THE UNIVERSITY OF HULL

"CONTINUOUS FLOW INJECTION ANALYSIS"

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by

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To

My wife Roonak

and

My daughters Leelave and Lena

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SUMMARY

In this thesis, new developments in flow injection analysis are presented.

In Chapter One, a description of the flow injection technique precedes a review of its analytical importance.

In Chapter Two, instrumental developments for monitoring chemiluminescence by flow injection analysis are described. A new detector was designed which was found effective in measuring weak chemiluminescent emissions. Using such a detector, a new method is proposed for the determination of hydrazine based on the enhancement by aluminium and some transition metals of its chemiluminescence on oxidation with sodium hypochlorite.

Chapter Three describes a new chemiluminescence method for hydrazine determination using a permanganate-polyphosphoric acid system. This method was found to be more selective than the previous method in that metal ions [except manganese(II)] did not interfere.

Chapter Four describes how a small ion-exchange column was incorporated into the chemiluminescence system for purifying the sample and carrier stream from metal ion impurities. Using a luminol system, it was found that a chelating resin (Dowex A-1) was best for sample purification. When hydrazine was passed through the column, however, chemiluminescence was completely prevented, indicating that certain metal ions are necessary in order to stimulate the luminescence. It was possible, however, to restore the emission by addition of traces of certain metal ions (especially aluminium).

Chapter Five describes the use of a reducing column for metal speciation by flow injection analysis. Reductions of iron(III) to iron(II) were achieved efficiently in a small Jones reductor column, and then the iron(II) determined spectrophotometrically via its 1,10-phenanthroline

complex. Using this reductor, iron(II) ($1x10^{-5}M$) and total iron can be determined by splitting the injected sample so that a portion passes through a reductor column and a delay coil. After recombination with the unreduced portion, the sample stream was mixed with the reagent for spectrophotometric detection. Two peaks are obtained for each sample, the first being a measure of iron(II), the second of total iron.

In Chapter Six, a method is described for the rapid determination of copper spectrophotometrically after its reduction to copper(I) by an on-line silver reductor using bathocuproine disulphonic acid as a reagent.

Chapter Seven describes the development of a new method for the determination of anions using on-line ion-exchange with spectrophotometric detection. The procedure comprises an exchange between the ion to be determined in a sample solution with another one held on the resin column. The spectrophotometric detection of the displaced anion is simple. Anions such as sulphate, nitrate, chloride and oxalate, which are difficult to determine spectrophotometrically, can be determined by exchange with an equivalent amount of thiocyanate, which is determined as its red iron(III) complex. The system was extended by incorporating a small column before the exchanger column to improve selectivity by removing several anions from the sample to be analysed. This allows the simultaneous determination of two anions such as chloride or sulphate and nitrate by splitting the sample and removing chloride or sulphate by a silver reduction column.

In Chapter Eight a preliminary investigation is described for the development of flow injection analysis for facilitating amplification reactions for sodium carried out on two ion-exchange columns.

CONTENTS

CHAPTER ONE

REVIEW OF FLOW INJECTION ANALYSIS

1.1. Historical development of the concept

The technique of flow injection analysis (F.I.A.) has won rapid acceptance in many laboratories due to its general applicability. It may be defined as an automated or semiautomated analytical process consisting of the repetitive injection of aliquots into an unsegmented continuously flowing stream. Following injection the aliquot may be delivered directly to a detector or dispersed in a carrier stream to facilitate sample conditioning prior to measurement. The capability to establish accurately and precisely a wide range of dispersion conditions in the system is the key to the operation and versatility of F.I.A.

Although the recent interest in this technique can be largely attributed to the work of Ruzicka and Hansen in Denmark (1) and Stewart et al. in the U.S.A. (2), individual concepts developed by earlier workers provided much of the basic understanding needed for the development of F.I.A. Since the introduction of gas and liquid chromatography, analyte samples have been injected into non-segmented flowing streams (3-6) and the concentration profile monitored with a suitable flow-through detector. Gas chromatography was already based on sample injection into a continuously flowing stream with continuous recording downstream. The development by Keulemans (4) introduced the concept of injecting the sample into the stream with a hypodermic needle.

Systems based on non-segmented flowing streams of liquid reagents have been described (7-14). The modern idea was introduced in 1970 by Nagy et al. (9). Their initial purpose was to use sample injection into a carrier stream as a means to deliver reproducible sample volumes to an electrode following stirred mixing, in order to effect high analysis rates.

They found the analytical readout to be in the form of transient peaks with baseline resolution between samples.

Intensive investigations in 1972 established basic concepts for flow injection analysis (10, 11). An important approach was enzymatic determinations which involved sample injection into a recirculating unsegmented flow stream. This was the first use of F.I.A. for enzymatic analysis (10).

The system devised by Feher and Pungor in 1974 (14) involved injection of electroactive and non-electroactive samples into a stream of supporting electrolyte. The signal responses were a linear function of analyte concentration in each case. Using a flow rate of 5.8ml min^{-1}, analyses of 60 samples per hour were achieved. The use of the peristaltic pump, sample syringe and area measurements, now familiar in F.I.A., can be traced back to these innovations.

Expanding on these efforts the workers who followed modified the technique. Their major discovery was that the dispersion of the injected sample in the carrier stream could be induced by the flow process alone and did not require any mechanical assistance. Ruzicka and Hansen (1) applied this principle for the spectrophotometric determination of methyl orange in a model system and of phosphate by the molybdenum blue method. A high sampling rate (360 per hour) and good precision(±1%) were obtained. Stewart et al. (2), on the other hand, used this idea for analysing discrete enzyme samples using an automated flow injection system. Their system comprised two flowing streams connected by a simple injection valve. Analysis rates of 100 samples per hour were readily obtained with a precision of better than 1%. Independently, these two groups have introduced many innovations and have greatly advanced the theoretical understanding of the dispersion process in non-segmented flow systems. Hence the theoretical framework slowly being established should facilitate the design of systems optimized for

sensitivity, sampling rate, precision and accuracy.

The new F.I.A. technique was significant, because it had long been thought that air bubbles had to be introduced at frequent and regular intervals in order for a continuous flow analyser to work. The purposes of air bubbles were to limit sample dispersion and generate turbulent flow to promote mixing and to scrub the walls of the analytical conduits to prevent carry-over and cross-contamination between samples. In fact, all three of these functions can be implemented in an unsegmented stream.

F.I.A. was useful in practical applications in fields as varied as water and environmental control and agriculture, pharmaceutical and clinical analyses. Recently F.I.A. equipment has become available commercially but most published work has been performed on "home-made" analysers. A simple but useful F.I.A. system is easily constructed from apparatus available in most laboratories. Subsequent development has been very rapid and a steadily increasing range of applications has now accumulated. Progress in this field is reflected in the huge volume of literature references and has been the subject of numerous general reviews (15-34).

1.2. Principles of F.I.A.

Flow injection analysis is based on three main principles: introduction of the sample plug into an unsegmented continuously flowing reagent (or carrier) stream; constant residence time of the sample in the manifold (reproducible timing); controlled dispersion of the sample zone. A typical diagram of a simple F.I.A. system is shown in figure 1.1. It consists of carrier and reagent streams propelled by a peristaltic pump and an injection valve, connected to a flow cell via a mixing coil. The sample plug injected is bracketed by the carrier stream, which is merged with the reagent stream to bring about a chemical reaction between the sample and the reagent. The reaction product is recorded as a peak as

it passes through the detector.

The operation of the injection valve must be regulated to ensure injection of a precise sample volume, which is required for reproducible results. The timing involved through the system is the most important parameter to be controlled in F.I.A. to achieve the required precision. The pump provides a constant flow, and no compressible air segments are present in the system. Accordingly, the residence time of the sample is undoubtedly constant. The residence time of the sample in the analytical conduits should be identical for each sample and the conditions to which the sample is exposed during processing should also be the same. Any fluctuation in flow rate that effects the residence time will result in imprecise peak heights. The degree of dispersion (or dilution) of the sample can be controlled by varying a number of factors such as sample volume, length and diameter of mixing coils, and flow rate. The dispersion is characteristic of the F.I.A. system. It can be manipulated easily to suit the demands of a particular analytical process.

1.3. Comparison between the continuous approaches of segmented flow and non-segmented flow injection analyses

It was widely believed that a regular pattern of air bubbles had to be present in the flow stream, as occurs in the AutoAnalyser. Skeggs' idea of continous air-segmented flow (35) was the basis on which this consideration was exemplified. The air bubble was purportedly essential to prevent excessive sample dilution, generate turbulent flow to promote mixing and rinse the walls of the analytical conduit to minimize carryover and cross-contamination. The effectiveness of this concept was so apparent that air segmentation, proportional sampling and steady state readout were the foundations of this kind of automation. AutoAnalyser systems are widely established in clinical and industrial laboratories, and this technique of continuous flow analysis has been reviewed at length (36, 37).

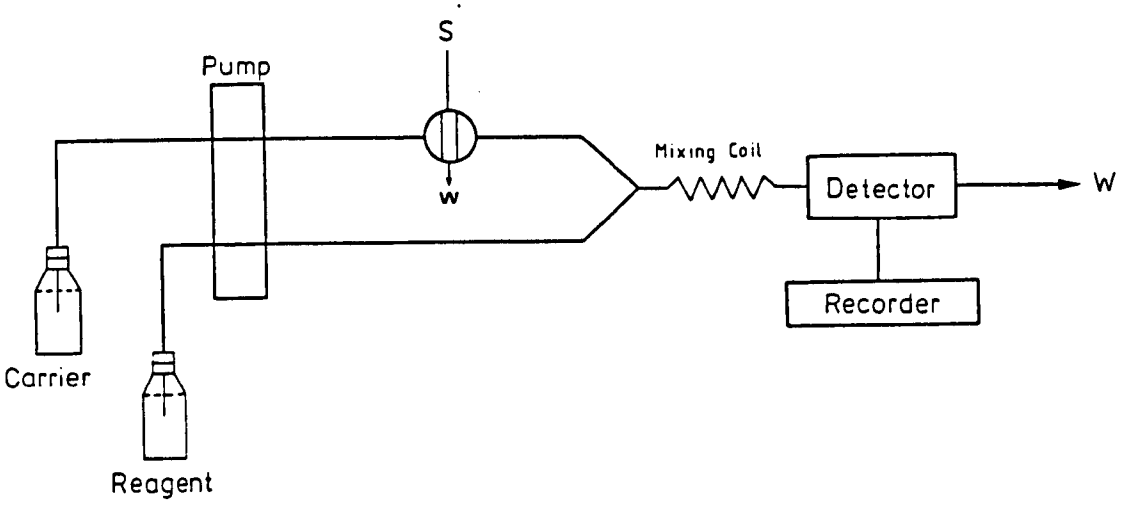


Fig. 1.1. Schematic diagram of a simple F.I.A. system.
S, sample injection, W, waste.

The benefit of this technique was limited because air must be added to and removed from the sample stream before detection and also because of the compressibility of air. This has led to rather elaborate and expensive apparatus.

Although F.I.A. bears a strong resemblance to the continuous flow analysis (C.F.A.) as implemented, for instance, in AutoAnalyser systems, F.I.A. has demonstrated that sample processing can be performed in an analytical stream which is not segmented by air bubbles. Hence a sample zone freely disperses when injected into a carrier stream of reagent. Figure 1.2 illustrates the main differences between C.F.A. and F.I.A. manifolds. (39, 47).

Basically, both techniques comprise the addition of sample to a reagent stream, the flow of the combined stream through a mixing coil for a

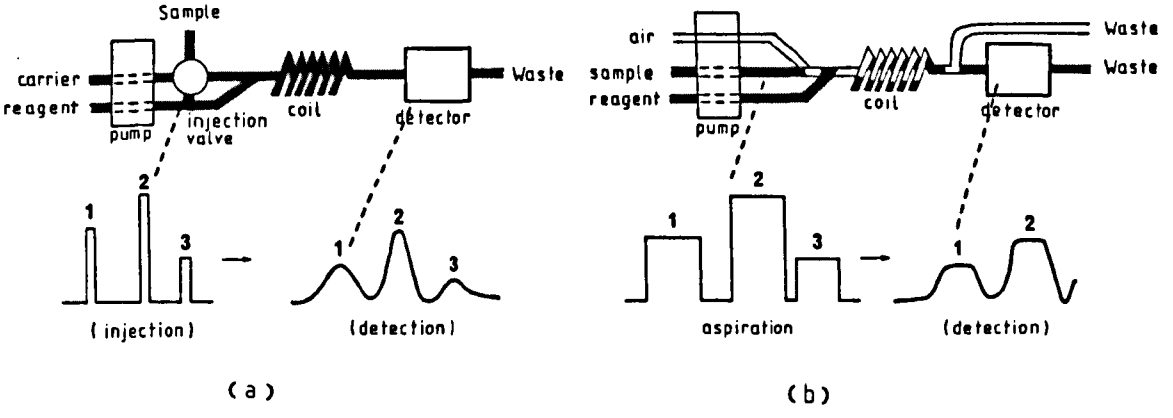


Fig. 1.2. Comparison of manifolds and output sensed by the detector for: (a) F.I.A. (b) C.F.A. (39, 47).

limited time followed by passage through a flow-through detector. In C.F.A. the sample is usually aspirated into a reagent line in a form of a broad slug, as shown in figure 1.2.(b). The combined stream is then segmented with air bubbles and the segmented stream flows through the mixing coil with debubbling of the final stream before detection. However, in F.I.A. the sample is injected rather than aspirated and there is no air-segmentation and hence no debubbling device in the manifold design (figure 1.2.a).

Depending on these characteristic features of C.F.A. and F.I.A. several comparisons of the two techniques have been made (19-21, 29, 38, 39). Table 1.1. summarizes a selection of major differences between the two approaches. There are successful applications for both methods. F.I.A. is more suited to most of the relatively rapid analytical methods due to its higher sampling rate, instant availability of the analytical readout, small sample volume and short start-up time.

TABLE 1.1.

COMPARISON OF THE MAIN C.F.A. AND F.I.A. CHARACTERISTICS

PARAMETER	C.F.A.	F.I.A.
Analytical flow stream	air-segmented	non-segmented
Sample introduction	aspiration	injection
Sample volume	large (200µl)	small $\leq$100µl
Manifold tubing	about 2mm i.d.	<1mm i.d.
System	complicated	simple
Sampling frequency	slow because of air segmentation (up to 80 sh^{-1})	high sample throughput 60-400 samples per hour.
Lag phase	significant	negligible
Reagent consumption	high	low
Sample mixing	within each segment as turbulent flow by interaction of bubbles with the carrier stream and coil walls	injected sample disperses into and thereby mixes with the analytical stream under laminar flow conditions.
Reproducibility (r.s.d.%)	good ($\simeq$ 1%)	good ($\simeq$ 1%)
Data reduction	recorded peak height	integration or peak height/ area.
Flow stream	because of compressibility of air, the stream tends to pulsate	constant flow stream and reduced pulsation because of absence of air bubbles.

1.4. Dispersion in F.I.A.

The F.I.A. approach has introduced a novel use of dispersion. When a discrete sample slug is placed in the moving stream it disperses as it passes downstream under laminar flow conditions, resulting in its dilution by the reagent stream. An understanding of dispersion is essential to achieve optimum conditions for a particular analytical process.

It is important to understand how excessive deposition on the conduit walls is prevented under the laminar flow conditions used in F.I.A. In a long straight tube two mechanisms illustrate the role of dispersion phenomena: Convection is the difference in velocity between the stream lines and diffusion is the movement of molecules along a concentration gradient. These characteristic profiles as stated by Tyson (17) are shown in figure 1.3.

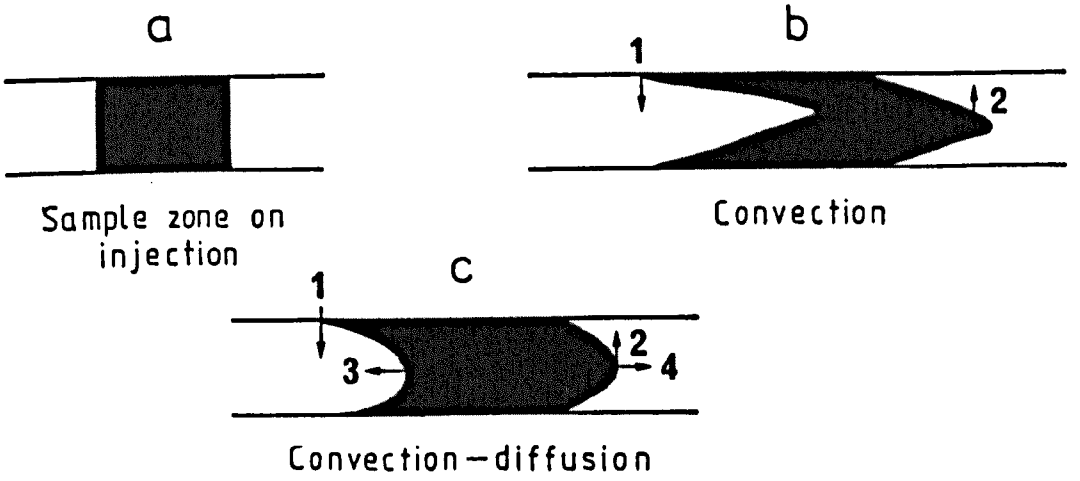


Fig. 1.3. Convection-diffusion mechanism for dispersion.

There is a viscous tug applied to the tube walls, therefore the velocity at the walls is zero. The velocity in the centre is twice the average, and a parabolic velocity profile is produced between the centre and walls, as indicated in figure 1.3.b. Dispersion arising from this mode of velocity causes tailing. Convection produces concentration gradients in the sample zone radially across the tube and axially along the tube. This phenomenon is illustrated in figure 1.3.b. Thus a molecule at position 1 (figure 1.3.c) in the tail of the profile experiences a concentration gradient towards the centre and thus tends to move off the tube wall and into a faster moving stream line. The opposite effect will occur with molecules at positions exemplified by 2 at the head of the profile, which will experience a concentration gradient moving the molecules towards the wall and thus from a faster to a slower stream line. Molecules will also diffuse under longitudinal concentration gradients as shown by the arrows for positions 3 and 4 (figure 1.3.c).

According to Taylor's theory (40), laminar flow in open bore tubular reactors results in plug flow whereby the linear velocity of the moving stream is zero at the liquid-wall interface. Taylor's equation, used to describe radial diffusion-controlled dispersion, is as follows:

$$C = \frac{M}{\pi r^2} \quad \frac{1}{2\sqrt{\pi \sigma L^2}} \quad e^{-(1-x)^2/4L^2\sigma} \qquad 1.1.$$

where σ is the diffusion coefficient, M, mass of material injected at point X = 0 and at time = 0, which moves through a narrow tube of length L and radius r. Equation 1.1 describes a Gaussian peak, which is actually found only with coiled tubing or with long tubes used to increase dispersion. Considering this equation for F.I.A. dispersion, no baseline resolution in output signals will be obtained. The idea of dispersion, however, is successfully reviewed by Vanderslice et al. (41)

They confuted the Taylor theory for interpreting the dispersion phenomena in F.I.A. Taylor's theory, in their consideration, is applicable for a particular case of chemical reactor where either convection or diffusion is dominant in the mixing process as indicated in the above investigations. Basically, in F.I.A. a combination of radial diffusion and convection phenomena is likely to exist (figure 1.3.c).

In the Vanderslice model dispersion in F.I.A. was explained by two equations that describe the sample travel time from injection port to detector (t_a) and the baseline-to-baseline time (t_b) for the peak (41). Four variables affect the dispersion phenomena; they are sample volume, flow rate, tube length and tube diameter:

$$t_a = \frac{109\ r^2\ D^{0.025}}{f}\ \frac{L}{q} \qquad 1.2$$

$$t_b = \frac{(35.4)r^2 f}{D^{0.36}}\ \left(\frac{L}{q}\right)^{0.64} \qquad 1.3$$

Where D is the molecular diffusion coefficient, q, the flow rate and f, the concentration/sensitivity constant. Equations 1.2 and 1.3 might be beneficial for establishing conditions in operating F.I.A. for a particular process.

Dispersion in F.I.A. is measured as the dispersion coefficient D_t (15) defined as the ratio of the initial analyte concentration (C_o) to the concentration of the analyte at the peak maximum (C_{max}) after time t:

$$D_t = \frac{C_o}{C_{max}} = \frac{h \cdot H_o}{h \cdot H_{max}} \qquad 1.4$$

where H is peak height and h is a constant.

Dispersion is most commonly described in terms of the three categories of limited (D_t = 1-3) , medium (D_t = 3-10) and large (D_t >10)

dispersion, each of which is used for different purposes. Limited dispersion is achieved by injecting a relatively large sample volume into a short length of tubing which connects directly to a detector. This is the case when the original composition of the same solution is to be measured. Limited dispersion ensures that the readout at the centre of the sample zone is only slightly affected by mixing with the surrounding carrier stream (e.g. for measurement of pH, conductivity). When this kind of dispersion is operative a large number of samples can be processed as highly transient peaks per unit time. For example the pH of samples (30µl) could be measured at a sampling frequency of 240 per hour (38) using a flow-through capillary glass electrode.

Medium dispersion is employed to create sufficient mixing of the sample and reagent for a chemical reaction to occur. Such mixing must be done in a strictly controlled manner while the sample moves towards the detector. In this case sufficient time should elapse to attain an adequate concentration of reaction product before the detection stage. Colorimetric, and fluorimetric reactions are the major examples. Such dispersion makes it possible to incorporate various analytical techniques to modify the F.I.A. manifold such as liquid - liquid extraction, ion-exchange, dialysis and a reactor column in order to achieve sample treatment.

Large dispersion means large sample dilution. This is particularly used to perform continuous flow titrations (42). This technique was subsequently automated, in which a fixed volume of sample is injected and thoroughly mixed with titrant in a mechanically stirred chamber to form an exponential concentration gradient (43). Usually for the purpose of a titration the width of the sample zone is measured as it passes through the detector, which is proportional to the titrant composition. This is described in more detail in section 1.7.3.

Mathematical analyses of the dispersion concept always contradict

the experimental conditions in such a way that they do not give rise to equations which allow the correct selection of experimental values to attain the required dispersion. Most F.I.A. procedures require a necessary simplifying assumption which lead to inadequate mathematical equations. Many factors are responsible for this inadequacy. For example, the detector may cause dispersion by acting as a small mixing chamber. In addition complications always arise between the short lengths of the dispersed sample zone of most flow-through detectors, and because of the theoretical assumptions that the concentration is measured in an infinitely thin slice across the tube (17). It is rather difficult to attain completely smooth connection between different pieces of tubing in the manifold, which undoubtedly leads to a little turbulence that effects the moving stream. Besides, coiling of the tubing introduces a secondary flow pattern in the fluid due to centripetal forces, i.e. a circulation from the walls to the centre. Many other parameters practically effect the dispersion and as a result such mathematical concepts become inappropriate.

1.5. Optimization of dispersion conditions in F.I.A.

Before any chemical reaction can be adapted to F.I.A., several practical considerations have to be taken into account in order to achieve an optimized manifold design and the necessary dispersion. An important choice is the parameters to which the dispersion (D_t) should be related, such as sample volume, flow rate, tube length, tube internal diameter, effect of coiling diamter and other paramters (15, 44). The dispersion can be simply measured in a simple F.I.A. system, assuming no reactions are involved, by injecting an exact volume of a coloured solution. Visual inspection of the chart recorder tracing at a fast chart speed gives an indication of the effect of the above parameters on peak shape and allows the optimum conditions for dispersion to be selected. At constant

pumping rate the influence of sample volume and tube length is shown in figure 1.4 which illustrates peak height as a function of sample volume and tube length. At any point the fronts of all peaks are the same as the rising part of the steady state curve obtained for a very large sample volume. If reproducibility of flow rate and sample volume is attained there is no need to achieve the steady state to obtain good precision. Therefore sample volume is a critical factor for dispersion control, that needs to satisfy the following equations:

$$C = C_o (1-e^{-kS}) \qquad 1.5$$ (for the rising part of the peak)

$$\text{i.e. } S_{\frac{1}{2}} = 0.693/k \qquad 1.6$$

where S is the sample volume and $S_{\frac{1}{2}}$ is the volume of the sample solution required to reach 50% of the steady state volume, i.e. that gives a value of $D_t = 2$. If the particular situation demands a very low dispersion, e.g. $D_t = 1.3$, a volume of $2S_{\frac{1}{2}}$ is necessary so as to give a signal which is 75% of C_o (a steady-state signals as indicated in figure 1.4.a). Ruzicka and Hansen (15), who considered the above interpretations, also indicated that as the achievement of a steady-state is not necessary in F.I.A., a sample volume of $2S_{\frac{1}{2}}$ is satisfactory for limited dispersion and $< S_{\frac{1}{2}}$ achieves greater dispersion. Therefore, the value of $S_{\frac{1}{2}}$ is apparently dependent on the geometry and volume of the flow-through cell in the detection instrument. Certainly, when using manifold tubing of small diameter, the sample volume required becomes very small because the sample volume occupies a larger pathlength and the centre of the sample zone will be less easily mixed and dispersed. Accordingly, the tube radius must be small if low or medium dispersion is required. However, there is bound to be a minimum tube radius that can be used. Despite the fact that the dispersion is actually decreased when microbore tubes are

used (<0.1mm i.d.) disadvantages such as the ease of blockage and large back pressure retard analytical use, or in some cases make it necessary to use a h.p.l.c. pump instead of a simple peristaltic pump (45).

Ruzicka and Hansen (44) stated that the dispersion increases with the square root of the distance travelled through an open narrow tube (L) and decreases with decreasing flow rate (q):

$$D_t = K_1 \times L^{\frac{1}{2}} \qquad 1.7$$

$$D_t = K_2 \times q \qquad 1.8$$

Where K_1 and K_2 are constants. For a constant flow velocity the residence time (T) is proportional to the dispersion to the second power:

$$D = K_3 \times T^{\frac{1}{2}} \qquad 1.9$$

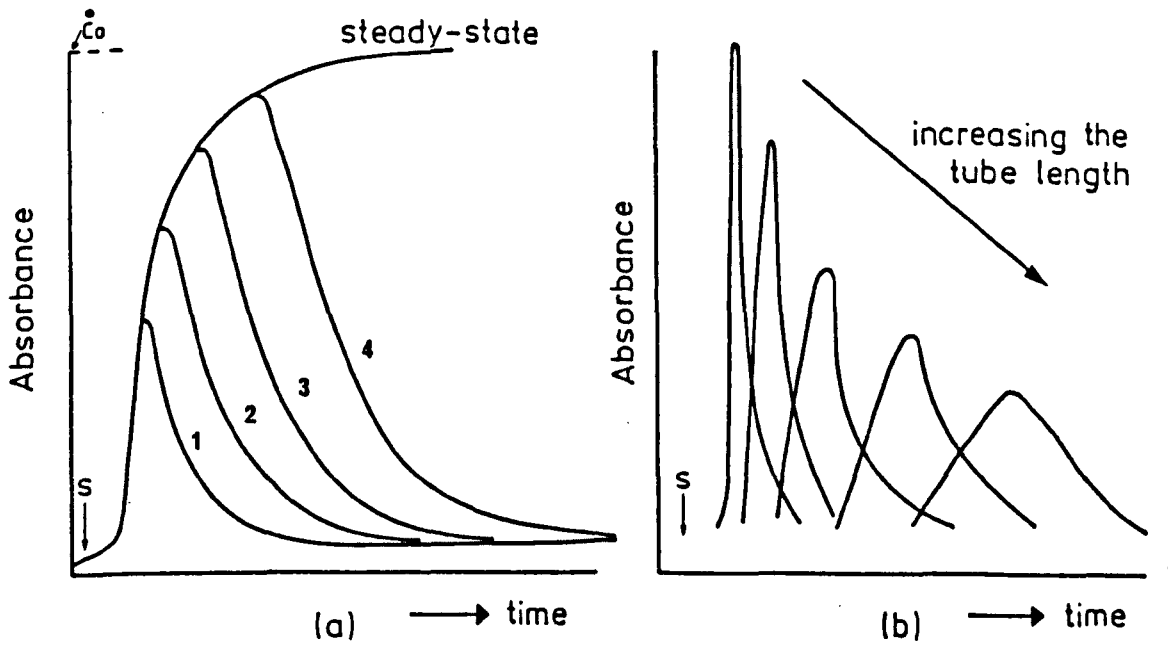


Fig. 1.4. F.I.A. responses as a function of: (a) sample volume (b) tube length. S is the point of injection. In (a) sample size increases in the order 1<2<3<4 ..

Equations 1.7, 1.8 and 1.9 lead to an important conclusion, that the residence time can be readily adjusted by regulating the flow rate. If it is inevitable that a larger residence time is required for a particular chemical reaction to be complete, the tube length can be kept short and the flow rate decreased. Accordingly, dispersion is almost completely prevented when the flow is stopped. Hence the concept of stopped-flow analysis as a means of increasing sensitivity (section 1.7.2.).

A radial flow model in the liquid flowing in the tube which is known as secondary flow is attributed mainly to centrifugal force (45, 46). This phenomenon as described by Van der Linden (47) exhibits similar effects as radial diffusion in that the velocity with which the molecules pass through the reactor is averaged. This was expressed as λ (aspect ratio), which represents the ratio of coil diameter to tube diameter. The expected effect of coiling is to attenuate dispersion (or peak broadening) by enhancing the axial pressure gradient which causes a sample and a reagent to mix faster relative to a straight open tube of the same dimensions in which this factor is of less importance.

Attractive results have been obtained by manipulating dispersion using packed reactors. The particle diameter and the ratio of column diameter to particle diamater are important factors. It was found that the smaller the particle diameter the smaller the dispersion (47, 48). However, it is essential to ensure firm connections between different points in the manifold to eliminate the effect of a large pressure drop across the reactor. A recent development in F.I.A. is the single bead string reactor (S.B.S.R.) (49). Its basic characteristic is that it produces a dispersion which is about 10 times smaller than for an open tube of the same dimensions.

Dispersion on the other hand, can be controlled effectively by variations in manifold design. The target may be to add reagents in an appropriate manner, to decrease reagent consumption and to decrease the

peak widths for high dilutions. An example of this approach is the use of confluence points that produce different mixing pattern to the diffusion-convection process described for a single line dispersion mechanism, and therefore give different dispersion values. Merging zone designs can possibly be achieved with one pump and two injection valves or one injector with two pumps. Splitting the stream before or after the pump and zone sampling can produce a particular dispersion required. Bergamin et al. (50) outlined the possibility of zone sampling applications for simultaneous analysis of species that require a different degree of sample dispersion. This idea was applied to the determination of aluminium and iron in plant digests (51, 52). An extensive review on simultaneous approaches recently appeared in the literature (34).

The fact that dispersion can be controlled, along with the possibility of exact timing of zones, gives opportunities of introducing different sample treatment units such as gas diffusion, liquid-liquid extraction, ion-exchange and dialysis units.

It would be possible to continue at length with the quantitative treatment of these and other effects relevant to dispersion optimization in F.I.A., but it is more convenient to refer to the sources that produced such informations. A rational starting point is a concise review by Betteridge (16). This includes a simple explanation of the convection-diffusion and the tanks-in-series model and a set of basic equations and guidelines for F.I.A. Ruzicka and Hansen (44) have already predicted D values for the simple F.I.A. manifold. Although no obvious distinction was made between the tanks-in-series model and the convection-diffusion concept, they set out rules for the manipulation of dispersion. As pointed out in the preceding discussion, a more successful mathematical approach has been presented by Vanderslice et al. (41). Their basic calculations and estimation of D were derived in terms of peak widths which have led to easier mathematics. Tyson et al. (53) recently

suggested that a simplified version of the tanks-in-series model (one tank) could be a useful basis for describing dispersion behaviour. Finally the dispersion phenomenon itself has been the subject of many papers (39, 45, 48, 54).

The above theoretical considerations are of great importance in optimizing an F.I.A. manifold, but practical investigation remains essential.

1.6. Flow Injection Instrumentation

The ideal set-up for F.I.A. depends on the special needs of the user. In order to ensure adequate operation, thoughtful design of the analytical manifold is necessary. Since sample processing by F.I.A. is based on controllable dispersion which effects specific functions including sample mixing, reaction and dilution, unnecessary fixed system dead volume must be minimized. This enables superior operator control over dispersion by modifying the manifold arrangement, sample volume and flow rates.

Despite the availability of some commercial F.I.A. systems as complete analysers most workers have preferred the use of a home-made analyser. Such an experimental flow injection analyser can be quickly assembled from standard equipment found in most laboratories. The manifold should consist of a pump to propel the flowing stream, an injection valve, a sample treatment component including transport and reaction tubes, a detector fitted with a low volume flow-through cell, and a recorder. In some circumstances, to maximize the benefit of F.I.A., the injection port can be automated and a microprocessor incorporated to achieve a system control and data reduction function.

Since F.I.A. in the following chapters has been used in various analytical developments, it is essential to scrutinize the components of an F.I.A. system and to give observations on the ways in which these

components have developed to their present day form.

1.6.1. Pumps

These are important devices which provide an easy and appropriate way to deliver the analytical stream through the manifold at a constant flow rate. Different types of pumps have been used including those originally devised for liquid chromatography and continuous flow analysis.

The simplest form of transporting a solution is a reagent stream fed by gravity (55). The instrument was designed specifically for the routine assay of enzymes or substrates by which glucose is determined by the glucose oxidase-peroxidase coupled system. The reagent stream is stored in a 200-ml reservoir, fitted with a rubber stopper and air-inlet tube. When the stopper is tight, so that air can enter the reservoir only through the air-inlet tube, the reagent head is maintained at the lower end of the inlet tube, regardless of the liquid level in the reservoir. The reagent flow rate is then constant, being determined principally by the length and geometry of the flowing system. A given rate may be obtained by adjusting the position of the outlet at the end of the entire system. This device was satisfactory for transporting large volumes of reagent at a constant flow rate over a long period of time.

The most relevant means of driving solutions in F.I.A. is the peristaltic pump which is based on providing a low pulsation flow. The operating characteristic of this kind of low pressure pump is based on squeezing the flexible tube held tightly across rollers which are fixed on a rotor. Transporting the analytical stream is accomplished by propelling solutions through these connected tubes as a result of the rollers' movement when the rotor turns. As each tube might either be used for aspirating or delivering the solution, a propelling as well as a withdrawing motion may be expected from the same pump. Most of the

F.I.A. manifolds described in the literature use this type of pump. Examples of the most popular peristaltic pump used is the Ismatec Minipuls (56) and Gilson Minipuls (57) which have up to four channels.

Kelly and Christian (58) used compressed air to drive the analytical stream when F.I.A. was applied to monitor oxidase-dependent reactions with fluorimetric detection. Their pumping device was based on driving separately regulated streams from respective reservoirs through teflon tubing of 0.8mm i.d.

The constant, steady flow necessary for effective F.I.A. may be achieved in many ways. As air bubbles are absent, pulsation is never a major problem. But, however, some workers considered it as a minor effect and depulsing devices have been incorporated in the manifold design. Stewart (59) has referred to this subject extensively and developed one such depulsing system for a positive displacement pump using a flow resistance network with a needle valve as the flow restrictor and pressure gauges as the mechanical ballast. This depulsing system was found to be adaptable to different operating pressures and different flow rates (60). Leach et al. (61), on the other hand, designed a flow injection manifold for trace metal determination in which the carrier and reagent streams are propelled by pressurised helium. This pumping pattern is chosen to avoid flow pulsation which affected the detector response through flactuations in the stream composition at the mixing point.

A disadvantage of syringe and reciprocating pumps is that an individual pump will be needed for each channel and some designs may not be suitable for use with corrosive reagents. Controllable and constant flow rates can be attained upon driving the plunger of a large syringe slowly. Rule and Seitz (62) have used such a low pressure syringe pump in their flow injection-cheiluminescent system. However, the main limitation of this device is its low capacity, arising from difficulties associated with refilling the syringe when all the reagent has been

completely expelled. This might cause an interruption in the analytical process.

1.6.2. Injection Port

The design of the injection device is critical for the effectiveness of any F.I.A. system. It is vital for the injector to be capable of virtually instantaneous flow switching to interfere minimally with the flow. The volume of the sample should be easily selectable and it is essential that a given volume can be injected reproducibly. Basically, two methods of sample injection are in use, syringe injection and valve injection.

Syringe injection through a septum was employed by many workers. Ruzicka and Hansen (1) started this mode of sample introduction by injecting samples from a 1-ml disposable syringe provided with a standard hydrodynamic needle (0.5mm diameter, length 10mm). Filling of the syringe and injection of an 0.5ml sample solution was performed with great precautions for avoiding air bubbles. During chemiluminescent measurements by F.I.A. for zinc and cadmium determination using ion-exchange, the sample (100µl) and eluting solution (50µl) were injected from a glass syringe through a self-sealing rubber septum into a solvent stream (63). Burguera et al. (64) used a particular type of injection assembly where undiluted serum samples were to be injected into a water stream. The sample was injected manually with a Hamilton glass microsyringe (capacity 10µl) through the self-sealing rubber septum of the special sample injector. Their procedure allowed the determination of sodium and potassium in blood serum by using the coupled F.I.A. atomic absorption spectrometric technique.

Many types of injection valve designs have been used in F.I.A. For a variety of applications, all these designs seem to exhibit a similar capability. The main requirement for selecting a valve for a

particular analysis is smooth injection. Flap valves were common during the early development of F.I.A. (65-68). Samples were injected manually by means of disposable plastic syringe through a valve located on a plastic tube through which the carrier stream of a reagent was pumped (66). The valve had a dead volume of less than 3µl, and the samples were forced through it at maximum speed. This approach was simpler to use than the previously described syringe technique (1). This injection procedure was modified later so that sample introduction was attained semi-automatically through a sampling loop which was controlled by two magnetic valves which synchronously opened and closed two pairs of tubes (68). This later design formed the basis of most popular rotary valves.

The methods of sample injections for chromatography (both syringe and valve design) were found suitable in some circumstances for F.I.A. Liquid chromatography septum injector ports were used with manual injection of the sample from a microsyringe (69). This method has been used to determine the therapeutic concentration of lithium in 10µl of serum (70). A liquid chromatographic internal sampling valve (Valco CFSV-4 H P_a-HC) was used to inject the sample into the supporting electrolyte stream. A similar chromatographic injector with a volume of 50µl was used with the manifolds used in a study of phase separators (71). A sample introduction system which resembles the injection block of a gas chromatograph has been designed by Virtanen (72) in a F.I.A. system with multiple ion-selective electrode detectors. The sample was injected with a syringe through a rubber membrane into the flowing background solution or an injection block which employs no membrane. The rate of solution flowing into the injection block was higher than that drawn out by the pump through the cell so a part of the solution flowed backwards out of the injection block. This design, which is shown in figure 1.5, allows very easy injection of sample.

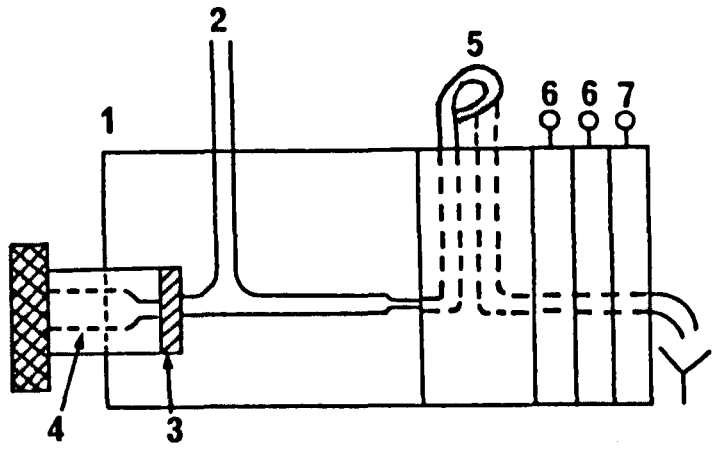


Fig. 1.5. 1. Injection block 2. background solution from pump. 3. rubber membrane 4. tightening nut 5. delay module 6. ion-selective electrodes 7. reference electrode.

Chromatographic valves are still applied in F.I.A. (73).

Stewart et al. (2) have applied a slider valve of the kind commonly used for liquid chromatography. In fact most of these valves are not provided with a bypass, which hinders the analytical streams for a short time during the injection and when the valve returns to its original position. Generally, such valves are applicable with those detectors which are not so sensitive to stream obstructions, such as photometric detectors. This type of valve, however, has been modified by incorporating the loop between the vacant parts of the slider valve and replacement of the existing flow tube with the required tube (74).

A great advance was the use of a rotary valve with a bypass which offers simplicity and higher precision. Figure 1.6 illustrates the operating principle for such a rotary valve (75). It consists of a centre part made of white Teflon sandwiched between two stators made of Perspex. Fig. 1.6.A. shows the case in which the sample fills the bore

b while the carrier stream is connected to the moving stream via bore c. The valve bore is filled with a syringe or by aspiration with a peristaltic pump. Upon returning the centre part manually or automatically all bores are inaccessible to any of the streams and the presence of the bypass will allow the transfer of the carrier stream in an uninterrupted manner as illustrated in Fig. 1.6.B. As a result of turning the centre through 90°, the sample in bore b is introduced to the carrier stream and transferred to the next chemical stage (Fig. 1.6.C). When the sample is in the injection position the stream in the bypass ceases because of its higher hydrodynamic flow resistance than the volumetric bore due to its smaller diameter. The designated arrow in position (IV) indicates the return of the centre part to the filling position again.

Many types of these valves have been described (2, 38, 76) and in some cases their operation is computerized via a microprocessor control system (77). Commercially, a variety of these valves has been constructed. The variable volume valve (one channel) which allows injection of sample volumes of 20-500µl and the two channel injection valve with a fixed volume (30µl) which allows injection of both sample and reagent into separate carrier streams are manufactured by Tecator (78). Although these valves are rather expensive (about £600), they have a high reproducibility of sample injection. Rheodyne (79), however, produce simpler and more practical rotary valves. The body of these valves consists of Altex's totally inert, high performance resin, with low flow passage holes drilled in the body to produce the desired switching pattern. These valves are available in two bore sizes and tube end fittings are colour coded for ease of connection. In the present work, the two-channel injection valve (30µl volume) is used with an F.I.A. system for monitoring chemiluminescent reactions (Chapter 2 - 4) and a Rheodyne-Type 50 Teflon rotary injection valve is used for the rest of the work (Chapter 5-8).

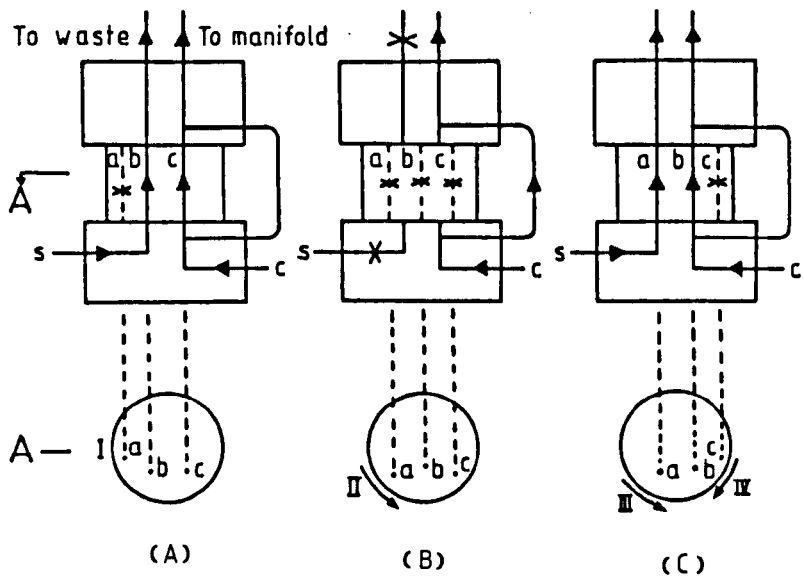


Fig. 1.6. Principle of the function of a rotary valve.

1.6.3. Manifold Components in F.I.A.

1.6.3.1. Basic Components

The manifold is mainly the transport and reaction section between the injection port and detector. Manipulation of sample dispersion in the analytical conduit can easily be accomplished by an attentive design of the manifold.

Different types of manifold pump tubing are now available for use with the peristaltic pump to propel the carrier stream with constant flow rates (80). These include standard pump tubing, accurate flow, standard-running feet pump tubing, solvent resistant, silicone and acid resistant pump tubing. Each of these is available in 20 different sizes.

The manifold is usually made of Telfon, Polyethylene or polypropylene, with an internal diameter of 0.3 - 1mm depending on the

dispersion pattern requisites. In practice Teflon tubing of 0.5 or 0.8mm i.d. is used in most of F.I.A. Tubing of less than 0.3mm i.d. is less practical due to its easy blockage and producing back pressure, as indicated in section 1.5, and a tube radius more than 1mm is liable to cause an inordinate carry-over of the dispersed sample.

Tubing connections can be achieved in many ways, for example by flanging the tubes and then joining them using precision-bore blocks or T-pieces. Today, the Altex plug and its corresponding coupling (Anachem) are the most practical connectors. In practice, this type of connector is the most reasonable fastening device as they provide a complete alignment of the adjacent tubes which leads to a firm connection, and lengthening of the manifold is usually avoided. This mean of connection, however, is applicable mainly when Teflon tubing is used because of the simplicity of flanging. For polyethylene or polypropylene tubes, other means of connection should be applied.

Flangless Omnifit variable-bore connectors provide an alternative means of flexible and reliable connection in F.I.A. Instant leak-tight connections can be made of tubing of diameter from 0.5mm to 11mm. Because flanging is not required, these connectors can be used with rigid or flexible tubing of virtually any material. The connector comprises the following parts; the body is machined of virgin Teflon, end-cups are of different colours, the standard O-ring seals are elastomers and the Teflon cones are used in a few cases where Teflon tubing are connected. Different types of these connectors are available ranging from two-way to eight-way types. Use is often made of these connectors in the work presented in this thesis. In a few circumstances, however, it is impracticable to use any of the above connectors because the experimental conditions required a very short set up. This problem is solved in the present work by using another connection pattern based on inserting two adjacent Teflon tubes through a short silicone rubber tube (about 7mm

long) of a suitable diameter that provides a tight connection. The area around the end of each tube is moistened with one or two drops of cyclohexanone before pushing inside the connector tube. This helps to achieve firm connection and prevents loosening of the tube ends inside the connector, and thus degrading its alignment.

For most practical applications the reaction coils are made by winding the required length of manifold tubing (Teflon or otherwise) round a piece of Perspex or Teflon rod or perhaps glass tubing with a suitable outside diameter (about 2cm).

Progress in the application of F.I.A. demands the incorporation of many devices in order to solve difficulties in performing some complicated mode of analysis, particularly those that require interchange between two streams or splitting the sample stream, changing the direction of one stream to another line, on-off fluid control and interfacing several systems having different input/output terminals. Omnifit was superior for this task because different valve designs could be used whose operations are controlled by movable keys. For example, a two-way valve single key can be used as a connector with an on-off control feature. It may be inserted at any point in the analytical stream to provide linear flow control. By means of two 3-way valve single keys it is possible to divert the fluid stream from one position to another one in the same line without any interruption in the flow. This idea is useful for examining the effects of packed reactors such as reducing columns and ion-exchange columns. In cases where it is essential to study dispersion (for example) in the absence or presence of a column reactor there is no need to stop the pump each time and disconnect the column. An arrangement such as shown in Fig. 1.7 in which two 3-way valve single keys are used facilitates this sort of operation. This mode of manifold operation has been applied throughout the work presented in this thesis in situations which required such devices. It is possible to stop the mixing between

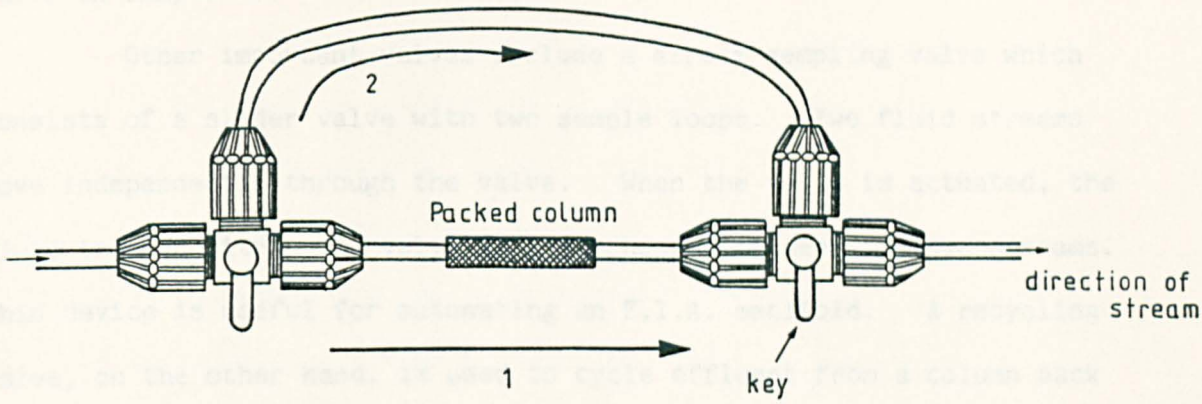


Fig. 1.7. Diversion of a stream by means of two 3-way valve single keys. 1,2 indicates flow paths.

two reagents for a particular chemical reaction without any interruption in the flow. This is achieved by using a 3-way valve 3-key. The same valve can possibly be used to recombine samples by closing one stream line. A combination of these valves can be employed for manipulating any required manifold design that make the F.I.A. system more reliable and accordingly the area of application can easily be widened.

Another interesting tool is a switching valve (4- or 6- way rotary valve which interchanges between two streams. Figure 1.8 illustrates the operational scheme of a 4-way switching valve. The best example of this approach is the application of ion-exchange for separating and determining different species based on sequential elution. By turning this valve the carrier stream is exchanged for an eluting solution stream or vice versa. Malamas et al. (81) used this device when designing

a manifold for on-line trace metal enrichment and matrix isolation by a column containing immobilized 8-quinolinol. The same kind of valves have been applied in a flow manifold used for the speciation of iron(II) and iron(III) using an ion-exchange resin (82). Use is made of a 4-way valve in Chapter 8.

Other important valves include a stream sampling valve which consists of a slider valve with two sample loops. Two fluid streams move independently through the valve. When the valve is actuated, the fluid trapped within the valve is interchanged between the two streams. This device is useful for automating an F.I.A. manifold. A recycling valve, on the other hand, is used to cycle effluent from a column back into the system pump, and out through the column again.

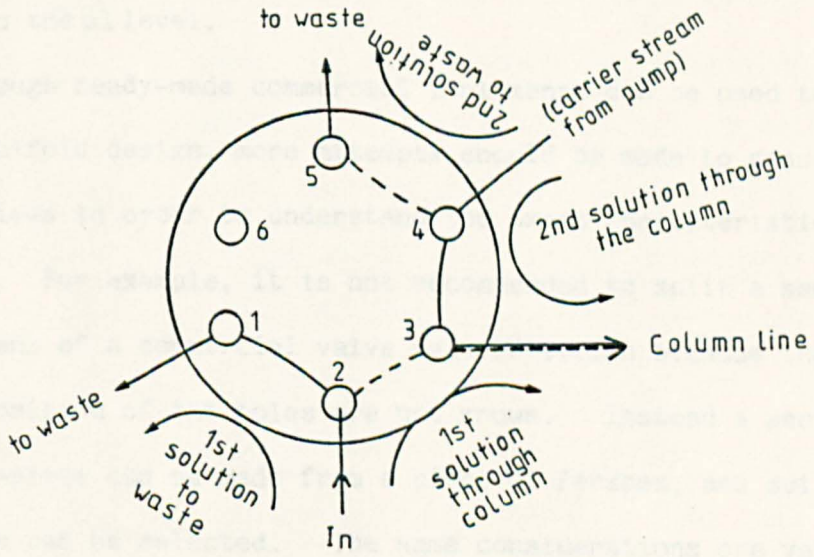


Fig. 1.8. Interchange between two streams by means of a 4-way switching valve. The picture shows a cross-section of the valve.

Some commercial compact manifolds have been designed that perform several analytical processes. An example is the Chemifold, which is a manifold section of the 5020 Flow Injection Analyzer (Tecator) for addition of two reagents (23).

The latest development in F.I.A. is an integrated microconduit (83). The manifold is situated in a permanent, rigid, planar structure. The grooves, forming the flow channels, are engraved into a transparent plate and then are closed by a flat layer, thus forming a structure of conduits with a semicircular cross section. This device was used as a means of integrating a number of solution-handling tasks such as in sequential reagent addition module. A more complex module has been designed for preconcentration of lead and cadmium using a microcolumn packed with Chelex-100 chelating resin (84). The small dimensions of the integrated microconduits allow further reduction of sample and reagent consumption to the μl level.

Although ready-made commercial implements can be used to optimize a manifold design, more attempts should be made to construct home-made devices in order to understand the exact characteristics of such devices. For example, it is not recommended to split a sample segment by means of a commercial valve made of Teflon because the geometrical position of the holes are not known. Instead a perfect geometrical Y-piece can be made from a piece of Perspex, and suitable diameter holes can be selected. The same considerations are valid for a T-piece used to achieve efficient and rapid mixing of two streams. Many such considerations are put forward throughout this thesis.

1.6.3.2. Other Manifold Components

Packed reactors have significant importance in recent F.I.A. development, either for monitoring a particular chemical phenomenon or for optimizing the dispersion conditions in the manifold. Van den Berg et al.

(48) have indicated, on the basis of theoretical considerations, that remarkable results can be obtained with this type of reactor. The most important example of a reactor which has optimized dispersion is the single bead string reactor (49). It consists of a tube filled with glass beads which have a diameter of about 75% of the internal diameter of the tube. Uniform packing is always established and the dispersion was found to be less than in an open tube with the same dimensions. In fact the great advantage of such a reactor is that the residence time can be increased without any change in dispersion because the peak height are almost independent of the flow rate (between 0.2 and 1.5ml min^{-1}) (47). This reactor was recently introduced into many F.I.A. manifolds for increasing the residence time and stabilizing the output baseline (85). A similar reactor is applied in the present work (Chapters 5 and 6).

Different kinds of chemical phenomena have been monitored by using a small column in the F.I.A. manifold filled with particles of a reactive compound. An example of such reactors are ion-exchange resins (63, 82, 86), oxidizing agents (87), enzymes (88-91) and reducing materials (85, 92). A recent development is the introduction of a strongly reducing column in the manifold design (85) in order to produce unstable lower oxidation states of metal ions, which are rather difficult to produce and handle by conventional analytical techniques.

A liquid-liquid extraction unit, which has been described for continuous flow systems, was introduced successfully into the F.I.A. manifold (93, 94). The extraction coils were made of PTFE tubing, which is strongly hydrophobic, with an inner diameter of 0.5mm (95-98). Such an extraction apparatus promotes contact and efficient separation of two immiscible liquids. A typical application was the determination of thiamine (Vitamin B_1) (97). Samples are injected into an aqueous stream of buffered potassium hexacyanoferrate(III) and then combined with chloroform to give alternating aqueous and organic segments to effect bolus

flow through the extraction coil. The phases are then separated, and a portion of the organic stream is carried through the detector (fluorimeter). This demonstrates the adaption to F.I.A. of a relatively complex analytical manifold containing both a reaction and an extraction step. A sampling frequency of 70 per hour was achieved.

Incorporation of membrane separation into the F.I.A. manifold was an elegant development, particularly in the field of clinical and pharmaceutical analysis. Among important applications are gas diffusion and dialysis units. In the first method the gas generated from the sample in one stream passes through a membrane into a collector stream in which its concentration is then measured. Baadehuijsen et al. (99) made a simple manifold for determination of carbon dioxide in plasma. The sample is injected into a carrier stream of sulphuric acid and the carbon dioxide released diffuses through the membrane into a buffered cresol red indicator stream which is detected colorimetrically, with a sample throughput of 90 per hour. The gas diffusion membrane, in other circumstances, may be replaced by an air gap. This technique is called isothermal distillation (100). This was found to be less selective than the membrane method. A dialyzer was first used for chloride and phosphate determination in serum by F.I.A. (68). Other examples are the estimation of glucose in serum (101) and the turbidimetric determination of urinary sulphate after its dialysis into a stream of barium chloride (102).

1.6.4. Methods of Detection in F.I.A.

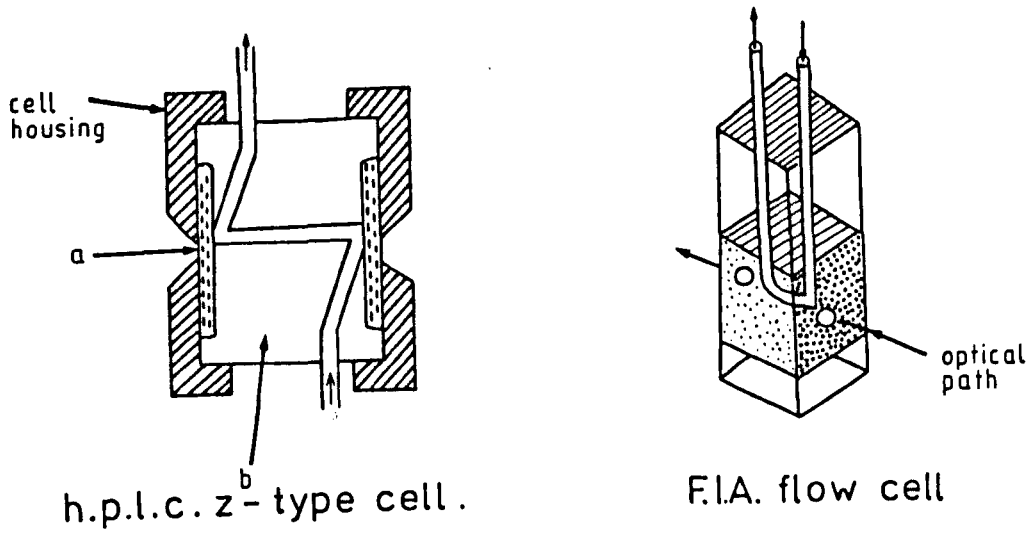
Today, any detector that can be equipped with a flow-through cell is applicable for F.I.A. purposes. The characterization and design of liquid phase flow-through detector systems have been contemplated by Poppe (103). He stated that in order to achieve higher rates of analysis it is necessary to miniaturize the systems. For the detection side this means that low volume detectors, capable of fast response, have to be

developed. Principles for liquid phase flow-through detectors e.g. using uv-visible absorption photometry, fluorimetry or electrochemistry have also been discussed in the same article.

A wide range of novel detection principles in combination with F.I.A. have been developed (44, 104), among which are spectrophotometry, fluorimetry, ion-selective electrodes, chemiluminescence, atomic absorption spectrometry, flame photometry, ICP (inductively coupled plasma), nephelometry, refractometry, voltammetry and polarography.

Photometric methods in the visible range are the most widely employed for detection so far in F.I.A. Commercial spectrophotometers with flow cells of volumes 8-40µl are the most common. It is possible, however, to modify an ordinary double beam spectrophotometer to a flow-through type by fixing a commercial flow cell in the sample beam and an ordinary cell which is covered with a black tape (or black painted), except for a precisely located cell, in the reference beam. Betteridge et al. (105) have constructed a small, inexpensive colorimetric detector. A light emitting diode and a phototransistor sensor are mounted at opposite ends of a Perspex block through which a flow channel and perpendicular inlet and outlet channels are drilled. Application of such a device depends on the range of emission wave-lengths of the diode. The characteristics of these diodes in providing a very stable light source with emission of a narrow bandwidth make them very useful. An improved flow-through phototransducer has also been reported by Sly et al. (106).

Generally, two types of flow-through cells have been used in most photometers. Figure 1.9 illustrates their design (44). The h.p.l.c. z-type cell is made of a transparent window (a) of glass (quartz or sapphire) surrounding both ends of the optical void, forming an optical path 10mm long and 1.5mm diameter. The window and the z-shaped path are fixed in a Teflon body (b). This cell will be connected such that the carrier stream enters at the lower end, to help in removing any air bubble that might be trapped in the cell path.



h.p.l.c. z-type cell.

F.I.A. flow cell

Fig. 1.9. Flow-through cells used in F.I.A. photometers.

Most of the commercial spectrophotometers are provided with the second type, which is a low-pressure flow through cell. They are available with small hold-up volumes of standard dimensions to fit any photometer. This cell is now an important features of F.I.A. as far as spectrophotometric detection is concerned.

Despite the excellent stability of modern single beam spectrophotometers, which have simple optics and use fixed sample cells, some workers in F.I.A. refer to a problem associated with refractive index disturbances arising from temperature gradients or sample introduction, or stability and reproducibility of the reagent blank. Ham (107) first pointed out this phenomenon by investigating the effects of different flow-through cell designs. Leach et al. (61), on the other hand, presented several combined concepts for manipulating the sample to compensate for refractive index and blank absorbance effects on trace level spectrophotometric detection in F.I.A. Their work was based on

injecting samples into a matched carrier stream followed by merging with a reagent stream and combined with series differential detection to overcome optical perturbations from sample introduction and to provide baseline stability. This technique was used for trace metal determinations at the ppb level. In the present work, however, the instability of the baseline was solved by introducing a minicolumn of glass beads (Chapters 5 and 6).

The normal mode of detection used in most spectrophotometric applications is simply passage of the dispersed sample through a single flow cell. In some situations, however, two flow cells have been used either for stream splitting, nonsynchronized detection of two components in the sample or kinetic assays (108).

Ion-selective electrodes have been found to be versatile detection devices in F.I.A. (109, 110, 111). A cascade-type flow cell is used within a very short manifold that situates the electrode as close as possible to the coils and injection port. The ion-selective electrode was inclined at about 30^{o} and the lower edge of the sensing surface was immersed in a pool of carrier stream in which the reference electrode was situated. Differential pumping or a drainage tube can be used to maintain a constant level of liquid in the reservoir. The liquid film covering the surface (about 10µl) is renewed many times per second if the flow rate of 2ml/min is adopted.

Many types of flow through electrodes have been applied such as a capillary-type pH electrode (112) or a flat surface-type glass electrode which was utilized in a F.I.A. titration system (113). Winkel et al. (114) described the characterization and performance of a fluoride selective electrode in continuous and discontinuous systems. They devised a home-made timer which enables samples of the older generation to be coupled to modules for F.I.A. Ion-sensitive field effect transistors have gained many applications in F.I.A. because of their small size and

fast response. They comprise, for example, two sensors, for pH and calcium activity measurement. Ramsing et al. (115) gave details of such a detection device. Various investigations with F.I.A.-ion selective electrodes have been summarized (104).

The use of atomic absorption spectrometry (A.A.S.) as the detection method in F.I.A. has also been reported. The advantages of flow injection for sample introduction are exemplified by Wolf and Stewart (60) who achieved good precision (2% r.s.d.), low detection limits, rapid analysis (120-180 samples per hour) and improvement in nebulizer performance achieved when the flow rate of the carrier is controlled by a suitable pump rather than by the oxidant flow rate. Their automated multiple flow injection analysis allowed determination of 3ng of zinc and 4ng of copper. Yoza et al. (116) also described a manifold which permits the analysis of less than 1ml of sample solution. Reviews of F.I.A. - A.A.S. have been published (53, 75) and most of the applications summarized (104).

Among important applications is the use of organic solvents. Fukamachi and Ishibashi (117) recommended the use of an organic carrier stream for the determination of trace elements. Aqueous solutions of several metal ions are injected into a stream of an immiscible solvent (n-butyl acetate or methyl isobutyl ketone) propelled by the suction of the nebulizer. An extraction process which allowed continuous liquid-liquid extraction of aqueous solutions of metal ions has been carried out effectively (118). A fraction of the organic stream is separated and is led into the sample loop of an injector. The carrier stream is pure water so that the organic solution injected enters the nebulizer as a slug and is not dispersed into the carrier stream. A preconcentration factor of 5.5 has been obtained for copper.

Interesting combinations of A.A.S. and other techniques with F.I.A. have found wide applications. For example on-line flow injection spectrophotometry followed by atomic absorption spectrometry has been

used for metal speciation (119, 120). It enabled simultaneous determination of iron(II) and total iron. Iron(III) was measured spectrophotometrically with a chromogenic reagent and total iron is determined in the same flow line by A.A.S. Other important applications include the possibility of using zone sampling injection and merging zones (117, 121), clinical applications such as the determination of zinc and copper in micro-samples of serum (112) and flow injection calibration methods for atomic absorption spectrometry (53).

A convenient combination of fluorescence and chemiluminescence detectors with F.I.A. systems is a major step forward in the field of flow injection analysis. Kina et al. (123) first used a fluorescence detector in conjunction with F.I.A. for the determination of potassium at the $\mu g\ ml^{-1}$ level. This included the extraction of a cationic complex formed between potassium and a macrocyclic compound, into 1, 2-dichloroethane as an ion-pair with anilinonaphthalene sulphate (ANS). This ion-pair is extracted as the injected aquous plug travels through the tubing; as a result of this extraction the ANS becomes strongly fluorescent. It is important to note that most common flow cells adapted for fluorescence detection in h.p.l.c. are easily applied in F.I.A. Flow cells of 10 - 40µl volume will be adequate for most straightforward applications which are now available such as single channel systems (118, 124-126). More complex systems, such as those based on fluorescence immunoassays, have been developed which demand incorporation of stopped-flow and merging zone techniques (127, 128), and an extraction unit incorporation in the F.I.A. - fluorescent system has also been reported (97, 123, 129).

F.I.A. -chemiluminescent systems are a promising means of detection because of the simplicity of constructing a light-tight detector which enables in some cases $10^{-10}M$ levels of some analyte to be detected. In chapter two, extensive investigations involving this important

combination will be discussed.

Flow injection systems incorporating an inductively-coupled plasma (I.C.P.) emission spectrometer is another elegant combination (30, 130, 131). Jacintho et al. (130) introduced fundamental considerations about this technique by using both the simple single line manifold and merging zones to add either an internal standard or to make standard additions. Greenfield's review (30) gave a comparison between ways of introducing solutions into an I.C.P. by segmented flow analysis and flow injection analysis. A simple manifold was used,and it demonstrated the usefulness of the flow injection analogue of the standard addition method in which the sample is pumped continuously into the nebulizer (carrier stream). In this method simultaneous multielement analyses were performed with a frequency of 100 samples per hour.

As mentioned previously, F.I.A. has been used extensively in combination with a variety of analytical methods, and has nearly unlimited potential. These applications have been thoroughly reviewed (15, 44, 104).

1.7. Some application modes of F.I.A.

1.7.1. Reagent Conservation

An important feature of F.I.A. is normally a continuous flow of reagent stream even when no sample is present. This creates an obvious inconvenience in some circumstances, particularly when an expensive reagent, such as an enzyme, is being employed. This uneconomical approach can be overcome by the merging zone principle, which was first proposed by Bergamin et al. (50). By use of this technique, not only the sample, but also the reagent is injected into a carrier stream, which then meet in a controlled manner and the combined zone thus proceeds into the rest of the manifold. This can also be accomplished by means of a multi-injection

valve (50, 132) which allows simultaneous injection of two or more zones and their efficient merging in an appropriate manner. As the carrier stream consists of a buffer or even of distilled water the sample and reagent consumption in one analysis will be limited to, say, 30µl. Ruzicka and Hansen (20) stated that the merging zone approach to monitoring enzymatic assays might reverse the idea of using insolubilized enzymes not only because of the high sampling rate but also of easier blank and rate measurements.

An advantage of merging zones as illustrated by Ranger (31) is to eliminate several problems associated with sample injection alone, such as eliminating the high blank values and negative peaks when analyzing dilute solutions upon combining the sample and reagent at confluence point instead of injecting the sample into the reagent stream. By this procedure the reagent immediately reaches the centre of the sample zone and hence results in an increase in reaction efficiency and higher sensitivity.

An example of merging zone application is the determination of calcium, magnesium and potassium in plant material using atomic absorption and flame emission spectrometry at 300 samples per hour with a r.s.d. of 0.5% (133). Another is simultaneous determination of aluminium and iron in plant digests by a zone sampling approach (52). Abdullahi et al. (127) used this principle to study the characteristics of drug-protein binding using fluorescent detection. Their experiment includes enhancing the fluorescence of ANS by a factor of many thousands when it bound to a specific site on the albumin molecule.

A combination of merging zones and stopped flow has also been applied. Ruzicka and Hansen (38) used this principle for the determination of glucose. Only 10µl a sample and 26.5µl of reagent were required. The sample-reagent zone was stopped for 20 seconds and the reaction rate recorded. This procedure saved 95% of the enzyme required for the Auto-

Analyser method. The same combined technique was applied by Lim et al. (128) for the determination of albumin by a homogenous fluorescence energy transfer immunoassay.

Another way of conserving reagents is "intermittent pumping", first established by Ruzicka and Hansen (38). This was based on using two independently operating pumps programmed so that one pump operates first until the peak maximum has been reached, then the second pump is activated while the first pump is stopped. The importance of this method is that sample throughput can be increased when a wash cycle is performed with a separate pump of a higher pumping rate, and expensive reagent can be saved by using one pump for reagent addition only when required. Karlberg (75) summarized the main advantages of this technique. Zagatto et al. (134) used this method for the determination of nitrite in water.

An improved flow injection system employing alternating streams of reagent has been designed for the turbidemetric determination of sulphate (135). The procedure includes injection of a sample into a suitable chemically inert carrier stream with further addition of a fixed level of barium chloride followed by flow-through measurement of the turbidity. The performance is improved by the intermittent replacement of the reagent stream by a solution pumped at hight flow rate and the alternating streams are controlled by an electronically operated proportional injector commutator; 120 samples/hr with a precision of less than 1% was achieved.

1.7.2. Stopped-flow F.I.A.

This F.I.A. mode can be used when it is required either to increase the sensitivity of a slow reaction with the aim of allowing time for the development of sufficient reaction product, or to observe a reaction rate when a part of the sample is situated in the flow cell. The first

attempt along this line was established by Ruzicka and Hansen (15, 136) who developed enzymatic methods for the determination of glucose and urea in serum. To achieve only the increase in the residence time it is essential, however, to stop the flow immediately after the injected sample and reagent have mixed. After the stop period, the stream is restarted and passed through the detector. In other cases, e.g. to study reaction rate, the movement of the carrier stream can be exactly controlled so that the same section of the sample zone is exactly held in the flow cell on each occasion. This idea can be explained using the determination of sulphur dioxide in wine as an example (38). Here the sample is injected into a mixture of p-rosaniline and formaldehyde, which with sulphur dioxide forms an intensely red product. Using an electronic timer the mixture is stopped in the flow cell for a fixed period of time until the colour has developed sufficiently. This procedure was found to be very useful in eliminating background problems. Furthermore, stopping the flow allows the reaction to proceed without further dispersion, which would otherwise result in a loss of sensitivity.

The combination of stopped flow with merging zones is an economical way of analysis (50, 132). Great advantages of this combination have been found in enzymatic analysis (38, 128).

1.7.3. F.I.A. Titrations

The ability to titrate an analyte in F.I.A. is important due to the precision, specifity, sensitivity and simplicity of operation. Large dispersion forms the principle of this approach. Discrete samples are passed through a gradient device and are then mixed with a continously flowing stream of titrant of fixed concentration. Ruzicka et al. (42) in their original description of an F.I.A. titration, applied potentiometric as well as spectrophotometric end-point indication. Since

that time a number of authors have studied titrations in detail using F.I.A. methodology (38, 113, 138).

In an F.I.A. titration the use of peak width rather than peak height is predominant for the quantitation of analytical data. The peak width measured in units of time is linearly related to the logarithm of the analyte concentration (42). Hence in order to use peak width as the experimental measurable, a large dispersion is advantageous because it serves to increase precision. A mixing chamber is used in conventional F.I.A. titration. This technique has been automated so that a fixed volume of sample is injected and thoroughly mixed with titrant in a mechanically stirred chamber to form an exponential concentration gradient (43). Ramsing et al. (137), however, replaced the mixing chamber by use of a gradient mixer in the form of a simple flow channel, which led to a high-speed F.I.A. titration. In this method, by monitoring the duration of the titration with an electronic timer, it is feasible to carry out the entire procedure within 10 sec., with a r.s.d. of $< 0.5\%$. The precision was limited by factors such as detector efficiency and the effect of flow rate. Most applications in this area have been summarized (104).

1.7.4. Gradient Principles in F.I.A.

The concentration gradient of the sample slug in F.I.A. is found to have significant potential, with which the role of dispersion can be followed in space and time. By using this principle a suitable element of the dispersed sample zone can be selected and a particular concentration of that element exploited for analytical purposes. This concept has led to designs involving gradient dilutions and calibration (138), stopped-flow reaction rate procedures (44, 139), a gradient F.I.A. titration (44, 138), gradient scanning (44, 140), pH gradients (22, 140-142), zone sampling (121) and a selectivity evaluation method (143).

A comprehensive review of most of these topics has been presented by Ruzicka and Hansen (144).

Usually, evaluation of dispersion is achieved by using a maximum concentration (peak maximum), and no attention is paid to the shape of the sample profile. The proportions between the sample and the reagent clearly vary across the sample zone. The use of this property was put forward by the inventors of F.I.A. (15, 136) on the basis of a combination of stopped flow and gradient dilution. A modern generalization of this approach (144) stated that it is not essential that the read-out is obtained only from the peak maximum, but if desired it is possible to be replaced by the read-out at any other part of the peak, provided that they are always taken at exactly the same delay time after injection. Calibration graphs obtained from data collected at fixed delay times are linear. Some authors believe that certain conditions are essential to manipulate this kind of calibration such as the availability of sufficient reagent until all measurement is accomplished, no side reactions can take place and use of an effective detector that produces a constant response.

Betteridge and Fields (140) developed the use of pH gradients in F.I.A. for multielement analysis in a single sample slug. They stated that if a mixture of metal ions is present in the sample solution and the carrier stream is a reagent solution which reacts with the metal ions, if the sample and carrier are at different pH values, a well-defined sequence of colour forming reactions is to be expected across the interfacial region as the reagent and metal ions react and the pH changes. This phenomenon will lead to the construction of so-called pH-absorbance curves which are characteristics for any given metal ion. The authors utilized this idea by injecting a sample containing lead and vanadium ion at pH 2 into an alkaline carrier of 4-(2'-pyridylazo) resorcinol (PAR) at pH 9. Lead reacts with PAR at pH 9 while vanadium reacts at pH $\geq$ 2. The resulting output was a set of three peaks, the two lateral

ones belong to lead and the middle peak to vanadium. This putput was a result of a mutual discrimination at the two extreme pH values. Applications of this approach were recently reported (22, 141).

1.7.5. Data Acquisition and System Control

Despite the fact that F.I.A. is a very simple technique, some authors believe that the precision in sample introduction and timing between two injections is subject to variation between two different analysts. Also the reading of the output, whether it is digitial or measurement of peak heights varies with different analysts, so in special circumstances the F.I.A. system needs some kind of automation, of at least the injection and readout processes. The progress in some F.I.A. approaches such as the application of merging zones or stopped flow requires a well-controlled system if good precision is to be attained. Microcomputers and solid-state electronics facilitate such control. The results are higher sampling frequency, better reproducibility and elegant instrumental design. Different F.I.A. automation schemes have been employed including control of the various components of the system such as the injection port, sample changer and confluence points regulating residence time and data processing which allows the evaluation of results via peak height, kinetic data, linear regression and standard calibration graph. Microcomputers have been linked to the F.I.A. system in order to facilitate data acquisition and control (44, 77, 137). Stewart et al. (77) described a microprocessor controlled system for automated multiple flow injection analysis. Simple, short and straightforward programming has been established. Programs for routine analyses are easily developed and stored on magnetic tape so that by changing the number of samples on the loaded cassette any given analysis can easily be run. Versatile microcomputer-controlled flow injection systems have been described for the total automation of the stopped-flow technique (138,

145). For example, the work of Kagenow and Jensen (145) involves a controlled method for simultaneous determination of magnesium and calcium in solution.

Such controlled F.I.A. systems have been successfully adapted for pharmaceutical analysis (127, 146, 147). The automatic system devised by Strandberg and Thelander (146) for drug analysis has a microcomputer controlling the sampler and injection valve, reading the output from the detector (spectrophotometer) and evaluating the results. Monitoring of immunoprecipitin reactions using F.I.A. has been accomplished through an automated system based on the application of merging zones with turbidimetric detection (147). In this method the timing of events, the activation of the injection valve, the switching on and off of the pump and reagent introductions are all controlled by an Apple IIe microcomputer through a 6522 VIA board.

Nearly all the methods of automation which have been reported used very expensive equipment which contrasts to some extent with the idea of flow injection analysis being a cheap and simple technique.

PART I

(FLOW INJECTION ANALYSIS FOR MONITORING CHEMILUMINESCENCE DETECTION)

CHAPTER TWO

CONSTRUCTION OF A NEW DETECTOR FOR MONITORING CHEMILUMINESCENCE BY F.I.A. AND SOME ANALYTICAL APPLICATIONS

2.1. Introduction

2.1.1. Fundamentals of Chemiluminescence

Chemiluminescence(CL) may be defined as the emission of light from excited molecules where their excitation energy is supplied by a chemical reaction. Chemiluminescence resembles fluorescence once excitation has taken place. Usually the emitter is the product of a chemical reaction. The product emitter being formed in the excited state.

The possibility of the conversion of chemical energy into radiative energy depends on the type of reaction, temperature effects, and properties of the reactants and products of the reaction, and the medium in which the reaction takes place. Classification of chemiluminescent reactions may be based either on the class of compound, or on the phase in which luminescence occurs, i.e. gas, liquid, or solid. Liquid and solid phase CL reactions are of great importance in analytical chemistry (148-151). Methods of relating light output to analyte concentration in the luminescence area has led to a variety of instrumentation, which has also been the subject of many reviews (151-154).

There are three conditions that must be met so that CL occurs and is detectable. These conditions are:

(1) The chemical reaction must be energetically favourable, i.e. sufficient energy must be released by the reaction so that the formation of the product in the excited state is possible.

(2) The presence of a kinetically feasible pathway leading to the excited state of the product. When the reaction energy is released in the vicinity of the electrons being excited, the resulting electronic excited state of the product is more probable than other excitation modes. The redistribution of the energy released by the reaction among the other

modes is slower than the electronic excited state formation. Conditions 1 and 2 are essential and sufficient for the CL to occur.

(3) A reasonably high quantum efficiency must exist for the chemiluminescent reaction to be useful. The quantum efficiency is defined as:

$$Q_{CL} = \frac{\text{number or rate of photons emitted}}{\text{number or rate of molecules reacted}} \qquad 2.1$$

Although the number of reactions satisfying these conditions is small, many different types of reactions can lead to chemiluminescence. Liquid phase chemiluminescence has been observed for the oxidation of organic compounds such as luminol, from electron transfer reactions of metal chelates and from anion radical-cation radical annihilation reactions. Sensitized CL has been observed for energetic liquid phase reactions that do not lead to an emitting product, by using fluorescent molecules that can accept the energy from the excited product.

Q_{CL} values vary widely, with non-biological systems rarely exceeding 0.01 as compared to bioluminescence for which Q_{CL} is near to unity.

The rate of CL emission is related to the concentration of the analyte by equation 2.2.

$$I_{CL}(t) = Q_{CL} \cdot dC/dt \qquad 2.2$$

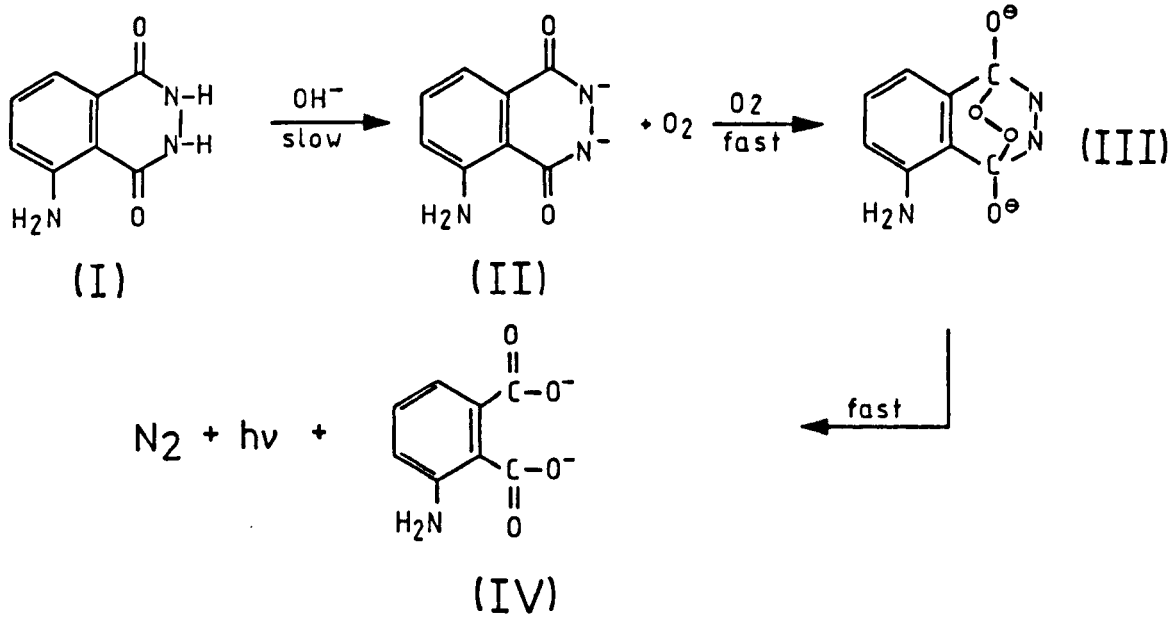
where $I_{CL}(t)$ is the observed instantaneous CL intensity, C is the concentration of the analyte and t is the time elapsed. Either the instantaneous or integrated intensity of the CL is proportional to the concentration of the analyte.

The minimum concentration detectable by CL depends on how sensitively light can be distinguished from the background (i.e. signal/noise ratio). With a good photomultiplier tube, this can easily be as low as a few hundred photons per second. Therefore CL methods are

generally very sensitive. Seitz and Neary (150) concluded that the minimum detectable concentration usually depends on reactant purity.

2. 1. 2. Some examples of Chemiluminescence

Chemiluminescence from the alkaline oxidation of luminol (3-aminophthalhydrazide, I, first described in 1928 (155) is very popular. Many attempts have been made to identify the emitter (148, 150, 156), now established as 3-aminophthalate(IV):



The fact that the luminol oxidation is catalysed by various metal ions allows very sensitive trace metal analysis. There has been speculation about the catalytic effects of metal ions and no agreement was made about any particular mechanism (150, 157, 158).

The oxidation of luminol by hydrogen peroxide in the presence of certain transition metals is attractive as a means for the determination of such metals (148, 150) and of hydrogen peroxide (159). Cobalt(II), for example, is the most effective catalyst and as little as 10^{-6}-10^{-7}%

of cobalt in high purity zinc has been determined (160). Further discussion of this catalytic effect is given in section 2.1.4.

Other chemiluminescence systems include oxidation of lophine (2,4,5-triphenylimidazole) (161) and lucigenin (N,N-dimethyl-q,q-biacridinium dinitrate) (150). The important feature of the latter is that it is catalysed by metal ions, some of which do not catalyse the luminol reaction, such as Pb(II), Bi(III), Tl(III) and Hg(II) (162). Therefore the lucigenin reaction could provide the basis for analytical applications not possible with luminol.

Finally, hydrazine in alkaline solution has been found to give chemiluminescence with sodium hypochlorite (163). This was a direct determination which allowed detection of $\geq 5 \times 10^{-8}$M hydrazine.

There are numerous other examples, which are comprehensively discussed in various review articles (148-151, 162-165).

2.1.3. Light-measuring Devices for Chemiluminescence Measurement

Different techniques have been applied for measuring light intensities including chemical and photoelectric methods. Among the chemical methods the photographic film technique was one of the original artificial means of detecting luminescent reactions (166). The disadvantages of this method are lack of precision (about 10%), the restricted range of light intensity which can be detected, particularly when using very sensitive film, or by extending exposure times to increase sensitivity, a non-linear response is achieved. With the advent of 'instant' emulsions (e.g. the Polaroid system) potentially very convenient luminescent quantitation has become available. Carter et al. (167) have incorporated this kind of photographic detection for chemiluminescent measurements of glucose in which the luminescent reactions were carried out in disposable plastic tubes. The assembly was placed in

intimate contact with Polaroid black and white film.

Direct measurements of the light intensity in the form of electrical signals are an important characteristic of photoelectric methods. The fundamentals of these photoemissive detectors have been reviewed extensively (168). A photoresistor is the cheapest of these devices. It is made of a substance such as cadmium sulphide whose electrical resistance is proportional to the light intensity that falls on its surface. This device is found suitable only for detecting strong CL emissions such as those emanating from the catalytic oxidation of luminol (169).

The basic photoemissive detector is the vacuum phototube. It consists of an evacuated envelope, usually glass or quartz, containing a photocathode and an anode (168). Electrons are produced by the action of light on the photocathode which are collected by the anode. Two basic groups of phototubes have been developed, low-voltage vacuum phototube and high-voltage biplanar phototubes. The performance characteristics (spectral responses) of these tubes depend strongly on the nature of the photocathode. The dark current which flows between anode and cathode is a fixed feature which results from thermal emission, leakage between cathode and anode contacts, field emission and luminescence of the envelope (168). The use of a simple vacuum photocell is again recommended only for measuring intense CL emissions.

The photomultiplier tube (PMT) is admirably suited for measuring low radiant power. This sensitive photoemissive detector contains a photocathode, an electron optical input system, a secondary emission multiplier and an anode. They are two basic types of geometrical arrangement of the photocathode. In an end-on photomultipliers, the photocathode is deposited on the inside of the tube window and is semitransparent. The alternative arrangement employs an opaque cathode situated some distance within the glass envelope. A comprehensive survey

and main characteristics of photomultipliers including types of PMT, photocathode and dynode systems, spectral properties, responsivity, linearity, stability, gain-related characteristics, dark-current and noise and applications is available (153, 168, 170, 171).

The use of photomultipliers for CL detection is advantageous in that they have a large linear range and they are not very expensive. The external equipment necessary is simple, consisting of a power supply (up to 1500V) and a resistive network to provide the accelerating voltage demanded; no complex electronics are required for the output signal, a normal chart recorder being used.

Other devices called multichannel detectors based on instantaneously scanning of the spectrum such as the silicon-vidicon computerized detector (172) and fast scanning spectrophotometers including intensified diode array spectrometers (173) are also used for CL detection.

2.1.4. Significance of metal inhibition and activation in CL analysis

Inhibition and activation effects are of particular interest in chemiluminescence. Trace metal analysis based on the effects of these metals on CL measurements has received much attention. The intensities of luminol and lucigenin systems are often substantially modified by the presence of some metal ions (148, 150, 174). Paul (175) has stated that substances which lower the CL intensity without affecting the total light output are termed inhibitors, but if the quantum yield is also suppressed the material is referred to as a quencher. The contribution of these metal ions is either by chemically removing a reactant or by "mopping up" radicals. Several metal ions, however, increase CL emissions via pronounced catalytic effects. Frequently, the maximum light intensity is strongly increased and the duration decreases.

Several metal ions are known to catalyse or inhibit the CL of luminol and lucigenin, and good reviews of their use in analytical

chemistry have been presented (148-151, 175). In general, while the catalytic mechanism remains unclear, luminol and lucigenin have been employed for the determination of many trace metals which catalyse their oxidation. However, the only problem associated with these determinations is the lack of selectivity in that at least 25 different metals enhance the luminol system and 20 different metals enhanced light emission from the lucigenin system.

Inhibition by metals is analytically less applicable than catalysis, but a few trace metal analyses based on inhibition have been reported (63, 176, 177). The fact that the emission intensity of the enhanced CL reaction is proportional, over a wide range, to the concentration of the trace metal ion is an important characteristic for analytical applications. Most effort has been concentrated on the luminol system. Methods using photographic detection have been reported for Co, Cu and Fe (160, 178, 179). Selective methods for Cr(III) and Fe(II) have been developed using photoelectric detection and have been applied in both environmental and biological samples down to ppb levels (180-182).

The great sensitivity of metal catalytic and inhibiting effects has led to the development of instrumental modifications. The use of ion-exchange separation has solved to a great extent the non-selectivity of metal catalysis and this of course has widened the scope of the applications. Ion-exchange has been applied in procedures for luminol CL detection of two chromatographically separated metal ions (183, 184). These procedures, however, have not found any further applications.

The catalytic process has also proved capable of resolving different chemical forms of trace metals. For example Cr(III) catalyses the luminol system efficiently while Cr(VI) does not catalyse the reaction at all (180).

There is a demand for other CL systems to avoid the limitations of the luminol system with regard, for example, to selectivity.

Lucigenin is the second most popular CL reagent for trace metal determination (185, 186). The advantages over the luminol system are lower detection limits for some metal ions (Co, Ru, Os, Ag, Cu and Cr) and improved conditions for determination of some elements such as Mn(II), Tl(III), Pb and Fe(II).

Another CL reaction system is lophine with H_2O_2 in alkaline solution, which has been investigated for use in the determination of traces of metal ions and other inorganic species (173, 187). Generally, the detection limits were found to be worse than for luminol or lucigenin systems. Only 11 species show enhancement, therefore the lophine reaction shows greater selectivity than the above systems. Nevertheless, lophine is unlikely to become a popular reagent because of its fairly high cost and poor stability in solution.

2.1.5. Chemiluminescence Instrumentation Based on Continuous Flow and Flow injection analysis

The rapid growth of flow injection analysis which utilizes chemiluminescence techniques reflects their many advantages relative to conventional procedures. As CL reactions usually produce transient emissions and because flow injection methods provide for rapid reproducible mixing, it is possible, therefore, to generate such emissions in a flow system, and the emitting species can be instantaneously measured as it flows through small coils of tubing in view of the detector. By appropriate choice of flow rate and tubing diameter it is possible to achieve rapid mixing without extensive sample spreading.

For F.I.A. or any other continuous flow analyses, two parameters are important, detector efficiency and mixing. The detector should be capable of efficiently collecting the light expected. To achieve that the reaction cell and the detector should be as close as possible. The rate of mixing, on the other hand, depends on the rate of the luminescent

reaction. Figure 2.1. illustrates the effects of mixing and reaction rate on peak height and shape produced by CL in F.I.A. (150). It shows that mixing must be efficiently controlled so as to achieve reproducible and sharp emission signals. As most of the CL reactions take place over a time scale of a few seconds, the above parameters can be improved by using an instrument that provides rapid mixing. Hence, modified F.I.A. fulfils these CL requirements.

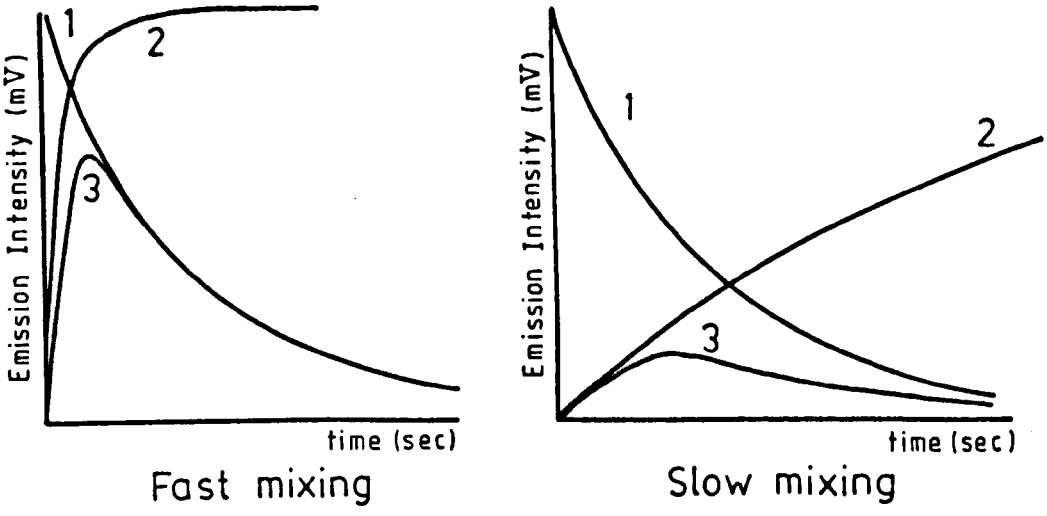


Fig. 2.1. Mixing and reaction rate profile: (1) reaction rate as a function of time, (2) extent of mixing Vs. time, (3) CL signals vs time.

Progress in monitoring CL reactions in flowing streams has been made in continuous unsegmented (188) and air-segmented flow systems (180, 189) and in liquid chromatography detectors (183, 184, 190). Such continuous modes of detection, while generally popular, require elaborate or expensive instrumentation to deliver the analyte and reagent stream and

are not very conservative of reagents. Besides, a typical relative standard deviation (r.s.d.) range of 3-5% and low sample throughput are associated with these methods. The main disadvantages of air-segmented continuous flow analysis were that mixing is slow, and it is necessary to remove the air bubbles before detection, which of course produce an obvious interval between mixing and measuring the emission. An example of this approach is the work carried out by Snyder et al. (189) in which the mixing of sample and reagent is brought about by the natural flow pattern in each segment of solution.

However, with the aim of optimizing conditions for continuous mixing and detection of low CL emissions, different types of flow systems have recently been designed (191-194). Although each system was particularized for a given analytical measurement, all these systems provided better CL detection (151).

The CL emission measurements for triethylamine determination were carried out in an F.I.A. system, the sample being injected by means of a 1.0ml glass syringe through a self-sealing rubber septum (195). The analyte and oxidant were mixed in the reaction cell in front of detector (Cecil CE spectrophotometer with light source removed). The problem was the presence of the mixing cell of large dead volume which caused excessive peak tailing. Another system for peroxide determination has been designed for an unsegmented flow system (62). The flow is driven by a compact infusion pump capable of variable flow rates. The detection system consisted of a coil of tubing (41cm long) pressed against the photocathode of a RCS 9558QA PMT. The system is sealed from background light by draping the PMT and coil in layers of black cloth and by wrapping exposed positions of tubing with black tape to prevent a light-piping effect.

Burguera et al. (196) have reported some further improvements in

using F.I.A. for monitoring CL reaction in which the mixing chamber (195) is again replaced by various coils placed in front of the detector. The flow system using a coiled cell provides an extremely sensitive and simple means for CL reactions. A great advantage was the very small blank emission.

F.I.A. with CL detection has been used to determine traces of cobalt(II) by means of the gallic acid-H_2O_2-NaOH system, containing small amount of methanol to increase sensitivity (197). The detection flow cell consists of flexible, transparent PVC tubing (0.8mm i.d., 90cm length) spiralled to a diameter of 42mm on the adhesive surface of a piece of gummy tape. The gummy tape is stuck on a PMT cooler, so that the flow cell faces the PMT. This work permits the determination of 40ng l^{-1} of Co(II) at a sampling rate of 20 per hour with a r.s.d. of 6% for 0.6ng of Co(II).

Olsson (198) determined H_2O_2 with a flow system using micro-peroxidase as a catalyst for the luminol CL reaction. The F.I.A.-CL detector was an LKB-Wallac Luminometer 1250 modified to accept a flow through cell. The cell (80µl volume) was a W-shaped glass tube, 1mm i.d., mounted close to the PMT with a mirror behind. Mohant et al. (199) also modified a fluorimeter by removal of its light source and replaced it with a 100µl silica flow cell for the determination of fluorescein and fluorescein-labelled species using CL detection.

Finally, the idea of a flow coil as developed by Rule and Seitz (62) and a T-piece joint as a means of rapid mixing during CL measurements (198) were applied by Wheatley (163) to construct an F.I.A.-CL detector for monitoring some chemiluminescent reactions. Basically, the detector was modified from those reported previously (197, 198), which are based on a mixing-junction, flow coil and a PMT as a detector. In the latter case (163) the T-piece junction was positioned inside the light-tight box and connected to the flow coil with a minimal distance

between them. This allowed the dispersed sample and the reagent to mix completely at a T-piece, before travelling a very small distance to a spiral flow cell in front of a PMT.

2.1.6. Summary and Research Objective

So far, the majority of CL measurements by F.I.A. were made using a modified spectrophotometers or fluorimeter, with their light sources were blocked or removed. The presence of a monochromotors (either filter or grating) impedes the monitoring of CL either by preventing a large proportion of the light from reaching the detector and by requiring a significant distance between the reaction cell which the light is produced and the detector.

Owing to the nature of the CL process the emission of light often starts immediately upon mixing the reactants, so if the distance between the mixing point and the detection unit is significant the intensity maximum is likely to have occurred before the emitting solution has reached the detector, thus leading to insensitive measurements. Therefore modifying commercial instruments is in general improper for CL monitoring, particularly for detecting weak CL.

In other cases where a PMT is used as a detector in F.I.A., difficulties of providing efficient mixing and maximum light collection were important when a single-line manifold was used (62) or when the mixing point (T-piece junction) was positioned outside the detector housing with a somewhat large distance between this and the flow cell (197, 198). The latest attempt (163) seemed to be a compromise by situating the T-piece and the reaction coil in a housing in front of a PMT. Although this system has been used for some CL detections, it had some defects, for example, it was suspectible to mechanical damage, and there was a ~~continuous~~ background noise due to inefficient design of the housing, so that some stray light reached the PMT.

In this laboratory, at the time the project of combining CL with F.I.A. was put forward, no detector existed to accomplish that. Therefore it was decided to design and construct a new F.I.A.-CL intensity monitor to be used as a reliable detector not only for the work described here but also for monitoring other research investigations. The inspiration of the detector design is due, in part, to the latest investigation of Wheatley (163) in which the T-piece junction and the flow coil are put in a light-tight housing. It was intended that the new detector would overcome to a great extent the major disadvantages associated with the preceding attempts mentioned above and to use the new detector for monitoring some analytical applications of low intensity CL reactions (e.g. oxidation of hydrazine by hypochlorite).

2.2. Experimental

2.2.1. Construction of a continuous flow injection detection system for monitoring chemiluminescence (F.I.A.-CL detector)

2.2.1.1. Detector Housing

This is the most important feature of the apparatus. Care must be taken in designing the detection device in order to achieve maximum collection of the transient emissions produced by the CL process and to give full protection to the detector, especially from stray light and mechanical damage. The housing (30 x 16 x 42cm) is a compact stainless steel box covered tightly with thick black insulating tape. Figure 2.2. shows the detector compartment which includes the following constituents:

1. A Perspex T-piece for mixing the streams.
2. A glass flow coil.
3. A P.M.T.
4. Simple electronic equipment for the operation of the P.M.T.

The aim of using the Perspex T-piece junction is to create fast and efficient mixing of the sample and reagents; this minimizes inordinate tailing produced if other means of mixing are employed. The T-piece used was constructed simply by drilling accurately a selected Perspex fragment (32 x 16 x 7mm). Figure 2.3 illustrates the design. The internal diameter of the drilled channel bores is 1mm, to which Teflon tubing of suitable diameter is forced inside so that tight connection is achieved. The Teflon inlet tubing is painted black outside and covered tightly with black silicone rubber tubes. The two tubes pass through two holes in the detector housing, and are attached thereto with a strong adhesive (Permabond), so that any movement of the tubes outside the detector housing does not affect the part inside; in this way a rigid position for the T-piece is established.

The coiled flow cell is similar to those used previously (163, 196). It was made by winding a desired length (about 39cm) of 0.8mm i.d. glass tubing. The coil is fixed by means of a transparent adhesive (Bostik 1) to the surface of a black plate, the central area of which had previously been covered with a sheet of aluminium foil (60 x 40mm). The foil enhances the collection of the emitted light. Figure 2.4 is a schematic diagram of the reaction coil as it appears inside the detector housing. One end of the coil is connected tightly to Teflon tubing which is then covered with a black silicone rubber tube, to discharge the final solution to waste via another hole in the housing. The other end was passed through a hole at the centre of the plate, and was connected to the Perspex T-piece by a very short piece of Teflon tubing which was pushed inside the T-piece and the coil. An adhesive (Bostik 1) was also used to cover the small gap between the T-piece and the coil to ensure a durable connection. The distance between the centre of the coil and confluence point of the T-piece was about 6mm.

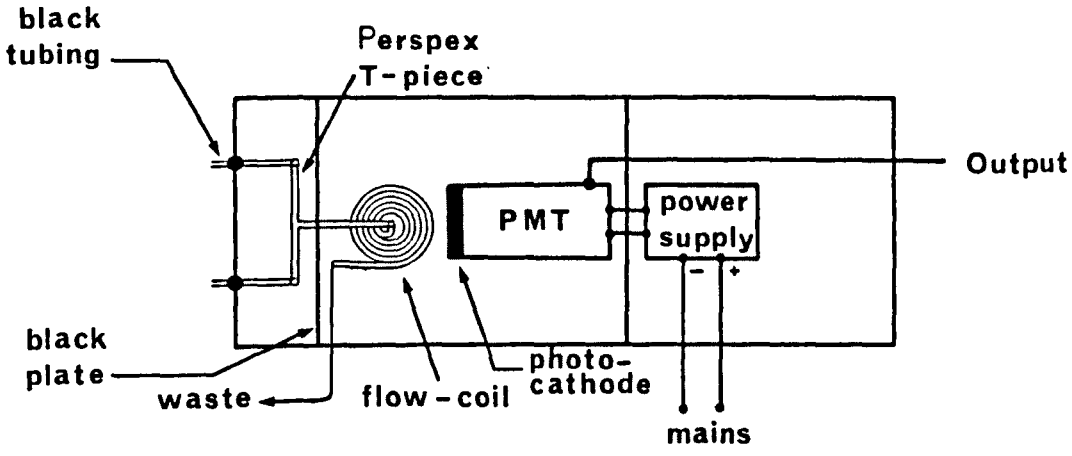


Fig. 2.2. Detector housing (the flow coil is turned through 90^{o} to illustrate its design)

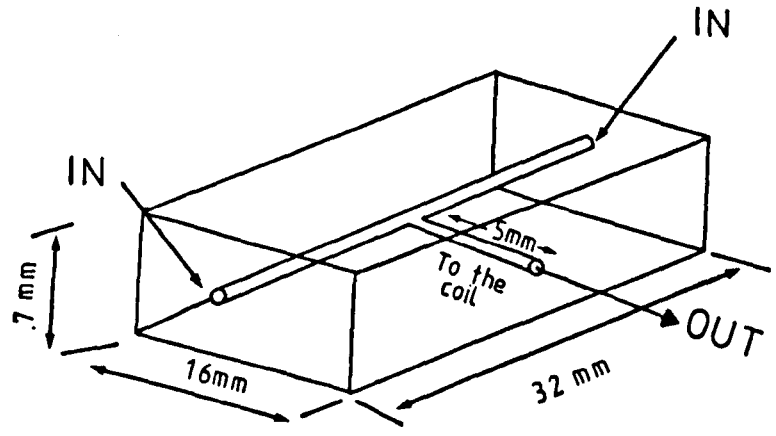


Fig. 2.3. Perspex T-piece.

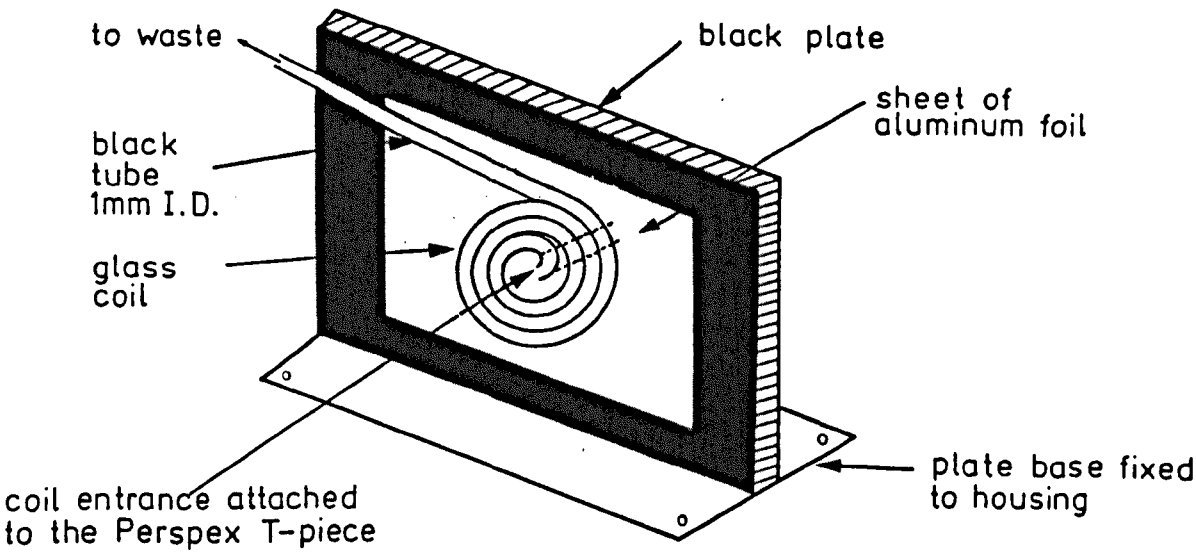


Fig. 2.4. Reaction coil and its location in the housing.

Because this distance is so short, the emitting solution arrives almost instantly in the coil and remains in front of the detector for a sufficient time to measure most of the emitted light.

A photomultiplier as a detector in chemiluminescence measurements is very satisfactory because of its sensitivity to the very low light levels anticipated. For the present work a semitransparent end-window type P.M.T. (EMI, No. 9844B) was used. Because a P.M.T. is easily damaged by exposure to external radiation and therefore to prevent changes in its performance, arrangements were established inside the housing so that the P.M.T. was separated from most of the other components in another light-tight space. Other electrical equipment occupied a separate part inside the detector housing. It consisted of an E.H.T. stabilized power supply (up to 1500V) and a sensitive network to provide the accelerating voltage essential to give the high gain.

An end-window type P.M.T. used as a detector in F.I.A.-CL system offers several advantages such as the large area of its photocathode

is capable of detecting very weak emissions, there is efficient and uniform collection of light over the entire front surface of the tube, and it is quite small (94 x 52mm diam.). The spectral response of the bialkali photocathode was suitable for monitoring the CL reactions studied. Maximum response between 300-500nm was typical with this PMT tube (200).

As discussed above the presence of black tubing eliminated light piping effects. It was also easy to open the lid of the detector housing to work on the flow system in daylight as an internal cover protected the photomultiplier tube. The housing was earthed, and it was safe to remove the lid while the power supply was connected. A photograph of the open detector is shown in figure 2.5.

2.2.1.2. Flow apparatus for the F.I.A.-CL System

Modern high sensitivity monitors such as chemiluminescence detection require a low pulsation flow to give a really stable baseline. A 4-channel peristaltic pump (Minipuls 2, Gilson) was found to accomplish this, so it is used in the F.I.A.-CL system, and 0.051in i.d. standard pump tubing is used with this pump to propel carrier and reagent(s) streams. The sample is introduced into the carrier stream through an injection port of an accurately made dual channel rotary valve (Tecator) with a bore of volume 30µl and furnished with a bypass of higher flow resistance.

In all experiments Teflon tubing of 0.5mm i.d. was used for the flow system. The emission signals produced are recorded by means of a TE200 Labwriter recorder (Tekman LTD) with an output sensitivity range 0.05mV - 100V and speed control range betweeen 10mm/hr and 600mm/hr. The new system has been used throughout all the experiments, in a semidark room in which the daylight and artificial light are at a low level, thereby diminishing further the possibility of stray light piping effects. The complete F.I.A.-CL system is shown in figure 2.6.

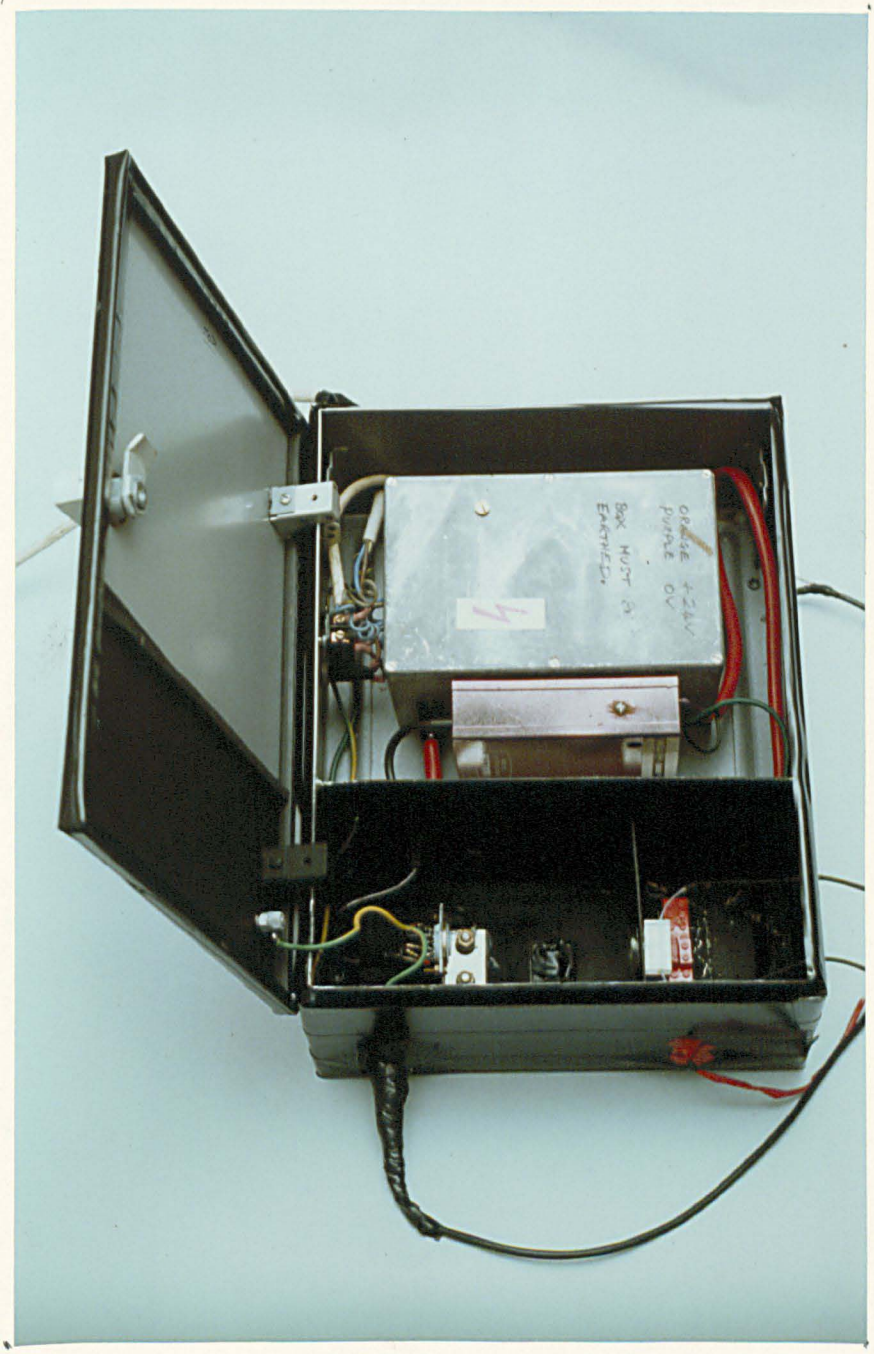


Fig. 2.5. Photograph of the detector housing for the F.I.A.-CL system (The P.M.T. is removed).

Fig. 2.6. The complete F.I.A.-CL system.

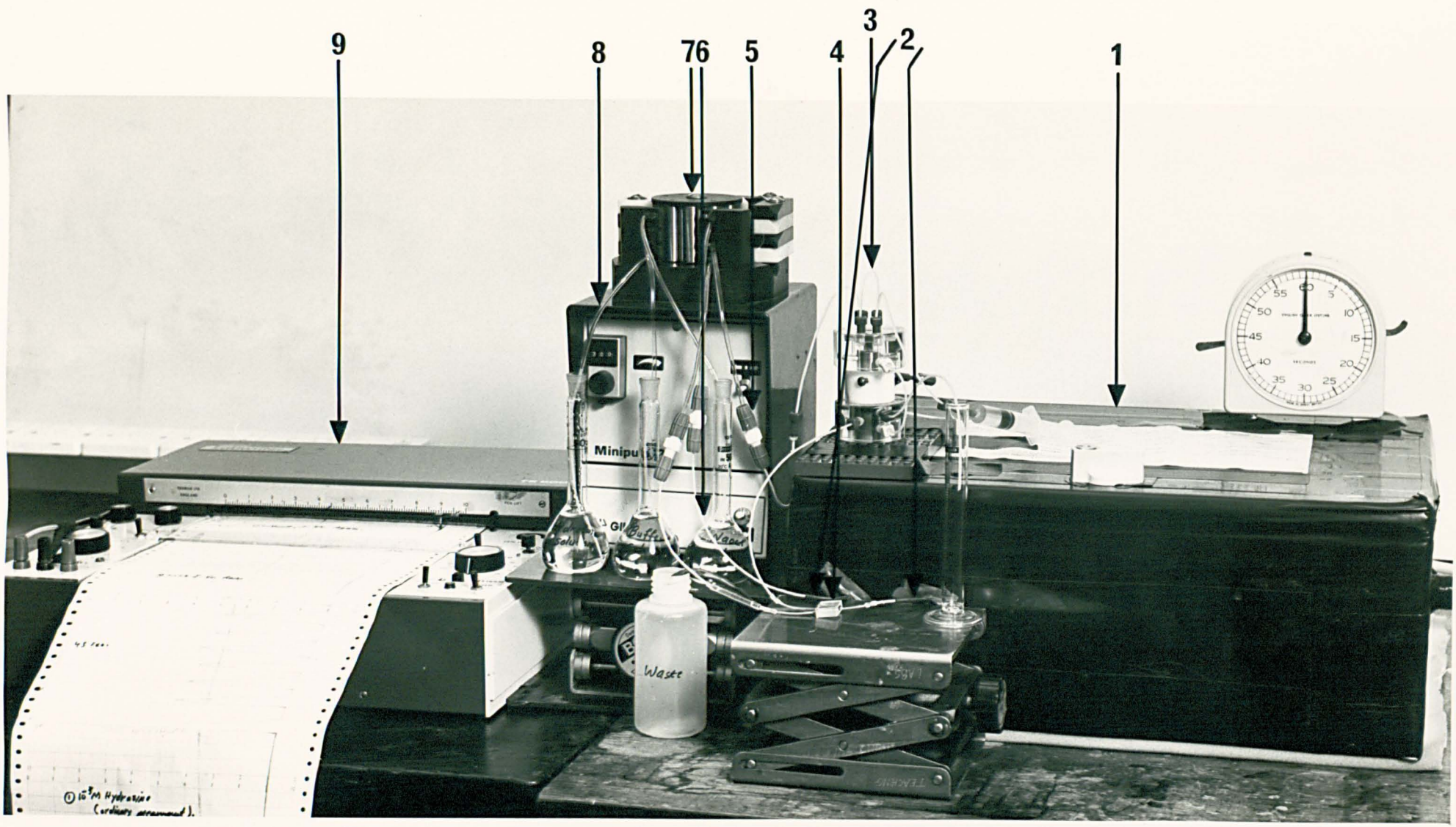
(1) Detector housing. (2) Black tubing (0.8mm i.d.).

(3) Injection valve (Tecator). (4) A home-made Y-piece.

(5) Omnifit connector. (6)Teflon tubing (0.5mm i.d.).

(7) Gilson Minipuls 2 peristaltic pump. (8) Pump tubes.

(9) Recorder.


9
8
7
6
5
4
3
2
1
Waste

During all analytical applications with this detector, an "Elgastat Spectrum deionizer" was used to provide ultra pure deionized water and all reagents employed were analytical grade unless otherwise stated.

The new detector has found rapid acceptance in this laboratory. Soon after the aims of the present project were accomplished, the detector was used for monitoring other F.I.A.-CL procedures. Worsfold and Nabi (201) monitored bioluminescence assays; for the determination of ATP using firefly luciferase, a detection limit of 10^{-14} mole ATP was achieved. Al-Tamrah and Townshend (202) employed the detector to determine sulphite on the basis of its oxidation with acidic permanganate in the presence of riboflavine or 3-cyclohexylaminopropane sulphonic acid; 1.2-8ng of sulphite can be determined with a relative standard deviation of 2%. Sulphur compounds (sulphite, thiosulphate, metabisulphite and dithionite) have been determined by oxidation with cerium(IV) in the presence of 3-cyclohexylaminopropanesulphonic acid (203). A detection limit of 0.4ng for these ions was achieved. The detector is continuously in use by other workers for examining various analytical phenomena based on flow injection chemiluminescent detection.

2.2.2. Testing the new F.I.A.-CL System

2.2.2.1. Determination of cobalt using the chemiluminescent oxidation of luminol

The cobalt(II)-catalysed oxidation of luminol by hydrogen peroxide (195) has been studied as a typical chemiluminescent reaction of analytical utility to investigate the performance of the new system. The manifold used is shown in figure 2.7.

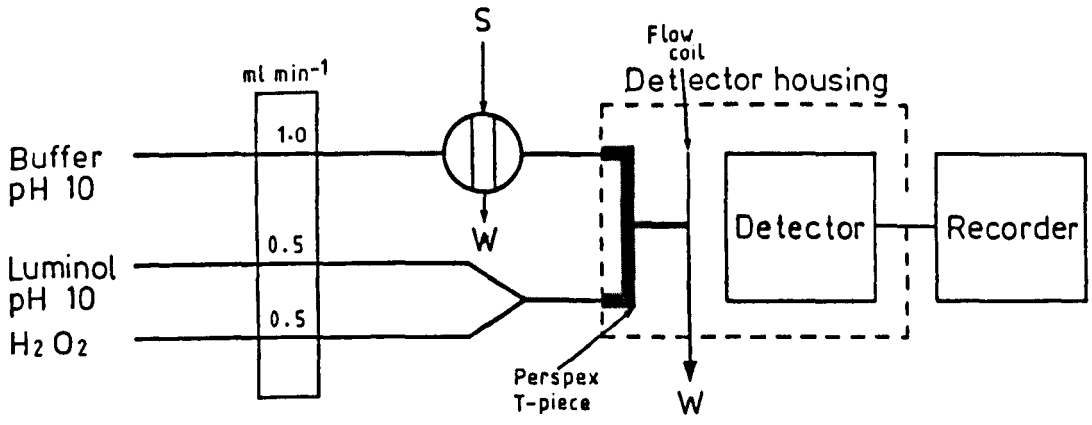


Fig. 2.7. Manifold for the determination of cobalt using luminol. S, sample (30µl); W, waste.

Reagents

0.1M carbonate buffer solution was prepared by dissolving 10.59g of Na_2CO_3 (B.D.H.) in water, adjusting the pH to 10 with 0.5M NaOH and making up to exactly 1 litre with water.

Stock luminol solution (1×10^{-3}M) was prepared by dissolving 0.0443g of 3-aminophthaloylhydrazide (B.D.H.) in 250ml of 0.1M sodium carbonate, pH 10. Other solutions were prepared by appropriate dilution of the stock solution with the buffer solution.

Hydrogen peroxide (0.01M) was prepared from 100 volume H_2O_2 solution (B.D.H.) by diluting 0.28ml to 250ml with water.

Stock cobalt(II) solution (1000µg ml^{-1}) was prepared by dissolving 0.0403g of $CoCl_2.6H_2O$ in 100ml of water. Working solutions (0.0015 - 25µg ml^{-1}) were prepared daily by appropriate dilution with water.

Apparatus

The F.I.A.-CL system shown in figure 2.6 was used.

Procedure

According to the arrangement shown in figure 2.7, the luminol solution (1×10^{-5}M) was mixed first with 1×10^{-2}M hydrogen peroxide. This is rapidly mixed in the T-piece with the carbonate buffer (pH 10). A stable baseline was recorded. The reaction was started by injecting 30µl of cobalt(II) solution into the carbonate buffer stream. The emission intensity was recorded as a function of time (over 18 sec).

Results and Discussion

The peak height increased with increasing concentration of cobalt in the injected sample. The results obtained are shown in table 2.1 and figure 2.8.

TABLE 2.1.

RESULTS FOR THE DETERMINATION OF COBALT USING THE CHEMILUMINESCENT OXIDATION OF LUMINOL

$[Co^{2+}]$ ($\mu g\ ml^{-1}$)	$\log_{10}[Co^{2+}]$ ($\mu g\ ml^{-1}$)	Mean Emission Intensity (mV)	$\log_{10}$(Emission Intensity)	R.S.D.(%) for 4 replicates
0.0015	-2.8	12.5	1.1	2.8
0.003	-2.5	25	1.4	2.5
0.006	-2.2	51	1.7	1.6
0.012	-1.9	159	2.2	1.5
0.024	-1.6	510	2.7	1.6
0.048	-1.3	1585	3.2	1.1
0.096	-1.0	2800	3.4	1.1
0.195	-0.7	4100	3.6	0.9
0.39	-0.4	5200	3.7	0.4
0.78	-0.1	5700	3.75	0.4
1.56	0.19	5850	3.76	0.4
3.12	0.49	5850	3.76	0.3

Mean R.S.D. = 1.2%

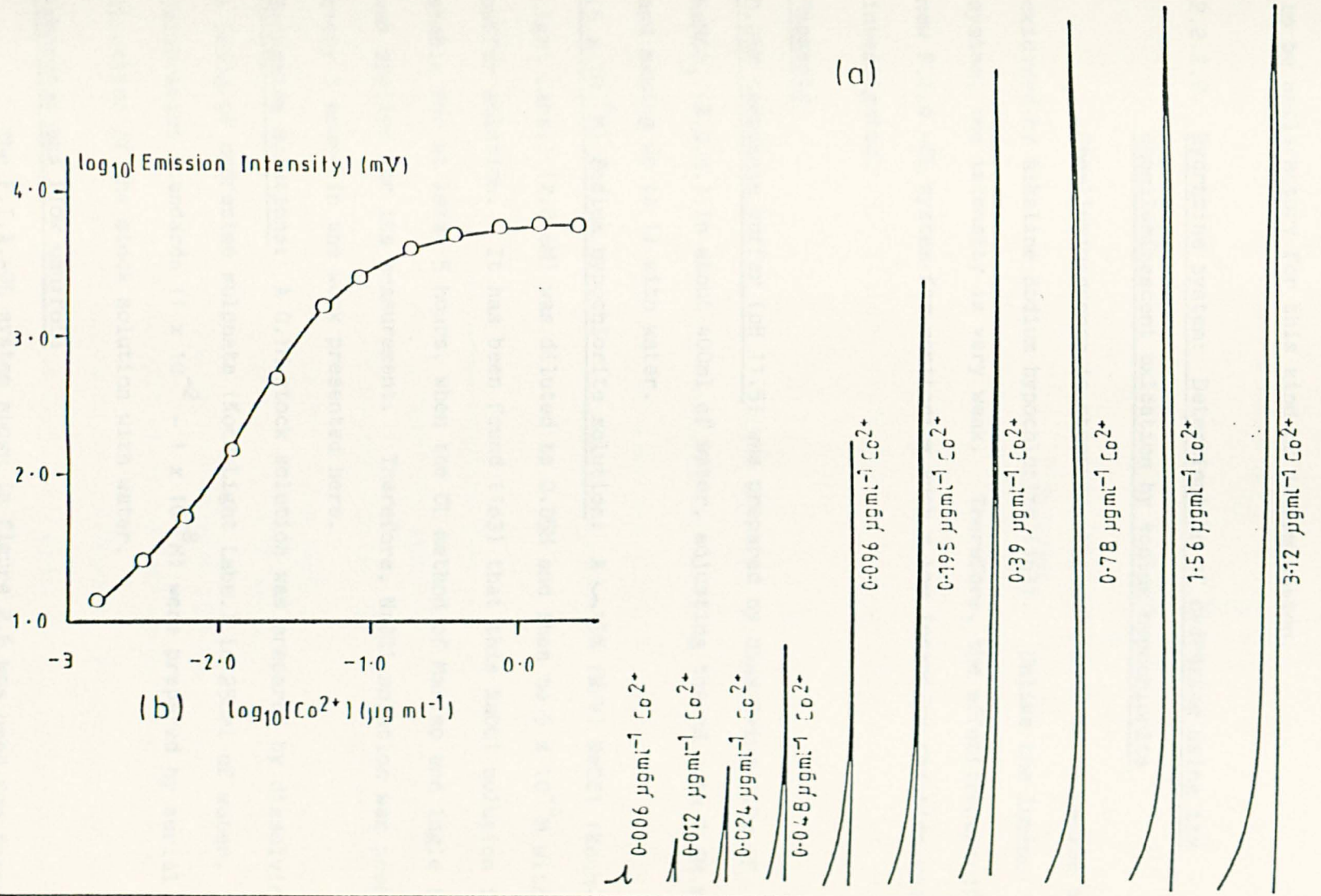


Fig. 2.8. (a) Calibration peaks for determination of cobalt using Co(II)-luminol-H_2O_2 system. (b) The corresponding log-log graph.

The relative standard deviation for six replicates of 0.006 $\mu g\ ml^{-1}$ Co^{2+} was 1.2%. Accordingly the new F.I.A.-CL detector was found to be satisfactory for this kind of determination.

2.2.2.2. Hydrazine system: Determination of hydrazine using its chemiluminescent oxidation by sodium hypochlorite

Chemiluminescence is observed when solutions of hydrazine are oxidized by alkaline sodium hypochlorite (163). Unlike the luminol system, the intensity is very weak. Therefore, the effectiveness of the new F.I.A.-CL system for monitoring such a low intensity reaction was investigated.

Reagents

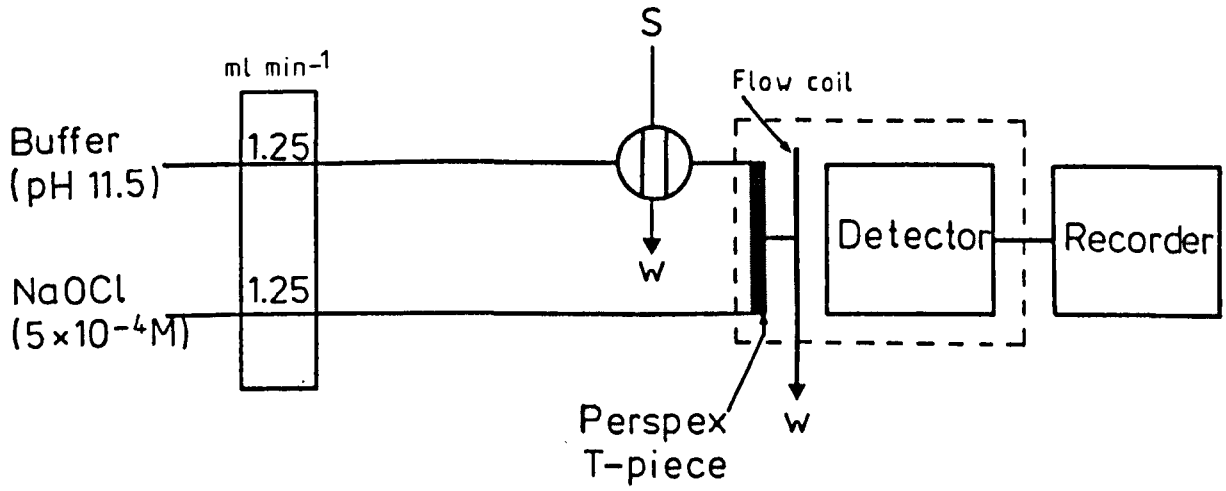
0.05M carbonate buffer (pH 11.5) was prepared by dissolving 4.2g of $NaHCO_3$ (B.D.H.) in about 400ml of water, adjusting the pH with 0.5M NaOH and making up to 1l with water.

(5×10^{-4}M) Sodium hypochlorite solution: A ~15% (W/V) NaOCl (Koch-Light Labs.) (2.016M) was diluted to 0.05M and then to 5×10^{-4}M with the buffer solution. It has been found (163) that this NaOCl solution is stable for at least 5 hours, when the CL method of Marino and Ingle (204) was applied for its measurement. Therefore, NaOCl solution was prepared every 5 hours in the work presented here.

Hydrazine solutions: A 0.1M stock solution was prepared by dissolving 3.5253g of hydrazine sulphate (Koch-Light Labs.) in 250ml of water. Calibration standards ($1 \times 10^{-2} - 1 \times 10^{-8}$M) were prepared by serial dilutions of the stock solution with water.

Apparatus and flow manifold

The F.I.A.-CL system shown in figure 2.6 was used for these measurements. The manifold used is shown in figure 2.9.



2.9. Flow injection chemiluminescent system used for the determination of hydrazine, S. sample, W. Waste.

Procedure

30µl of hydrazine solution was injected into the buffer stream which immediately mixed with sodium hypochlorite in the T-piece. The resulting emission peak reached a maximum within 4 sec. and the time from injection to completion of the peak was 15 seconds.

Results and Discussion

The chemiluminescent reaction from the oxidation of hydrazine by sodium hypochlorite was successfully monitored by the new detector. Despite the low intensity of the emission the detector responded to all hydrazine concentrations injected in the range 5×10^{-8} - 1×10^{-2}M. The data obtained from 5×10^{-8} - 1×10^{-5}M hydrazine is shown in table 2.2 and the recorder tracing of the emission signals produced is shown in figure 2.10. Generally the peaks are well-shaped, and increase in height with increasing hydrazine concentration. The log-log calibration graph in figure 2.10(b) is linear with a slope of 0.5, and therefore the concentration range studied is analytically useful. In table 2.3 a comparison is made between the important parameters used in this procedure and that used previously by Wheatley (163).

TABLE 2.2.

EMISSION SIGNALS OBTAINED FROM THE CHEMILUMINESCENT OXIDATION OF HYDRAZINE WITH SODIUM HYPOCHLORITE

Hydrazine Conc.(M)	log[hydrazine] (M)	Emission Intensity (mV)	$\log_{10}$(Emission Intensity)(mV)	R.S.D.% for 3 replicates
2.5×10^{-8}	-7.6	0.36	-0.40	2.6
5×10^{-8}	-7.3	0.86	-0.07	2.4
2.5×10^{-7}	-6.6	2.1	0.32	1.1
5×10^{-7}	-6.3	3.7	0.60	0.5
2.5×10^{-6}	-5.6	7.0	0.85	1.7
1×10^{-5}	-5.0	18.4	1.26	0.9

Mean R.S.D. = 1.5%

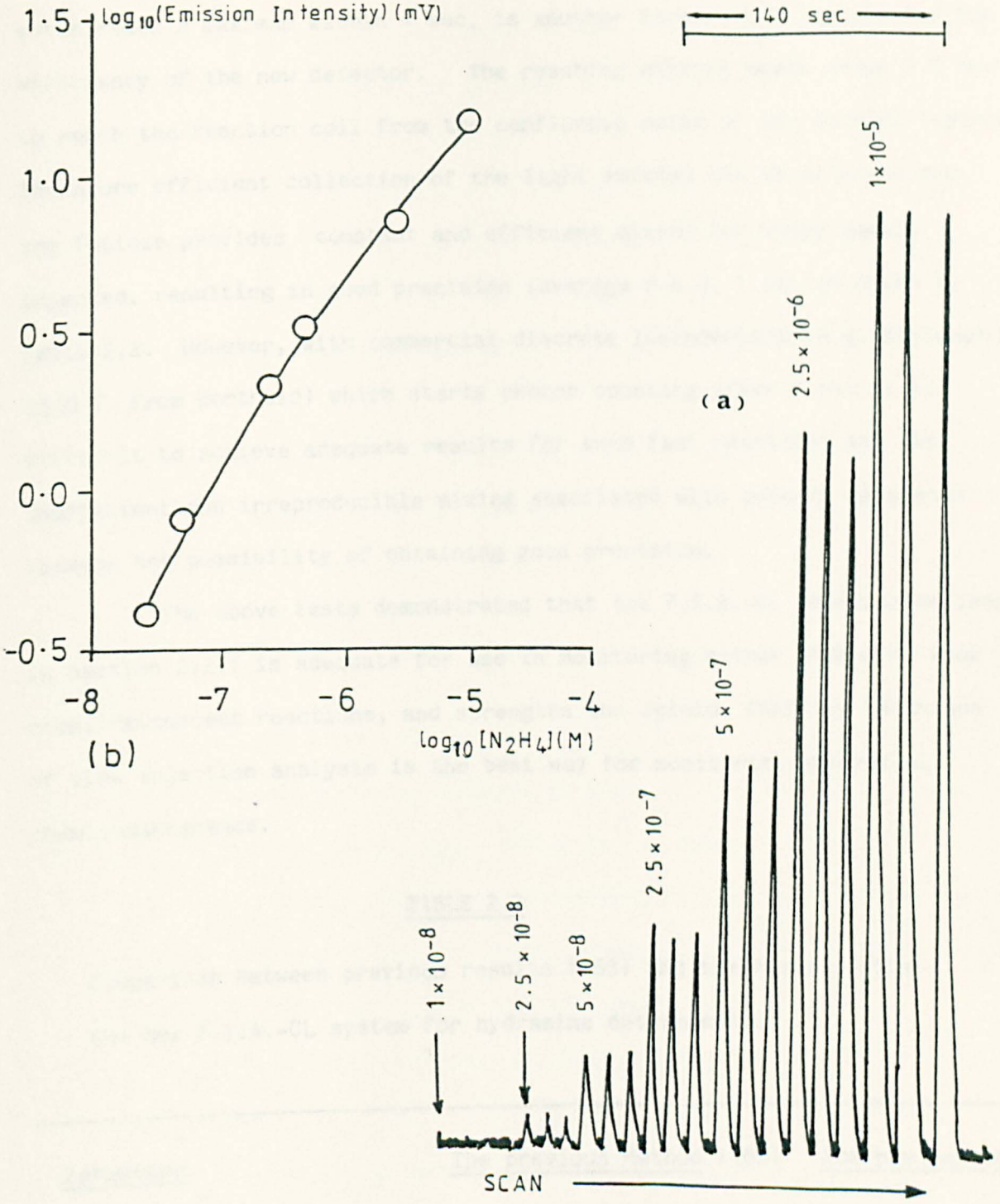


Fig. 2.10(a) Typical calibration peaks for hydrazine in the range 2.5×10^{-8} - 1×10^{-5}M. (b) corresponding log-log graph.

The transient nature of hydrazine chemiluminescent emissions, which reach a maximum within 4 sec, is another factor that illustrates the efficiency of the new detector. The reacting mixture needs about 0.5 sec to reach the reaction coil from the confluence point of the perspex T-piece. Therefore efficient collection of the light emitted can be achieved and the T-piece provides constant and efficient mixing for every sample injected, resulting in good precision (average r.s.d. 1.5%) as shown in table 2.2. However, with commercial discrete luminometers (e.g. Biolumat LB 9500 T from Berthold) which starts photon counting after 2 sec it is difficult to achieve adequate results for such fast reactions and the inefficient and irreproducible mixing associated with such an apparatus lessens the possibility of obtaining good precision.

The above tests demonstrated that the F.I.A.-CL detector devised in section 2.2.1 is adequate for use in monitoring either strong or weak chemiluminescent reactions, and strengths the opinion that the technique of flow injection analysis is the best way for monitoring analytical chemiluminescence.

TABLE 2.3

Comparison between previous results (163) and the results with the new F.I.A.-CL system for hydrazine determination

Parameter	The previous Method (163)	The new method
Flow Rate (ml min^{-1})	3 - 3.7	2.0 - 2.5
Detection Limit (M)	3×10^{-8}	2.5×10^{-8} M
Mid-range precision (r.s.d.)	2%	1.5%
Sample rate (h^{-1})	50	120

2.2.3. Metal Analysis with Hydrazine-hypochlorite CL system

In the flow injection chemiluminescence procedure for hydrazine determination (163), a number of species, including metal ions Fe^{2+}, Ba^{2+}, Mg^{2+} and Pb^{2+}, were found to interfere by suppressing the emission. In any realistic method for hydrazine it will be necessary to prevent such interfering effects. It was decided, therefore, to investigate such metal ion effects in detail, and, on the basis of the information obtained, to attempt to eliminate metal ion interferences in the determination of hydrazine.

For the present work some common metal ions, which have exhibited enhancement or depression of the luminol or lucigenin systems, were selected for study. They were Cu^{2+}, Co^{2+}, Mn^{2+}, Cr^{3+}, Cd^{2+}, Zn^{2+}, Ni^{2+} and Al^{3+}.

Reagents

Carbonate buffer (0.05M, pH 11.5), sodium hypochlorite ($5x10^{-4}$M) and hydrazine solutions were prepared as described in section 2.2.2.2.

Standard solutions of metal ions: 100μg ml^{-1} stock solutions of each metal ion were prepared from their corresponding AnalaR salts by dissolving the following weights of salt in 100ml of water: 0.0494g of $Co(NO_3)_2.6H_2O$, 0.0393g of $CuSO_4.5H_2O$, 0.0446g of $(CH_3COO)_2Mn.4H_2O$, 0.0769g of $Cr(NO_3)_2.9H_2O$, 0.0237g of $(CH_3COO)_2Cd.2H_2O$, 0.0335g of $(CH_3COO)_2Zn.2H_2O$, 0.0495g of $Ni(NO_3)_2.6H_2O$ and 0.1390g of $Al(NO_3)_3.4H_2O$. The working solutions were prepared by serial dilution of each stock solution with water.

Apparatus and Flow manifold

The F.I.A.-CL system shown in figure 2.6 was used throughout. Different manifolds are arranged to add the metal ions into the hydrazine CL system. The manifold shown in figure 2.11 is typical.

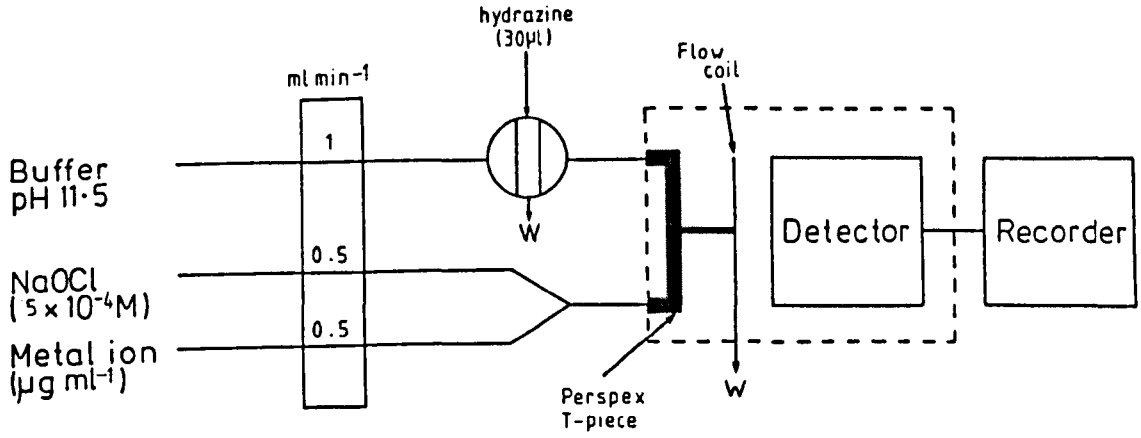


Fig. 2.11 F.I.A.-CL system used for studying metal ion enhancement or inhibition effects on hydrazine CL.

Procedure

Introducing the metal ion to the hydrazine CL system was the main requirement. Three different procedures were used for this purpose. Firstly, to a fixed hydrazine concentration different amounts of metal ion were added and the resulting mixture was immediately injected to the system shown in figure 2.9. Secondly, the manifold in figure 2.9 was modified so that a metal ion solution was pumped through a separate line and mixed with the buffer stream before entering the detector. Finally a separate stream of metal ion solution was mixed with the hypochlorite stream before reaching the detector, as shown in figure 2.11. For each ion a range of 0.01-25µg ml^{-1} was examined.

Results and Discussion

Preliminary experiments were made to examine the efficiency of the three different arrangements mentioned above. Cobalt(II) and

cadmium(II) (0.01-10μg ml^{-1}) were used for this purpose. The results obtained with the three arrangements were very similar and reproducible which means that the metal ions can be introduced to the system in different ways depending on what conditions are required during the analytical process, e.g. to take account of side reactions between the metal ions and the buffer solution or sodium hypochlorite. For simplicity and rapidity of analysis the system shown in figure 2.11 was used for further work.

The results obtained showed that the metal ions can be grouped into two categories, inhibitors (depression of the emission intensity) and catalysts (enhancement of the emission intensity). Both effects were studied by gradually increasing the metal ion concentration in the range shown.

Inhibiting Effects

Figure 2.12(a) illustrates the negative effects of Co^{2+}, Cr^{3+}, Cu^{2+} and Mn^{2+}. Their effects were in the order $Co^{2+} < Cr^{3+} < Mn^{2+} < Cu^{2+}$. No effects were observed for any of these ions when their concentrations were less than 0.1μg ml^{-1}. Generally, the emission signals obtained were as reproducible as those obtained in the absence of metal interferent.

Copper(II) ($\geq$ 5μg ml^{-1}) and Mn^{2+} ($\geq$0.1μg ml^{-1}) completely prevented the emission. Moreover, the previous presence of Cu^{2+} and Mn^{2+} in the flow system disturbed the responses from subsequent hydrazine samples injected. For example after the effect of 5μg ml^{-1}(Cu^{2+} or Mn^{2+}) had been investigated, if a pure hydrazine solution was injected, no signal was obtained. Therefore, reliable calibrations of hydrazine were difficult to obtain after metal inhibition experiment had been run, without conditioning the manifold by passing the carbonate buffer (pH 11.5) for at least five minutes. This was due to the adsorption of

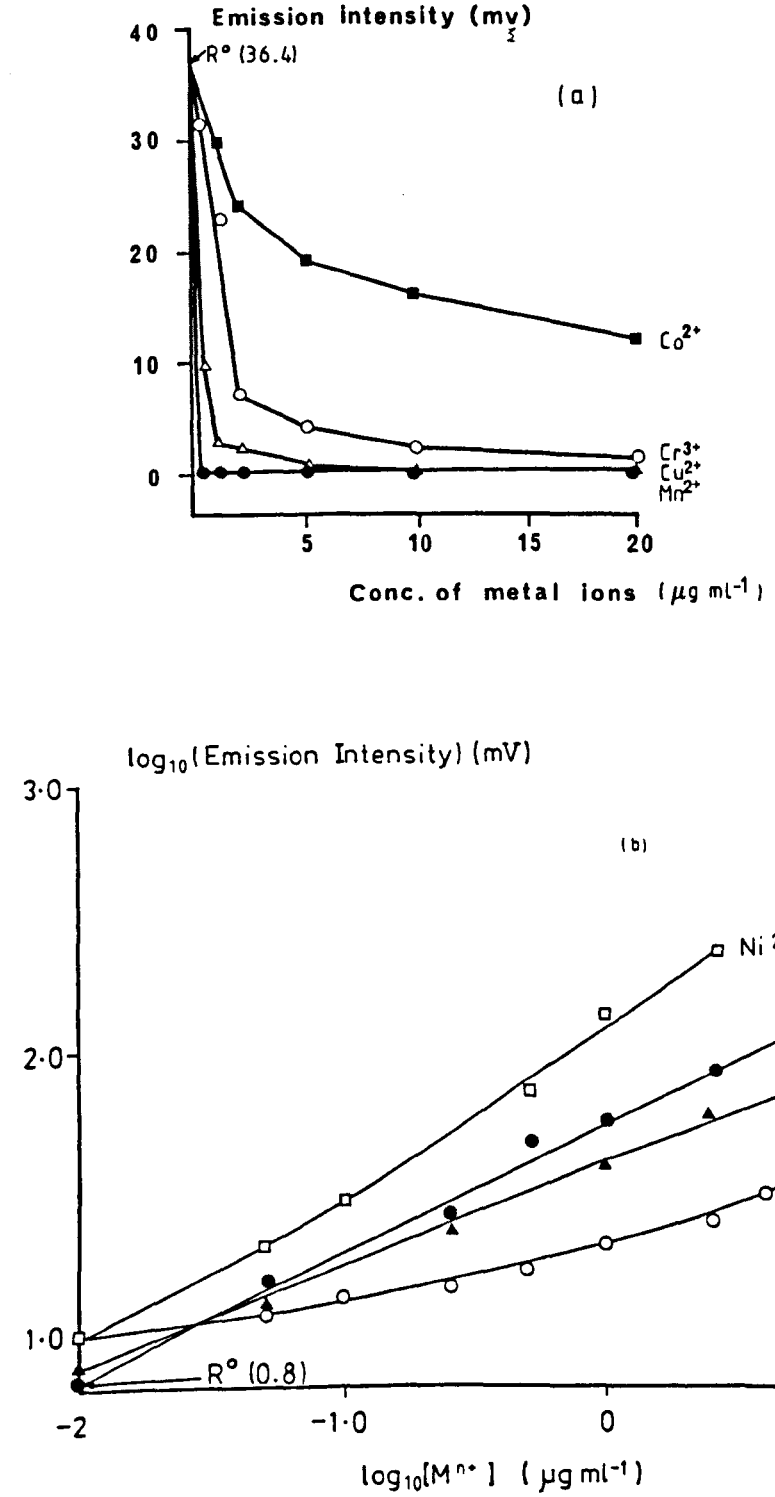


Fig.2.12 Effects of metal ions on the chemiluminescent reaction of hydrazine with NaOCl: (a) inhibiting effects, (b) Enhancement effects. $[N_2H_4]$ in a and b are $5x10^{-5}$M and $5x10^{-6}$M respectively, ▲ and ● are responses for Cu^{2+} and Mn^{2+} respectively. R^o, responses in the absence of added metal ion.

these metals on the manifold tubing. No significant change was observed in the peak width or peak shape during the inhibition process.

As the presence of these metal ions strongly affects the chemiluminescent reaction between hydrazine and sodium hypochlorite, means of eliminating them are essential. This might be done by using a masking agent or by including a separation process such as ion exchange in the F.I.A.-CL manifold. The latter is described in Chapter 4.

Enhancement Effects

The enhancement of the CL intensity by some common metal ions was also of great importance as far as hydrazine determination was concerned. These effects eventually led to improved conditions for the determination of hydrazine. Four common metal ions (Al^{3+}, Cd^{2+}, Zn^{2+} and Ni^{2+}) exhibited substantial enhancing effects on the emission signals obtained which are illustrated in figure 2.12(b). The effects increased in the order $Al^{3+} < Cd^{2+} < Zn^{2+} < Ni^{2+}$, with log-log slopes of about 0.2, 0.40, 0.40 and 0.57, respectively. Despite the marked enhancement of the signals peak width was unchanged.

Such effects will also interfere with hydrazine determination and must, again be controlled. There is also the possibility that they could be used for trace metal determinations (in the range 0.01-10$\mu g\ ml^{-1}$) although there will be several interferences.

The results in figure 2.12(b) show a pronounced increase in sensitivity for $2x10^{-6}M$ hydrazine, the increase being 22.5 in the presence of $1\mu g\ ml^{-1}$ Ni^{2+}, and therefore further experiments were carried out to investigate the effect of these metals ions in the concentration range 0.05 - $12.5\mu g\ ml^{-1}$ on a wide range of hydrazine concentrations ($1x10^{-10}$ - $5x10^{-5}M$). The results are shown in table 2.4 and illustrated as figures 2.13 - 2.20.

TABLE 2.4

RESULT OF EFFECTS OF METAL ION ON THE CL OF HYDRAZINE

Metal ion conc. ($\mu g\ ml^{-1}$)	Emission Intensity (mV)							
	Hydrazine Concentration (M)							
	$1x10^{-10}$	$1x10^{-9}$	$1x10^{-8}$	$1x10^{-7}$	$1x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$	$5x10^{-5}$
Ni^{2+}								
0.031	1.0	1.0	2.1	3.9	19.9	41.6	63.0	-
0.158	1.0	1.4	3.1	9.5	50.0	151	178	-
0.316	1.0	1.9	4.3	18.2	96	251	389	-
0.63	1.1	2.0	5.0	28.8	151	437	794	-
1.26	1.2	2.5	5.1	50.1	263	794	1580	-
3.5	1.2	2.5	6.9	79.4	398	174	3020	-
6.3	1.0	1.5	3.1	10.0	25.1	100	159	-
Cd^{2+}								
0.1	0.0	0.0	0.4	1.1	2.5	14.0	-	26.2
0.63	0.0	0.0	1.0	3.1	6.3	31.6	-	100
3.25	0.0	0.0	1.9	7.9	19.9	63.0	-	252
6.3	0.0	1.4	2.7	10.9	25.1	126	-	331
10.0	0.0	1.4	3.2	12.5	28.8	110	-	346
12.5	0.0	0.0	2.5	10.0	23.9	72	-	252
Zn^{2+}								
0.1	0.0	0.0	0.5	1.5	3.8	30.5	44	-
3.3	0.0	0.0	2.2	8.7	63	241	351	-
12.5	0.0	0.2	3.5	16	128	550	725	-
25	0.0	1.3	4.7	22	174	660	958	-
50	0.0	1.9	7.6	40	321	129	192	-
Al^{3+}								
0.15	0.0	0.0	0.60	1.40	6.0	13.8	-	27.5
0.31	0.0	0.0	0.70	1.58	7.9	17.8	-	36.3
0.63	0.0	0.0	0.80	1.86	8.7	20	-	45.7
1.25	0.0	0.0	0.96	1.95	10.0	24	-	60
5.1	0.0	0.0	1.00	2.50	12.0	38	-	83
10.0	0.0	0.0	1.40	3.16	15.8	56	-	166
12.5	0.0	0.0	1.26	2.80	14.4	55	-	100

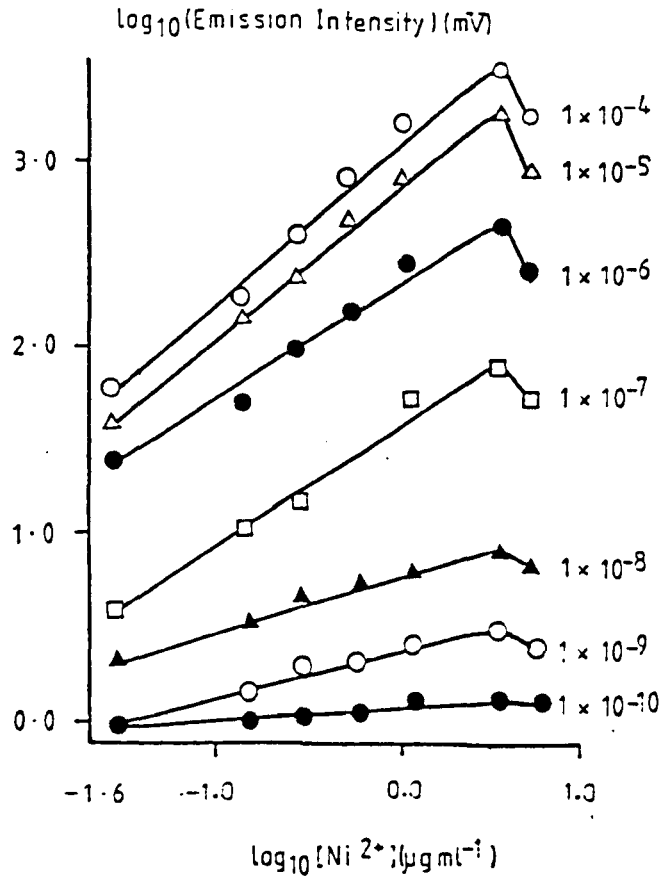


Fig. 2.13 Effect of nickel on CL of hydrazine (concentration shown)

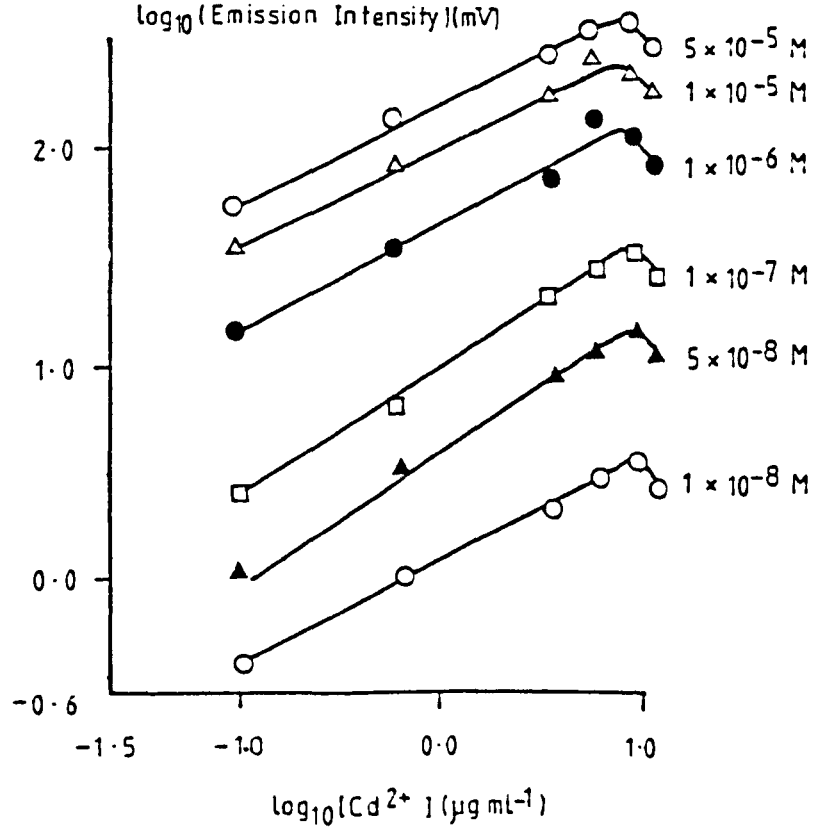


Fig. 2.14 Effect of cadmium concentration on CL of hydrazine (concentration shown)

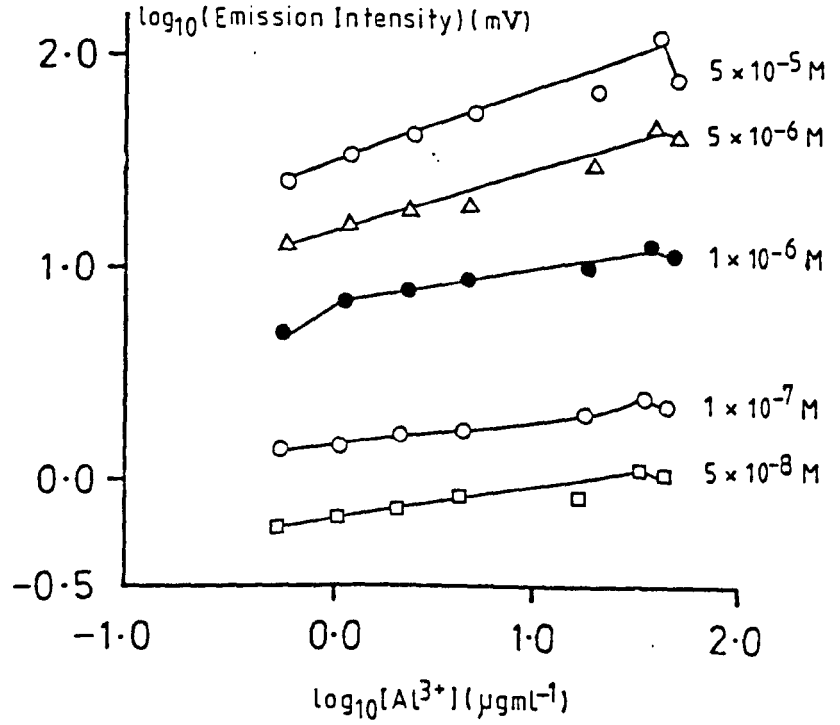


Fig. 2.15 Effect of aluminium concentration on CL of hydrazine (concentration shown)

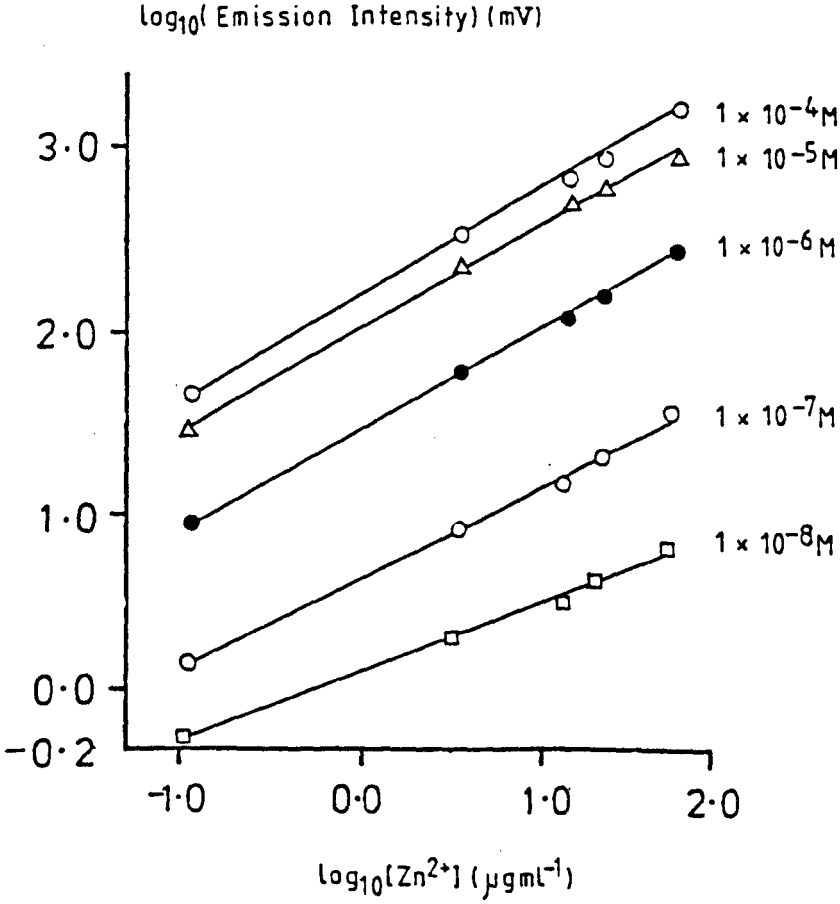


Fig. 2.16 Effect of zinc concentration on CL of hydrazine (concentration shown).

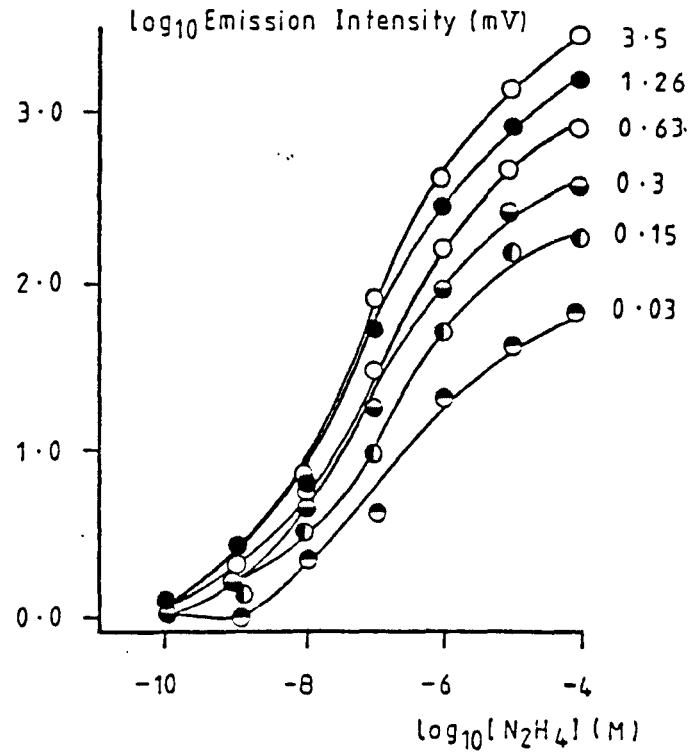


Fig. 2.17 Calibration graphs of hydrazine with nickel (at concentrations in μg ml^{-1} given)

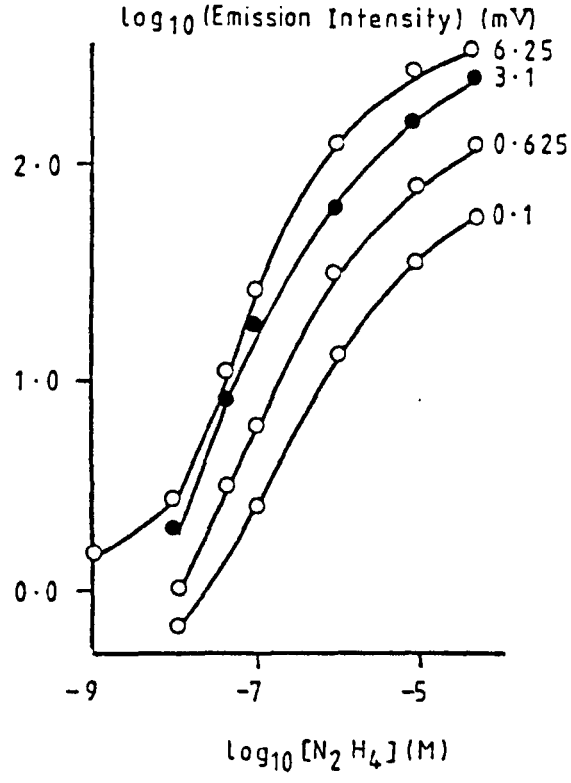


Fig. 2.18 Calibration graphs of hydrazine with cadmium (at concentrations in μg ml^{-1} given)

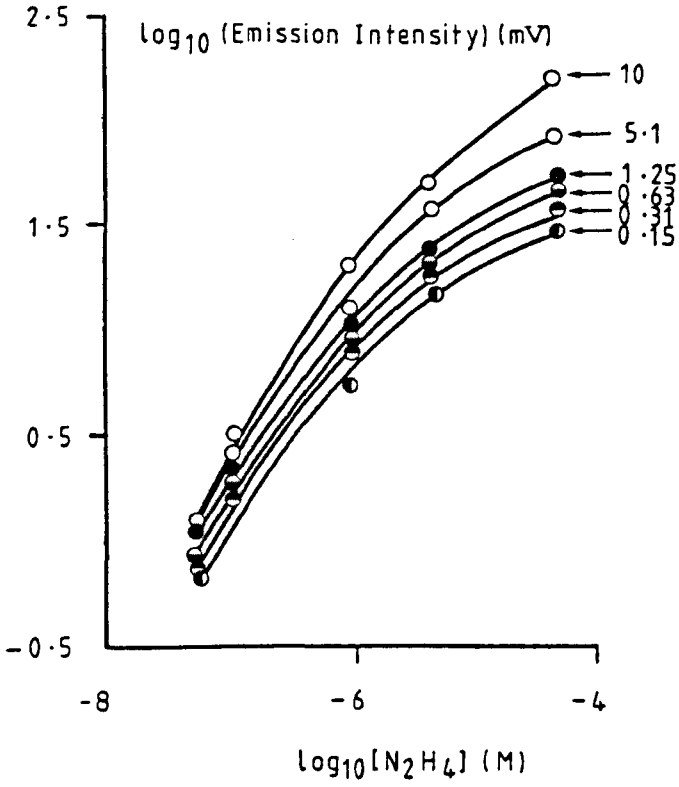


Fig. 2.19 Calibration graphs of hydrazine with aluminium (at concentrations in μg ml^{-1} given)

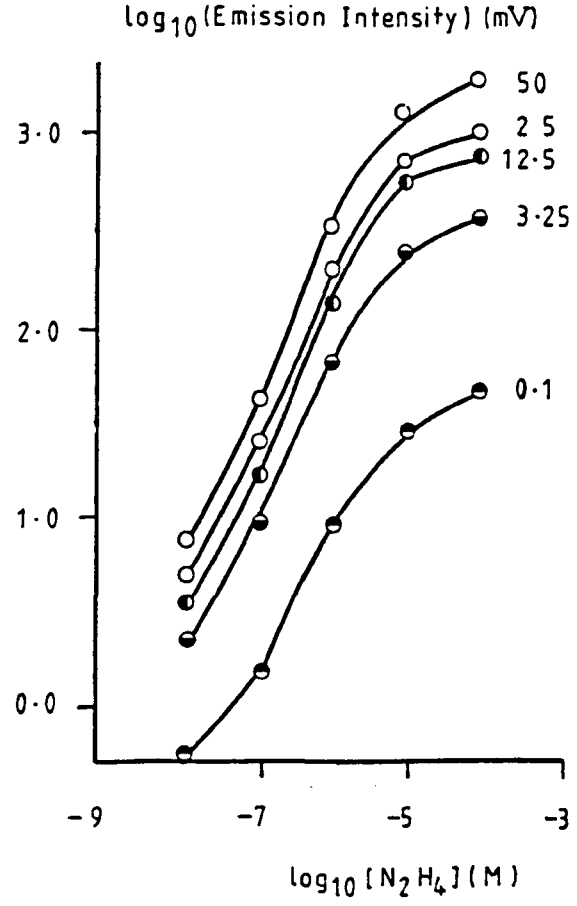


Fig. 2.20 Calibration graphs of hydrazine with zinc (at concentrations in μg ml^{-1} given)

Despite the differences in sensitivity, all the metal ions exhibited a similar pattern of effects, which reached a maximum at a certain metal concentration and then sharply decreased as a more concentrated metal ion solution was employed. A detection limit (2 x noise) of $5x10^{-10}M$ hydrazine was obtained in the presence of 0.15μg ml^{-1} nickel, while for 6.25μg ml^{-1} cadmium (and 10μg ml^{-1} zinc) it was $1x10^{-9}$ and $1x10^{-8}M$ for 5.1μg ml^{-1} aluminium compared with $2.5x10^{-8}M$ in the absence of these metals (section 2.2.2.2).

Except for Zn^{2+} (figures 2.16, 2.20) which continously caused enhancement of the signals as its concentration increased up to 50μg ml^{-1}, Ni^{2+}, Cd^{2+} and Al^{3+} showed maximum effects at certain concentrations, namely 3.5μg ml^{-1} for Ni^{2+}, 6.3μg ml^{-1} for Cd^{2+} and 10μg ml^{-1} for Al^{3+} at all hydrazine concentrations (figures 2.13 - 2.15, 2.17 - 2.19). This situation resembled the Co(II) and luminol-H_2O_2 system (section 2.2.2.1).

These studies show, therefore, that enhanced sensitivity for hydrazine can be achieved in the presence of certain metal ions. Because the metal ion concentrations in real samples are not controlled, however, metal ion interferences remain a problem. Two possible means of solving this problem were investigated. One was to change the oxidant in an attempt to find a more selective reaction; this is discussed in Chapter 3. The other is to remove metal ions by on-line ion-exchange, as described in Chapter 4.

CHAPTER THREE

NEW CHEMILUMINESCENT REACTION OF HYDRAZINE WITH PERMANGANATE

3.1. Introduction

The possibility of generating chemiluminescence by reactions of hydrazine with other oxidizing reagents was investigated. Hydrogen peroxide and potassium periodate in alkaline media and potassium permanganate in acidic media, were used in the system shown in figure 2.9. Those oxidants were selected because of the previous contribution of some of them in producing chemiluminescence such as hydrogen peroxide with luminol (148-150) and potassium permanganate with sulphide (205) and to test the capability of other oxidants such as KIO_4 which is an oxidant in alkaline medium (e.g. pH 9). Using hydrazine concentrations of $5x10^{-6}$ and $5x10^{-5}$M, no signals were observed with hydrogen peroxide (0.01M) at pH 10.0 - 11.5 using carbonate buffer even with some metal ions (2.5μg ml^{-1} of Ni^{2+}, Co^{2+} and Fe^{3+}) present. No signals were produced either by potassium periodate (0.01M) at pH 9.0 using carbonate buffer also. However, when potassium permanganate ($1x10^{-4}$M) was used in nitric acid (pH 1.5), a 2mV signal was obtained with $5x10^{-5}$M hydrazine. Therefore attempts were made to improve the sensitivity in acidic media.

Abbott (206) has found that polyphosphate ions enhanced the chemiluminescence produced by some alkaloids in the presence of permanganate, possibly due to the stabilization of managanese(III). It is interesting that solid complexes such as $Mn(H_2PO_4)_2$, $Mn(H_2PO_4)_2$, $MnH_2P_2O_7$ and C- $Mn_2P_4O_{12}$ were recently reported by Kaplanova et al. (207) to show intense luminescence. Therefore it was decided to use a permanganate-polyphosphate system to improve the selectivity for hydrazine.

3.2. Experimental

3.2.1. Reagents

Distilled-deionized Water (from a combined distillation apparatus and Elgastat spectrum deionizer) was used for the preparation of all solutions and unless otherwise stated, all chemicals were analytically grade reagents.

MnO_4^- solutions. A stock solution of 1×10^{-2}M $KMnO_4$ was prepared by dissolving 0.3950g of $KMnO_4$ (Hopkin and Williams) in 250ml of water. Other solutions were prepared by serial dilution of the stock solution with water.

Polyphosphate solutions. A stock solution of 0.5M polyphosphoric acid (BDH) was prepared by diluting 8.6585g of concentrated solution (82% P_2O_5) in 100ml of water. Other solutions were prepared by appropriate dilution of the stock solution with water.

Hydrazine solutions were prepared as described in section 2.2.2.2.

Standard solutions of metal ions. Except for Fe^{2+} (100μg ml^{-1} stock solution) which was prepared by dissolving 0.0355g $FeCl_2 . 4H_2O$ in 100ml of water, the stock solutions of other metal ions are prepared as described in section 2.2.3. For each metal the required concentration was prepared by diluting the stock solution with water.

3.2.2. Apparatus and Flow manifold

The F.I.A.-CL system shown in figure 2.6 was used. The flow injection manifold for this purpose is shown in figure 3.1.

3.2.3. Procedure

30μl of hydrazine solution was injected into a stream of polyphosphoric acid which was then mixed with a $KMnO_4$ solution inside the T-piece and passed into the reaction coil. The emission peaks reached a maximum 14 seconds after injection. The time from the point of

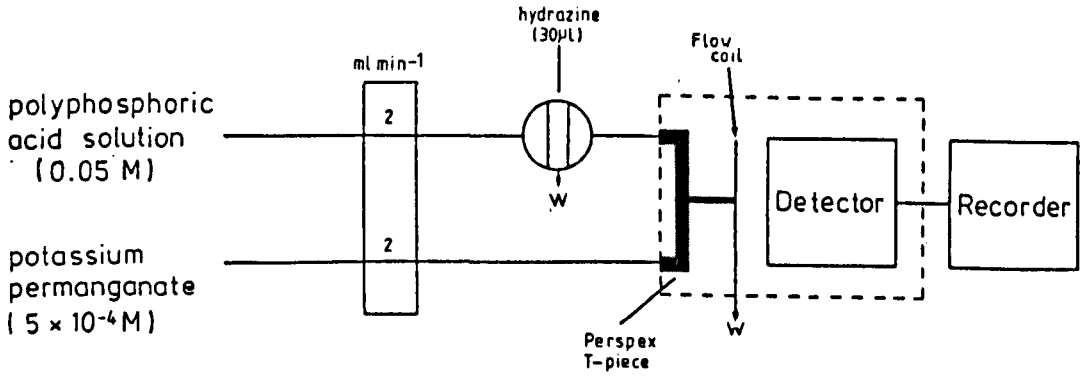


Fig. 3.1. F.I.A.-CL system used for determination of hydrazine using the permanganate/polyphosphoric acid system.

injection to the completion of a peak was 40 seconds at a flow rate of 3ml min^{-1}.

3.2.4. Results and Discussion

3.2.4.1. Optimization

Effect of potassium permanganate concentration

The effect of $KMnO_4$ concentration on the intensity (peak height) was investigated by varying the permanganate concentration from $1x10^{-2}$ to $1x10^{-6}$M while keeping all other variables constant. It was studied for two hydrazine concentrations, $1x10^{-3}$M and $1x10^{-4}$M, with $5x10^{-2}$M polyphosphoric acid as carrier stream. The results are shown in figure 3.2 which indicate that $5x10^{-4}$M $KMnO_4$ gives the largest intensity at both hydrazine concentrations. The decrease in the signals at higher permanganate concentrations is undoubtedly due to the permanganate which absorbs the emitted light. Therefore $5x10^{-4}$M $KMnO_4$ was used in further studies.

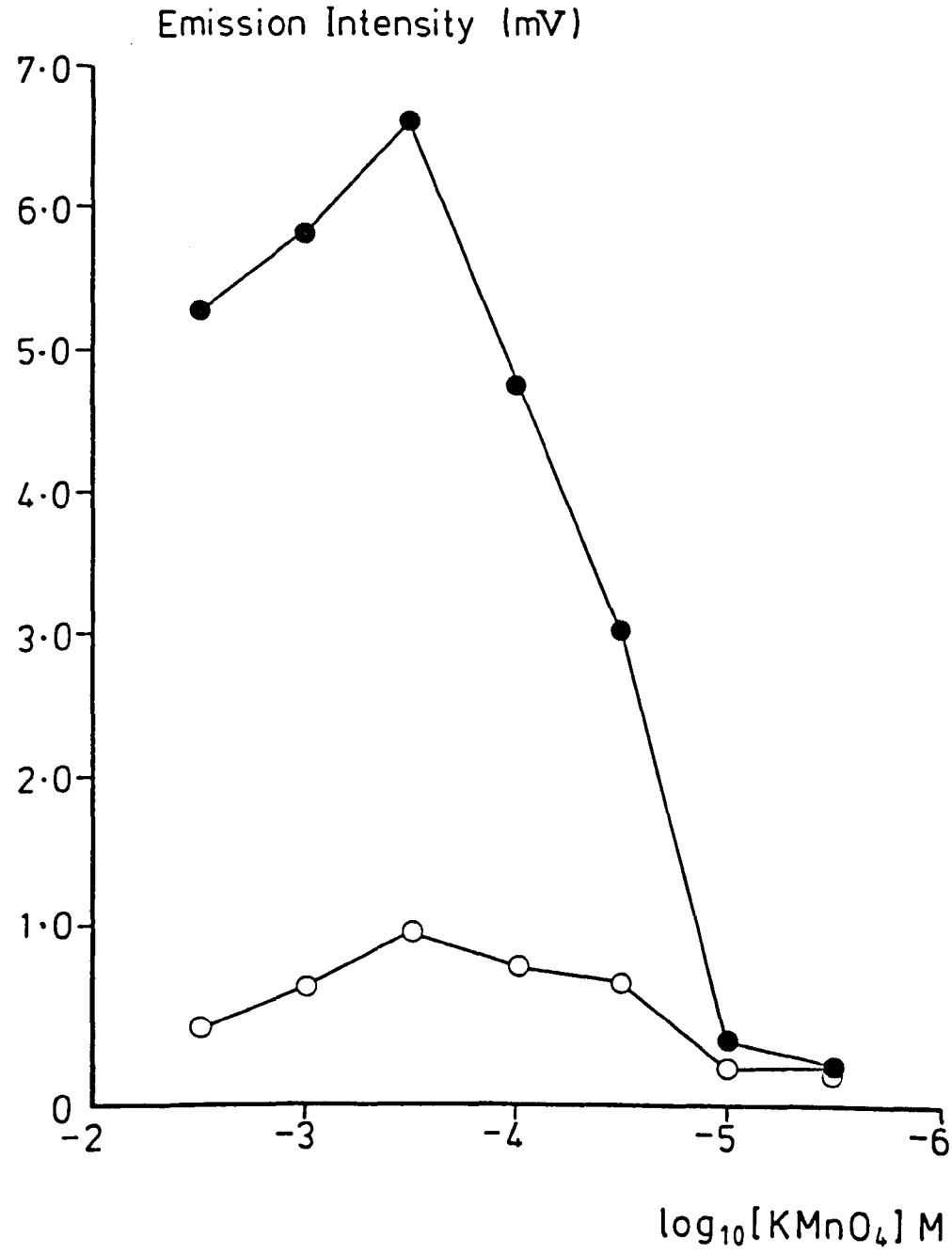


Fig. 3.2. Effect of potassium permanganate concentration

Effect of polyphosphoric acid concentration

With constant permanganate concentration ($5x10^{-4}$M) the effect of polyphosphoric acid concentration was tested in the range $1x10^{-4}$ - 1M, at two hydrazine concentrations, $1x10^{-4}$ and $5x10^{-3}$M. Table 3.1 shows the results obtained, in which the concentration of polyphosphate and the associated change in pH produced a pronounced effect on the intensity; $\geq 5x10^{-2}$M polyphosphoric acid (at pH ≤ 1.6) gave maximum intensity,

therefore $5x10^{-2}$M polyphosphoric acid at pH 1.6 was used in further studies.

Effects of flow rate variation

While holding the concentration of $KMnO_4$ at $5x10^{-4}$M and the polyphosphoric acid concentration at $5x10^{-2}$M (pH 1.6), the flow system shown in figure 3.1 was operated at different flow rates and the effect on the emission signals from $1x10^{-3}$M hydrazine recorded. Figure 3.3 shows a continuous increase in peak height as flow rate increases up to 5.5ml min^{-1}. Despite the increase, no significant changes were observed in the peak shape. However, the duration for obtaining one peak was decreased as flow rate increased as illustrated in table 3.2.

In monitoring chemiluminescence by F.I.A. it is of great importance that a sufficiently high flow rate is used in order that the excited product reaches the detector in a minimum time and hence maximum collection of the emitted light can be achieved. Therefore the results shown in figure 3.3. and table 3.2. confirm that the present CL reaction of hydrazine is very fast and the excited product produced at the T-piece needs rapid transport to the coil for maximum light output to be monitored. The use of very high flow rates, however, in many circumstances is not desirable as it causes high reagent consumption, which may be uneconomic. In this case a flow rate of 4ml min^{-1} was found to be satisfactory and is recommended for hydrazine determination using the permanganate-polyphosphate system.

3.2.4.2. Calibration

Having carried out the necessary optimization of the important experimental parameters, two calibration graphs were constructed. The log-log calibration of hydrazine over the range $1x10^{-6}$ - 0.5M is shown in figure 3.4. The graph is linear from $1x10^{-6}$ - $3x10^{-4}$M with a slope of 1.2.

TABLE 3.1

EFFECT OF POLYPHOSPHORIC ACID CONCENTRATION

Polyphosphoric acid Conc.		Emission Signals (mV)	
(M)	pH	$1x10^{-4}$M hydrazine	$1x10^{-3}$M hydrazine
$5x10^{-4}$	3.5	0.0	0.4
$1x10^{-3}$	3.0	0.15	1.7
$5x10^{-3}$	2.5	0.6	7.6
$1x10^{-2}$	2.2	1.0	10.6
$5x10^{-2}$	1.6	2.8	22.0
0.1	1.4	2.85	22.4
0.5	1.3	2.9	22.7

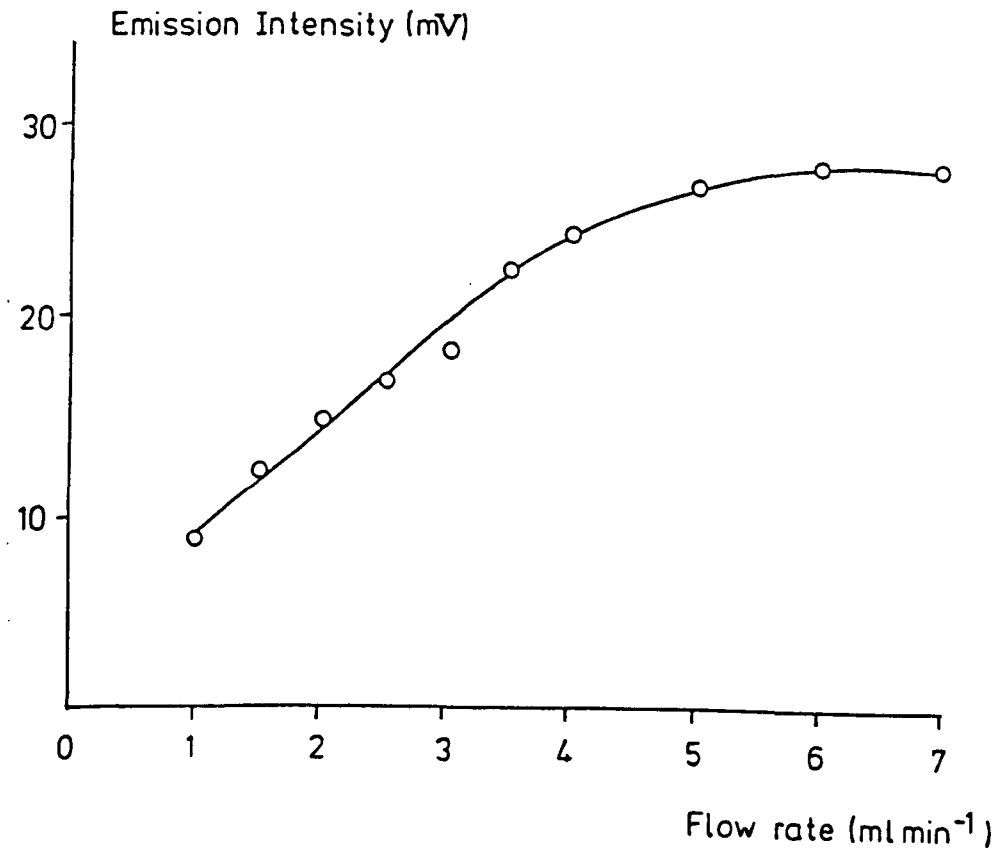


Fig. 3.3. Effect of flow rate on peak height.

TABLE 3.2

EFFECT OF FLOW RATE ON DURATION OF SIGNALS OBTAINED FOR HYDRAZINE

Flow rate ($ml\ min^{-1}$)	Time (in sec.) required from injection point:	
	to reach maximum	for complete peak
1.0	40	100
1.5	30	70
2.0	20	50
2.5	17	40
3.0	14	40
3.5	12	30
4.0	12	30
5.0	11	28
6.0	11	28
7.0	10	25

The limit of detection (signal twice the blank noise) was $3x10^{-7}M$ (0.4ng) hydrazine which give rise to a signal of 0.08mV.

To illustrate the reliability of the procedure, results for a typical short range calib ration are shown in table 3.3 and figures 3.5 (a and b). The linear calibration graph in figure 3.4 has a regression coefficient of 0.9988.

3.2.4.3. Interfering effects from manganese (Mn^{2+})

Among several metal ions examined for their interfering effects, including Ni^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, Ag^{+}, Fe^{3+}, Al^{3+}, Cd^{2+} and Mn^{2+} (in the concentration range 1 - 100$\mu g\ ml^{-1}$), Mn^{2+} (as $MnCl_2$ solution) was the only cation that exhibited a significant effect on the present CL system for

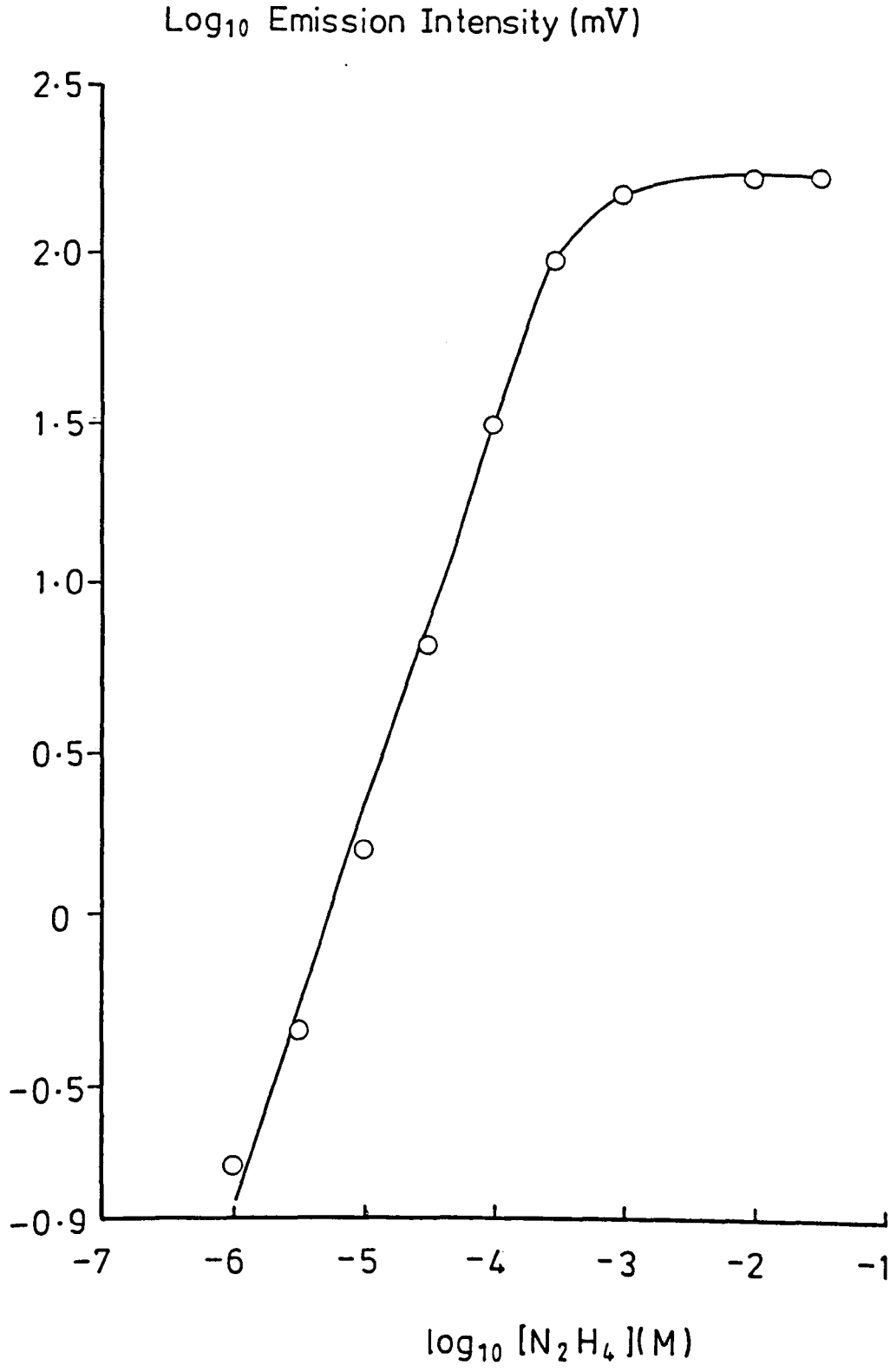


Fig. 3.4 Long range log-log calibration graph for hydrazine.

TABLE 3.3

CALIBRATION RESULTS FOR HYDRAZINE

Hydrazine Conc. (M)	Emission Intensity (mV)	Mean Value (mV)	R.S.D. (%)
$1x10^{-5}$	1.64 1.70 1.62	1.65	2.5
$2x10^{-5}$	3.84 3.72 3.84	3.8	1.8
$3x10^{-5}$	7.2 7.2 7.4	7.3	1.8
$4x10^{-5}$	10.4 10.2 10.4	10.3	1.2
$5x10^{-5}$	13.2 13.4 13.3	13.3	0.8
$6x10^{-5}$	16.4 16.3 16.5	16.4	0.6

Mean r.s.d. = 1.5%

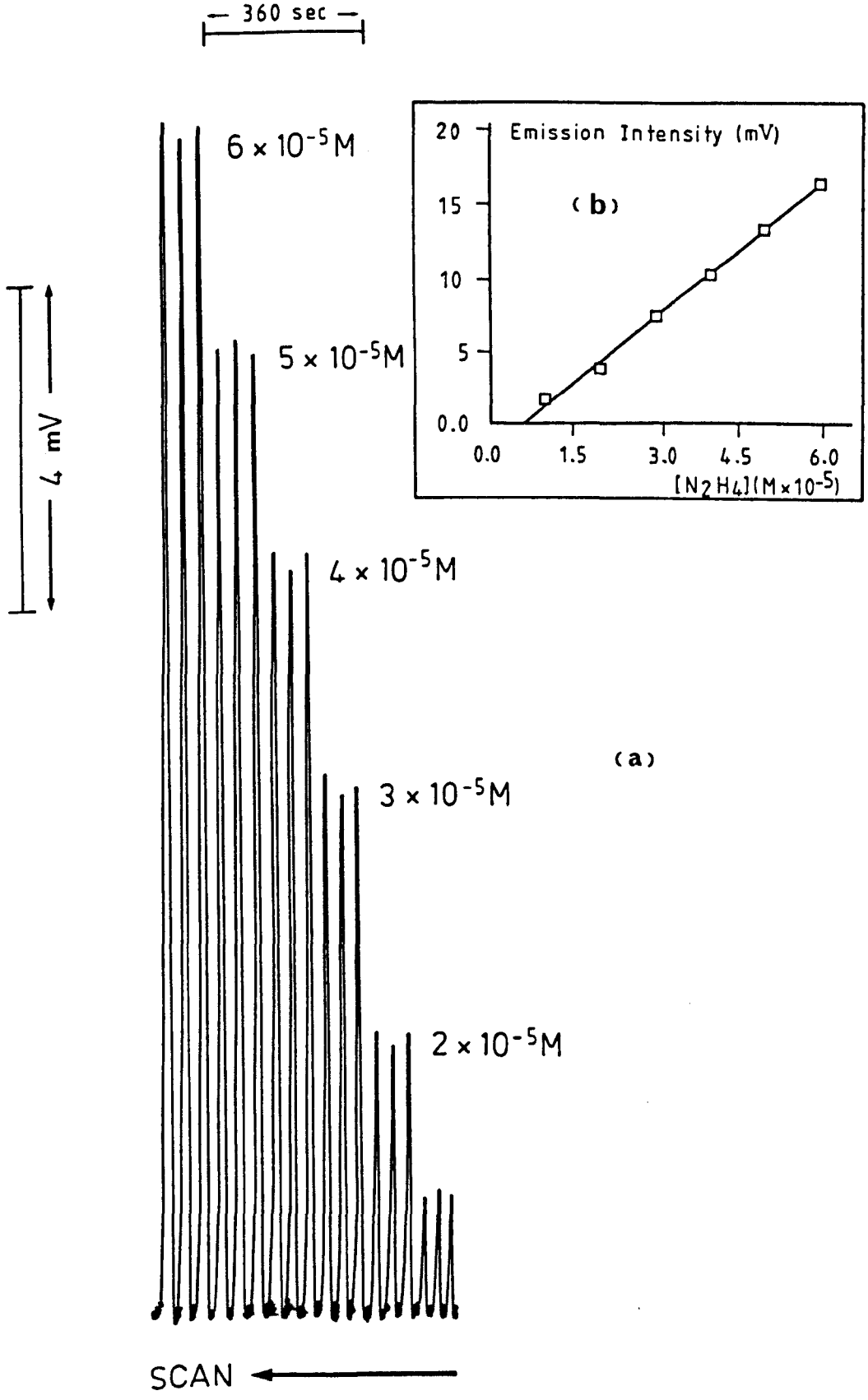


Fig. 3.5. (a) Short range calibration peaks for hydrazine concentrations shown. (b) the associated calibration graph.

hydrazine. Therefore further studies were made to investigate the effects of Mn^{2+} in detail. Firstly, a range of Mn^{2+} concentrations (0.1 - 60$\mu g\ ml^{-1}$) was prepared with a constant concentration of hydrazine, and the final solutions injected into the system. Secondly, the manifold in figure 3.1 was modified by introducing a stream of Mn^{2+} to combine with the $KMnO_4$ before mixing with hydrazine/polyphosphate stream inside the detector housing. The same range of Mn^{2+} concentrations was applied in this case.

In both procedures Mn^{2+} inhibited the chemiluminescence. The results are shown in table 3.4 and figure 3.6.

The Mn^{2+} inhibiting effect was independent on the way it was added to the reaction system. Therefore, means of removal of Mn^{2+} from hydrazine samples should be established before analysis, which can be carried out on real samples which might contain manganese. The use of a chelating resin, which is discussed in detail in chapter 4, is a good way to attain quantitative elimination of Mn^{2+} from hydrazine samples.

3.2.4.4. Conclusions

The new chemiluminescent system which involves a reaction system of hydrazine/permanganate/polyphosphate is a direct and successful method for the determination of hydrazine. The optimum conditions investigated are summarised in table 3.5.

Although this CL system provides a simple and precise method for the determination of hydrazine, more investigations are still needed in order to improve its sensitivity. However, this CL system is less susceptible to metal ion interferences than hydrazine/NaOCl system (section 2.2.2.2.). This may partly be due to the complexing properties of polyphosphate. It should also be borne in mind that other substances produce CL (206) in this system, and this may cause a selectivity problem. If this is the case, isolation of hydrazine before analysis may be

TABLE 3.4

RESULTS FOR INHIBITING EFFECTS OF MANGANESE ON THE CL SYSTEM OF HYDRAZINE-PERMANGANATE-POLYPHOSPHATE

Mn^{2+} added ($\mu g\ ml^{-1}$)	Emission Intensity (mV)	
	Mn^{2+} added to hydrazine before injection	Mn^{2+} added via additional line
0	18.5	18.5
0.5	17.0	18.0
1.0	14.0	16.3
2.5	10.0	14.0
5.0	7.5	12.5
10	6.0	9.5
20	5.0	7.0
30	4.5	5.5
40	4.5	5.2
60	4.0	4.0
(1000)	4.0	4.0

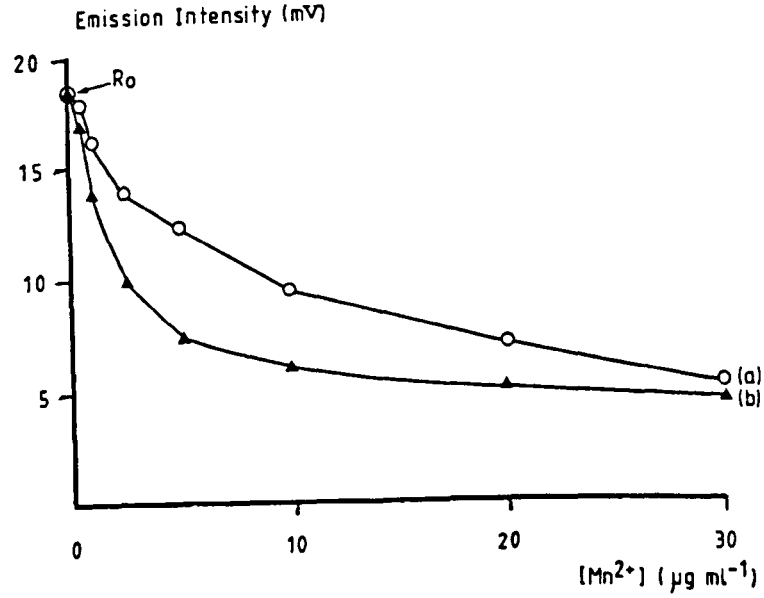


Fig. 3.6. Inhibiting effect of manganese ion (a) Mn^{2+} added to N_2H_4 sample (b) added via manifold; $[N_2H_4] = 6x10^{-5}M$. R_o is initial response of hydrazine before Mn^{2+} added.

necessary. However, the chemiluminescence methods are much simpler and cheaper than other methods with similar detection limits that require expensive and elaborate instrumentation with a very poor sampling frequency.

TABLE 3.5

OPTIMUM CONDITIONS FOR HYDRAZINE DETERMINATION USING CL SYSTEM OF HYDRAZINE/PERMANGANATE/POLYPHOSPHATE

PARAMETER	VALUE
Carrier stream	0.05M polyphosphoric acid
pH	1.6
Potassium permanganate	$5x10^{-4}M$
Sample volume	30µl
Flow rate (combined)	4ml min^{-1}
Detection limit	$5x10^{-7}M$
Mid-range precision	1.5%
Sample rate	90 h^{-1}
Free Mn^{2+} ions should be absent	

Finally a comparison between the major characteristics of this new method and other CL methods noted in chapter 2 (sections 2.2.2.2 and 2.2.3) is summarised in table 2.6.

TABLE 3.6

A COMPARISON BETWEEN DIFFERENT CL METHODS FOR HYDRAZINE DETERMINATION

Parameter	Reaction system contain hydrazine with		
	NaOCl	Metal-NaOCl	$KMnO_4$-polyphosphate
Reaction medium pH	11.5	11.5	1.6
Limit of detection (M)	$5x10^{-8}$	$5x10^{-10}$ (Ni^{2+})	$5x10^{-7}$
R.S.D. (%)	1.5	1.5	1.5
Sample rate	120 h^{-1}	120 h^{-1}	90 h^{-1}
Selectivity	many common cations either inhibit or enhance		only Mn^{2+} interferes seriously

CHAPTER FOUR

INCORPORATION OF A MINIATURE ION-EXCHANGE COLUMN IN THE FLOW INJECTION CHEMILUMINESCENT SYSTEM.

4.1. Introduction and Research Objectives

Foreign ions often interfere in an analytical procedure. These interferences can sometimes be removed before the determination of the analyte ion by using a suitable ion-exchanger. The use of a complexing or chelating resin is especially advantageous in this respect.

Because of the high sensitivity of chemiluminescence analyses interfering effects are always important. For example, a practical difficulty in using the luminol system for transition metal analysis at the trace level is its lack of specifity since many samples drawn from natural sources contain many of the metals which react to give chemiluminescence with luminol. This difficulty may be overcome by masking the interfering metals. However, trace metal contamination always causes significant background chemiluminescence and, in some cases, organic complexing agents are used to depress such effects (150). Little attention has been paid to solve these problems in real situations, and this has undoubtedly limited applications of chemiluminescence for the analysis of real samples.

Flow injection analysis provides a versatile means for monitoring chemiluminescence detection. The simplicity of the instrumentation (Chapter 2) faciliates manifold design so as to solve the selectivity problems and purification processes necessary for viable chemiluminescence monitoring. As ion exchange chromatography has proved the best available means of resolving the difficulty of specifity, the use of a minicolumn of a suitable exchanger resin into the F.I.A.-CL system was thought to be an appropriate solution to interference problems. The inspiration for this kind of application arose, in part, from the need to prevent metal

inhibition of the chemiluminescent reaction of hydrazine with sodium hypochlorite. The aim of this work was to originate an F.I.A.-CL manifold incorporating a miniature column to eliminate such inhibition phenomena and to establish conditions for column performance that are compatible with the conditions required for the CL reaction.

4.1.1. Fundamentals of Ion-Exchangers

Ion-exchangers have an important place in analytical chemistry. They are mostly used in the separation of mixtures of ions (208-211), but also can be used in the preconcentration of ions (212-215), in preparing and purifying solutions (216-218) and in many other ways. Many monographs on ion exchangers and their applications are available. (219-225)

Ion-exchange is the reversible interchange of ions between a solution and a solid, insoluble body (224). The solid material contains a network or matrix that is fixed and chemically insensitive to the surrounding electrolyte. It acts like a semipermeable membrane through which charged species can diffuse. The matrix always carries fixed ions via covalent bonds and exchangeable or mobile ions via electrostatic forces. The latter are called counter ions when they have charges opposite to those of the fixed ions. These counter ions can be exchanged when brought in contact with an external solution containing ions of suitable charge and size. A schematic drawing of the structure of an ion-exchange resin bead is shown in figure 4.1 (224).

If the network carries a negative charge, the mobile ions will be positively charged, and the material is a cation exchanger. Conversely, for a positively charged network the mobile ions are negatively charged and the material is an anion exchanger. Typical exchangeable ions for cation and anion exchangers are shown in table 4.1.

Most commercially available ion exchange resins are spherical

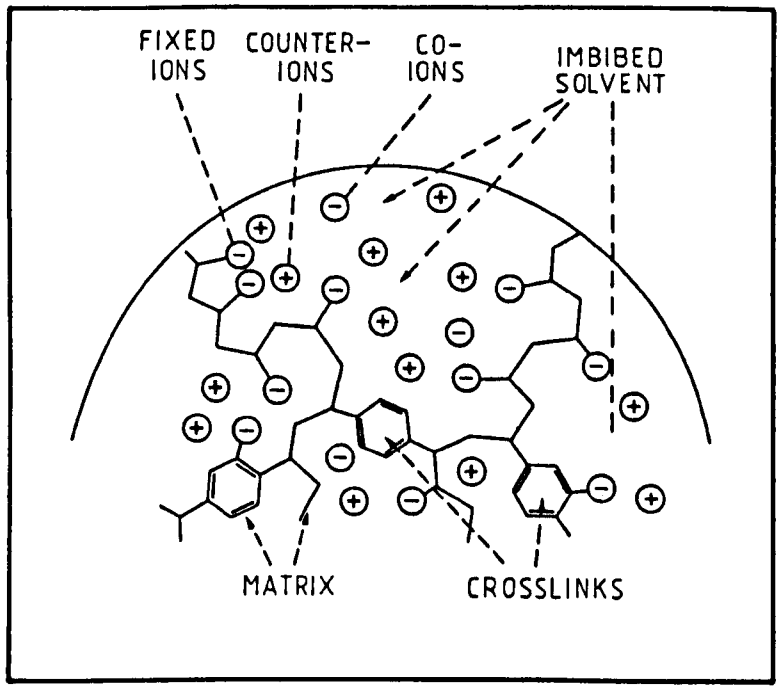


Fig. 4.1. Diagram of an ion exchange resin bead.

beads. When placed in water, the beads swell because water diffuses into the interior. The amount of swelling is directly related to the type and number of functional groups affixed to the matrix, since different groups have different degree of hydration.

TABLE 4.1

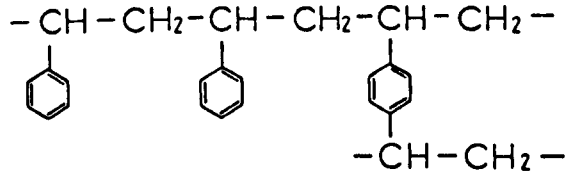
EXCHANGEABLE IONS IN CATION AND ANION EXCHANGE RESINS

Cation exchanger	Anion exchanger
H^+, Na^+, Ag^+, Pb^{2+}	OH^-, Cl^-, $SO4^{-2}$, $HSO4^-$, $PO4^{-3}$,
Ca^{2+}, Zn^{2+}, Fe^{3+}, Th^{4+}	$HPO4^{-2}$, H_2PO4^-, SCN^-, I^-, ---
----- etc.	--- etc.
Complexes, e.g. $FeCl^{2+}$	Complexes, e.g. $FeCl_4^-$, $ZnCl_4^{2-}$,
$Cu(NH_3)_4^{2+}$.	etc.
Organic ions RNH_3^+, R_4N^+	Organic ions: $RCOO^-$

Swelling is a reversible process and the equilibrium is determined by the two opposing forces (226); the elastic forces of the polymer matrix and the osmotic pressure within the resin bead which is a measure of a resin's capability to undergo hydration. There is also an inverse relation between the swelling and the degree of cross-linking. Therefore as the degree of cross-linking increases the resin structure becomes stiffer, the amount of swelling decreases and thus exchange phenomena will be slower.

The extent of swelling of an ion-exchange resin is inversely related to the concentration of the electrolyte with which the resin has made contact (or to charge on the ions present in the electrolyte) (221). Therefore it is essential to consider the change in resin volume accompanying the absorption of the solvent when used as a column in a flow system to prevent the extra pressure which might interrupt the manifold connection.

Typical ion-exchange resins are made from styrene and divinylbenzene. The crosslinked polymer structure (V) is the product:



V

The introduction of the ionic functional groups into the crosslinked matrix is accomplished by appropriate chemical reactions. Table 4.2. summarizes some properties of organic ion exchangers.

Resins are almost always provided in the form of spherical beads, ranging from 1μm to 1mm in diameter. Although a relatively high pressure gradient is produced during column operation with smaller resin particles, these resins have a higher exchange efficiency and exhibit faster equilibration; thus the use of a smaller quantity of such resins may be permissible.

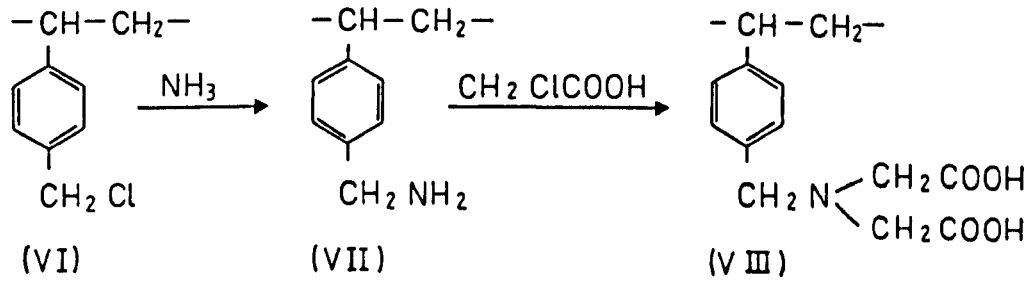
TABLE 4.2

SOME CHARACTERISTICS OF ORGANIC ION-EXCHANGERS

Resin Type	Functional Group	Exchange Capacity ($meq\ ml^{-1}$ wet)	Max. Temp. (^{o}C)	pH range	Commercial Resins
(i) Cation:					
Strongly acidic	$-SO_3^-$	1.9	120	0-14	Dowex 50 Amberlite IR-110 Lewatit S100
Weakly acidic	$-CO_2^-$	3.5	100	4-14	Amberlite IRC-50 Permutit H70 Wofatit CP-300
(ii) Anion:					
Strongly basic	$-NR_3^+$	1.2	60	0-14	Dowex 1 Amberlite IR-400 Ostion AT Anex AV-19 Lewatit M500
Medium and Weakly basic	$-R_2$	1.3	70	0-9	Zerolit MPH Amberlite IRA 93 Wofatit AD41
(iii) Chelating	$-N\begin{matrix}\diagup CH_2COO^- \\ \diagdown CH_2COO^-\end{matrix}$	3.9	110	4-10	Dowex A-1 Chelex 100 Wofatit CM50 Lewatit Tp-207

4.1.2. Chelating ion-exchangers

Chelating ion exchangers are normally based on a styrene-divinylbenzene copolymer with low cross-linking or with a macroreticular structure. They are able to form inner complexes with numerous ions. The functional group of these resins is iminodiacetic acid [$-N(CH_2COOH)_2$] introduced as follows (227)



These exchangers are suitable for selective sorption and separation of heavy metals and some other transition elements (228-232). The affinity of these resins towards metal ions is reported as the following series (222, 233):

doubly charged ions: Hg > U(VI) > Cu > V(V) > Pb > Ni > Cd > Zn > Co > Fe > Mn > Be > Ca > Mg > Ba > Sr.

triply charged ions: Cr > In > Fe > Co > Al > La.

alkali metals: Li > Na > K > Rb > Cs.

Dowex A-1 is a typical chelating resin which was used throughout the work in this chapter. The resin is in the form of spherical grains of diameter 315-400μm (40-50 mesh). Two other varieties are produced, 100-200 mesh and 200-400 mesh, which are called Chelex-100. They are obtained by grinding down the Dowex A-1.

Fundamental research carried out so far on Dowex A-1 has dealt mainly with synthesis (227), kinetics (234), equilibrium studies (228, 235), separation possibilities (233, 236) and the effects of the medium (237).

The variation in the resin's capacity with concentration of some cations has been studied (233). Figure 4.2 shows that, for doubly-charged cations which give very stable complexes with the resin (Cu, Ni, Cd, Co, Mn, Ba, Ca, Mg and Sn), the capacity increases more or less linearly with concentration. This is due to the penetration of the electrolyte into the grains as a result of Donnan equilibrium. The slopes of the lines vary according to the element in question which is explained by the fact that the Donnan constants vary according to the element. In case of H^+ and Pb^{2+} the capacity increases greatly but non-linearly with concentration, while for alkali metal cations the increase in capacity with concentration is small, since these cations exist in the interior of the resin largely as free ions and not as complexes with the exchange groupings.

Knowledge of the variation of distribution coefficient values with pH is important in planning retentions utilizing Dowex A-1. The distribution coefficient D is defined by:

$$D = \frac{\text{amount of metal ion per g of dry resin}}{\text{amount of metal ion per ml of solution}}$$

The pH dependence of the distribution coefficient has been investigated extensively by Leyden and Underwood (235). All cations show similar behaviour, exhibiting D values which gradually increase with pH above 4 and become maximal at pH 7. These results have been confirmed recently by Nakashima et al. (238), who found that the absorption of some metal ions (e.g. Mn^{2+}) above pH 9 decreases whereas for some others (Pb^{2+}) the absorption increases above pH 8. The decrease in absorption of manganese was attributed to hydrolysis of the metal ion and, conversely, the increase in iron retention above pH 8 may suggest absorption or adherence of hydroxo complexes.

Regeneration of the exchanger is usually carried out with $>1M$ HCl and H_2SO4. The exchanger resists mild oxidizing agents; on heating

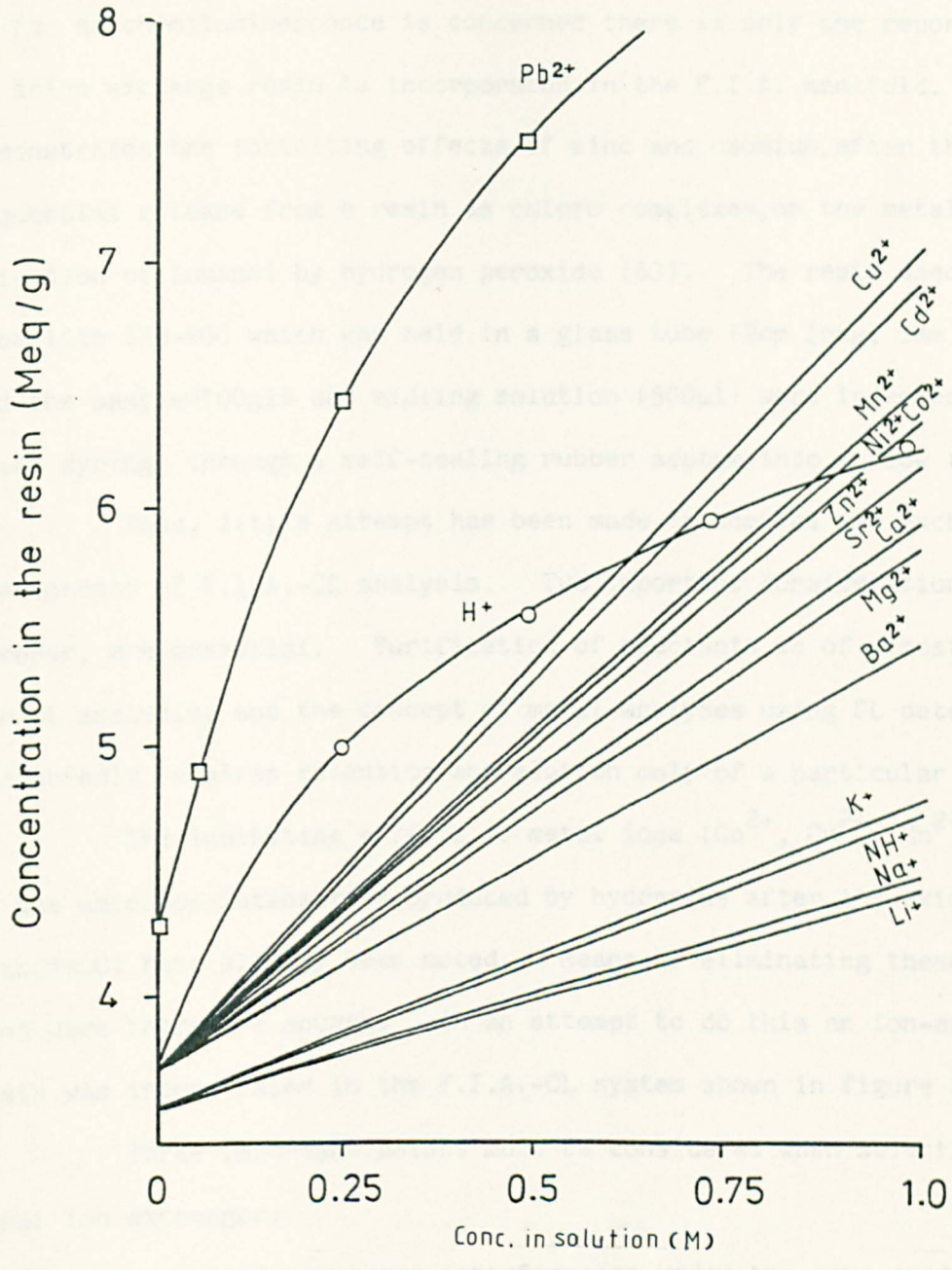


Fig. 4.2. Capacity interpretation of Dowex A-1 resin for metal ions (233)

in water the resin shows a little degradation at 75°C after 100 hours.

4.1.3. Ion exchange in flow injection chemiluminescent systems

In F.I.A. the incorporation of ion exchangers has not been much reported despite its potential. The little work that has been done is discussed in detail in Chapter 7. The aim of this section is to deduce the important features of a combination of F.I.A.-CL with ion-exchange.

As far as chemiluminescence is concerned there is only one report in which an anion exchange resin is incorporated in the F.I.A. manifold. It demonstrates the inhibiting effects of zinc and cadmium, after their sequential release from a resin as chloro complexes, on the metal-catalysed oxidation of luminol by hydrogen peroxide (63). The resin used was Amberlite IRA-400 which was held in a glass tube (2cm long, 3mm i.d.) and the sample (100µl) and eluting solution (500µl) were injected from a glass syringe through a self-sealing rubber septum into a flow tube.

Thus, little attempt has been made to combine ion exchange with the concept of F.I.A.-CL analysis. Two important considerations, however, are essential. Purification of reactants is of utmost importance for CL analysis, and the concept of metal analyses using CL detection undoubtedly requires retention and elution only of a particular metal ion.

The inhibiting effects of metal ions (Co^{2+}, Cu^{2+}, Mn^{2+} and Cr^{3+}) on the emission intensities produced by hydrazine after its oxidation with NaOCl have already been noted. Means of eliminating these metal ions were therefore sought. In an attempt to do this an ion-exchange resin was incorporated in the F.I.A.-CL system shown in figure 2.11.

Three important points must be considered when selecting an ideal ion exchanger:

1. The resin should eliminate interferences under the same conditions applied for the original CL reaction.
2. The operation of the exchanger should be controlled by providing conditions that do not disturb the acidity or alkalinity of the reaction medium.
3. Dispersion is a most important parameter in F.I.A. (page 8).

Thus every effort should be made to minimize additional dispersion when the exchanger column is incorporated. Therefore an extensive study is necessary to establish the effects of the physical characteristics of the resin particles as well as effects of column length, internal diameter etc.,

on the dispersion.

Accordingly, some ion-exchange resins have been examined for their suitability as a purification device in the F.I.A.-CL system, as described in the following experiments.

4.2. Experimental

4.2.1. Preparation of the ion-exchange columns

Four different ion-exchange resins were examined to identify which was most suitable. They were:

1. Amberlite IRC-718, wet mesh 16-50, approx. moisture 56% (Sigma Chemical Co.), capacity 1meq ml^{-1}.
2. Dowex A-1 chelating resin, dry mesh 50-100, moisture content 72% Na^{+}-form (Sigma Chemical Co.), capacity 3.9meq ml^{-1}.
3. Amberlite IRA-400, mesh size 16-50, moisture content 45% OH^{-}-form (B.D.H, AnalaR), capacity 1.2meq ml^{-1}.
4. Resin of polystyrene-supported poly(maleic anhydride) prepared by initiation of the polymerization of maleic anhydride by polystyrylethylpyridine) (239). It was supplied by Dr. Rowley, University of Edinburgh. Dry mesh, 20-35.

All resins were suspended in deionized water for at least 24 hours to allow swelling. The resin was then stirred well with fresh deionized water and the supernatant liquid was decanted repeatedly until it became completely clear.

The exchanger columns were prepared by adding an appropriate amount of the swollen resin in a glass tube (2.5cm long, 2mm i.d.). An electronic vibrator (Pifco 50Hz) was used to homogenize the settlement of the particles inside the column and to exclude the entry of air into the column during filling. The presence of air may lead to the formation of channels in the exchanger bed. A thin layer of glass wool was put at

both ends of the column to prevent movement of the resin particles by the carrier stream. A small piece of silicone rubber tube (0.8mm i.d.) was pushed into each end of the column so as to achieve a very tight connection, and a suitable adhesive (Bostik 1) was applied from outside onto the column-silicone rubber tube junction. Figure 4.3 is a photograph of the prepared exchanger minicolumns.

4.2.2. Efficiencies of the ion-exchanger minicolumns

One of the most efficient non-biological CL reactions, the oxidation of luminol by hydrogen peroxide in the presence of certain transition metal ions, is attractive as a means for sensitive determination of some transition metals and hydrogen peroxide (page 65). Therefore the Co(II)-luminol-H_2O_2 system was chosen to estimate the efficiency of each exchanger minicolumn by its effectiveness in retaining the Co(II) required for producing CL.

4.2.2.1. Reagents

Luminol, hydrogen peroxide, carbonate buffer (pH 10) and cobalt(II) standard solutions were all prepared as described in section 2.2.2.1), and deionized water from an Elgastat Spectrum deionizer was used in all preparations. All reagents (unless otherwise stated) were analytical grade.

4.2.2.2. Apparatus and Flow manifold

The apparatus shown in figure 2.6 (Chapter two) was used. The flow injection manifold in which the ion exchanger is incorporated is shown in figure 4.4. The miniature column was linked to the carrier stream by means of two 3-way valve one keys, as shown in figure 1.7. This was used to divert the direction of the sample stream whenever necessary without

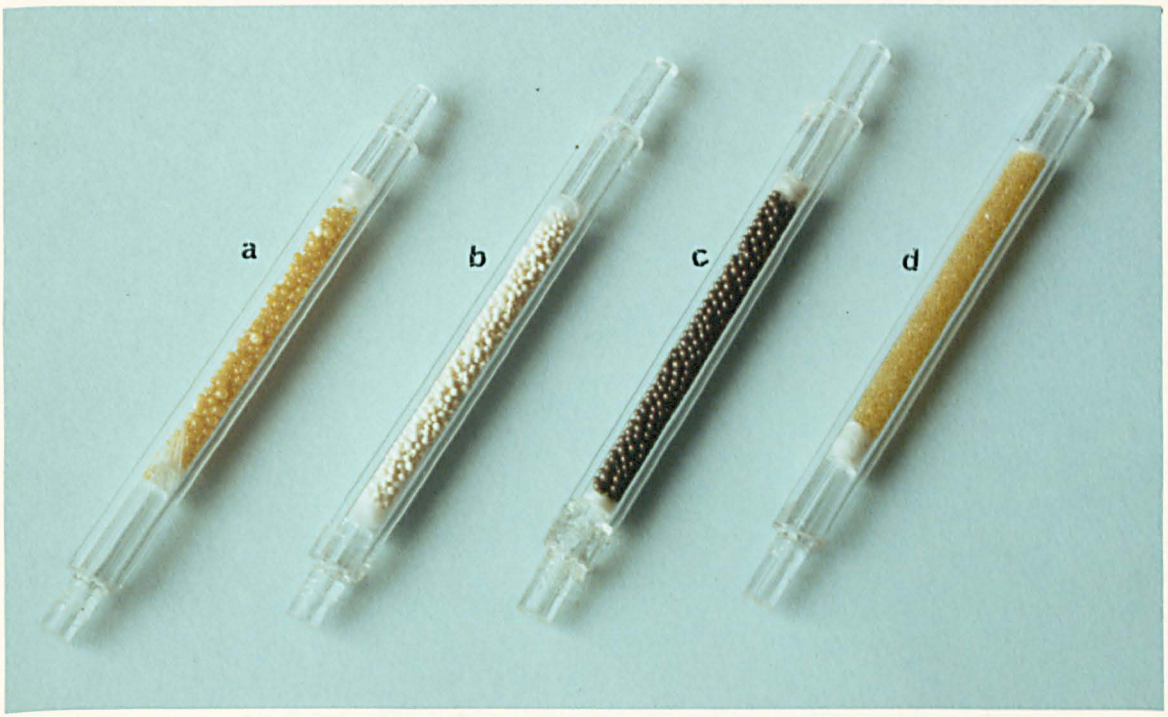


Fig. 4.3. Photograph of the prepared ion-exchange minicolumns. (a) Dowex A-1, (b) Amberlite IRC-718, (c) Resin of polystyrene-supported poly(maleic anhydride), (d) Amberlite IRA-400.

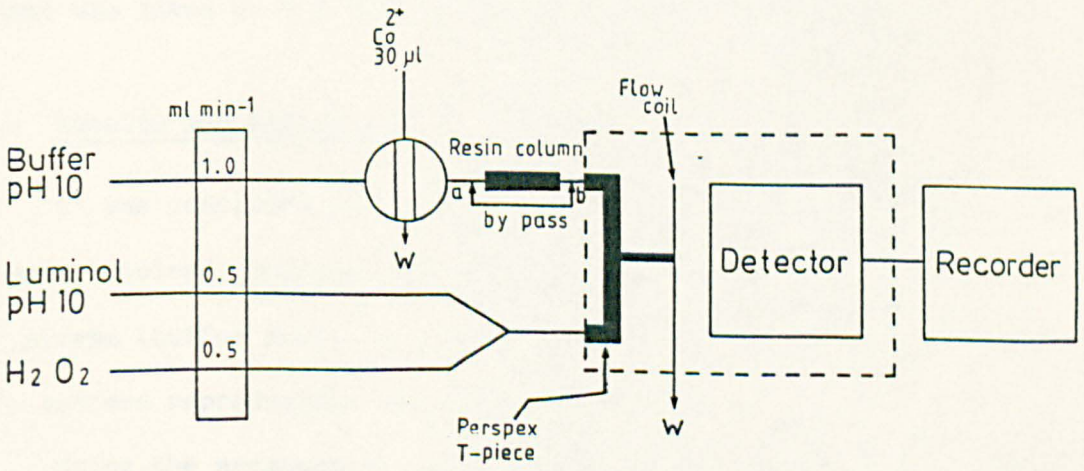


Fig. 4.4. F.I.A.-CL system combined with ion-exchange minicolumn as a purification device for the buffer and injected sample. W, waste, a, b, 3-way valve - single key.

causing any disturbance of the flow and keeping the experimental conditions invariable.

4.2.2.3. Procedure

A similar procedure was applied as that described on page (67) with respect to the CL reaction which is produced when Co(II) is added to a mixture of luminol and H_2O_2. The effect of incorporation of the exchanger column, however, was the main object of investigation, and its operation altered the experimental results. For all tests a 30µl sample solution of Co(II) was injected and was allowed to flow through a by-pass, mixed with a luminol/H_2O_2 mixture and hence lead to a CL emission, the intensity of which depended on the Co(II) concentration. Using key-valves at both ends of the column, the direction of the carrier stream was diverted towards the resin column and 30µl of the same Co(II) concentration was injected again and the resulting signal recorded. These two operations were applied with each concentration of metal ion injected. In all cases the output was in a form of an emission peak and its height was taken as the basis of the analytical measurements.

4.2.2.4. Results and Discussion

It was concluded as a result of preliminary investigations that the columns required conditioning for at least 15 minutes by passing a carrier stream (buffer solution) at 1ml min^{-1} through the column, in order to achieve reproducible results.

Using the arrangement shown in figure 4.4, Dowex A-1 chelating resin was the only effective resin column to retain quantitatively the amount of cobalt injected at any concentration level applied (0.01 - 25µg ml^{-1}), so that no CL reaction occurred, as indicated by a stable baseline on a chart recorder. For the other three resin columns the

emission signals obtained for a particular cobalt concentration were identical with and without the column. This showed the inefficiency of such columns under the present experimental condition provided by the pH 10 buffer solution. Therefore it was difficult to compare all the resin columns. The alternative was to modify the manifold so that the resin column was connected through an additional line in which deionized water was used as a carrier and this stream was combined later with the buffer stream. The rest of the manifold was unchanged.

Results obtained using this modified manifold are shown in figure 4.5. No emission signals were obtained for Dowex A-1, due to its removal of all the cobalt injected, but Amberlite-IRC, poly(maleic anhydride) and Amberlite IRA-400 were less effective. Amberlite IRA-400 was the most ineffective. The reasons for the inefficiencies of some of the ion exchanger beads may have arisen from their relatively large particle sizes (16-50 mesh).

The pH of the adsorbing medium, also, might have a significant effect. An acidic pH at which metal ions retained as chloro complexes (223) might be an alternative to improve their exchange capacity, but this would need further pretreatment of the resin column and the addition of new chemicals as a carrier stream in the manifold which might strongly influence the conditions established for the CL reaction. For example the best working pH for catalytic oxidation of luminol by hydrogen peroxide is 10 (195) and that of hydrazine oxidation by sodium hypochlorite is 11.5 (163), therefore an acidic stream might decrease the pH to a value at which the CL reaction become weak or non-existent. Introducing further reagents required for column operation may produce side effects such as the possibility of introducing more interferences. Similar interpretations may be made in the case of mixed solvents or organic solvent used for the column process.

The results in figure 4.5 gave rise to great confidence that the chelating resin (Dowex A-1) would effectively retain metal ions over

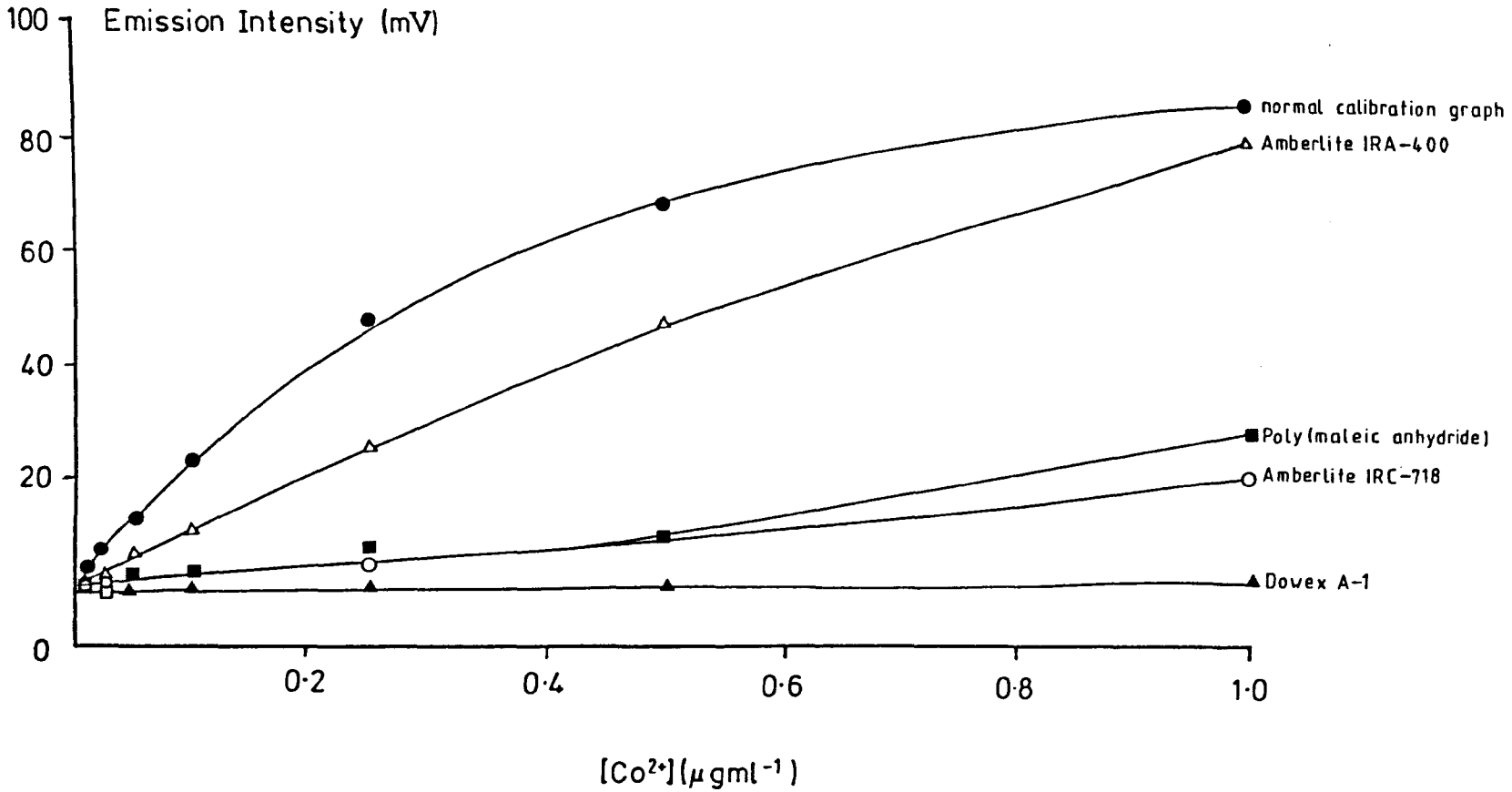


Fig. 4.5. Comparison between the efficiency of different ion-exchange resins in retaining Co(II) ions required for producing chemiluminescence with the luminol/H_2O_2 reaction system.

a wide pH range, so that it is the best resin column for sample and reagent purification during CL analysis, particularly when the retained metals are not required for further analyses. Nevertheless, more extensive studies have been made so as to improve the efficiency of other resin columns.

Effect of flow rate

The effect of flow rate on the efficiency of the resin columns was studied on the Dowex A-1 column (2.5cm length, 2mm i.d.) by applying conditions of the F.I.A.-CL system in figure 4.4. Figure 4.6 illustrates the effect for 0.1μg Co^{2+} ml^{-1}. The resin shows maximum efficiency in removing Co^{2+} from the injected sample up to a flow rate of 1ml min^{-1} through the column. Therefore this flow rate was used for further studies.

Effect of pH

Each resin column was studied in three different carrier streams, pH 4, obtained by adding 0.005M HCl to deionized water, pH 6.15 (deionized water) and pH 10 (0.1M carbonate buffer) (section 2.2.2.1). The F.I.A.-CL system shown in figure 4.4 and its associated procedure in section 4.2.2.3 were used. The carrier stream was replaced every time depending on the pH required for the column operation, but the rest of the manifold was unchanged. Figure 4.7 shows the effects of pH on the retention efficiency of the various resin columns.

The results show that the efficiency of Dowex A-1 is not affected by pH changes. Thus its wide pH range of operation makes it more reliable for CL purposes. Amberlite IRC-718, however, has better retention at pH$<$7, but even under these conditions the retention of Co^{2+} is not quantitative. The resin column of polystyrene-supported poly(maleic anhydride) has poorer retention at all pH values.

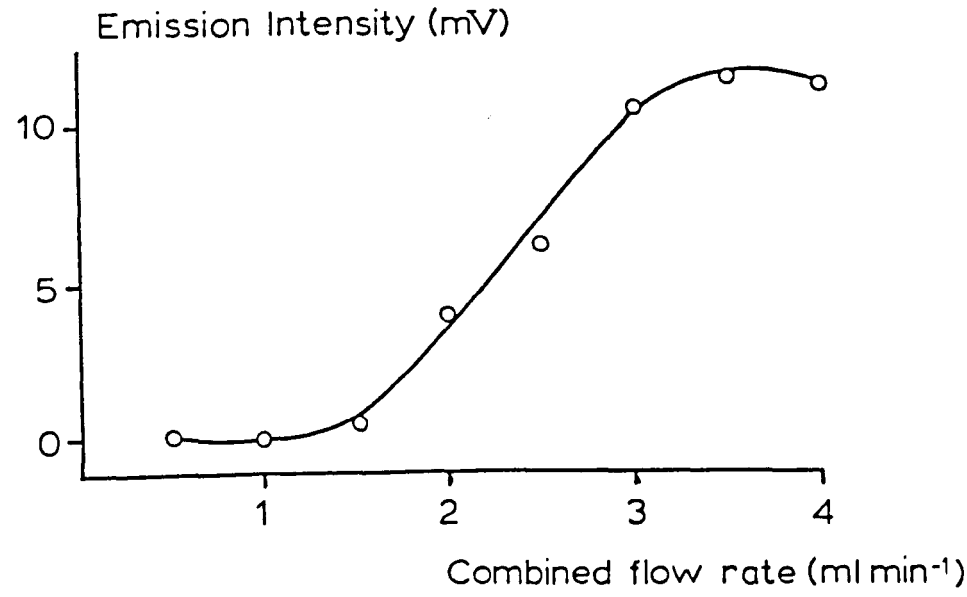


Fig. 4.6. Effect of flow rate on the efficiency of the Dowex A-1 column (0.1μg Co^{2+} ml^{-1})

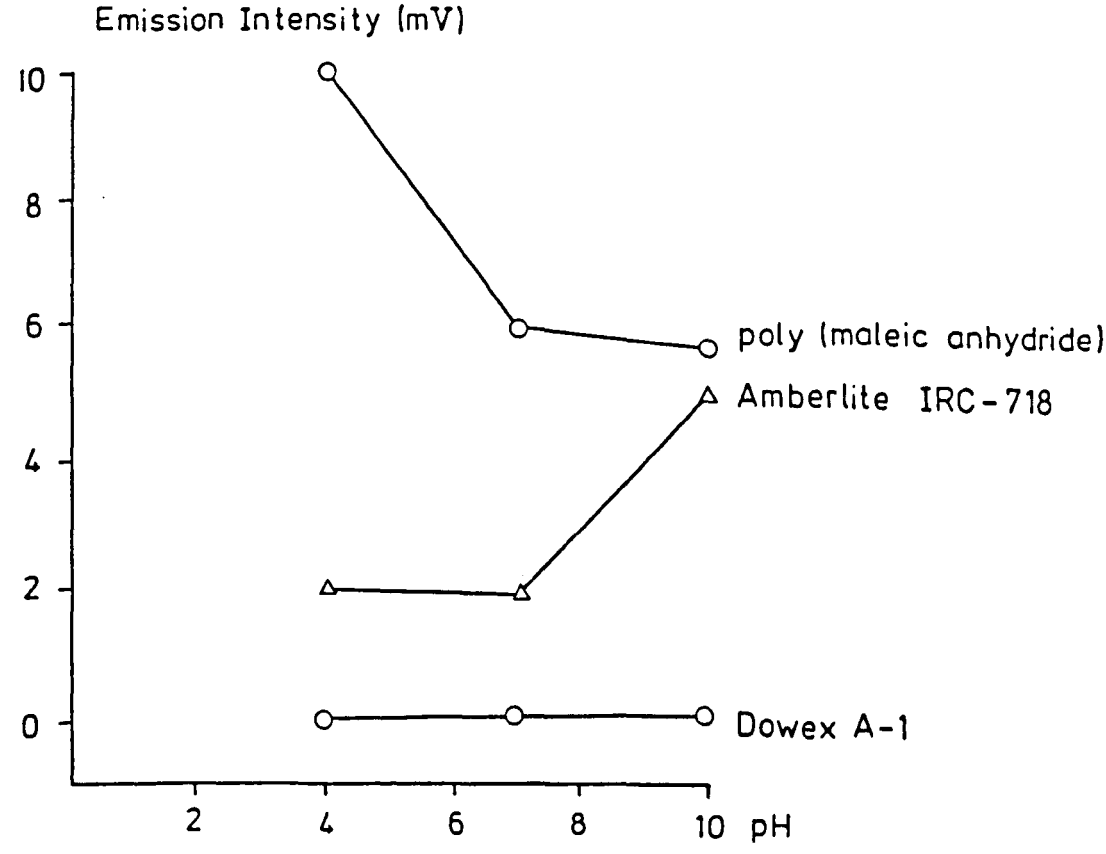


Fig. 4.7. pH effect on retention of 0.25μg Co^{2+} ml^{-1}.

Effects of Column Length and Internal Diameter

Different types of resin columns were prepared of various lengths and internal diameters, as follows:

(a) Keeping the internal diameter constant at 2mm, five columns of 1, 2, 3, 4 and 5cm long were prepared for each resin type.

(b) With a constant column length (3cm), three columns of 1, 2 and 2.5mm internal diameter were prepared for each resin type. Swelling and column packing was as described in section 4.2.1 and the studies were made in accordance with the procedure explained in section 4.2.2.3, using the F.I.A.-CL system shown in figure 4.4.

(a) Column Length

Table 4.3 shows emission signals obtained as a result of using different column lengths of each resin type. Dowex A-1 gave similar results with the three longer column lengths. It completely held the cobalt injected, thus no emission signals were produced. The 1cm and 2cm columns were less effective, and after retention of about $98\mu g\ Co^{2+}$ through successive injections of $30\mu l\ Co^{2+}$ samples, small emission signals were produced when $\geq 1\mu g\ Co^{2+}\ ml^{-1}$ was injected.

For the other ion-exchangers, longer columns were more efficient. Amberlite IRC-718 illustrates this effect, as shown in figure 4.8. Table 4.3 also indicates that increasing column length to more than 3cm did not improve the efficiency of the column further.

(b) Internal Diameter

According to the basic principles of F.I.A., the use of large diameter columns was avoided to prevent excessive dispersion. However, it was difficult to achieve adequate packing with columns of a very small internal diameter (<1mm). Therefore for the present investigations an internal diameter range of 1.0-2.5mm was studied as a compromise. The

TABLE 4.3

EFFECT OF COLUMN LENGTH

Resin Type	Column Length(cm)	Emission signals (mV) for 30µl Co^{2+} (µg ml^{-1})					
		0.01	0.05	0.1	0.5	1	2.5
Dowex A-1	5	0.0	0.0	0.0	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0
	2	0.0	0.0	0.0	0.0	0.0	1.0
	1	0.0	0.0	0.0	0.0	0.05	4.0
Amberlite IRC-718	5	0.0	0.45	1.2	1.8	-	-
	4	0.0	0.5	1.3	1.8	-	-
	3	0.0	0.5	1.5	2.0	-	-
	2	0.0	1.0	5.0	9.0	-	-
	1	0.5	2.0	8.0	14.0	-	-
Poly(maleic anhydride)	5	0.0	1.1	1.2	3.3	-	-
	4	0.0	1.1	1.25	3.3	-	-
	3	1	1.3	1.5	3.5	-	-
	2	1	16	3.0	18	-	-
	1	1.5	2.8	4.0	23	-	-

efficiencies of all the ion exchange columns were not significantly affected by changing their internal diameter. Table 4.4 illustrates such observations.

Column Capacity

Finally, it was easy during the present investigations to detect whenever a particular column started to lose its capacity.

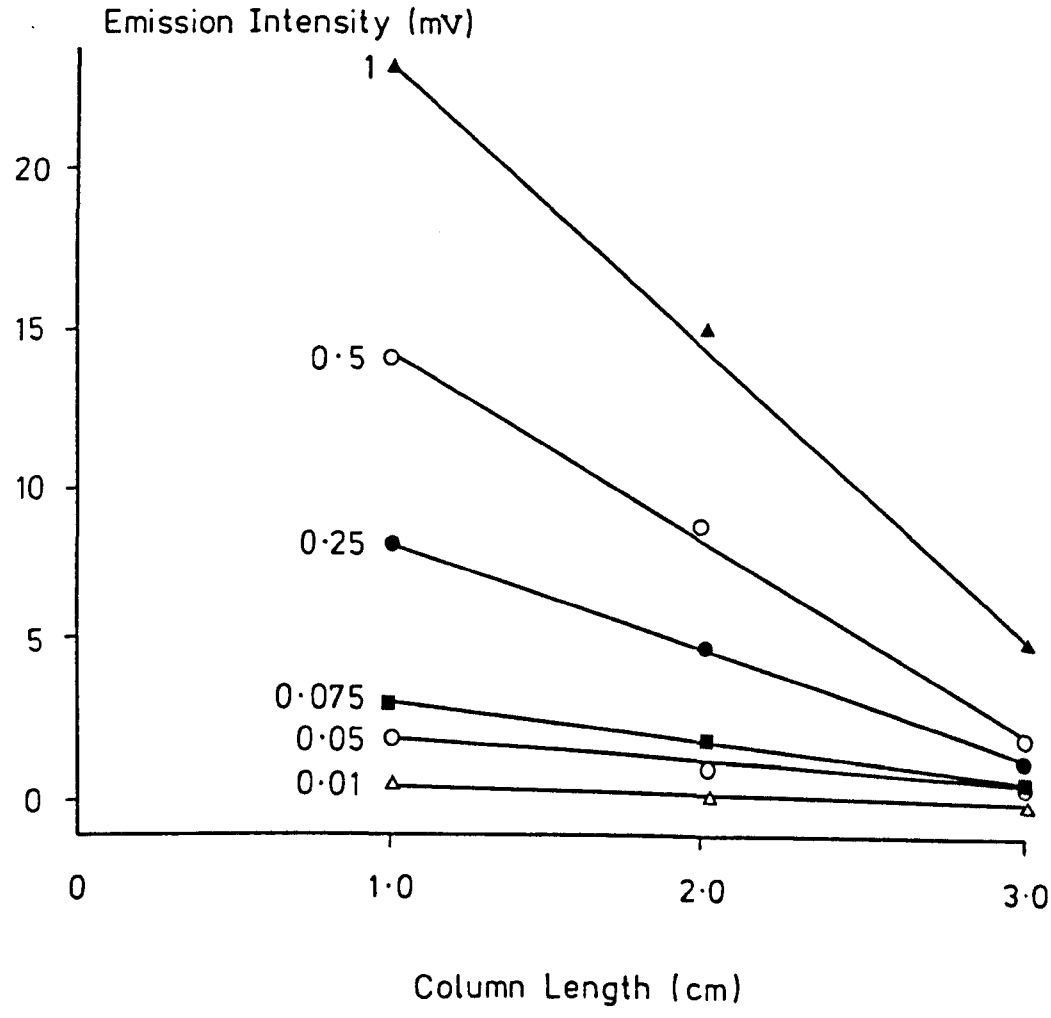


Fig. 4.8. Efficiency of Amberlite IRC-718 minicolumn as a function of column length for the Co^{2+} concentrations shown ($\mu g\ ml^{-1}$)

Figure 4.9 illustrates this phenomenon for a consumed column of Dowex A-1. When the column starts to lose its efficiency it no longer retains completely the injected cobalt and therefore the unbound metal produces some CL. As the capacity becomes increasingly saturated, the amount of metal passing through increases and hence produces higher signals.

Dowex A-1 chelating resin, therefore, is found to give the best column for sample and reagent purification from trace metal impurities in different pH media. Its operation is very simple and did not disturb the CL reaction.

TABLE 4.4

EFFECT OF INTERNAL DIAMETER

Resin Type	Column i.d. (mm)	Emission signals (mV) obtained for 30µl Co^{2+} (µg ml^{-1})				
		0.01	0.05	0.1	0.5	1.0
Dowex A-1	2.5	0.0	0.0	0.0	0.0	0.0
	2.0	0.0	0.0	0.0	0.0	0.0
	1.0	0.0	0.0	0.0	0.0	0.0
Amberlite IRC-718	2.5	0.0	0.54	1.45	1.95	-
	2.0	0.0	0.6	1.53	1.9	-
	1.0	0.0	0.6	1.5	2.0	-
Poly(maleic anhydride)	2.5	0.95	1.4	1.58	3.4	-
	2.0	1.0	1.4	1.62	3.4	-
	1.0	1.2	1.45	1.7	3.95	-

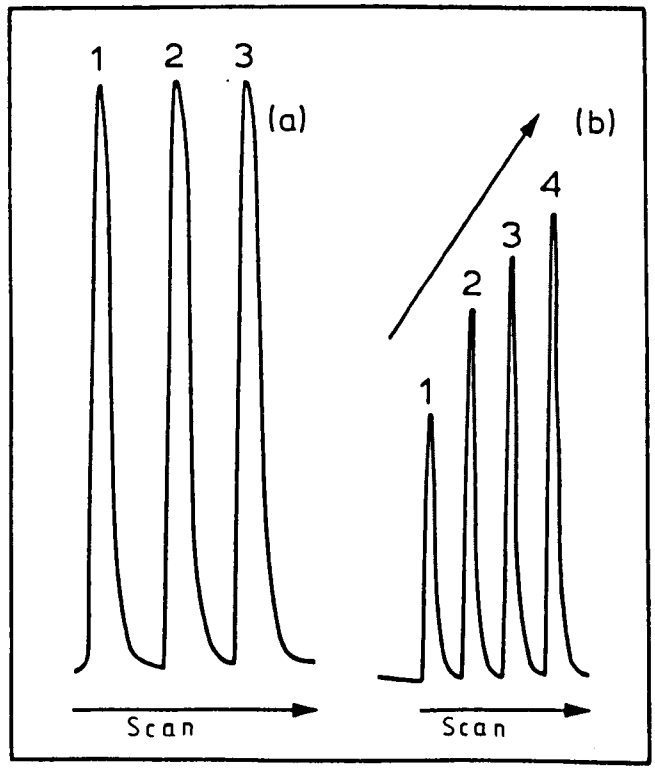


Fig. 4.9 Behaviour of Dowex A-1 as it loses its capacity. (a) Normal emission signals for 12.5µg ml^{-1} Co^{2+} injected to luminol-H_2O_2 mixture using F.I.A.-CL system in figure 4.4. (b) Signals obtained with the use of nearly consumed resin.

4.2.3. Application of Dowex A-1 chelating resin for purification in hydrazine determination.

4.2.3.1. Fundamental View

The miniature Dowex A-1 column was thought to be suitable for eliminating those metal ions that strongly affected the CL reaction of hydrazine with NaOCl (page 76) so as to establish a procedure in which hydrazine in different real samples could be determined despite variation in their metal impurity content. Therefore a small column containing the Dowex A-1 chelating resin (prepared as in section 4.2.1.) was incorporated in the system shown in figure 2.9 after the injection port by means of two 3-way valves each provided with a single key, as described in figure 1.7. These valves enable column disconnection whenever required without interruption of the flow.

Initially, 30µl of $1x10^{-6}$M hydrazine was injected into the carrier stream using the modified manifold. Allowing the injected sample to by-pass the column, the usual calibration emission signals were obtained, but when the hydrazine sample passed through the column the emission signal was completely extinguished. It was thought that this might be attributed to destructive interaction of the resin column with such a low concentration of hydrazine, but the fact that the same phenomenon occurred with more concentrated hydrazine solutions ($1x10^{-5}$ and $1x10^{-4}$M) defied such a possibility. No signals were obtained when different metal ions (Co(II), Ni(II), Zn(II), Cu(II), Cr(III) and Cd(II)) in the concentration range 0.1-25µg ml^{-1} were added to each hydrazine sample ($1x10^{-7}$-$1x10^{-2}$M). It appeared, therefore, that traces of some metal ions in the hydrazine sample were stimulating the CL reaction, and without these impurities the CL reaction did not occur. This resembles the oxidation of luminol by hydrogen peroxide in which the presence of traces of metals is required to generate CL emissions (section 2.2.2.1).

Therefore, it was decided to add metal ions to the reaction system beyond the position of the resin column to observe whether the diminished peaks reappeared again. This would undoubtedly elucidate whether hydrazine is destroyed by the column, or the effect is due to removal of metal impurities as described in the following experiments.

4.2.3.2. Reagents

Deionized water from an Elgastat Spectrum deionizer was used throughout and all chemicals used were analytical grade.
Hydrazine solutions, sodium hypochlorite and carbonate buffer were prepared as described in section 2.2.2.2.
Standard solutions of metal ions were prepared according to the procedure in section 2.2.3.

4.2.3.3. Apparatus and Flow manifold

The F.I.A.-CL manifold used is as shown in figure 4.10. The items are the same as in figure 2.6. A Dowex A-1 resin minicolumn was prepared as illustrated in section 4.2.1.

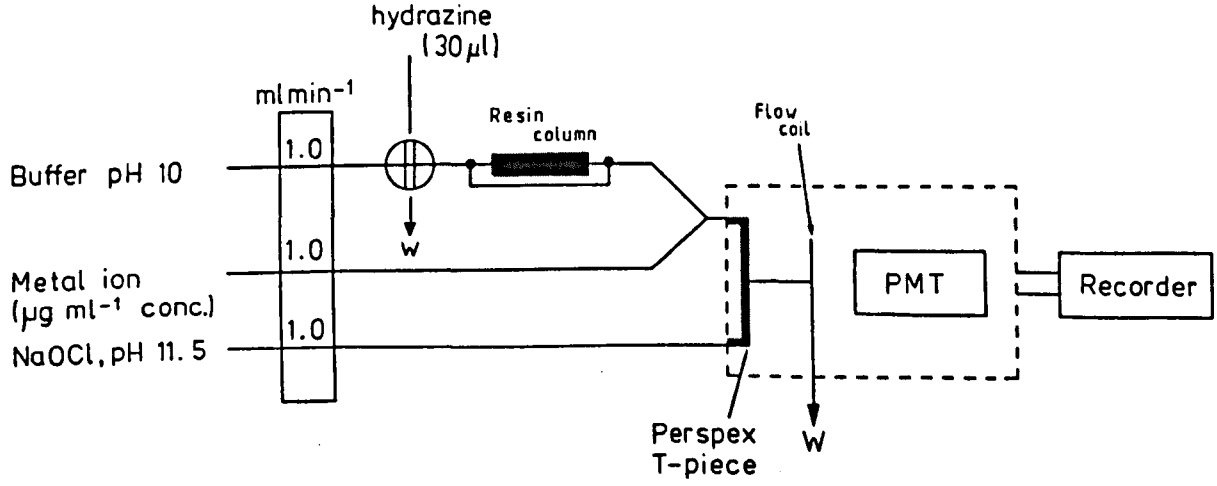


Fig. 4.10. Combined ion-exchange + F.I.A.-CL system used for purification and determination of hydrazine. W, waste.

4.2.3.4. Procedure

According to figure 4.10, the procedure included an immediate purification of hydrazine by means of a Dowex A-1 minicolumn, followed by mixing the purified hydrazine with an appropriate amount of metal ion of known concentration which then mixed with the hypochlorite stream in a Perspex T-piece to produce a CL emission.

4.2.3.5. Results and Discussion

The metal ions were examined in the concentration range between 0.04-25μg ml^{-1}. Preliminary experimental results indicated that no emission signals were obtained when Cu^{2+}, Co^{2+}, Mn^{2+} or Cr^{3+} were added to the purified hydrazine. This is probably due to the inhibiting characteristics of these metal ions towards the hydrazine/NaOCl system reported above (page 76). Cd^{2+}, Ni^{2+}, Zn^{2+} and Al^{3+}, however, which were previously found to enhance the CL, caused the CL peaks to appear when added to the purified hydrazine. This is important evidence that the hydrazine sample is purified rather than destroyed as it passes through the resin minicolumn.

Accordingly, such metal ion addition was used in an alternative method for the determination of hydrazine, which should be free of metal ion interferences. Hence, some optimization was necessary.

(a) Effect of pH

The Dowex A-1 minicolumn had shown efficiency in retaining metal ions in the pH range 4-10 (page 114), and pH 10.0 carbonate buffer is recommended as a carrier stream. As the hydrazine gave maximum CL emission at pH 11.5 (Section 2.2.2.2), it was necessary, therefore, to study the effect of the pH of the NaOCl stream when pH 10 carbonate buffer was used in the sample stream. When the F.I.A.-CL system in figure 4.10 was used, the pH profile resulting from the injection of

30µl of $1x10^{-4}$M hydrazine with 2.5µg Al^{3+} ml^{-1} as a catalyst shown in figure 4.11 was obtained. Sodium hypochlorite solution, pH 11.5, again gave maximum peak height, and was not affected by changing the pH of the carrier stream to 10.0.

(b) Effect of sodium hypochlorite concentration

The effect of NaOCl concentration on the CL intensity was measured by varying the concentration while keeping all other variables constant. The emission signals from two different hydrazine concentrations were measured and the results are shown in figure 4.12. The optimum concentration of sodium hypochlorite was ca. $5x10^{-4}$M.

(c) Effects of metal ion concentration

Using the F.I.A.-CL system in figure 4.10, the effects of Cd^{2+}, Ni^{2+}, Zn^{2+} and Al^{3+} ions were studied in the concentration range 0.04-12.5µg ml^{-1}, keeping the other variables constant. The emission intensities produced for hydrazine concentrations of 10^{-7}-10^{-4}M using cadmium or nickel as a catalyst are shown in table 4.5 and figures 4.13 and 4.14. These two metals in the concentration range 0.025-10µg ml^{-1} demonstrated similar characteristics despite the difference in their concentration levels that produced maximum emission signals. For all hydrazine solutions studied, maximum intensity was produced by 6.25µg ml^{-1} Cd^{2+} and 0.195µg ml^{-1} Ni^{2+}. A linear relationship was attained between metal concentration and the emission signals obtained in the range 0.04-6.25µg ml^{-1} for Cd^{2+} and 0.04-0.195µg ml^{-1} for Ni^{2+}. In both cases, further addition of metal ion diminished the signals.

For zinc, on the other hand, the emission responses increased continuously by increasing the Zn^{2+} ion over the range studied. The results are shown in table 4.6. The log-log graph of intensity versus zinc concentration was linear over the range studied (figure 4.15). For

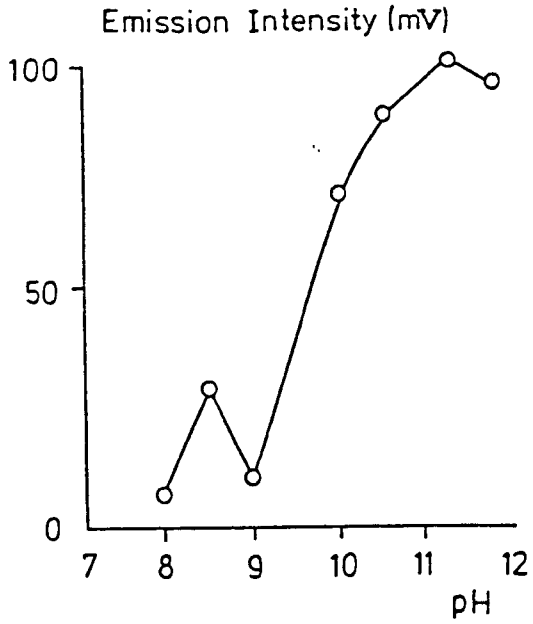


Fig. 4.11. Effect of pH of NaOCl on the CL emissions.

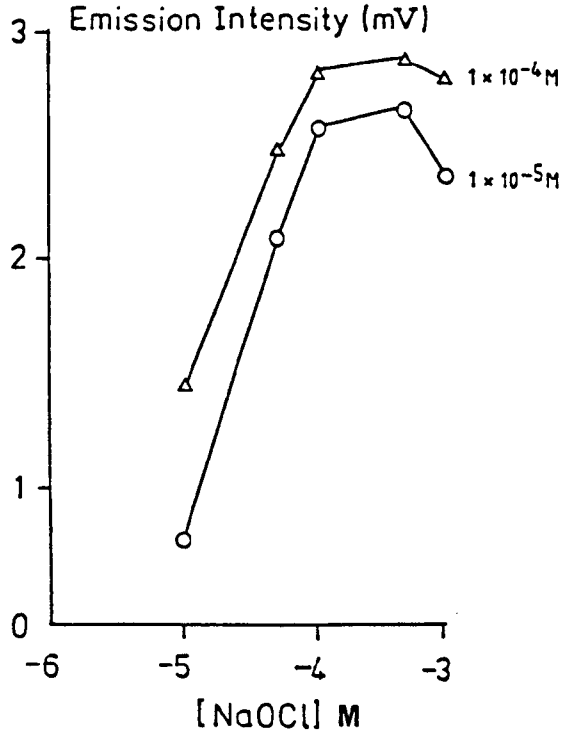


Fig. 4.12. Effect of sodium hypochlorite concentration. $[Al^{3+}]$ is 0.78μg ml^{-1} at the hydrazine concentrations shown.

TABLE 4.5

EMISSION SIGNALS FROM THE CHEMILUMINESCENT REACTION OF Cd^{2+} and Ni^{2+} WITH HYDRAZINE/NaOCl SYSTEM

Metal ion Conc. ($\mu g\ ml^{-1}$)	Emission Intensity (mV)						
	Hydrazine Concentrations (M)						
	$1x10^{-7}$	$5x10^{-7}$	$1x10^{-6}$	$2.5x10^{-6}$	$5x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$
a. Cadmium							
0.04	0.0	0.0	1.75	3.2	5.3	8.5	17.4
0.095	0.0	1.3	2.4	4.4	6.8	11.4	23.0
0.39	0.0	1.75	3.0	5.3	8.7	14.5	28.5
3.13	0.0	2.65	4.2	6.4	13	22.5	36.5
6.25	0.0	3.5	6.0	9.2	14.6	25.6	46.4
12.5	0.0	2.5	4.0	7.6	12	23.5	45.7
25	0.0	1.45	2.0	3.5	6.4	8.5	16.0
b. Nickel							
0.04	0.0	1.4	2.96	4.5	7.0	12.5	26.0
0.095	0.0	1.8	3.5	6.0	8.0	14.0	30.0
0.195	0.0	2.0	4.0	7.5	10.0	17.0	35.0
0.39	0.0	2.0	3.0	5.0	7.6	13.5	32.0
0.78	0.0	1.0	2.0	3.0	4.5	7.5	22.0
1.56	0.0	0.0	1.0	1.5	2.5	3.0	5.0

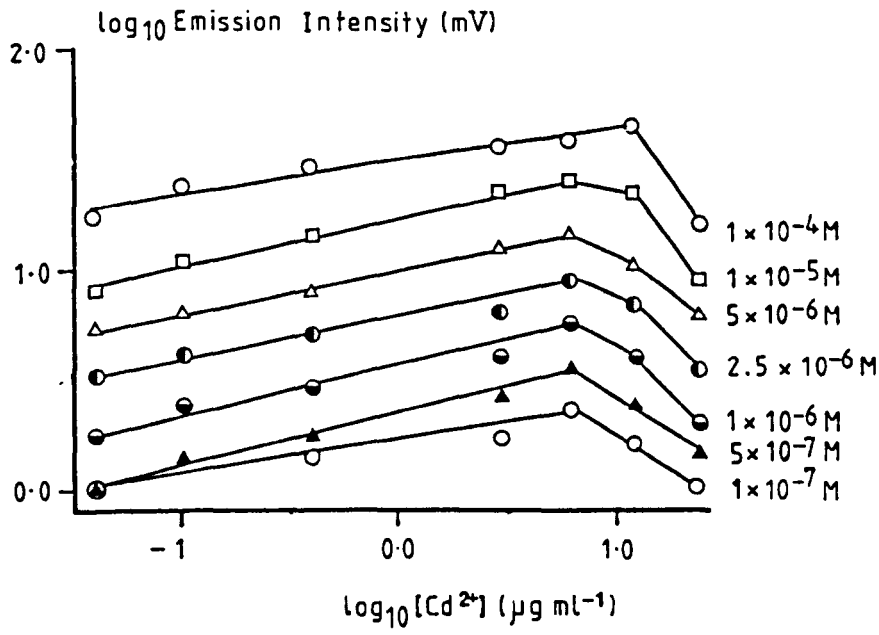


Fig. 4.13. Effect of cadmium in stimulating the CL of purified hydrazine/NaOCl system at the hydrazine concentration shown.

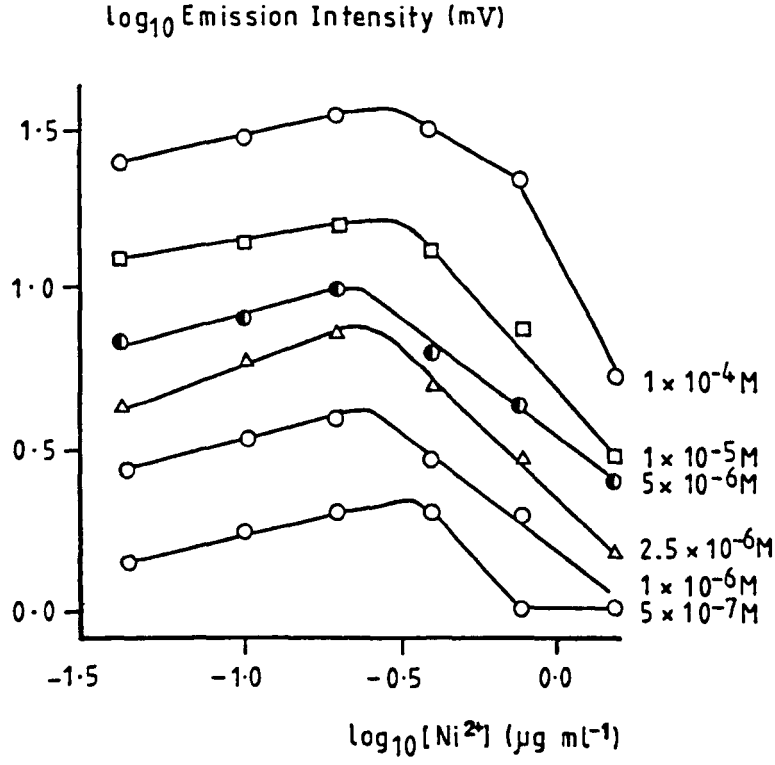


Fig. 4.14. Effect of nickel in stimulating the CL of purified hydrazine/NaOCl system at the hydrazine concentrations shown.

TABLE 4.6

EMISSION SIGNALS FROM THE CHEMILUMINESCENT REACTION OF Zn^{2+} WITH PURIFIED HYDRAZINE/NaOCl SYSTEM

Metal ion Conc. ($\mu g\ ml^{-1}$)	Emission Intensity (mV) Hydrazine Concentrations (M)						
	$1x10^{-7}$	$5x10^{-7}$	$1x10^{-6}$	$2.5x10^{-6}$	$5x10^{-6}$	$1x10^{-5}$	$1x10^{-4}$
a) Zinc							
0.090	0.0	0.8	1	2.0	3.5	7.0	13
0.195	0.0	1.0	1.5	3.0	-	9.5	18
0.39	0.0	1.0	1.8	4.0	5.5	10	20
0.78	0.0	1.2	2.2	4.5	6.6	13.4	24
1.56	0.0	1.5	3.0	5.0	8.7	19	34
3.13	0.0	2.0	3.5	6.5	10.0	22	52
12.5	0.0	3.0	4.0	7.5	11.0	31	61
25	0.0	4.5	8.0	16	24	50	96
100	0.0	6.0	11.5	21	40	60.5	160
b) Aluminium							
0.04	0.0	3.5	9.0	21	38	68	184
0.1	0.0	22	70	128	236	376	820
0.4	0.0	26	70	135	230	446	1320
2.5	0.0	26	70	145	260	450	1620
6.25	0.0	260	760	1440	2280	3800	796
12.5	0.0	1.0	6.0	16	28	52	132
25	0.0	2.0	6.0	12	22	44	102

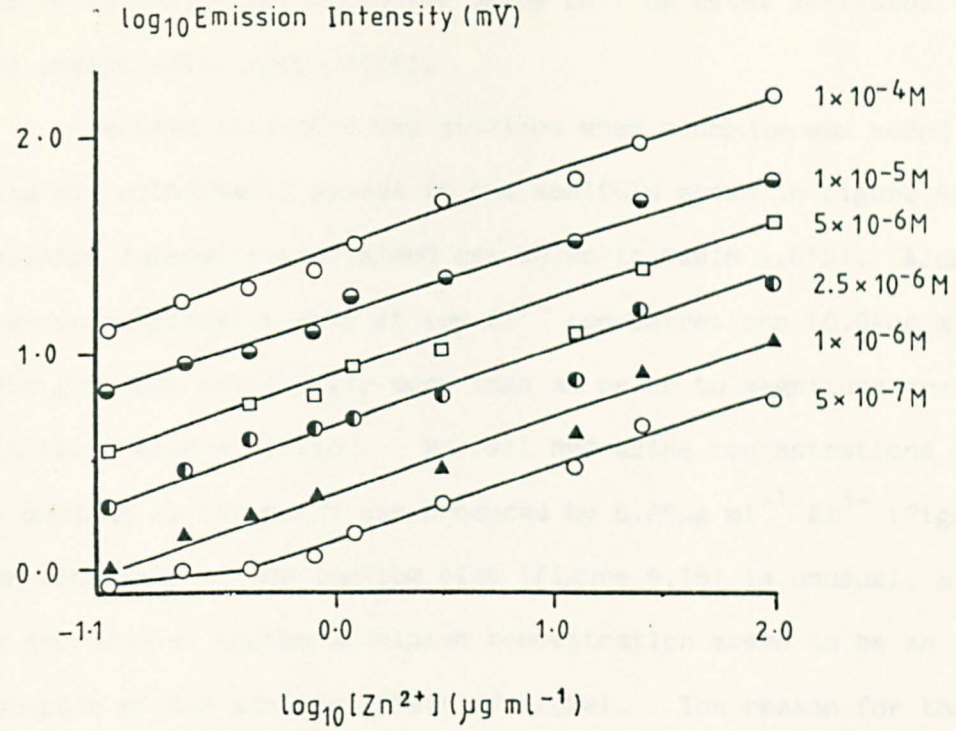


Fig. 4.15. Effect of zinc in stimulating the CL of purified hydrazine/NaOCl system at the hydrazine concentrations shown.

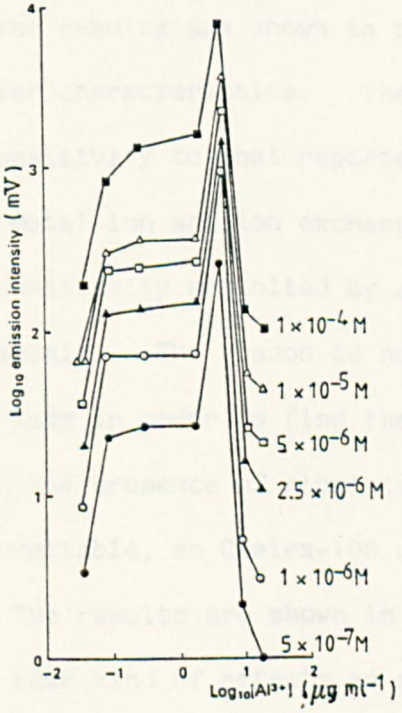


Fig. 4.16. Effect of aluminium in stimulating the CL of purified hydrazine/NaOCl system at the hydrazine concentrations shown.

the aim of hydrazine determination using Zn^{2+} as metal activator 10μg ml^{-1} Zn^{2+} is analytically appropriate.

Greatest intensity was attained when aluminium was added to the purified hydrazine/NaOCl system in the manifold shown in figure 4.10. The emission intensities obtained are shown in table 4.6(b). Aluminium gives great sensitivity even at low Al^{3+} concentrations (0.04μg ml^{-1}), with the greatest sensitivity more than an order to magnitude greater than in the presence of zinc. For all hydrazine concentrations ($5x10^{-7}$-10^{-4}M) maximum CL intensity was produced by 6.25μg ml^{-1} Al^{3+} (Figure 4.16). The non-linearity of the log-log plot (figure 4.16) is unusual, and the sudden decrease at higher aluminium concentration seems to be an exaggeration of the similar effect of nickel. The reason for these effects of Al^{3+} is not known.

Finally, using the data in tables 4.5 and 4.6, a comparison has been made between the resulting calibration graphs of hydrazine in the presence of 6.25μg Al^{3+} ml^{-1}, 12.5μg Zn^{2+} ml^{-1}, 6.25μg Cd^{2+} ml^{-1} and 0.195μg Ni^{2+} ml^{-1}. The results are shown in figure 4.17. Generally, the graphs have similar characteristics. The graph in the presence of zinc has a similar sensitivity to that reported by Wheatley (163) in the absence of added metal ion and ion exchange columns.

The great sensitivity exhibited by aluminium was rather unusual compared with other metals. The reason is not understood. However new experiments were made in order to find the lowest possible detection limit of hydrazine in the presence of aluminium. Unfortunately, no more Dowex A-1 resin was available, so Chelex-100 chelating resin was used as an alternative. The results are shown in figure 4.18. Aluminium showed the same kind of effects as when Dowex A-1 was used (table 4.6). However, the baseline noise increased also with increasing aluminium concentrations, so that under the present conditions a detection limit of $1x10^{-7}$M hydrazine was achieved (2 x noise),

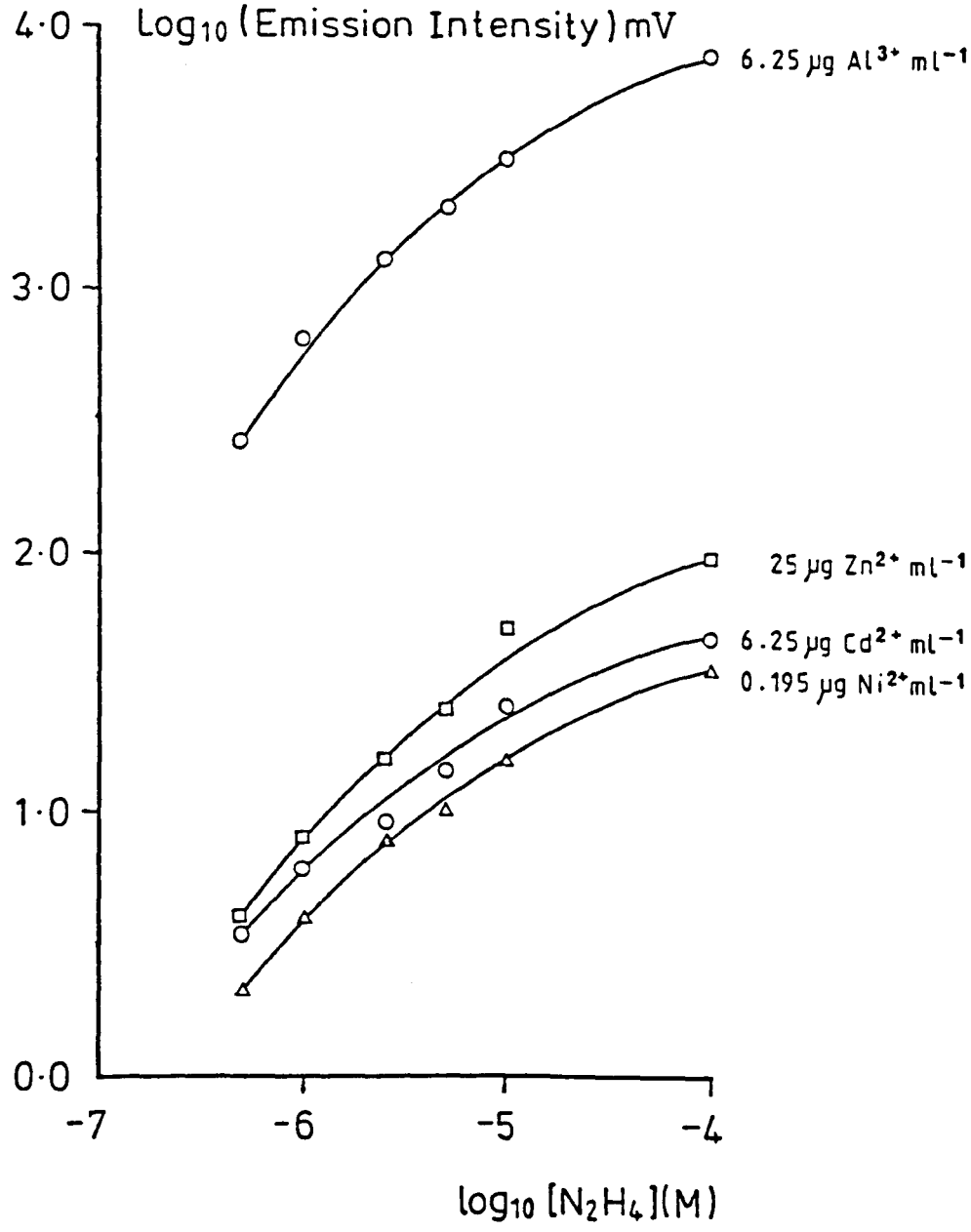


Fig. 4.17. A comparison between calibration graphs obtained from catalysed oxidation of hydrazine by sodium hypochlorite. Metal ions are at their optimal concentrations.

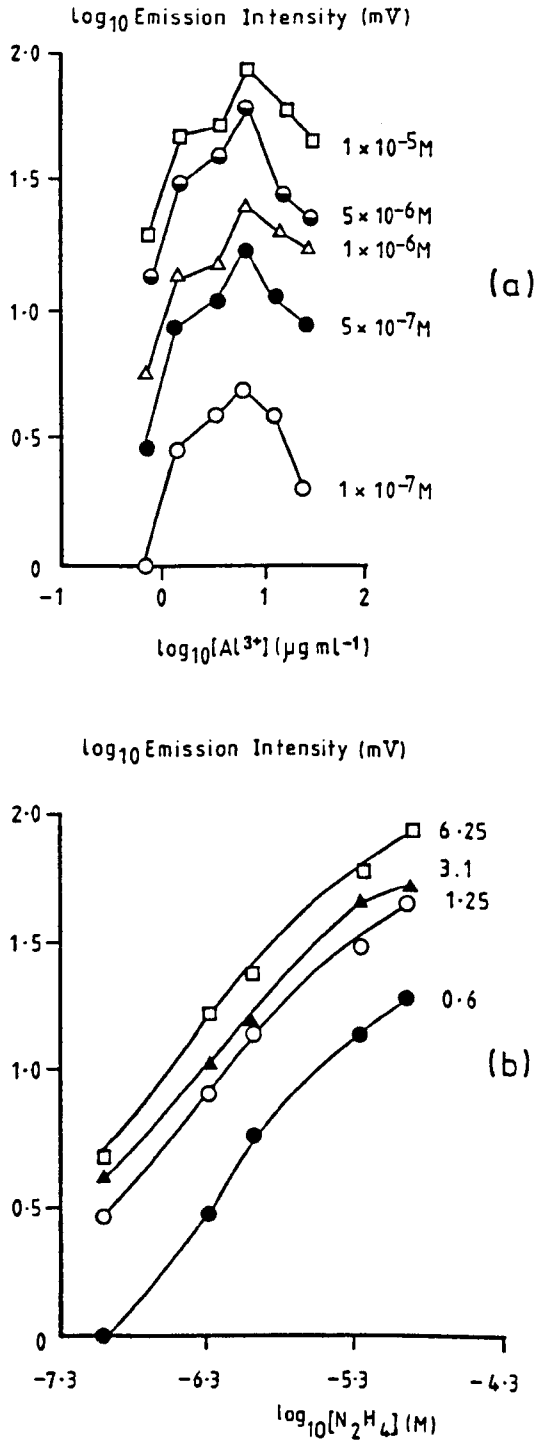


Fig. 4.18(a) Effect of aluminium in stimulating the CL of purified hydrazine/NaOCl system at the hydrazine concentration shown (b) Effect of $[Al^{3+}]$ in $\mu g\ ml^{-1}$ on the calibration graph of hydrazine. (Chelex-100 used as a resin-column).

irrespective of the aluminium concentration. This kind of effect is shown in figure 4.19.

The average relative standard deviation of 10 replicates of determination of hydrazine at concentrations of $5x10^{-7}$M and $5x10^{-6}$M was 2.4% using 0.78μg Ni^{2+} ml^{-1} for stimulating the emissions. Again the characteristics of the emission signals were not basically different from the previous procedures in which it reaches maximum within 6 sec. and a time required to obtain a complete signal was 20 sec. This allowed determination of about 120 samples per hour.

(d) Interferences

As far as the efficiency of the Dowex A-1 minicolumn is concerned the effect of some important metal ions was investigated. The interferences effects were measured by adding different amounts of each substance to a constant hydrazine concentration and comparing the emission intensity with that from a sample with no interferent. Metal ions (Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+} and Al^{3+}) in the concentration range 1μg ml^{-1} to 100μg ml^{-1} (5 steps) were examined with $5x10^{-6}$M hydrazine using 6.25μg Al^{3+} ml^{-1} as a catalyst. Except for Al^{3+} ion which caused a depression when its level exceeded 50μg ml^{-1}, no significant changes were obtained with other metal ions. This confirmed the efficiency of the Dowex A-1 column in removing these metal ions from the hydrazine sample.

4.2.3.6. Conclusion

The emission signal which was diminished as a result of passing hydrazine through a minicolumn of Dowex A-1 chelating resin is produced again after the purified hydrazine is mixed with a certain concentration of some transition metals (cadmium, nickel, zinc)or aluminium before it combines with the NaOCl stream. This ensured that hydrazine is purified from trace metal impurities as it passes through the resin column, and

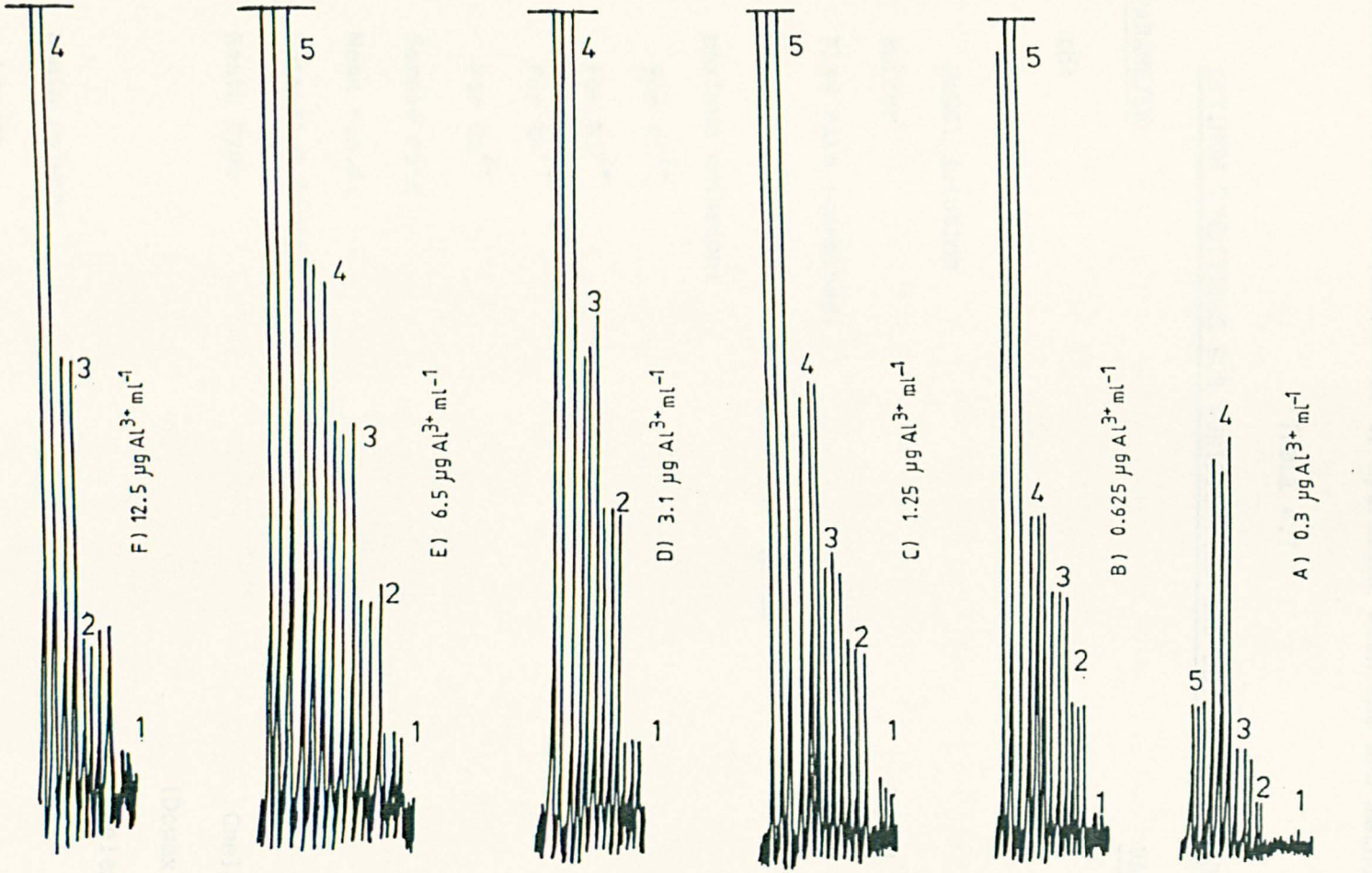


Fig. 4.19. Effect of increasing aluminium concentration on baseline disturbance of hydrazine CL. 1, 2, 3, 4 and 5 are hydrazine concentrations $1x10^{-7}$, $5x10^{-7}$, $1x10^{-6}$, $5x10^{-6}$ and $1x10^{-5}$M respectively.

indicates that the oxidation of hydrazine with NaOCl is a metal catalysed process, similar to the CL of luminol. Table 4.7 summarizes optimum conditions for the determination of hydrazine with the new method.

TABLE 4.7

OPTIMUM CONDITIONS FOR PURIFICATION AND DETERMINATION OF HYDRAZINE

PARAMETER	VALUE
pH:	
through the column	10
NaOCl solution	11.5
Buffer	0.05M $NaHCO_3$
Flow rate (combined)	3ml min^{-1}
Metal concentration ($\mu g\ ml^{-1}$) that gives maximum emission:	
For Al^{3+}	6.25
For Ni^{2+}	0.195
For Zn^{2+}	12.5
For Cd^{2+}	6.25
Sample rate	120 h^{-1}
Mean r.s.d.	2.4%
Detection limit	$5x10^{-7}M$
Resin type	Chelating resin (Dowex A-1) or (Chelex-100)
Resin column:	
Length	30mm
Internal diameter	2mm

The efficiencies of the metal ions in regenerating the emission signals are in the order $Al^{3+} > Zn^{2+} > Cd^{2+} \geq Ni^{2+}$. This

sequence is slightly different from the investigation in the absence of the resin column (Section 2.2.3).

Flow injection analysis as used throughout these experimental studies was found to be a very versatile technique for monitoring a chemiluminescence reaction. Sample treatment (such as purification in this case) can be executed instantaneously during the determination process using a miniature column of a chelating resin which does not effect the conditions required for the CL reaction.

The nature of the catalytic effect is not known. With the exception of nickel, the catalytic metal ions do not readily participate in redox reactions, and are more likely to be involved in hydrolysis or chelating reactions. Those metal ions that would commonly catalyse redox reactions (Cu^{2+}, Co^{2+}, etc) inhibit the present reaction.

PART II

APPLICATIONS OF REDUCING COLUMNS IN FLOW INJECTION ANALYSIS

CHAPTER FIVE

APPLICATION OF A REDUCING MINICOLUMN FOR METAL SPECIATION BY FLOW INJECTION ANALYSIS: SPECTROPHOTOMETRIC DETERMINATION OF IRON(III) AND SIMULTANEOUS DETERMINATION OF IRON(II) AND TOTAL IRON

5.1. Introduction

5.1.1. The current status of metal speciation studies

Interest in the measurement of metal speciation has assumed importance in recent years mainly because metal ions and their complexes are extensively involved in environmental and biological processes. In many circumstances it is necessary to know not only the total amount of an element of interest, but also the amounts present in different oxidation states, compounds or mineralogical forms, principally because of their different relative toxicities. For one particular metal, one oxidation state may be toxic while another is not. An example is the high toxicity of Cr(VI) and the inert character of Cr(III) (240). Albert (241) has extensively discussed the selective toxicity of metal ions depending on their oxidation states. Some undergo complex formation to produce more powerful oxidizing agents than the free metal ions. Such complexes could cause the destruction of vitral metabolites e.g. copper diethyldithiocarbamate destroys thiocitric acid. Iron(II) also undergoes such complexation.

Metal speciation is therefore an active area of research. Anodic stripping voltammetry (242), differential pulse polarography (243), neutron activation analysis (244), X-ray spectrometry (245), chemical methods (246) and other techniques (247) have been employed. An extensive survey is available in several important monographs (240, 248, 244). Metal speciation studies using metal-specific detectors for

chromatography are also important (250).

5.1.2. Methods of Speciation for Iron

There are more methods available for the speciation of iron than any other element. This is undoubtedly due to the various industrial applications of iron and its compounds and its wide distribution in nature. The chemical forms Fe(II) and Fe(III) in rocks are very important in geological and mineralogical analysis (248). Iron is essential for the functioning of animals and plants. It binds to protein, is present in haemoglobin and myoglobin and it causes certain diseases when its storage level is greatly increased (240).

Individual analyses for iron species Fe(II), Fe(III) and total iron have been achieved by using various analytical techniques. Spectrophotometric methods using a selective chromogenic reagent, atomic absorption spectrometry, neutron activation analysis, atomic fluorescence, potentiometric methods, etc. have been applied (240, 248, 249, 251, 252).

Methods based on selective leaching and refining operations have been described for the determination of iron(II) and iron(III) (253, 254). Equivalent concentrations of iron(II) and iron(III) were determined titrimetrically in electrolytes for galvanic production of iron-nickel alloys (255). After oxidizing iron(II) to iron(III) with H_2O_2 the end point for titration of total iron with 0.1M ascorbic acid was determined amperometrically or potentiometrically. In another portion of the sample the iron(III) content was determined spectrophotometrically at 480nm with NH_4SCN; iron(II) was calculated by difference. Simultaneous determination of iron(II), iron(III) and total iron in sphagnum moss peat has been accomplished by programmable voltammetry at a tubular graphite electrode (256). A single oxidation-reduction wave was obtained for a mixture of iron(II) and iron(III), the total height of this

wave and the anodic and cathodic parts of the wave are directly proportional to the concentration of total iron, iron(II) and iron(III) respectively.

Polarographic methods are useful in determining iron(II) and iron(III) in rocks (257) whereas controlled potential coulometry has been used for the determination of iron(II) and iron(III) in slag (258).

The rapid spectrophotometric analysis of total and ionic iron in natural waters has been described (259). Ferrozine is used for the determination of total iron at pH 3.0-5.5 in the presence of a reducing agent [$Na_3FeNH_3(CN)_5$]. In the absence of the reductant only the iron(II) ~~only~~ was determined spectrophotometrically with the same reagent, and the iron(III) found by difference. A calibration graph was linear over the range 0.025-1.75μg Fe(III)ml^{-1} and no interference was found from 20-fold amounts of Na, Ca, Mg, Al, Pb, Ni, Mn(II), K, SO_4^{2-}, Cl^-, NO_3^-, Cu(II), Zn and Cd. Silver, Au, Co(II) and Hg(II) interfered seriously.

Automated spectrophotometry has been applied for the determination of iron(II) and total iron in silicate rocks (260). Iron(II) is determined in an AutoAnalyzer after buffering a solution of the sample at pH 5 in the presence of NH_4VO_3 and measurement of the absorbance 520nm of the complex of iron(II) with 2,2′-bipyridyl. Total iron was determined similarly, after reduction of iron(III) with hydroxylamine. Iron(II) and total iron in glass were determined spectrophotometrically with 1,10-phenanthroline (261), after dissolution in HF. In this procedure, iron(III) was reduced with quinol.

Combined spectrophotometry-ion exchange was used for determination of iron(II), iron(III) and total iron (262). Iron(II) (1-50μg) was adsorbed from up to 1 litre of water as its complex with 1,10-phenanthroline, on to 0.5g of Dowex 50W-X2 (100-200 mesh) or 50W-X4 (200-400 mesh) cation exchange resin by stirring the test solution and the

resin together for 30 minutes at pH 5. Iron(III) was masked by the addition of diammonium hydrogen citrate which also acted as a buffer. The resulting coloured resin was removed and the absorbance of its slurry measured at 517nm and at 630 or 700nm (resin background). For total iron the same procedure was applied after the addition of hydroxylamine to the iron sample. The coefficient of variation of 10 determination of 0.2μg ml^{-1} total iron was 4.6%. The method has been applied to natural waters, but interferences from Cu, Zn, Co are significant.

Finally a preliminary study of the continuous analysis approach for iron(II) and iron(III) in a process stream has been published by Parry and Anderson, using pulse polarography (263).

5.1.3. Flow Injection Analysis for Iron

Several F.I.A. procedures are available for the determination of iron in different samples. A method of repetitive injection of a sample into a ferrozine solution continuously passing through a spectrophotometric cell was applied to the determination of $1\text{-}9\times10^{-8}$ mole of iron(II) (264). The method was applied to various water samples with a frequency of 360 h^{-1} at a flow rate of 80ml min^{-1}.

Redox potential detection has been applied to the determination of iron(II) (as a reducing agent) injected into a non-segmented stream of cerium(IV) solution (265). The injected sample was mixed with the reagent and the consequent redox potential shift, determined by the ratio of cerium(VI) to cerium(III), was detected by a platinum or graphite electrode with suitably placed indicator and reference electrodes. The effect of different detector configurations, flow rates and coil lengths were investigated. Iron(II) at the millimolar level was determined and a rate of 45-60 samples per hour was achieved.

Zagatto et al. (52) proposed a procedure for simultaneous

determination of aluminium and iron in plant material by applying a flow manifold involving zone sampling and merging zones. Two detectors were used; a spectrophotometer for determining aluminium after complexation with eriochrome cyanine R and atomic absorption spectrophotometry for determination of iron. This method permits analysis of about 120 samples per hour with a r.s.d. of > 2%.

The relative merits of F.I.A. methods for the determination of iron(III) by complex formation with β-resorcyclic acid, salicylic acid, sulphosalicylic acid and KSCN were examined in a simplified flow injection apparatus with a single channel manifold (266). This method allowed analysis of 250 samples per hour; it was found that the thiocyanate and β-resorcyclic acid methods were the most sensitive.

Total iron in silicate rocks has been determined spectrophotometrically by F.I.A. (267). Rock samples are opened up by fusion with a mixture of lithium carbonate and boric acid, the melt is taken up in 1M HCl and the resulting solution is used for the determination of both iron and aluminium with two separate F.I.A. manifolds. The flow system for iron determination needs no particular reagents but the U.V. absorption of iron(III) in HCl (as $FeCl_4^{2-}$) at 335nm was measured. The r.s.d. was 0.1-0.7% for three replicate measurements.

The spectrophotometric determination of iron in natural waters and plant material by the ascorbic acid-1,10-phenanthroline method has also been carried out by F.I.A. (268). Iron in the range 0.1-30μg ml^{-1} was determined at a sampling rate of 180 h^{-1} with a r.s.d. of < 1%. Iron is included with other elements during a multielement determination by F.I.A. with inductively coupled plasma atomic emission spectroscopy using a micro injection technique (269).

Iron was among several metals determined spectrophotometrically at sub-trace levels (0.7ppb) by flow injection and serial differential

detection (61). In this work, injecting samples into a matched carrier followed by merging with a reagent stream was combined with series differential detection to overcome optical perturbations from sample introduction and to provide baseline stability in spite of a variable blank. A reagent solution was a mixture of hydroxylammonium chloride (22mM), sodium acetate (18mM) and 1,10-phenanthroline (0.1mM) after it had been purified from trace iron impurities by means of an Amberlite XAD-2 ion-exchange column.

Determinations of iron based on its complexation with new organic reagents (270) and differential catalytic-fluorimetric assays (271) have been described recently, using F.I.A. In clinical analysis F.I.A.-atomic absorption spectrophotometry was used for determination of iron and of total iron binding capacity in serum (272). The deproteinized sample (150µl) was injected into a continuously flowing stream of deionized water which was propelled, via a capillary tube, to the nebulizer of an atomic absorption spectrophotometer. The analytical readout was obtained in the form of transient peaks 6 seconds after sample injection.

The increasing interest in flow injection analysis (F.I.A.) is due to its characteristics of simplicity, versatility, cost, precision and high sampling rate. It is also well suited for speciation. The possibility of performing various chemical processes by suitable modification of the F.I.A. manifold, as well as the small sample volumes, minimal reagent consumption and high sampling throughput are major advantages for speciation over the conventional techniques in which there are frequently difficulties in obtaining a rapid quantitative measure of the oxidation states present.

Despite the rapid growth in the number of analyses done by F.I.A. since 1975, applications for metal speciation were rare until 1983. There are only a few publications on this area (82, 119, 120,

273-275). The earliest attempt was the determination of iron(II) and iron(III) with amperometric detection (274). An electrochemical flow-through cell provided with a glassy carbon electrode was used for the detection by d.c. voltammetry. The two oxidation states were differentiated by an appropriate choice of indication potentials. Calibration graphs were linear over the ranges 10^{-3}-10^{-5}M iron(III) and $5x10^{-4}$-10^{-5}M iron(II).

Bubnis et al. (273) incorporated a two-channel switching valve in the flow injection manifold for on-line control of the speciation of iron(II) and iron(III), using spectrophotometric detection with 1,10-phenanthroline. The valve allowed introduction of ascorbic acid to one of a pair of sample injections, thus giving peaks for iron(II) and total iron. This rather complicated system, requiring two sample injections, has been simplified by incorporating sequential spectrophotometric and atomic absorption spectrophotometric detectors into a simple F.I.A. manifold (119, 120). This allowed iron(II) to be determined spectrophotometrically with 1,10-phenanthroline and the effluent from the spectrophotometric cell was nebulized into an atomic absorption spectrometer for determination of total iron.

A recent approach for the speciation of mixed oxidation state metal ions is described in the spectrophotometric determination of iron(II) and iron(III) by synchronized sample injection into two parallel flow systems, in which iron(II) was determined with 1,10-phenanthroline and iron(III) with thiocyanate (120b). Two spectrophotometric detectors were used. Finally two other approaches for speciation of iron(II) and iron(III) have recently been reported (82). The first was to a great extent similar to the work of Bubnis et al. (273) in which switching valves controlled the introduction of ascorbic acid for iron(III) reduction. The system was very complicated, and comprised two pumps, two injectors and several mixing coils. In the second approach, however,

a packed-bed separation column (Dowex-1 anion exchange resin) was placed between opposite channels of the injection valve. An atomic absorption spectrophotometer was used as a detector. Using 6M HCl as a carrier stream iron(II) (as $FeCl_2$) in the sample passed through the column and was detected while the iron(III) (as $FeCl_4^-$) was retained by the resin. On turning the switching valve a stream of water, as a result of operating another programmed pump, was introduced, which eluted the iron(III) into the detector. This method was automated; pump programming was used to introduce selectively the desired carrier stream.

5.1.4. Conclusion and Research Objectives

From the above communications it may be concluded that the spectrophotometric determination of iron using 1,10-phenanthroline as the chromogenic reagent is readily carried out by F.I.A. for the determination of iron (61, 269) particularly for the speciation of iron and for total iron determination (119, 120, 273, 275). The use of ascorbic acid in some cases (82, 269, 273) for reduction of iron(III), however, has resulted in complicated manifolds that required many pumps, injection valves and also some kind of automation to control several operations in sequence.

Application of a solid reductant in the form of small column was therefore thought to provide a simple F.I.A. manifold for quantitative reduction of iron(III) which would result in a better procedure with higher efficiency. Minireductor columns have been found to be particularly effective in F.I.A. Den Boef et al. have shown that unstable oxidation states of metal ions such as chromium(II) (85a), vanadium(II) (85a) and uranium(III) (85c) are readily produced, and that nitrate and nitrite can be reduced by chromium(II)-EDTA produced in such a column (85b). Application of such a column for the speciation of iron has not yet been reported.

The use of two detectors has been another route which was applied for the determination of iron species (119, 120). The work of Lynch et al. (120b) based on synchronized sample injection into two spectrophotometers, is of particular interest. There would be obvious advantages if the two flow streams could pass through a single detector, as described by Kagenow and Jensen (276), or if a single injection could be split into two channels for separate analyses (67, 277). Most advantageous would be a combination of the two, in which a single injection is split into two channels, then returned to a single stream to pass in sequence through a single detector. The general idea of sequential determinations has been reviewed by Luque de Castro and Valcarcel (34) and the simultaneous determination of species that produce a colour at different rates (278) (e.g. nickel and cobalt with 2-hydroxybenzaldehyde thiosemicarbazone). Masoom and Townshend (91) used a similar principle for the simultaneous enzymatic determination of sucrose and glucose.

It is possible, therefore, to apply this principle to the simultaneous spectrophotometric determination of iron(III) and total iron by flow injection analysis. The sample is split into two in the manifold; one portion proceeds directly to react with 1,10-phenanthroline, the other is diverted through a mini-reductor column and a delay coil. It rejoins the original flow stream after the first portion has passed for spectrophotometric determination. Both portions pass through the same detector. In this way, successive peaks for iron(II) and total iron are obtained. The development of this procedure is described in this chapter.

5.2. Experimental

5.2.1. Reagents

All chemicals were AnalaR and deionized water was used throughout.

1,10-phenanthroline solution: A 0.25% (W/V) solution was prepared by dissolving 0.625g of 1,10-phenanthroline hydrochloride (BDH) in 250ml of 0.05M hydrochloric acid. This solution was prepared every 4 days.

Buffer solutions: Citrate buffers of different pH values were prepared by mixing appropriate volume of 0.1M citric acid and 0.1M sodium citrate to give the desired pH values between 3.0 and 6.2 (279a).

Acetate buffer, pH 5.2, was prepared by mixing 10.5ml of 0.2M acetic acid and 39.5ml of 0.2M sodium acetate, and diluted to 100ml (279b).

Universal buffer, pH 5.2, was prepared by adding 17.7ml of 0.2M sodium hydroxide to 100ml of BDH universal buffer solution and then diluting to 200ml with water.

Phosphate buffer, pH 5.8, was prepared by mixing 50ml of 0.1M potassium dihydrogen phosphate and 3.6ml of 0.1M sodium hydroxide and diluting the mixture to 100ml with water (279c).

Iron(II) and iron(III) solutions: A 100ml stock solution containing exactly 0.1M each of iron(II) and iron(III) was prepared by dissolving 1.9881g and 2.7030g, respectively, of their chlorides in 0.1M hydrochloric acid. Other solutions were prepared by appropriate dilutions with the same acid concentration.

The iron(II)-iron(III) mixtures were prepared by transferring appropriate volumes of 1×10^{-3}M solutions of each ionic form to a 50ml-volumetric flask and the final volume completed with 0.1M HCl solution.

5.2.2. Apparatus

5.2.2.1. Preparation of the reductor

The Jones reductor was prepared as follows (280). Zinc shot (BDH, 20-30 mesh) was sieved through a 22-mesh sieve, 4g was covered with 1M hydrochloric acid and stirred for 1 min. The liquid was decanted and 30ml of 0.25M mercury(II) nitrate (or chloride) solution added. The

mixture was stirred thoroughly for 3 minutes. The liquid was again decanted and the amalgam was washed three times with water. The amalgam was added slowly to a 3.0-cm long glass tube (2mm i.d.) until the required packing was achieved. A thin layer of glass wool was put at both ends of the column to prevent movement of the amalgam by the carrier stream. A small piece of silicone tubing (0.8mm i.d.) was pushed into each end of the column so as to achieve a very tight connection, and a suitable adhesive (Bostik 1) was applied from outside onto the column-silicone tube joint. An electronic vibrator (Pifco, 50Hz) was used to settle the particles uniformly in the column. Water was passed through the column and the reductor was stored in this condition until required for use.

5.2.2.2. Flow manifold

The system was first set up for iron(III) determination only. The manifold used for the determination of iron(III) is shown in figure 5.1. A 4-channel peristaltic pump (Gilson Minipuls 2) was used, and iron(III) solutions were introduced via a Rheodyne RH 5020 injection valve (Anachem) with a sample loop of 40µl. Two packed reactors (3cm long, 2mm i.d.) filled with glass beads (0.25mm diameter) were inserted as shown in order to give a stable baseline (85). Teflon tubing (0.5mm i.d.) was used for the rest of the manifold.

The absorbance was measured at 512nm using a Cecil CE 373 linear readout spectrophotometer connected to a Teckman Labwriter TE 200 recorder. The complete F.I.A. system is shown in figure 5.2.

Simultaneous determination of iron(II) and iron(III) was achieved by modifying the manifold shown in figure5.1 by splitting the sample injected into two streams. The complete assembly is shown in figure 5.11.

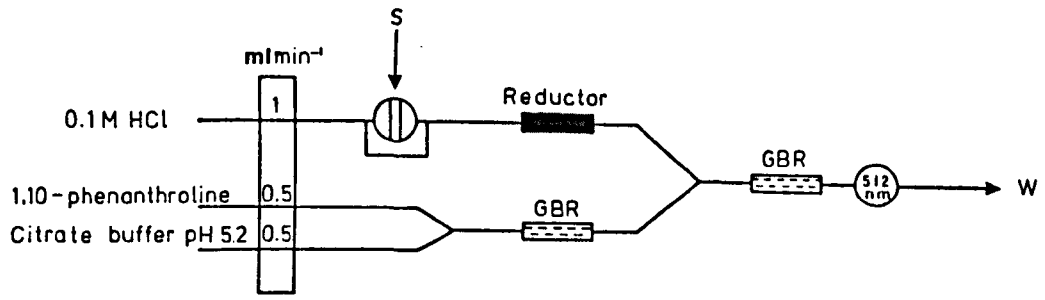


Fig. 5.1. Flow diagram of the system used for the determination of iron(III). S, sample. GBR, glass beads reactor. W, waste.

5.2.3. Results and Discussion

5.2.3.1. Optimization of variables

Carrier stream

When deionized water was used as a carrier stream for iron(III) a precipitate formed in the reductor after a short time due to the formation of hydrated iron oxide. Early reports about the formation of hydrogen peroxide when water was used as solvent in the zinc reductor (281, 282, 283) also ensured the unsuitability of water as a medium for the reduction process. Therefore it was necessary to use an acidic solution in order to prevent precipitation inside the reductor. Due to the powerful reducing character of amalgamated zinc (i.e. because of its highly negative reduction potential) the effect of acid concentration on its efficiency is not critical (284). Taking account of the conditions required for complex formation between 1,10-phenanthroline and iron(II), it was the concentration of the acid capable of preventing precipitate formation during the reduction process that was most important. The use

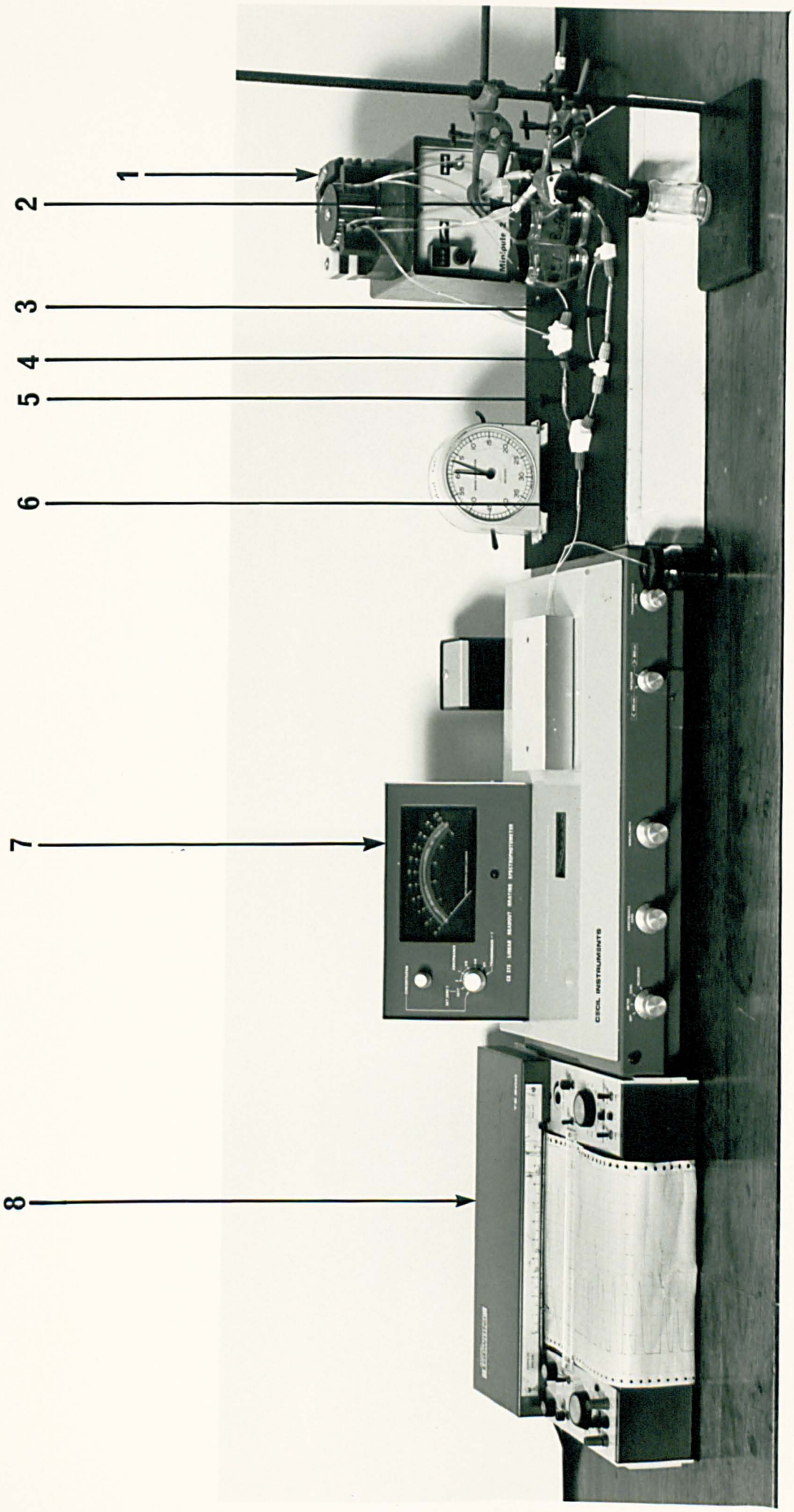
Fig. 5.2. The complete F.I.A. system used for the spectrophotometric determination of iron(III).

(1) Gilson Minipuls 2 peristaltic pump. (2) A Rheodyne RH 5020 injection valve.

(3) Zn-amalgam minireductor column. (4) Two-way valve single key.

(5, 6) GBR (single beads reactors). (7) Cecil CE 373 spectrophotometer.

(8) Recorder.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CECIL INSTRUMENTS

of 0.1M hydrochloric acid was found to be satisfactory, therefore it was used as a carrier stream through the reductor line.

Reagent concentration and wavelength

Because 1,10-phenanthroline forms stable cationic complexes with many metals (285), which do not absorb at the wavelength of the iron(II) chelate, nevertheless a sufficient excess of reagent must be added to chelate these metals as well as iron, otherwise they will interfere by causing a deficiency of reagent. When F.I.A. has previously been used for the determination of iron (120, 268, 273) a 1,10-phenanthroline concentration of 0.25% m/V was typical, therefore this concentration was used in the present work.

In figure 5.3 the absorption spectrum of the iron(II)-1,10-phenanthroline complex is shown. Maximum absorbance was at 512nm and this wavelength is recommended for use.

Buffer solution

Four buffer solutions were examined for use in the determination of iron(III) by reduction to iron(II): acetate, phosphate, universal and citrate buffer solutions. These solutions were examined in the pH range 3 - 6. Acetate buffer was not preferred because it produced a pale greenish yellow colour with iron(III) (λ_{max} = 382nm). Citrate buffer did not produce such a colour. In addition, citrate buffer can mask certain potential interferences (286, 287). Phosphate and universal buffers were also suitable, but small amounts of calcium interfered by precipitation when phosphate was used and the large number of constitutents of universal buffer increases the opportunity for interfering effects. Therefore citrate buffers were prepared in the pH range 3.0 - 6.2 (279a) and their effects on different iron(III) solutions studied using the F.I.A. system shown in figure 5.1. The iron(III) responses were the same throughout this range, therefore pH 5.2 was selected for

further work.

Effect of flow rate

The effect of flow rate in the sample line was studied at pH 5.2. The results are shown in figure 5.4. At the beginning, increasing the flow rate through the reductor column up to 1ml min^{-1} was accompanied by increasing peak height. This was thought to be attributed either to physical (change in dispersion) or chemical factors. For this purpose the flow injection system in figure 5.1 was operated by injecting a 0.01% (W/V) methylene blue to study the effect of flow rate on dispersion without performing any chemical reaction. The result is shown in figure 5.5 (a and b), which indicates that increasing flow rate has led to higher, but broader signals.

In figure 5.4, the effect of flow rate on the iron response is shown. At flows > 1ml min^{-1}, the peak height (absorbance) decreases gradually, in spite of the counter effect of less dispersion as indicated in figure 5.5. This must be due to increasingly incomplete reduction of iron(III) as flow rates increase above 1ml min^{-1}. Therefore as maximum peak height was required, a flow rate of 1ml min^{-1} through the reductor is recommended.

Effects of glass bead columns

It was necessary to include the two glass beads columns (GBR) into the F.I.A. system shown in figure 5.1 in order to overcome optical perturbations and to provide baseline stability caused by variable blanks. Investigations concerning these effects are illustrated in figure 5.6. Figure 5.6a shows the baseline in the presence and absence of both GBR reactors. The extent of baseline instability was so pronounced it was rather difficult to proceed with the analysis. This was greatly improved by introducing these two reactors as shown. However, in the absence of one of these reactors the baseline was less perturbed

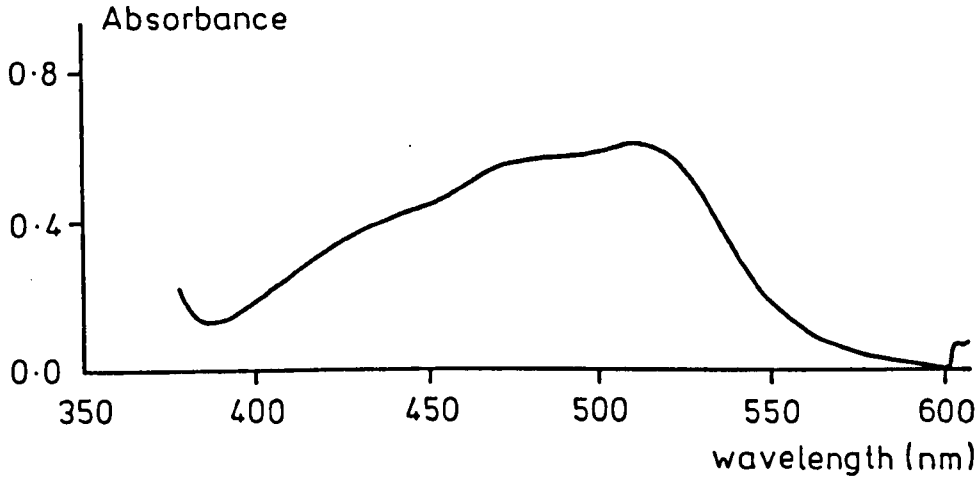


Fig. 5.3. Absorption spectrum of $5x10^{-5}$M iron(III) - phenanthroline complex in citrate buffer pH 5.0 against the buffered reagent as a blank in a 1cm-path length cell.

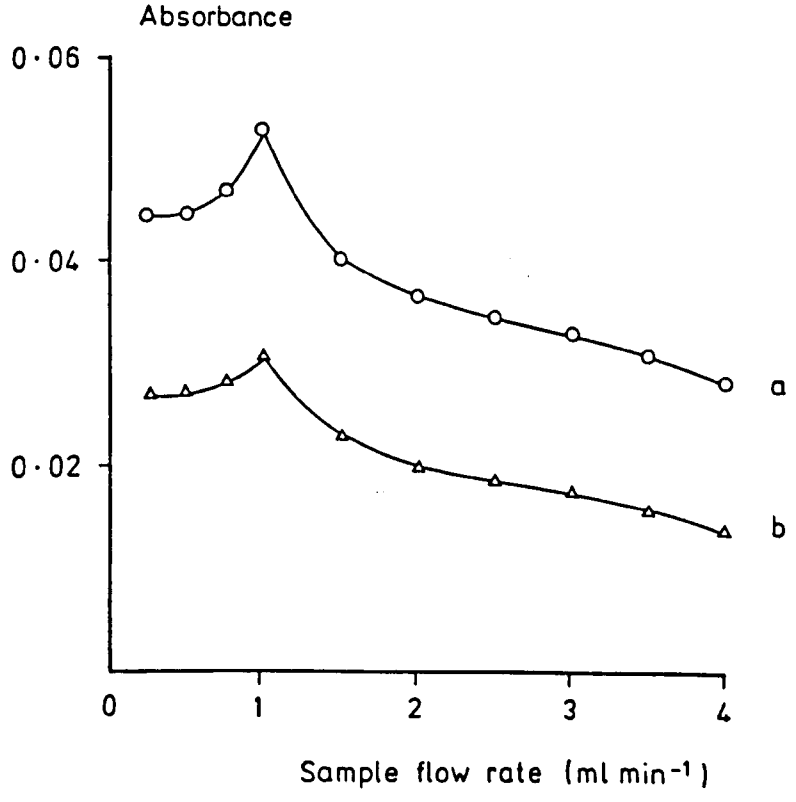


Fig. 5.4. Signal height for iron(III) as a function of flow rate through the reductor column for: (a) $8x10^{-5}$M, (b) $5x10^{-5}$M iron(III). (0.25% of reagent, 0.1M HCl)

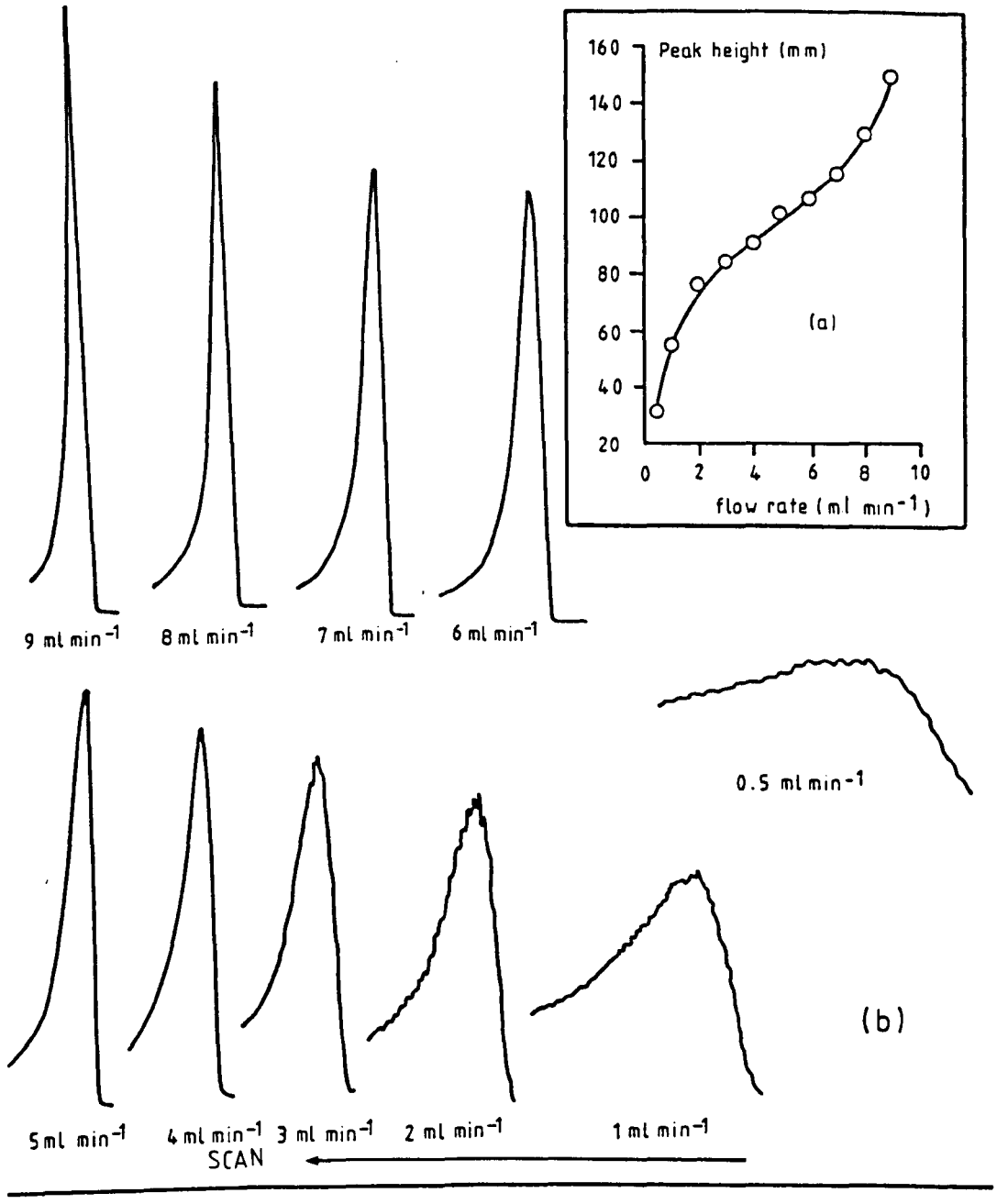


Fig. 5.5. (a) Effect of Jones reductor on sample dispersion at different flow rates in absence of chemical reaction
(b) Effect of flow rate on peak height and shape obtained.

and therefore an experiment was done to show some peaks in the absence and presence of one reactor. Figure 5.6b shows the absorption signals obtained for $1x10^{-5}$M iron(III) by operating the manifold in figure 5.1 with and without the second GBR reactor through which the combined streams were passed. These results show that the baseline is very unstable even when one of these reactors is removed. Therefore, both columns are important in order to achieve a most stable baseline; best GBR columns in this case were 3cm long, 2mm i.d. because shorter columns (e.g. 1cm or 2cm) were not capable of eliminating the noise.

Effect of reductor length and internal diameter

Keeping the internal diameter constant at 2mm, the effect of different lengths of reductor column was investigated. Figure 5.7 shows the effects of various columns on the absorbance of $1x10^{-4}$M iron(III). The extent of the reduction of iron(III) to iron(II) reaches a maximum at 3cm length. A 2mm i.d. column was the minimum diameter which could be suitably packed with the amalgam particles, and therefore no attempt was made to use a wider column, to avoid possible increase in dispersion. Hence a 2mm i.d. reductor is recommended.

Reductor Capacity

The 3cm-long, 2mm i.d. mini-reductor column exhibits high reduction capacity as concluded by repetitive 40µl injections of $1x10^{-3}$M iron(III) using the manifold described in figure 5.1. The result of 128 injections each of 2.23µg iron(III) is shown in figure 5.8. Reproducible measurements are obtained up to injection of 178.4µg iron(III) (80 injections). When the amount of iron(III) injected has reached about 180µg the column has lost about 10% of its reducing capacity, which has increased to 17% when 245.3µg of iron(III) has been reached. Therefore this column is capable of efficiently reducing about 180µg iron(III), after which regeneration with 0.25% nitric acid or replacement is necessary.

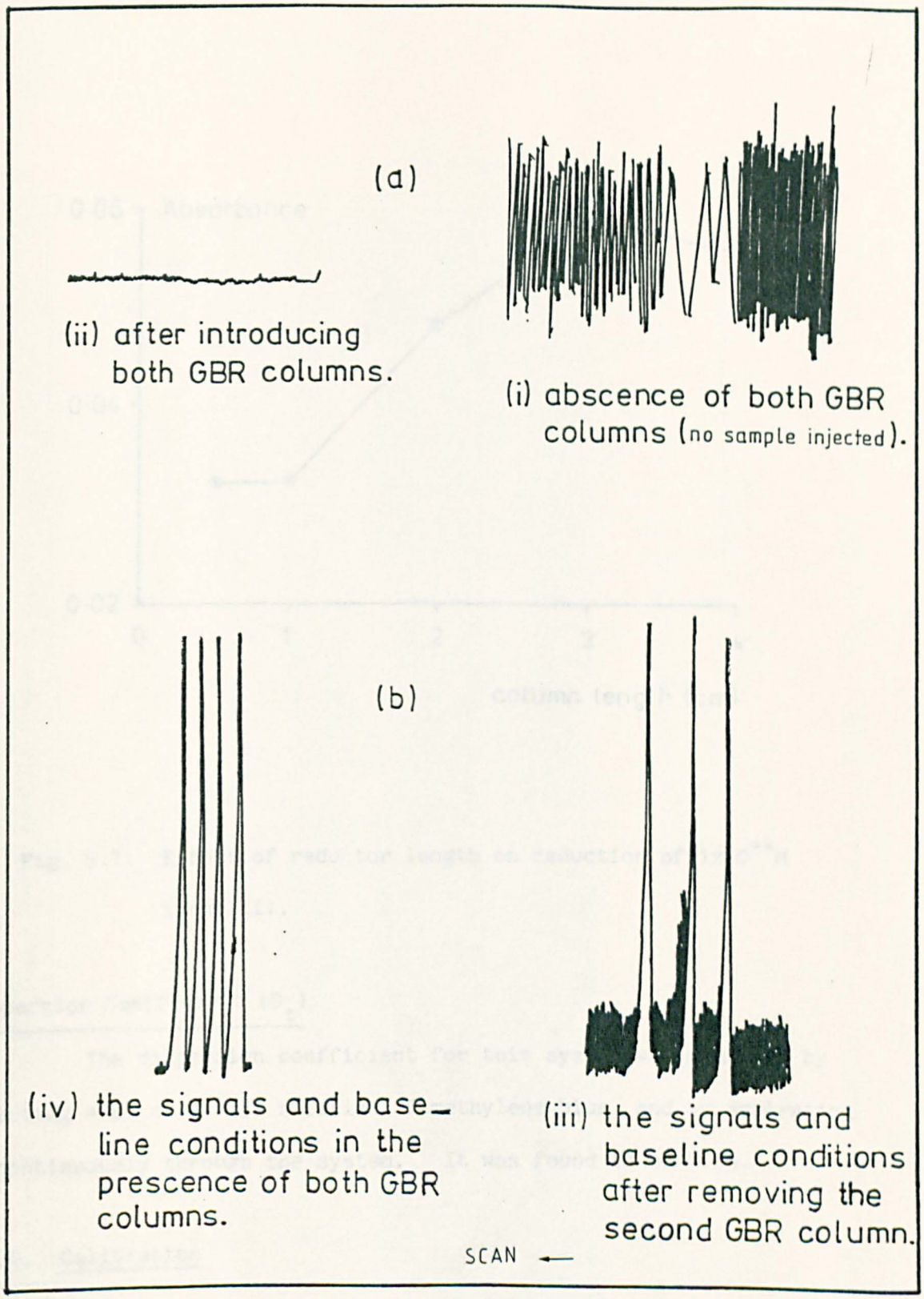


Fig. 5.6. (a) Effect of both GBR columns on the stability of base-line before injecting iron(III) sample. (b) Signals and baseline conditions in the absence and presence of the second GBR column. Iron(III) conc. is 5×10^{-5}M.

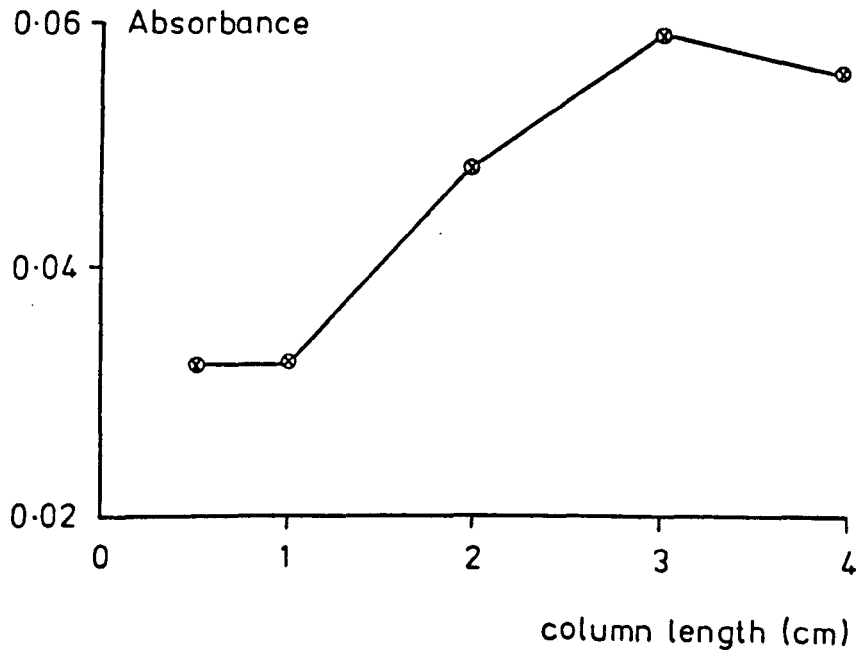


Fig. 5.7. Effect of reductor length on reduction of $1x10^{-4}$M iron(III).

Dispersion Coefficient (D_t)

The dispersion coefficient for this system was measured by injecting 40µl of 0.015% solution of methylene blue, and by aspirating it continuously through the system. It was found to be 7.65.

5.2.4. Calibration

Under the conditions established, calibration graphs for iron(III) were constructed. The calibration graph is linear for $3x10^{-6}$-$5x10^{-4}$M iron(III). Typical calibration results are shown in table 5.1 and figure 5.9.

The linear graph has a regression coefficient of 0.9975 (6

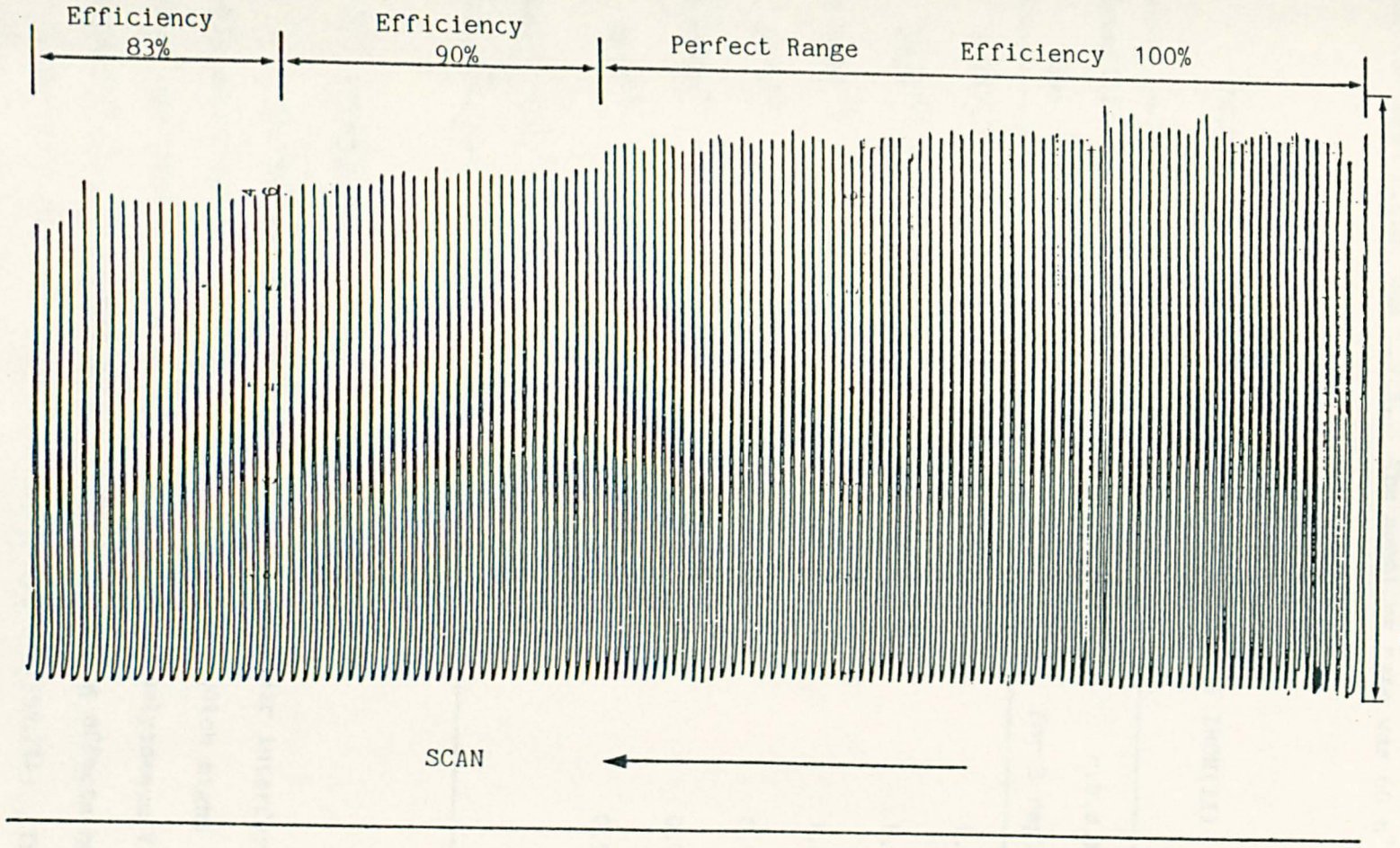


Fig. 5.8. Capacity-profile of the reductor column. The Fe(III) concentration used was 1×10^{-3}M (40µl).

points) and the graph satisfies the least squares equation A = 575.238 $[Fe^{3+}]$ + 0.00113. The detection limit (2 times blank noise) was $3x10^{-6}$M, and the mean r.s.d. was 0.91%. The sampling rate was 60 h^{-1}.

TABLE 5.1

CALIBRATION RESULT FOR THE DETERMINATION OF IRON(III)

Iron(III) Conc. (M)	Mean Absorbance	r.s.d.% for 3 replicates
$1.5x10^{-5}$	0.010	1.3
$3x10^{-5}$	0.020	1.2
$4.5x10^{-5}$	0.027	1.2
$6x10^{-5}$	0.035	0.7
$7.5x10^{-5}$	0.043	0.7
$9x10^{-5}$	0.054	0.5

Mean r.s.d. = 0.91%

5.2.5. Interferences

Two groups of metals were studied for their interfering effects, firstly those elements in higher oxidation states which might be reduced as they pass through the reductor (chromium(III), molybdenum(VI) and vanadium(V)) and, secondly, metals whose interfering effects have already been reported (copper(II), cobalt(II) and nickel) (269,273). The interference effects were measured by adding different amounts of each metal in the range 1-100μg ml^{-1} to $1x10^{-4}$M iron(III) solutions and comparing the absorption peaks with that from a sample with no interferent present.

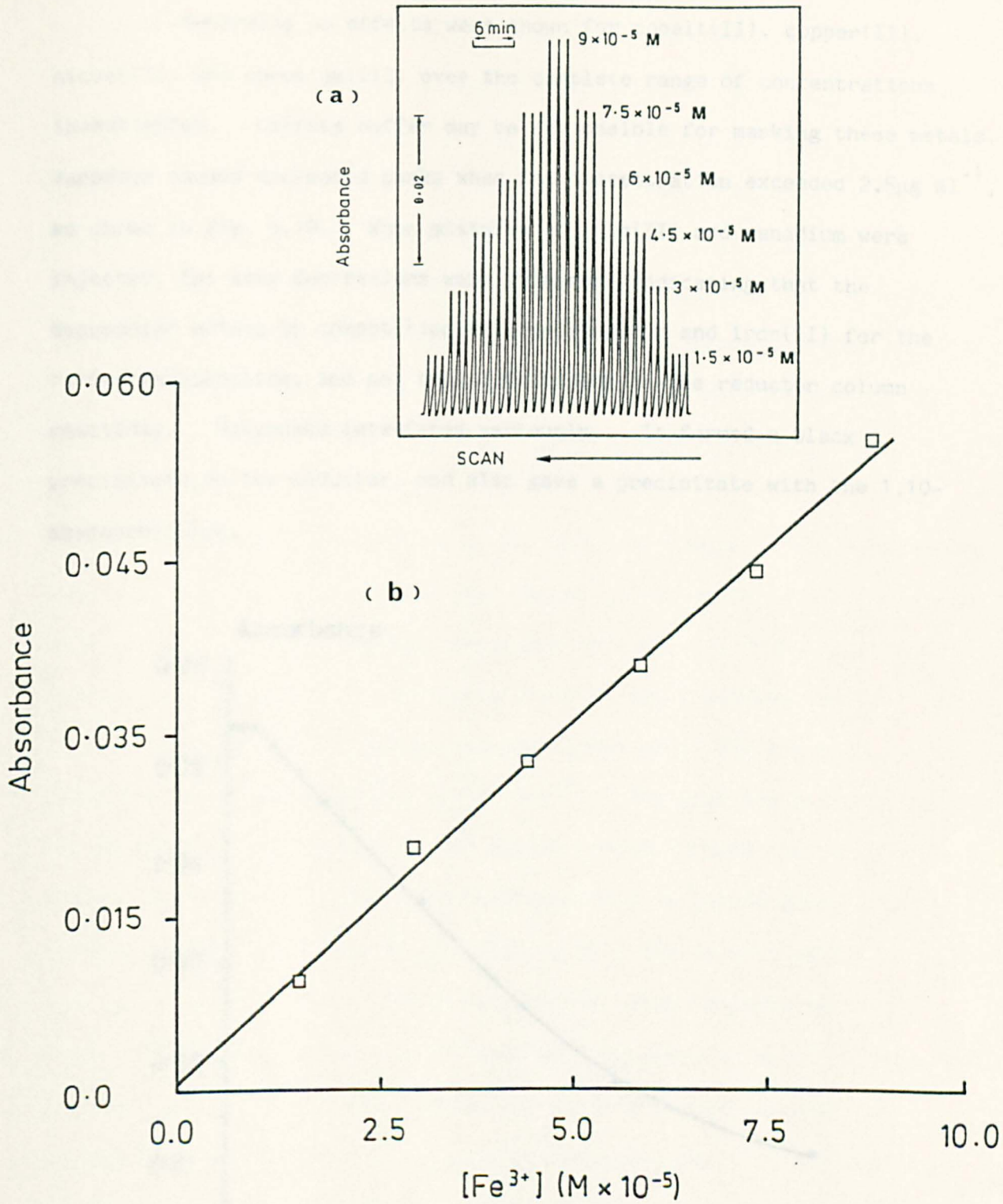


Fig. 5.9. (a) Peaks obtained by injecting triplicate iron(III) standards in the concentration range shown in the manifold in figure 5.1 (sampling rate 60 h^{-1}). (b) The corresponding least squares calibration graph.

Generally no effects were shown for cobalt(II), copper(II), nickel(II) and chromium(III) over the complete range of concentrations investigated. Citrate buffer may be responsible for masking these metals. Vanadium caused decreased peaks when its concentration exceeded 2.5μg ml^{-1}, as shown in Fig. 5.10. When mixtures of iron(II) and vanadium were injected, the same depressions were obtained, indicating that the depression arises by competition between vanadium and iron(II) for the 1,10-phenanthroline, and not from involvement in the reductor column reactions. Molybdate interfered seriously. It formed a black precipitate on the reductor, and also gave a precipitate with the 1,10-phenanthroline.

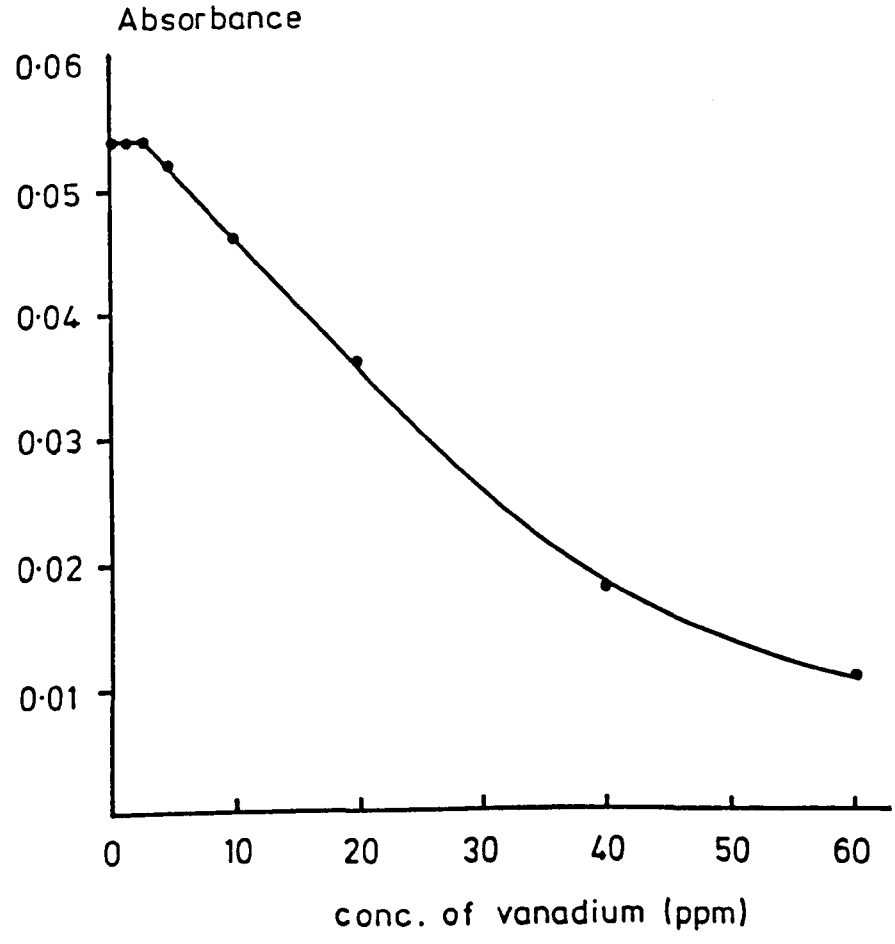


Fig. 5.10. Effects of vanadium on the absorbance of $1x10^{-4}$M iron (III).

5.2.6. Simultaneous determination of iron(III) and total iron

The simultaneous determination of iron(III) and total iron can be achieved by splitting the injected sample into two streams. The manifold is shown in figure 5.11. One stream reacts directly with 1,10-phenanthroline, and then passes to the detector. This measures the iron(II) concentration. The other portion flows through the reductor column and a delay coil, and then rejoins the 1,10-phenanthroline stream to give a second peak completely resolved from the iron(II) peak. This peak is a measure of total iron, i.e. iron(II) and iron(III), in the original sample.

Reproducible splitting of the injected sample is not necessarily simple to achieve. Many means were investigated, which to a great extent were similar to those reported previously (34, 91, 277, 278). In one case the splitting device (a Perspex Y-piece, angle between the two outflow lines 90°, i.d., 0.7mm) was positioned after the point of injection, with the flow rate at 1ml min^{-1}. The pump was placed before the injector. One portion of the sample passed through the reductor and was immediately mixed with 1,10-phenanthroline before passing to the detector. The other portion was delayed by a 400-cm coil before mixing with the reagent. This arrangement gave an irreproducible splitting ratio, varying between 5:1 and 3:1 and in some cases no splitting occurred. A 60-cm long coil was placed before the injection point to make the sample speed as constant as possible before splitting (67) and also the splitting was controlled by introducing a 3-key 3-way valve (Anachem) at the convergence point of the two streams before mixing with the reagent. However, these modifications were not successful nor was the use of the 3-way valve as a splitter.

The failure of these common splitting procedures is attributed mainly to the relatively slow flow of the sample stream which is required

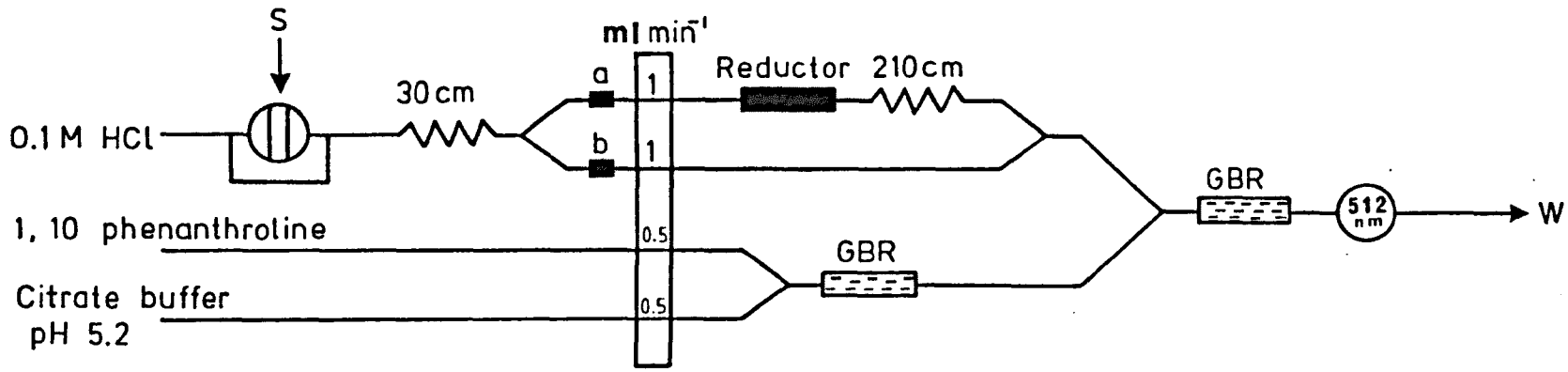


Fig. 5.11. Flow injection system for the simultaneous determination of iron(III) and total iron. S, sample. GBR, glass bead reactor. a & b are pulse suppressors. W, waste.

for complete reduction, compared to the flow rates (8.6 and 17.0ml min^{-1}) used for splitting reported previously (67, 277). It might be possible to design a splitting manifold suitable for slow flow but it would require many long coils in many parts of the system, which would have very detrimental effects on the dispersion of the sample.

After extensive investigation, the arrangement shown in figure 5.11 was found to give the best results, in which the pump is placed after the splitter. The design of the splitter is not critical, and the splitting ratio is controlled by the use of pump tubes of various sizes.

5.2.6.1. Splitting Outfit

Figure 5.12 shows a picture of the splitting section. The 40µl sample containing iron(III) and iron(II) is injected by means of a Rheadyne RH 5020 injection valve after which a 30cm coil (0.5mm i.d.) is placed to provide a steady movement of the sample before splitting. Preliminary results indicated that constancy of sample flow is essential to achieve reproducible splitting, therefore two lengths of pulse suppressor tubing (0.508mm i.d., Sterilin) were connected between the splitter and pump tubes to eliminate any effect of pulsing on the splitting ratio.

The splitter was a Y-piece, the geometrical characteristics of which were critical. This was the main reason why the use of commercial Y-shaped connectors are not successful because as these devices are made of Teflon the interior channels are not clearly visible and the angles between the two outflows and the confluence point are thus not easily measurable. Therefore in the present work, splitting was achieved by means of a home-made Perspex Y-piece described in section 5.2.6.

The splitting to give peaks of equal height for a given iron concentration was achieved by connecting one pump tube of 0.8mm i.d. to

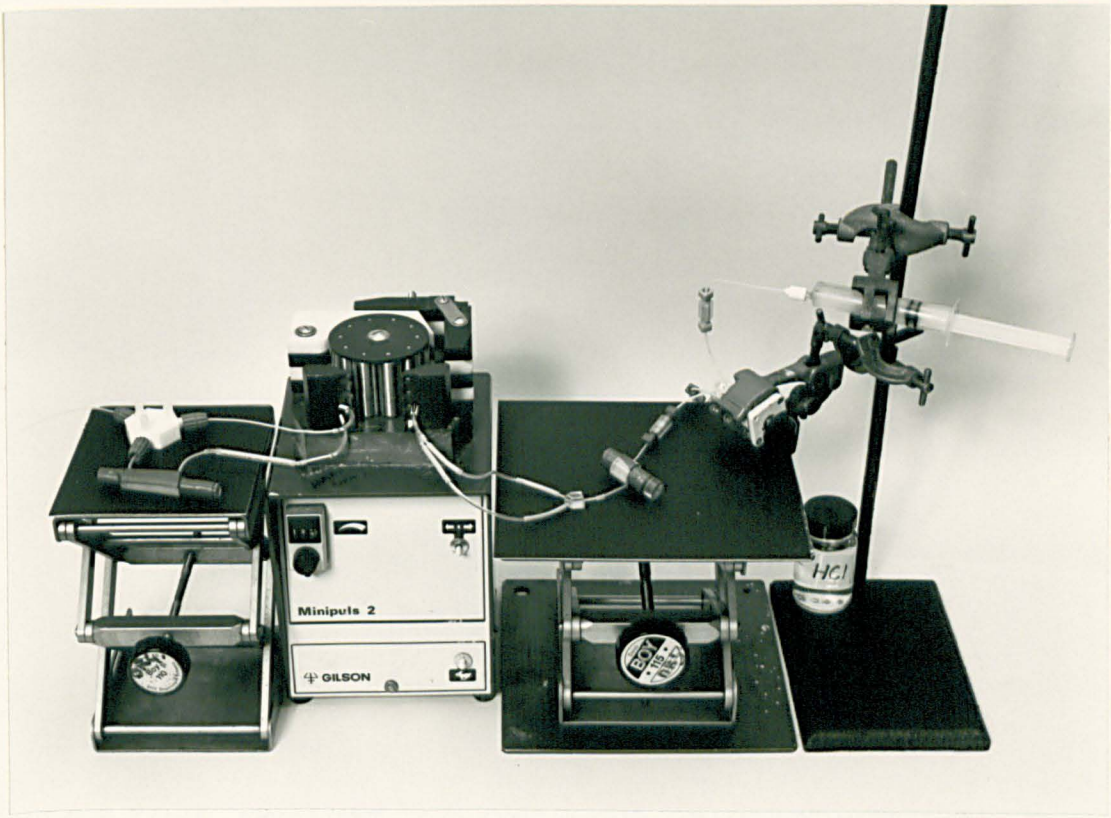


Fig. 5.12. Photograph of the splitting section used in simultaneous determination of iron(II) and iron(III).

the reductor line and one of 0.5mm i.d. to the other channel. This splitting ratio 1:1 was confirmed by injecting a standard solution of iron(II) before analysing an iron(II)-iron(III) mixture. Under the optimized conditions given in figure 5.11 the essentially 1:1 splitting obtained is shown in figure 5.13, as well as the fact that the second peak is slightly broader than the first, as would be expected due to the passage of this sample portion through the reductor and delay coil.

5.2.6.2. Calibration graph

Using the optimal conditions for the reduction of iron(III) and splitting of the sample (to give a 1:1 peak height ratio), calibration for iron(II)-iron(III) mixtures was successfully achieved. Typical calibration results for 40µl injections of iron(II)-iron(III) standards are shown in figure 5.14 for the concentrations shown in table 5.2.

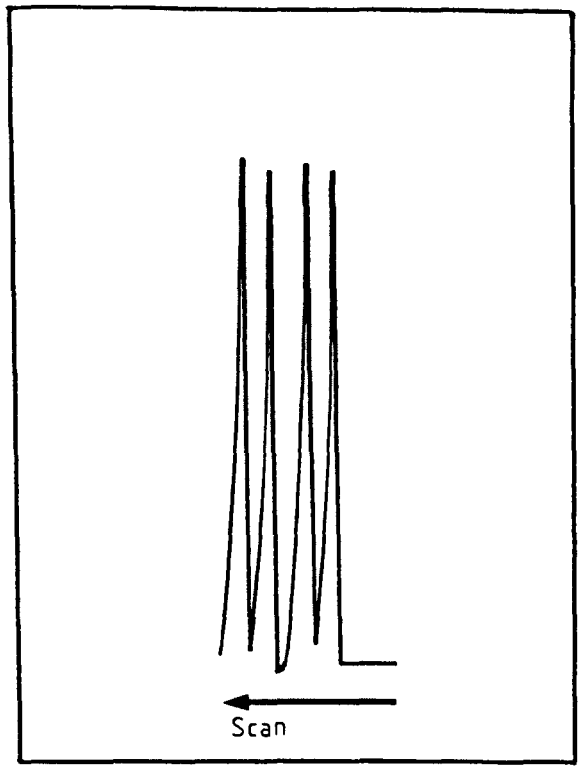


Fig. 5.13. Splitting of 4.5×10^{-5}M iron(II) solutions.

In figure 5.14 the first peak is a measure of iron(II) and the second of total iron. The sample throughput was 40 h^{-1} with a r.s.d. of 0.7-1% over the range shown in table 5.2.

TABLE 5.2.

SAMPLE CONSTITUENTS (Fe(II) and Fe(III)) USED THROUGHOUT SIMULTANEOUS DETERMINATIONS

Sample no.	Iron(II) Conc. (M)	Iron(III) Conc. (M)
1	9.0×10^{-5}	5.5×10^{-5}
2	8.5×10^{-5}	4.5×10^{-5}
3	8.0×10^{-5}	3.5×10^{-5}
4	7.5×10^{-5}	2.5×10^{-5}
5	7.0×10^{-5}	1.5×10^{-5}
6	6.5×10^{-5}	0.5×10^{-5}

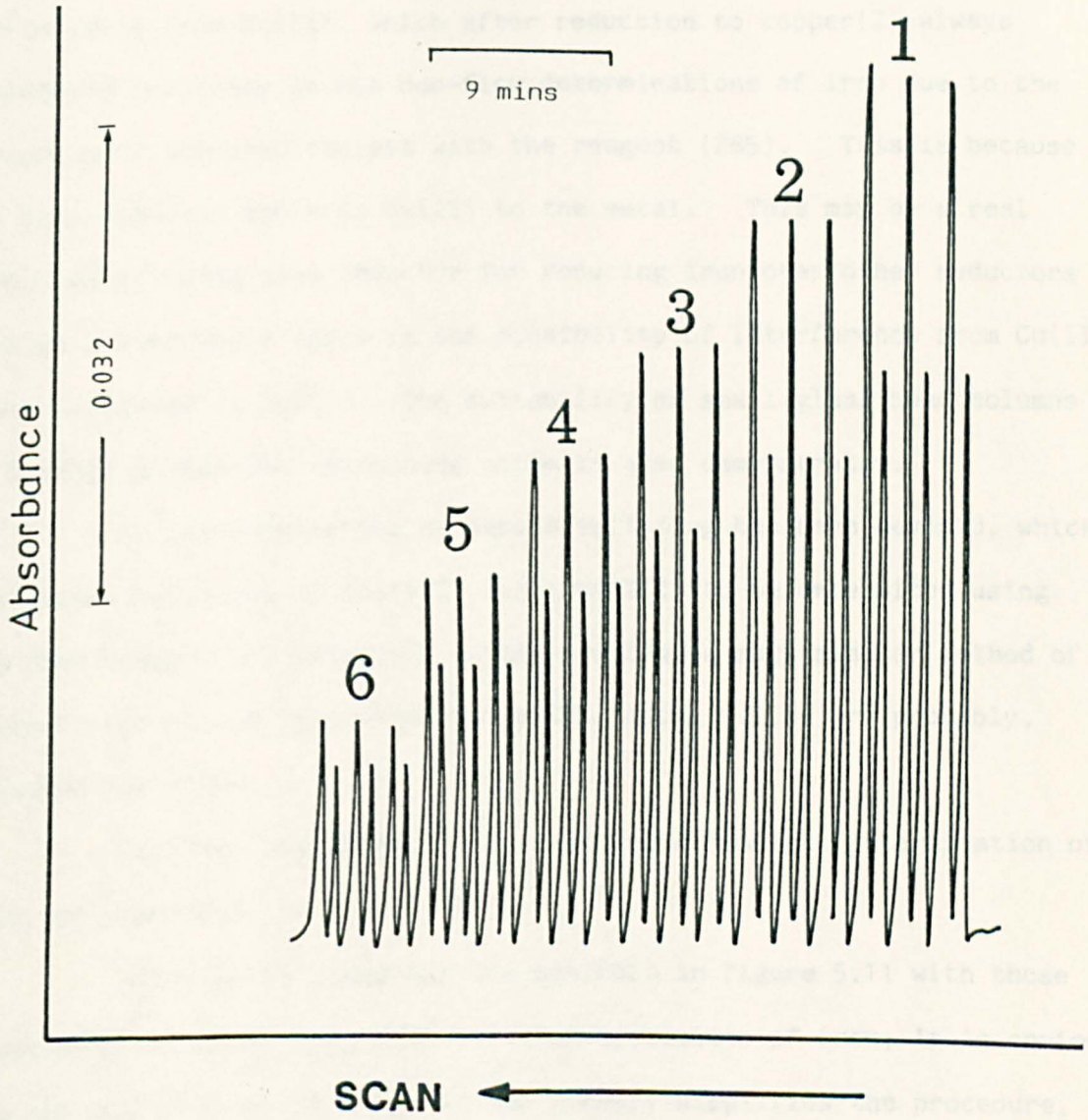


Fig. 5.14. Typical calibration responses for simultaneous determination of iron(III) and iron(II) for the concentrations shown in Table 5.2.

5.3. Conclusions

The incorporation of a mini-reductor column into the F.I.A. system provides a simple means of determining iron(III) with 1,10-phenanthroline. There were no interferences from most common cations particularly from Cu(II), which after reduction to copper(I) always interfered seriously in the non-flow determinations of iron due to the formation of coloured chelate with the reagent (285). This is because the zinc reductor converts Cu(II) to the metal. This may be a real advantage of using zinc reductor for reducing iron over other reductors such as silver where there is the possibility of interference from Cu(II) when it reduced to Cu(I). The suitability of small glass bead columns to promote mixing and decreasing noise is also demonstrated.

An improved method of sample splitting has been devised, which has allowed mixtures of iron(II) and iron(III) to be determined using the same reagent and detector. This provides a much simpler method of analysis than those described previously, using F.I.A. or, probably, any other technique.

Optimum conditions for the spectrophotometric determination of iron are summarised in table 5.3.

Finally, by comparing the manifold in figure 5.11 with those reported previously (119, 120, 273) for speciation of iron, it is obvious how the use of a minireductor column greatly simplifies the procedure, by avoiding the need of two injection valves, different pumps and confluence points, mixing coils or two detectors.

TABLE 5.3

OPTIMUM CONDITIONS FOR THE DETERMINATION OF IRON

PARAMETER	VALUE
Carrier stream	0.1M HCl
1,10-phenanthroline	0.25%
Buffer solution	Citrate, pH 5.2
Total flow rate	2ml min^{-1}
Flow rate through reductor	1ml min^{-1}
Reductor dimensions	3cm long, 2mm i.d.
Sample volume	40µl
Linear calibration	$3x10^{-6}$ - $5x10^{-4}$M Fe^{3+}.
Detection limit (2 x blank noise)	$3x10^{-6}$M Fe^{3+}
r.s.d.	$\leq$ 1%
Sample rate	60 h^{-1}

CHAPTER SIX

SILVER REDUCTOR IN AN ON-LINE COLUMN FOR SPECTROPHOTOMETRIC DETERMINATION OF COPPER BY FLOW INJECTION ANALYSIS

6.1. Introduction

6.1.1. Principles of metallic reductors

The low reduction potential of some pure metals indicates that such substances would serve as excellent reductants. They are zinc, silver, cadmium, aluminium, lead and copper, and they have been widely used in analytical procedures (288, 289). The value of these reductants is attributed to the selectivity of their reducing action, which is a function of the electrode potential of the metal-metal ion couple and to the ease of removing excess of the reducing agents by filtration, washing or dissolving in acid (284).

Metals in different physical forms, such as a rod, wire coil, shot and powder, can be used but the most elegant means involves the use of a reductor in which the acidified solution passes through a column packed with granules of the metal. Zinc (or zinc amalgam) and silver reductors are the most common. Some of these metals (e.g. zinc, cadmium, aluminium) are very strong reductors, therefore they not only reduce the analyte but also have a tendency to evolve hydrogen as they dissolve in the acidic solutions. As a result the metal is consumed rapidly and undesirable amounts of metal ions are introduced into the test solution. Amalgamation of these metals with mercury overcomes these problems and provides a better opportunity to use a high acidity medium which in some cases is needed for complete reduction. The Jones [i.e. Zn(Hg)] reductor and cadmium reductor fall in this category.

Due to its strongly negative potential, the Jones reductor is

a non-selective reductor. It reduces many species to lower oxidation states, including Fe(III), Mo(VI), V(V), Cr(III), Ti(IV) and U(VI). Several others are reduced to the metal such as Ag(I), Cu(II), Pb(II), Hg(II), Ni(II), Pt(II) and Sn(II). Factors that affect reductor efficiency (290), the effects of atmospheric oxygen (282-284, 291) and the effects of addition of metal catalysts (292) have already been studied.

The use of a silver reductor, originally developed by Walden et al. (293), has found wider applications due to its milder reducing character and is hence the more selective reducing properties. Therefore reductions such as Cu(II) to Cu(I), which was not possible with the Jones reductor, is an important use of the silver reductor. Use is made of this reductor in the present work and it is discussed in detail in section 6.1.2. Table 6.1 illustrates the difference in the reduction process for certain substances with zinc amalgam and silver reductors (294).

Reductor column packings involving other metals have been used for specific applications, such as the lead reductor in the reduction of U(VI) to U(IV) (291), cadmium reductor in the reduction of NO_3^- to NO_2^- (295), mercury reductor to examine the behaviour of U(VI) (296) and mixed reductors such as the zinc-bismuth reductor for the determination of inorganic and organic species (297).

6.1.2. Silver Reductor

In addition to the selectivity of the silver reductor its production appears to be very convenient. Preparation of silver by reaction between silver nitrate and copper metal is very simple (293). In a few circumstances, however, this method can be modified, e.g. in an attempt to prepare silver of larger size particles (298, 299). Generally these methods are inefficient, and have low yield, and as in

TABLE 6.1

A COMPARISON OF SILVER AND JONES REDUCTORS

Silver Reductor	Jones Reductor
$Ag(S) + Cl^- \rightleftharpoons AgCl(S) + e$	$Zn(S) \rightleftharpoons Zn^{2+} + 2e$
$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$	$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$
$Cu^{2+} + e^- \rightleftharpoons Cu^+$	$Cu^{2+} + 2e^- \rightleftharpoons Cu(S)$
$H_2MoO_4 + 2H^+ + e^- \rightleftharpoons MoO_2^{+} + 2H_2O$	$H_2MoO_4 + 6H^+ + 3e^- \rightleftharpoons Mo^{3+} + 4H_2O$
Ti^{4+} not reduced (in HCl medium)	$Ti^{4+} + 2H^+ + e^- \rightleftharpoons Ti^{2+} + H_2O$
$UO_2^{2+} \rightleftharpoons U^{4+}$	$UO_2^{2+} \rightleftharpoons U^{3+} + U^{4+}$
Cr^{3+} not reduced	$Cr^{3+} + e^- \rightleftharpoons Cr^{2+}$
$VO_4^{3-} + 6H^+ + e^- \rightleftharpoons VO^{2+} + 9H_2O$	$VO_4^{3-} + 8H^+ + 3e \rightleftharpoons V^{2+} + 12H_2O$

case of the method of Salam Khan and Stephen (299), the product was not pure silver, but contained only 45-50% of Ag after a complicated procedure. Therefore in this work the silver was prepared in accordance with Walden's method (293).

The reduction processes carried out in the silver reductor involve hydrochloric acid media. The acidity is critical in order to achieve quantitative reduction as the reducing properties of the metal are enhanced during silver chloride formation as concluded from equation 6.1.

$$Ag(S) + Cl^- \rightleftharpoons AgCl(S) + e^- \qquad E^{\circ} = 0.2223V \qquad (6.1)$$

In column procedures hydrochloric acid concentrations from 1M to 5M have been applied for reduction of different metal ions (300). Also, the low reduction potential of the Ag/AgCl couple ensures that ions such as Ti^{4+} and Cr^{3+} are not reduced.

Investigations are reported for the silver reductor about the possibility of formation of hydrogen peroxide (301), reduction in bromide media (302), comparison with other reductors (303), obtaining a mixture of metal oxidation states from the reduction process (304, 305) and effects of acidity on the reduction of some metals (306).

Because of the selective reducing properties of the silver reductor it has widely been used for the determination of several metal ions such as iron (293, 302), uranium (302, 306), vanadium (304) and molybdenum (305). Despite the fact that titanium is not reduced in hydrochloric acid, it is reduced by a silver reductor in H_2SO_4 (307) and on heating to 90^{o} in mixed acid solutions (7M HCl - 3.2M CH_3COOH) (308), and can be determined on this basis. Determination of copper with a silver reductor was rather uncommon, possibly due to the instability of Cu(I) produced owing to unavoidable air oxidation. Birnbaum and Edmonds (306) reduced Cu(II) in 2M HCl, collected the reduced copper [Cu(I)] in iron(II) alum solution and titrated the resulting iron(II) with cerium(IV) sulphate using ferroin as indicator. Wilson (309) collected the Cu(I) from the silver reductor in standard dichromate solution and determined the excess dichromate by titration with iron(II) with barium diphenylamine sulphonate as indicator. The method allowed determination of 5-20 mg of copper. The work of Salam Khan and Stephen (299) also involves the application of their particular silver reductor for the reduction of copper(II).

6.1.3. Flow injection analysis for copper

Two modes of analyses for copper have been applied with the flow

injection technique, methods in which the determination of copper was taken as an example to demonstrate the suitability of some new F.I.A. ideas or techniques without extensive investigations of the application to copper. The determination of copper by use of a hghly sensitive flow-through phototransducer devised for F.I.A. purposes is described by Betteride et al. (105). The copper in the samples (3-10 ng ml^{-1}) is complexed first with 4-(2′-pyridylazo) recorcinol (PAR) and after the colour has developed aliquots of the solution are injected into a stream of doubly distilled water buffered with sodium tetraborate. Wolf and Stewart (60) developed an automated multiple F.I.A. for flame atomic absorption spectrometry. It was applied to the determination of 4ng of copper, in order to demonstrate the automation of sample introduction in flame atomic absorption spectroscopy. With this automated model an analysis speed of 120-180 samples per hour with a 2% r.s.d. was achieved.

The effects of different organic solvents as carrier streams are investigated on the sensitivity of the determination of copper using combined F.I.A.-atomic absorption spectrometry (117). Enhancement factors were obtained by injecting aqueous copper(II) solutions (2μg ml^{-1}) into a stream of methanol, MIBK or n-butyl acetate and comparing the peaks obtained with those resulting from water as a carrier stream. Improved sensitivity was achieved using n-butyl acetate, which allows the determination of 0.1-3μg ml^{-1} copper at a sampling rate of 300 h^{-1} using a flow rate of 6ml min^{-1}. The r.s.d. was 5%.

A flow injection system with merging zones was used to demonstrate the method for multielement analysis (Ni/Cu/Zn) using inductively-coupled argon plasma atomic emission spectrometry (131). The generalized standard addition method applied was found to be useful to overcome matrix and spectral interferences. A sample volume of 500μl was applied.

The determination of copper was used to study a manifold which allowed liquid-liquid extraction of aqueous solutions of metal ions using atomic absorption spectrophotometric detection (118). Copper-MIBK test solutions were prepared by shaking aqueous solutions of copper-pyrrolidinedithiocarbamate (APDC) with MIBK. The calibration graph for copper was linear up to 0.15μg ml^{-1}. The r.s.d. was 0.6% for 10 replicate injections for 1μg ml^{-1} Cu-MIBK solution at a carrier flow of 5.9ml min^{-1}.

Many direct and indirect methods have been applied for the determination of copper. Van der Linden and Oostervink (310) described the construction and characteristics of micro flow-through copper(II)-selective electrode. The prepared electrode was used for continuous monitoring of the copper content in water which arises from copper pipes. Copper solutions (0.13ml) were injected into an acetate buffer solution (pH 4.75) and a glass bead column (5cm long, 2mm i.d.) was used as a mixing device. Although the copper determination was not affected by interferences from many transition elements, the procedure was unable to provide a linear calibration graph and it was essential to introduce a constant copper concentration level as background to fix the potential at a certain level between different injection peaks.

Potentiometric stripping analysis in conjunction with F.I.A. has been described for the determination of Cu, Cd and Pb in ground water (311). The purpose of the work was to demonstrate the automated scheme of their computerised system. Peak area measurements are used to evaluate experimental data. Linear calibration graphs over two ranges of copper standards (20-200 and 200-1000μg ml^{-1}) were obtained with correlation coefficients of 0.995. The r.s.d. was 2% for 1μg ml^{-1} copper. Another F.I.A. method for copper using potentiometric stripping has also been described (312).

The method described by Alexander and Akapongkul (313) involves

the direct electroreduction of metal ions in ammonical buffer solution at a copper amalgam electrode using an F.I.A.-amperometric system. The detection of 2-3ng of Cu is achieved.

Determination of copper via its catalytic effects is of considerable interest in F.I.A. (314-316). The method of Ramasamy and Mottola (314) is based on the catalytic effect of copper(II) ions in the oxidation of thiosulphate by iron(III) in acidic medium. This conventional method was monitored by a F.I.A. method for the determination of copper in human serum with a detection limit of 0.25μg ml^{-1} and a sampling rate of $325h^{-1}$, with a r.s.d. of 2%. Lazaro et al. (316) applied a stopped-flow injection method for Cu(II) determination in the range 0.2-300ng ml^{-1} which was based on the catalytic effect of this ion on the 2, 2/-dipyridylketone hydrazone-hydrogen peroxide system. The oxidation product shows an intense blue fluorescence. Calibration intervals of 0.2-2ng ml^{-1} and 2-10ng ml^{-1} were obtained. The significant disadvantage of this method is the interfering effects of many cations and anions. The sampling rate was $72h^{-1}$ with a r.s.d. of 0.6%.

Spectrophotometric methods (U.V. and visible) using various chromogenic reagents have been suggested for the direct determination of copper (140, 141, 142, 317, 318). Methods by Betteridge et al. (140, 142) based on the application of a pH gradient are discussed in chapter one. The procedures of Kuroda et al. involved the measurement of the absorbance of the copper(II) aquo-complex at 805nm (317, 318) or of the Cu(II)- EDTA complex at 730nm (317). In both cases, accurate results were obtained for several types of brass, beryllium copper, deoxidised copper, german silver and aluminium bronze. Nitric acid (1.4M) was used to prepare the test solutions and as a carrier stream in the F.I.A. manifold. A sample volume of 318μl was necessary when a

flow rate of 5ml min^{-1} was used. This allowed the analysis of 280 samples per hour with a r.s.d. of 0.5%.

Leach et al. (61) have applied series differential detection for the determination of copper and iron. As the aim of the method was to demonstrate the importance of the technique, most of the analytical processes were executed conventionally. Therefore the copper samples were injected into a prepared reagent solution (a mixture of 0.1mM bathocuproine, 1mM sodium acetate and 22mM hydroxylammonium chloride).

Chemiluminescence in conjunction with F.I.A. is described for the determination of copper(II) by means of the FMN-hydrogen peroxide-phosphate buffer system (319). This method was found to be more selective than other CL analyses and the detection of 30pg of Cu(II) was achieved.

6.1.4. Summary and Research Objectives

The above discussion indicates that no methods have been reported for the determination of copper by F.I.A. using a metallic reductor. Methods based on copper reduction are very important conventionally for its determination, especially because the specific reactions between Cu(I) and some chromogenic reagents are the basis of major spectrophotometric methods for the determination of copper (320, 321). These reduction processes almost all involve the use of soluble reductants (e.g. hydroxylamine or ascrobic acid). It is evident, however, that reduction by soluble reductants requires a complicated flow injection manifold (82, 120, 273).

Reduction of copper by the metallic reductor (silver reductor) in a conventional system has been reported in one or two circumstances (306, 309), but the combination of this reduction process with direct spectrophotometric determination of the reduced form of copper has not been reported. The reason is certainly the instability of the Cu(I) species produced, owing to air oxidation, and the difficulty of

establishing suitable reaction conditions. Titrimetric methods are normally applied when copper is reduced by a silver reductor (306, 309).

In order to demonstrate further advantages of F.I.A., it was decided to combine the use of a silver reductor with the spectrophotometric determination of copper. Using a metallic reductor will provide more efficient reduction and overcome the need of excess of concentrated soluble reducing agent, and also simplifies the design of the flow injection manifold. The use of bathocuproine disulphonic acid will provide a very selective and sensitive method (320, 321). The copper(I) produced should remain much more stable due to the use of a closed apparatus.

6.2. Experimental

6.2.1. Reagents

All chemicals were of analytical-reagent grade, and deionized water was used throughout.

Bathocuproine disulphonic acid solutions: A 0.01-0.5% W/V solutions were prepared by dissolving the desired weight of bathocuproine disulphonic acid (Koch-light Labs.) in 250ml of water. The solution was prepared every three days.

Buffer Solutions: Acetate buffers of different pH values were prepared by mixing appropriate volumes of 0.2M acetic acid and 0.2M sodium acetate, and diluting to 100ml with water (279b). Citrate buffers were prepared as described in section 5.2.1.

Copper(II) Solutions: Stock 0.100M copper solution was prepared by dissolving 4.2622g of $CuCl_2.2H_2O$ (BDH, AnalaR) in 250ml of 0.1M hydrochloric acid solution. Other copper(II) standards were prepared by appropriate dilution of the stock solution with 0.1M HCl.

Vanadium(V), Iron(III), Chromium(III), Cobalt(II), Nickel, Lead, Mercury(II) and Molybdenum(VI) solutions: A stock solution (200μg ml^{-1}) was prepared for each metal ion by dissolving the respective chloride salts, ammonium metavanadate and ammonium molybdate in 0.1M HCl, or 0.4M HNO_3 for molybdate.

6.2.2. Apparatus

6.2.2.1. Preparation of the silver reductor minicolumn

Silver for the reductor was prepared (293) by suspending a piece of electrolytic copper sheet (BDH, AnalaR), 15cm long and 10cm wide, in 300ml of 0.4M silver nitrate. The reaction was allowed to proceed with continuous vigorous mechanical stirring until tests (with chloride and p-dimethylaminobenzalrhodanine in acidic media) showed the absence of silver ions in the solution. The precipitated silver was washed by decantation with dilute sulphuric acid until almost all of the copper was removed (test with ammonia). About 15g of silver was produced.

To a required length of column (length between 1-5cm were tested) some of the silver was added slowly until the required packing was achieved. A thin layer of glass wool was put at each end of the column to prevent movement of the silver particles by the carrier flow, and a small piece of silicone rubber tube (0.8mm i.d.) was pushed into each end of the column so that a firm connection was made and a suitable adhesive (Bostik 1) was also applied from outside to eliminate the possibility of air diffusion into the column. During packing the use of an electronic vibrator (Pifco, 50Hz) was used to uniformly settle the silver particles and expel air bubbles.

The column was incorporated in the manifold by means of two 3-way valve single keys so that a kind of connection similar to that in Fig. 1.7 was established with which the sample flow through or by-passing

the column could be achieved easily. Water was passed through the column until no copper blank signal was obtained when used in the F.I.A. system, and the reductor was stored in this condition unit required for use.

6.2.2.2. Flow manifold and detection unit

A flow injection system similar to that described in figure 5.1 was used for the determination of copper. It was modified by incorporating a silver reductor minicolumn in place of the Jones reductor, and inserting some glass bead columns with different dimensions. The manifold is shown in figure 6.1, together with the optimal conditions and concentrations.

Copper(II) solutions were added by means of a Rheodyne RH 5020 injection valve (Anachem) with a 40µl sample loop, and a 4-channel peristaltic pump (Gilson Minipuls 2) was used to propel the solutions.

Glass bead columns, 2cm long, 2mm i.d., filled with 0.25mm diam. glass beads, were used to stabilize the baseline. Teflon tubing (0.5mm i.d.) was used for the rest of the manifold.

The complex formed between copper(I) and bathocuproine disulphonate was measured at 483nm using a Cecil CE 373 single beam linear readout spectrophotometer (provided with an 18µl flow-through cell) connected to a Teckman Labwriter model TE200 recorder. The detection apparatus is shown in figure 5.2.

6.2.3. Results and Discussion

6.2.3.1. Optimization of experimental conditions

Elimination of background noise

When the flow injection system shown in figure 6.1 was operated, an unstable baseline was obtained before injecting copper(II)

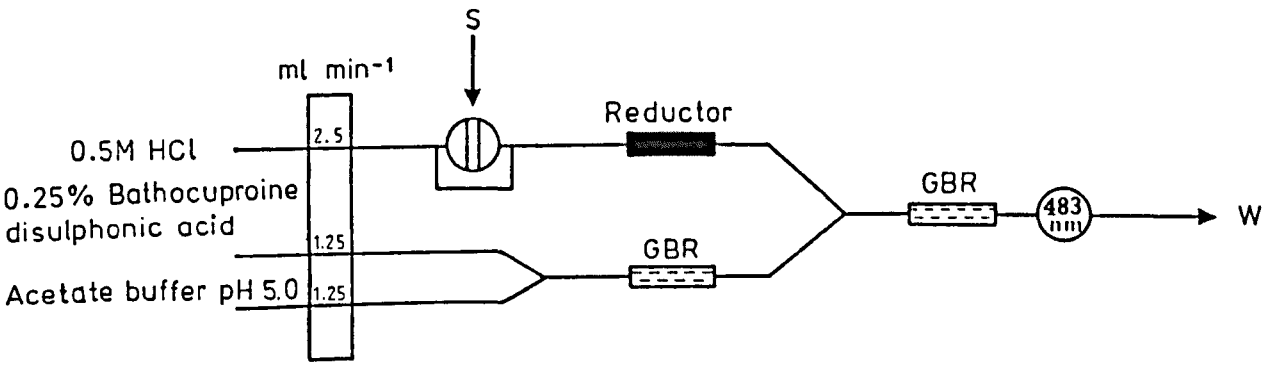


Fig. 6.1. Manifold used for the determination of copper : (S), sample injected: (GBR) glass beads reactor : (W) waste.

standards. This was again attributed to inadequate mixing and optical disturbance of the different reagents employed. This continued even when different proportions of reagents were used. The instability, however, was less significant than for the F.I.A. system applied for the determination of iron (page 155), therefore smaller glass beads columns were found to be satisfactory to eliminating the noise. Two glass-beads minicolumns, 2cm long, 2mm i.d. filled with 0.25mm diameter, glass beads were found to be suitable, without causing any significant increase in dispersion. Figure 6.2 shows the signals obtained in the absence and presence of both mixing columns (GBR) when 1×10^{-5}M Cu(II) solution was injected according to the conditions shown in figure 6.1.

Carrier stream

When a silver reductor column was employed for reduction of copper(II), hydrochloric acid up to 1 or 2M was essential for quantitative reduction (306, 309). The electrode potential varies with the chloride concentration, therefore the HCl concentration is more critical than with the Jones reductor (284). The effects of hydrochloric and sulphuric

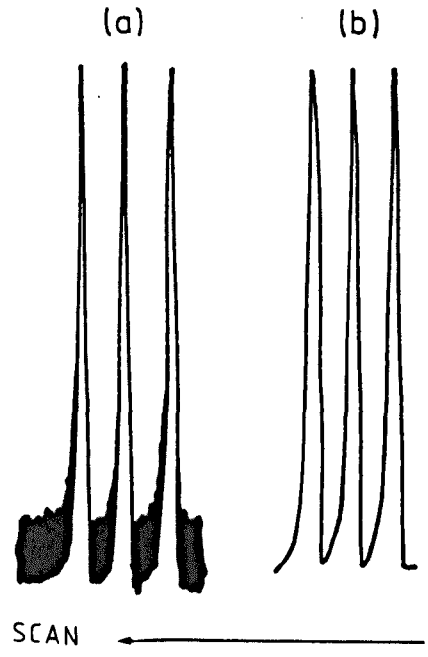


Fig. 6.2. Effect of using columns containing glass beads on the signals obtained from $1x10^{-5}$M copper: (a) without column; (b) with columns. Citrate buffer was used in both cases.

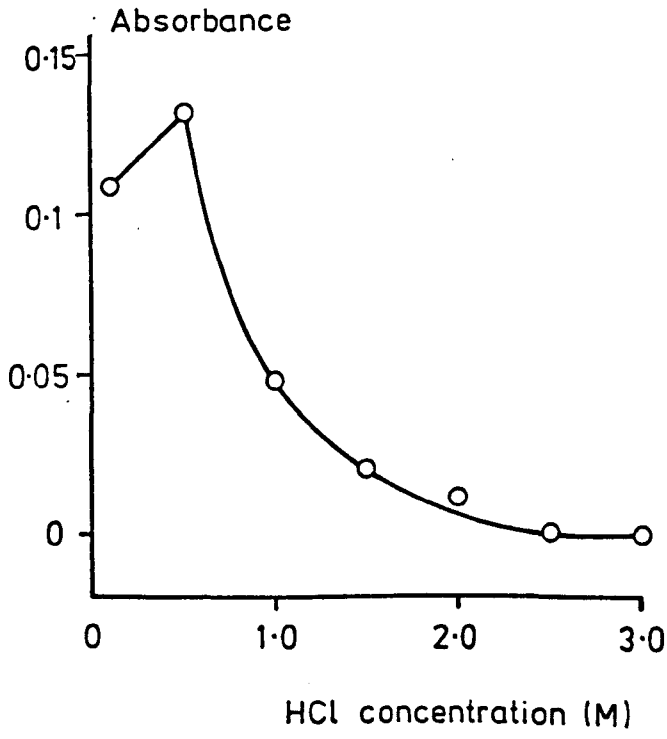


Fig. 6.3. Effect of hydrochloric acid concentration on the signal height from $2.5x10^{-5}$M copper.

acids were therefore investigated using the F.I.A. system shown in figure 6.1. The effects of different hydrochloric acid concentrations on the copper signals are shown in figure 6.3. They show an increase in absorbance as the hydrochloric acid concentration increases from 0.1 to 0.5M and then decreases as it increases to 2.5M. For copper, a high chloride concentration favours formation of soluble $CuCl_2^-$ ($\beta_2=6.5\times10^{-7}M$) rather than insoluble CuCl, which is produced at lower chloride concentrations, and deposits on the silver particles leading to smaller signals. Hydrochloric acid at $>$ 0.5M gives greater production of $CuCl_2^-$ (306), but the peaks obtained are smaller because the high acidity dissociates the Cu(I)-bathocuproine disulphonate complex. Therefore 0.5M hydrochloric acid was chosen as optimal for the determination of copper in this system.

Almost no response was obtained when 0.01-2M sulphuric acid was used in place of hydrochloric acid, probably because the copper(I) produced deposits on the column as it is unable to form a soluble complex under these conditions. During these investigations a 3cm long reductor column was used.

Buffer Solution

The copper(I)-bathocuproine disulphonate complex is reported to show constant absorbance at 483nm between pH 4 and 8 (251, 321), therefore acetate buffers pH 3-6 were initially chosen to optimize experimental conditions. But as citrate buffer also provides the same pH range, it was also examined. Both buffers gave similar results, and the absorbance obtained for a particular copper(II) concentration was the same over the pH range 3-6 examined. Therefore as citrate buffer did not seem to have any particular advantage, acetate buffer, pH 5.0, was used in all further experiments.

Reagent Concentration

For 0.5M hydrochloric acid and using acetate buffer pH 5.0, the effect of different concentrations of bathocuproine disulphonic acid solutions in the range 0.01%-0.5% (W/V) was investigated. Figure 6.4 illustrates such an effect for copper concentrations of $5x10^{-5}$M and $8x10^{-5}$M. In both instances a significant increase in absorbance was obtained up to 0.25% reagent, which was used in subsequent experiments.

Flow rate and Related conditions

The effect of flow rate, using a reductor column 3cm long and 2mm i.d., was studied by operating the flow injection manifold shown in figure 6.1 and using optimal conditions for carrier stream (0.5M HCl), reagent concentration (0.25% W/V) and buffer solution (pH 5.2). The results for two copper(II) concentrations ($2x10^{-5}$M and $4x10^{-5}$M) are shown in figure 6.5. It shows that the minicolumn performs efficiently even at high flow rates of 4ml min^{-1} (8ml min^{-1} combined flow), unlike the Jones reductor, which required a low flow rate (1ml min^{-1} through the reductor) for greatest sensitivy (chapter five). Thus, rapid flow can be used, greatly decreasing the time needed for an analysis, and increasing sample throughput rate. For example at 1.5ml min^{-1} through the reductor, the time required for one analysis was 60 sec compared with 30 sec at 2.5ml min^{-1}.

The reason for the increase of peak height as flow rate increases was of particular interest. Almost no change in absorbance was observed for a fixed concentration of copper by using normal or deareated solutions, therefore the increase in absorbance was not attributed to any contribution of dissolved oxygen to reoxidize the Cu(I) produced. It also confirms that there is no need to deareate the reagent and sample solutions before analysis.

To examine the possibility of oxygen diffusion through the tubing into the sample stream, the whole F.I.A. manifold including the

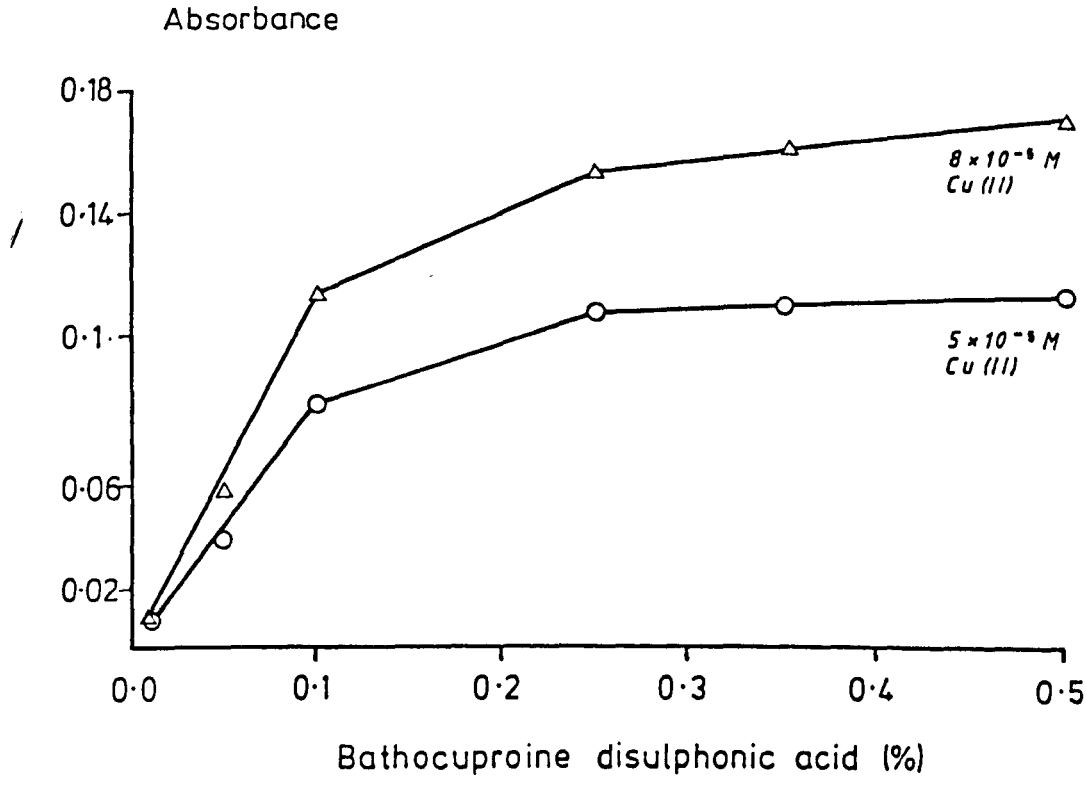


Fig. 6.4. Effect of bathocuproine disulphonic acid concentration. Flow rate 3ml min^{-1} (combined).

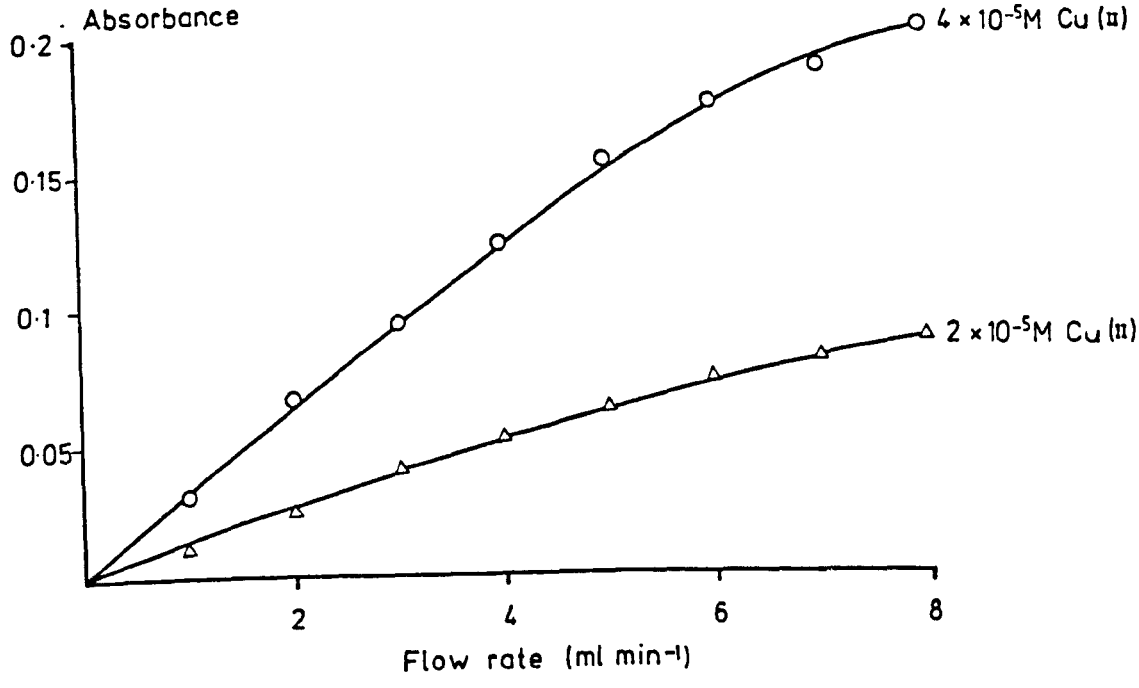


Fig. 6.5. Effects of low rate.

peristaltic pump and injection valve were placed inside a gas-tight box, and the box was filled with nitrogen gas. Under this condition, an increase in flow rate again led to a greater sensitivity in the same order as in the absence in nitrogen. Figure 6.6 shows a flow rate-dependence of the peak obtained in an atmosphere of nitrogen. Therefore oxygen diffusion into the system is not a factor, and the increase of peak height in figure 6.5 and 6.6 as flow rate increases were either due to the effect of the reductor on the extent of dispersion or to chemical factors that related to the reduction process.

Taking figure 6.6 as a basis, some mathematical calculations were made in order to interpret the reason of the increase of peak height as flow rate increases. Figure 6.7 is a time dependence graph which satisfies the basic rule of F.I.A. that a rapid flow greatly decreases the time needed for an analysis.

To study the effect of the reductor on the sample dispersion at different flow rates the flow injection system in figure 6.1 was operated without a chemical reaction, by injecting a 40µl solution of methylene blue (0.01% W/V). The results are shown in figure 6.8(a and b) which shows (as for the Jones reductor) that peak height increases with flow rate. However the peaks are broader and lower than for the Jones reductor, reflecting the increased dispersion by the smaller particle sizes of the silver particles than those of zinc.

The increase of peak height in figure 6.5 is similar to that in figure 6.8, although the increase in copper signal is less than that for methylene blue, perhaps indicating a slight decrease in extent of reduction at high flow rates.

Effects of Column length and Internal diameter

By inserting different lengths of 2mm i.d. bore columns into the flow manifold shown in figure 6.1 it was possible to observe the

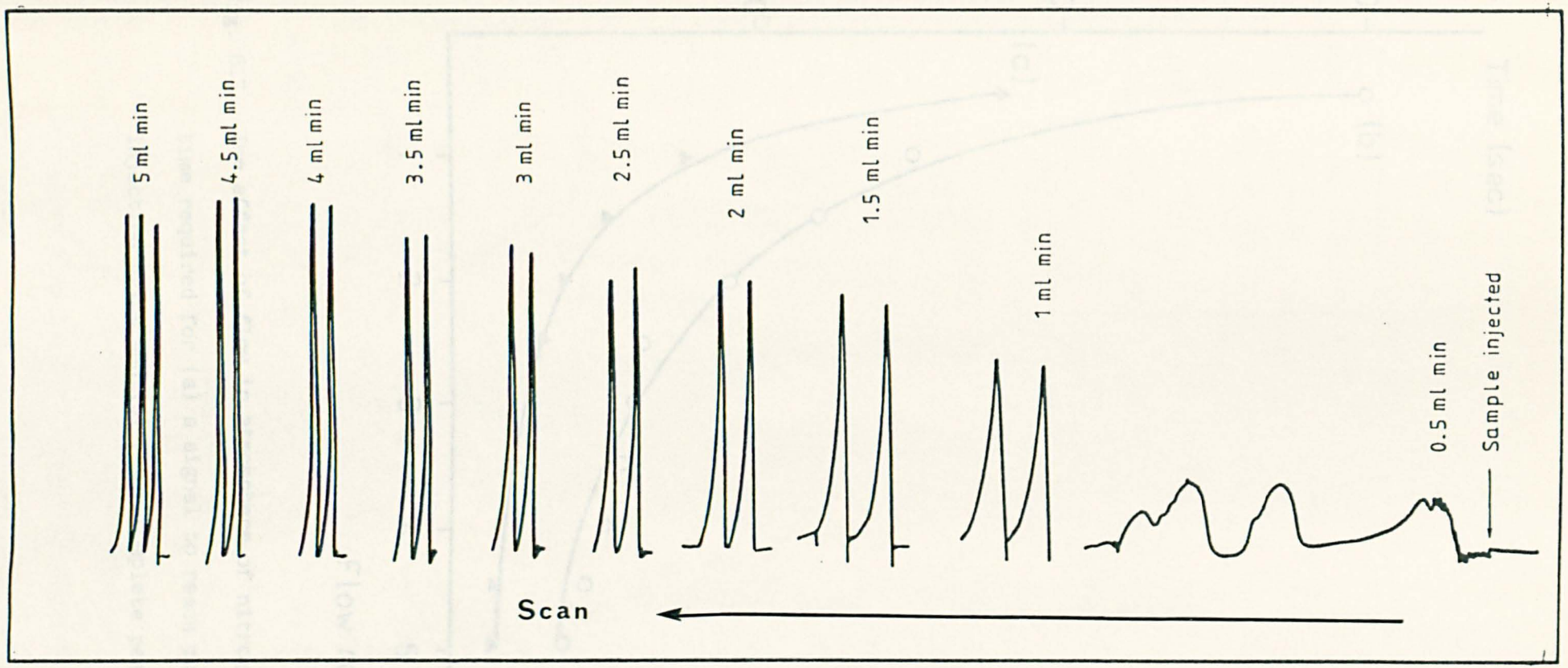


Fig. 6.6. Effect of flow rate on peak height and peak shape in an atmosphere of nitrogen.

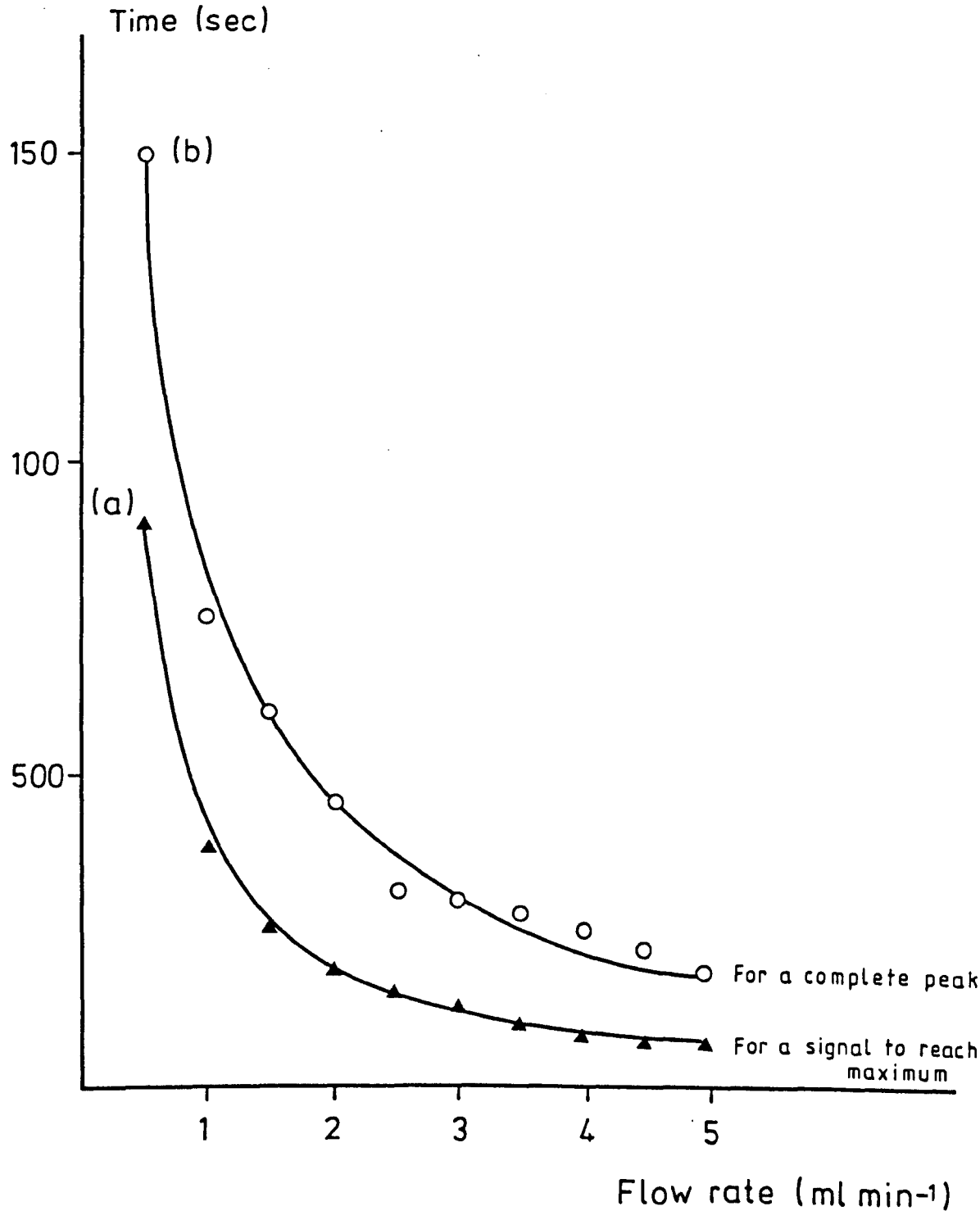


Fig. 6.7. The effect of flow in atmosphere of nitrogen on the time required for (a) a signal to reach maximum from injection point and (b) for a complete peak.

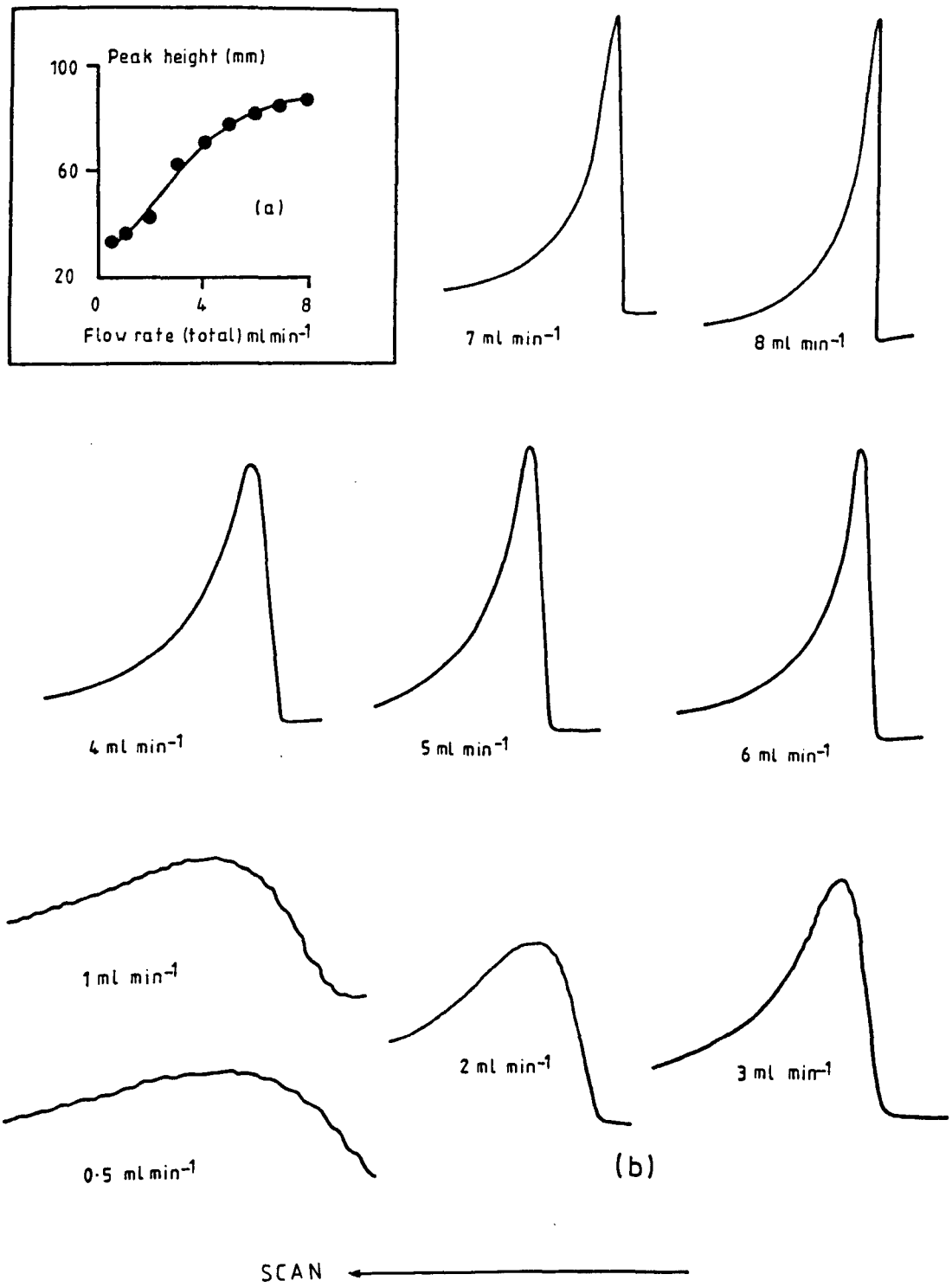


Fig. 6.8. (a) Effect of silver reductor on sample dispersion at different flow rates in absence of chemical reaction.
(b) Effect of flow rate on peak height and shape obtained.

effect of reductor length on the reduction and determination of copper. Column lengths 1cm, 2cm, 3cm, 4cm and 5cm are used for this purpose and all reductors are prepared in a similar way as described in section 6.2.2.1. The effect of reductor length is shown in figure 6.9. The peak height increases with decreasing column length over the range investigated but with a column length smaller than 2cm the rate of decrease is less and it appears to reach a plateau at 1cm. The decrease in signal height is a consequence of dispersion increase as a result of introducing the longer column.

Therefore, as the use of very long column (5cm) reduces significantly the sensitivity of copper determinations (figure 6.9), a shorter column is preferred, particularly, as shown in figure 6.9, quantitative reduction is possible even with a smaller column (1cm long, 2mm i.d.). To compromise between the capacity required and the sensitivity of the determination, a 3cm long column is recommended.

With other variables at their optimum values, the effect of internal diameter of the reductor minicolumn was examined. Different reductors of a fixed length (3cm) and internal diamters of 1.5mm, 2mm, 4mm, and 5mm were incorporated into the F.I.A. system shown in figure 6.1. The results which relate the absorbance and residence time to the increase in internal diameter are shown in table 6.2. The signal height was depressed significantly as a wider column employed. There was little variation in signal height when an internal diameter less than 2mm is used. Therefore a 2mm i.d. reductor found has an advantage over a narrower reductor ($<$ 2mm) with which the possibility of blockage increases.

At constant flow rate, the time required for obtaining a complete peak from the point of injection is 70 sec when a reductor Of 5mm i.d. is used, compared with 40 seconds and 30 seconds with reductors of 4mm and 2mm i.d. respectively.

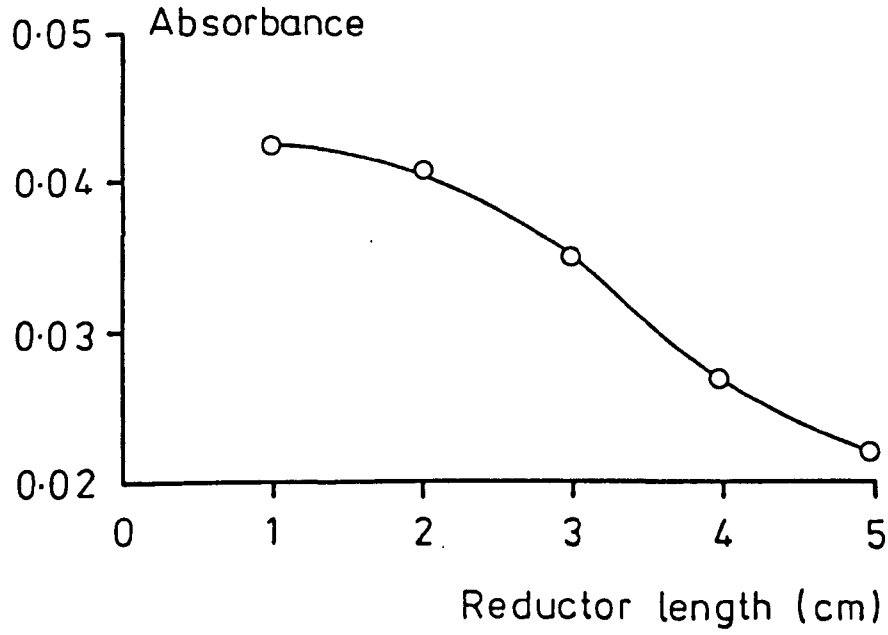


Fig. 6.9. Effect of the length of the reductor on the sensitivity of copper determination ($1x10^{-5}M$)

TABLE 6.2

EFFECT OF INTERNAL DIAMETER OF A 3-cm LONG REDUCTOR (COMBINED FLOW RATE 4ml min^{-1})

Internal diameter of the reductor (mm)	Absorbance	Base width (mm)	Time recorded from point of injection (sec)	
			To reach a maximum	for a complete peak
1	0.034	1.5	15	30
2	0.03	1.5	15	30
4	0.018	2	20	40
5	0.01	5	40	70

Dispersion Coefficient (D_t)

Having the necessary conditions optimized for an efficient reduction process, the dispersion in the flow injection system in figure 6.1 was measured as a dispersion coefficient by injecting 40µl of a 0.01% (W/V) solution of methylene blue, and by aspirating it continuously through the system. D_t was found to be 7.95.

Capacity of the Reductor

The capacity of silver reductor was measured in terms of a repetitive injections of 40µl of 10^{-3}M Cu(II) solution using the F.I.A. system in figure 6.1. The result of 75 injections (equivalent to 190.5µg of Cu(II)) is shown in figure 6.10 in which a column 3cm long, 2mm i.d. is used. No change in signal was observed. Like the Jones reductor, this silver minicolumn possesses high reduction efficiency over the injection range applied and therefore it is difficult to build a relation between the weight of silver particles used and the high capacity exhibited by the column. Having optimizing conditions, only one column was used throughout the work presented here.

Effect of sample volume

By inserting different lengths of 0.8mm bore tubing into the valve sample loop it was possible to observe the effect of sample size. The effect of sample volume on absorbance is shown in figure 6.11. The absorbance increases with sample volume over the range investigated but with volumes greater than 80µl the rate of increase is less than with smaller volumes. However as the sample volume increases the time required for one analysis increased significantly and the base width of the peak increased accordingly. Table 6.3 illustrates these investigations.

From the investigation of the effect of silver particles on sample dispersion inside the column and to avoid the use of a higher flow

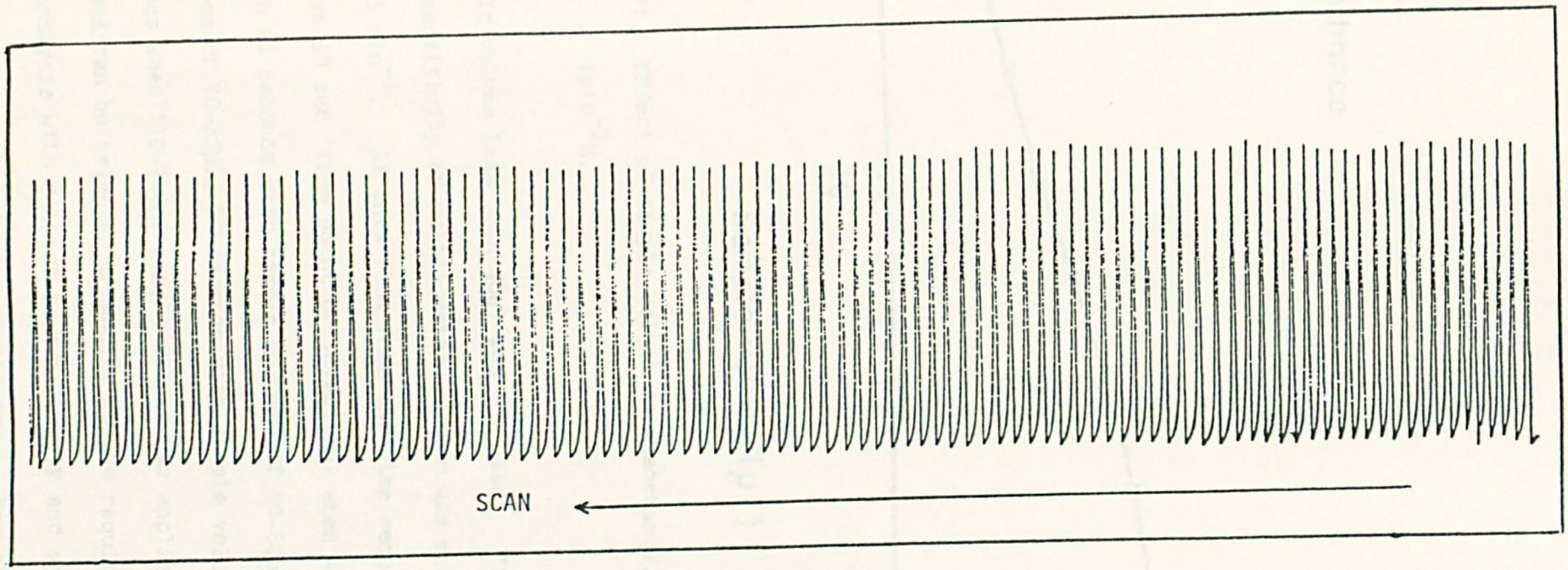


Fig. 6.10. Capacity-Profile of Silver reductor. Cu(II) concentration injected is 10^{-3}M (40µl).

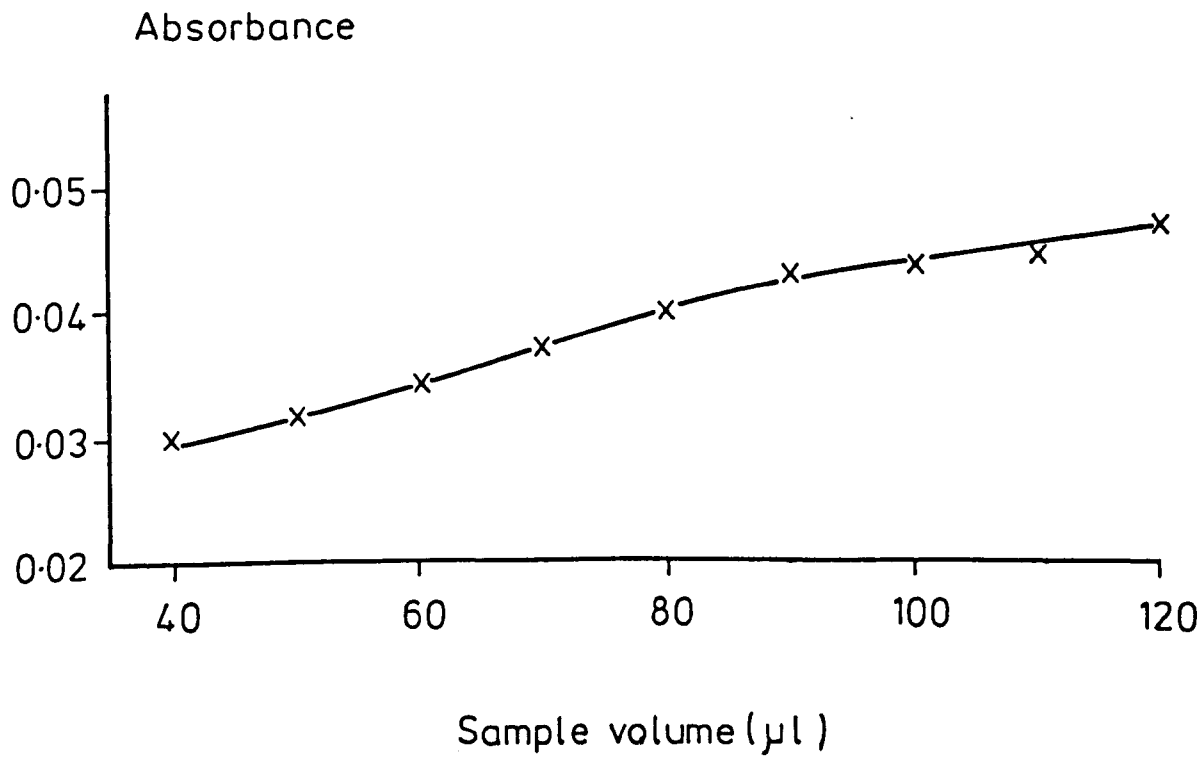


Fig. 6.11. Effect of sample volume on absorbance. Cu(II) conc. $1x10^{-5}$M.

rate, a sample volume less than 40µl was not used. The effect of sample size on the sensitivity of Cu(II) determination was measured at a flow rate of 2.5ml min^{-1}. At this flow rate the time required for analysing one sample was 30 sec (from point of injection) when 40µl sample used compared with 40 seconds with sample volumes of 50-60µl, 45 seconds with sample volumes of 70-80µl, 50 seconds with sample volumes of 90-100µl and 55 seconds when 110-120µl sample volume was applied. Therefore sample volumes can be selected depending on the requirements of a particular analysis with respect to sensitivity and sample throughput, and there should be a compromise between the two. For the present work however a 40µl sample was used.

TABLE 6.3

EFFECT OF SAMPLE VOLUME ON TIME AND PEAK WIDTH

Sample Volume (μl)	Time required for one injection (sec)	Base-width of the peak (mm)
40	30	2.5
50	40	3.0
60	40	3.0
70	45	3.5
80	45	3.5
90	50	4.0
100	50	4.0
110	55	5.0
120	55	5.0

5.2.3.2. Interferences

There are almost no metal ion interferences when copper is determined by using bathocuproine disulphonic acid in conventional spectrophotometry using soluble reducing agents (321). For the new F.I.A. procedure in which the reduction via a minicolumn is combined with spectrophotometric detection, two groups of metal ions were tested for possible interfering effects. They were those metal ions in a higher oxidation state (Fe^{3+}, Cr^{3+}, V(V), Mo(VI)) which might be reduced by the reductor, and those metals normally present in copper samples (Co^{2+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Hg^{2+}). For each potential interferent a series of solutions was prepared that contained a fixed concentration of copper(II) and different concentrations (1 - 150$\mu g\ ml^{-1}$) of the other ions. None of these ions interfered at any concentration up to 150$\mu g\ ml^{-1}$. This selectivity pattern is most advantageous over methods in which the effects of other metals are significant (105, 316).

5.2.3.3. Calibration graph

Having carried out necessary optimization, a calibrations graph of copper were constructed. The graph was linear up to $1x10^{-4}M$. Typical calibration peaks for copper in that linear range are shown in table 6.4 and figure 6.12. The linear relation between peak height absorbance and copper concentration is expressed by the least-squares equation $Abs=6.65x10^{-4} + 3191.3[Cu]$ where the copper concentration is in mol l^{-1} ($r = 0.9999$, 5 points. Fig. 6.12). The limit of detection (2 x noise) was $5x10^{-7}M$ (3.4ng) and the r.s.d. of 12 replicate injections of $6x10^{-5}M$ copper was 0.83%. Analyses of 120 samples h^{-1} were achievable when a flow rate of 2.5ml min^{-1} through the reductor was used.

TABLE 6.4

CALIBRATION RESULT FOR THE DETERMINATION OF COPPER

Sample	Cu(II) Conc. (M)	Absorbance	R.S.D.% for 3 replicates
1	$1x10^{-5}$	0.03	1.8
2	$3x10^{-5}$	0.10	0.6
3	$6x10^{-5}$	0.19	0.85
4	$9x10^{-5}$	0.29	0.5
5	$12x10^{-5}$	0.38	1.5

Mean R. S. D. = 1%

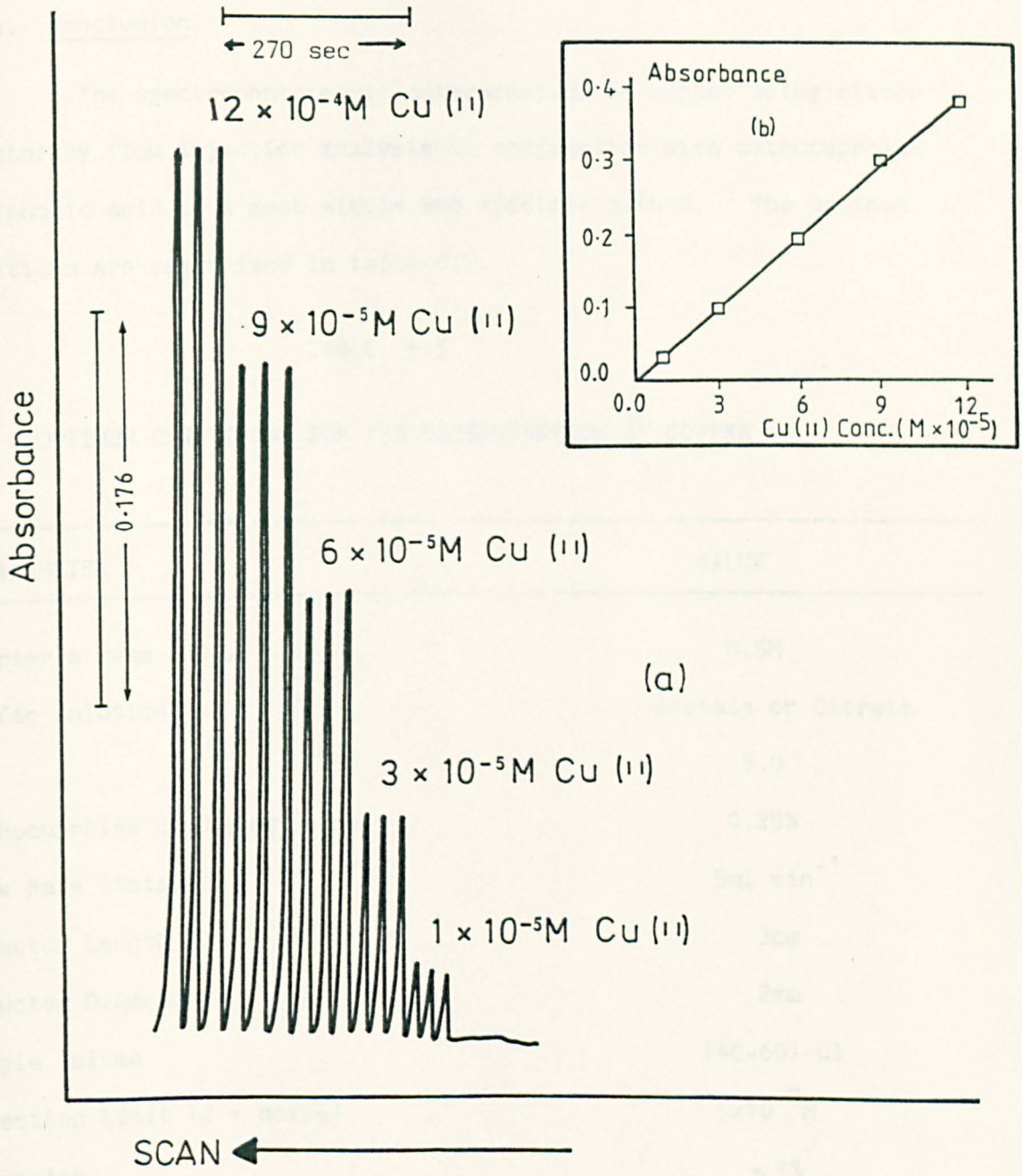


Fig. 6.12 (a) Peaks obtained by injecting triplicate copper(II) standards in the concentration range shown using the manifold in figure 6.1 (Sampling rate $120h^{-1}$); (b) the corresponding calibration graph.

5.2.4. Conclusion

The spectrophotometric determination of copper using silver reductor by flow injection analysis in conjunction with bathocuproine disulphonic acid is a most simple and specific method. The optimum conditions are summarised in table 6.5.

TABLE 6.5

OPTIMUM CONDITIONS FOR THE DETERMINATION OF COPPER

PARAMETER	VALUE
Carrier stream (HCl)	0.5M
Buffer solution	Acetate or Citrate
pH	5.0
Bathocuproine disulphonic acid	0.25%
Flow Rate (Total)	5ml min^{-1}
Reductor Length	3cm
Reductor Diameter	2mm
Sample volume	(40-60) µl
Detection Limit (2 x noise)	$5x10^{-7}M$
Precision	< 1%
Sample rate	120 s. $hour^{-1}$
Selectivity	No interferences from other metal ions

The use of standard F.I.A. systems as a means of modifying classical assays has been remarkably successful. The combination of F.I.A. with such procedures has resulted in a marked increase in sample

throughput rate, usually accompanied by increased precision. Reduction of copper by a silver column is found to be efficient and reproducible, and its use, in conjunction with bathocuproine disulphonic acid provides a very effective means of determining copper by F.I.A. with great selectivity and sensitivity. The use of the reductor minicolumn eliminates the need for use of a soluble reductant, thus simplifying the manifold.

It is concluded that F.I.A. has solved to a great extent some chemical problems that obscured the combination of this reduction with spectrophotometric detection. For example using F.I.A. 0.5M HCl was found sufficient for efficient reduction compared with 1 to 2M conventionally. By increasing the flow rate it was also possible to overcome the extent of increased dispersion caused by the reductor. This eliminates the need to prepare silver of larger particle sizes (298, 299) as a means of reducing sample dispersion which might be more expensive.

PART III

APPLICATION OF ION-EXCHANGE IN FLOW INJECTION ANALYSIS

CHAPTER SEVEN

A COMBINATION OF ION-EXCHANGE COLUMNS WITH SPECTROPHOTOMETRIC DETECTION FOR THE DETERMINATIONS OF ANIONS BY FLOW INJECTION ANALYSIS

7.1 Introduction and Research objectives

In flow injection analysis the use of insoluble compounds in the form of packed reactors is of particular interest. The use of reducing columns was described in chapters 5 and 6.

The use of solid ion-exchangers is also possible in F.I.A. (82, 86, 322-324). An example is the determination of the sum of either anions or cations in an aqueous solution (322). The method is based on measuring the pH change caused by the hydroxide ion (or hydrogen ion) released from the strongly basic anion exchanger (or strongly acid cation exchanger) by the anions (or cations). The pH is detected by the change of colour of an acid-base indicator.

The incorporation of a miniature ion-exchange column in the F.I.A. system for monitoring chemiluminescence has been extensively investigated (323) [chapter four]. A chelating resin (Dowex A-1 or Chelex-100) was most suitable for removing metal ion impurities from hydrazine samples. Kamson and Townshend (324) used an anion-exchange column in a F.I.A. system to eliminate the interfering effects of phosphate and sulphate on the determination of calcium by a.a.s. Here two anion exchange resins were tested (Amberlite IRA-400 and De-Acidite FF, both in Cl^- form). A strongly basic anion exchange resin was used for separation and determination of iron(II) and iron(III) using a.a.s. (82). Using 6M HCl as an eluent, iron(II) is retained by the resin column while iron(III) is eluted. Iron(II) is released later by replacing the acid stream by distilled water.

The importance of anion determination is well recognized. Many

of the common anions (e.g. sulphate, chloride, oxalate and nitrate) form few colour systems, so that there is a dearth of direct spectrophotometric methods for them. Indirect methods often depend on the displacement of a chromogenic species from insoluble compounds such as the mercury thiocyanate method for the determination of chloride (325) and the barium chloranilate method for spectrophotometric determination of sulphate (326). Ion-exchange has been used for separating these anions or for their determination by exchanging with another anion which can be determined spectrophotometrically. An example on this latter concept is the work carried out by Ducret and Ratouis (327) in which sulphate exchanged with thiocyanate on a strongly basic anion exchange column in the thiocyanate form, the thiocyanate being determined by extracting its methylene blue ion pair into 1,2-dichloroethane.

The aim of the present work is to combine ion-exchange with spectrophotometric detection in F.I.A. to develop a new method for the determination of those anions whose spectrophotometric determinations are uncommon. These anions include sulphate, chloride, nitrate and oxalate. It involves a rapid exchange of these anions with another present on the resin such as SCN^-, $H_2PO_4^-$ and I^-. Spectrophotometric determinations of these anions are straightforward (328-330). Thiocyanate immediately forms a red complex with iron(III) which can be measured at 465nm (328), phosphate forms a blue colour with a mixture of ascorbic acid and ammonium molybdate (329) and iodide ion complexes with palladium chloride and can be measured at 400nm (330). All these procedures are accomplished in aquous media, which is advantageous for F.I.A.

It is also intended to examine the use of packed columns to improve selectivity of anion determinations. To achieve this, a principle of ion chromatography which involves the use of a suppressor column is considered in order to establish a selective detection procedure.

The fundamental characteristics of the suppressor columns have been described extensively (331-333), and can involve one of a number of reactions. One is the conversion of a highly conductive eluent to a form which has low conductivity. Equations 7.1–7.3 illustrate some such reactions for eluent constituents commonly used in anion analysis (333):

$$\underset{\text{resin phase}}{H^+ - \text{resin}} + \underset{\text{eluent}}{Na^+ + OH^-} \rightleftharpoons \underset{\text{resin phase}}{Na^+ - \text{resin}} + \underset{\text{final eluent}}{H_2O} \qquad (7.1)$$

$$H^+ - \text{resin} + Na^+ + HCO_3^- \rightleftharpoons Na^+ - \text{resin} + H_2O + CO_2 \qquad (7.2)$$

$$2(H^+ - \text{resin}) + Na_2CO_3 \rightleftharpoons 2(Na^+ - \text{resin}) + H_2O + CO_2 \qquad (7.3)$$

A second reaction of the suppressor is conversion of the anions to a single, highly conductive form as shown in equation (7.4).

$$H^+ - \text{resin} + Na^+ + \text{Anion} \rightleftharpoons Na^+ - \text{resin} + H^+ - \text{Anion} \qquad (7.4)$$

Suppression via precipitation has also been used during ion chromatography (334, 335). One example is the precipitation of Cl^- from an HCl eluent with Ag^+ (from the suppressor) and by exchanging the H^+ onto the vacant resin site. The silver chloride precipitate is held in the resin network and therefore regeneration is required by dissolving the AgCl with NH_3 and water followed by resilvering with $AgNO_3$ and washing again with water. The regeneration can be avoided by using a commercial disposable plastic column (332). Its expended portion is cut off after a day of operation to eliminate the continuous pressure increase because of precipitation of AgCl.

Therefore, it is possible to design a flow injection manifold similar to that used in ion chromatography in which two columns are used in the operation for anion determination, a suppressor column and an

exchanger column. As the aim of the suppressor is to remove interferences, it is possible to prepare a variety of these columns in different forms depending on the type of anion to be removed e.g. cation exchange resin in Ag^{+}, Ca^{2+}, Pb^{2+} forms.

7.2. Experimental

7.2.1. Reagents

All chemicals were of analytical grade and distilled-deionized water was used throughout.

Thiocyanate Solution: a 2M stock stolution was prepared by dissolving 48.59g of KSCN (AnalaR) in 250ml of water. A 0.1M SCN^{-} solution which was used for the preparation of the SCN-form anion-exchange resin was prepared by diluting the stock solution with water.

Iron(III) Solution: an 0.01M Fe(III) solution was prepared by dissolving 0.6757g of $FeCl_3.6H_2O$ (AnalaR) in 250ml of 0.1M HCl. Other solutions were prepared by appropriate dilution.

Standard solutions of Anions: Stock solutions (0.1M) of sulphate, phosphate, oxalate, nitrate and chloride were prepared by dissolving 4.3562g of K_2SO4, 3.4022g of KH_2PO4, 4.6057g of K_2C_2O4, 2.5275g of KNO_3 and 1.8640g of KCl (all AnalaR) in 250ml of water. Calibration solutions were prepared by appropriate dilution of each stock solution with water.

7.2.2. Apparatus

7.2.2.1. Preparation of the exchanger column

The strongly basic anion exchange resin Dowex 1-x4(200-400 mesh) in the Cl^{-} form was used and converted to the SCN^{-} form in the following way (327). 2g of resin was placed in a 100ml round-bottomed flask and shaken with 50ml of 0.1M KSCN for 20 min twice and then 3 times

with 50ml of 2M KSCN for the same period. The resin was filtered and washed with water between each shaking. The resin was twice shaken again for 10 min. with 50ml of water and filtered and left for 24 hours in water. The resin was filtered again and added to a glass tube (3cm length, 2mm i.d.) until the required packing was achieved. A thin layer of glass wool was put at both ends of the column to prevent movement of the resin particles by the carrier stream. A small piece of silicone rubber tubing (0.8mm i.d.) was pushed to each end of the column so as to achieve a very tight connection and a suitable adhesive (Bostik1) was applied from outside onto the column-silicone tube joint. Using the flow injection system in figure 7.1, water was passed through the exchanger column until a stable baseline shows the absence of thiocyanate in the eluent and the column was stored in this condition until required for use.

The same procedure above was used to convert the resin to the iodide or phosphate form by shaking the resin with potassium iodide or potassium hydrogen phosphate, respectively. The packed columns were prepared in the same way described for thiocyanate-form resin.

7.2.2.2. Preparation of the suppressor columns

The suppressor columns used were in a cation form for depressing anions. Therefore a strongly acidic cation exchange resin in the H^+ form (Dowex 50W X8, 4% Crosslinked, 100-200 mesh) was used. The resin was stored in water for 2 days to achieve the required swelling.

Ag^+-form resin was prepared by passing 25ml of 1M $AgNO_3$ through the H^+ form column (1.5cm long, 2mm i.d.) for 25 min. at 1ml min^{-1}. Water was passed until the test (with chloride) shows the absence of silver ions in the effluent.

Jones reductor (amalgamated zinc), 1.5cm long, 2mm i.d., was prepared as previously described (chapter five).

The body of the suppressor columns were made of Perspex tubing. Therefore combined suppressor columns could be prepared by screwing two different column forms (each 1.5cm long, 2mm i.d.) onto each other. Glass wool was used to separate the contents of each column. This combination technique facilitates the separate regeneration of each column. Fig. 7.2 shows a picture of the suppressor columns combination used in this work.

7.2.2.3. Flow manifold

The F.I.A. system used for the determination of anions is shown in figure 7.1. A 4-channel peristaltic pump (Gilson Minipuls 2) was used, and anion solutions were introduced by means of a Rheodyne RH 5020 injection valve (Anachem) with a sample loop of 40µl. Teflon tubing (0.5mm i.d.) was used for all connections.

The absorbance was measured at a suitable wavelength (465 nm in case of thiocyanate-iron(III) complex), 660nm (for phosphate) and 400nm (for iodide) using a Cecil CE 373 linear readout spectrophotometer (with a 18µl flow cell) connected to a Teckman Labwriter TE 200 recorder. This apparatus is the same as shown in figure 5.2.

7.2.3. Results and Discussion

7.2.3.1. Optimization of Variables

In acidic medium (pH 1.8) standard solutions of iron(III) were mixed with thiocyanate solutions. The red complex formed instantly and showed maximum absorbance at 465nm for all iron(III) concentrations in the range (10^{-2}-10^{-5}M). Therefore this wavelength was chosen for further studies.

Having operated the pump in figure 7.1, the baseline is ensured to be stable before injecting an anion sample. Standard solutions of different anions (SO_4^{2-}, $C_2O_4^{2-}$, NO_3^-, Cl^- and $H_2PO_4^-$) were injected into

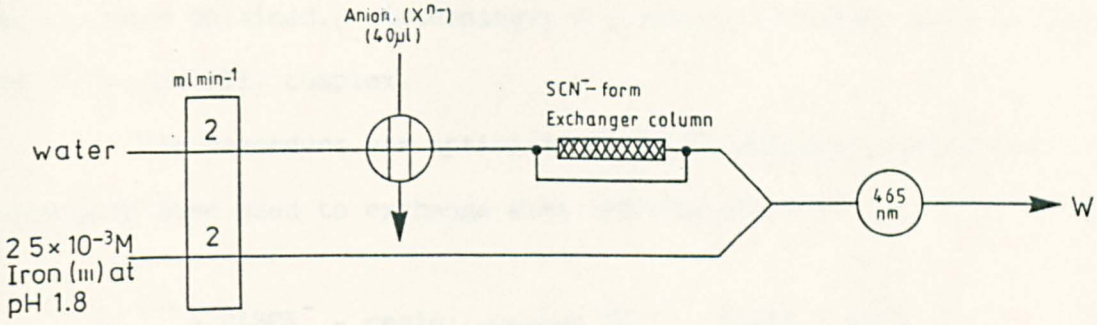


Fig. 7.1. Flow diagram of the system used for the determination of anions. W, waste.

Fig. 7.2. Suppressor column bodies and the mode of combination.

the carrier stream, with the exchanger column bypassed, and no absorption signals were obtained. Accordingly any signals obtained will be due to the SCN^--iron(III) complex.

The procedure was optimized by using sulphate, which had previously been used to exchange with thiocyanate (327).

$$SO_4^{2-} + 2(SCN^- - \text{resin}) \rightleftharpoons SO_4^{2-} - \text{resin} + 2SCN^- \qquad (7.5)$$

Effect of iron(III) concentration

The effect of different iron(III) concentrations in the range $5x10^{-5}$ - $1x10^{-2}$M were examined using the system in figure 7.1, with the bypass closed. The results obtained for $5x10^{-5}$M sulphate are shown in figure 7.3. The absorbance increased on increasing the iron(III) concentration. For $5x10^{-3}$M iron(III) the absorbance approaches a plateau. Therefore the use of $5x10^{-3}$M is recommended.

pH

The formation of iron(III)-thiocyanate complexes predominantly takes place at pH below 1.5 (329). Above pH 2.0, precipitation of hydrated iron(III) oxide is possible. Hence a solution that gives the pH range required (1.0-2.2) was prepared from HCl and KCl (279d), prepared by mixing 25ml of 0.2MKCl and 67.0ml, 26.6ml, 10.2ml or 3.9ml of 0.2M HCl to give solutions of pH 1.0, 1.4, 1.8 and 2.2, respectively. These were used to prepare a $2.5x10^{-3}$M iron(III) solution which was used in the F.I.A. procedure. The results obtained (mean of 3 injections) are shown in figure 7.4. The absorbance increases as pH increased up to pH 1.8 and decrease on further increase in pH. In the lower pH solutions the Cl^- concentration is increased and dissociation of the $[Fe(SCN)]^{2+}$ upon replacing some SCN^- by Cl^- becomes most probable, and this results in lower signals. It should be noted also that ionic-

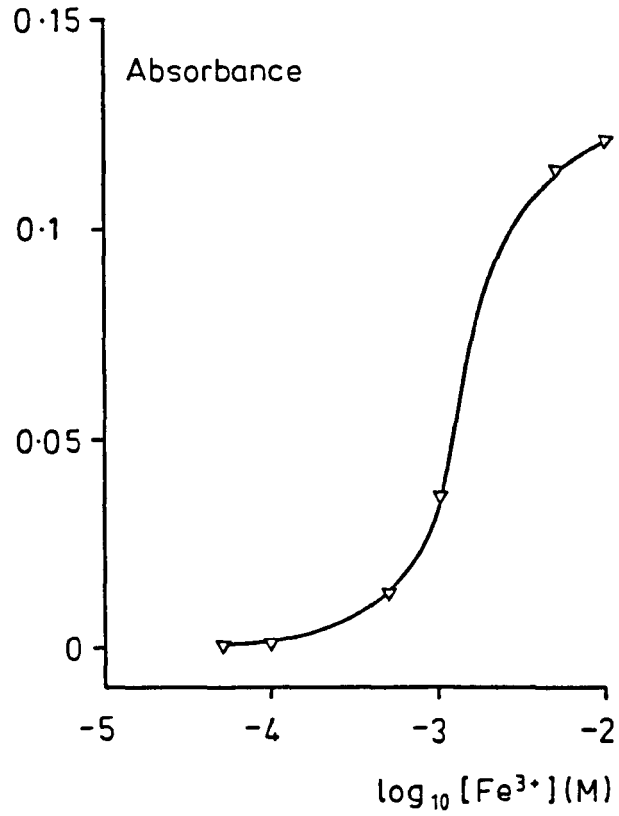


Fig. 7.3. Effect of iron(III) concentration ($[SO_4^{2-}] = 5x10^{-5}M$, flow rate = 1.5ml min^{-1}).

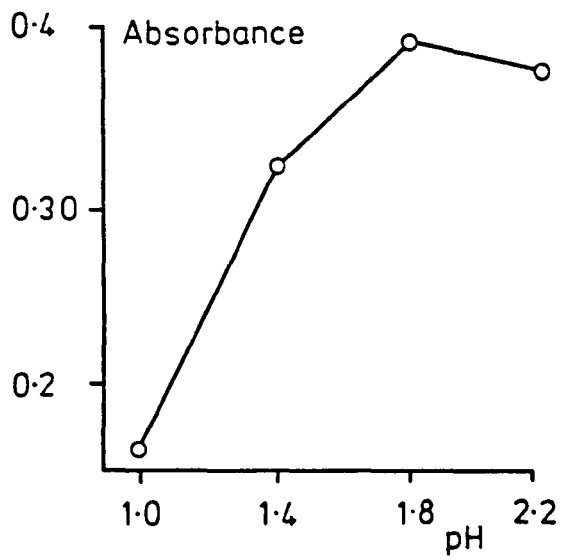


Fig. 7.4. Effect of pH $[SO_4^{2-}] = 5x10^{-4}M$.

strength which is greater at lower pH (0.216, 0.117, 0.068 and 0.049 for pH 1, 1.4, 1.8 and 2.2 respectively) might be an additional factor that causes the increase in signals as the pH also increases. As the pH exceeds 1.8 the hydrolysis of iron(III) becomes more effective and causes a significant decrease in absorbance.

Effect of flow rate

Keeping some conditions such as pH (1.5) and iron(III) concentration (2.5×10^{-3}M) constant, the effect of flow rate in the sample stream was studied. The results are shown in figure 7.5. Increasing the flow rate through the exchanger column from 0.5 to 5ml min^{-1} is accompanied by an increase in the peak height. This phenomenon is similar to the preceding investigations concluded for Jones and silver reductors (chapter five and six). The increase in absorbance by increasing flow rate is expected because the resin particles are 200-400 mesh, which resembles the size of the silver particles, shows the same sort of effect on sample dispersion inside the column. These considerations are shown in figure 7.6 and 7.7 which confirmed that the peaks get broader (dispersed sample volume) as flow rate increased. A curvature of the curve in fig 7.6 at high flow rate (>8ml min^{-1}) is mainly attributed to incomplete exchange of thiocyanate.

It should be noted that at a very high flow rate (>8ml min^{-1} combined) the displacement of SCN^- might not be complete. To avoid this and excessive reagent consumption a flow rate of 2ml min^{-1} through both exchanger and reagent line (total 4ml min^{-1}) is satisfactory which allows analyses at 120 h^{-1}.

Effect of column length

It was also of interest to study the effect of column on the exchange of thiocyanate by sulphate. Five exchanger columns were prepared having lengths of 1.5, 3.0, 4.5, 7.5 and 10.0cm, all with

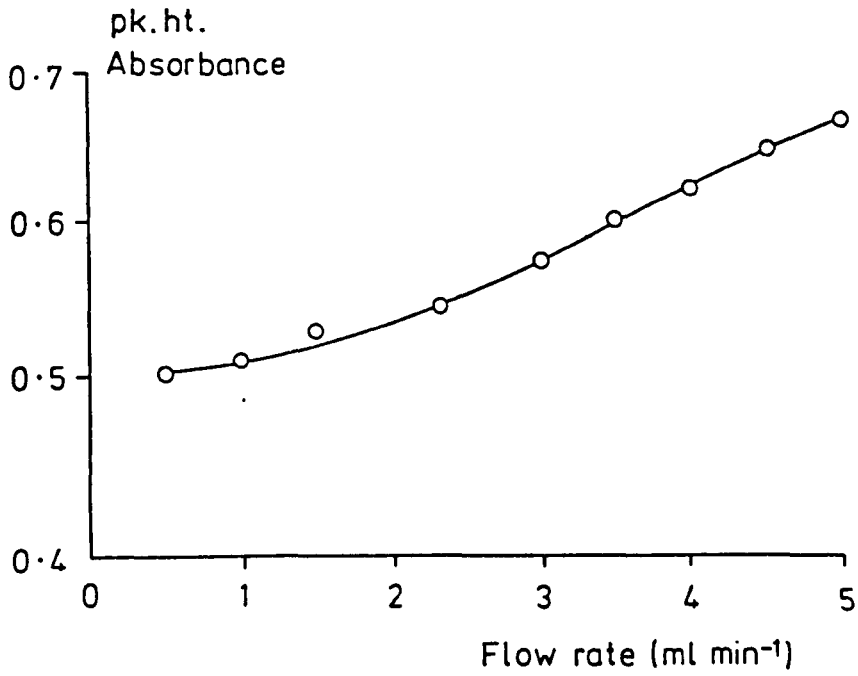


Fig. 7.5. Effect of flow rate through the exchanger column. $[SO_4^{2-}] = 2 \times 10^{-4} M$.

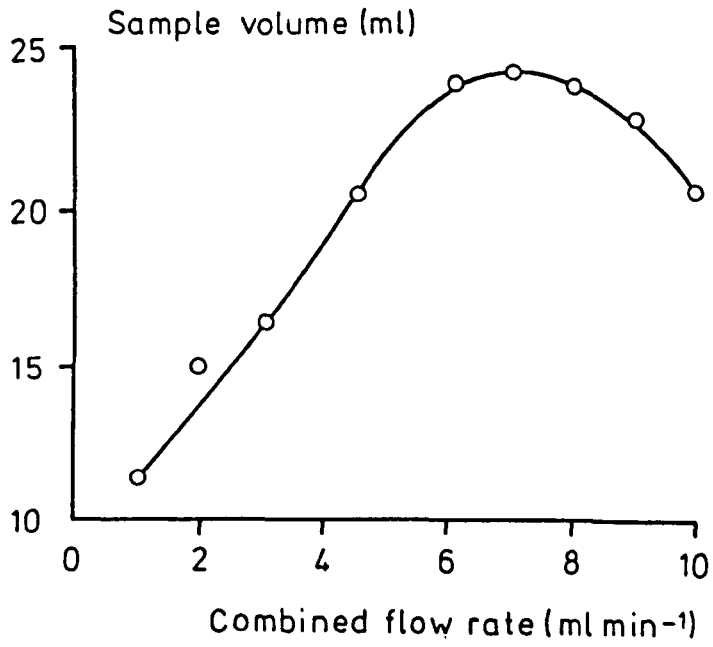


Fig. 7.6. Change in sample volume as flow rate increases.

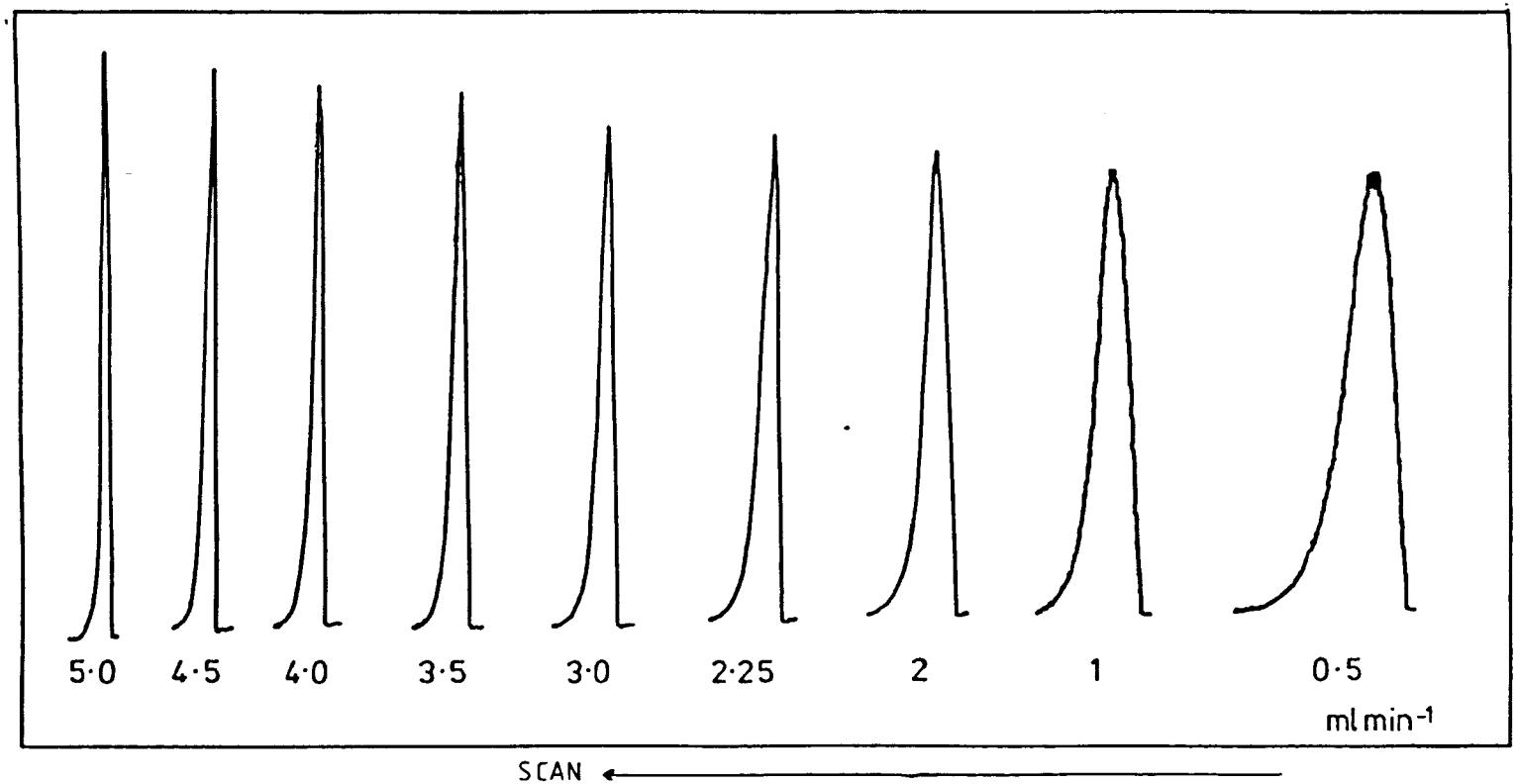


Fig. 7.7. The height and shape of the peaks obtained by increasing flow rates (through the exchanger column) in ml min^{-1}. $[SO_4^{2-}] = 2x10^{-4}M$.

internal diameter 2mm. The results obtained for each column by injecting different sulphate concentrations are shown in figure 7.8.

Two conclusions can be drawn from these results. First, it is not possible to achieve complete exchange by using a short column (e.g. 1.5cm long) at the optimized flow rate of 2ml min^{-1} used. This parameter seems to influence the peak height absorbance until a column length 3cm is reached with which the greatest peak height is achieved. Further increase in column length is not advantageous for the exchange phenomenon but causes additional dispersion of the sample which results in a lower signal.

Effect of crosslinking of the exchanger resin

The effects of using different crosslinked resins for preparing the exchanger column were investigated. The strongly basic anion exchange resin Dowex-1 (Cl^- form, 200-400 mesh) with three different crosslinking of 2%, 4% and 8% were converted to thiocyanate form as described in section 7.2.2.1 and packed into individual columns each 3cm long, 2mm i.d. Sulphate of concentrations $5x10^{-5}$ - $15x10^{-5}$M was used for each column. The results are shown in figure 7.9. They show that 4% crosslinking gives greatest sensitivity, although all columns gave reasonable sensitivity and a linear calibration graph.

7.2.3.2. Calibration graph

A calibration graph for the determination of sulphate was obtained under the optimized conditions. The calibration graph is linear over the range $5x10^{-6}$ - $2.5x10^{-4}$M SO_4^{2-}. The results for a typical calibration are shown in table 7.1 and figure 7.10. The calibration graph in figure 7.10(b) has a regression coefficient of 0.9970. The least square equation is Abs = 2340 $[SO_4^{2-}]$ - $5x10^{-3}$. The limit of detection (2 x blank noise) was $5x10^{-6}$M SO_4^{2-}. The mean

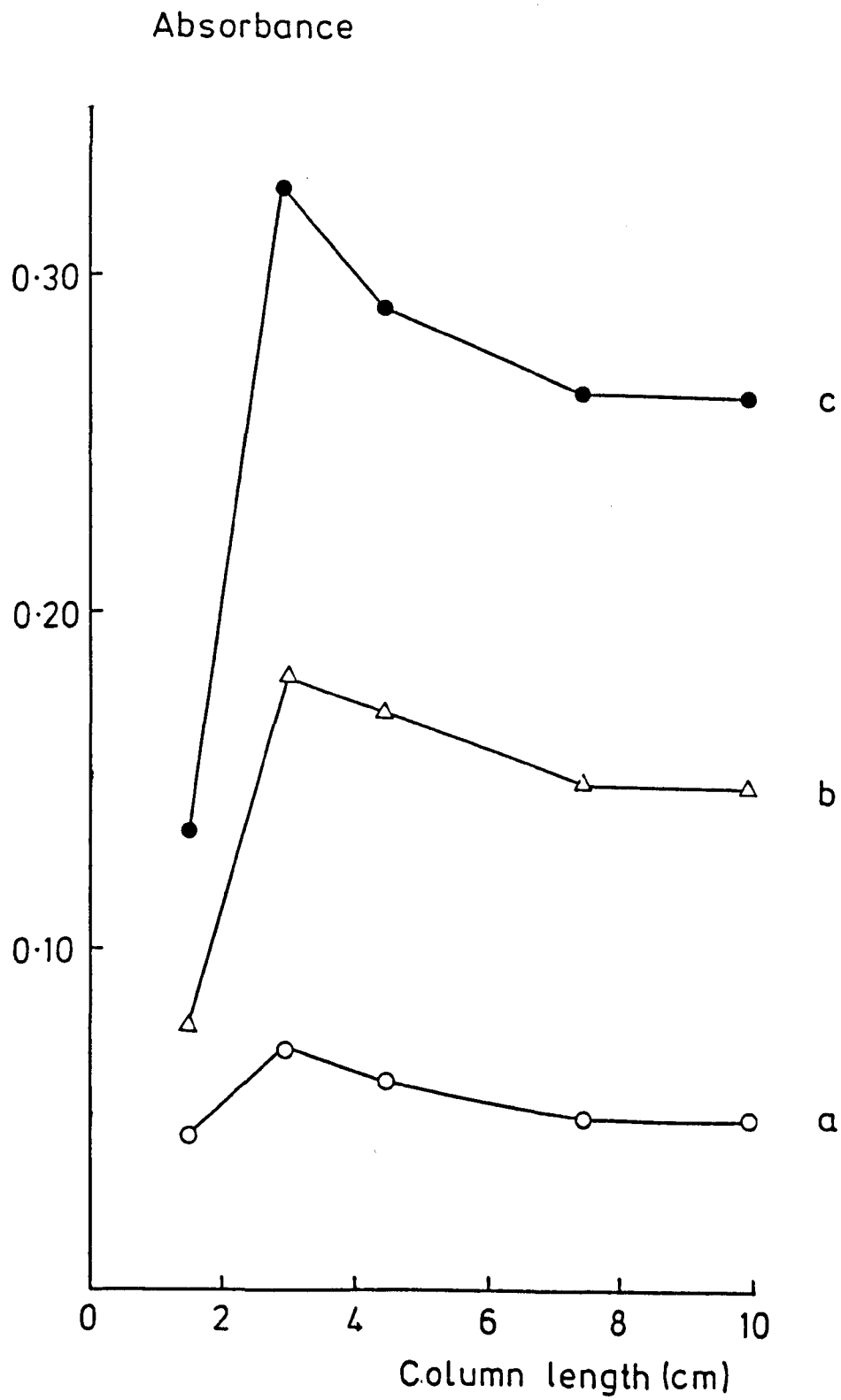


Fig. 7.8. Effect of the length of exchanger column. $[SO_4^{2-}]$ are: (a) 4×10^{-5}M, (b) 9×10^{-5}M, (c) 15×10^{-5}M. pH 1.8, flow rate 4ml min^{-1} (total).

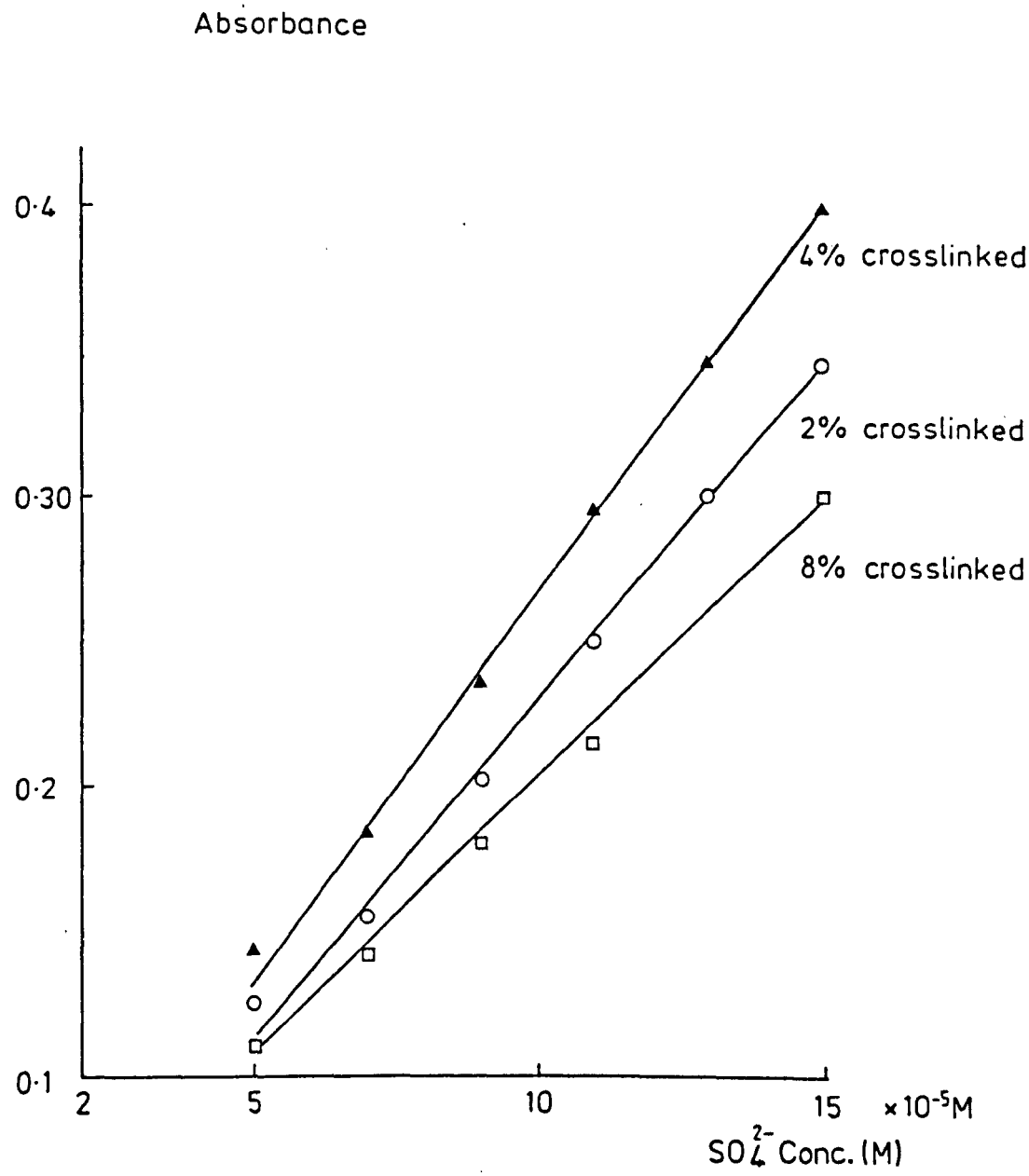


Fig. 7.9. Effect of degree of crosslinking.

r.s.d.% of these measurements was 1%. The sampling rate of 120 h^{-1} when a flow rate of 2ml min^{-1} was used through the exchanger column (4ml min^{-1} total).

TABLE 7.1.

CALIBRATION RESULTS FOR SULPHATE

SO_4^{2-} Conc. (M)	Mean Absorbance	R.S.D. % (3 replicates)
$1x10^{-5}$	0.020	2.2
$4x10^{-5}$	0.078	2.1
$8x10^{-5}$	0.168	1.4
$12x10^{-5}$	0.260	0.6
$16x10^{-5}$	0.364	0.7
$20x10^{-5}$	0.468	0.2
$24x10^{-5}$	0.567	0.6

Average r.s.d. 1.1%

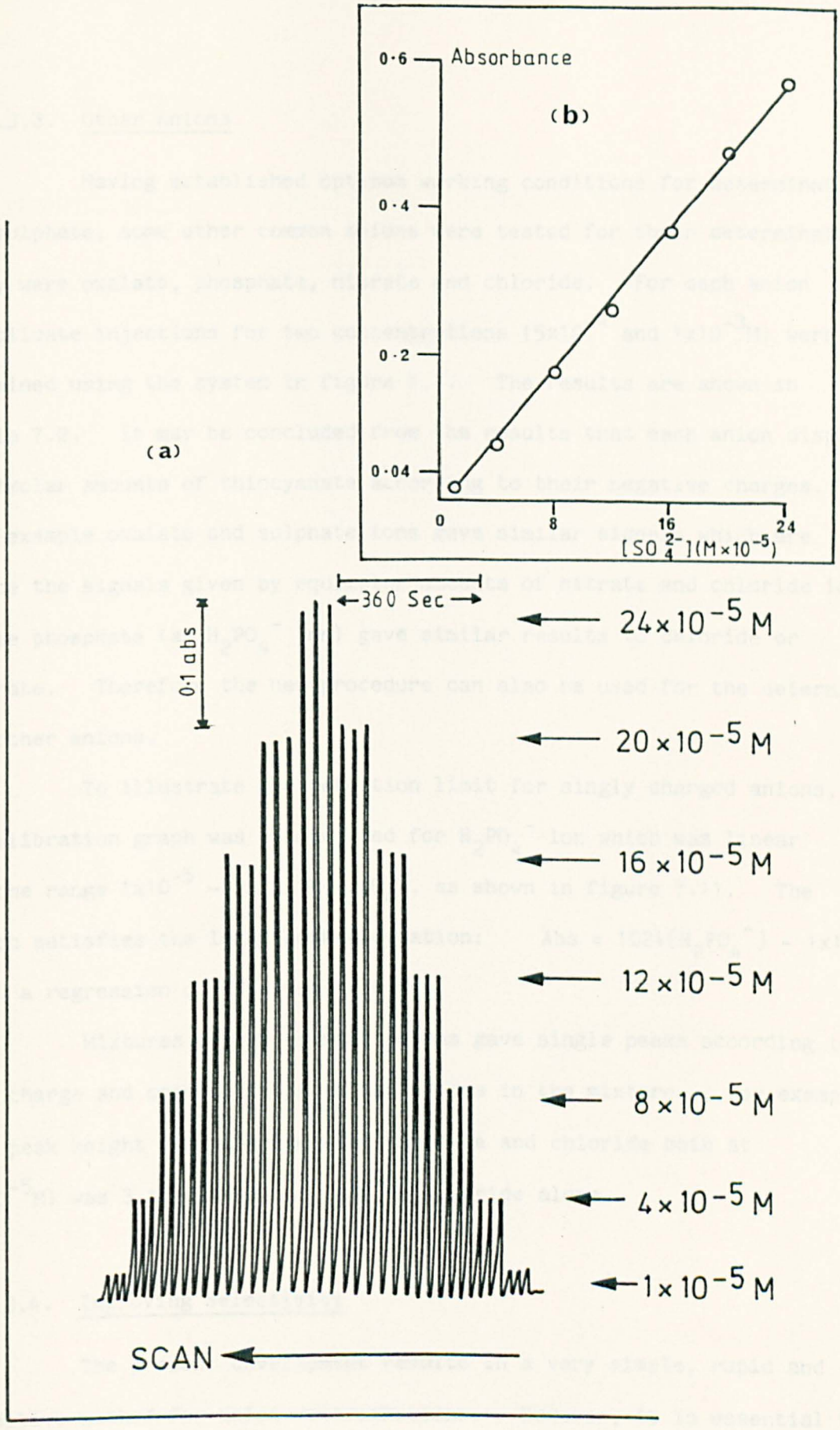


Fig. 7.10 (a) Peaks obtained by injecting triplicate sulphate standards of the concentrations shown; (b) the corresponding calibration graph.

7.2.3.3. Other Anions

Having established optimum working conditions for determination of sulphate, some other common anions were tested for their determination. They were oxalate, phosphate, nitrate and chloride. For each anion triplicate injections for two concentrations ($5x10^{-4}$ and $1x10^{-3}$M) were examined using the system in figure 7.1. The results are shown in table 7.2. It may be concluded from the results that each anion displaces equimolar amounts of thiocyanate according to their negative charges. For example oxalate and sulphate ions gave similar signals which are twice the signals given by equimolar amounts of nitrate and chloride ions, while phosphate (as $H_2PO_4^-$ ion) gave similar results to chloride or nitrate. Therefore the new procedure can also be used for the determination of other anions.

To illustrate the detection limit for singly charged anions, a calibration graph was constructed for $H_2PO_4^-$ ion which was linear in the range $1x10^{-5}$ - $3x10^{-4}$M $H_2PO_4^-$, as shown in figure 7.11. The graph satisfies the least squares equation: $Abs = 1024[H_2PO_4^-] - 1x10^{-3}$ with a regression coefficient of 0.998.

Mixtures of two or more anions gave single peaks according to the charge and concentration of the anions in the mixture. For example the peak height from a mixture of sulphate and chloride both at $1x10^{-5}$M) was 3 times that of $1x10^{-5}$M chloride alone.

7.2.3.4. Improving Selectivity

The present development results in a very simple, rapid and sensitive method for anion determinations. However, it is essential to improve its selectivity so that one or two anions can be determined in the presence of others. Several attempts have been made to do this as is indicated in the following sections.

TABLE 7.2

RESULTS OBTAINED FOR THE EXCHANGE OF THIOCYANATE BY SOME COMMON ANIONS

Anion	Absorbance	
	Anion Concentration (M)	
	5×10^{-5}	1×10^{-4}
$C_2O_4^{2-}$	0.15	0.3
SO_4^{2-}	0.14	0.29
$H_2PO_4^-$	0.06	0.14
Cl^-	0.067	0.14
NO_3^-	0.065	0.15

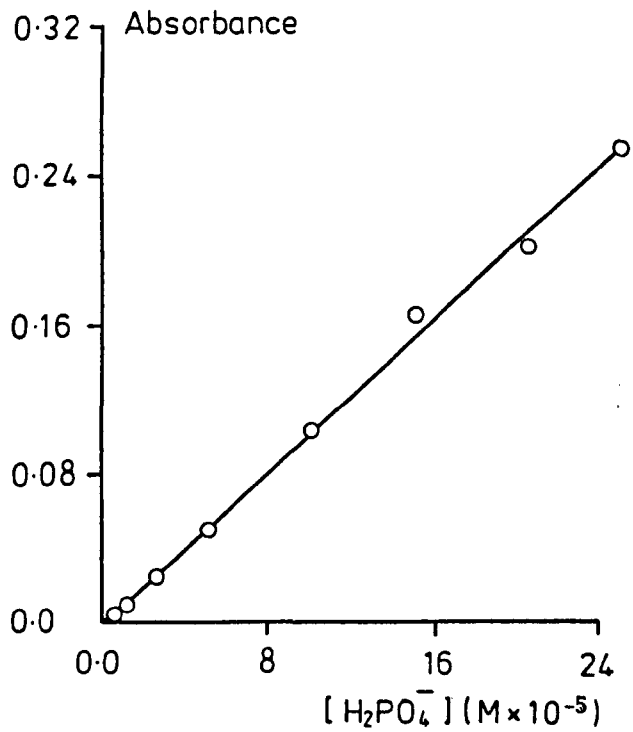


Fig. 7.11. Calibration graph for phosphate.

Using other exchanger columns

Strongly basic-anion exchange resin, Dowex 1 (Cl^--form, 4% crosslinked, 200-400 mesh) was converted into phosphate or iodide forms as described for thiocyanate (section 7.2.2.1) by shaking the resin particles with 2M potassium dihydrogen phosphate or 2M potassium iodide solution. Minicolumns, 3cm long, 2mm i.d., were made of each form as for the thiocyanate form.

Two flow injection manifolds depended on the detection method. For phosphate its spectrophotometric detection was based on molybdophosphoric acid formation and its conversion to molybdenum blue (336); for iodide by complexation with palladium chloride (330). The procedures and manifolds used are shown in figure 7.12. Both systems behaved similarly to the thiocyanate system in exchanging all other anions depending on their charges. Therefore no selectivity was found by replacing the thiocyanate column, but they could be used for the determination of anions.

Using suppressor columns

The idea of a suppressor column was applied to remove selected anion interferents by passing the anion sample through a small ion exchange column in a particular cationic form before exchanging with thiocyanate. For example a cation exchange column in the lead form could be used to remove sulphate leaving nitrate unaffected. This approach was investigated by Ryabinin and Bogatyrev (337) in which lead sulphate was precipitated and retained on the column and nitrate eluted as $PbNO_3$. A cation exchange resin in the Ag^+ form has been widely used as a post-suppressor column in an ion-chromatographic manifold to depress anions (332, 338). A similar column has been used to remove anions such as sulphate, carbonate, halide, oxalate and phosphate (329), a cadmium-form column for removing sulphide, oxalate, carbonate and hydroxide from sulphate or phosphate, a barium-form column to remove

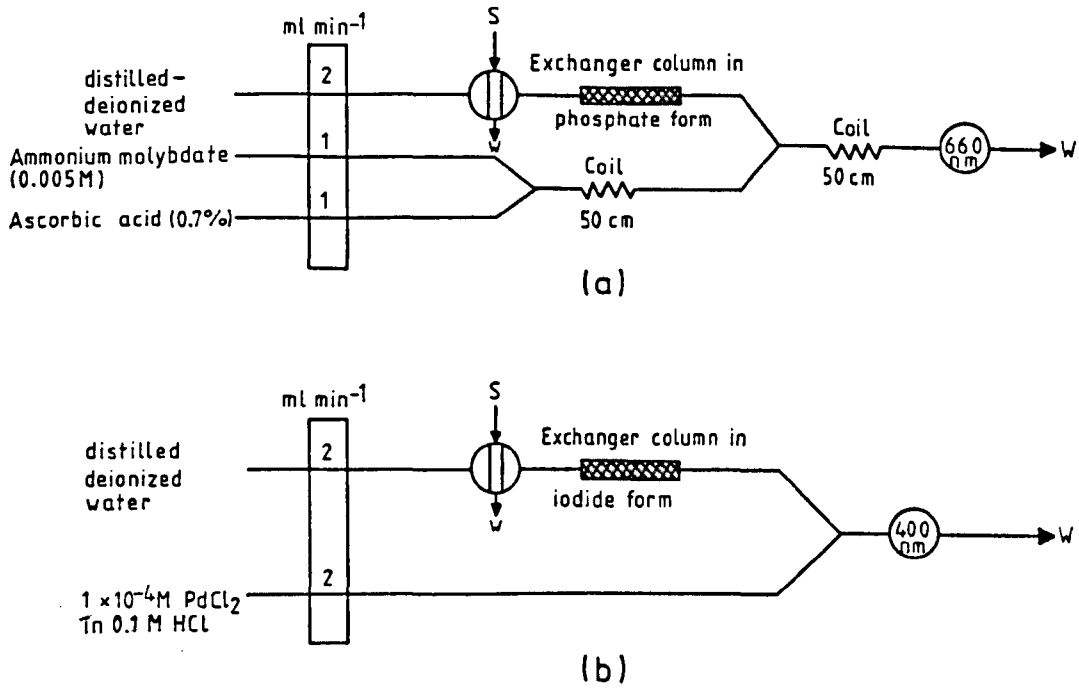


Fig. 7.12. Flow injection manifold for the determination of anions using (a) phosphate (b) iodide as exchanger. S, sample W, waste.

sulphate or a cadmium-form column to remove phosphate and oxalate. The ideas can easily be modified and applied to F.I.A.

In the present work two applications were studied to illustrate the performance of the new technique; they are the simultaneous determination of two anions (e.g. $Cl^-+NO_3^-$ and $SO_4^{2-}+NO_3^-$) and the determination of nitrate.

7.2.3.5. Simultaneous determination of chloride and nitrate

7.2.3.5.1. Optimizing conditions for the suppressor column

For this determination it is necessary to use a suppressor to remove chloride and allow the other ion to reach the exchanger column. A suppressor column (3cm long, 2mm i.d.) in the Ag^+ form was prepared as described in section 7.2.2.2. and incorporated after the sample injection port in the system in figure 7.1. It was found that when chloride or nitrate solutions were injected, no signals were obtained for either anion, indicating that both anions were suppressed by the column. This can be explained as follows. Chloride forms a precipitate with silver on the resin; nitrate must elute from the column, but in a form that does not displace free thiocyanate ions or perhaps the Ag binds SCN^- displaced by NO_3^-. Similar investigations indicated that when a mixture of sulphate and nitrate ions was passed through a cation-exchange column in the Pb^{2+} form (337), nitrate is eluted with Pb^{2+} ion also.

Accordingly, it was necessary to remove silver ions from the eluted nitrate solution. This was achieved by trapping the Ag^+ ion by small Jones reductor column (Chapter Five). Under these conditions the nitrate displaced the SCN^- from the exchanger column. The effectiveness of this method of removing Ag^+ was proved by Siemer (339) who used amalgamated zinc for quantitative trapping of Ag^+ coeluted with the halide in an ammonical solution from the silver-loaded resin used for separation of halides.

Therefore, in addition to the post-suppressor column in the Ag^+ form for removing Cl^-, an additional column is required to trap the displaced Ag^+ from this column. Amalgamated zinc is recommended as it has little effect on dispersion (Chapter 5) and its regeneration by passing a solution of 0.25M $Hg(NO_3)_2$ until the reduced Ag^+ is removed,

is very simple.

In order to accomplish both operations (removing Cl^- and trapping displaced Ag^+) it was decided to prepare a combined column (4cm long, 2mm i.d.) from two Perspex columns (see figure 7.2), each 2cm long, 2mm i.d., screwed together firmly so that to form a compact continuous unit, half of which was packed with Ag^+-form resin to remove chloride and other half with amalgamated zinc to remove displaced Ag^+. Figure 7.13 shows the combined suppressor minicolumn.

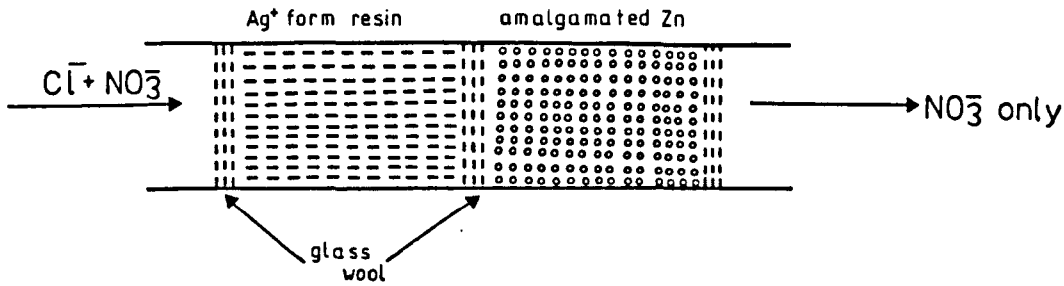


Fig. 7.13. Combined suppressor used for simultaneous determination of Cl^- and NO_3^-.

7.2.3.5.2. Effect of flow rate on the operation of the suppressor column

The effect of flow rate on the efficiency of the combined suppressor column was investigated by modifying the flow injection system in figure 7.1 by incorporating the suppressor between the injection port and the exchanger column and by injecting a chloride+nitrate solution, and using different flow rates. The results are shown in figure 7.14. Generally, at slow flow rates the peak area increased on increasing the flow rate and then dropped gradually at high flow rate (>3.5ml min^{-1}) due to the contribution of chemical factors, most probably incomplete trapping of Ag^+ which inhibited some NO_3^- reactions. This led to a change in the height and width of the peaks obtained. Increasing the flow rate

caused a marked decrease in the absorbance which illustrates two important phenomena. First the removal of chloride by the Ag^+ form resin seems to be effective even at higher flow rates otherwise higher signals would be expected, which was not the case. Therefore the function of the suppressor column seems to be limited by the efficiency of the Jones reductor (the second part of the suppressor). The inefficiency of the reduction properties of Jones reductor at high flow rates is similar to that found in the previous investigations of the reduction of iron(III) (chapter 5). Therefore, a flow rate of 1ml min^{-1} is recommended.

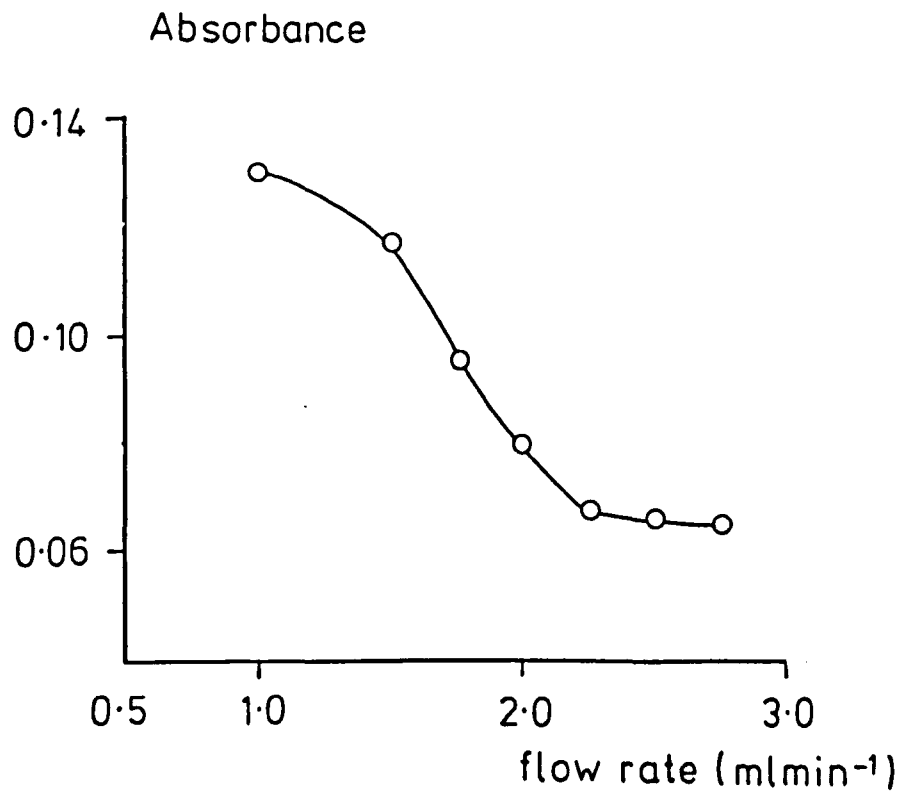


Fig. 7.14. Effect of flow rate on the efficiency of the combined suppressor minicolumn.

6.2.3.5.3. Procedure and manifold design

The simultaneous determination of chloride and nitrate can be achieved by splitting the injected sample into two streams. The manifold

is shown in figure 7.15. One stream passes through the suppressor column and then through the exchanger column. This produces the first peak which is a measure of the concentration of nitrate only. The other portion flows through a 200cm delay coil (0.5mm i.d.), and then rejoins the stream before the SCN^--form exchanger to give the second peak, completely resolved from the NO_3^- peak. This peak is a measure of chloride+nitrate. Because the combined suppressor requires a slow flow rate (1ml min^{-1}), splitting was achieved by situating the pump after the splitter as mentioned in chapter five. The splitting was achieved by means of a Perspex Y-piece (angle between the two outflow lines 90^o, i.d. 0.7mm) and in this case 1:1 splitting, to give peaks of equal heights, was achieved by using one pump tube of 0.8mm i.d. connected to the suppressor, and one of 0.5mm i.d. for the other channel, as shown by splitting a standard nitrate solution (figure 7.16). Pulse suppressors (0.508mm i.d., Sterilin) were connected between the splitter and pump tubes to eliminate any effect of pulsing on the splitting ratio.

7.2.3.5.4. Calibration

Having optimized the necessary condition for the combined suppressor operation and applying the optimum conditions investigated for the determination of anions with the manifold in figure 7.15, the construction of a calibration graph for simultaneous determination of Cl^- and NO_3^- was achieved. In these calibrations the proportions of chloride and nitrate were varied. Typical calibration results for 40µl injections of Cl^-+NO_3^- standards are shown in figure 7.16. The first peak is a measure of NO_3^-, the second of Cl^-+NO_3^-. The sample throughput was 90 h^{-1}. The resulting calibration graphs were linear for nitrate and chloride with regression coefficients of 0.999 and 0.997, respectively. The least squares equations are Abs = $521[NO_3^-] + 7.5\times10^{-3}$ and $870[Cl^-] - 2.3\times10^{-2}$.

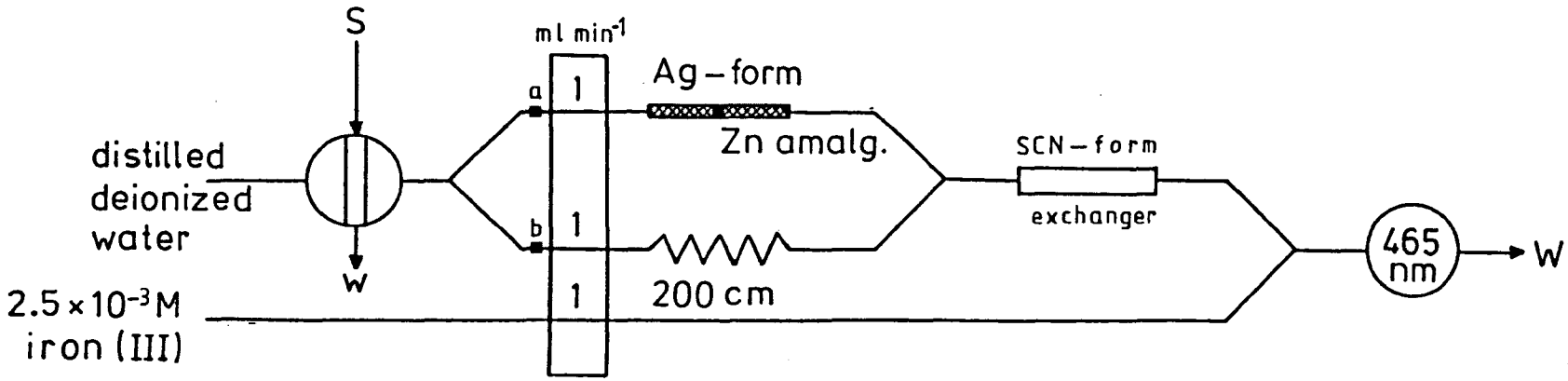


Fig. 7.15. Manifold used for simultaneous spectrophotometric determination of Chloride and Nitrate. S, sample, a & b are pulse suppressors, W, waste.

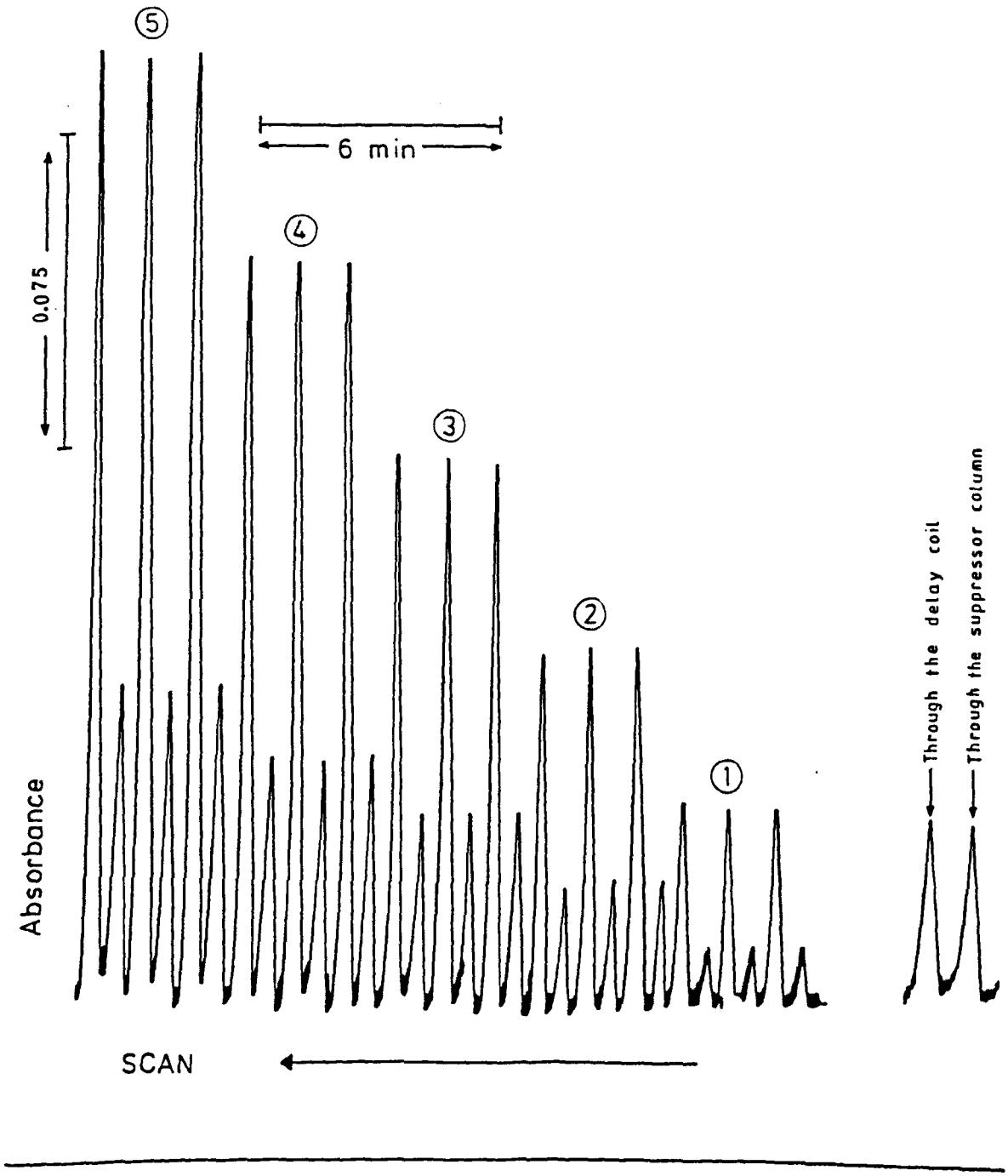


Fig. 7.16. Typical calibration responses for simultaneous determination of chloride and nitrate at the following concentrations: (1) 5×10^{-5}M, 2×10^{-5}M; (2) 8×10^{-5}M, 5×10^{-5}M; (3) 11×10^{-5}M, 8×10^{-5}M; (4) 14×10^{-5}M, 11×10^{-5}M; (5) 17×10^{-5}M, 14×10^{-5}M.

7.2.3.6. Simultaneous determination of sulphate and nitrate

The flow injection system shown in figure 7.15 was also operated to determine sulphate and nitrate. Table 7.3 shows the results for a typical range of sulphate and nitrate concentrations analysed, and the calibration graphs obtained are shown in figures 7.17 and 7.18. The regression coefficients were 0.9989 and 0.980 for nitrate and sulphate, respectively. The least square equations for the nitrate and sulphate calibrations were Abs = $540[NO_3^-] - 1.3 \times 10^{-2}$ and Abs = $855\ [SO_4^{2-}] - 3.2 \times 10^{-2}$, respectively. Sampling throughput was 90 h^{-1} with a midrange r.s.d. for 5 analyses of 1.5% for NO_3^-.

TABLE 7.3

CALIBRATION RESULTS FOR SIMULTANEOUS DETERMINATION OF SULPHATE AND NITRATE

Sample No.	SO_4^{2-} Conc. (M)	NO_3^- Conc (M)	Abs. for NO_3^-	Abs. for $SO_4^{2-}+NO_3^-$
1	2×10^{-5}	5×10^{-5}	0.03	0.06
2	4×10^{-5}	8×10^{-5}	0.05	0.09
3	6×10^{-5}	11×10^{-5}	0.07	0.12
4	8×10^{-5}	14×10^{-5}	0.095	0.15
5	10×10^{-5}	17×10^{-5}	0.12	0.195

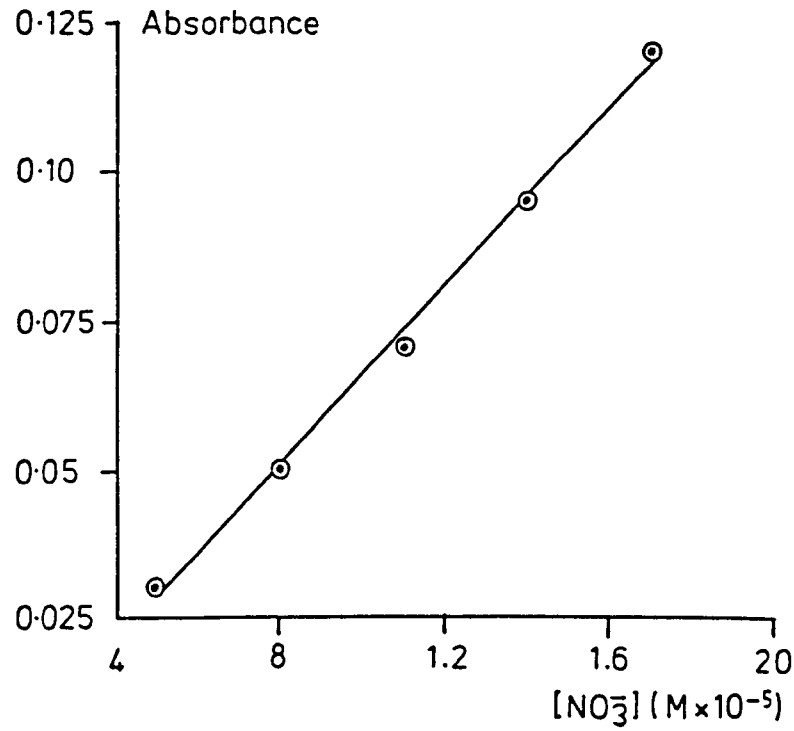


Fig. 7.17. Calibration graph for nitrate.

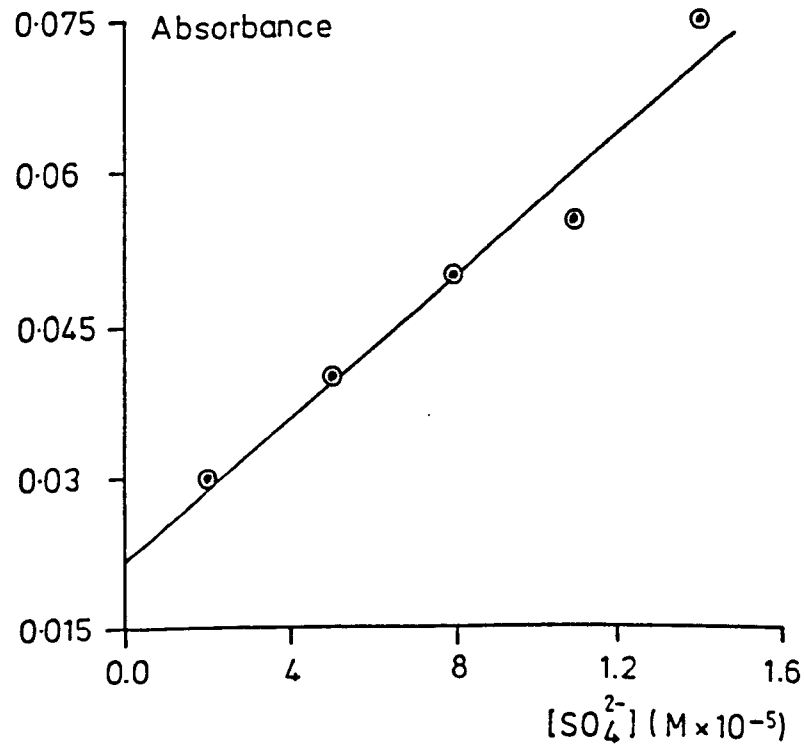


Fig. 7.18. Calibration graph for sulphate.

7.2.3.7. Removing interfering effects of carbonate and chloride (or sulphate) in the determination of nitrate

This is another application of the new method for anion determination using a suitable small combined suppressor for removing interferences. It is based on eleminating the effects of chloride (or sulphate) and carbonate during the determination of nitrate.

Ion-exchange has already been used to separate nitrate from chloride, hydrogen carbonate and organic anions (338). Two cation-exchange resins were used. The first, in silver form, removed chloride and the second, in hydrogen form, removes the silver displaced from the first. Displacement of some Ag^+ may be due to the other cationic species or to the affinity of Ag^+ for the anionic species in preference to the resin base. Solutions leaving the second column are acidic thus eliminating the hydrogen carbonate present. This idea is used in devising a flow injection manifold for the determination of nitrate.

7.2.3.7.1. The design of the suppressor column

Figure 7.19 shows the suppressor columns used during the optimization of conditions of operating the columns to eliminate chloride, sulphate and carbonate. The flow injection system shown in figure 7.1 was used by introducing the suppressor into the manifold between the injection valve and the exchanger column (in SCN^- form).

Procedure and Discussion

Three different mixtures were prepared containing different proportions of Cl^-, CO_3^{2-} and NO_3^-, as shown in table 7.4. Using suppressor (a), 40µl of any of solutions no. 1 which contained only carbonate was injected into the system. No signals were obtained. This indicates that the suppressor eliminates completely the effect of carbonate. With the same suppressor, when 40µl of 2×10^{-4}M NO_3^- was injected, a

measurable signal was obtained [Fig. 7.20(A)]. This was confirmed when comparable signals were obtained by injecting the four samples of mixture no. 2 (table 7.4) and gave evidence that suppressor type (a) successfully removed carbonate interference as shown in figure 7.20(B).

When 40µl of the four solutions of mixture 3 ($Cl^-+NO_3^-+CO_3^{2-}$) were injected into the system containing suppressor column (b), the peaks obtained were smaller than those obtained from a mixture of CO_3^{2-} and NO_3^- [figure 7.20(C)]. This is mainly attributed to the presence of displaced Ag^+ in the final eluent produced during chloride retention in the first column and inefficiency of the H^+-form part of the resin towards retaining the eluted Ag^+. Therefore the presence of some Ag^+ depressed the NO_3^- signal (as discussed in section 7.2.3.5.1). This problem was solved by connecting a third part to the column with suppressor (b) 1.5cm long, 2mm i.d., filled with amalgamated zinc to trap any Ag^+ ion in the final stage. This is suppressor form (c) in figure 7.19, and was found to be satisfactory to remove the interfering effects of chloride and carbonate from nitrate [figure 7.20(D)].

7.2.3.8. Application of the suppressed F.I.A. system for determination of nitrate in tap water

The low solubility product of most anion silver salts gave an important opportunity for the determination of nitrate in tap water. It was assumed that the presence of the combined suppressor columns (7.19C) is capable of removing most of the anions present in tap water. This column was therefore incorporated in the flow injection system in figure 7.1 and used for this purpose.

During preliminary investigations deducted from injecting tap water directly into the system a signal ratio of about 7:1 was achieved in the absence and presence of the suppressor column. This ratio

TABLE 7.4

VARIOUS MIXTURES USED TO OPTIMIZE CONDITIONS FOR SUPPRESSORS USED IN THE DETERMINATION OF NITRATE

Solution No.		Carbonate Conc.(M)	Nitrate Conc.(M)	Chloride Conc. (M)
(1)	a	$2x10^{-4}$	0	0
	b	$4x10^{-4}$	0	0
	c	$6x10^{-4}$	0	0
(2)	a	$2x10^{-4}$	$2x10^{-4}$	0
	b	$4x10^{-4}$	$2x10^{-4}$	0
	c	$6x10^{-4}$	$2x10^{-4}$	0
	d	$8x10^{-4}$	$2x10^{-4}$	0
(3)	a	$2x10^{-4}$	$2x10^{-4}$	$2x10^{-4}$
	b	$4x10^{-4}$	$2x10^{-4}$	$4x10^{-4}$
	c	$6x10^{-4}$	$2x10^{-4}$	$6x10^{-4}$
	d	$8x10^{-4}$	$2x10^{-4}$	$8x10^{-4}$

indicates the capability of the suppressor column in removing most of the anions in water.

A standard addition method was used for nitrate determination. Figure 7.20 shows a calibration graph for the standard addition method applied for this purpose. The amount of nitrate found was 23.7μg ml^{-1} NO_3^- which indicates a high level nitrate in that particular storage tank water. The r.s.d. in these determinations for 3 replicate samples

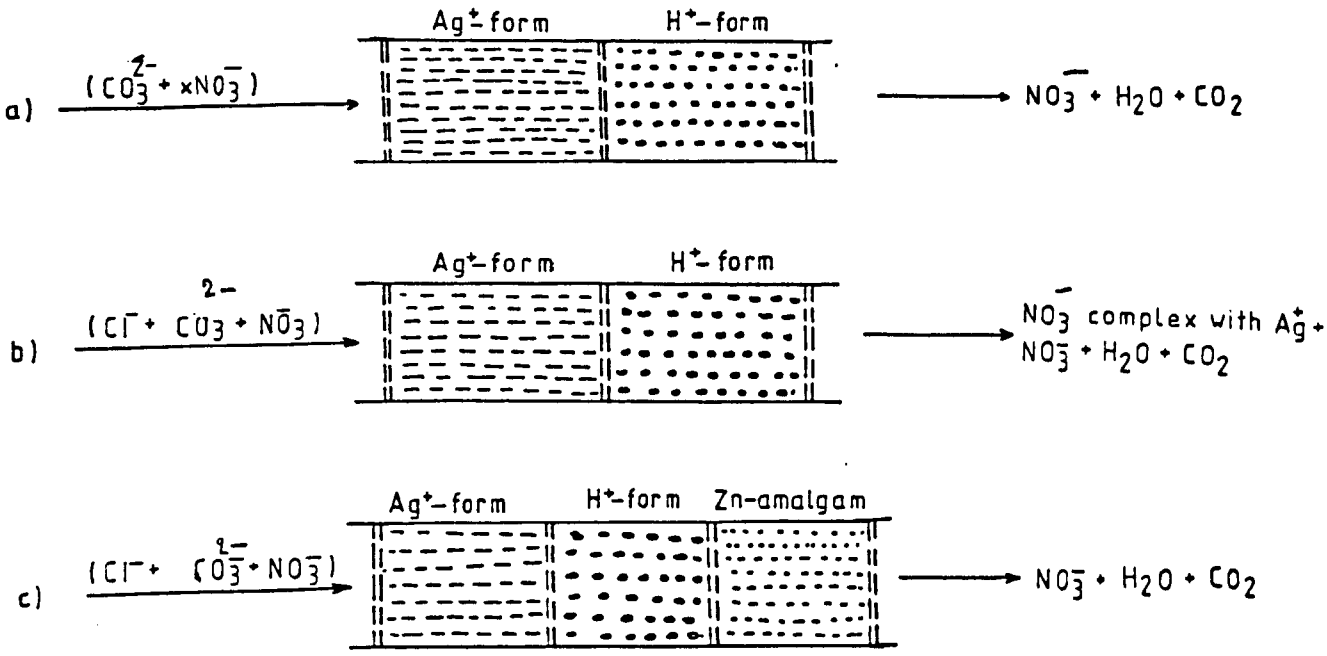


Fig. 7.19. Arrangements of suppressor columns used for eliminating interfering effects of chloride and carbonate in nitrate determination.

is 1.3%. The same water was also analysed for nitrate by the method of Hoather and Raikam (340). The procedure was particularly for high level nitrate samples, with absorbance measurement at 210nm. This measurement was simply accomplished by measuring the absorbance of the same solutions analysed by the F.I.A. method at 210nm using a LKB Ultrospec 4050 spectrophotometer. The amount of nitrate found by measuring one sample was 22.5μg ml^{-1}.

Therefore, the idea of suppressed F.I.A. method developed here is suitable for the determination of high levels of nitrate in samples by eliminating the interferences of other anions in lower concentration level than nitrate.

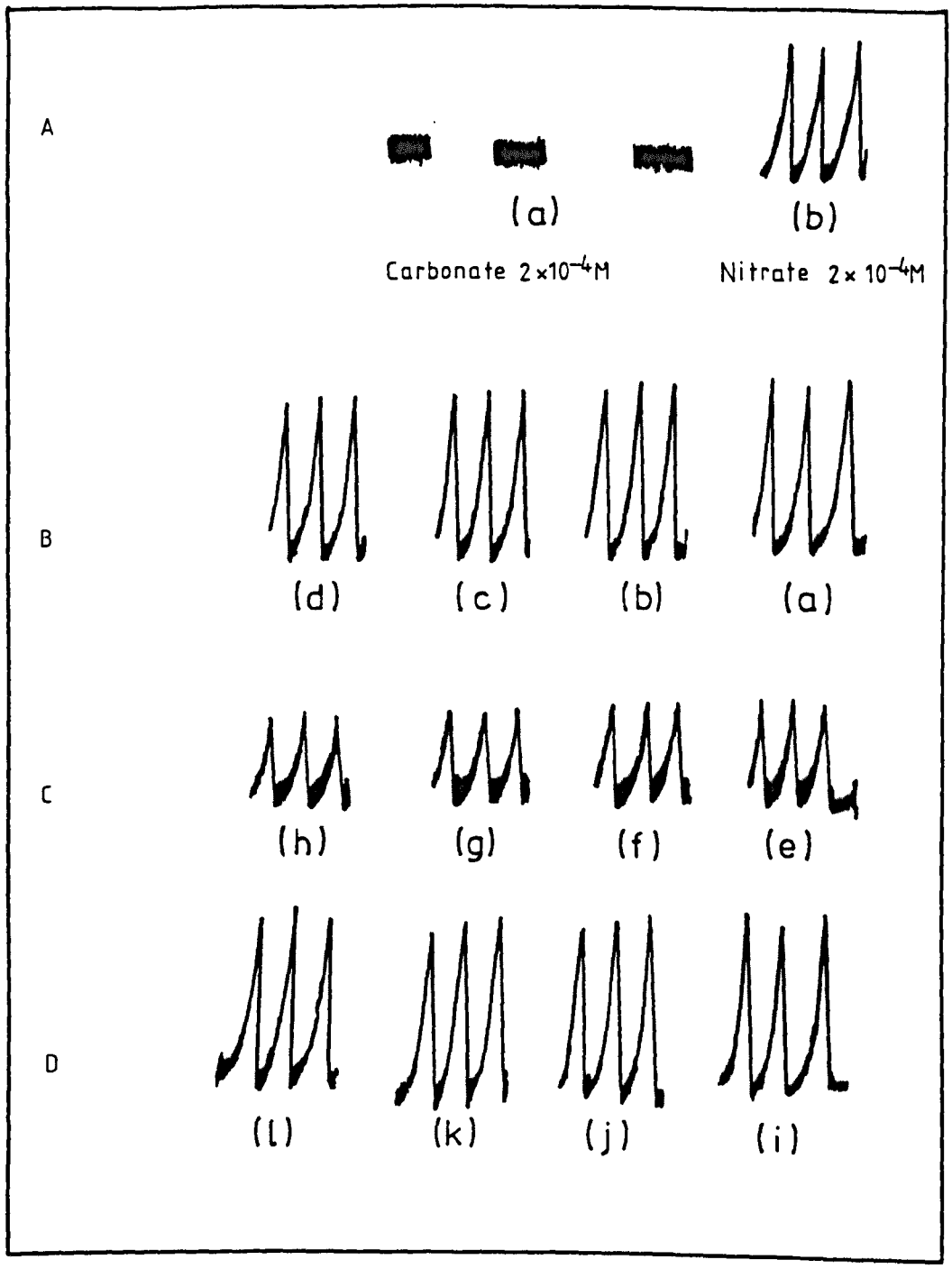


Figure 7.20. Peaks obtained for interpreting the efficiencies of different forms of suppressor columns. a, b, c, d are signals obtained from injecting the corresponding solutions (No. 2) in table 7.4. e, f, g, h and i, j, k, l are signals deduced from injecting the corresponding solutions (No. 3) in table 7.4.

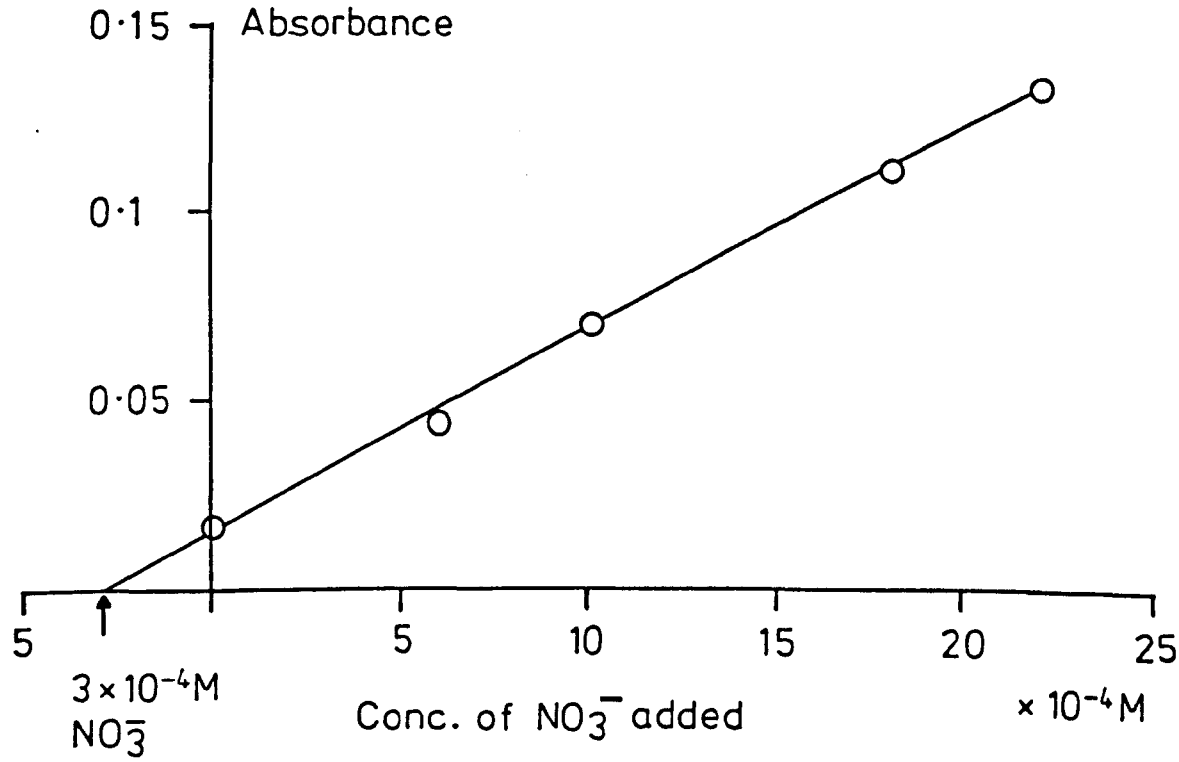


Fig. 7.21. Standard addition result for the determination of nitrate using suppressed F.I.A. technique.

CHAPTER EIGHT

AMPLIFICATION BY FLOW INJECTION ANALYSIS

An amplification reaction is a means of improving the sensitivity of an analytical procedure. A comprehensive survey has been made by Belcher (341). A well-known example is the amplification of iodide by means of periodate oxidation (342) which is a direct amplification process. Indirect amplification reactions are most widely used, which are based on amplifying and measuring a particular species to determine another stoichiometrically related species (343).

Most amplification reactions reported involve precipitation or removal of the excess of added reagents by extraction or heating for a long period and therefore are difficult to modify for use in F.I.A. In the present work, however, initial steps are made to demonstrate the possibility of using amplification in F.I.A. It is based on the procedure of Weisz and Fritsche (344) for amplifying Na^+ using two ion-exchange columns. It involves the passage of a sample solution containing Na^+ through a H^+-form column to release an equivalent amount of H^+ which then passes through a Na^+-form column, and the released Na^+ is passed back through the first column. With each cycle, a definite amount of Na^+ ions is added to the first column. After a definite number of cycles the Na^+ ions are eluted with HCl and determined.

8.1. Reagents

Deionized water (from an Elgastat Spectrum) was used in all preparations and all chemicals were of analytical grade reagents.

Na^+ solution: A 200μg ml^{-1} stock solution was prepared by dissolving 0.395g of NaCl (B.D.H.) in 250ml of water. Other solutions were prepared by appropriate dilution with water.

Hydrochloric acid solutions used as eluents were prepared by diluting the concentrated solution (10.18M, B.D.H.) with water.

8.2. Apparatus

The strongly acidic cation exchange resin (Dowex 50W X8, 50-100 mesh) in H^+ and Na^+ forms was used. A minicolumn (4cm long, 2mm i.d.) for each resin-form was prepared as described in the previous chapters. The injection valve and peristaltic pump were as described in preceding chapters. The eluted sodium was detected using a Petracourt PFP1 flame photometer connected to a Tekman Labwriter TE200 recorder.

8.3. Flow manifold

The manifold design is the most important part of the development because the exchanging of ions, recycling processes, elution and regeneration of the resins all contribute to the success of the amplification. The detector is of secondary importance; it depends on the type of species analysed. After extensive investigations, the flow injection manifold shown in figure 8.1 was devised. The procedure involved the following steps:

1. Washing the manifold

Water (or washing solution) was cycled through the manifold and detector. To achieve this key-valve (a) was turned so that the HCl flow is stopped and only water flowed through the H^+-form resin, the Na^+-form resin (using key-valve C), the injection valve and by means of switching valve (b), to waste (W_2). Meanwhile the calibrating solution continuously flowed through the detector by means of switching valve (d).

2. Amplification

Switching valve (b) was turned so that the cycle line was closed and the washing solution was directed to waste (W_1). The amplification was started by injecting the sample (Na^+ solution) at a precise time

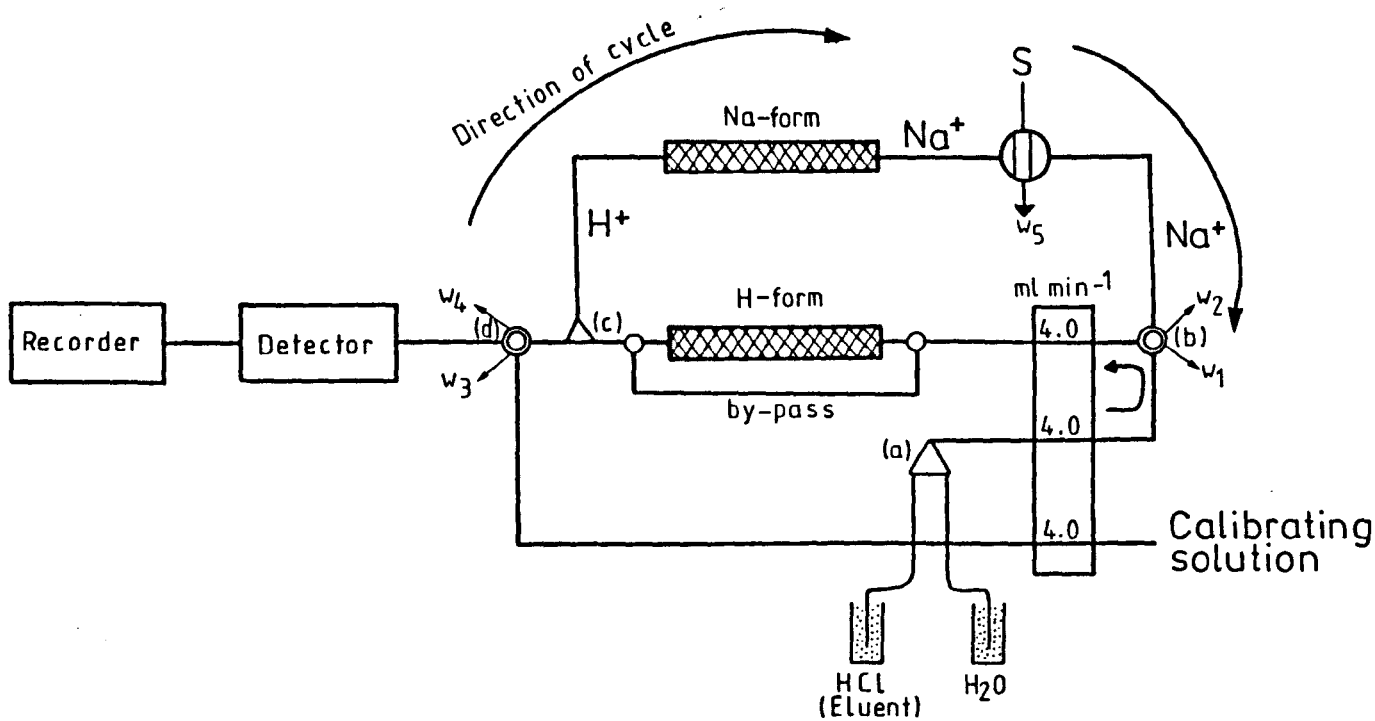


Fig. 8.1. Flow injection system for monitoring amplification reaction. (a), (c) are 3-way valve-single keys, (b) and (d) are switching valves to interchange two streams, S. sample W. waste.

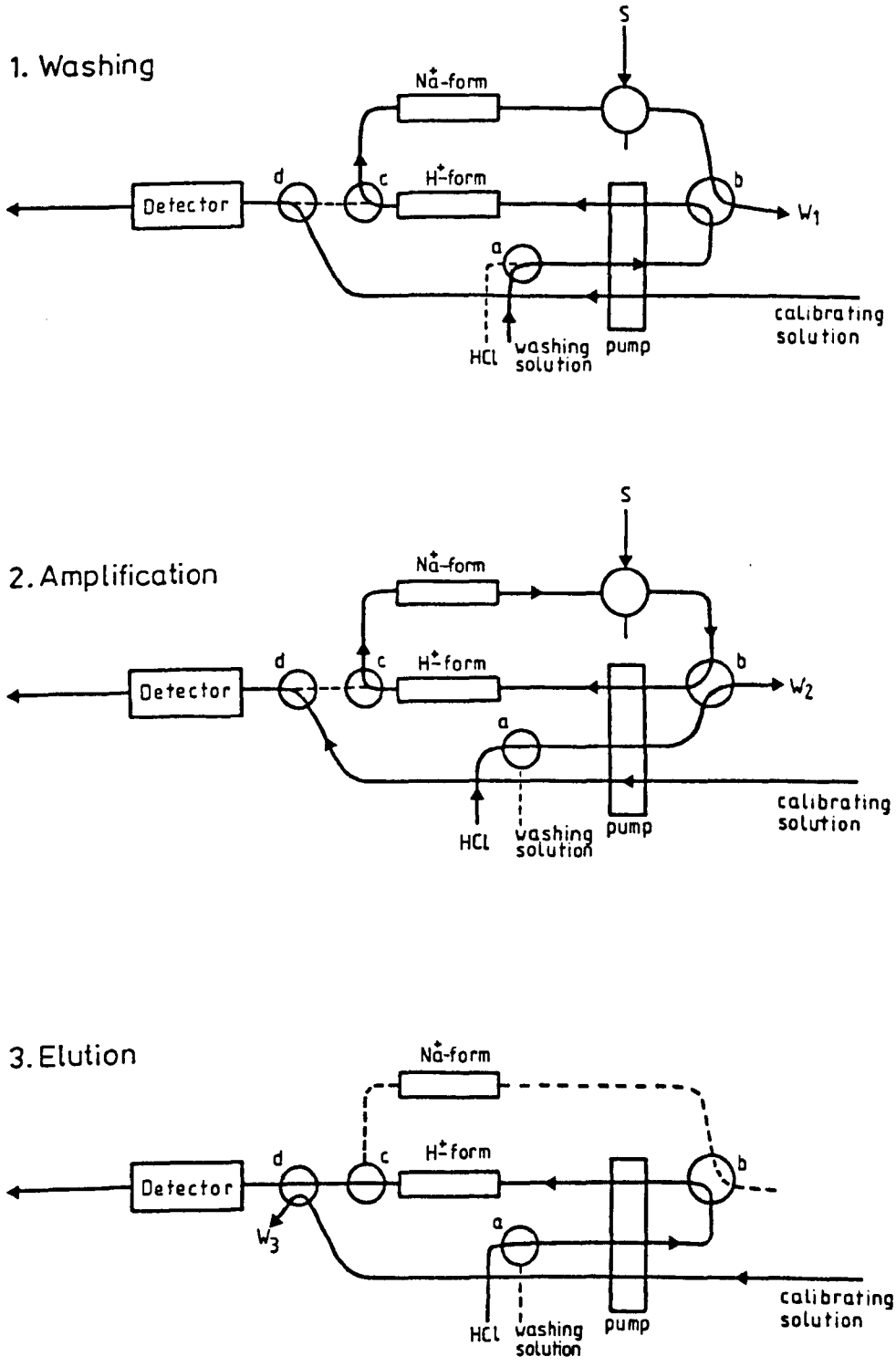


Fig. 8.2. Main operations during amplification monitoring by F.I.A.

Dotted lines illustrate interruption of the flow in that position.

(Upon injecting a dye solution [0.01% methylene blue], it was found that the time required for the dye to pass through both columns, return to the injection valve and enter the line of H^+-form resin column after key-valve (b) is about 30 sec which could be regarded as one cycle). This can be applied for amplifying the Na^+ sample as follows. During that time (30 sec) the injected Na^+ displaced H^+, and H^+ displaced Na^+ which passes the injection port and point (b) to accumulate at the H^+-form resin. Accordingly the injected Na^+ was doubled so this was one cycle of amplification. Each cycle adds a similar amount of Na^+ to the column.

While the amplification was in progress it was necessary to move key-valve (a) so that the eluent HCl filled the tubing between valves (a) and (b), and was ready for elution.

3. Elution

The elution of the amplified species was achieved rapidly by instantly turning valves b, c and d to their alternative positions. This allowed the HCl eluent to flush the Na^+ deposited in the H^+-form resin to the detector until the signal was complete. The operation of valves b, c, d during elution had the following effects. By turning valves (b) and (c) the line which contained the Na-form resin and the injection valve was completely disconnected and the HCl flow was diverted from W_1 to the detector. The movement of valve (d) directed the calibrating solution from the detector to waste (W_3). The eluent was allowed to flow for a limited time after which it was replaced by water (using key-valve (a)) in order to remove all the eluent between b and c before starting a new amplification process.

The repositioning of the manifold to the washing state was achieved by turning valves (b), (c) and (d) to their initial positions. Steps 1, 2 and 3 were repeated for each amplification process. In the present work a 15 sec washing cycle, 30 sec amplification cycle and about 20 sec elution was required for one analysis.

4. Regeneration of the ion-exchange columns

The H^+-form resin column is automatically regenerated after each elution process by passing HCl. The Na^+-form resin, most of which is converted to the H^+-form resin during the amplification process, could simply be regenerated when the system was in its first position (step 1 washing-state). By using the bypass (turning valves 1 and 2) of the H^+-form column it was possible to pass a solution of 1M NaOH in place of water through the Na^+-form resin for a desired length of time. Then the stream was changed to water again and the by-pass of the H^+-form column disconnected again so that the manifold was returned to its washing position again.

8.4. Optimization

Carrier stream for the amplification process

Before injecting a Na^+ sample noticeable signals were obtained when the elution process was repeated several times. This was attributed to the Na^+ present in the water used and therefore it was not satisfactory for use as a carrier stream during Na^+ amplification. When a series of ethanol/water mixtures were used, figure 8.3 shows how the background signals diminished as the water proportion in the carrier stream decreased. Therefore it was recommended for this particular study to use ethanol instead of water for the amplification process.

Effect of the HCl concentration in the eluent

Using ethanol as a carrier stream, the effect of hydrochloric acid concentration on the elution of 2.5μg ml^{-1} Na^+ injected was investigated. The results are shown in figure 8.4. The peak height increased on increasing the hydrochloric acid concentration up to 1M after which the effect appeared to be reaching a plateau. Therefore 1M HCl was recommended.

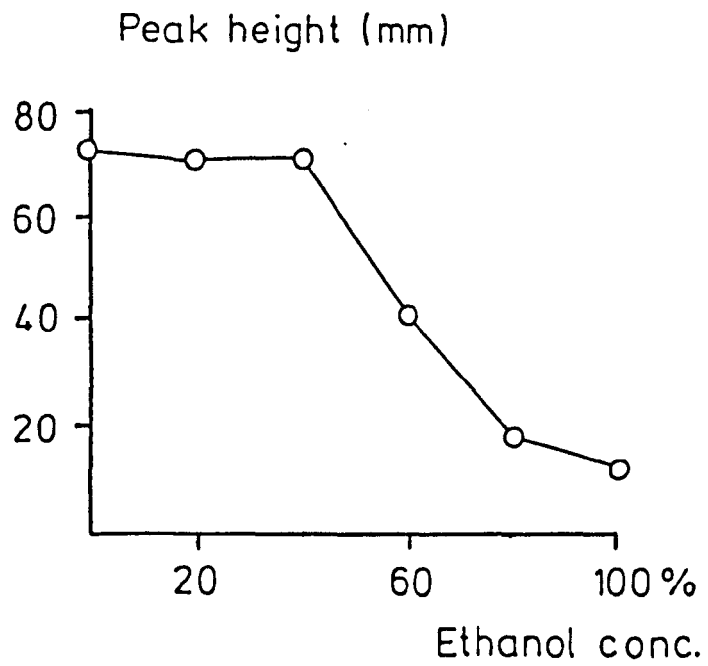


Fig. 8.3. Effect of the ethanol/water composition on the background signal.

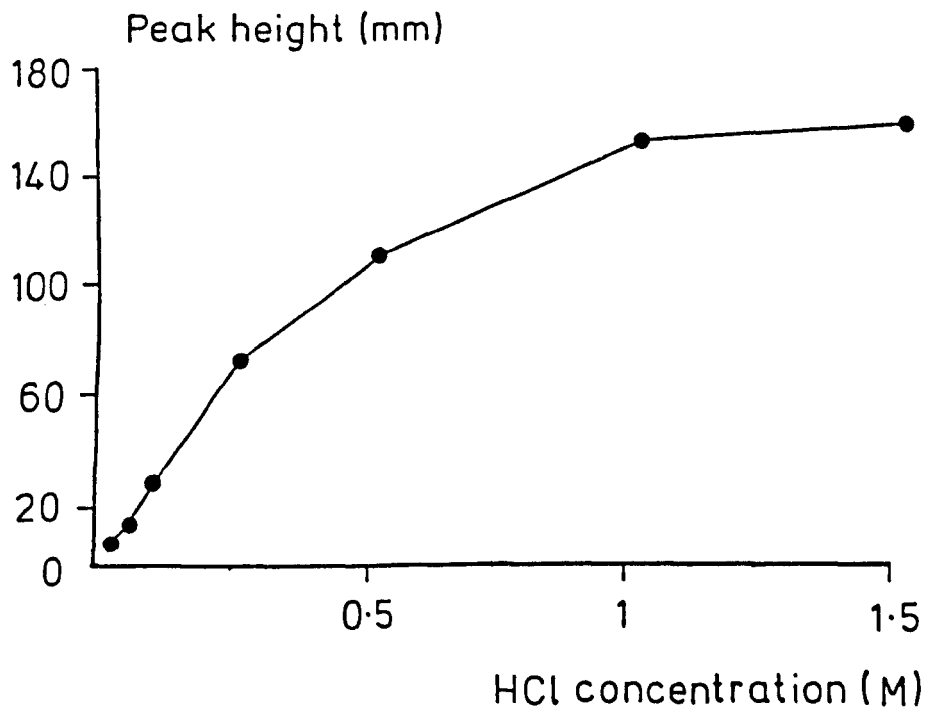


Fig. 8.4. Effect of hydrochloric acid concentration.

Effect of the duration of the amplification

The effect of cycling time on the sodium amplification was investigated. Figure 8.5 illustrates the effect for 1μg Na^+ ml^{-1}. The amplification time was directly proportional to the signal height (mm) in the range shown (30 sec - 150 sec). Longer periods caused a deviation of the graph, possibly because the large amount of sodium accumulated saturated the flame spectrometer response. In figure 8.5 the signals were found to be proportional to the number of cycles applied. i.e. two cycle amplification (60 sec) produced signals twice the one cycle amplification (30 sec) and the latter was approximately twice the expected response (dotted line) when no amplification takes place.

Mainly there were no significant changes in peak width as amplification increased.

Precision

The precision of the results was established for 1μg Na^+ ml^{-1} within a 30 sec amplification periods. The results of 10 successive replicates is shown in figure 8.6. Despite the fact the timing was controlled manually, good precision was obtained (r.s.d = 4.8%). The Na^+ column exhibited good capacity, so there was no need to regenerate it after each amplification.

8.5. Conclusion

Monitoring the amplification reaction by F.I.A. is a new development that enables amplification of species by using ion-exchange phenomena. It would be relatively simple to automate the system so as to achieve a predetermined number of cycles, and thus a given amplification factor.

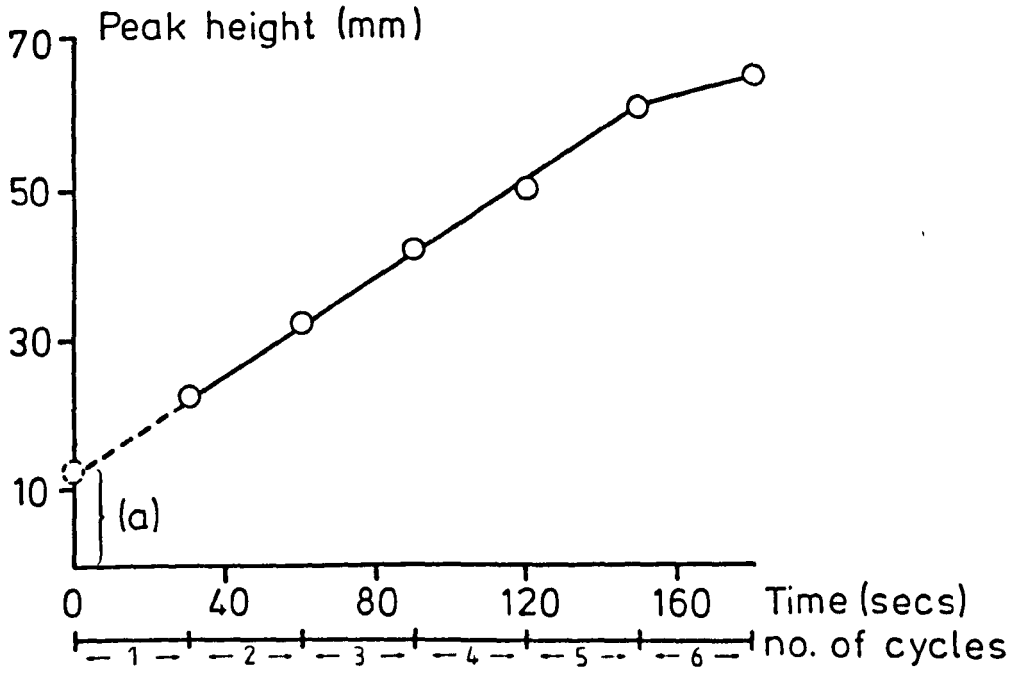


Fig. 8.5. Effect of amplification period (no. of cycles). (a) refers to the signal from injected sample without amplification.

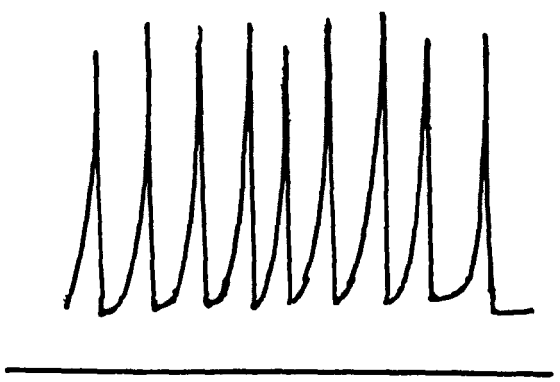


Fig. 8.6. Precision of the amplification process by flow injection analysis.

CHAPTER NINE

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

In the work presented in this thesis most recent developments in flow injection analysis technique are presented. Some of the work arose from initial suggestions by a predecessor whereas most of it is new, the inspiration of some of which is difficult to trace. The new developments established have opened remarkable opportunities for future studies and therefore the following suggestions are made.

1. F.I.A. for CL detection

The detector devised in this work proved to be feasible for continuous and rapid analysis by measuring chemiluminescence emission. However it is tempting to suggest further modifications of such a device in order to improve its analytical performance such as using a more sensitive PMT and constructing an assembly so that the T-piece and the flow coil can easily be removed for cleaning without exposing the PMT front window to stray light. If a particular design enables the T-piece and flow coil to be movable with respect to each other, it would be possible to study many effects of these two devices on the emission signals. The overall results will be higher sensitivity and smaller residence time, to obtain higher sampling frequency if desirable.

For hydrazine, three methods have been developed for its determination, via metal activation or ion-exchange purification or by the permanganate/polyphosphate system. Many areas of application are expected:
(i) A linear relationship between the emission intensity and metal ion concentration makes hydrazine chemiluminescence useful for the determination of different transition metals. The selectivity can undoubtedly be solved by using a suitable technique with the F.I.A. manifold such as an ion-exchange column for retaining and eluting one particular

metal ion before it is added to the purified hydrazine/NaOCl system.

(ii) In the present work the effects of common metals are investigated. It is possible to study other metals, and also it is worthwhile, generally, to obtain further information about the mechanism of the catalysed hydrazine chemiluminescent reaction and to identify the emitter species. This suggestion is also relevant to the reaction of hydrazine in the permanganate/polyphosphate system.

(iii) If a method of separating hydrazine from other species that give CL with the $KMnO_4$/polyphosphate system is developed, its combination with permanganate/polyphosphate system will be ideal for its determination which is much less subject to metal interferences than the NaOCl/metal ion system.

(iv) The combination of ion-exchange for separating and stepwise release of several metal ions followed by their CL detection is not yet reported by F.I.A. The type of detector developed here is ideal for that combination. Other applications such as the use of a reducing column for metal speciation with CL detection are of interest.

2. Reducing Columns in F.I.A.

The incorporation of a reducing minicolumn enables the same reagent and detector to be applied for a complicated analytical process such as metal speciation. Although determination of iron was exemplified in this work, other speciations could be performed such as for chromium and uranium using a suitable reductant.

The depressive effect of the silver reductor in chapter 6 is minimized by increasing the flow rate but, it will be important to study the effect of different particle sizes of the reductant. This, of course, requires new attempts for preparing silver of different particle sizes. A suggestion is to convert the silver particles into a pellet under high pressure and then grained it so that different particle sizes are obtained.

It is possible to prepare reducing or oxidizing columns by loading a suitable metal ion [Fe^{2+}, Ag^{+}, Tl(III)] on an ion-exchange resin. This idea might be more applicable for reducing or oxidizing organic compounds such as reducing organic compound containing nitro-groups to the corresponding amines and vice versa. This provides an important method for organic analysis.

It should be noted that reduction of anions is also an important area of application (such as sulphate to sulphide, iodate to iodide, chlorate to chloride) for their determination.

3. Ion-Exchange in F.I.A.

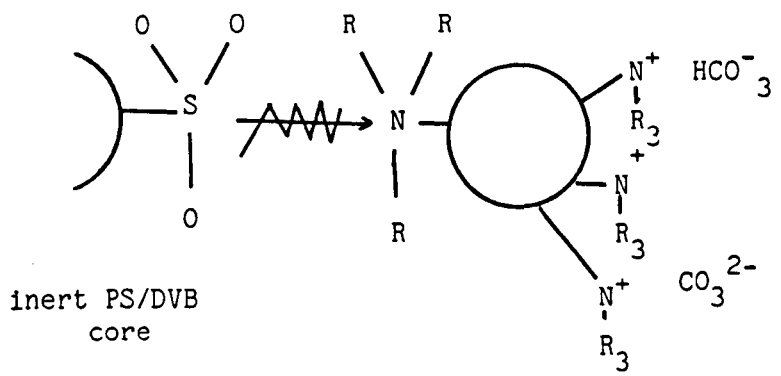
An attractive method has been developed for anion determination where a particular anion in a sample is exchanged with another in the resin-form. The application of a suppressor column in solving selectivity problems was successful and many more applications are possible. For example, an ion-exchange suppressor column is a convenient method of separating phosphate from both cations and anions. Vanadium(V) interferes by forming the vanadomolybdophosphate complex. Using a combined suppressor made of silver reductor (or Zn-amalgam) and H^{+}-form resin, it is possible to reduce V(V) to V(IV) or V(II) and removed it completely on a H^{+}-form resin before phosphate complexation. For other cations a cation exchange suppressor is an adequate mean for removing their interfering.

Another example is removing cation interferences from fluoride using lanthanum-alizarin fluorine blue. Metal ion such as Pb^{2+}, Zn^{2+}, Al^{2+}, and Fe^{3+}, which either compete with La^{3+} or form a complex with F^{-} can be removed with a suitable suppressor column.

Several forms of suppressor columns can be made for simultaneous determinations of two or more anions depending on their solubility product with the species loaded onto the resin column. Sulphate

and chromate, sulphate and phosphate, sulphate and thiosulphate (or sulphite), chloride and iodide, nitrate and nitrite, ... etc and a huge number of anion determinationsare possible.

The kind of development accomplished in chapter 7 in which on-line suppressor and exchanger columns are used for anion determination has led to an inspiration about the possibility of using a similar principle of ion-chromatography for separation and determination of a mixture of anions. The material for a separator column can be prepared on a small scale and packed in small glass columns. The whole preparation is a combination of an inert polystyrene/divinylbenzene (PS/DVB) core and anion exchange beads on a latex as shown below (332).



Latex

The kind of selectivity required can be chosen according to the latex employed. These latex groups are commercially available or the whole separator material, known as AS1, AS2, AS3 and AS4, are produced by Dow Chemical Company. Having prepared the column it is connected to the exchanger column in the SCN^--form (or any other form) which is then connected to a spectrophotometric detector, depending on the type of anion in the exchanger column.

Amplification by F.I.A. is an interest application. This can be applied to various fields. The arrangement shown in chapter 8 can be modified and used to amplify many other cations or anions using two resin

columns in different ionic form. Despite the simplicity of operating the arrangement in figure (8.1), its automation will exhibit reproducible timing and hence higher precision.

Among other important applications of ion exchange in F.I.A. which have not yet been considered is the possibility of combining separation and stepwise elution process of a mixture of metal ions with spectrophotometric detection for their sequential determination. The stepwise elution technique is particularly useful when the ions to be resolved have very different distribution coefficients. Eluents can be chosen so that each gives a sharp peak for one species, the others being retained on the column. Conventionally the application of anion exchange (222, 285, 341) for metal analysis using stepwise elution is well known. Despite the success of some chemical phenomena for sequential determination of metal ions by F.I.A. using combined ion-exchange-atomic absorption spectrophotometry, preliminary experiment showed that the application of this concept using spectrophotometric detection is not simple. The main problems are attributed to various parameters including the distribution of the sample on the resin column during the adsorption process and the optical purturbations which arises as a result of the sudden introduction of the eluent. Figure 9.1 shows the F.I.A. arrangements used for these preliminary investigations and kind of signals obtained in all cases. Dual peaks were obtained in all instances. Such a complicated analysis can be executed by use of a gradient pump similar to those used in HPLC, programmed so that after adsorption of the elements is complete a gradual change takes place automatically to a first eluent concentration and to the second and so on.

Finally, F.I.A. for work in different analytical fields is highly recommended because it is convenient, reproducible and fast. The overall significance of these points is that the analyst can use a simple

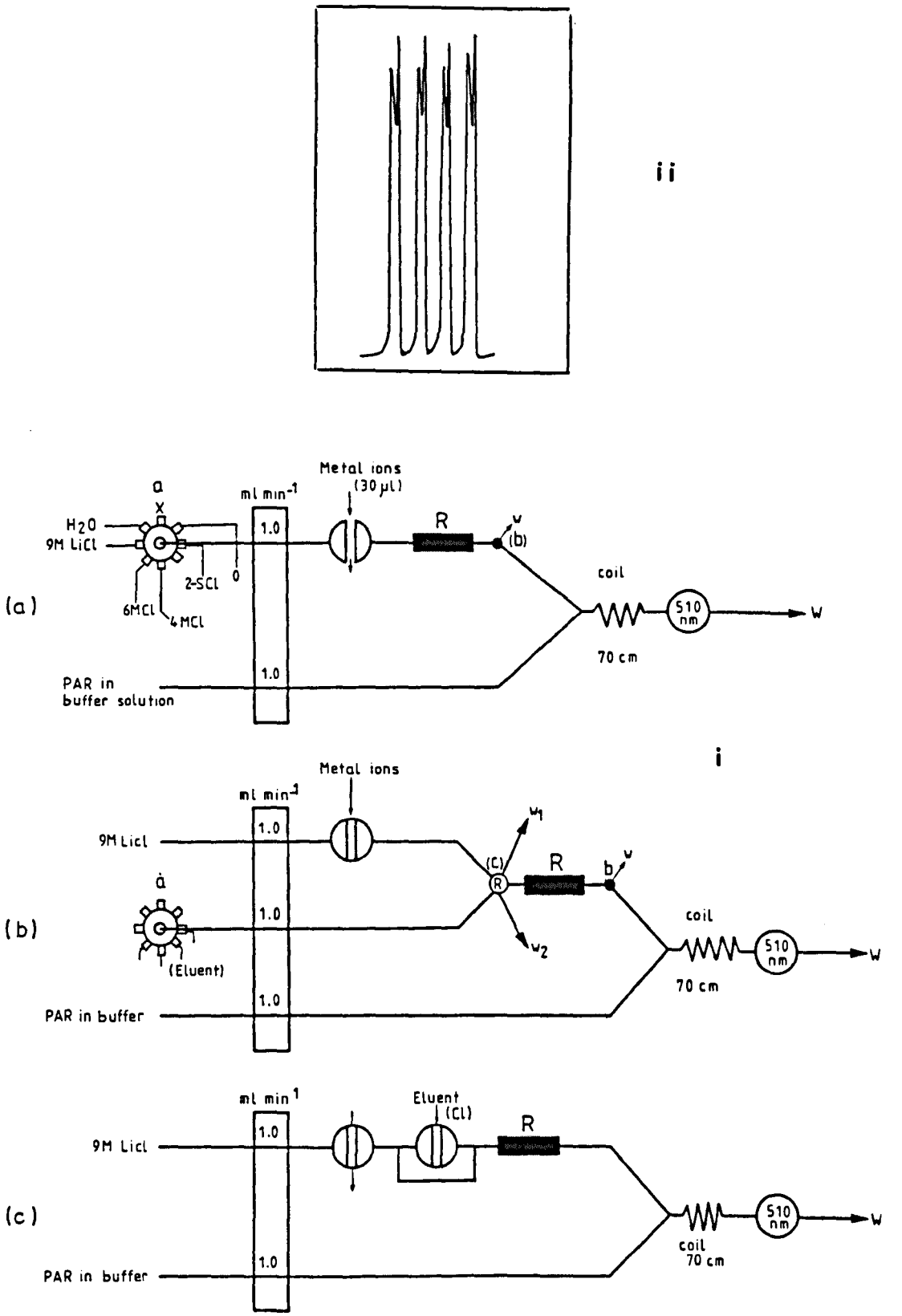


Fig. 9.1. (i) Flow manifolds used for separation and sequential spectrophotometric determination of metal ions. R, resin column. (a) is an eight-way valve with keys (Omnifit), (b) Two-way valve single key and (c) is a switching valve to interchange between two streams (Anachem).
(ii) The general shape of a response signals from 1×10^{-5}M Co^{2+} using the F.I.A. arrangements shown.

manifold design and operating conditions to obtain a wide variety of conditions for solving a given analytical problem. The seemingly trivial point of F.I.A. is a major advantage in method development where a great deal of time can be consumed in performing a particular analytical process.

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Appendix I

POSTERS AND PUBLICATIONS

The work described in this thesis has been presented as follows:

a. As posters

1. "Inorganic trace analysis by flow injection using chemiluminescent detection"

   A. T. Faizullah, A. Townshend and A. Wheatley

   SAC 83 Int. Conf. on Anal. Chem., Edinburgh 1983; in [cf Anal. Proc. 20 (1983) 313].

2. "Flow injection analysis for monitoring CL detection"

   A. T. Faizullah

   "Research and Developments Topics in Analytical Chemistry, Analytical Division Royal Society of Chemistry, Manchester, June 26/27 (1984).

3. "Use of reductor and ion-exchange minicolumns in F.I.A."

   A. T. Faizullah and A. Townshend

   "Flow Analysis III", Birmingham, September 6/9 (1985).

b. The F.I.A-CL detector was demonstrated in the practical sessions in the following conferences:

(i) SAC 83; Edinburgh (1983)

(ii) F.I.A. Short Course; Loughborough University of Technology, September 14/16 (1983).

c. Publications:

1. "Flow injection analysis with chemiluminescence detection"

   A. T. Faizullah and A. Townshend

   Anal. Proc. 22 (1985) 15.

2. "Application of a reducing column for metal speciation by flow injection analysis"

   A. T. Faizullah and A. Townshend

   Anal. Chim. Acta 167 (1985) 225

3. "Spectrophotometric determination of copper by flow injection analysis using on-line reduction column"
A. T. Faizullah and A. Townshend
Anal. Chim. Acta 172 (1985) 291.

4. "Application of ion-exchange with spectrophotometric detection for anion determination by flow injection analysis"
A. T. Faizullah and A. Townshend
Anal. Chim. Acta, in press.