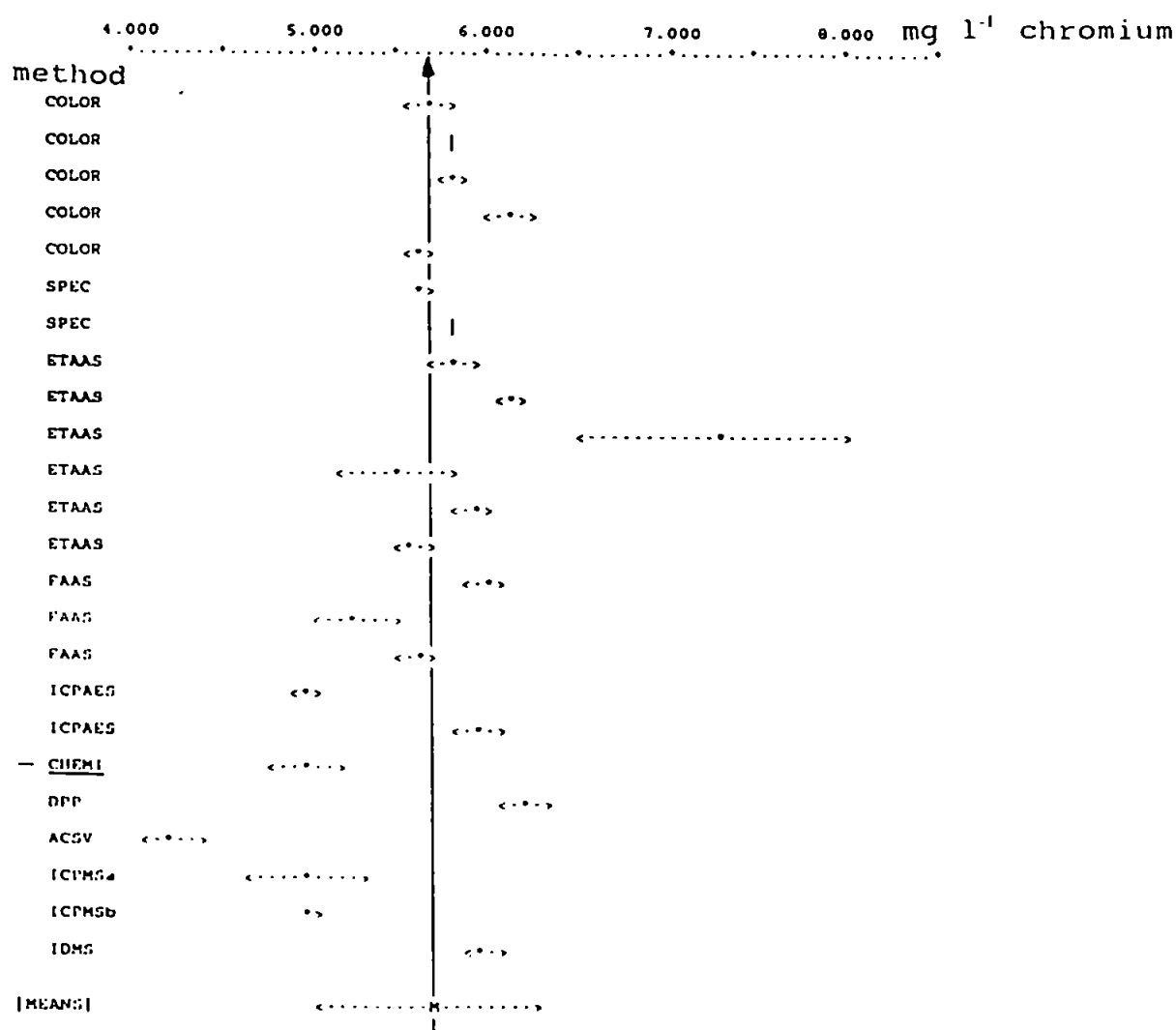
Figure 25   Comparison of results obtained by this method to those obtained by other laboratories for the determination of chromium (VI) in lyophillised water sample type **B.** Arrow indicates concentration of chromium in sample.

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not stable and was not wholly converted to the simple chromium (III) species, again as with the determination of chromium (III) by this system, only chromium eluting at the expected time was determined. However, no spurious peaks were observed, this may indicate that a non CL active complex was formed in the sample and would require further study of the chemistry occurring in the sample.

3.6   Conclusion.

The developed system successfully resolved chromium (III) and chromium (VI) and separation was achieved on one column. No sample pretreatment (except pH adjustment) was required before injection into the IC system.

In the SFW sample the requirement for pH adjustment was because of instability of the chromium (VI) species and seems, so far, to be limited to the SFW and is probably due to the presence of readily oxidizable species in the sample coupled with increased oxidising power of chromium (VI) at low pHs. Instability of chromium (III) at higher pHs was found and is shown in Figure 22 and the Pourbaix diagram for chromium speciation, Figure 20.

The need for pH adjustment in the reconstituted lyophilised samples was due to another problem, namely the higher ionic strength of the sample in the carbonate buffer at pH 6.5 and the elution of both chromium species on the solvent front if carbonate was not first removed. However, once the carbonate buffer was removed, both chromium species were stable in the reconstituted lyophilised samples at the lower pH (pH 2.2).

Thus the need for pH adjustment in the two samples was different, in the SFW it was because there were some easily oxidised species present and in the lyophilised sample it was because the choice of buffer interfered with the chromatography.

The method was shown to be sensitive to both the CL active chromium (III) and the non CL active chromium (VI) after reduction, with limits of detection of 50 ng l^{-1} and 100 ng l^{-1} respectively.

The good separation and low detection limits achieved with the developed system will allow a more detailed study of chromium speciation in natural waters and effluent, as presented in Chapter 6.

CHAPTER FOUR.

4.0   DEVELOPMENT OF AN IC SEPARATION AND CHEMILUMINESCENCE DETECTION SYSTEM FOR SILVER.

4.1.1   Introduction.

The nuclear industry has a need to determine trace amounts of silver in some types of pressurized water reactors (PWRs). Most of these PWRs are situated in former Eastern Block countries. The design is similar to Western PWRs and they are called VVERs. Silver together with other metals can be mobilized in the primary coolant water, irradiated and then deposited in out-of-core areas. These processes are discussed below.

4.1.2   Operation of PWRs.

PWRs, including VVERs, have two coolant water circuits, the primary and secondary coolant circuits, as in Figure 1 (3). The primary coolant circuit is used to provide cooling and moderation to the nuclear core. PWRs' primary circuit contains water which is pressurized to about 2000 psi and heated to a temperature of 300°C, with the temperature of VVERs being slightly less. At these temperatures and pressures the water in the primary circuit does not boil, instead the energy is transferred to the secondary coolant circuit via a heat exchanger. The secondary circuit is kept at a lower pressure than the primary circuit and turns to steam, this steam is used to drive

turbines which generate electricity.

4.1.3   Radioactive isotopes out-of-core.

Water in the primary circuit enters areas of high radioactivity and neutron flux, where
dissolved species and fine particulates are irradiated and may pass out of the core to
other parts of the primary coolant circuit. The presence of highly radioactive species
in out-of-core areas is of concern when it is time to carry out maintenance work, such
as refuelling, where the increased radioactivity leads to increased occupational
exposure. The extra precautions that may have to be taken will therefore considerably
increase the maintenance costs. These safety and economic factors have led to
increased research effort into the causes of release and deposition within PWRs and the
ability to measure extremely low levels of dissolved metal is very important to the
understanding of these processes.

4.1.4   Trace elements in primary coolant water.

Trace elements may be present in the primary coolant water as a result of wear or
solubilisation of the components and materials that the circuit is constructed from or as
impurities in the water or chemicals. Wear and corrosion of alloys used in the
construction of the primary coolant circuits of PWRs have resulted in elevated levels of
trace metals in the coolant water. For example nickel from Iconel 600 used in
construction of steam generation tubes in the heat exchanger and cobalt from Stellinite

used as hard facing in high wear components.

Other elements may be present in the coolant water as a result of the underlying geology of the water source (94) although Ferrett points out that internal sources form the major contribution of radioactive products in PWRs (8), other sources result from impurities in the chemicals used in the coolant water (136). These inputs are minimized by the use of high purity chemicals and the ion exchange scrubbing of coolant in the circuit and as water is added to the PWR system.

The source of the silver in VVERs, either as a result of corrosion or wear or by addition with reagents, such as boric acid, is not known. This ignorance must be due, in part, to the present inability to measure the presence of very low levels of silver by non-radiochemical means.

4.1.5   Monitoring of trace elements.

In the last ten years IC has been used in the nuclear industry to monitor various soluble trace elements in reactor coolant in PWRs and VVERs (137, 57, 30, 31). The IC systems often employed spectrophotometric detection which is not sensitive enough on its own and so the IC system needed to incorporate a preconcentration column to achieve the very low detection limits required (137). Detection limits of transition metals are often limited by the elution of other metals at similar retention times and sometimes this has led to ambiguous identification (137). However, the use of more selective very sensitive CL detection has enabled the unambiguous detection of

extremely low levels of cobalt (II) without the need for preconcentration (38, 137).

The ability to monitor trace analytes, particularly those that form highly radioactive daughter products, has led to an understanding of the processes that lead to their presence and mobilization. This has led to an ability to control these processes and decrease the levels of radionuclides in the out-of-core areas (137).

4.1.6   Silver in VVER coolant.

Recently silver has been found as a radionuclide in some VVER reactors' primary coolant (138). As mentioned above, IC has been shown to be a suitable method for following and determining trace first row transition metals in reactor coolant with various types of detection. There is no known IC method for silver at present. Therefore, the development of an IC system for silver will involve the development of an ion exchange method for the separation of silver from other metals in the PWR coolant and a very sensitive detection method.

4.2.1   The Ion Chromatography of Silver.

Many IC systems use resins as the stationary phase and these, especially the low capacity columns, have been used in separations of transition metal cations. The increased use of IC has been reported to be due, in part, to the use of low capacity

resin columns enabling sensitive detection of ionic species. However, the retention of silver on resin columns has been reported to be great and there have been few studies regarding the IC of silver.

The retention of silver on macroporous divinyl benzene resin when using perchloric acid as the eluent was reported by Strelow and Sondorp to be very strong (139). Therefore, the elution of silver from polystyrene divinyl benzene columns requires strong acids or complexing agents. Strong acids, particularly hydrochloric acid or hydrobromic acid eluents are used, as soluble silver halo complexes can be formed in excess halic acid. However, this requires the use of concentrated acid solutions (140, 141, 142, 143, 144). The use of concentrated acid solution as eluent is not desirable when using CL detection. The CL reaction occurs in alkaline solutions and would require substantial buffering. Such buffering would lead to an increased risk of contamination, also there may be an effect on the CL signal due to the increased ionic strength of the solution.

Elution of silver has also been achieved by the addition of a chelating agent to the eluent (145, 146, 147, 148) and by ion interaction chromatography (143). Thiourea or ammonium thiocyanate have been used to complex the silver in classical column chromatography. The formation of the cyano complex prior to separation by ion interaction chromatography was applied to the analysis of gold processing solutions (143). Again these types of eluent are undesirable as the CL reaction requires that the silver is in a free ionic state and able to form a complex with a polyamine. The complexes used for separation purposes, above, are very stable and therefore silver present in these complexes would not be free to form a complex with the polyamine

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and so could not be easily detected, increasing the detection limits.

4.2.2   Present determination methods for silver.

Sensitive determination of silver (I) has been achieved using electrothermal atomic absorption spectrometry (ETAAS), anodic stripping voltammetry (ASV), inductively coupled plasma-mass spectrometry (ICP-MS) and neutron activation analysis (NAA), reported detection limits are given in Table 13. However, none of these techniques are either easily coupled to or available for coupling to a separation system. Also, the detection limits of coupled systems are often considerably higher due to band broadening of the analyte on the column and passage through the interface. Importantly, many of these instruments are not suitable for use in a PWR plant.

Spectrophotometric methods for the determination of silver (I) are less sensitive than other instrumental methods, some examples are also given in Table 13. In addition these spectrophotometric methods require long reaction times which make them unsuitable for use in a PCR detection system.

Sensitive CL determination of silver has been achieved in a batch system using simple instrumentation. CL detection systems have been successfully applied to IC for the detection of other cations, most notably cobalt (II). Therefore, the most promising approach would be to develop a CL detection system for silver after IC separation.

Table 13   Detection limits for silver by various methods.

Method	Detection Limit for Silver ($\mu g\ l^{-1}$) * = sensitivity ^{1} = $\mu g\ kg^{-1}$	Reference
Spectroscopic		
Graphite Furnace AAS	0.09	149
*	0.16	150
*	1	151
*	$1 \times 10^{13}\ g$ *	152
*	2	153
Electrothermal AAS	0.001	154
*	0.00037	155
ICP-MS	1	156
Flame AAS	100	147
*	3	150
*	2	157
Flow injection FAAS	4	158
Neutron Activation Analysis	1^1	159
*	0.0022	148
Electrochemical		
Voltammetry	1	157
Chemically Modified Electrodes	1	160
Differential Pulse Polography	1	161
Potentiometric	10	162
Flow injection Potentiometric	1000	163
Piezoelectric	20	164
Spetrophotometric		
Spectrophotometric	1000	165
*	100	166
*	5	86
*	1	167
*	50	157
*	1000	168
Kinetic Spectrophotometric	200	169
*	30	170
*	100	167
Catalytic Spectrophotometric	15	171
Catalytic Indirect	30	172
Catalytic Fluorometric	5.4	173
Extraction Fluorometric	1	174
Extraction Spectrophotometric	100	167
Indirect Spectrophotometric	5000	175
Chemiluminescence	2	86

4.2.3   CL reactions involving silver.

CL reactions that are catalysed in the presence of silver include the oxidation of gallic acid (101, 83), lucigenin (107, 99), lophine (102, 106) and luminol. In common with other transition metals the luminol reaction offers the greatest sensitivity. The luminol reaction is the preferred choice for other reasons and the reader is referred to Section 1.12.1 in the introduction.

A method developed by Pilipenko *et al.* was shown to be sensitive for the batch determination of silver in mineral waters, with limit of detection of 2 μg l^{-1} (86). The CL reaction used was the oxidation in alkaline solution of luminol by persulphate. This reaction can be catalysed by silver(I) in the presence of an activator, the polyamine, triethylenetetramine (NH_2-CH_2-CH_2-NH-CH_2-CH_2-NH-CH_2-CH_2-NH_2), (TETA).

4.3   Aim of this Study.

The main aim is to develop an IC method capable of determining silver at the sub μg l^{-1} level in PWR coolant. This requires the development of a CL detection system capable of detecting such levels of silver in the column effluent. It is also necessary to investigate novel ion exchange systems to enable the separation of silver from other ions in PWR coolant. The separation system will need to be compatible with CL detection.

4.4   Experimental.

The lack of previous IC investigations in the literature required that the CL detection and chromatographic systems were developed together. Thus, initially the CL detection conditions reported by Pilipenko *et al.* were used in order to obtain a working IC system using simple IC on Dionex low capacity cation exchange columns. With simple IC of silver on the Dionex columns it was possible to develop the CL detection system. The chromatography and detection systems could then be more fully investigated. This included the effect of the ionic strength of the eluent on the CL detection system, the use of triethylenetetramine (TETA) in the eluent and the use of preconcentration of silver onto the Dionex IC column. Polymethacrylate resin based IC packing material was examined as an alternative to the more typical polystyrene divinyl-benzene resin based packing material used in IC. Large, 50 μm, particle size resin was used initially. Increase in column efficiency was expected with smaller 10 μm particle size resin and a short 2.5 cm column was also investigated. The effect of iodide and other halides on the silver response was investigated. A standard reference material was analyzed for silver and the result obtained compared to the given value. The equipment and reagents used are detailed below.

4.4.1   Chromatograph and reagents.

As shown in Figure 26 the chromatograph set up consisted of a 4000i Dionex gradient pump (Dionex, Sunnyvale, CA, U.S.A.) which was used to deliver the eluent to the column at 1 ml min⁻¹. The CL reagent was added just prior to the detection coil at 1

ELUENT PUMP

INJECTION VALVE

ION EXCHANGE COLUMN

LUMINOL PUMP
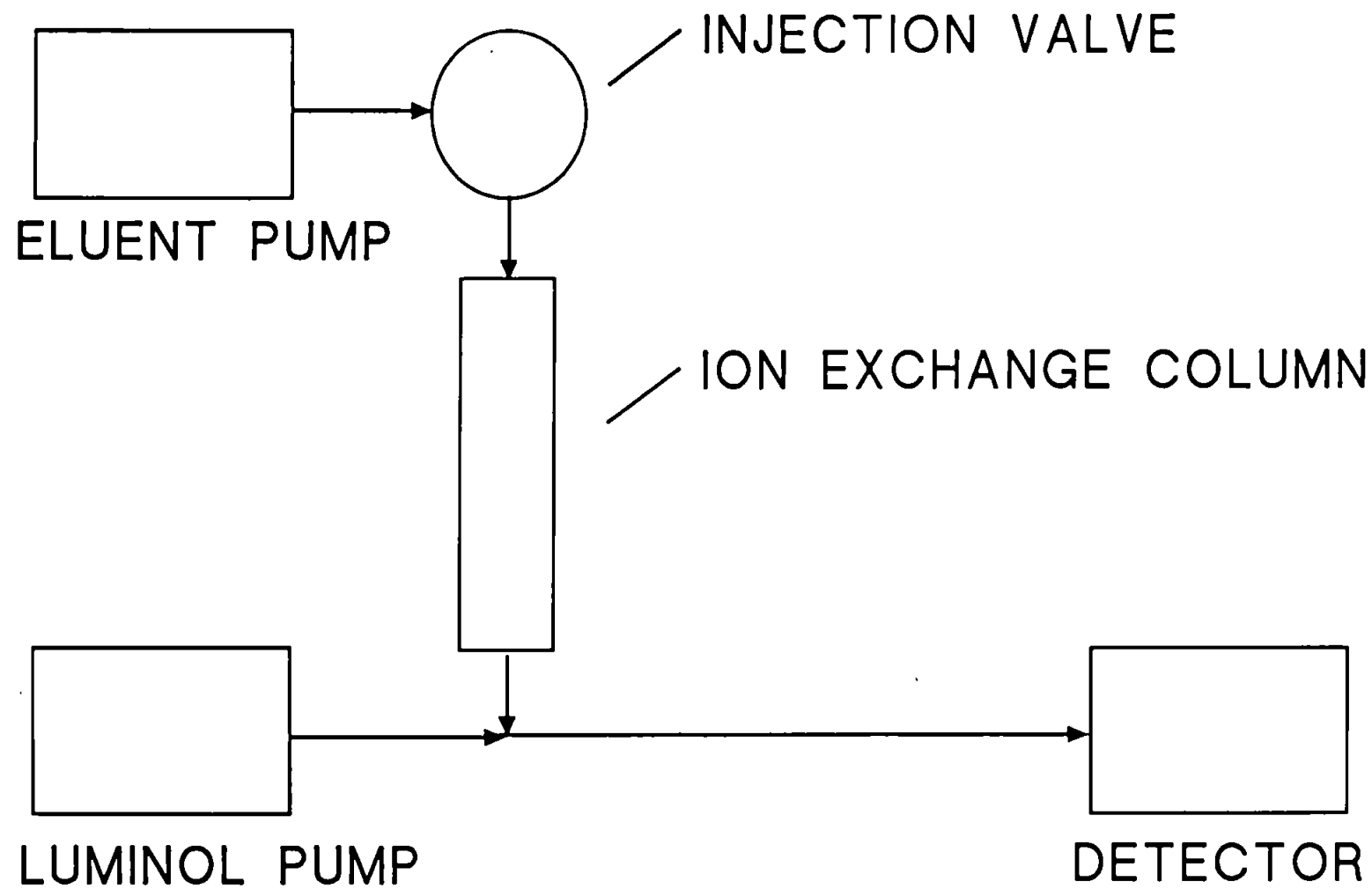
DETECTOR

Figure 26   Schematic diagram of chromatograph employed.

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ml min^{-1} using an inert plastic pump (Dionex 2000i). The chemiluminescence was monitored with a modified FD-100 fluorescence detector (Spectrovision, MA, U.S.A.). The modification involved replacing the fluorescence cell with a flat PTFE coil, volume 30μl, pressed against the face of the photomultiplier tube.

Sample injection was carried out via an inert PEEK Rheodyne injection valve (Model 9010, Cotati, CA, U.S.A.) with a PEEK 100 μl sample loop. Later experiments utilised a PTFE 1 ml sample loop. PTFE connecting tubing, 0.3 mm i.d., was used throughout. All eluents were degassed with helium prior to use.

Milli Q deionized water (Millipore Corp., Bedford, MA, U.S.A.) was used for all solutions. All reagents were AnalaR (Merck, Poole, U.K.) except luminol (Fluka, Poole, U.K.) and Aristar potassium hydroxide (Merck).

4.4.2 Certified reference material.

Certified reference material NIST 1643C (Simulated River Water) was used as a reference material, containing 2.21 $\pm$ 0.30 μg l^{-1} silver.

4.4.3 Pressurised water reactor coolant.

Simulated PWR coolant was prepared from 0.0691 g potassium persulphate, 0.148 g ammonium chloride, 2.1 g orthoboric acid and 0.026 ml ammonia solution (25%) made

up to 2 l gave the concentrations of the major ions found in PWR coolant. These are given in Table 14. Typical concentrations of trace metals found in PWR coolant are also given in Table 14 (58).

4.5   Results and Discussion.

4.5.1   Preliminary investigations.

As discussed in the introduction, the previous reported methods for the elution of silver are not compatible with a CL detection system based on luminol. Therefore, it was necessary to develop a suitable separation system. However, the previous reported methods had used classical column chromatography which relied on high capacity columns. Thus, it was decided that initial experiments were to be made on lower capacity Dionex resin based columns. The low capacity gave the possibility of eluting silver using simple ion exchange principles and relatively low concentration of counter ion as the eluent.

Due to the interdependence of the separation and detection systems an iterative approach was necessary, particularly in the preliminary stages.

4.5.2   Optimisation of the CL system.

The CL detection system reported by Pilipenko *et al.*, Table 15, was modified for

Table 14   Typical concentrations of major ions and trace metals in PWR coolant.

Concentration of Major Ions in PWR Coolant

Ion	Concentration
Potassium	10 mg l^{-1}
Ammonium	25 mg l^{-1}
Borate	1000 mg l^{-1}
	pH 6.0 to 6.5

Typical Concentrations of Trace Metals in PWR Coolant

Metal	Concentration
Zinc (II)	0.18 μg l^{-1}
Nickel (II)	1.8 μg l^{-1}
Cobalt (II)	0.21 μg l^{-1}
Iron (II)	60 μg l^{-1}
Manganese (II)	2.6 μg l^{-1}

Table 15   Conditions used by Pilipenko *et al.* (86) for the determination of silver by a batch CL method.

Solution	Concentration of Solution Added (M)	Reagent Volume (ml)	Concentration in Final Solution, 5.25 ml (M)
Test solution		4	
EDTA	5×10^{-3}	0.2	1.9×10^{-4}
Boric Acid pH 9.0	0.1	0.5	9.5×10^{-3}
Luminol	10^{-3}	0.25	4.8×10^{-5}
Sodium Persulphate	2×10^{-2}	0.3	1.1×10^{-3}

adaption to a flow system. This made it possible to determine whether silver (I) was eluted from the column and separated from the solvent front when using a simple salt eluent. A 0.15 M potassium nitrate, 0.03 M nitric acid eluent was used and this enabled initial optimisation of the CL parameters.

The parameters that were considered important for optimisation of the CL system were:

1)   concentration of polyamine activator;

2)   most suitable polyamine activator;

3)   pH of the solution in the detection coil;

4)   concentration of luminol;

5)   concentration of potassium persulphate;

6)   concentration of orthoboric acid;

7)   time taken from luminol addition to entering the detection cell.

Figures 27-34 show the variation in sensitivity for a range of reagent concentrations required in the luminol solution and the pH required in the detection coil. Figure 31 shows the maximum peak height at pH 11.5 when using triethylenetetramine (TETA) activator. However, as the final pH increased over 10.5 the signal to noise ratio decreased giving optimum detector performance at pH 10.5. A similar increase in response with increasing pH was observed when using the other polyamine activator, diethylenetriamine (DETA), but at a lower pH. Comparison of peak heights for the two activators across a range of pH values showed that the greatest response was given by TETA, as reported by Pilipenko *et al.* (The detector was used on a setting ten times more sensitive for the determination of DETA peak heights than for the

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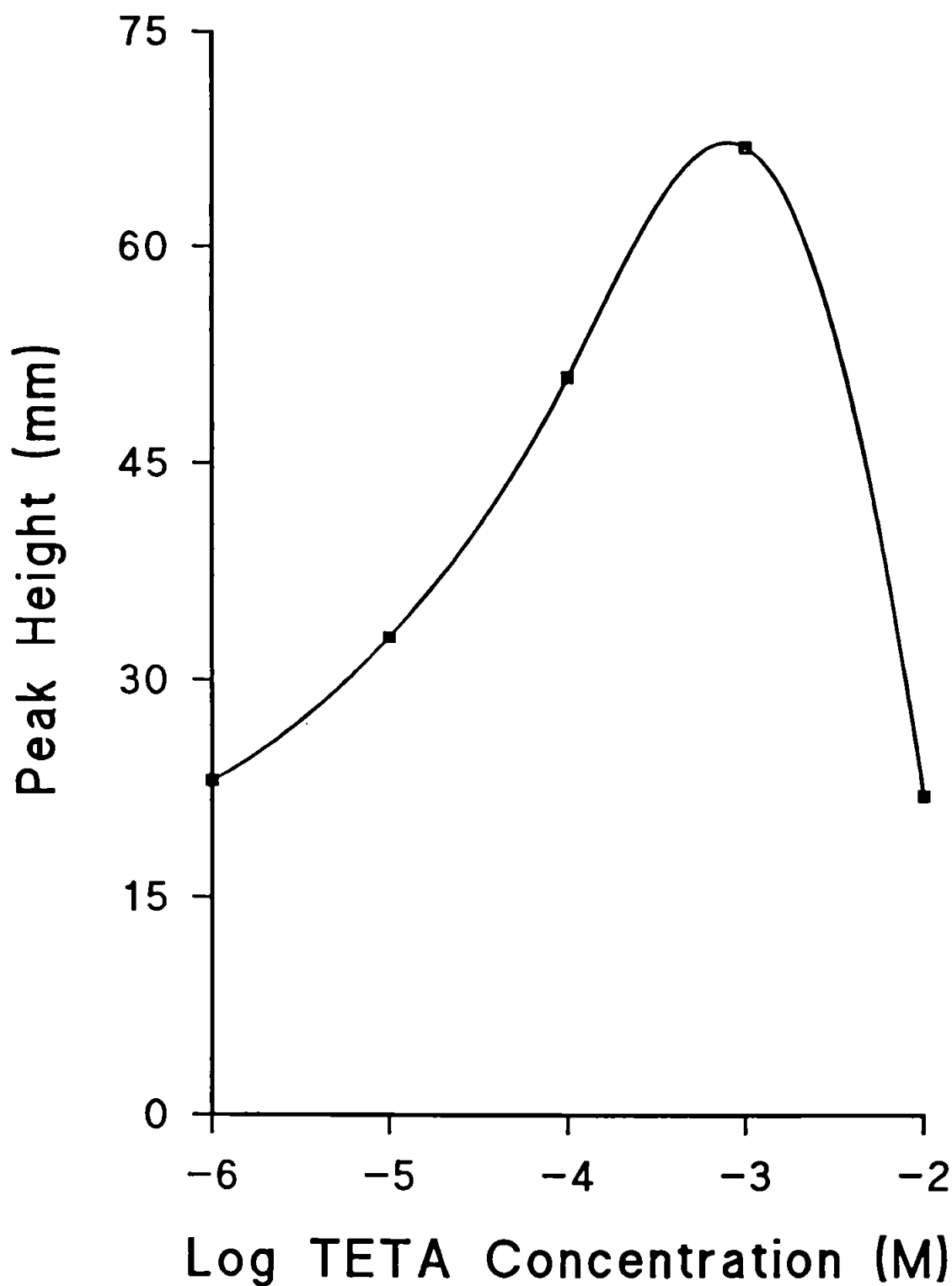
Figure 27   Effect of TETA concentration on the CL response to 100 μg l^{-1} silver at pH 10.5, with: 5 x 10^{-5} M luminol, 0.1 M orthoboric acid and 0.003 M potassium persulphate.
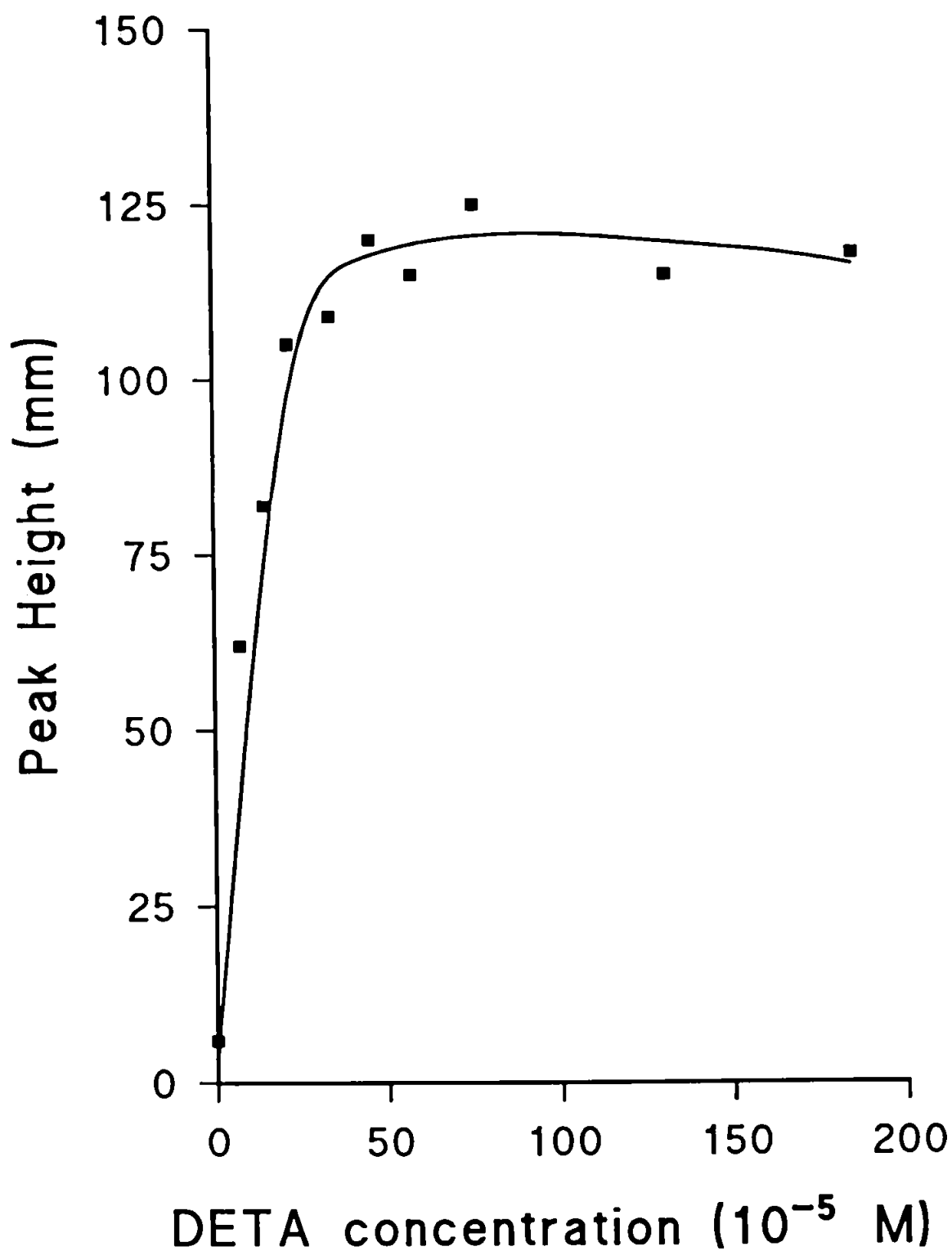
Figure 28   Effect of DETA concentration on the CL response of 200 μg l^{-1}
silver at pH 10.3, with: 5 x 10^{-5} M luminol, 0.1 M orthoboric
acid and 0.003 M potassium persulphate.
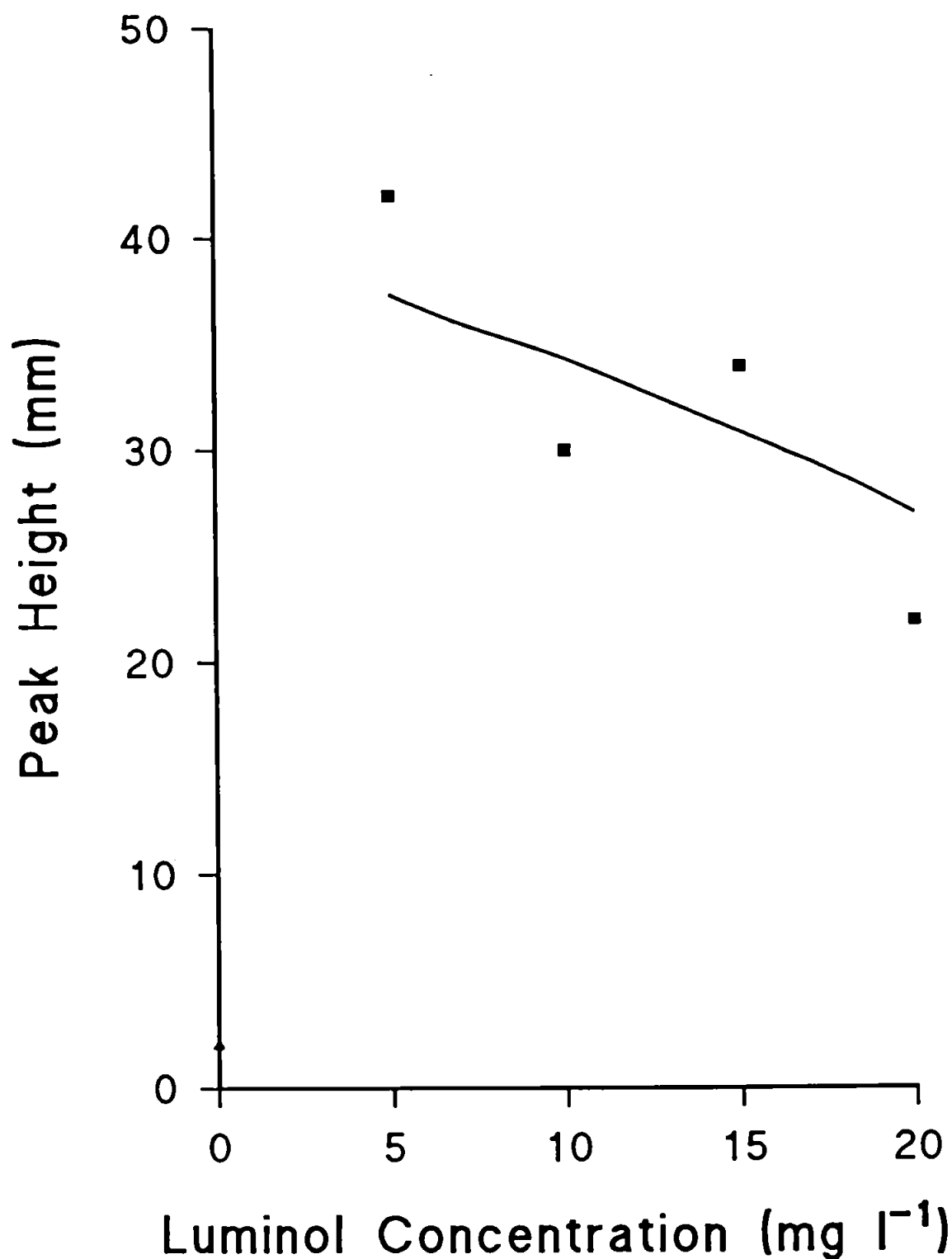
Figure 29   Effect of Luminol concentration on the CL response of 10 μg l^{-1}
                   silver at pH 10.5. 0.001 M TETA, 0.003 M potassium
                   persulphate, 0.1 M orthoboric acid. Wide calibration range.
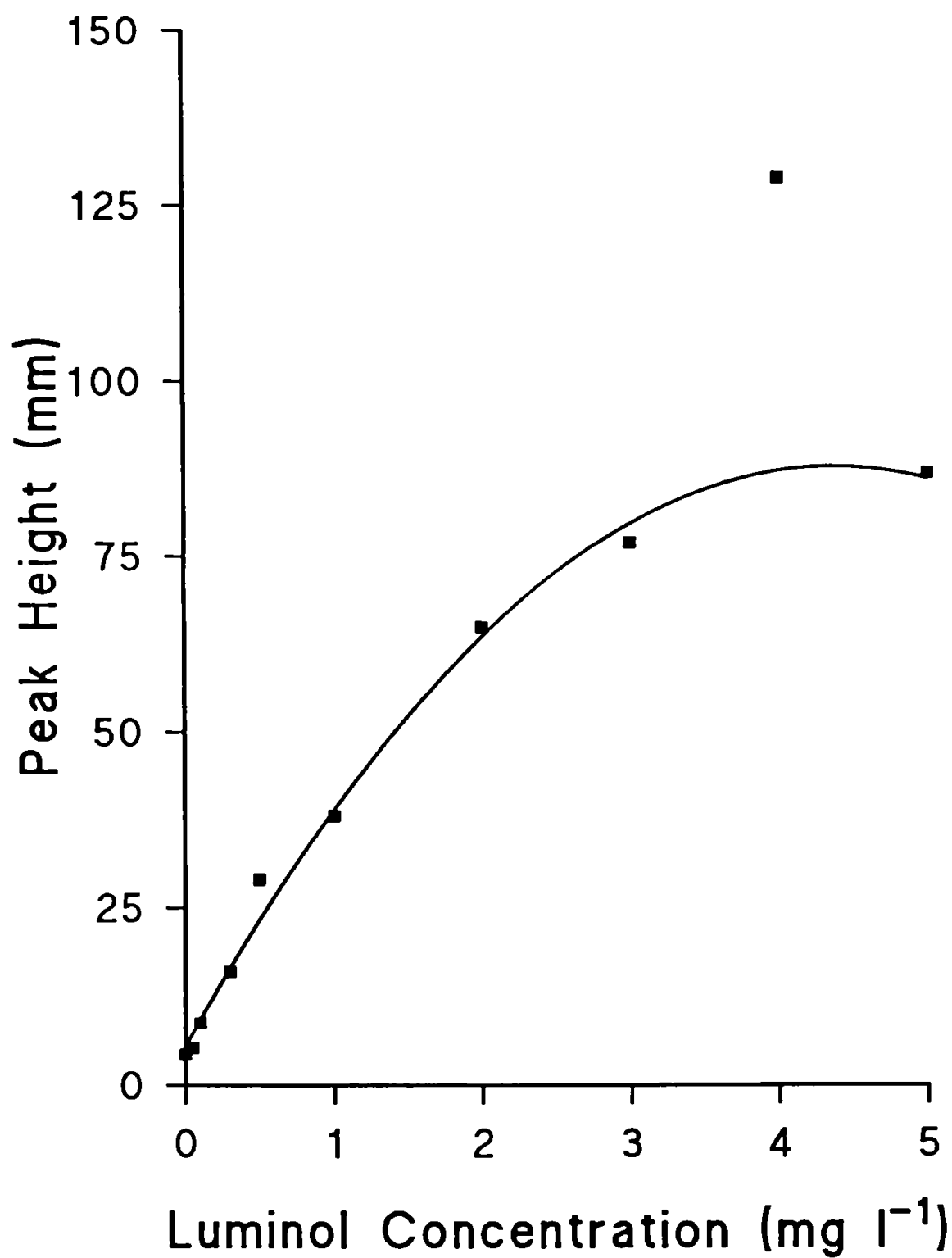
Figure 30   Effect of Luminol concentration on the CL response of 10 μg l⁻¹

silver, other conditions as Figure 31.   Narrow calibration range.

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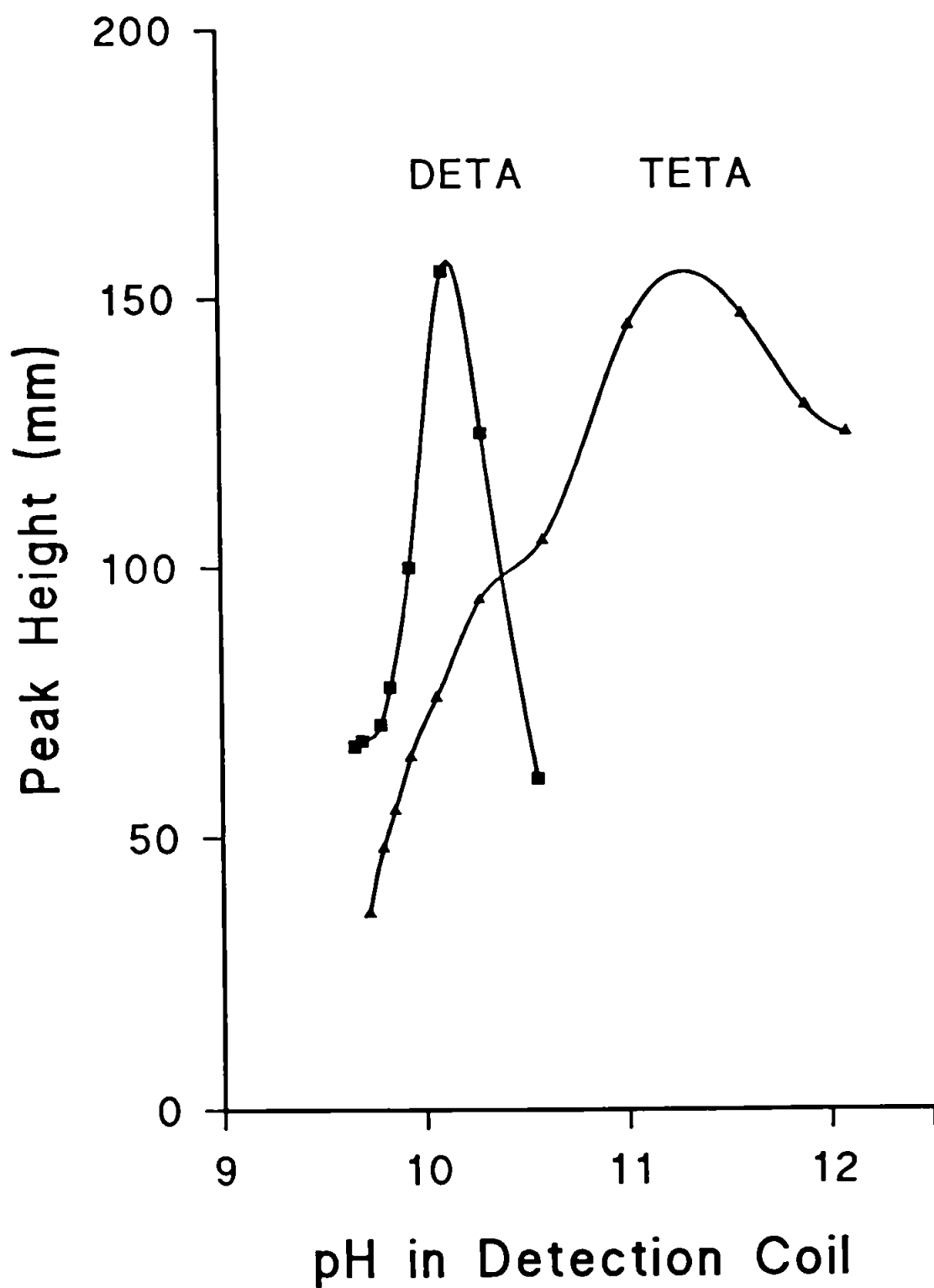
Figure 31   Effect of pH on the CL response of 100 μg l^{-1} silver, with 0.001
M TETA or 5 x 10^{-5} M DETA as the activator. 5 x 10^{-5} M
luminol, O.003 M potassium persulphate and 0.1 M orthoboric
acid.
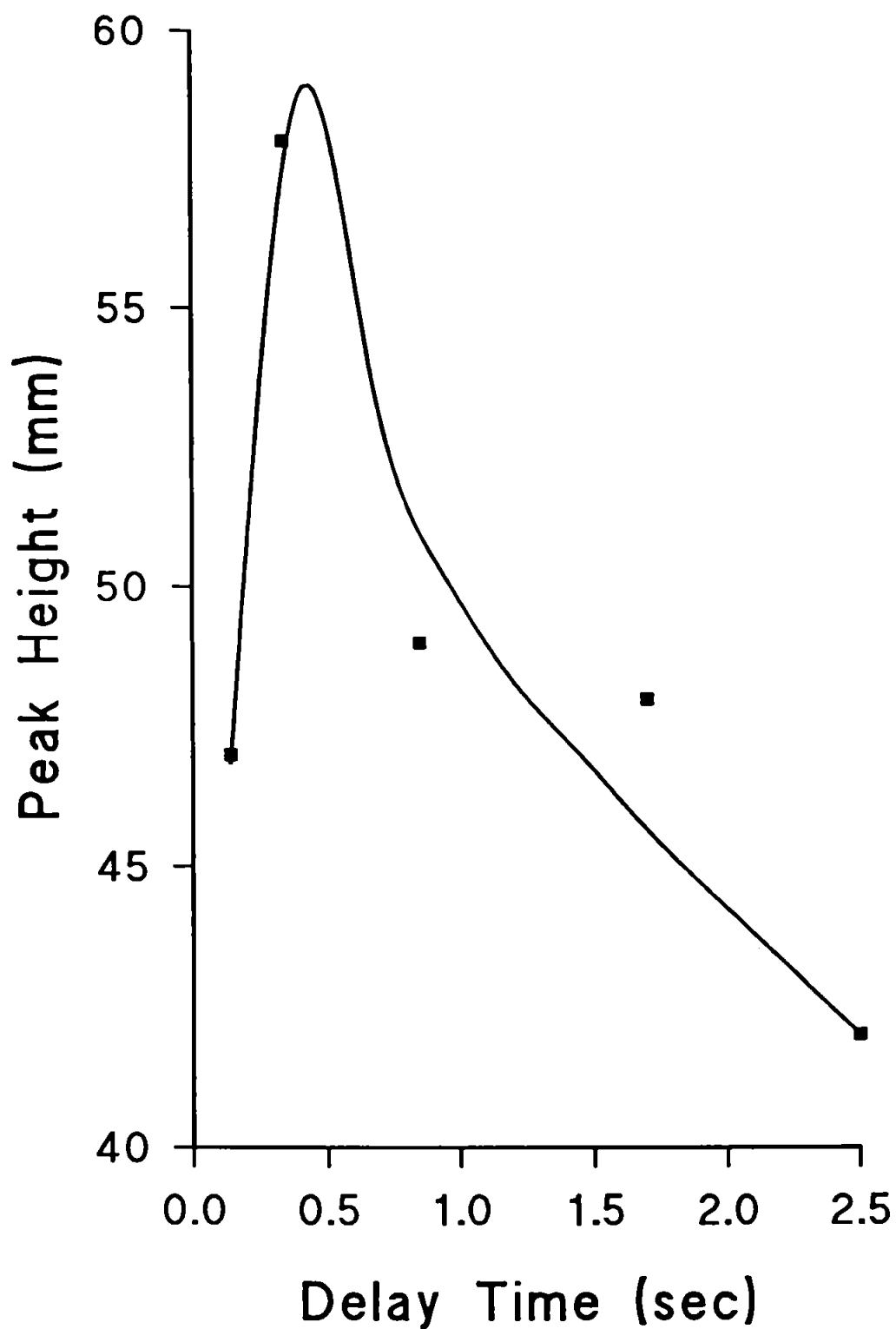
Figure 32   Effect of delay between mixing of the column effluent with the
luminol solution and the detector on the CL response of 100 μg l^{-1}
silver at pH 10.5, with: 0.1 M orthoboric acid, 5 x 10^{-5} M
luminol, 0.001M TETA and 0.003 M potassium persulphate.
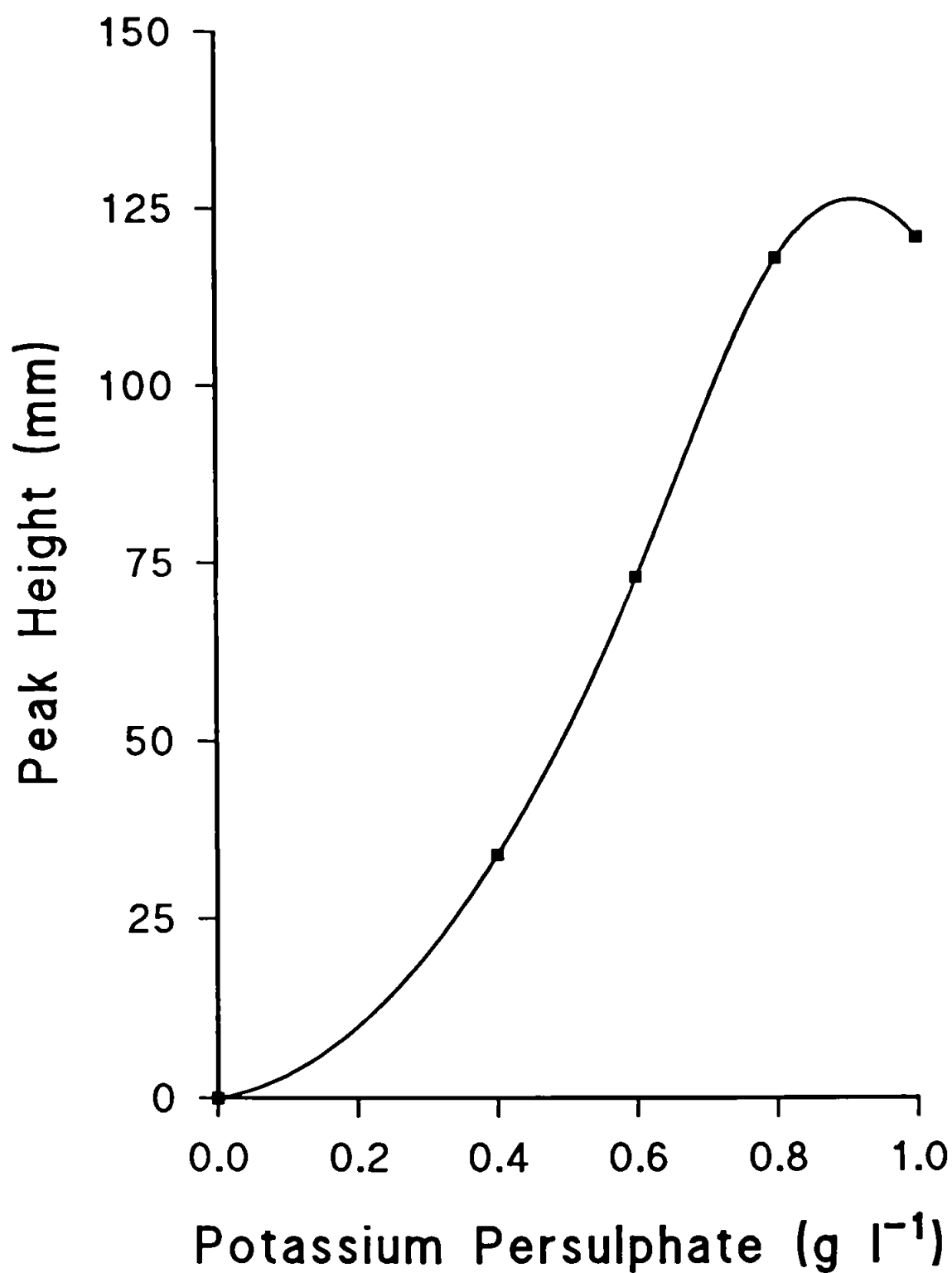
Figure 33   Effect of potassium persulphate concentration on the CL response .
of 100 μg l^{-1} silver at pH 10.5, with: 0.1 M orthoboric acid, 5 x
10^{-5} M luminol and 0.001M TETA.
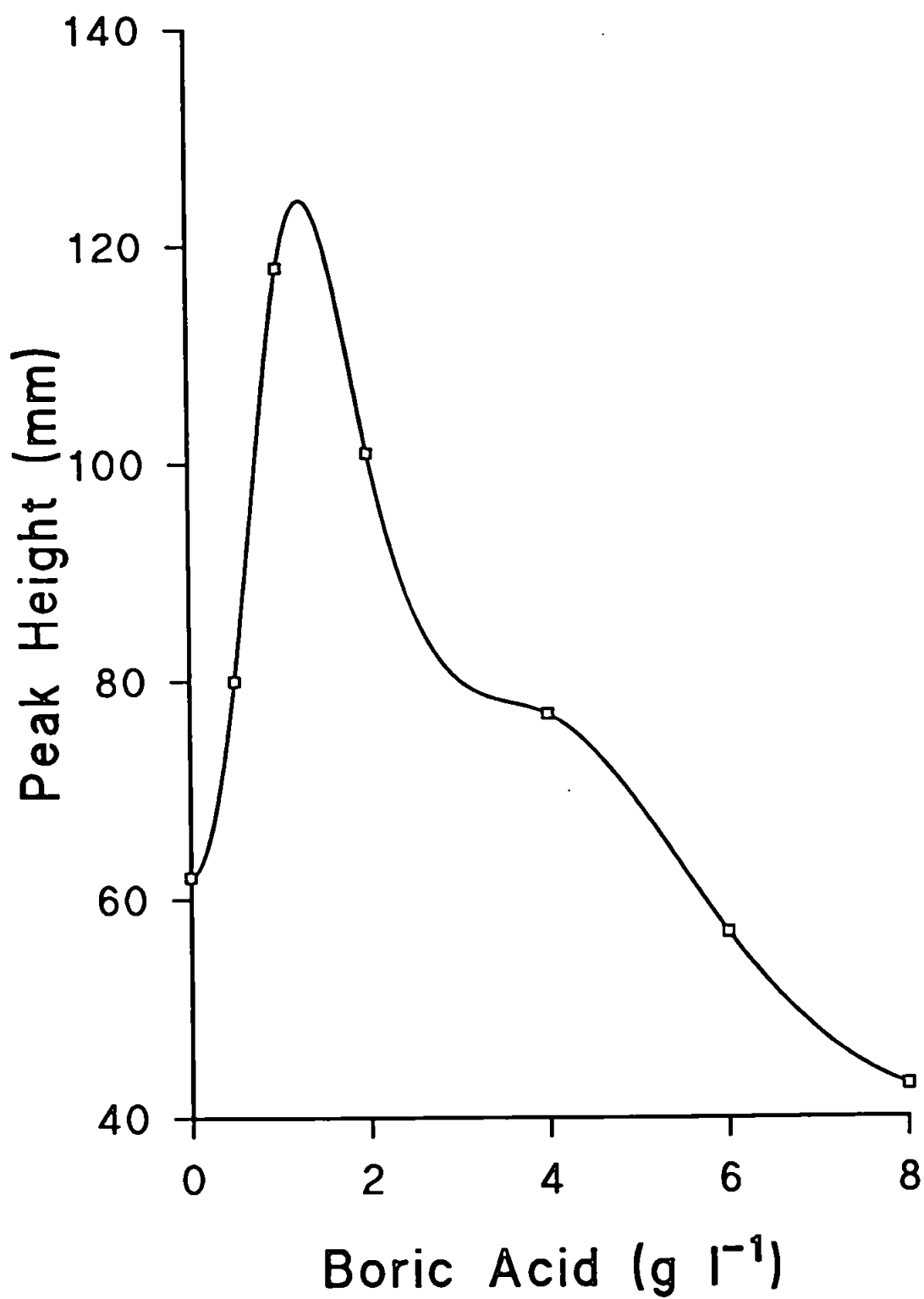
Figure 34   Effect of orthoboric acid concentration on the CL response of 10
μg l^{-1} silver at pH 10.5, with: 5 x 10^{-5} M luminol, 0.003 M
potassium persulphate and 0.001M TETA.

determination of TETA peak heights).

Once the detection system was optimised for the chosen eluent the detection limit decreased. With the increase in sensitivity the peak shapes that had initially looked symmetrical showed a great degree of tailing, Figure 35. During detector optimization it became apparent that the concentration of potassium nitrate used for elution probably caused some quenching of the silver signal and that the system response was highly non-linear.

4.5.3   Effect of salt concentration on sensitivity.

In order to determine whether the concentration of salt had an effect on the CL system it was necessary to remove the column and so operate in flow injection mode. The concentration of the salt in the eluent was increased whilst observing the effect on the CL response for silver.

Table 16 shows the effect increasing concentrations of potassium nitrate and potassium sulphate have on the CL signal. This table also shows that the different potassium salts have different degrees of effect on the CL response. Sodium acetate and acetic acid were investigated as possible alternatives to either potassium nitrate or potassium sulphate. However, both caused serious quenching of the signal, for example, 1 mg l^{-1} silver was not determined with acetic acid in the eluent. Another possibility is the use of divalent cations as the counter-ion in the eluent. The greater selectivity coefficient of the more highly charged ions would mean lower concentrations would be required.
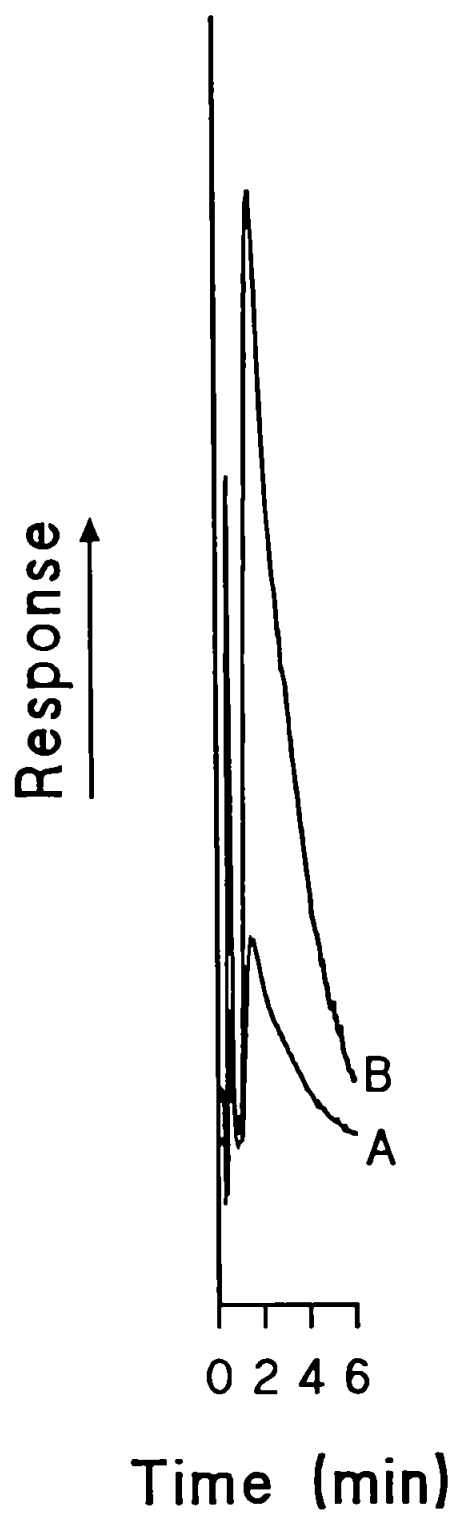
Figure 35   Typical chromatogram of 30 μg l^{-1} (A) and 100 μg l^{-1} (B) silver.

0.15 M potassium nitrate, 0.03 M nitric acid eluent. Dionex

CG2 column. 100 μl injection.

Table 16   The decrease in response for silver CL with increasing salt

concentration.

Potassium Nitrate		Potassium Sulphate	
Concentration (M)	Relative Peak Height (%)	Concentration (M)	Relative Peak Height (%)
0.00	100	0.00	100
0.01	55	0.006	92
0.02	52	0.012	86
0.04	43	0.024	79
0.05	32	0.046	69
0.13	9		
0.17	5		
0.21	2		

However, this was not possible due to the high pH of the detection system, which would result in the formation of the insoluble hydroxides of the divalent cations.

4.5.4   The use of polyamine in the eluent.

It was discovered whilst optimising the CL system that if either DETA or TETA were added to the eluent then the silver peaks became sharper and moved to the solvent front, even though the molar concentrations of TETA or DETA added were considerably less than the concentration of potassium nitrate required.

The use of a polyamine without potassium nitrate was investigated in order to develop a low ionic strength eluent. TETA was used in the eluent as it was required in the PCR CL detection system and response to silver was greater with TETA as the activator than with DETA as the activator. Figure 36 shows a typical chromatogram with 0.001 M TETA eluent.

The use of a TETA eluent enabled the detection of lower concentrations of silver, and the linearity of the response improved. However, the improved detection limits also emphasized the amount of peak tailing. With the less sensitive CL detection given with a potassium nitrate eluent the peaks in Figure 35 show some asymmetry. However, with the greater sensitivity detection, using a TETA eluent, it was possible to detect concentrations of silver that were below the previous detection limit. This enabled determination of silver in the tails of the eluting silver peaks, which would previously . gone unnoticed
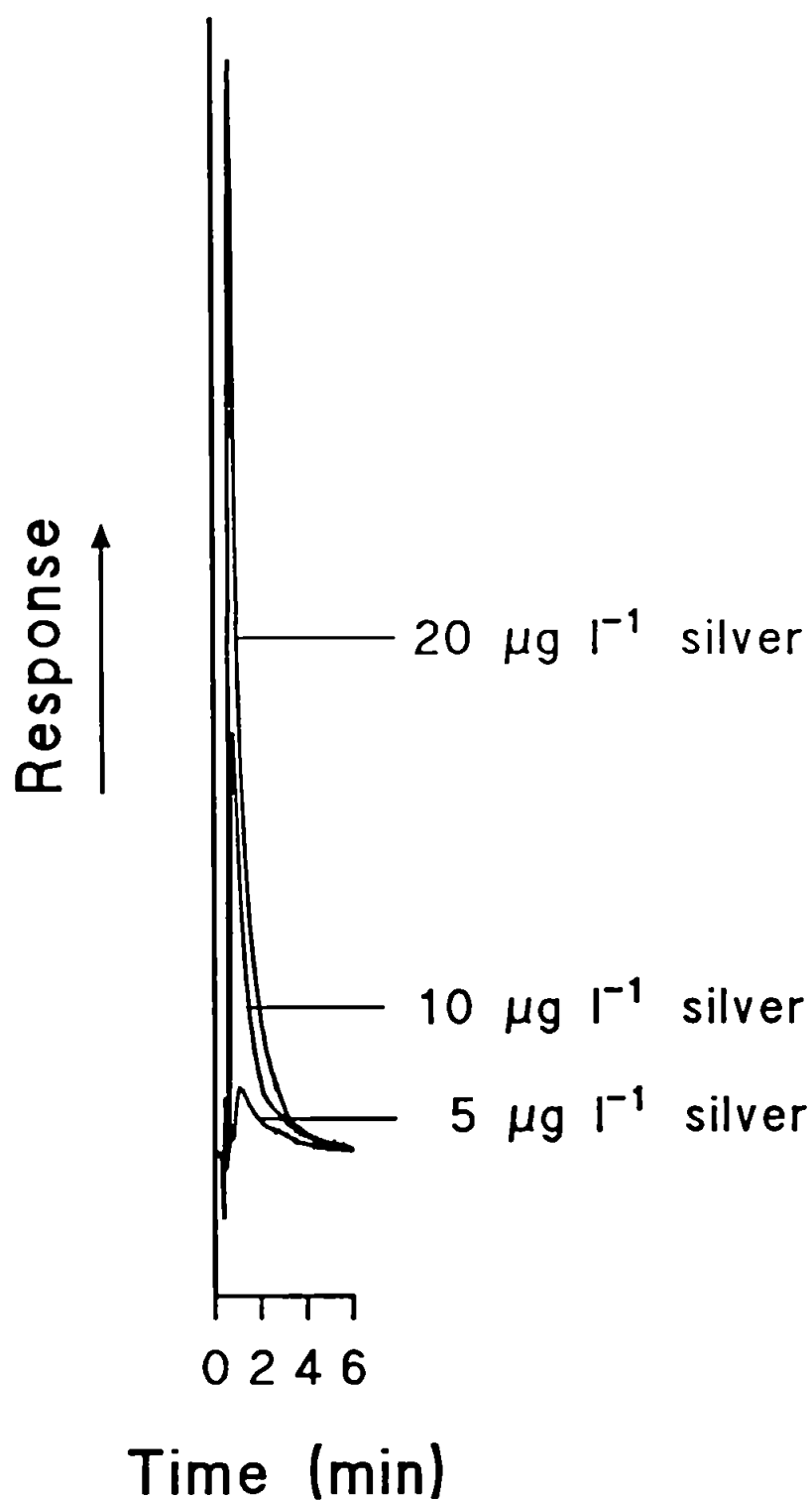
Figure 36   Typical chromatogram of 5 μg l^{-1}, 10 μg l^{-1} and 20 μg l^{-1} silver.

0.001 M TETA, pH 2.25. Dionex CG2 column. 100 μl

injection.

However, the concentration of TETA used in the eluent for these preliminary investigations was the concentration of TETA required by the detection system. It was now necessary to investigate the effect of concentration and eluent pH on the elution of silver when using TETA in the eluent, to ascertain whether conditions could be chosen to obtain significantly better elution characteristics.

4.5.5 The protonation of triethylenetetramine.

The protonation of TETA was investigated and the effect of decrease in pH in particular can be attributed to the successive protonation of the amine moieties of the TETA molecule. Figure 37 shows that at low pH TETA is quadruply protonated and hence carries a +4 charge. The relationship between charge and selectivity coefficients make it a very strong cation exchanger.

4.5.6 Effect of pH with triethylenetetramine eluent.

The effect of pH on silver elution with 0.1 M, 0.01 M and 0.001 M TETA eluents was studied from pH 10 to 2.

With the aid of the pH against TETA speciation plot, Figure 37, and the pH against overall charge on the TETA molecule plot, Figure 38, it is possible to explain and predict the change in elution time as the pH of the eluent changes. Table 17 and Figure 39 show the retention times of silver at different pH's and TETA

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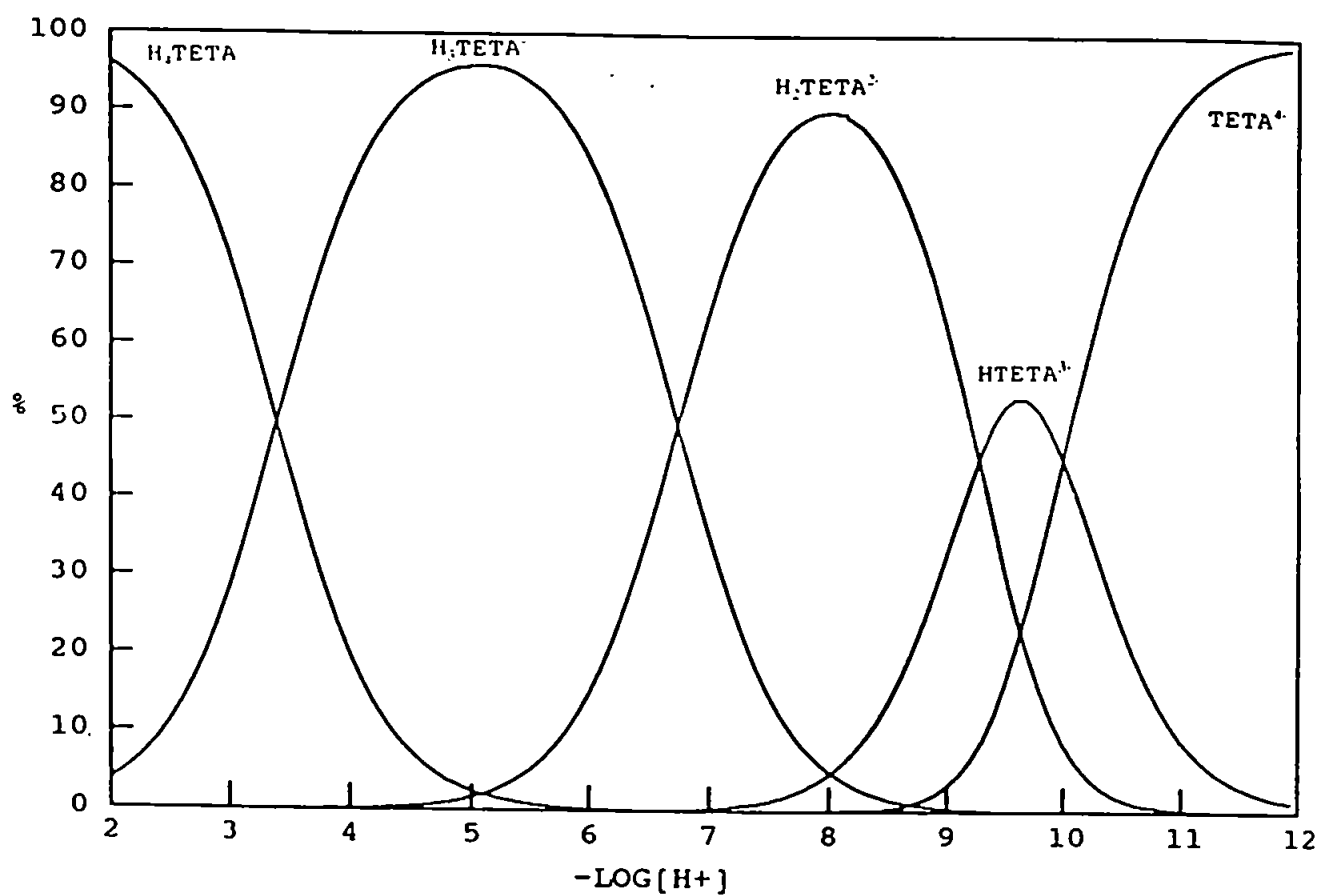
Figure 37   Speciation of TETA as a function of pH.
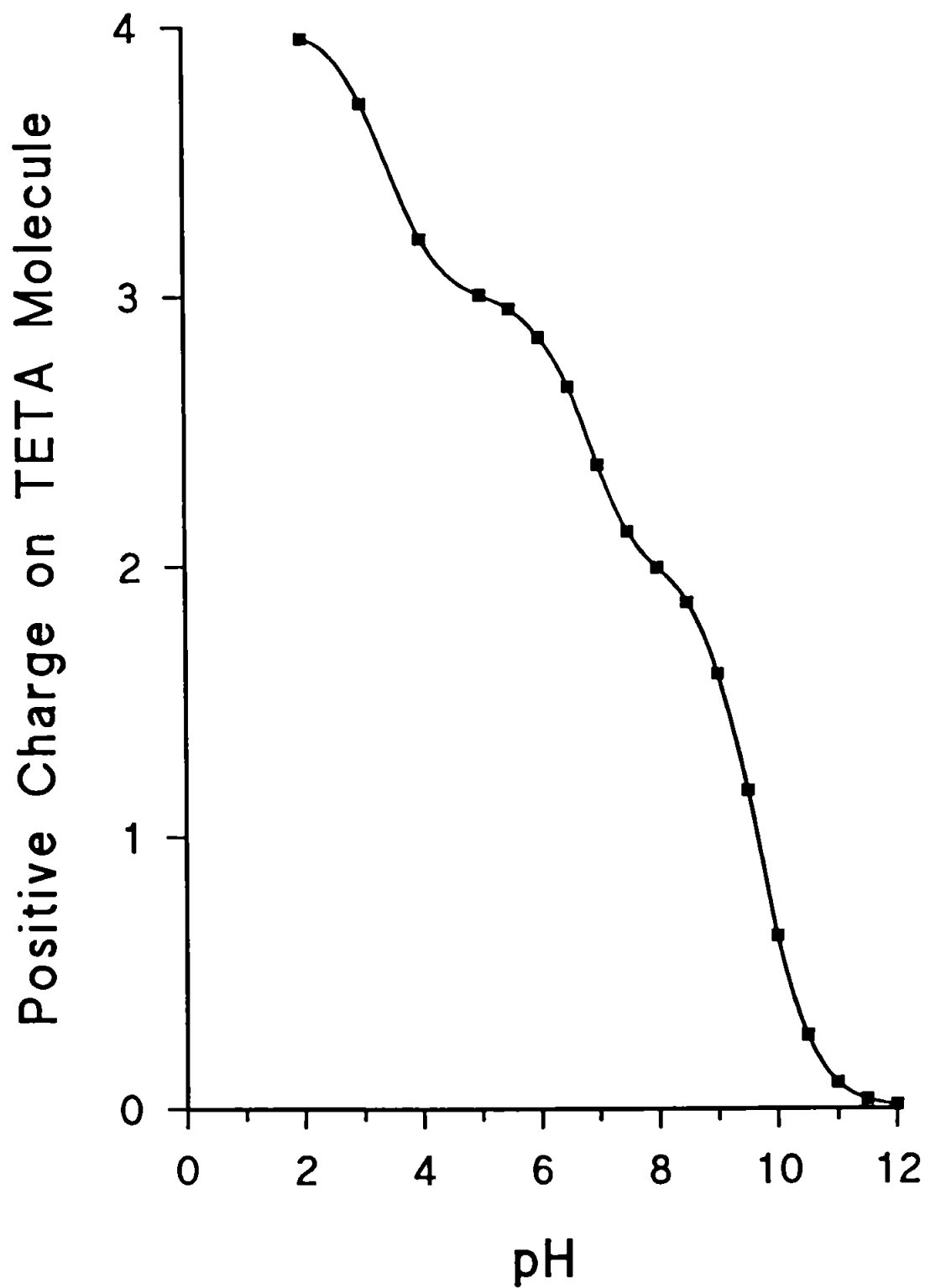
Figure 38   Average positive charge on the TETA molecule as a function of

pH.

Table 17   Effect of TETA concentration and pH on the elution of silver from a
            Dionex CG2 column.

TETA Concentration (M)	Eluent pH	Retention Time (min)
0.1	10	1.5
	9	2.0
	8	3.5
	7	2.75
	6	1.75
	4	1.00
	2	0.75
0.01	10	6
	8	22.5
	6	7
	4	1.5
	2	0.75
0.001	10	29
	4	2
	2	0.75
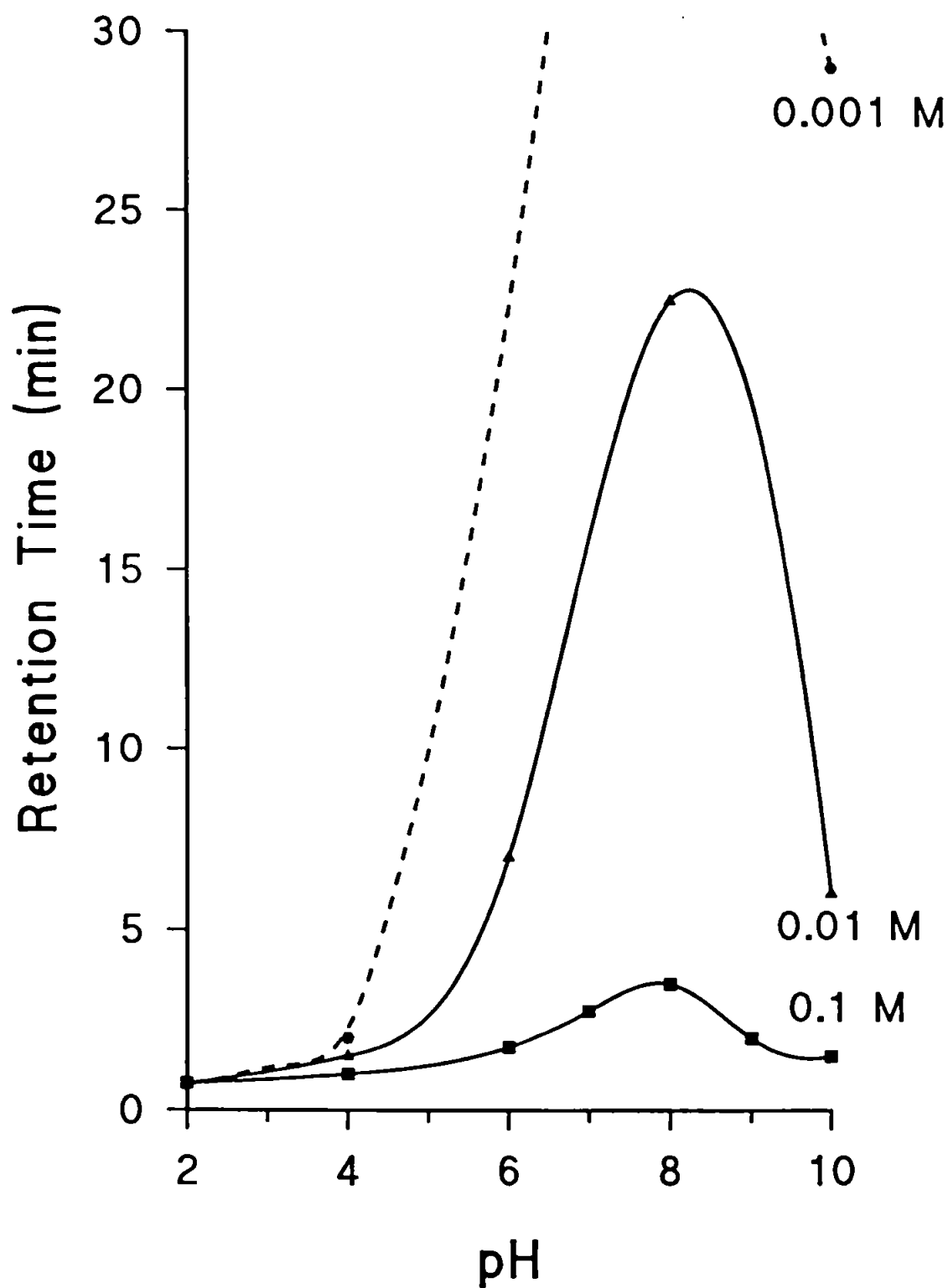
Figure 39   Effect of pH on the retention of silver using TETA eluents.
Concentration of TETA either 0.1 M, 0.01 M or 0.001 M.
Dashed line (---) shows the predicted elution behaviour of silver
at 0.001 M TETA.

concentrations. It can be seen that the retention of silver increases as the pH decreases from pH 10 to about pH 8, but after this the retention time decreases with decreasing pH and this effect is most apparent with lower TETA concentrations.

The effect of pH can be explained by the successive protonation of the TETA molecule with a decrease in pH, the subsequent increase in overall charge on the molecule and the affect this has on the selectivity coefficient. The overall charge on TETA is the average charge of all the different TETA species that are present at any one pH. Thus as the pH decreases the resultant change in TETA speciation leads to an increase in the overall charge on the TETA molecule from essentially zero at high pHs to essentially +4 at low pHs. The formation of silver hydroxide is significant only when the pH is greater than 10 and so can be ignored (117).

Silver forms a complex with TETA, and so with a TETA eluent silver (I) is not the eluted species but a silver (I)-TETA complex. This complex will carry a single positive charge from silver (I) in addition to any charge due to the protonation of TETA. However, as silver occupies two nitrogen sites the maximum charge that this complex can attain is +3. Thus, initially at higher pHs the silver (I)-TETA complex will have a greater charge than the TETA molecule, therefore a greater selectivity coefficient. The increase in overall charge on the silver-TETA complex and the TETA molecule with decreasing pH will reach a point where the retention of the silver-TETA complex is greatest, this will be a balance between the selectivity coefficients which is related to the overall charge on the complex or molecule and their relative concentrations. Once this maximum retention time has been reached, further decrease in pH will lead to an increase in the overall charge of the TETA molecule but not the

silver (I)-TETA complex, as it is at pH 8.0 that the maximum concentration of H_2TETA^{2+} is reached. The rather complex relationship between selectivity coefficients, retention and charge means that the maximum selectivity coefficient for the silver (I)-TETA complex is achieved at pH 8.0. Figure 39 shows that maximum retention of silver is obtained at pH 8.0.

4.5.7   The effect of TETA concentration.

The effect on the elution of silver was investigated over a wider range of TETA concentrations. The concentrations of TETA used were 0.1 M, 0.01 M, 0.001 M, 0.0001 M and 0.00001 M.

As shown in Figure 39 TETA concentrations between 0.01 M and 0.001 M showed a change in silver retention with a change in pH, with greater change in retention of silver being observed at lower TETA concentrations. 0.1 M and 0.01 M TETA both show the greatest retention of silver at pH 8, the predicted retention of silver with 0.001 M TETA eluent is shown by the dotted line. At the lowest pH (pH 2.0) the elution times were almost the same regardless of the TETA concentration. At pH 4.0 slight differences in retention is observed with different TETA concentrations, whilst at pH 8.0 silver eluted much more rapidly in 0.1 M TETA eluent than in 0.01 M TETA eluent. The two lowest concentrations used, 10^{-4} M and 10^{-5} M TETA, resulted in silver eluting as broad peaks, even at pH 2.

It was decided therefore to use 0.001 M TETA as the eluent, which happens to be the

optimum concentration of TETA for use with the detection system. The elution of

silver could be controlled by adjustment of the pH in 0.001 M TETA, whereas pH has

less effect on more concentrated TETA solutions.

4.5.8 Calibration of system.

Three Calibration curves were prepared using an eluent of 0.001 M TETA adjusted to

pH 2.2 with sulphuric acid. Separation was on a 5 cm Dionex CG2 guard column.

The luminol solution was as optimised. The calibration curves, Figures 40 to 42 are

highly non-linear at the lowest concentrations. This was considered to be in part due to

the high degree of tailing still present when using the Dionex CG2 polystyrene resin

based columns. Figure 36 shows the tail forms a larger proportion of the peak at the

lowest concentrations. The use of peak area should have offset the effect of tailing but

the proportion of the peak excluded from the measurement is likely to be greater at the

lowest concentrations. There is also a possibility that an interferent present in the

eluent may have prevented some of the silver from catalysing the CL reaction and this

would have a greater effect at the lowest concentrations. This possibility is discussed

more fully in Section 4.8.1.

4.5.9 Interferences.

As TETA is a very powerful cation exchanger at low pHs this could cause the divalent

cations that could be expected in PWR coolant water, to co-elute with silver. Increase
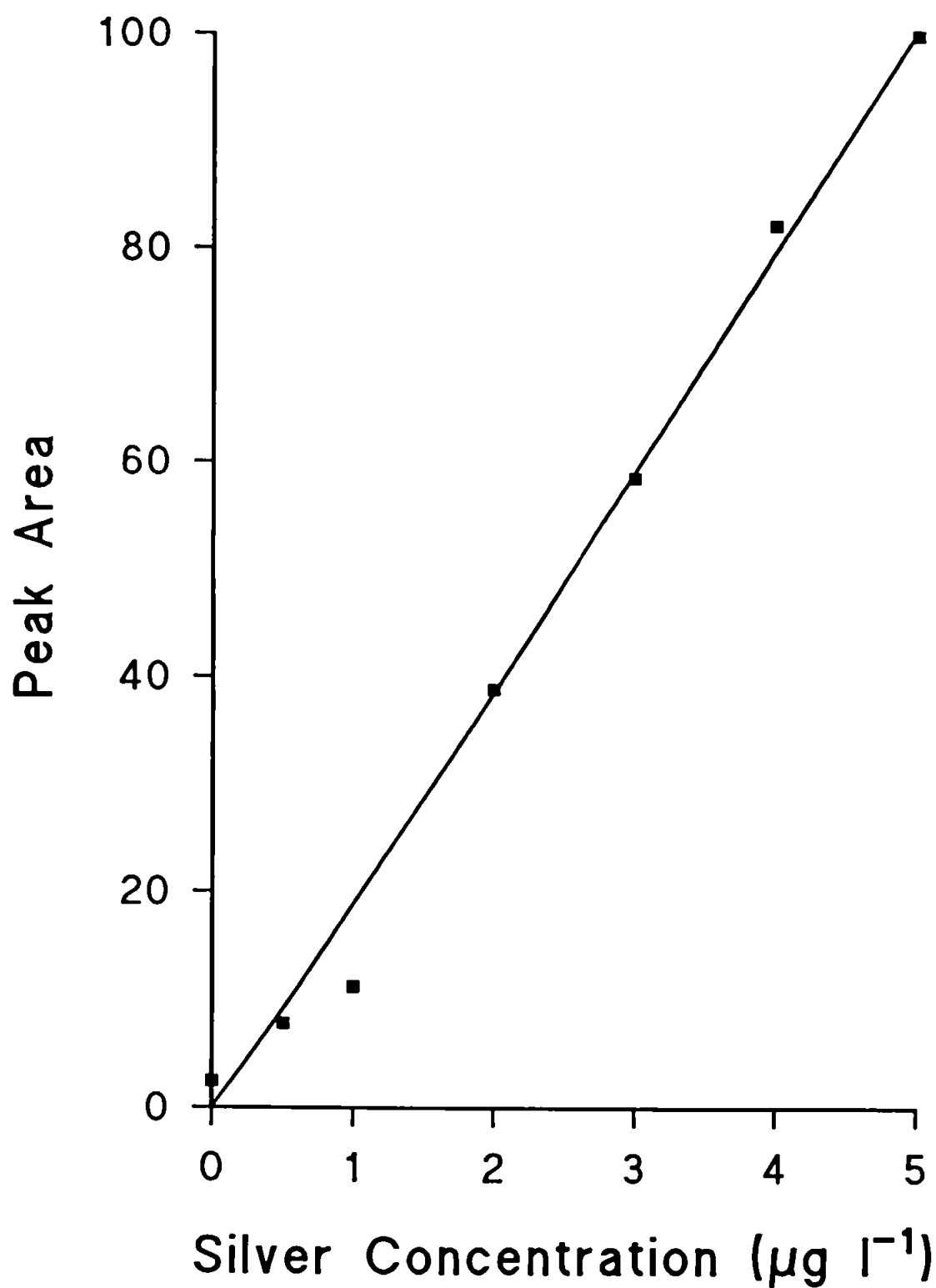
Figure 40   Calibration curve between 0.5 μg l^{-1} and 5 μg l^{-1} silver. 0.001 M

TETA, pH 2.25. Dionex CG2 column, 100 μl injection.
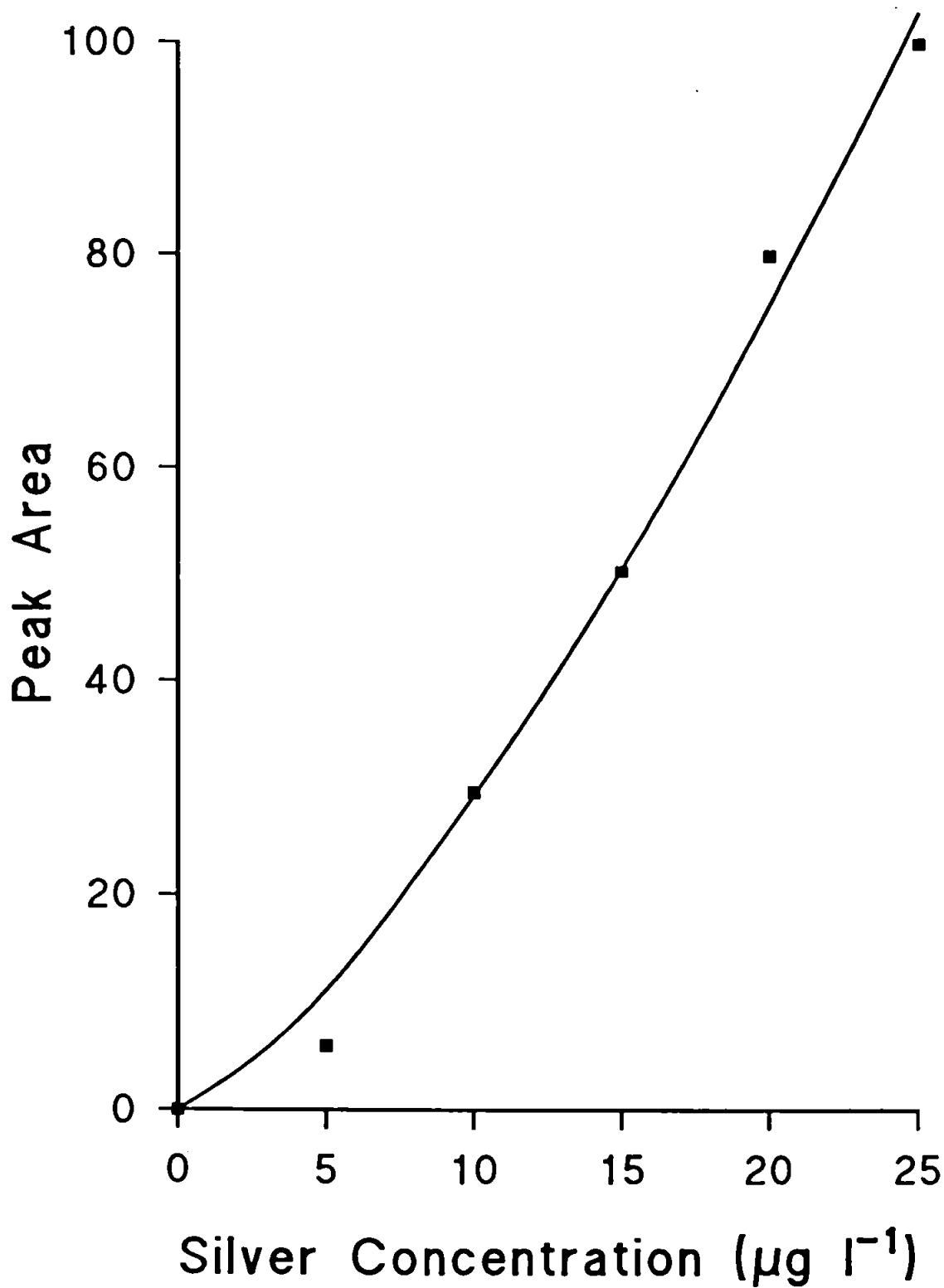
Figure 41   Calibration curve between 5 μg l⁻¹ and 25 μg l⁻¹ silver. 0.001 M

TETA, pH 2.25. Dionex CG2 column, 100 μl injection.
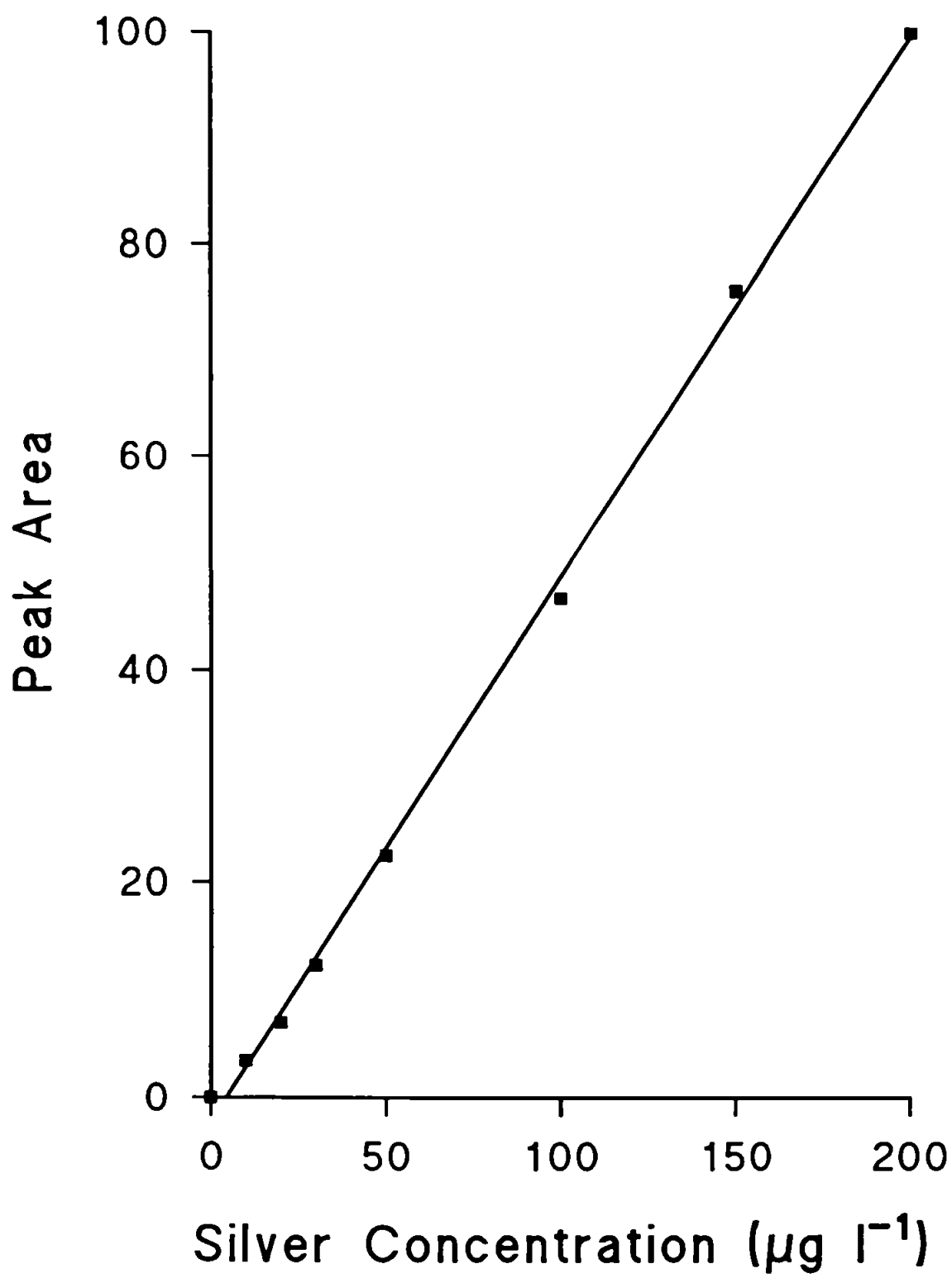
Figure 42   Calibration curve between 10 μg l^{-1} and 200 μg l^{-1} silver. 0.001

M TETA, pH 2.25. Dionex CG2 column, 100 μl injection.

in the CL signal was found with 100 μg l^{-1} nickel (II), cobalt (II) and iron (III). On the other hand 100 μg l^{-1} manganese (II) caused serious signal suppression.

4.5.10   Conclusion after initial experiments.

CL detection coupled to IC has again shown that it is possible to achieve very low detection limits. However, the detection of silver is still not at the level required to determine silver in PWR coolant water. Also, the use of TETA as an eluent has serious limitations due to the very strong cation exchange capability and the concurrent lack of separation of silver from other cations. The use of ethylenediamine, a similar polyamine (containing only two amine moieties and consequently a maximum charge of +2) was investigated for use in the eluent at various concentrations, as a possible replacement for TETA. It also showed a lack of separation ability and was not such a good activator as TETA resulting in much higher detection limits.

4.6.1   The Way Forward.

Improved detection limits could be achieved in two ways using IC. One, was to take advantage of the strong affinity of silver to polystyrene columns by preconcentration of silver on the column. Preconcentration of analytes in samples of low ionic strength is widely used in IC in order to determine analytes that are present in samples that would otherwise be below the detection limits of the detection method (34, 57, 137). The second was to determine a separation procedure that both separated silver from divalent

cations and also eluted silver as sharp peaks. When injecting low levels of silver onto the column with either a potassium nitrate eluent or a TETA eluent much of the silver was contained in the significant peak tails. Developing a chromatographic system with much less peak tailing would inevitably lead to lower limits of detection. However, it would be necessary to find an ion exchange resin that has less affinity for silver than polystyrene, the polymethacrylate resins seemed to offer such a possibility. However, these resins are only recently appearing on the market and small particle size resins are expensive, although Biorad produce a reasonably priced 50μm resin.

Before moving to a new substrate it was decided to explore the possibility of preconcentration on a polystyrene divinyl-benzene based resin.

4.6.2 Preconcentration studies.

First row transition metals at trace levels in PWR coolant have been successfully preconcentrated onto cation exchange columns to enable the determination of cobalt (II) (34, 57, 137). It was envisaged that silver in PWR coolant could be similarly preconcentrated. In addition it was envisaged that as divalent cations would be preconcentrated along with silver and at this time there was no means of separating them chromatographically without severely affecting the detection limits, the divalent cations could be eluted prior to silver. Washing the column with an eluent that contained a ligand which preferentially complexed and removed the divalent cations would enable silver to be eluted later using a TETA eluent.

4.6.3   Initial preconcentration studies.

Initial preconcentration studies were carried out using Dionex columns that had been used in previous preliminary studies.

In the first preconcentration studies, silver in deionized water was preconcentrated on a Dionex CG2 column. The column was then washed and the silver eluted with a TETA eluent. However, the preconcentration of silver was found to be erratic. This was attributed to the use of TETA as the eluent. TETA in the eluent carries a +4 charge and therefore is strongly retained on the column, essentially converting the column to the TETA form. The selectivity coefficient of silver is expected to be considerably less than that of the TETA. Silver would not then displace TETA and therefore would not preconcentrate significantly on the column.

It was therefore necessary to introduce a prewash to remove TETA prior to the preconcentration of silver. Lithium is considered to be the weakest counter-cation on strong cation exchange resins and so would be the easiest cation for silver to displace. In addition, lithium added to the eluent as the hydroxide would increase the pH and cause the deprotonation of TETA. When the pH is high enough to cause the total deprotonation of TETA the column will have no attraction for it and TETA will be replaced by lithium. A solution containing 5×10^5 M lithium hydroxide was used as the prewash. Whilst the preconcentration of silver was improved by the addition of a prewash the preconcentration of less than 1 μg l^1 proved to be difficult. The deionized water used to prepare the reagents was later analyzed and the presence of iodine was determined to be about the 1 μg l^1 level. The solubility product of silver iodide, (log S

175

= -16.08), shows that even at the μg l^{-1} level the solubility product is likely to be exceeded resulting in formation of insoluble silver iodide. This is discussed in more detail later.

4.6.4   Post preconcentration wash.

Preconcentration of ions from very dilute solutions results in the preconcentration of both the analyte ion and interfering ions. As explained previously it is necessary to remove these ions from the column prior to the elution of silver by the strong TETA eluent. To do this the preconcentrate was washed with solutions containing chelating agents that have a significantly greater affinity for the interfering divalent cations than the silver ions. Various organic acids were tried including lactic acid, citric acid, salicylic acid and ethylenediaminetetraacetic acid (EDTA) as the disodium and dilithium salt.

4.6.5   Lactic acid.

The first wash solution to be investigated contained lactic acid. Lactic acid had been used previously to elute divalent cations from the Dionex columns. 0.1 M and 0.05 M lactic acid solutions were adjusted to pH 8 by addition of lithium hydroxide. These solutions were used as eluents for the direct injection of silver or divalent cations to see if elution of silver and the divalent cations was produced.

Nickel and manganese can be detected by the CL detection system as either an increase or a decrease in CL respectively. Although detection is not sensitive it allows the determination of whether typical divalent cations are likely to be eluted. 0.1 M lactic acid caused rapid elution of nickel, manganese and silver. 0.05 M lactic acid eluted manganese in a slightly longer time, however the response to nickel was not observed. Silver did not seem to be eluted by 0.05 M lactic acid and so this solution was investigated as a wash solution.

Unfortunately, as can be seen in Figure 43 significant amounts of silver were being preconcentrated from the lactic acid. Lengthening the time of the wash cycle simply led to even larger system blank response. Lactic acid is prepared industrially from plants (176), and as it is possible for plants to preconcentrate silver between 0.06 to 0.28 mg l^{-1} (177) it is quite likely lactic acid is contaminated. The concentration of silver in the lactic acid solution may not be great but was significantly greater than the concentration of silver likely to be present in a PWR sample and therefore would obscure any silver preconcentrated from a PWR sample.

Attempts to remove silver from the lactic acid solutions by passing the lactate solution through an ion exchange column of Dowex 50W-X8 (2 x 15 cm) proved unsuccessful.

4.6.6   Baseline change.

The use of TETA in the eluent resulted in a decrease in background CL when the TETA eluent was removed during preconcentration. To some extent this made it
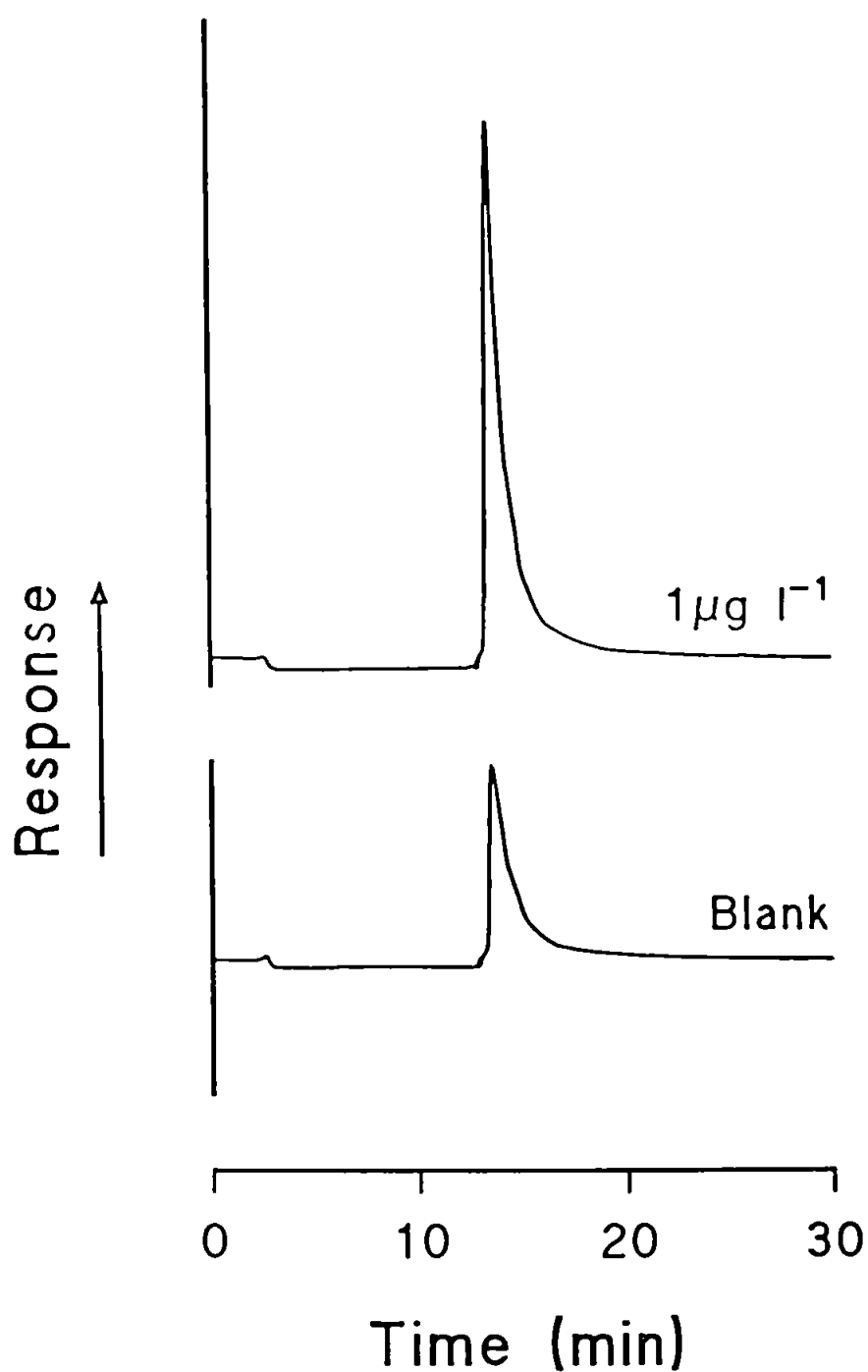
Figure 43   Chromatogram showing the preconcentration of silver on a

Dionex CG2 column for a system blank and 1 μg l^{-1} silver.

Lactic Acid Wash 10 min. Eluent 0.001 M TETA, pH 2.25.

difficult to determine whether silver was eluted by the wash reagents. The reintroduction of TETA in the eluent to elute silver resulted in an increase in the background signal. The introduction of a prewash to enable the preconcentration of silver to occur meant that the column was no longer in the TETA form and thus when the TETA eluent was again used, TETA would be retained initially to re-establish the equilibrium. Rapid elution of silver was required in order to prevent even worse tailing on the polystyrene divinyl-benzene column. This resulted in the elution of silver on a slope as the background CL increased as shown in Figure 44 and prevented the exploitation of silver preconcentration on the Dionex column. Although this avenue had to be abandoned a lot of information was gained on the elution characteristics of the silver ion.

It was apparent that TETA was not a suitable eluent and that if another eluent were to be used a column that showed significantly less affinity and non-specific interactions than the polystyrene divinyl benzene columns would be required. Although not reported anywhere hydrophilic polymethacrylate resin matrix columns were considered as a possible alternative for investigation as $p\pi$-$d\pi$ interactions would be considerably less than with polystyrene.

4.7   Hydrophilic Resins.

4.7.1   Polymethacrylate resins.

The polymethacrylate polymer is composed of methacrylic acid monomers
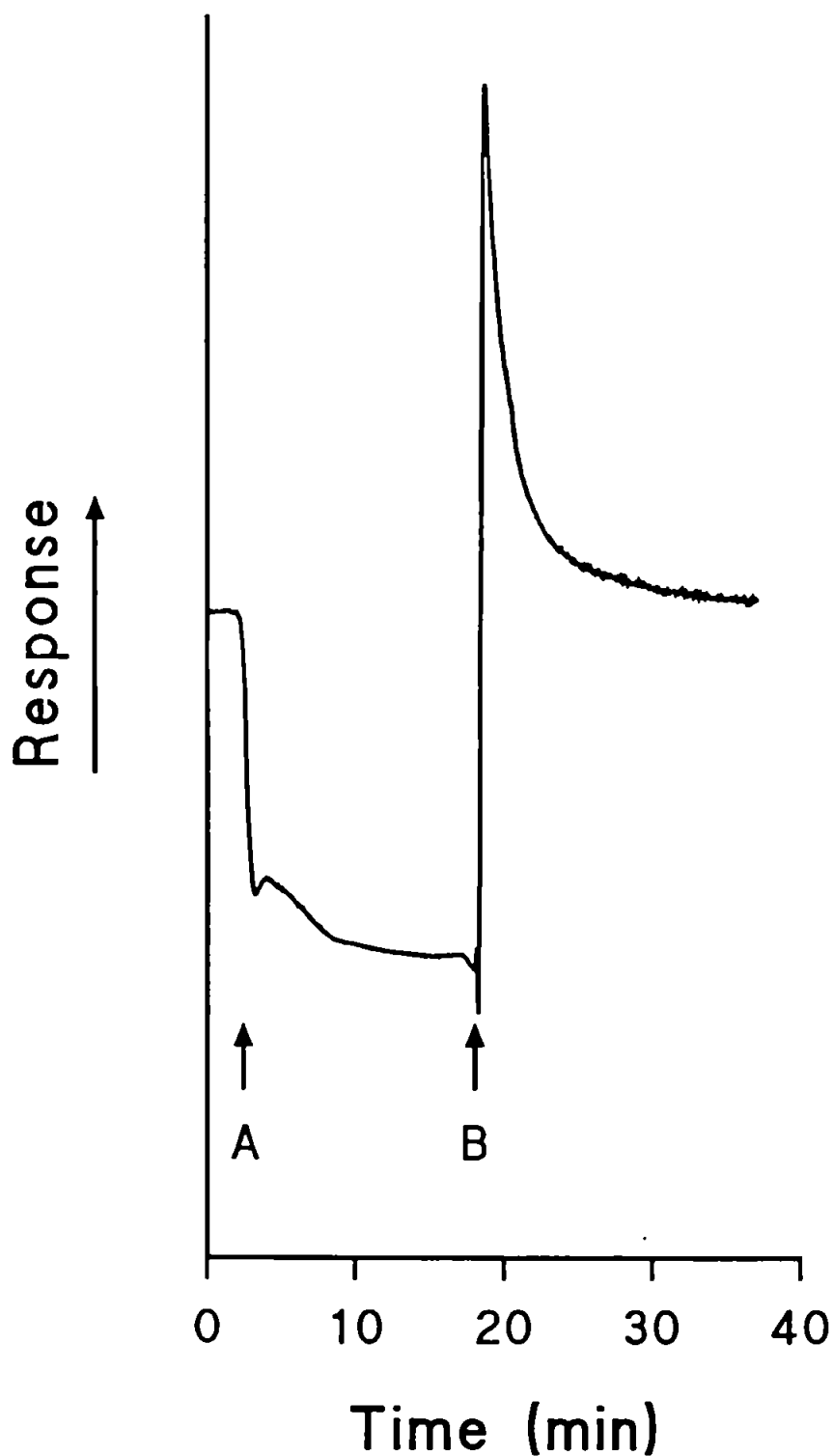
Figure 44   Chromatogram showing the change in baseline when
preconcentrating lactic acid for 15 mins at 1 ml min⁻¹. Point A
lactate wash solution reaches the detector with no TETA in the
Eluent. Point B shows the increase in baseline with TETA eluent
and the rapid elution of preconcentrated silver as the baseline
increases.

(2-methylpropenoic acid) ($CH_2=C(CH_3)COOH$). The polymer does not contain carbon-carbon double bonds, or therefore delocalized pi bonding systems such as the aromatic ring structures, found in polystyrene divinyl-benzene resin columns. These delocalised pi bonding systems have been given as the cause of non specific interactions with polarizable metals on such columns (16). Two types of chromatographic grade resin were available, one was Macroprep and the other HEMA-IEC. Macroprep resins were available with either strong or weak acid functionality as 50 μm particle size low capacity packing material. The capacity of the strong cation exchange material is 160 $\pm$ 40 μeq ml^{-1} and the weak cation exchange material 210 $\pm$ 40 μeq ml^{-1}. Each 5 cm column contained approx 0.26 ml packing material.

The cation exchange capacity of the weak cation exchange material can be controlled by pH. Thus both silver and divalent cations would co-elute as the pH is lowered. This would simplify the elution regime and could be used with the previous preconcentration procedure.

4.7.2   Macroprep CM weak cation exchange resin.

Functional groups on weak cation exchange resins are composed of carboxylic acid moieties. As the pH decreases the weak carboxylic acids become protonated releasing the cation. In order to dispense with the TETA eluent the use of a weak cation exchange resin may facilitate the preconcentration of silver, followed by the removal of divalent cations by the use of complexing agents and then the elution of silver by a drop in pH.

The carboxylic acid moieties will tend to buffer pH changes of the eluent. It was therefore necessary to prepare the column prior to preconcentration to ensure that the pH was greater than the pK_a of the carboxylic acid moiety. Citric acid can be used to alter the pH of the column to pH 6 prior to preconcentration. However, citric acid, like lactic acid gave very large blank values and could not be used to adjust the pH. Unless the pH of the column is increased by a buffer, large volumes of unbuffered eluent will need to be passed before the pH of the column is significantly altered. Adjustment of a solution of silver to pH 9 by the addition of lithium hydroxide resulted in limited retention of silver after 5 or 10 minutes preconcentration with significantly greater retention after 15 minutes preconcentration. Table 18 shows the slow change in pH as the silver solution is passed through the column. The inability to rapidly increase the pH of the column limited the use of the weak cation exchange column. Also, the use of 0.01 M sulphuric acid eluent used to obtain a rapid drop in pH required that the CL solution was quite strongly buffered. Therefore, as the eluent is introduced there is a great change in the baseline. This is a similar but reversed effect observed when using TETA as the eluent with the Dionex CG2 column.

4.7.3   Macroprep 50S strong cation exchange resin.

With the introduction of a strong cation exchange resin based on polymethacrylate it was thought possible to use dilute salt solutions to elute silver rather than the powerful TETA eluent. A comparison on the elution of silver using different 0.01 M salt solutions and sulphuric acid was made. The solutions were prepared by neutralizing 0.01 M sulphuric acid with the relevant hydroxide and then adding sulphuric acid to

Table 18   Change in pH with time of the column effluent as the silver solution
            adjusted to pH 9 with lithium hydroxide is preconcentrated on a
            Macroprep CM weak cation exchange column for 30 minutes. 0.01 M
            sulphuric acid eluent is introduced at 30 minutes.

Aliquot	Time Period (minutes)	pH
1	0-2	2.12
2	2-4	2.36
3	4-6	3.67
4	6-8	4.45
5	8-10	4.87
6	10-12	4.43
7	12-14	4.60
8	14-16	4.77
9	16-18	4.84
10	18-20	5.04
11	20-22	4.84
12	22-24	4.86
13	24-26	4.97
14	26-28	5.03
15	28-30	5.11
16	30-32	5.00
17	32-34	2.46
18	34-36	2.07
19	36-38	2.07
20	38-40	2.06

give a final acid concentration of either 0.01 M or 0.001 M sulphuric acid. In each

case the column was converted to the relevant form by passing 0.05 M salt solution

(0.1 M cation) through the column for 10 minutes. The 0.05 M salt solution was

prepared in the same way as the 0.01 M solution. The results in Table 19 show that

the potassium ion elutes the silver most rapidly. Acid was required in the eluent in

order to keep divalent cations in solution. The elution of silver was investigated with

0.01 M potassium sulphate eluent with varying concentrations of sulphuric acid present.

Table 20 shows the effect different concentrations of sulphuric acid have on the

retention of silver and the peak height. The optimum concentration of sulphuric acid in

the eluent was 5×10^{-4} M sulphuric acid. Although 0.01 M potassium sulphate was

used in the optimisation of sulphuric acid, 0.05M potassium sulphate gave sharper

silver peaks and was used subsequently.

4.7.4 Retention of silver and divalent cations on the Macroprep 50S column.

The retention of silver was investigated using CL detection whilst the behaviour of

divalent cations was monitored using Calmagite inverse spectrophotometry. Details of

the principle of the Calmagite detection system are given in the introduction, Section

1.7.3.3.

The retention of silver on the Macroprep 50S column was less than the divalent

cations, 0.02 M potassium sulphate did not elute divalent cations but eluted silver in 5

minutes. 0.05 M potassium sulphate caused elution of silver after 3 min and the

divalent cations at 12 minutes. In view of the long retention times of the divalent

Table 19   Retention times of silver on a Macroprep 50S strong cation exchange

column with different cation eluents. *[1] very small peak.

Eluent	Concentration of Sulphuric Acid in Eluent (M)	Retention Time (minutes)
0.01 M Sulphuric Acid		20.5
0.02 M Sulphuric Acid		9
0.01 M Lithium Sulphate	0.01	10
	0.001	19
0.01 M Sodium Sulphate	0.01	9
	0.001	15
0.01 M Potassium Sulphate	0.01	7.5
	0.001	12
0.01 M Ammonium Sulphate*[1]	0.001	12.5

Table 20   Effect on the retention, peak height and peak width of silver with

increasing concentration of sulphuric acid in the eluent.

Concentration of Sulphuric Acid Added to 0.01 M Potassium Sulphate	Retention Time (Minutes)	Peak Height (mm)	Peak Width (mm)
0.0001 M	18.5	4	Very Broad
0.0005 M	13.5	14	12
0.001 M	12.5	9	6
0.005 M	8.75	8	5
0.01 M	7	33	3.5

cations, up to 64 minutes for manganese with 0.03 M potassium sulphate eluent, it was decided that in order to decrease the analysis times that these cations should be washed rapidly from the column after silver elution.

4.7.5a   Detection with complexing wash solutions as eluent.

The ability of the CL detection system to detect silver or the Calmagite system to detect other cations was prevented by the complexing agents in the wash solution. Both detection systems require that the cations complex with specific agents and are not complexed prior to this. Therefore, it was not possible to determine the elution of the cations in the wash solution directly but only whether the cations had been retained on the column for the period the wash solution had been used. This was achieved by switching the eluent back on line after a set time, to determine whether elution of the metals had occurred.

4.7.5b   EDTA wash reagents.

A wash reagent containing 0.0005 M EDTA dilithium salt was prepared by adding the appropriate amount of EDTA acid form to deionized water and then adding lithium hydroxide until the EDTA was dissolved to pH 6.5.

The elution of silver by an EDTA solution was investigated by injecting with the EDTA solution as eluent, then after 5 or 10 minutes changing the eluent back to

potassium sulphate (0.05 M) the elution of any remaining silver could then be determined. It was found that 0.0005 M EDTA did not cause the elution of silver after 10 minutes.

The effect of EDTA on the divalent cations was investigated in a similar manner. 10 mg l^{-1} divalent cation was injected onto the column with 0.0005 M EDTA solution as the eluent. After a set time the eluent was changed to 0.05 M potassium sulphate, this would elute any cation remaining on the column which could then be determined. 0.005 M EDTA eluted copper and manganese in less than 5 minutes.

4.7.6   Preconcentration of silver on the Macroprep 50S resin.

Silver solutions containing 1, 5 and 10 μg l^{-1} silver were preconcentrated onto the column, washed with 0.0005 M EDTA solution for either 5 or 10 minutes and then eluted with 0.05 M potassium sulphate eluent. However, less than 1 μg l^{-1} silver proved to be difficult to preconcentrate.

<u>4.8.1   Interference by Iodide in the Eluent.</u>

There had been some concern over the purity of the deionized water and the appearance of a large peak at a longer retention time than silver. This came to light when low concentrations of TETA had been used to recheck the concentration of TETA required in the CL reagent. The second peak was thought to be iron (II), a sample of

the standard used and de-ionized water were analyzed semi-quantitatively by ICP-MS. The results showed that iron was present in the contaminated standard but also that about 1 μg l^{-1} iodine was present. The semi-quantitative data provided may be only accurate to an order of magnitude but it showed that iodide was highly likely to be present in significant amounts. It was also likely that iodine would be present as iodide, and if present at about 1 μg l^{-1} then injection of silver at the same or greater concentration would lead to the solubility product of silver iodide being exceeded (log S =-16.01). In such an event a similar concentration of silver would precipitate and so less than about 1 μg l^{-1} silver would be difficult to determine.

In order to determine the effect of iodide in the eluent, the eluent was split into two parts. To one part 1 μg l^{-1} iodide was added and no iodide was added to the other part. Standards containing 30 μg l^{-1}, 40 μg l^{-1} and 60 μg l^{-1} silver were injected into the iodide "free" eluent and the peak heights were determined. The same concentration of silver was injected into the eluent with the added iodide. It was found that the 30 μg l^{-1} and 40 μg l^{-1} peaks had almost disappeared and the 60 μg l^{-1} peak was greatly reduced, as shown in Figure 45.

The interference was further investigated using 0.1, 0.5, 1, 5, and 10 μg l^{-1} iodide in the eluent. As the iodide concentration increased so the response of silver injected decreased. For example, the response of 5 μg l^{-1} silver largely unaffected by 0.5 μg l^{-1} iodide in the eluent, showed some decrease in response at 1 μg l^{-1} iodide, but at the 10 μg l^{-1} iodide level it could not be detected.

It was also observed that when using an eluent containing 1 or 5 μg l^{-1} iodide the
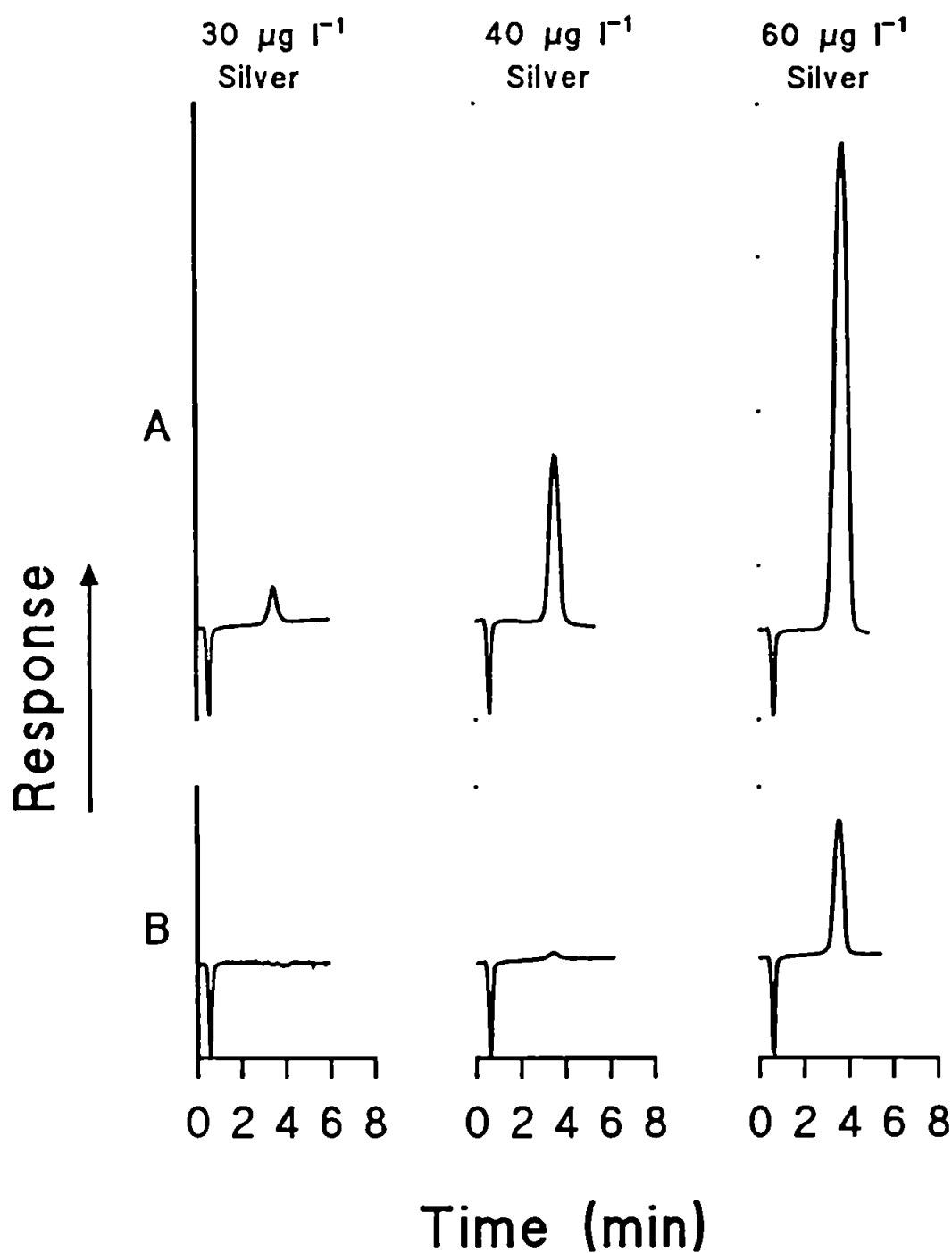
Figure 45   Effect of the addition of 1 μg l⁻¹ iodide to the eluent on the CL

response of 30 μg l⁻¹, 40 μg l⁻¹ and 60 μg l⁻¹ silver. 0.05 M

potassium sulphate, 5 x 10⁻⁴ M sulphuric acid eluent. 5 cm

column. Macroprep 50S Resin. Top set (A) no added iodide,

lower set (B) 1 μg l⁻¹ iodide added.

response to 20 μg l^{-1} silver increases with consecutive injections delivered in rapid succession, indicating that the iodide may be retained on the column.

4.8.2 The effect of chloride or bromide in the eluent.

Having found serious interference by iodide, it was important to check the other halides that form insoluble silver salts. Addition of increasing levels of chloride to the eluent resulted in both a decrease in peak area and retention time as shown in Table 21 and Figure 46.

The effect of bromide fell, as expected, between that of chloride and iodide, 1 μg l^{-1} bromide showing little effect on the response given by 20 μg l^{-1} silver but increasing the concentration of bromide in the eluent to 10 μg l^{-1} or 100 μg l^{-1} led to a decrease in response.

4.8.3 Addition of iodide, bromide and chloride to the Sample.

The effect of adding halide to the sample produced some rather surprising results. When iodide was added to a silver solution containing 20 μg l^{-1} silver the response was as expected. Thus, when 10 μg l^{-1} iodide was added the response decreased, and when 20 μg l^{-1} iodide was added the signal from the silver was almost completely suppressed.

The effect of adding either chloride or bromide did not necessarily cause suppression of

Table 21   Effect on the response and retention time of silver by the additon of

chloride to the eluent.

Concentration Chloride added (mg l^{-1})	Peak Area (Relative Units)	Change (%)	Retention Time (min)	Change (%)
0	311		3.85	
0.1	307	-1.5	3.78	-1.8
1.0	295	-5	3.64	-5.5
10	243	-22	3.45	-10
20	235	-24	3.18	-17
50	218	-30	2.49	-35
100	227	-27	1.75	-55
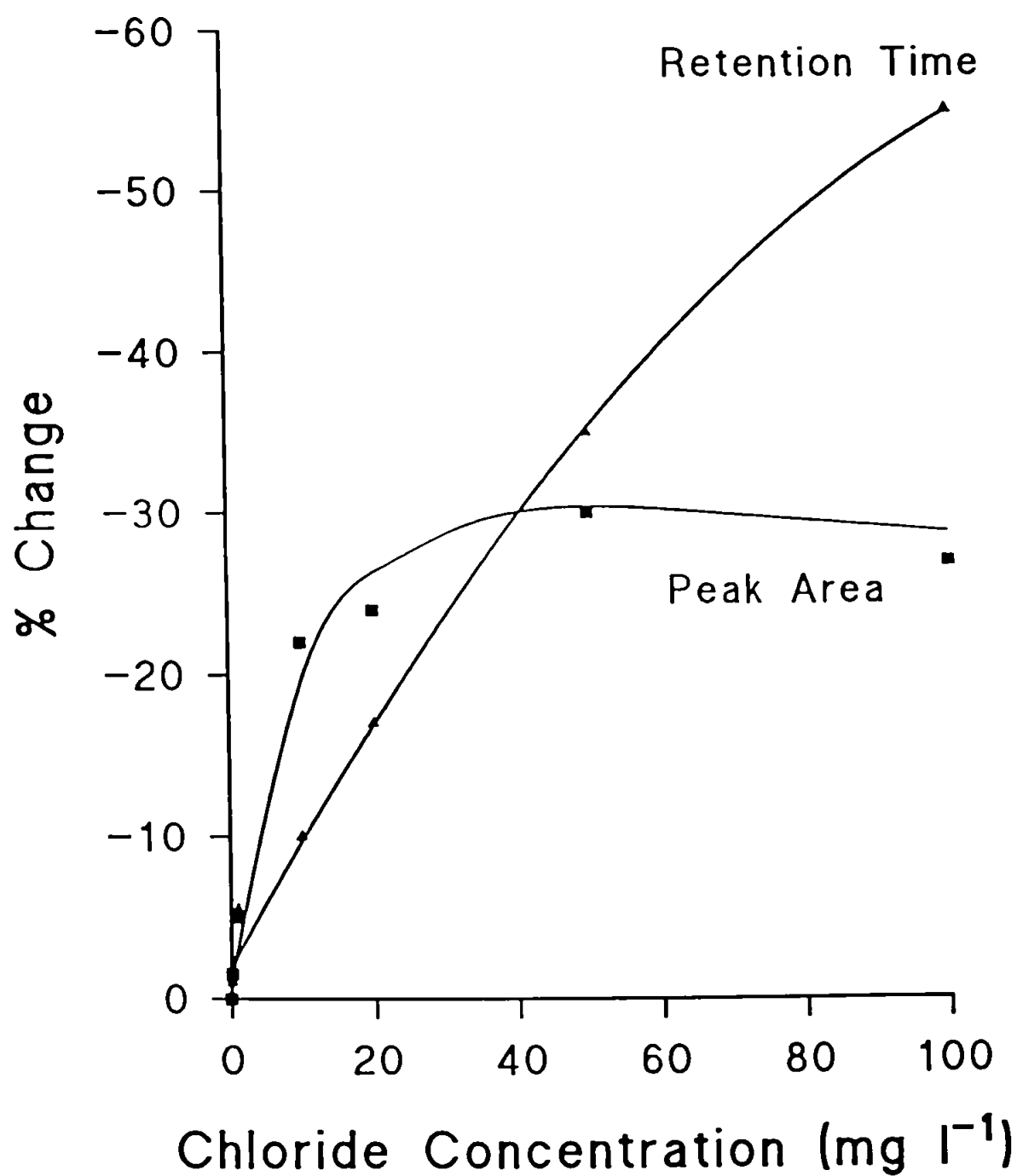
Figure 46   Effect of the addition of chloride to the eluent on the retention

time and peak height of silver.

the silver response and in some circumstances it led to signal enhancement. The

addition of chloride to the sample was investigated between 0.1 to 70 mg l^{-1}. It can be

seen from Table 22 that increasing addition of chloride up to 50 mg l^{-1} leads to

increasing enhancement of the response to silver but above this level the enhancement

starts to decrease.

Addition of bromide to the sample was also followed and showed a similar pattern but

at a lower concentration range, for example addition of 0.5 mg l^{-1} bromide led to

enhancement whilst addition of 1 mg l^{-1} bromide to the sample caused signal

suppression.

The enhancement shown by the addition of either bromide or chloride is thought to be

due to the formation of soluble ion pairs of silver-chloride/bromide which prevent the

formation of insoluble silver-iodide, thus increasing the silver that is present in the

detection system. It is not thought that the enhancement is caused by the removal of

iodide on the column by the larger concentrations of other halide present in the sample.

Injection of chloride onto the column directly prior to the injection of silver did not

result in the enhancement of the silver signal.

The different concentrations at which bromide and chloride show the enhancement is

thought to be due to the different solubility products of the respective silver halides.

The solubility products of iodide, bromide and chloride are given in Table 23, it can be

seen from the table that the solubility product increases substantially from iodide to

chloride. Enhancement of the silver response appears to increase by either chloride or .

bromide until the solubility product of that halide is exceeded. As observed this

Table 22   Effect on chloride added to the sample on silver response.

Concentration of Chloride Added to 20 μg l^{-1} Silver Sample (mg l^{-1})	Change in Signal (%)
0	
0.1	-3
0.5	+11
1.0	+12
10	+45
15	+59
25	+77
50	+77
70	+69

Table 23   Solubility products of silver chloride, bromide and iodide and the equivalent concentrations of silver and halide in μg l^{-1}, which would lead to the solubility product being exceeded.

Silver Halide	Solubility Product (log S)	Equivalent Molarity of Silver or Halide (log M)	Equivalent Concentration of Silver or Halide (μg l^{-1})	
			Silver	Halide
Chloride	-9.752	-4.876	1400	400
Bromide	-12.305	-6.153	70	60
Iodide	-16.08	-8.04	0.9	1.2

obviously occurs at lower concentrations for bromide than chloride.

It has been shown that iodide is a serious interferent in the system and is likely to be present in concentrations that are significant near the detection limit. It was therefore imperative that as much iodide as possible was removed from the system in order to detect silver at the lowest concentrations.

4.9   Addition of Potassium Persulphate to the Eluent.

It was thought that iodide could be removed by oxidation to iodine by potassium persulphate. A simple way of achieving this was by adding the potassium persulphate required by the CL detection system to the eluent instead of the CL reagent. The very low concentration of iodide present was unlikely to use up too much potassium persulphate and detrimentally effect the CL signal. Immediately potassium persulphate was added to the eluent the detection limit and the linearity of the system improved. Potassium persulphate in the presence of silver is a strong oxidising agent of organic compounds. Therefore, there was some concern regarding the stability of the column given that both silver and potassium persulphate would be present on the column at the same time. Potassium persulphate was added to the eluent whilst using the same column for the next month. No deterioration seemed to occur in column performance as a result of this addition.

4.10   Large Volume Injections.

Before the addition of potassium persulphate had been made it was decided to investigate the use of large volume sample as a means of increasing the sensitivity to silver. This would reduce the need for a complicated multi-step preconcentation system.

Two 1 ml sample loops were constructed from 0.7 mm i.d. PTFE tubing, these could be joined together if necessary to make a 2 ml sample loop. The filling of the 2 ml sample loop was problematic due to degassing of the sample which occurred as the sample was sucked up. The 1 ml sample loop was used without any degassing problem. The preliminary results were encouraging, the peaks that were obtained were narrow considering the volume of the injection indicating that the silver retained on the column was not eluted to any significant extent by the de-ionized water, or as to be seen later, simulated PWR coolant.

4.11.1   Qualitative Performance of the Optimized CL System.

Several advances had been made when using the Macroprep 50S strong cation exchange column. The discovery of iodine in the deionized water at sub μg l^{-1} levels and an understanding of the effect iodide could have on the silver response were important in explaining why low concentrations of silver did not preconcentrate. The addition of potassium persulphate to the eluent enabled the removal of iodide and the detection of .
lower concentrations of silver. The ability to separate silver from divalent cations on

this column enabled the use of large volume injections rather than a complete preconcentration system, simplifying the analysis. These advances led to the ability of the detection system to determine 0.1 μg l^{-1} silver in simulated PWR coolant.

The calibration curve shown in Figure 47 has a correlation coefficient (r) of 0.9995, the calibration shown in Figure 48 is slightly curved. The reproducibility of six repeat 1 ml injections of 5 and 0.5 μg l^{-1} silver in water was respectively 1.2% and 9.1% RSD. For 1 μg l^{-1} silver in simulated PWR coolant the reproducibility of six 1 ml injections was 2.6% RSD. Typical chromatograms for 10 μg l^{-1} and 1 μg l^{-1} silver on the Macroprep column with 0.05 M potassium sulphate and 5 x 10^{-4} M sulphuric acid eluent are shown in Figures 49 and 50.

4.11.2 Interferences by cations.

The possible interference of several divalent cations that are likely to be present in PWR coolant were investigated. Divalent cations were co-injected with 0.5 μg l^{-1} silver in separate 1 ml injections. 20 μg l^{-1} cobalt (II), nickel (II), manganese (II) and zinc (II) or 10 μg l^{-1} copper when co-injected did not cause interference with the silver signal although cobalt and manganese caused a decrease in the baseline on their elution, after silver. 100 μl and 1 ml injections of 100 μg l^{-1} iron (II) were made, the 1 ml injection of iron (II) was detected by the CL detection system and was off scale, a significant response was observed with the 100 μl injection. However, no interference with silver was observed.
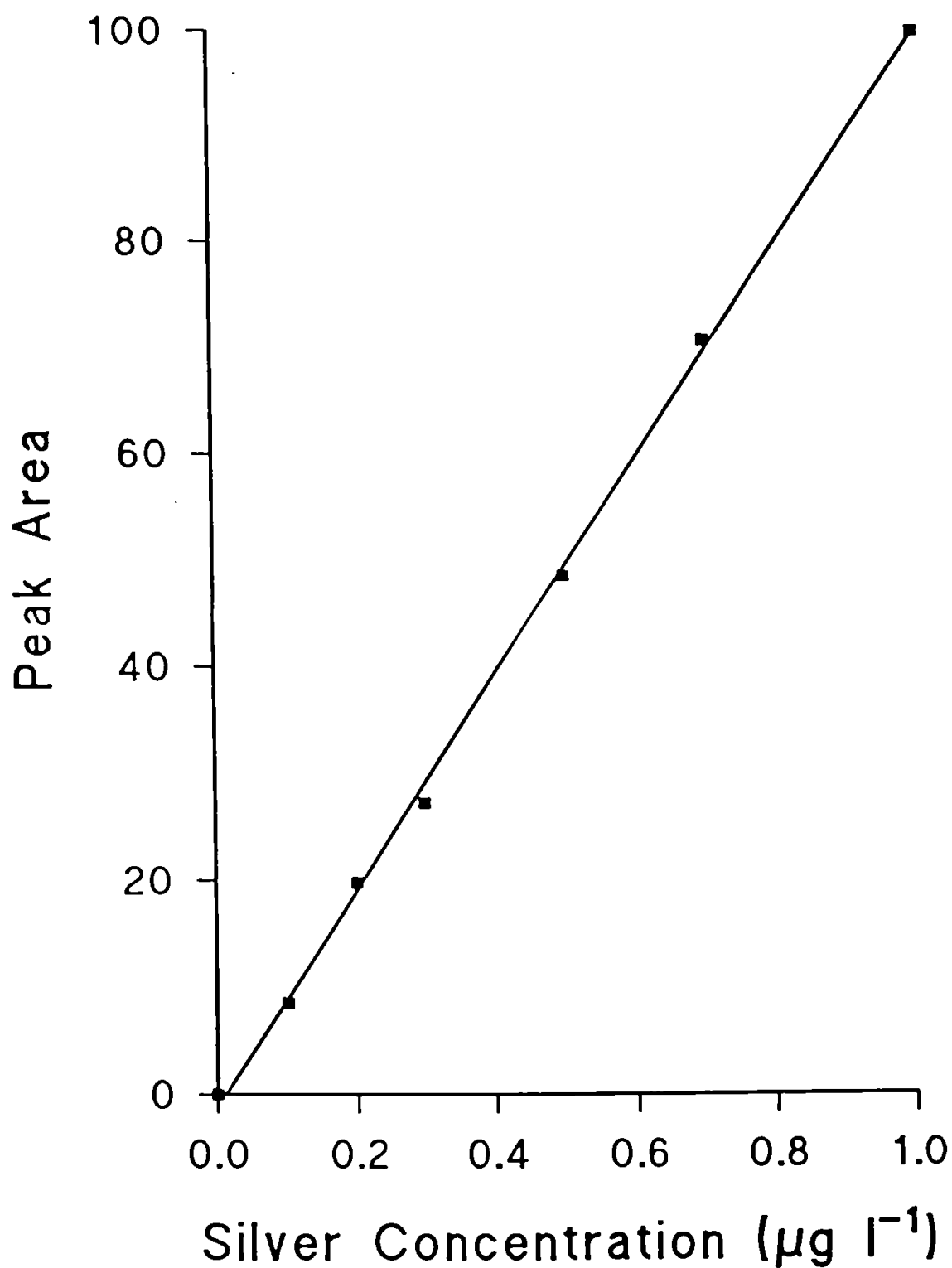
Figure 47   Calibration curve between 0.1 μg l^{-1} and 1 μg l^{-1} silver.

Macroprep 50S, 5 cm column. 0.047 M potassium sulphate,

0.003 M potassium persulphate and 5 x 10^{-4} M sulphuric acid

eluent. 1 ml injection.
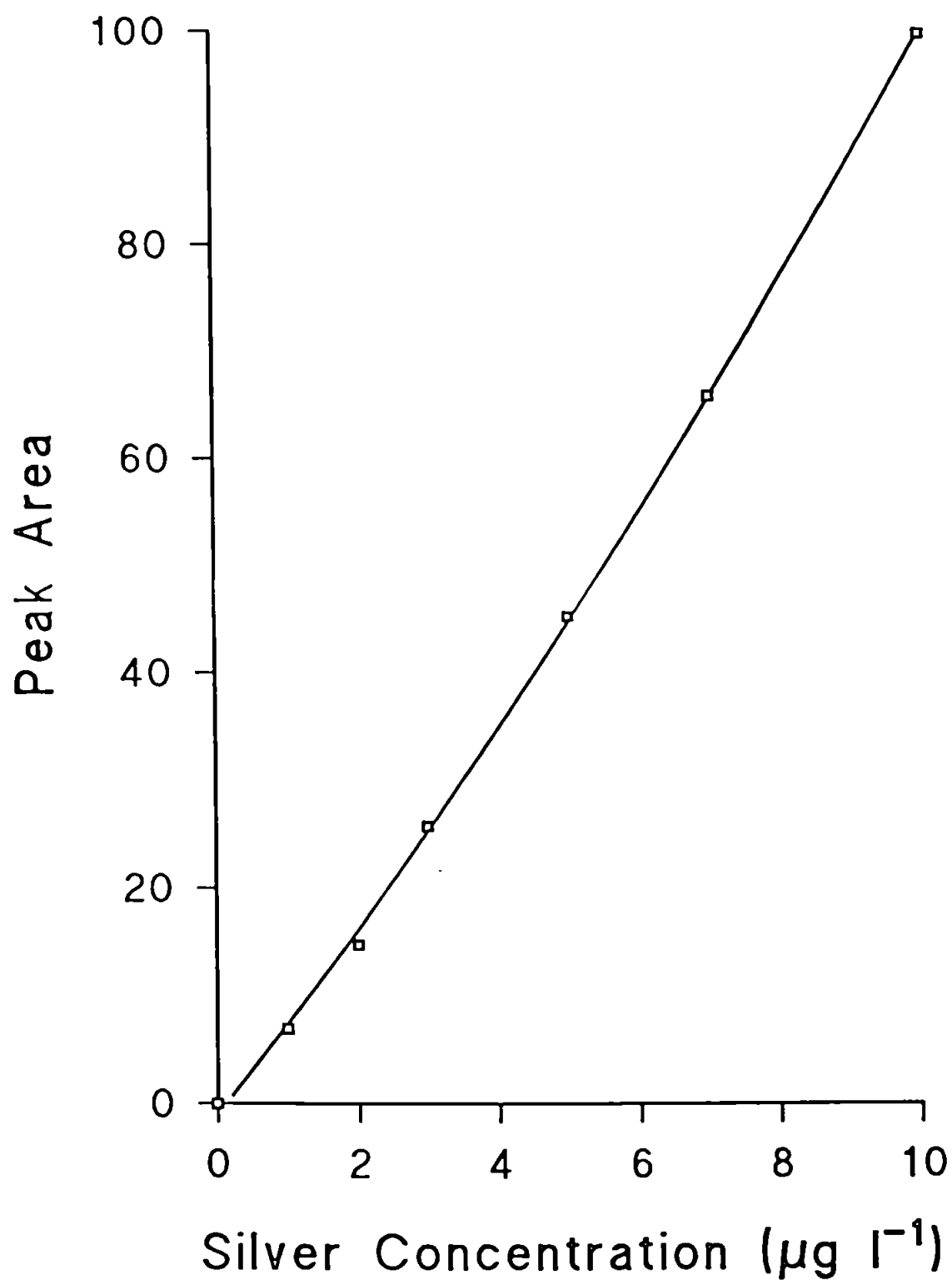
Figure 48   Calibration curve between 1 μg l⁻¹ and 10 μg l⁻¹ silver.

Macroprep 50S, 5 cm column. 0.047 M potassium sulphate,

0.003 M potassium persulphate and 5 x 10⁻⁴ M sulphuric acid

eluent. 100 μl injection.
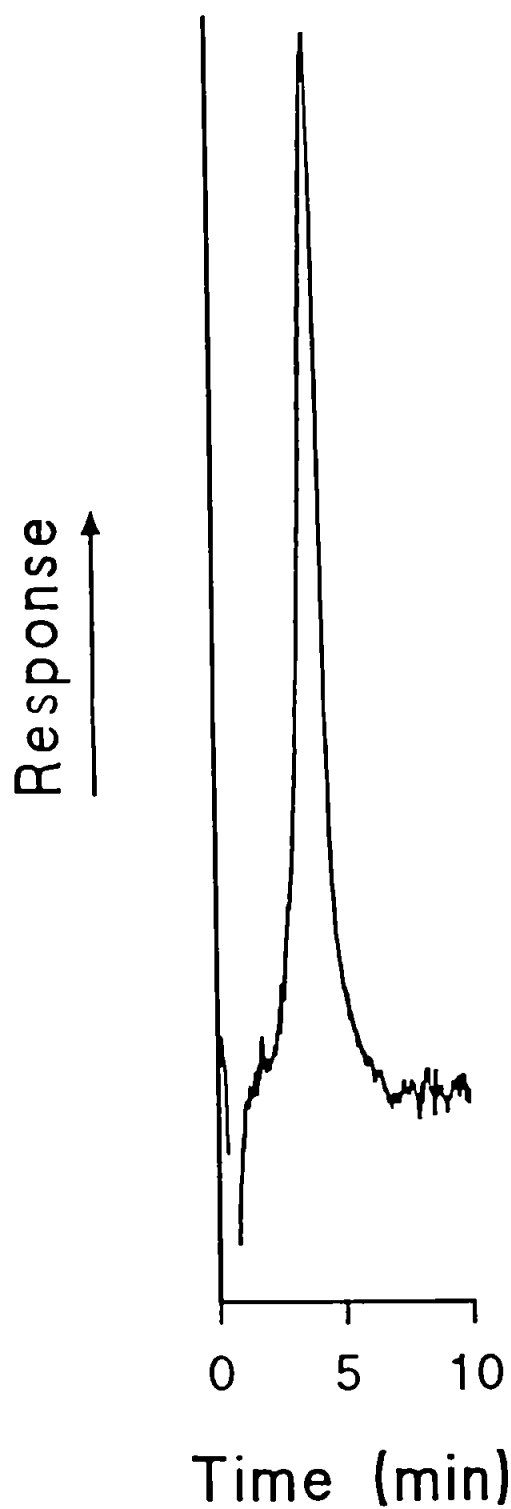
Figure 49   Typical chromatogram of 10 μg l^{-1} silver. Macroprep 50S, 5 cm

column. 0.047 M potassium sulphate, 0.003 M potassium

persulphate and 5 x 10^{-4} M sulphuric acid eluent. 100 μl
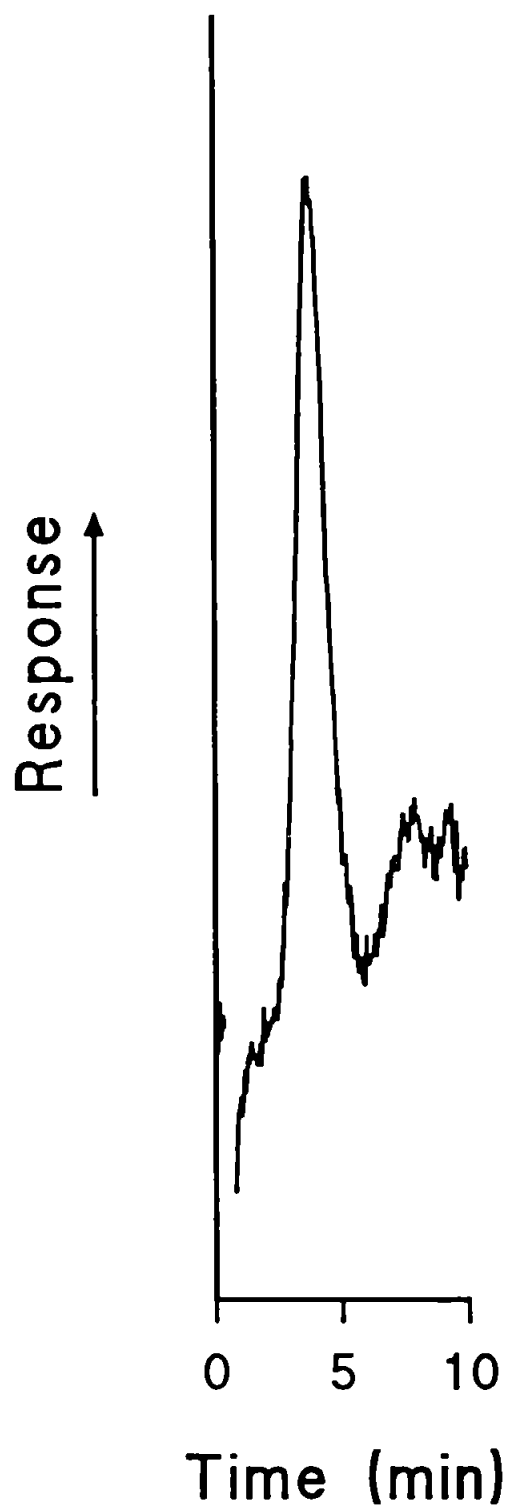
injection.

Figure 50   Typical chromatogram of 1 μg l^{-1} silver. Macroprep 50S, 5 cm
column. 0.047 M potassium sulphate, 0.003 M potassium
persulphate and 5 x 10^{-4} M sulphuric acid eluent. 100 μl
injection.

4.12.1   Investigation of HEMA-IEC High Efficiency Strong Cation Exchange Resin.

The Macroprep 50S strong cation exchange resin provided a cheap source of resin that could be used to study the elution of silver on a polymethacrylate resin. However, the particle size of 50 μm was too large to obtain the highest efficiency. Although silver was separated by a significant amount from divalent cations the use of a smaller particle size resin would give sharper peaks and therefore lower limits of detection. HEMA-IEC strong cation exchange resin is also a hydrophilic polymethacrylate based resin but with a particle size of 10 μm.

However, the higher capacity of the resin (1.2 - 1.6 meq g^{-1}) was expected to give problems as more concentrated eluents were likely to be needed. A 5 cm column was packed with HEMA-IEC and the retention of silver was indeed found to be great when using 0.05 M potassium sulphate eluent. Increasing the concentration to 0.1 M and then 0.2 M decreased the retention time significantly. The retention times for silver (I), copper (II) and nickel (II) are shown in Table 24. However, the peaks obtained with 0.2 M potassium sulphate eluent were smaller than expected, given the shorter retention time. It was therefore likely that the greater salt concentration was effecting the CL detection and so 0.1 M potassium sulphate was used as the eluent.

4.12.2   System performance with HEMA-IEC resin.

Figures 51 to 53 show the calibration of silver from 0.05 to 160 μg l^{-1} silver in

Table 24   Effect of potassium ion concentration on the elution of various metal ions

from a 5 cm HEMA-IEC column.

Metal Ion	Retention Time with 0.1 M Potassium Sulphate (min)	Retention Time with 0.2 M Potassium Sulphate (min)
Silver (I)	11.25	5.75
Copper (II)	39.25	10.25
Nickel (II)	37.25	
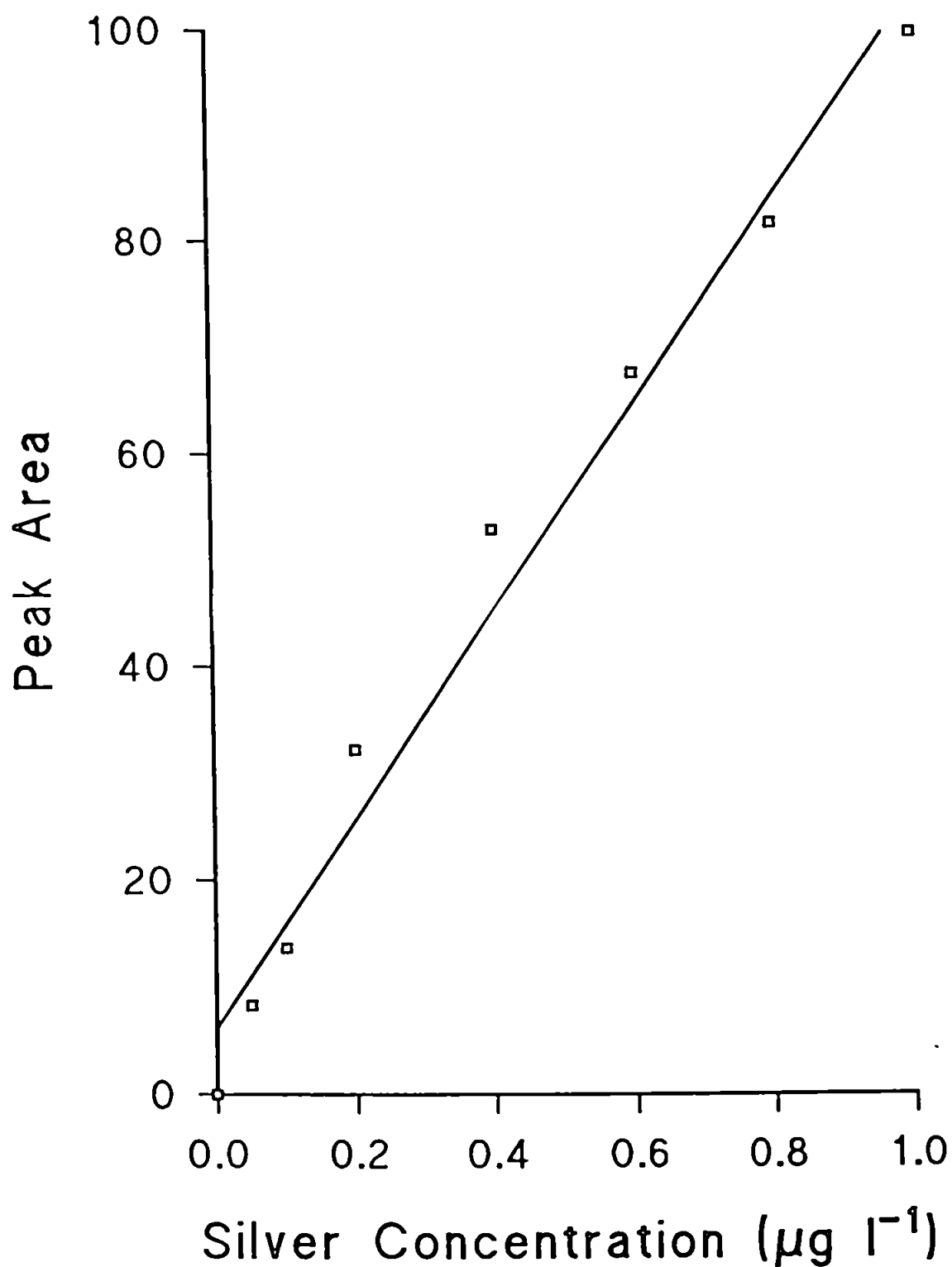
Figure 51   Calibration curve between 0.05 μg l^{-1} and 1 μg l^{-1} silver in

synthetic PWR coolant water.   HEMA-IEC SB, 5 cm column.

0.097 M potassium sulphate, 0.003 M potassium persulphate and

5 x 10^{-4} M sulphuric acid eluent.   1 ml injection.

Figure 52   Calibration curve between 1 μg l^{-1} and 10 μg l^{-1} silver in synthetic

PWR coolant water. HEMA-IEC SB, 5 cm column. 0.097 M

potassium sulphate, 0.003 M potassium persulphate and 5 x 10^{-4}

M sulphuric acid eluent. 100 μl injection.

Figure 53   Calibration curve between 10 μg l⁻¹ and 160 μg l⁻¹ silver in

synthetic PWR coolant water.   HEMA-IEC SB, 5 cm column.

0.097 M potassium sulphate, 0.003 M potassium persulphate and

5 x 10⁻⁴ M sulphuric acid eluent.   100 μl injection.

simulated PWR coolant. The lowest calibration curve, Figure 51, is linear with a correlation coefficient of 0.990 and was prepared using a 1 ml sample loop. The higher concentrations of silver required the small 100 μl sample loop, but gave curved calibrations and this may be due to the higher salt concentration of the eluent required with HEMA-IEC columns. It was shown in Section 4.5.3 that salt concentration has an effect on CL detection. The curved calibrations were thought to be due to the concentration of potassium sulphate used as eluent, which was higher with this column than with the Macroprep column. The use of a considerably shorter column with a more dilute eluent, using similar concentrations to the 0.05 M potassium sulphate used with the Macroprep column could possibly give more linear calibrations.

The reproducibility of six repeat injection was determined at 1 μg l^{-1} silver in simulated PWR coolant using 1 ml injections as 6.1% RSD and at 10 μg l^{-1} silver using 100 μl injections as 4.3% RSD. A typical chromatogram of 1 μg l^{-1} silver on the 5 cm HEMA-IEC column with 0.97 M potassium sulphate, 0.03 M potassium persulphate and 5 x 10^{-4} M sulphuric acid eluent is shown in Figure 54.

The divalent cations likely to be present in PWR coolant were co-injected with 1 μg l^{-1} silver using 1 ml injections. The divalent cations used were: 20 μg l^{-1} nickel (II), zinc (II), cobalt (II), copper (II), 5 μg l^{-1} manganese (II) and 100 μg l^{-1} iron (II). No effect on the silver response was observed by any of the co-injected cations. Nickel (II) gave a positive CL response at 38 min. Negative CL response were shown by cobalt (II) after 42 minutes, iron (II) and manganese (II) after 45 minutes, the response to manganese was much greater than that for iron (II). Copper and zinc did not show any CL response. These results are summarized in Table 25.

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Figure 54   Typical chromatogram of 1 μg l^{-1} silver in synthetic PWR coolant

water. HEMA-IEC SB, 5 cm column. 0.097 M potassium

sulphate, 0.003 M potassium persulphate and 5 x 10^{-4} M sulphuric
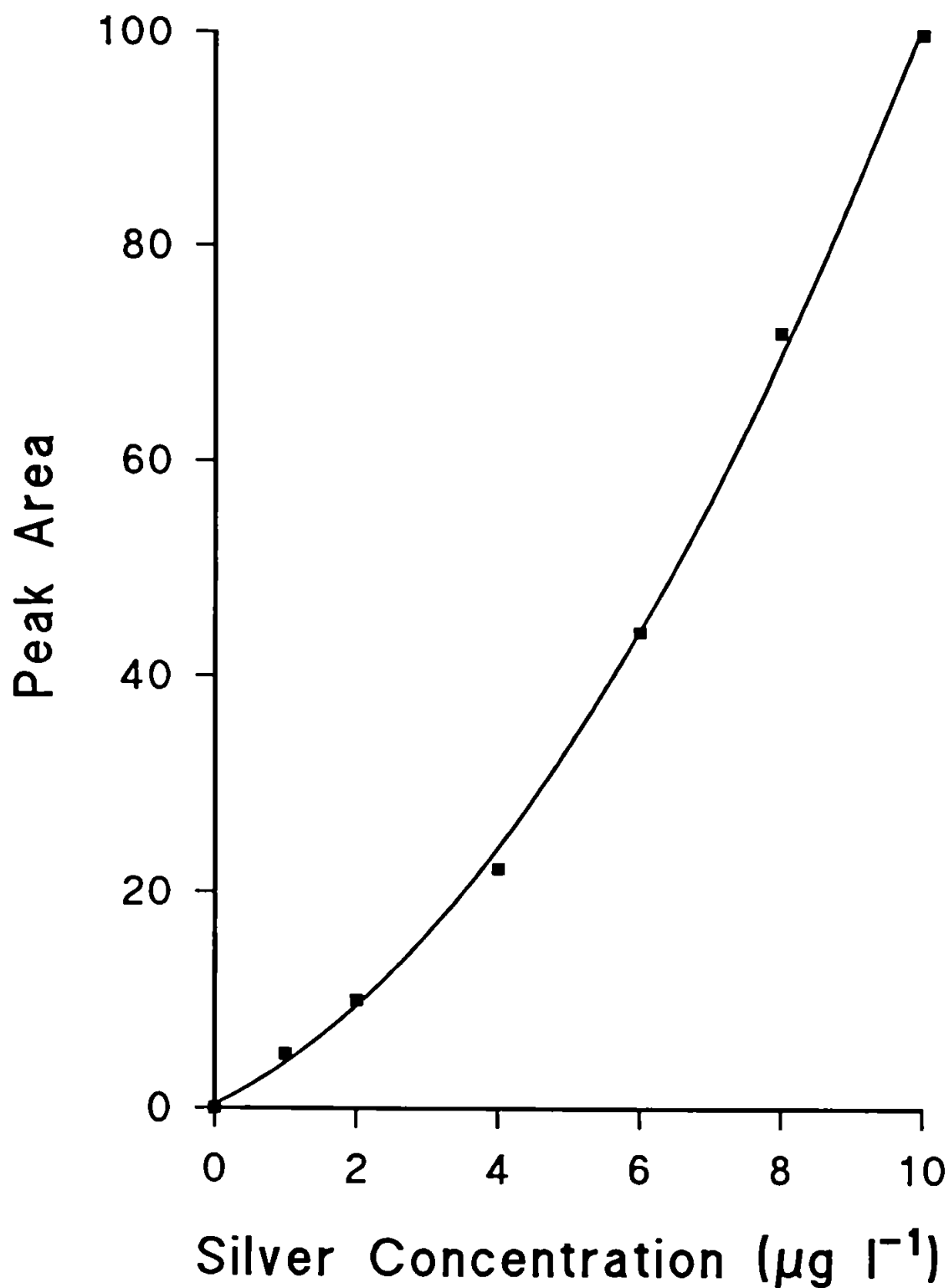
acid eluent. 1 ml injection.

Table 25   Response of the CL detection system to injections of various divalent

cations that may be present in PWR coolant. Co-injected with 1 ml of 1

μg l^{-1} silver. HEMA-IEC 5cm column.

Metal Ion and Concentration	Time of Maximium Response (min)	Response
Cobalt (II) 20 μg l^{-1}	42	Negative Peak
Nickel (II) 20 μg l^{-1}	38	Positive Peak
Zinc (II) 20 μg l^{-1}	/	No Response
Copper (II) 20 μg l^{-1}	/	No Response
Iron (II) 100 μg l^{-1}	45	Negative Peak
Manganese (II) 5 μg l^{-1}	45	Large Negative Peak

4.12.3   Shorter column length.

The use of 0.1 M potassium sulphate seemed to be tolerated by the CL detection system although the 0.2 M potassium sulphate eluent was not. The retention time of silver (I) was too great for routine analysis when using the 5 cm column given the need for also eluting the divalent cations at around 40 minutes. The large difference in retention times for silver, the divalent cations and the solvent front meant that it was possible to reduce the length of the column without risking the co-elution of silver with either the solvent front or the divalent cations. Reducing the column length meant that silver would be eluted in a shorter time, increasing the sharpness of the peaks and thus decreasing the detection limits. Divalent cations would also elute in a shorter time so decreasing the overall analysis time.

The length of the shorter column was limited by the length of the end caps, the minimum length that could be prepared was 2.5 cm. A 2.5 cm column was therefore used with a 0.1 M potassium sulphate eluent and gave elution of silver in the same time as achieved with 0.2 M potassium sulphate eluent with a 5 cm column. As expected this gave a lower detection limit of 0.025 μg l^{-1}. Typical chromatograms of 0.05 μg l^{-1} silver in synthetic PWR coolant, 1 ml injection and 5 μg l^{-1} silver in synthetic PWR coolant, 100 μl injection, 0.097 M potassium sulphate, 0.003 M potassium persulphate and 5 x 10^{-4} M sulphuric acid eluent on the 2.5 cm HEMA-IEC column are shown in Figures 55 and 56.
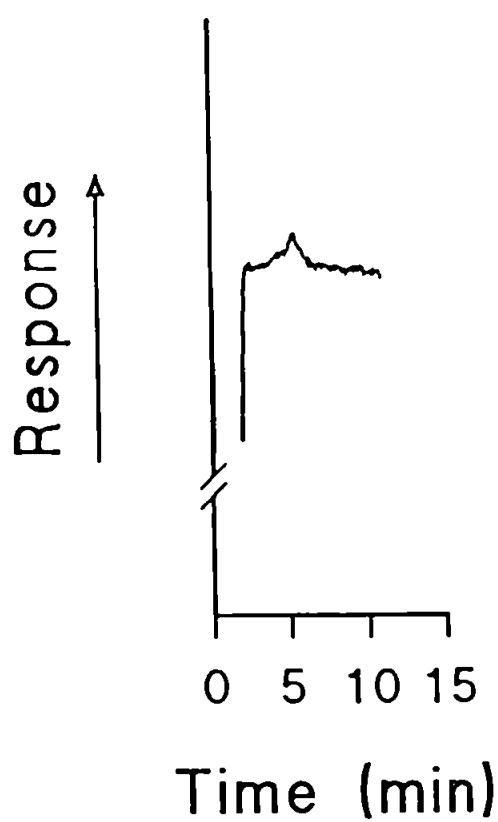
Figure 55   Typical chromatogram of 0.05 μg l⁻¹ silver in synthetic PWR

coolant water. HEMA-IEC SB, 2.5 cm column. 0.097 M

potassium sulphate, 0.003 M potassium persulphate and 5 x 10⁻⁴
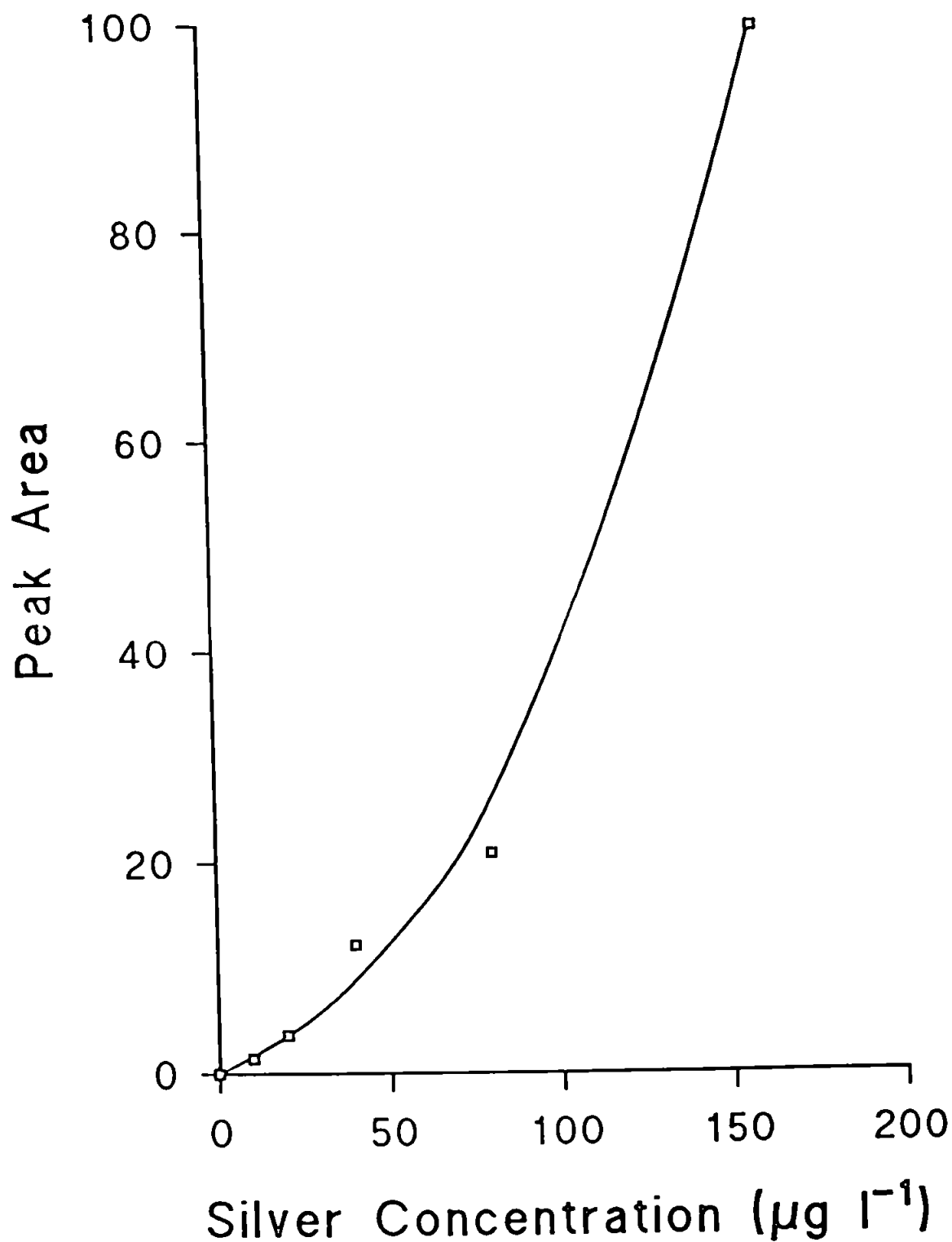
M sulphuric acid eluent. 1 ml injection.
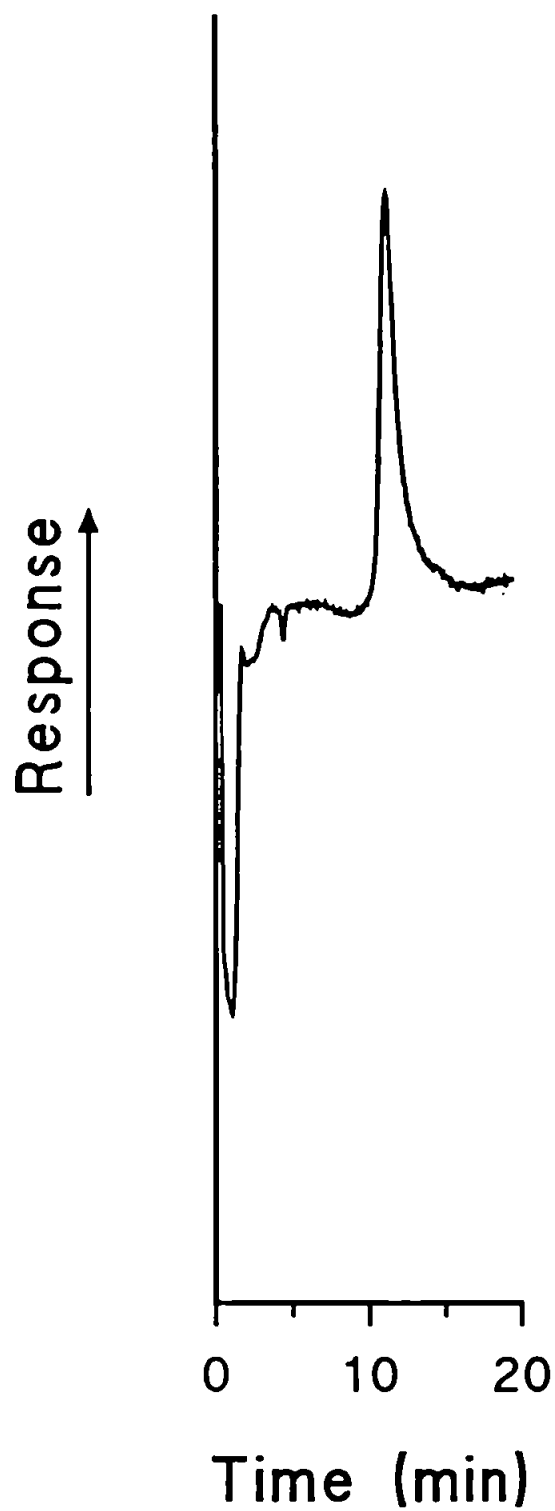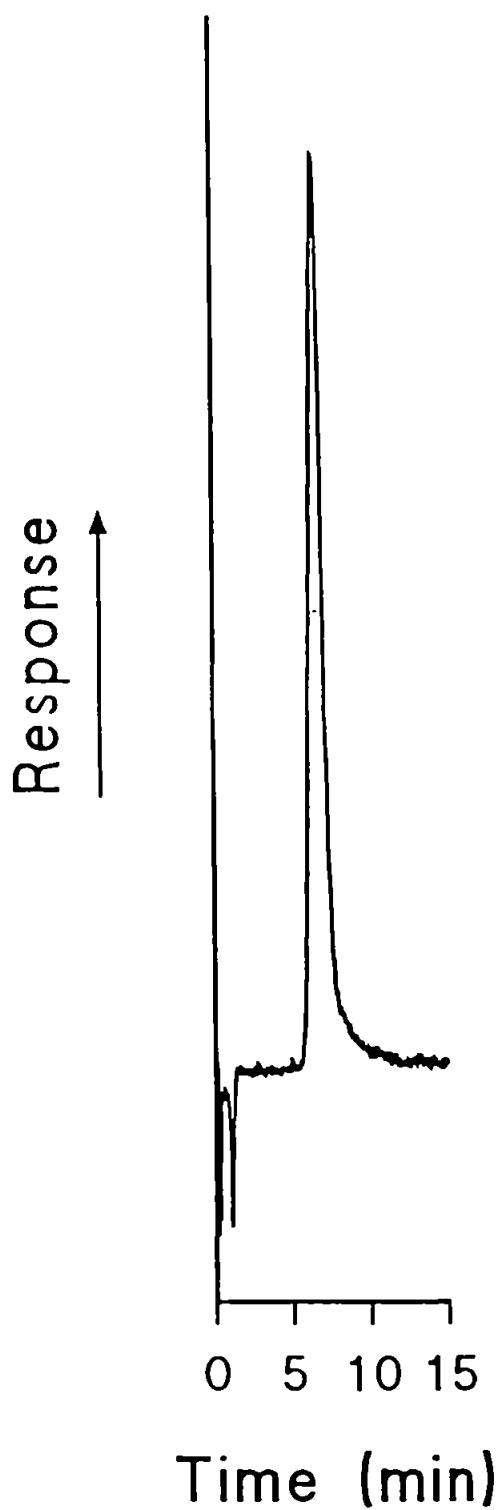
Figure 56   Typical chromatogram of 5 μg l^{-1} silver in synthetic PWR coolant water. HEMA-IEC SB, 2.5 cm column. 0.097 M potassium sulphate, 0.003 M potassium persulphate and 5 x 10^{-4} M sulphuric acid eluent. 100 μl injection.

4.13   Standard Reference Material.

Standard reference material, NIST 1643C, is a simulated fresh water sample containing 2.21 ± 0.30 μg l^{-1} silver. The concentration of silver in this sample was determined by constructing a calibration curve of silver concentration against peak area between 1 and 6 μg l^{-1} using 100 μl sample injections and by the standard addition method. The 2.5 cm HEMA-IEC column was used. Figure 57 shows the calibration curve and Figure 58 standard addition method, with correlation coefficients of 0.999 and 0.998 respectively. These calibrations were much less curved than those prepared with similar concentrations of silver, on the 5 cm HEMA-IEC column and may be due to the sharper peaks obtained with the shorter column. The standard addition method was found to be more accurate for this sample, giving 2.39 μg l^{-1} silver, compared to 1.33 μg l^{-1} silver by the standard curve method. The silver determined (2.39 μg l^{-1} silver) in the standard reference material was slightly greater than the referenced value but within the confidence limits. The reproducibility of the method on the NIST 1643C sample was 5.6% RSD on six repeat injections.

4.14   Conclusion.

When using a resin based on polystyrene divinyl benzene the retention of silver was considerable. The use of moderately concentrated salt solution affected the CL detection method leading to highly non linear calibrations and poor limits of detection. The tailing of the silver peak could not be assessed properly with the use of concentrated salt eluents and CL detection because of the poor detection limits. The
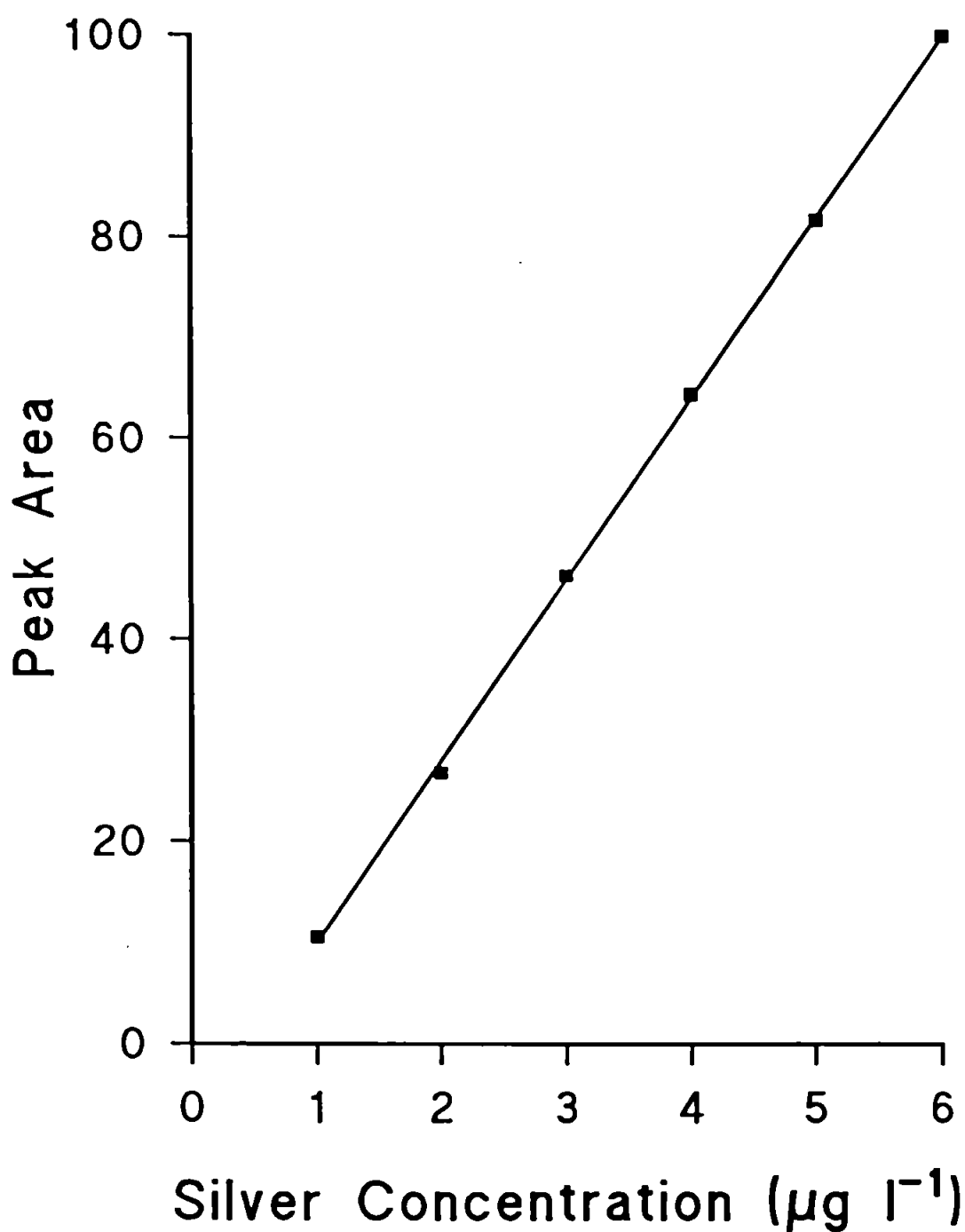
Figure 57   Calibration curve between 1 μg l⁻¹ and 6 μg l⁻¹ silver. Used for

the determination of silver in NIST 1643C. Conditions as Figure

56.
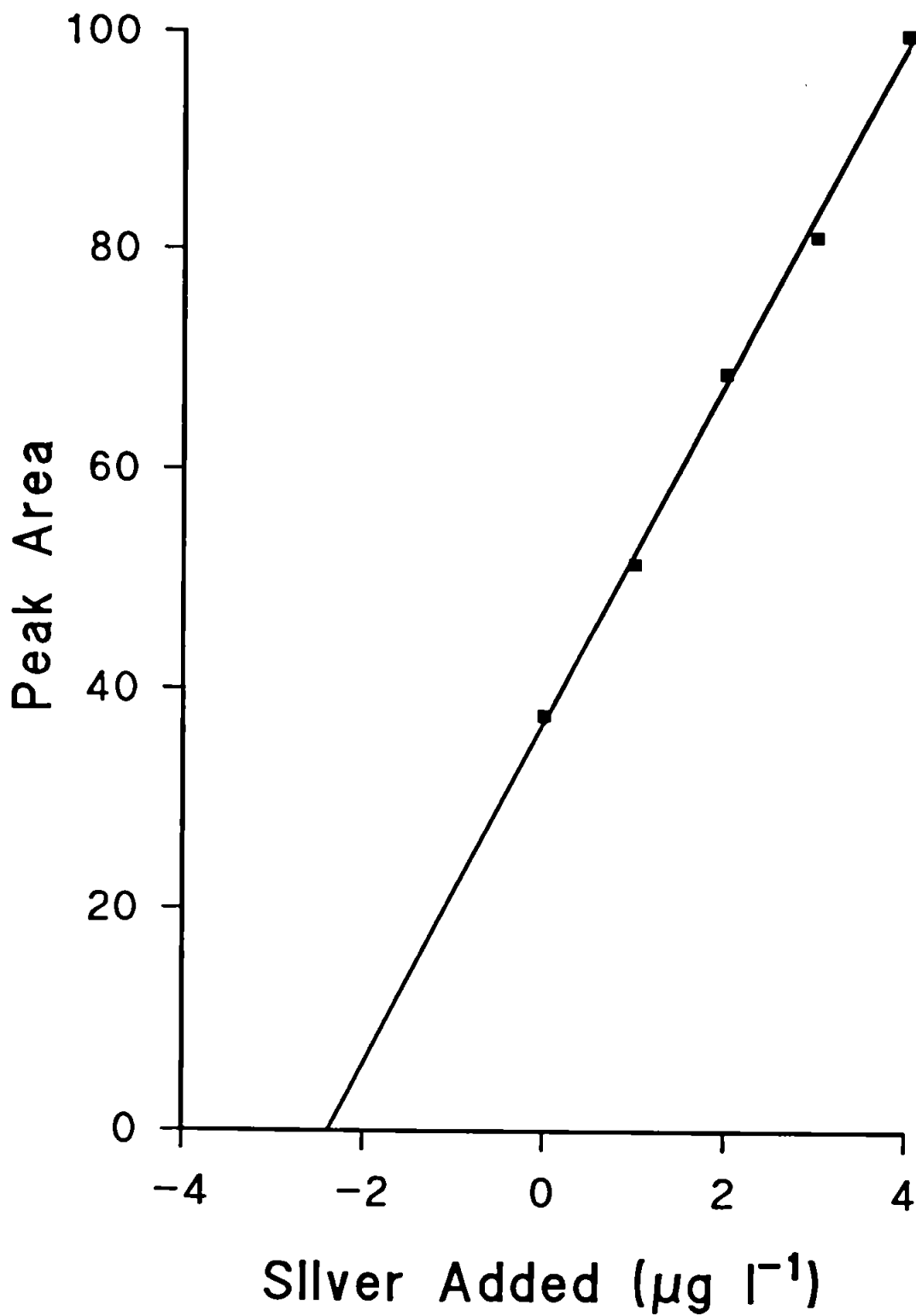
Figure 58   Standard addtion curve showing the addition of 0, 1, 2, 3 and 4

μg l^{-1} silver to NIST 1643C. Conditions as Figure 56.

use of the more powerful cation exchange eluent involving TETA, led to the use of more dilute eluents and improved detection limits and linearity. However, the lower detection limits made tailing of the silver peaks much more apparent and to a degree explained the non linear calibration curves. The use of strong eluents also caused the co-elution of silver with divalent transition metal cations and complicated attempts to preconcentrate the silver. All in all polystyrene divinyl benzene resin was found not to be a suitable substrate for high efficiency separations whatever eluent and preconcentration conditions were used.

The change of column resin matrix to one that had no delocalized pi bonding, polymethacrylate, led to a significant decrease in the peak tailing. However, the larger particle size of the first polymethacrylate resin investigated, Macroprep 50S, and the longer retention times it produced did not lead to a significant decrease in the detection limit. It did however allow the use of simple salt eluents that (i) were not so concentrated so as to detrimentally affect the CL system and (ii) allowed the separation of silver from divalent cations.

The use of large volume injections (1 ml) allowed the detection of silver in simulated PWR coolant down to 0.1 μg l^{-1}, with linear calibrations of silver in simulated PWR coolant between 0.1 to 1 μg l^{-1} and 1 to 10 μg l^{-1}. The reproducibility of silver determined at 5 μg l^{-1} was 1.2% RSD.

The use of smaller particle size hydrophilic polymethacrylate resin packing was investigated in order to achieve higher efficiency and therefore lower detection limits. The capacity of the resin was 1.2 - 1.6 meq g^{-1}, much greater than the larger particle

size resin and this resulted in the use of increased salt concentration in the eluent. Whilst it was possible that this adversely effected the CL detection system, it was considered an important step in developing the method with higher efficiency chromatography. The HEMA-IEC resin, as expected, did show increased efficiency over the Macroprep resin and it was possible to determine 0.05 μg l^{-1} silver in simulated PWR coolant. Increasing the salt concentration was shown to effect the CL detection system and this may account for the curved calibrations obtained using the HEMA-IEC column compared to the macroprep column.

The use of the small 2.5 cm length column with the eluent unchanged led to elution of silver in half the time, with the peak still resolved from the solvent front and from interfering cations. Also significantly sharper peaks were obtained thereby enabling a lower detection limit of 0.025 μg l^{-1} silver in simulated PWR coolant to be achieved.

The concentration of silver in a simulated fresh river water standard reference material, NIST 1643C, was investigated using this column. The concentration of silver determined by standard addition technique was 2.39 μg l^{-1} silver. This was within the limits of significance given as 2.21 $\pm$ 0.3 μg l^{-1} silver. The reproducibility of six replicate 100 μl injections of the standard reference material at 5.6 %RSD was very reasonable at this concentration level.

The final elution conditions used with the different column types are summarized in Table 26. These include, the elution of silver from a Dionex CG2 column with either a simple salt eluent or the more complex TETA eluent. The use of a potassium sulphate eluent or a potassium sulphate/potassium persulphate eluent with columns

Table 26   Optimium conditions of eluents and luminol post-column reagent used
with the different column types.

Column Type: **Dionex CG2.**

Eluent.   0.15 M potassium nitrate
0.03 M nitric acid

Luminol Post-Column Reagent.   0.1 M orthoboric acid
5×10^{-5} M luminol
0.001 M triethylenetetramine
0.003 M potassium persulphate

Column Type: **Dionex CG2.**

Eluent.   0.001 M triethylenetetramine, pH 2.25

Luminol Post-Column Reagent.   0.1 M orthoboric acid
5×10^{-5} M luminol
0.003 M potassium persulphate

Column Type: **Macroprep 50S, 5 cm column.**

Eluent.   0.05 M potassium sulphate
5×10^{-5} M sulphuric acid

Luminol Post-Column Reagent.   0.1 M orthoboric acid
5×10^{-5} M luminol
0.001 M triethylenetetramine
0.003 M potassium persulphate

Column Type: **Macroprep 50S, 5 cm column.**

Eluent.   0.047 M potassium sulphate
0.003 M potassium persulphate*
5×10^{-5} M sulphuric acid

Luminol Post-Column Reagent.   0.1 M orthoboric acid
5×10^{-5} M luminol
0.001 M triethylenetetramine

Column Type: **HEMA IEC, 5 cm or 2.5 cm column.**

Eluent.   0.097 M potassium sulphate
0.003 M potassium persulphate
5×10^{-5} M sulphuric acid.

Luminol Post-Column Reagent.   0.1 M orthoboric acid
5×10^{-5} M luminol
0.001 M triethylenetetramine

containing the polymethacrylate based resins, Macroprep 50S and HEMA-IEC, are also included.

This developed IC system has detection limits that are comparable to other methods but the main aim was to develop a monitoring system suitable for use in a PWR or VVER plant for the analysis of silver in coolant loop samples. Unfortunately there was no opportunity to set up a system at a VVER site. It will be some time well after this project before this can be arranged by the Atomic Energy Authority. Thus further work will need to be planned to evaluate the potential of the IC system for the monitoring of silver in a nuclear plant.

Nevertheless as far as the author is aware this is the first high performance IC method with sensitive detection that has been developed for silver. This has undoubtably been made possible by the introduction of polymethacrylate phases. Time did not permit more studies but further improvements can be made, as written in Chapter 6.

CHAPTER FIVE.

5.0 DEVELOPMENT OF CHEMILUMINESCENCE DETECTION FOR GOLD AFTER ION CHROMATOGRAPHY SEPARATION.

5.1 Introduction.

There are few sensitive methods for the determination of gold, Table 27 shows some of the most common methods and indicates that CL detection can be the most sensitive.

Detection of gold by luminol CL has been reported in either batch systems, by Zhang *et al.* (98) and Lukovskaya *et al.* (115) or flow injection systems, by Imdadullah *et al.* (84). However, ion chromatography (IC) with CL detection for the determination of gold has not been reported. The highly sensitive CL detection system reported by Imdadullah *et al.* requires the use of high concentrations of organic solvents in the mobile phase of the flow injection system (84).

Rocklin reported an IC method using a Dionex column for the separation of gold from other transition metals as its anionic chloro complex in aqueous solution (178). IC of gold as its chloro complex has also been presented by Jones and Schwedt (180). The IC method and the polystyrene-divinyl benzene Dionex column would not be compatible with high concentrations of organic solvent. In view of this incompatibility the aim of this work is to develop a CL PCR method for the determination of gold compatible with an IC separation method.

Table 27   Detection limits for gold by various methods.

Method	Detection Limit For Gold	Reference
Graphite Furnace AAS	0.1 μg l^{-1}	149
"	2 μg l^{-1}	84
"	0.1 μg l^{-1}	150
Flame AAS	1000 μg l^{-1}	84
"	6 μg l^{-1}	150
ICP-OES	300 μg l^{-1}	84
ICP-MS	0.1 μg l^{-1}	84
Spectrophotometric	2 mg l^{-1}	165
"	2 mg l^{-1}	84
Catalytic Spectrophotometric	50 μg l^{-1}	171
Direct injection IC	50 μg l^{-1}	178
160 fold preconcentration IC	1 μg l^{-1}	178
Capillary Zone Electrophoresis	3 mg l^{-1}	179
Chemiluminescence	10 μg l^{-1}	115
"	10 μg l^{-1}	98
"	0.01 μg l^{-1}	84

Gold was separated from other metal ions using the IC system developed by Rocklin (178). The effect of luminol concentration and pH on the gold CL response was examined initially. The use of co-oxidants, hydrogen peroxide and potassium persulphate, in the Cl reagents and the effect on the gold response were also investigated. Effect of EDTA addition to the eluent on the background signal was studied. Results obtained by this method and that given for a certified reference material were compared. The equipment and reagents used are detailed below

5.2.1   Chromatograph.

As shown in Figure 59 the chromatograph set up consisted of a 4000i Dionex gradient pump (Dionex, Sunnyvale, CA, U.S.A.) which was used to deliver the eluent to the column at 1.5 ml min^{-1}. The CL reagent was added just prior to the detection coil at 1.5 ml min^{-1} using an inert plastic pump (Dionex 2000i). The chemiluminescence was monitored with a modified FD-100 fluorescence detector (Spectrovision, MA, U.S.A.). The modification involved replacing the fluorescence cell with a flat PTFE coil, volume 30μl, pressed against the face of the photomultiplier tube.

Separations were performed on a Dionex HPIC-AG5 5 cm column and sample injection was carried via an inert PEEK Rheodyne injection valve (Model 9010, Cotati, CA, U.S.A.) with a PEEK 100 μl sample loop. 0.3 mm i.d. PTFE connecting tubing was used throughout.
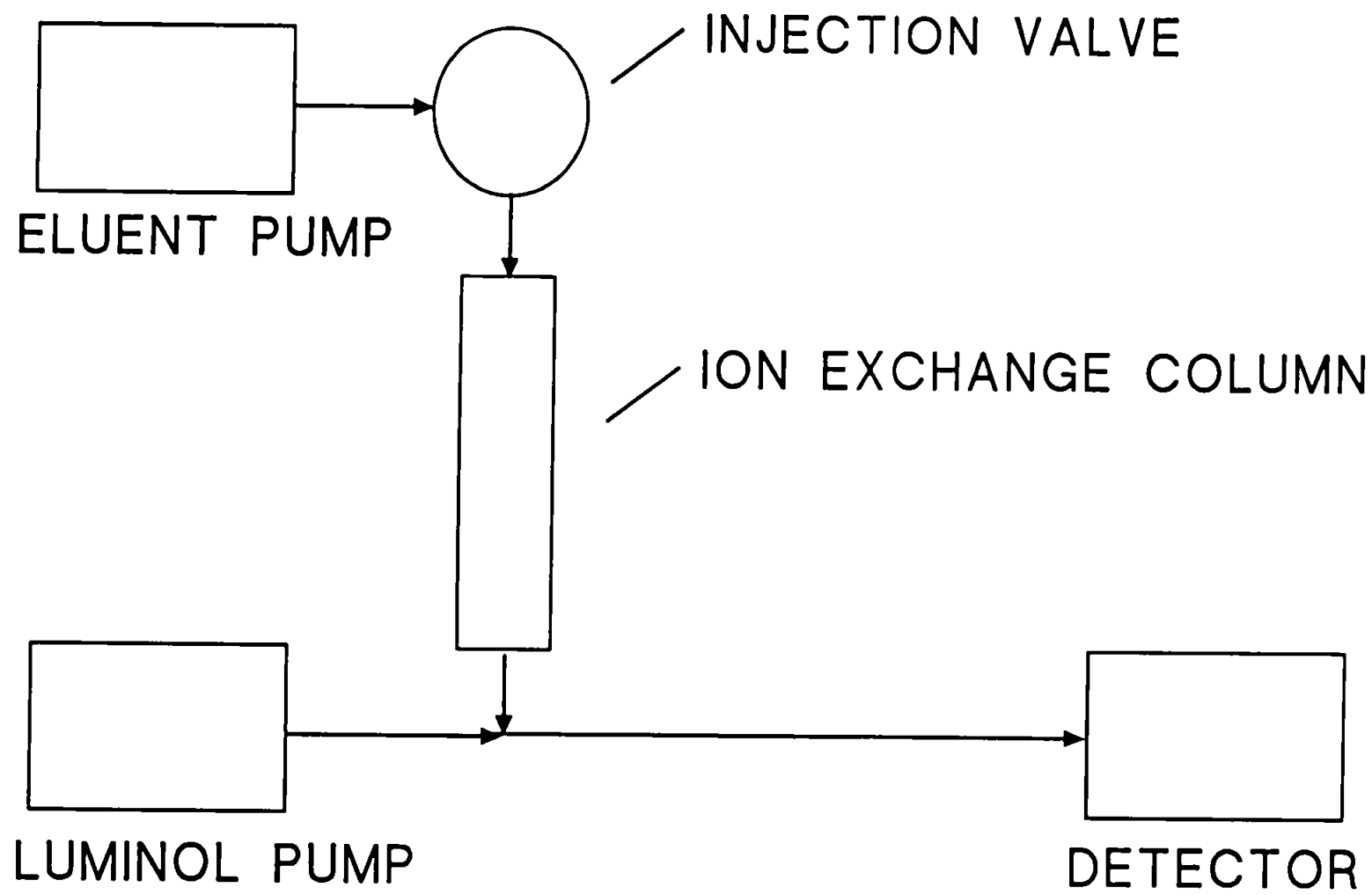
Figure 59   Schematic diagram of chromatograph employed.

5.2.2   Reagents.

The eluent consisted of 0.3 M sodium perchlorate and 0.05 M hydrochloric acid and 5 x 10^{-4} M EDTA. The post column reagent was a solution containing 5 mg l^{-1} luminol (3 x 10^{-5} M luminol) which was adjusted to a suitable pH by the addition of potassium hydroxide. All solutions were degassed with helium prior to use.

Milli Q deionized water (Millipore Corp., Bedford, MA, U.S.A.) was used for all solutions. All reagents were AnalaR (Merck, Poole, U.K.) except luminol (Fluka, Poole, U.K.) and Aristar potassium hydroxide (Merck). Working metal standards were prepared from 1,000 mg l^{-1} or 10,000 mg l^{-1} spectrosol standards (Merck).

5.2.3   Certified reference material.

Certified reference material BCS-CRM No. 347 electronic flowsolder was used as a reference material, containing 0.037% $\pm$ 0.004% gold. The electronic flowsolder was mainly tin (63%), trace metals accounted for 1% and the remainder was lead (26%).

5.2.4   Dissolution method for electronic flowsolder.

Dissolve a known amount of sample (about 0.4 g) in 10 ml concentrated hydrochloric acid and 2 ml concentrated nitric acid, warming gently if necessary. Boil the solution gently for about 5 minutes to expel nitrous fumes and chlorine. Allow to cool. Add

25.0 ml 0.2 M EDTA and boil for 1 minute. Cool and dilute 50 ml, further dilute 1 ml to 100 ml and inject.

5.3   Results and Discussion.

5.3.1   Optimisation of chemiluminescence detection.

It had been noted in preliminary studies, that when gold (III) was added to an alkaline solution of luminol, CL was observed even in the absence of added co-oxidant. Gold (III) as a chloro complex is a strong oxidising agent and so it is possible that it is capable of oxidising the luminol directly. In a detection system based upon this reaction there are only a few variables, pH of the solution and concentration of the luminol. In order to simplify this preliminary investigation into the development of an IC separation system with CL detection for gold the use of pH buffer was omitted. This is because the high pH required for maximum CL enabled the pH of the detection system to be maintained when gold was eluted with dilute acid eluent.

Figures 60 and 61 show the variation of CL response to 50 μg l^{-1} gold with change in pH and luminol concentration. The effect of increasing the pH leads to an increase in the CL response. However, at very high pH and therefore significant concentrations of potassium hydroxide the detection cell became blocked. The solubility of potassium perchlorate is given as 7 g l^{-1} at 0° C rising to 218 g l^{-1} at 100° C (181). 0.15 M potassium perchlorate present in the detection coil, as a result of potassium hydroxide used to adjust the pH and the sodium perchlorate eluent, is equivalent to 20 g l^{-1}.

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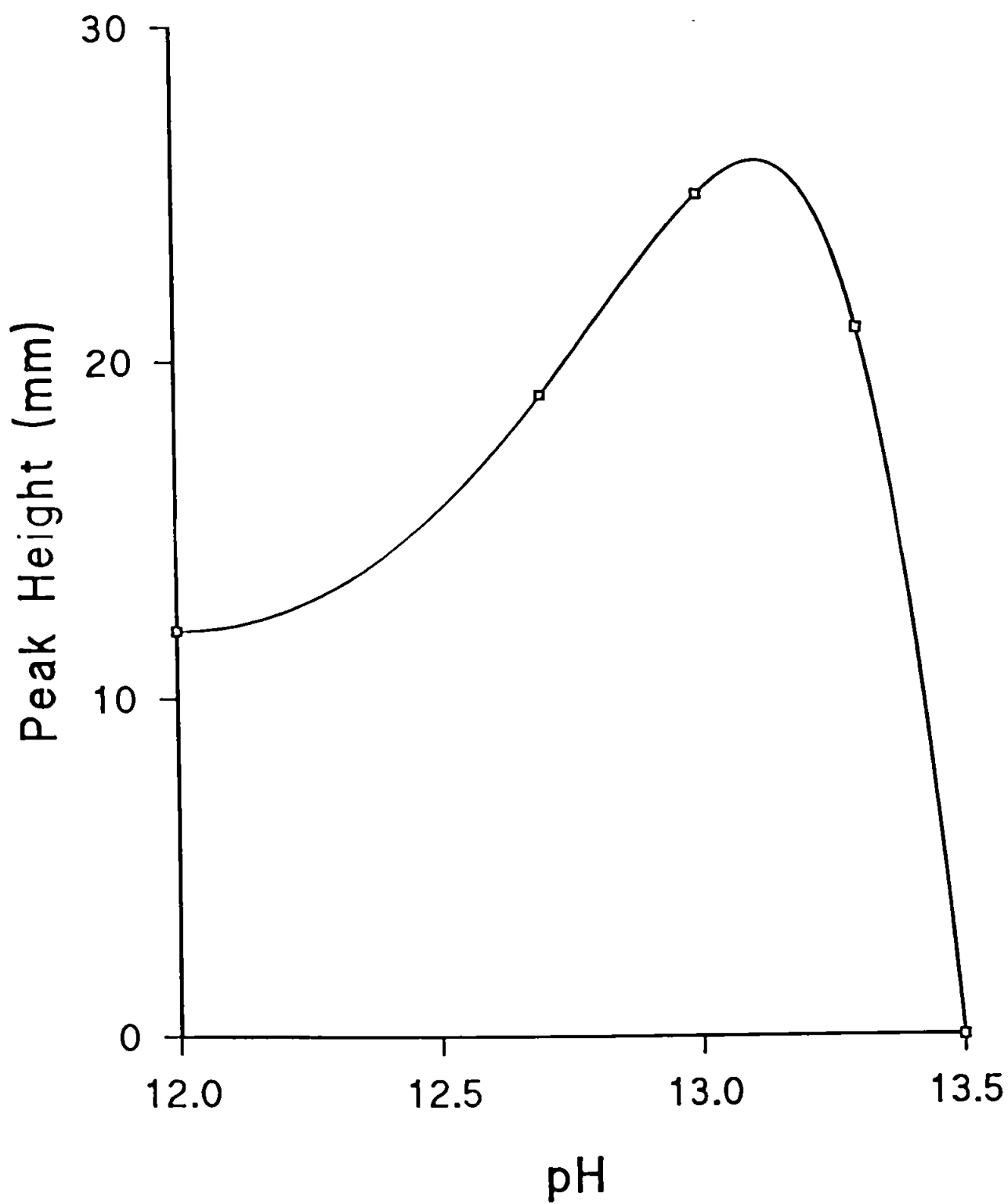
Figure 60   Effect of luminol solution pH on the CL response to 50 μg l^{-1}
gold.
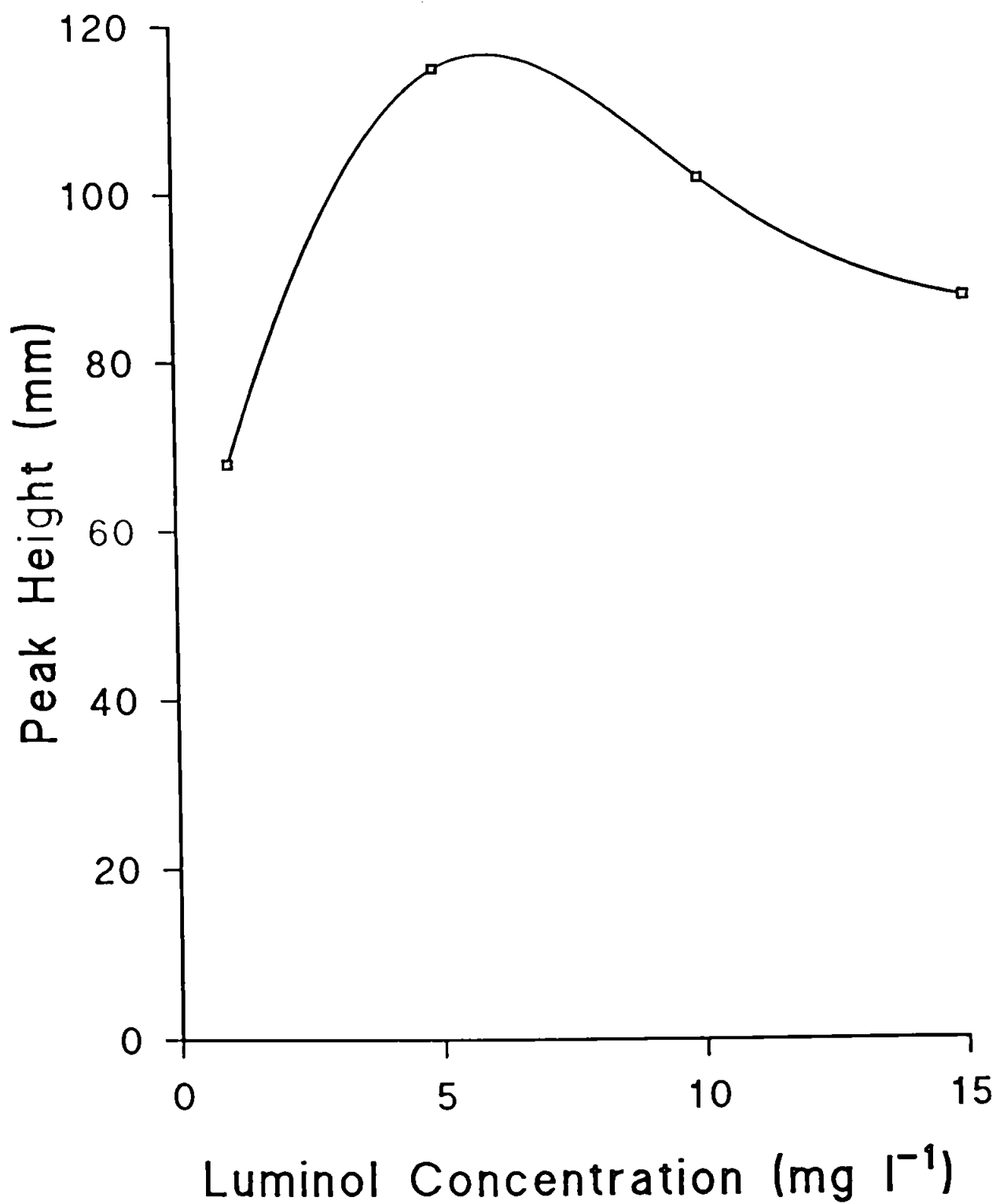
Figure 61   Effect of luminol concentration on the CL response to 50 μg l^{-1}

gold.

Therefore, it is probable that at ambient temperature this exceeded the solubility product of potassium perchlorate leading to blockage of the detector, hence the optimum pH for use was 13.0. The optimum concentration of luminol was 5 mg l^{-1} (3 x 10^{5} M).

It is not clear whether Zhang *et al.* (98) and Lukovskaya *et al.* (115) used co-oxidants in the CL determination of gold. In order to assess whether co-oxidants would lead to an increase in CL response addition of hydrogen peroxide or potassium persulphate was made. In addition, Rocklin had used hydrogen peroxide in the eluent when preconcentrating gold on the column to ensure gold was not reduced (178), it was important to ascertain whether this had any affect on the CL response to gold.

Figures 62 and 63 show the effect of adding hydrogen peroxide or potassium persulphate to the luminol solution on the CL response to gold. Addition of 0.001 M hydrogen peroxide leads to a much higher background signal and a decrease in the CL response to gold. 0.001 M Potassium persulphate has little effect on the CL response, 0.005 M potassium persulphate causing a slight decrease in CL response with a small increase in the baseline noise. Triethylenetetramine (TETA) was required as an activator in the previous CL system involving persulphate. TETA was therefore added to the CL reagent to see if any enhancement of the CL signal would be observed. However, 0.1% TETA caused severe quenching of the CL signal. From these results it was decided not to add any co-oxidant to the luminol solution.

Initially, the baseline noise of the gold CL system was found to be high. EDTA was added to the eluent in the chromium IC system with CL detection system to reduce the

Perchlorate
Eluent

+ 0.001M Hydrogen
Peroxide
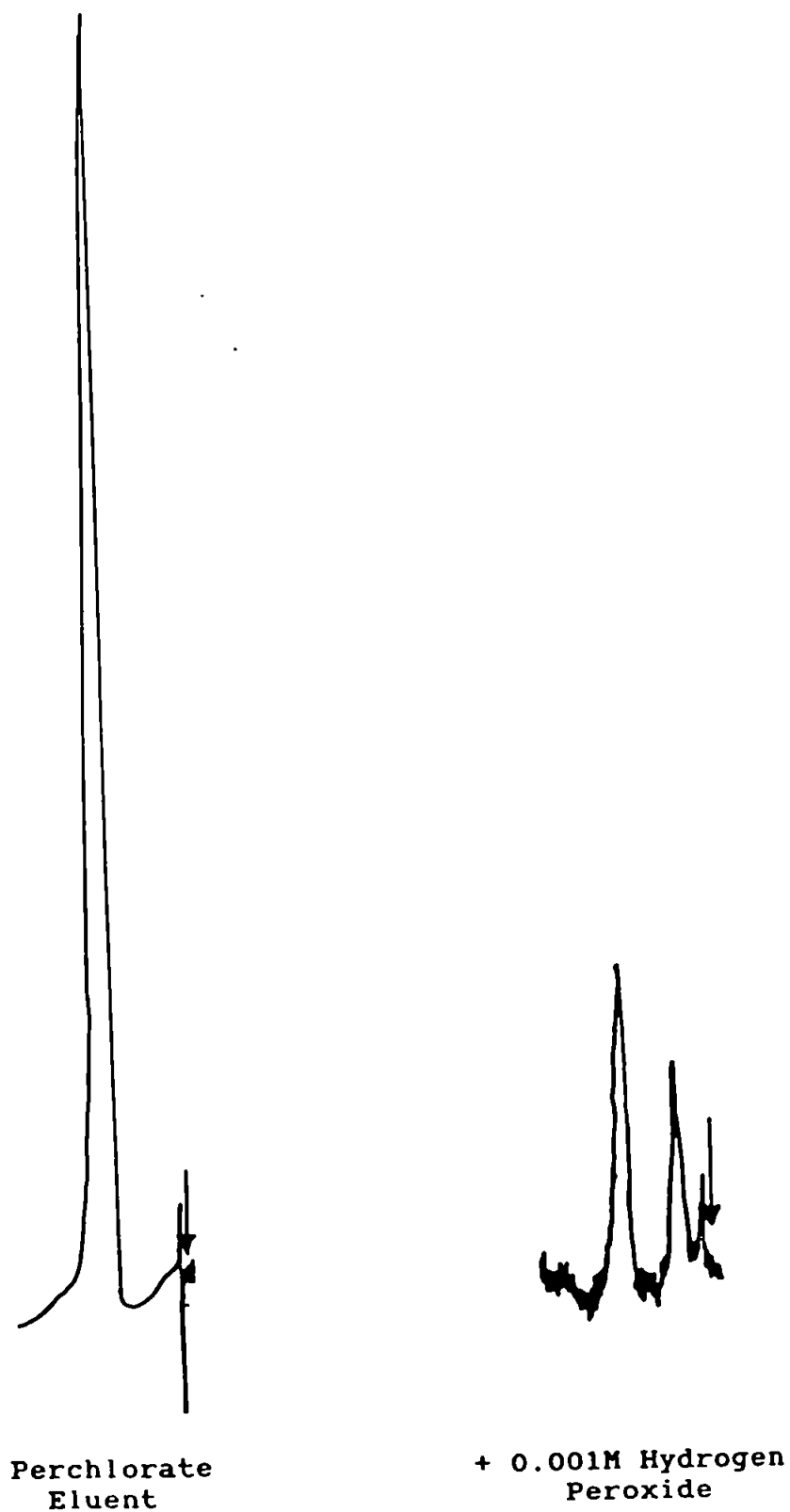
Figure 62   Effect on the CL response to 100 μg l^{-1} gold on the addition of

0.001 M hydrogen peroxide to the eluent.

Perchlorate
Eluent

+ 0.001 M
Potassium
Persulphate

+ 0.005 M
Potassium
Persulphate

+ 0.005M
Potassium
Persulphate
and 0.1%
Triethylene
Tetramine
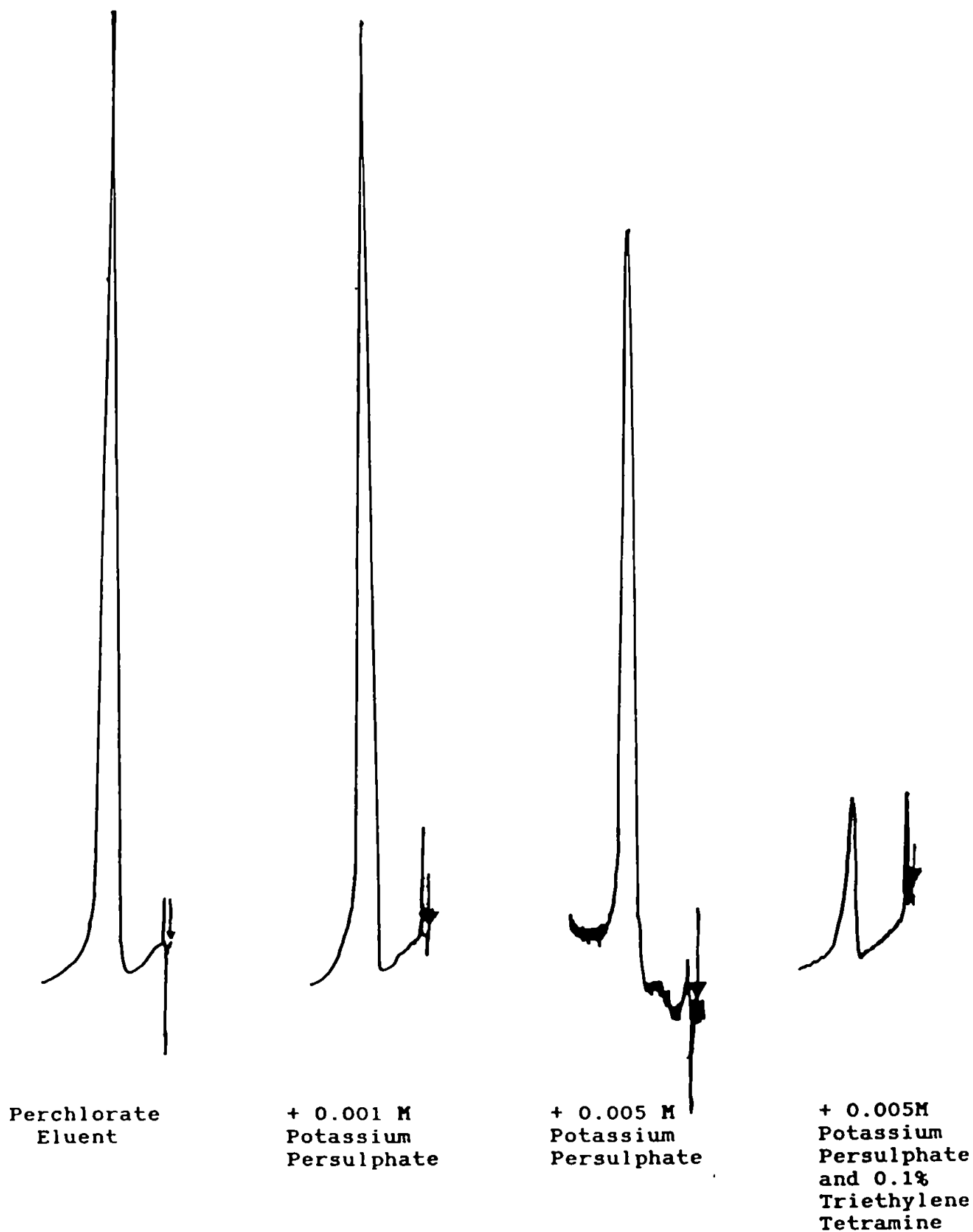
Figure 63   Effect on the CL response to 100 μg l^{-1} gold on the addition of
either 0.001 M, 0.005 M potassium persulphate or 0.005 M
potassium persulphate and 0.1% triethylenetetramine to the
eluent.

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baseline noise. The addition of EDTA to the perchlorate eluent was investigated to see if similar reduction in baseline noise could achieved. Figure 64 and Table 28 show the addition of EDTA to the eluent led to a reduction of absolute baseline noise as well as the pump noise component of the baseline noise. However, addition of 0.001 M EDTA to the eluent made the baseline unsteady, therefore the optimum concentration of EDTA added to the eluent was 5×10^{-4} M.

5.3.2 Optimisation of chromatography.

The IC method used by Rocklin required an eluent that contained 0.05 M hydrochloric acid to keep gold in its chloro complex (178). The use of a neutral chloride salt was investigated as a replacement for hydrochloric acid to make it more compatible with the high pH detection system. However, when 0.05 M sodium chloride was added to the eluent instead of the hydrochloric acid gold did not elute. This may be due to the formation of a gold hydroxy species at the higher pH of this eluent, the chloride complex being unable to prevent the formation of the hydroxy complex. However, when the eluent was acidified with 0.025 M sulphuric acid elution returned to normal.

The use of a short column as the analytical column meant the back-pressures were much lower than the recommended back-pressure for the Dionex pumps, resulting in poor seating of the check valves. Increase in eluent flow rate has two effects, it decreases the retention time in proportion to the increase and creates higher back-pressure as more liquid is forced through the column in the same time. In the IC system used by Rocklin gold was retained for 10 minutes and enabled the separation of
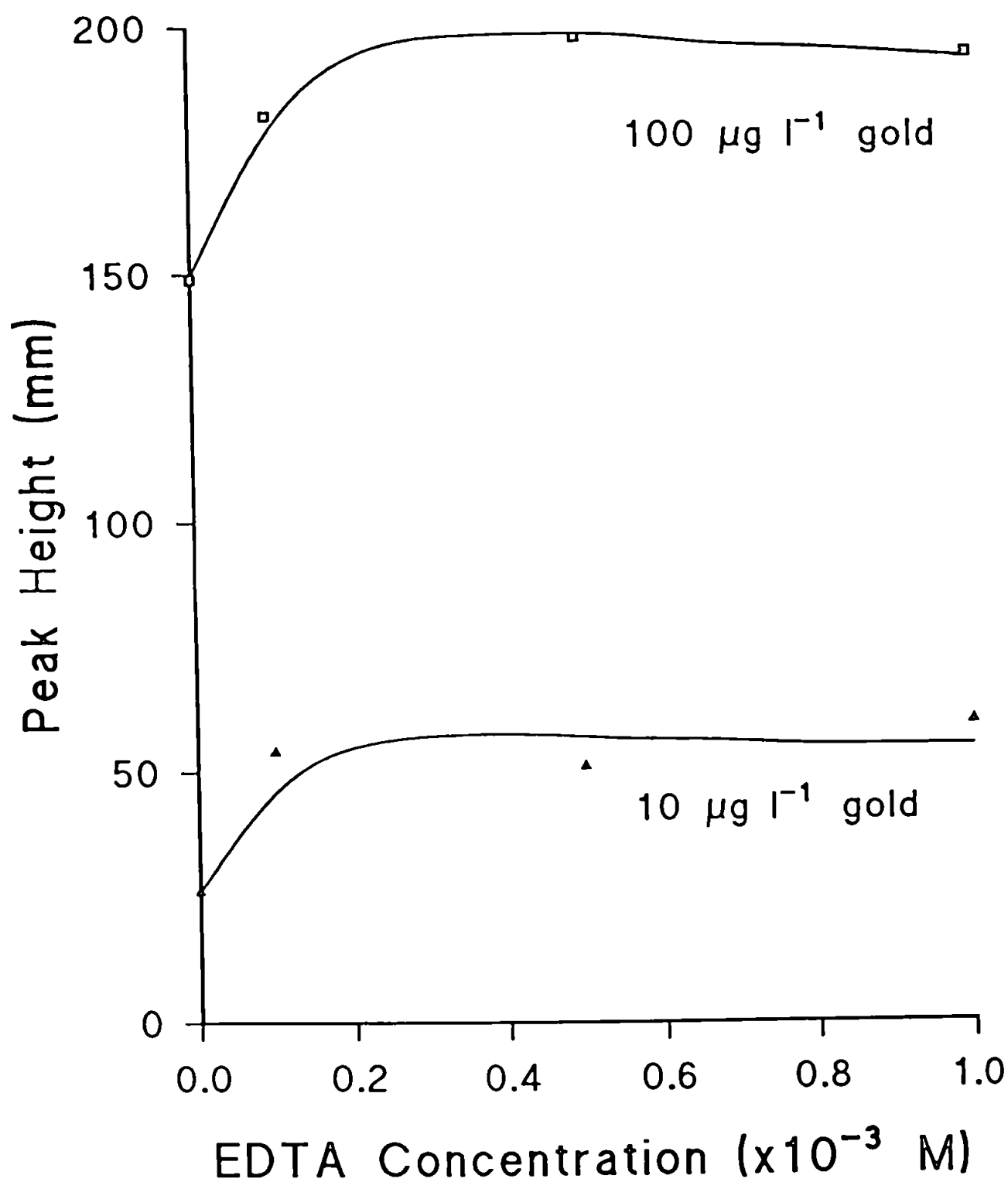
Figure 64   Effect of the addition of EDTA to the eluent on the CL response to 10 and 100 μg l^{-1} gold.

Table 28   Effect of the addition of EDTA to the eluent on the CL response to gold.

N/A = baseline noise too small to be measured.

EDTA Concentration (M)	Gold Concentration (μg l^{-1})	Peak Height (mm)	Baseline Noise
none	10	26	3 mm
	100	149	N/A
10^{-4}	10	54	N/A
	100	182	N/A
5 x 10^{-4}	10	51	N/A
	100	198	N/A
10^{-3}	10	60	unsteady
	100	195	unsteady

platinum (II), lead (II), gold (III) and iron (III) (178). In this work it was decided that a better compromise between separation of gold and more efficient working of the pumps could be achieved at a higher flow rate.

The effect on the retention and peak height by increasing the flow rate of one or both pumps is given in Table 29. The flow rate was increased to 1.5 ml min^{-1} for both pumps in order to keep the pH of the solution in the detector constant. The greater flow rate increased the back-pressure and enabled the Dionex pumps to operate more efficiently.

5.3.3   Interferences.

The effect of co-eluting cations was investigated by replacing the CL detection system with a Calmagite detection system. The principle of the Calmagite detection system is discussed in Section 1.7.3.3. Table 30 show the times of maximum response and the time when the peaks return to the baseline for a number of cations and gold (III), the flow rate of this initial study was at 1 ml min^{-1} and EDTA was omitted from the eluent. There was no overlap of peaks of the cations with the gold peak.

No interference was observed with 10 μg l^{-1} gold when it was co-injected with any of the following at 10 mg l^{-1} solutions: chromium (III), chromium (VI), manganese (II), tin (II), nickel (II), lead (II) or the sulphate anion (SO$_4^{2-}$).

Serious interference was observed when 10 μg l^{-1} gold was co-injected with, 0.005%

Table 29   Effect of increasing the flow rate of either the eluent pump alone or both

the eluent and the luminol pumps on the retention time and peak height

of gold (III).

Flow Rate (ml min⁻¹)	Increase Flow of Eluent Pump Only		Increase Flow in Both Pumps	
	Retention Time (min)	Peak Height (mm)	Retention Time (min)	Peak Height (mm)
1.0	7	52	7	70
1.5	5.5	105	5.5	121
2.0	3	124	Backpressure too high	

Table 30   Elution times for various cations that may interfere with the detection of

gold. The times given are the time at the maximum peak height and the

time taken for the ion to elute. Detection was with Calmagite inverse

photometry. Flow rate of the eluent and the post column reagent was 1

ml min^{-1}.

Metal Ion (10 mg l^{-1})	Time of Maximum Response (min)	Time Taken for Cation to Elute to baseline (min)
Gold (III)	7	
Copper (II)	immediate	2
Iron (III)	2	5
Manganese (II)	1	5
Zinc (II)	0.5	1.5

sulphur dioxide (SO_2), 10 mg l^{-1} iron (II) or 10 mg l^{-1} bromide. Interestingly, 10 mg l^{-1} iron (III) enhanced the gold signal by 20%. Interference by sulphur dioxide gave split peaks. Iron (II) caused the complete disappearance of the gold peak, presumably by reduction. An 88% reduction in the gold peak was observed on co-injection with 10 mg l^{-1} bromide. Co-injection with the more concentrated 50 mg l^{-1} bromide led to a 98% reduction of the 10 μg l^{-1} gold peak.

Very high backgrounds were observed when one of the inert plastic Dionex pumps was replaced with either a Gynkotch pump or a LKB pump fitted with titanium heads. Addition of titanium standard to an aliquot of the luminol solution gave a CL response. Titanium was reported by Alwarthan and Townshend to be CL active with luminol in the absence of co-oxidant (95). Hence these pumps were not used for further work.

5.3.4 System performance.

Figure 65 shows the calibration between 10 μg l^{-1} and 100 μg l^{-1} gold with a correlation coefficient of 0.9986. Figure 66 shows a typical chromatogram of 10 μg l^{-1} gold, while Figure 67 shows at typical chromatogram near the detection limit of 0.5 μg l^{-1} gold. The detection limit which can be defined as the concentration of gold that gives a peak with height twice the peak to peak noise of the baseline, was 0.25 μg l^{-1} gold. The reproducibility of the system was determined as 1.8% RSD for six repeat injections of 10 μg l^{-1} gold. Near the detection limit the reproducibility of six repeat injections of 1 μg l^{-1} gold was 6.2%.
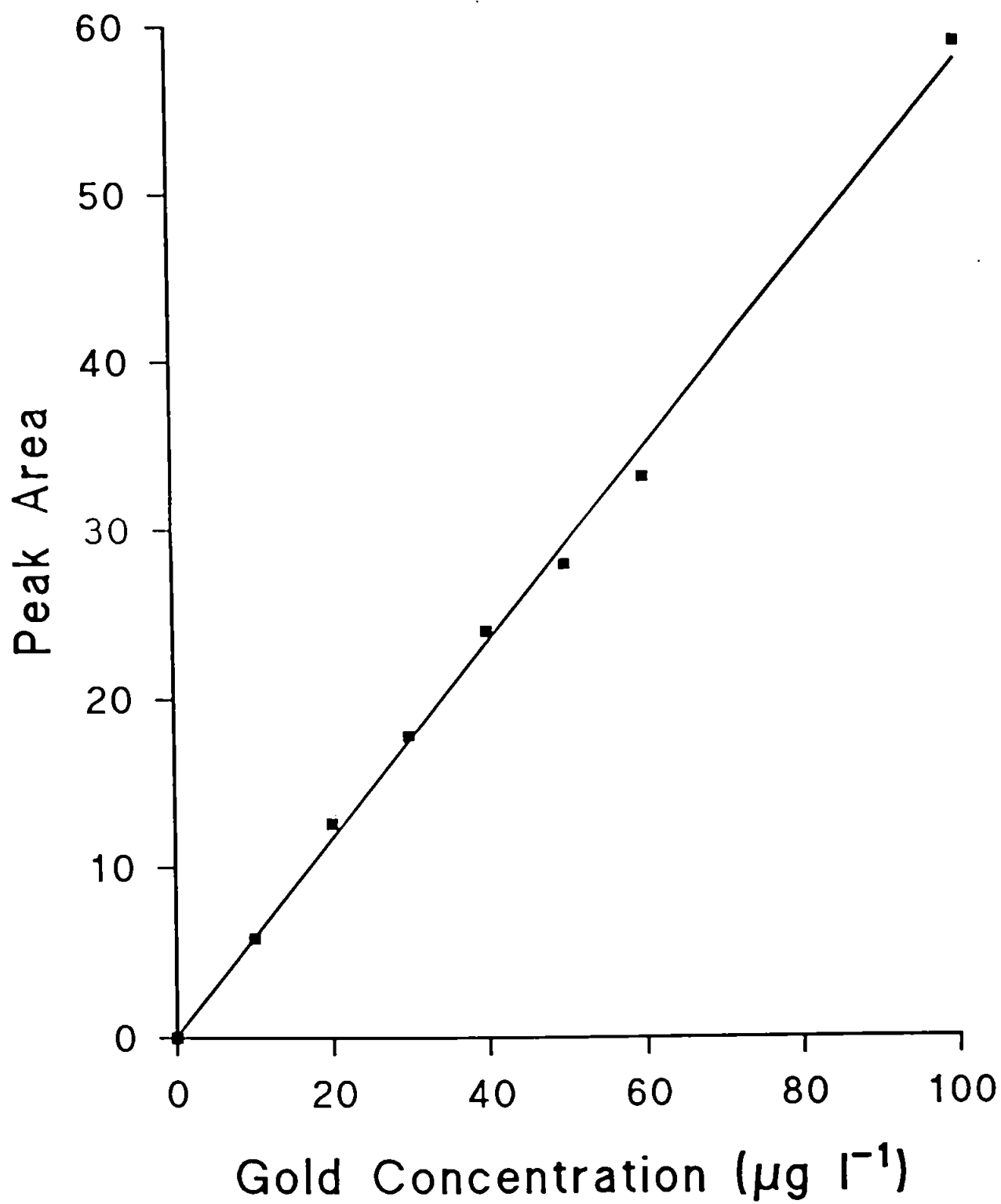
Figure 65   Calibration curve between 10 μg l⁻¹ and 100 μg l⁻¹ gold.

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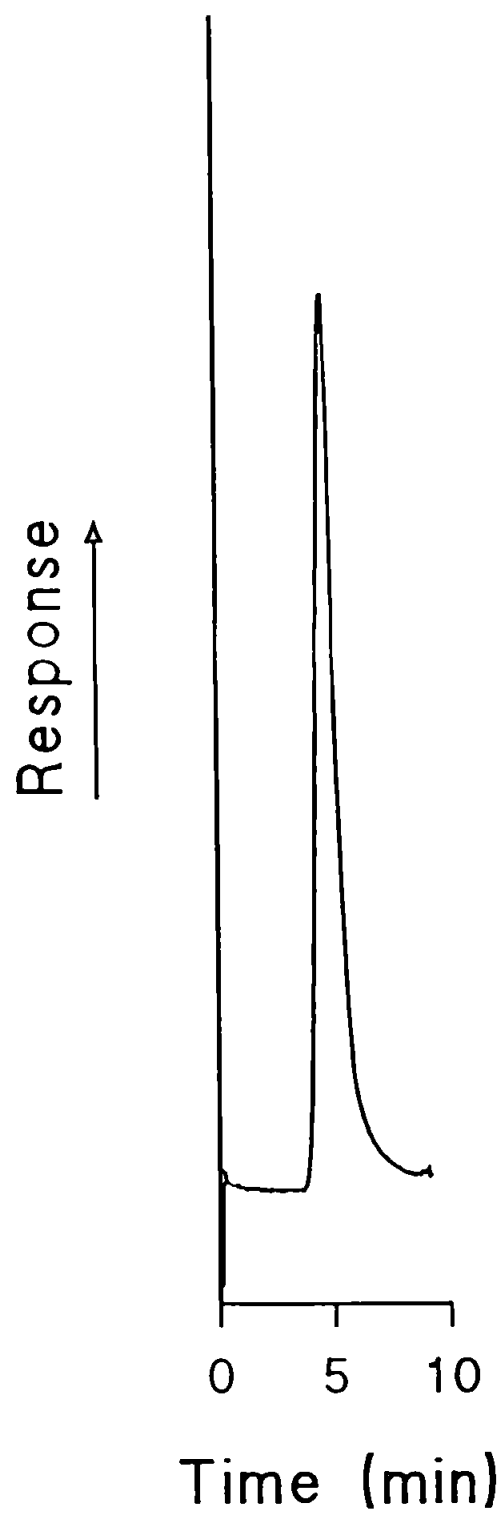
Figure 66   Typical Chromatogram showing 10 μg l⁻¹ gold.

Figure 67   Typical chromatogram near the detection limit showing 0.5 μg l^{-1}.

5.4　　　Standard Sample.

The choice of a standard sample was limited and it was only possible to obtain a standard reference material which contained gold in concentrations many times greater than the upper range of the detection system. However, it gave the opportunity to test the IC method in the presence of a large excess of matrix elements. The available sample, electronic flowsolder, was approximately 63% tin and 1% trace metals, the remainder being lead. Fortunately the large dilutions necessary gave tin and lead concentrations that were lower than the point at which these would precipitate in the detection system.

Figure 68 shows a calibration curve constructed in the determination of the gold in the electronic flowsolder standard reference material, the correlation coefficient being 0.9990. The concentration of gold determined in the CRM electronic flowsolder was 0.040% (average of 3 samples) which was within the certified limits provided, namely 0.037% ± 0.004%. The relative standard deviation (RSD) of the three samples was 1.5%.

5.5　　　Conclusion.

The expected high sensitivity of CL detection has been displayed with a sub μg l^{-1} detection limit of 0.25 μg l^{-1}, comparable to other more expensive instruments, such as ICP-MS with a detection limit for gold of 0.1 μg l^{-1}.
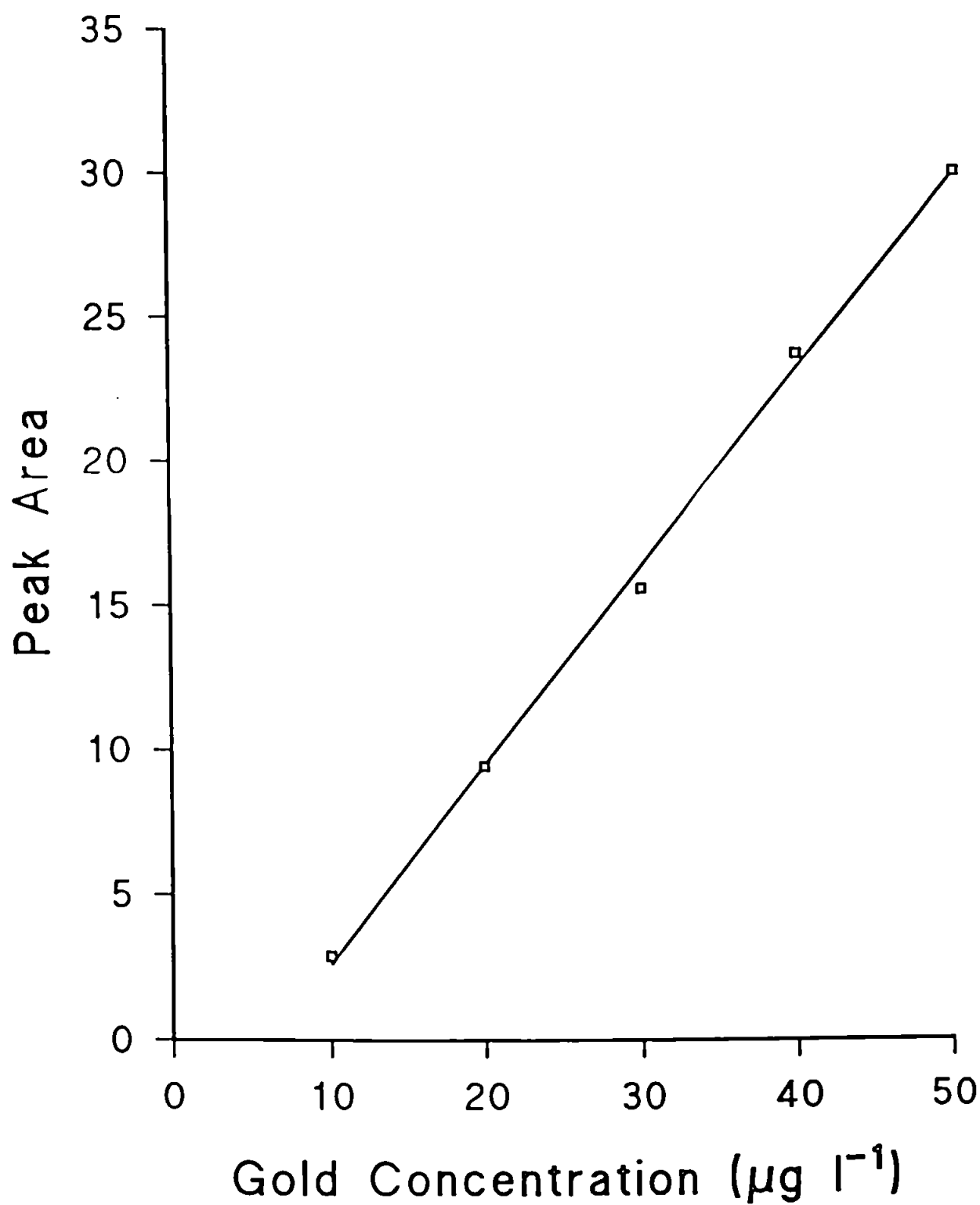
Figure 68   Cailbration curve for gold used for the analysis of CRM

electronic flowsolder.

The freedom from interference from divalent cations and sulphate may enable the use of preconcentration or large volume injections for the analysis of gold in complex matrices, such as rock, where there is interest in determining sub mg l^{-1} concentrations of gold in order to assess whether ore processing of the ore will be economic. Alternatively analysis for lower concentrations of gold in natural waters may be useful in the gold prospecting industry.

CHAPTER SIX.

6.0   OVERALL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK.

This work has dealt with different aspects of CL detection for IC and as such was divided into separate sections throughout. In continuing this trend, the final section shall be apportioned in a similar manner except where general conclusions can be drawn. Overall the objectives set have been met, CL detection for a range of metal ions was achieved that were highly sensitive and reproducible. Where appropriate the detection systems and IC systems developed have been tested for their ability to analyse for specific metals in standard reference samples. The results given by these methods compared favourably with the certified values for the metals analyzed.

The large number of variables in each of these systems, and the use of univariate optimisation, may lead to a situation where the system does not reach the universal optimum. Multivariate optimization techniques, such as simplex, were designed to enable optimisation of many variables simultaneously, to enable a universal optimum to be obtained. These may prove to be suitable for future optimisation of systems such as these. However, the need to allow chromatographic systems to stabilize for each new elution condition, change in CL response over time and the elution times of the chromatographic system will complicate and lengthen any attempts to optimise these systems using a multivariate technique.

The work described here made use of a pen recorder and a simple analogue to digital interface, enabling simple data handling to be carried out. More sophisticated digital signal processing (DSP) can be used to subtract regular baseline noise, such as pump noise, from the analytical signal. The systems described here exhibited regular pump noise. Therefore, future work could benefit from DSP, possibly enabling lower levels of analytes to be determined.

The first section dealt with an investigation into a multi-element CL detection system for cations. The developed method was less sensitive than single element CL detection, which is due mainly to the non-selective nature of the reaction producing relatively high background signals.

The system was based on two direct formation reactions between analyte, cobalt (II) and EDTA, whereas previous work had centred on exchange reactions. This approach, using more favourable kinetics, enabled the use of short reaction coils at ambient temperature. This was beneficial in terms of band broadening, complexity of the system and limiting metal-ligand exchange reactions.

The approach also gave a situation that with an increase in pH, metals that form relatively weak EDTA complexes are more sensitively determined. However, this also led to unsteady baselines as contaminating metals in the eluent produced high background signals. At still higher pHs a surface effect on the tubing caused band broadening limiting the pH increase.

Future work developing this detection system further would benefit from the use of

more selective complexing agents that can be used to target specific groups of analytes, rather than EDTA which forms complexes with many metals. Complexing agents that do not form complexes with alkaline earth metals such as 1,10-phenanthroline would limit the effect these cations, present as impurities, have on the baseline noise as the pH of the system increases. Further, the high background CL of this system, when using cobalt (II) may be decreased by changing to copper (II) as the catalyst. Copper (II) in general forms more stable complexes than cobalt (II) and should lead to an increase in the signal to noise ratio of analytes and therefore detection limits.

The second chapter involved the development of a single column IC system capable of separating the two chromium species chromium (III) and chromium (VI) simultaneously prior to CL detection. This was complicated by the fact that chromium (III) is a cation whereas chromium (VI) is an anion. This part of the study was successful and a method for separating these species without any sample pretreatment on a single column IC was developed. The use of isocratic pumps fitted with inert plastic pump heads and PTFE tubing throughout made the use of a potassium chloride eluent possible. This system was used successfully for the determination of chromium speciation in synthetic natural water standard and a lyophilised natural water in conjunction with the BCR programme. However, instability of the chromium species in the synthetic natural water was shown and the amount of chromium determined in the lyophilised samples was lower than expected, whilst still within the standard deviation of the collective results. Also, the collective BCR results for chromium (III) were lower than expected.

The separation system was geared towards separating free chromium (III). However,

complexes containing chromium (III) may be present which although slow to form are very stable. Also, speciation of chromium (III) and chromium (VI) alter with pH, as does the oxidising power of chromium (VI). Thus, future work in this area will involve a more thorough investigation into the chemistry of chromium (III) and chromium (VI) in natural waters, including the effect of pH and addition of complexing agents. Also, whilst this system has simplified the chromatography of chromium (III) and chromium (VI) the on-line reduction of chromium (VI) to chromium (III) needs to be simplified further and would be an area for future work. Improvements to this process may result from on-line electrochemical reduction post-column or by the post-column addition of sulphite from a pressurized container. Both these approaches would reduce the number of pumps required.

The third and largest section of this work involved the development of an IC system for the separation of silver (I) from possible cation interferents followed by CL detection. The application was to find sub μg l^{-1} concentrations of silver in PWR primary coolant. In this respect, the study was successful as it was possible to detect silver at 0.05 μg l^{-1} in simulated PWR primary coolant.

The separation of silver on columns with either polystyrene divinyl-benzene (PS-DVB) resin or polymethacrylate resin was investigated. Severe tailing of the silver peaks using the PS-DVB resin was observed but not with the polymethacrylate resin.

The large separation of silver from the solvent front and divalent cations that could interfere with the CL detection system allowed the use of short columns. The ionic strength of the eluent affected the CL detection system, reducing linearity. However,

the use of shorter columns allowed the use of more dilute eluents which are less
detrimental to the detection system.

Future work for the development of this CL detection system exists in two areas. First
the use of a very short, 1 cm long, column with a 0.05 M potassium
sulphate/potassium persulphate eluent is expected to give elution of silver in under 10
minutes coupled with more linear calibrations.

The second area of interest is to use this system on a VVER nuclear power station to
determine silver in the primary coolant. If this is successful then the next objective and
the targeted application of this system is the monitoring of silver in the primary coolant
throughout the reactor cycle and the monitoring of the reagents used in the primary
coolant to discover the source of the silver contamination.

The final section concerns the development of a CL detection system to be used in
conjunction with an existing IC system capable of separating gold (III) as its chloro
complex. Again like other CL detection methods that are selective, sub μg l^{-1} detection
limits were obtained. This method of determining gold gives detection limits that
compare favourably with other analytical techniques such as ICP-MS. Exploitation of
the IC's ability to preconcentrate analytes may lower the detection limits further. Gold
in certified electronic flow solder was determined to within the limits given on the
certificate, even though extremely large dilutions were necessary.

Extraction of gold in mine tailings at levels as low as 0.3 μg g^{-1} is considered to be
profitable. Future work should therefore include the development of this system to

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determine gold in rocks at the levels below this. Also, determination of gold in waters at much lower levels may be used in gold prospecting.

The above work has shown that sensitive selective CL detection can be extended to a greater range of metal ions. Future work should be aimed at providing selective and sensitive CL detection for even more metal ions or alternatively for non-metal ions that cannot at yet be determined sensitively by CL. For example this could involve exploitation of the formation of insoluble silver-iodide as a means of determining concentrations of iodide exceeding the stability constant of silver-iodide. The silver CL system may also be exploited to provide a detection system for compounds that form strong complexes with silver, for example sulphur and nitrogen containing compounds.

Alternatively, reducing compounds may be determined by the reduction of either gold (III) or chromium (VI). Obviously the latter is preferential as reduction of chromium (VI) to chromium (III) would lead to an increase in CL.

Finally, oxalate esters are being developed for use in aqueous solutions. As these become more compatible with the aqueous eluents used in IC then chemically initiated electron exchange luminescence (CIEEL) may be used. Thus, conventional post column reactions that incorporate metal ions into fluorescent compounds may then be more sensitively determined with CIEEL excitation than with radiative excitation.

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PRESENTATIONS AND CONFERENCES ATTENDED

Delegate at the Royal Society of Chemistry (RSC) (Analytical Division) Research and Developments Topics in Analytical Chemistry, Aberdeen, 1991.

Delegate at the RSC Symposium on Chemiluminescence Detection, Bristol, 1991.

RSC Chromatography Symposium, Chepstow, 11 March 1993. **Oral presentation titled: Simultaneous Determination of Chromium (III) and Chromium (VI) in River Water by Ion Chromatography with Chemiluminescence Detection.**

RSC Research and Development Topics in Analytical Chemistry, Birmingham, 1992, poster presentation titled: **Investigation into Multi-element Detection System for Ion Exchange Chromatography using Ligand Exchange Reactions and Chemiluminescence Detection.**

RSC Research and Development Topics in Analytical Chemistry, Bradford, 1993, poster presentation titled: **Simultaneous Determination of Chromium (III) and Chromium (VI) Speciation in River Water by Ion Exchange Chromatography with Chemiluminescence Detection.**