High Performance Liquid Chromatography, Ion Chromatography, Thin Layer and Column Chromatography of Water Samples 1983

Methods for the Examination of Waters and Associated Materials

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Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturer's catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection, 'Public Health Laboratory Service Monograph 6, HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio-chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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About this series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior analytical chemist, biologist, bacteriologist etc. to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Evironment set up in 1972. Currently it has seven Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
9.0 Radiochemical methods.

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover. Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No. 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in that booklet.

L R PITTWELL
Secretary

31 October 1983

About this booklet

This booklet contains two of the three essay reviews on chromatographic techniques issued as part of the series "Methods for the Examination of Waters and Associated Materials":

> High Performance Liquid Chromatography applied to the Analysis of Organic Compounds in Natural and Waste Waters 1981; (with a short note on the determination of Inorganic Substances).

and

> Ion Chromatography in the Analysis of Water Samples 1983.

The other review, which forms a separate booklet is:

> Gas Chromatography 1982.

The Standing Committee of Analysts had intended to produce a fourth booklet on paper, thin layer and simple packed column techniques; but as these latter techniques are sufficiently well described in existing texts, the Committee did not feel justified in adding to the available literature. However, for those requiring an introduction to these other chromatographic methods, a short note is appended to this booklet, which includes a bibliography. Chapter 8 of the companion survey on multielement and related methods also summarizes chromatographic methods[1].

The booklet begins with a general introduction to liquid chromatographic techniques.

Many of the recent developments in the fields covered by the reviews in this booklet have been made by commercial firms and it would be unfair not to mention them when appropriate. However, this does not mean that other similar equipment might not be equally suitable. Grateful thanks is also due to both the Water Research Centre and Imperial Chemical Industries plc for assistance in the preparation of these reviews. For other acknowledgements, see page 99. In this instance, member participation and interest in the preparation of a booklet was exceptionally high.

Reference:

1. A Survey of Multielement and Related Methods of Analysis for Waters, Sediments and other materials of interest to the Water Industry 1980. HMSO London.

Hazards

There are several special points which should be considered in addition to toxicity of materials and the other normal routine laboratory hazards. Firstly, consider the necessary precautions, safety regulations and legal requirements for the use of high pressure equipment, such as adequate safety shields, eye protection and proper maintenance. Secondly, consider the need for flame proof electrical equipment if flammable liquids are used. If columns are dismantled or packed, precautions must be taken against any risks from inhalation of harmful fine dusts, such as dry silica. Safe operation can be achieved by attention to ventilation, shielding commensurate with good control of the apparatus and protection in the event of a blowout of toxic, flammable or other hazardous material. Consideration should also be given to problems that might occur during maintenance work, waste disposal and the venting of fumes to atmosphere or drain. Attention is drawn to the last paragraph of **Warning to users**.

Introduction to Liquid Chromatographic Techniques

Chromatography is a physical technique for the separation of substances by means of their equilibrium distribution between two phases — one of which is stationary and the other mobile.

When a molecule of a substance is in the mobile phase, it moves at the speed of the mobile phase and has zero velocity when in the stationary phase. The speed of migration therefore depends on the partition coefficient Cm/Cs of the substance between the 2 phases. Separation is achieved by choosing stationary and mobile phases such that the different components of the mixture have different distribution (partition) coefficients between the 2 phases.

The stationary phase may be a solid or a liquid and the mobile phase may be a liquid or a gas. There are therefore four possible basic systems for chromatography.

Liquid — solid
Liquid — liquid
Gas — solid
Gas — liquid

There are further sub-divisions usually characterised by what is considered to be the predominant mechanism of separation — absorption, partition or ion-exchange. Separations can be carried out either in a column or along a planar surface (paper chromatography & TLC). All 4 modes may be carried out in a column but only liquid-solid and liquid-liquid may be carried out on a planar surface.

Paper Chromatography (PC)

PC is an example of liquid-liquid partition chromatography where the stationary phase is usually water, supported by the cellulose molecules of the paper and the mobile phase is usually a mixture of one or more organic solvents and water.

A drop of a solution containing the mixture to be separated is placed in a marked position on a sheet or strip of filter paper where it spreads out to form a circular spot. When the spot has dried, the paper is put into a suitable closed apparatus with one end immersed in the solvent chosen as the mobile phase. The solvent percolates through the fibres of the paper by capillary action and moves the components of the mixture to different extents in the direction of flow. When the solvent front has moved a suitable distance, the paper is removed from the apparatus and the sheet is allowed to dry. If the substances are coloured, they are now visible as separate zones or spots, otherwise they must be detected by physical or chemical means.

Thin-Layer Chromatography (TLC)

TLC in its simplest form is an example of liquid-solid absorption chromatography. A thin film of finely divided absorbant (stationary phase) is spread evenly onto a firm inert support (glass plates). A solution of the sample in a volatile solvent is applied to the plate. When the spot has dried, the plate is placed vertically in a suitable tank with its lower edge immersed in the selected mobile phase, and an ascending chromatographic separation is thus obtained as in paper chromatography. TLC is in general faster than PC and can in most cases give better resolution. TLC allows a wide choice of absorbant and by appropriate choice of stationary phase and mobile phase, liquid-liquid partition and ion-exchange mechanisms can also be involved for added separation selectivity.

Column Liquid Chromatography

This is similar to TLC except that the stationary phase is uniformly packed into a column of dimensions 5 mm — 5 cm id and 10 cm — 1 m length. The lower end of the column is drawn out so that it can be connected to a stopcock and the adsorbent is supported on a plug of glass wool or on a porous plate. The sample is applied as a concentrated solution to the top of the column. When all the sample has been absorbed on the top of the column, the vacant space above it can be filled with solvent. The supply of solvent can be replenished from a separating funnel. The flow of eluting agent usually continues until each component is washed completely from the column where it can be detected by suitable chemical or physical methods. This technique is particularly useful when large amounts of material have to be separated.

High Performance Liquid Chromatography

HPLC is an extension of column liquid chromatography using closed, reusable columns packed with microparticulate (3–10 m) stationary phases operated at high mobile phase flow rates and pressures giving major advantages in convenience, precision, speed and ability to carry out difficult separations. This technique is more capital but less labour intensive than the techniques outlined above.

Solvent flow is provided by high-pressure pumps giving controlled, reproducible flow which gives rise to greater precision and speed of analysis. Precise sample introduction is easily achieved by syringe injection or sample valve. Detection and quantitation is achieved with continuous detectors which yield a final chromatogram without intervention by the operator.

Recommended Texts for further reading

Chromatographic Methods by R. Stock & C.B.F. Rice, Science Paperbacks.

Introduction to Liquid Chromatography by L. R. Snyder & J.J. Kirkland, Wiley Interscience.

High Performance Liquid Chromatography Applied to the Analysis of Organic Compounds in Natural and Waste Waters 1981

An Essay Review by A. Waggott.

(Chapter 9 on Inorganic Applications contributed by other committee members)

1. Introduction

It is the intention in this paper to provide an overview of the application of high performance liquid chromatography (HPLC) to the field of water pollution analysis. HPLC now finds application in all branches of analytical chemistry, but in very few of these are the problems encountered as severe as in water analysis. Difficulties are usually associated with the complexity of the sample matrix and the low limits of detection which are generally required. The increasingly successful application of HPLC to water pollution analysis therefore is a good indication of its scope and power. Some other fields of application where the sample matrix is also complex, eg clinical chemistry, may also provide the basis for useful methods which may be applied to water analysis. These are covered in this review where appropriate equivalent methods for the analysis of water do not exist.

A separate section of the review deals with theoretical aspects of the application of HPLC. The intention is to provide basic information of use to the practical chromatographer rather than a detailed account of all the concepts of chromatography for the theoretical chemist. An in-depth understanding of the theory of chromatography is not an essential requirement for its successful application. However, a good basic knowledge of the theoretical aspects is essential for its efficient employment.

A further section of the review describes the available components and instrumentation. There is now a very varied selection and so only those freely available on the commercial market are dealt with.

The final section of the review describes applications of HPLC to the analysis of water samples. Virtually all applications in this field are for the determination of specific organic compounds which are of environmental concern. A much more limited amount of effort has been put into its application as a method of non-specific analysis for the determination of unknown unsuspected organic pollutants in the aqueous environment. This approach has been frequently adopted for producing characterisation profiles or fingerprints of various water samples but the actual identification of chromatographic peaks by either on-line or off-line identification techniques presents a problem which largely still awaits solution.

There are many reasons why HPLC is being increasingly applied to water pollution analysis. First of all it now satisfies all the accepted features of gas chromatography (GC) which make that technique such a powerful and popular tool for analysts. These include high resolving power, fast analysis, ease and simplicity of operation, continuous monitoring of column effluent, precise identification based on accurate retention measurement, accurate quantitative measurement, repetitive analysis with the same column, and, finally, automation of the complete analysis and data-handling operation.

While high resolution gas chromatography (HRGC) using capillary columns offers superior resolving power and in most situations is the preferred method where a choice is possible, HPLC in some respects offers certain advantages. First and foremost there is no molecular-weight or polarity limitation on the range of organic compounds which may be separated. Unlike GC where separations are effected in the vapour phase at temperatures up to several hundred °C, HPLC is generally carried out at ambient temperatures and there is no volatility limitation. It is, therefore, also relatively a much gentler technique which can often be applied to the separation of heat-labile compounds which are not amenable to separation by GC. For the same reason the application of derivatisation techniques to improve the volatile properties, and hence the chromatography, of some polar compounds in GC analysis is not required in the course of analytical techniques based on HPLC. Thus sample preparation procedures are likely to be simpler.

In practice, HPLC is a more flexible technique than GC. Virtually all GC separations depend upon partition of components between the gaseous mobile phase and the liquid stationary phase which is coated on an inert support. In HPLC there is a much wider selection of mechanisms from which to choose to effect chromatographic separations. These are partition, adsorption, ion exchange, and gel permeation or size exclusion chromatography. Slight variations on these mechanisms include ion pair chromatography, and affinity chromatography. In all four basic modes of operation, separation of mixtures of organic compounds depends upon their differing affinities for the stationary phase. Unlike GC, where the mobile phase is an inert gas, in HPLC the elution solvent plays an important part in the separation. Thus, as well as a greater choice of separation mechanisms, an additional parameter, the composition of the mobile phase, can be varied to increase the scope and flexibility of the technique, ie both the solvent and column packing material may be varied selectively to produce the appropriate separation.

A further advantage of HPLC compared with GC is that it is more amenable to operation on a preparative scale. Relatively large preparative-scale HPLC columns may be employed without serious loss of efficiency. Liquid-phase collection as opposed to gas-phase trapping of samples is also an easier proposition as is the application of stop flow techniques for full spectral analysis of eluted compounds. However, successful fractionation using HPLC also involves the application of techniques to remove the eluting solvent.

Among the relative disadvantages of HPLC are the need to operate expensive high pressure pumping systems in order to generate adequate flow rates through the high resistance columns employed, the additional precautions which must be taken to overcome the potential fire hazard of working with volatile and flammable solvents, and the expense of these solvents. There is also a shortage of practical sensitive specific detectors for some ranges of organic compounds.

2. Theoretical Aspects of HPLC

In order to achieve maximum efficiency when applying liquid chromatography, the factors controlling the resolution of the column must be understood. Most of the recent advances in the field have been in the area of improved resolution in shorter time. Resolution, which is expressed by Equation 3 in Table 1, may be defined as the difference in times of peak maxima divided by the mean peak widths at their bases between tangents to points of inflection. The concept is shown diagrammatically in Fig 1. It is clear that in order to achieve baseline separation, and no more, implies a resolution of unity. A situation of partial resolution may be avoided by either decreasing band widths without

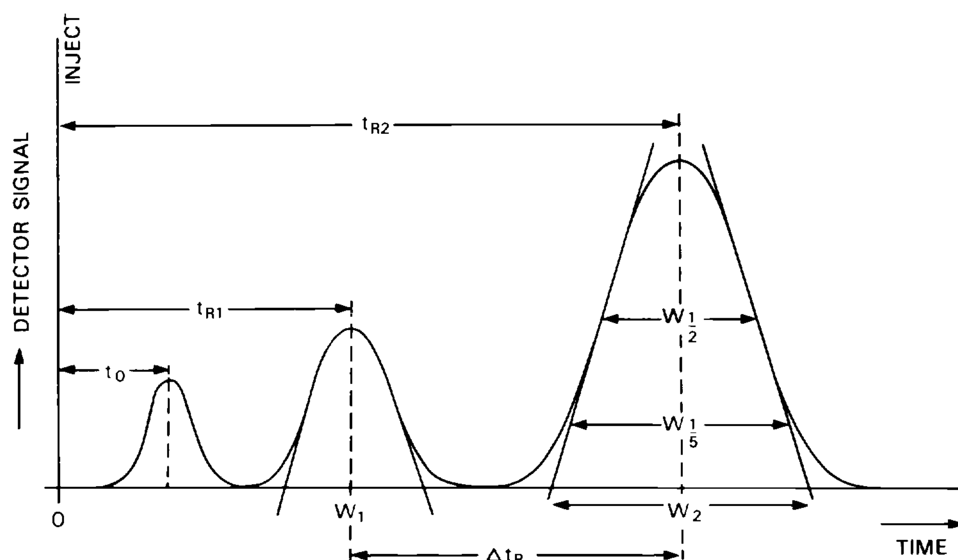


FIG 1 PARAMETERS USED TO CHARACTERISE A CHROMATOGRAPHIC SEPARATION (SEE TABLE 1 FOR EXPLANATION OF SYMBOLS).

changing the relative retention times of the peaks, or alternatively by increasing the peak separation without changing the widths. The latter variation requires either a change of the stationary phase (column) or the mobile phase (solvent) in order to effect a change in the relative partition ratios (defined by Equation 1 in Table 1) of the solutes between the two phases. Although partition ratios are not influenced by changing parameters such as the flow rate, particle size and column length, these factors do have an effect on the band width of peaks and their optimisation can therefore improve the resolution achieved. The first priority in designing and setting up a chromatographic system is to examine those kinetic features which can minimise band spreading.

A consideration of chromatographic theory can in practical terms lead to better design of columns and their more efficient application. The three mechanisms which give rise to band broadening are represented schematically in Fig. 2. These are differences in the length of flow paths, axial diffusion of solutes in the mobile phase, and resistance to transfer of solutes between mobile and stationary phases. An equation developed by van Deemter *et al*[1] links these three factors to the efficiency of the column, H, the height equivalent of a theoretical plate. H decreases in size as the efficiency of the column improves. Equation 7 in Table 1 shows two expressions for N, the number of plates obtained on a chromatographic column, in terms of measurable parameters. Equation 6 shows the relationship between N and H.

In its simplest form the van Deemter equation may be expressed as follows:

$$H = A + \frac{B}{u} + Cu$$

where A, B and C are constants and u is the mean linear velocity of the mobile phase through the column. The factor B, axial motion, is caused by random motion of the molecules and will increase in proportion to the time taken to pass through the column. The faster the linear velocity the less the time for this effect to become significant, hence the reverse relationship of H to u in the van Deemter equation.

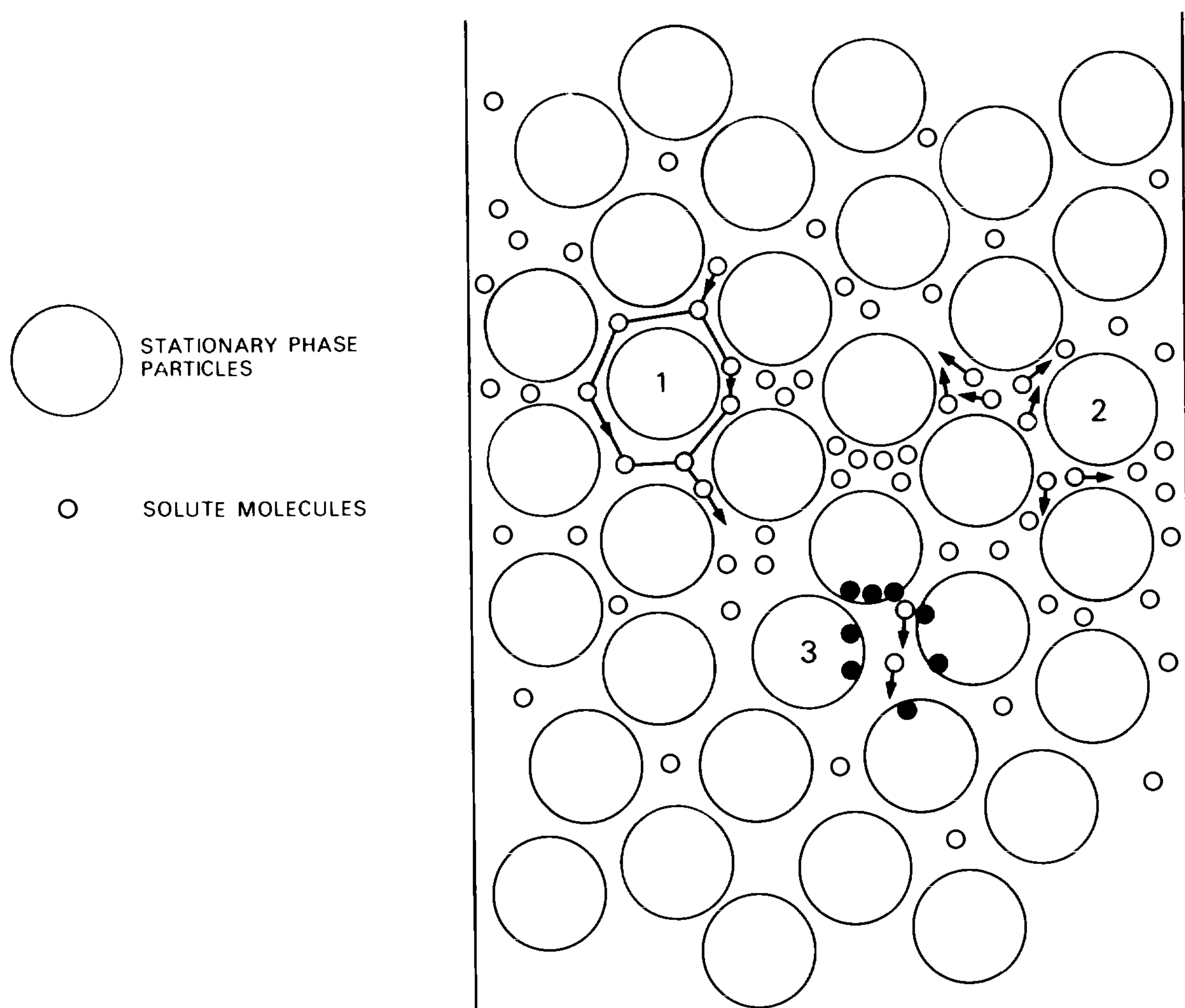
Table 1. Methods of measuring and optimising performance efficiency of the HPLC system †

Measuring performance efficiency			
1. Partition ratio, k'	$k' = \frac{t_R - t_o}{t_o}$	t_o:	retention time of solvent peak
2. Linear velocity, u	$u = \frac{L}{t_o}$	t_R:	retention time of solute peak
3. Resolution, R_s	$R_s = \frac{\Delta t_R}{W_1 + W_2}$	L:	column length
4. Separation number, n_{sep}	$n_{sep} = R_s - 1$	Δt_R:	retention time difference
5. Tailing factor, f	$f = \frac{W_{1/5}}{W_{1/2}} \times 5/8$	W:	peak width at base between tangents to points of inflection
6. Plate height, H	$H = \frac{L}{N}$		
7. Number of plates, N	$N = \frac{16(t_R)^2}{(W)}$ or $\frac{5.54(t_R - t_o)^2}{(W_{1/2})^2}$	$W_{1/2}$: $W_{1/5}$:	peak width at: half peak height, one fifth peak height
Optimising the separation conditions:			
1. Flow rate:	plot H vs u		
2. Column capacity:	plot H vs μl of sample introduced		

† The symbols adopted are those of Kirkland *et al*[A]

FIG 2 FACTORS LEADING TO BAND-BROADENING DURING DEVELOPMENT OF THE CHROMATOGRAM

1. DIFFERENCES IN LENGTH OF FLOW PATH
2. AXIAL DIFFUSION OF SOLUTES IN THE MOBILE PHASE
3. TRANSFER OF SOLUTES BETWEEN MOBILE AND STATIONARY PHASES



The interphase mass transfer process which is the most significant in considerations of HPLC theory (unlike GLC) has an effect on the column efficiency because solute molecules are effectively halted on entering the stationary phase and are therefore left behind. The greater the linear velocity of the solvent through the column the greater will be this gap and hence the direct relationship between H and u for this factor in the van Deemter equation. A fuller account of these factors and the many others which affect the chromatographic system has been given by Giddings.[B]

An expanded expression for the van Deemter equation may be written as follows:

$$H = 2\lambda d_p + \frac{2\gamma D_M}{u} + \frac{8}{\pi^2} \frac{k'}{(1+k')^2} \frac{d_f^2}{D_S} u$$

where
- λ = constant characteristic of packing irregularity
- γ = constant giving a corrector factor for the obstruction to diffusion by the packing
- d_p = mean particle diameter
- D_M = molecular diffusion coefficient of the solute in the mobile phase
- D_S = in the stationary phase
- k' = retention ratio of the solute
- d_f = depth of stationary phase

Several factors are equated over which some control can be exercised. These are the particle diameter, diffusion coefficients, mobile phase velocity, stationary phase

thickness, and the retention ratio. It can be immediately seen that reduction in the mean particle diameter and/or the thickness of the stationary phase can immediately and directly lead to increased column efficiency, and this has been one of the main features in the advent of HPLC in recent years.

Basic equations required for measuring the efficiency of an HPLC system as well as measuring column performance are shown in Table 1. In order to optimise the conditions of any separation, flow rate and column capacity effects should also be investigated. This is generally achieved by calculating plate heights respectively at various linear velocities and for injections of various sample volumes using appropriate standard compounds chromatographed under standard conditions. Optimum flow rates and column capacities are obtained by plotting graphs of H, the efficiency, versus linear velocity and volume of sample introduced.

Further factors must be considered in attempting to compare columns or column packing materials evaluated in different chromatographic systems, eg in inter-laboratory comparisons. In particular it is better to use reduced parameters which are independent of column dimensions. These factors have been discussed at length by Bristow and Knox[2].

3. Equipment and Materials

3.1 Introduction

The technique of HPLC has advanced considerably in recent years, mainly because of the development by the manufacturers of equipment of improved function and design. This has partly been due to an increased awareness of theoretical concepts leading to improved design of columns and equipment. The rather inflexible comprehensive approach to equipment design has now been almost completely superseded by the modular design approach. There is also increasingly a very varied choice of equipment available on the commercial market, fulfilling a variety of functions. The most modern equipment makes full use of new microprocessor technology to provide options for complete automation and control of the chromatographic system[3-5].

A schematic diagram of a typical HPLC system is shown in Fig 3. Degassed solvent is pumped at a pressure of generally up to 350 atmos. through a chromatographic column containing a microparticulate stationary phase (generally 5 to 20 micron in diameter) and then through the microcell of a suitable detection system. The sample is introduced at the head of the chromatographic column and is eluted through the system either under conditions of constant solvent composition (isocratically) or by the application of a solvent gradient which can be generated in several ways either at the high or low pressure side of the pump. In order to withstand the high pressures generated at the inlet of the column, components are generally constructed of high-grade stainless steel and column media are generally rigid in structure. Modern analytical columns are usually between 200 and 300 mm in length and 4 mm in internal diameter.

Factors which must be considered in designing and setting up an HPLC system have been discussed by Bombaugh *et al*[6] and commercially available equipment currently available on the market has been described and reviewed by Chandler and McNair in a series of updated papers. The latest was published in 1978[7].

3.2 Sample Introduction Techniques

In an ideal liquid chromatographic system, band broadening effects caused by the components of the apparatus are insignificant compared with those caused by elution on the column. This includes diffusion effects within the connecting tubes, the flowcell of the detector and the injection device. Of these the latter is the most important, since no matter how efficient the column or design of the rest of the system, an inefficient injection device will drastically reduce the overall performance. These factors have been discussed by Simpson[8].

3.2.1 Syringe Injection

Up to approximately 35 atmos. pressure, normal GC syringes may be safely used. However, quantitative treatment of results may be suspect owing to loss of a fraction of

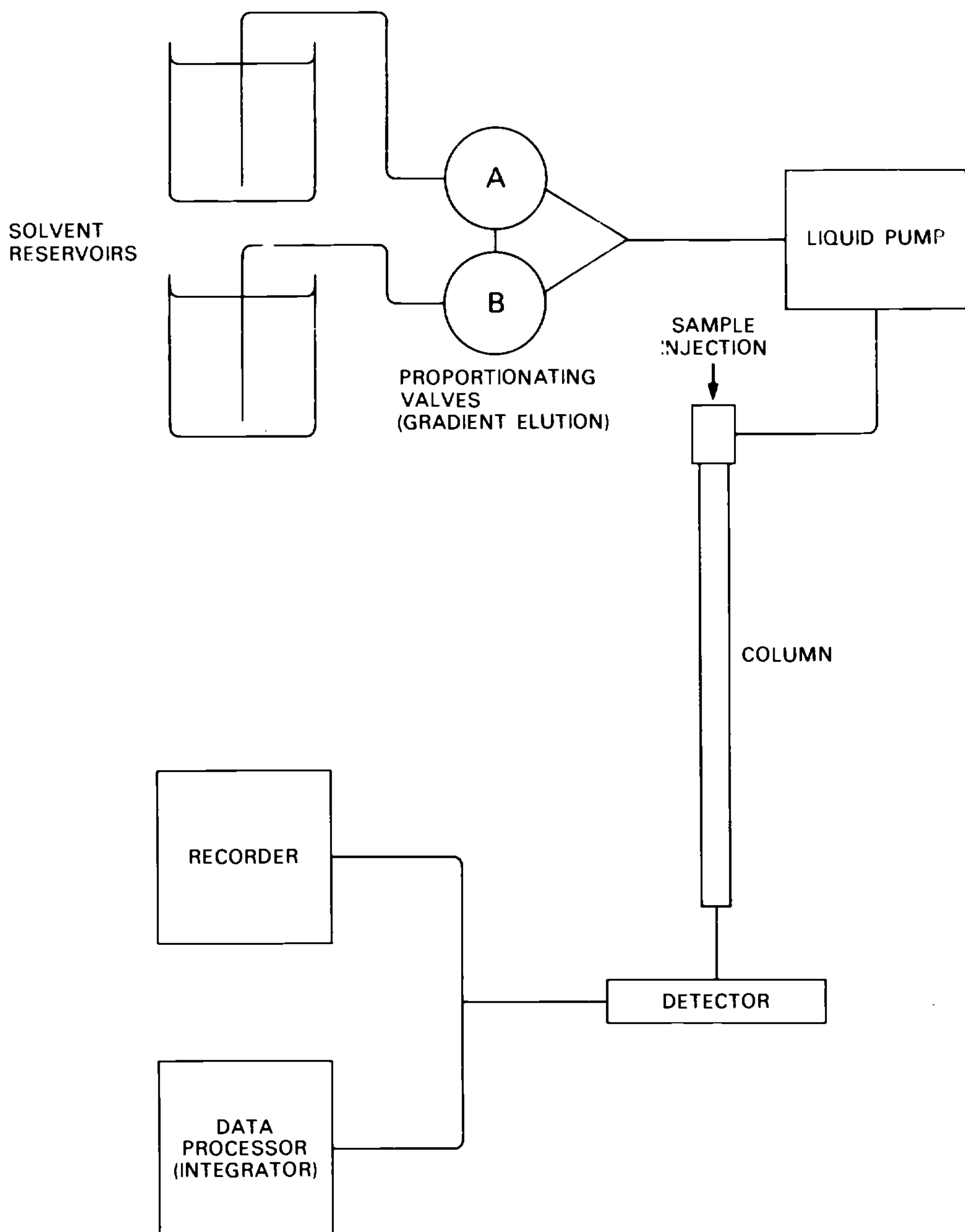


FIG 3 SCHEMATIC DIAGRAM OF A HPLC SYSTEM

the sample along the syringe barrel. Above a pressure of approximately 35 atmos, and for carrying out all quantitative determinations, special HPLC syringes are recommended.

In HPLC the choice of material of fabrication for the septum has to be made much more carefully than in gas chromatography. There must be sufficient strength in the elastomer to allow repeated needle penetration without total rupture or to prevent extrusion of the septum material through the needle hole. Such occurrences may be discouraged by the use of needle guides which allow the syringe needle to penetrate the same hole repeatedly. This also tends to increase septum life. For these reasons also, elastomers which swell in organic solvents are unsatisfactory. When operating in aqueous solvent media, silicone elastomers are generally suitable; this material swells in non-polar organic solvents, however, and perfluoroelastomers are therefore generally used in these phases. Septum materials which are covered with thin films of 'PTFE' are now available.

A further development of syringe injection techniques which precludes the disadvantages of the use of septa is that of septumless injection through valves of various design. Several commercial versions are available of which the Packard-Becker system is fairly typical[9]. Unfortunately, many of these are designed so that the sample is introduced directly into the mobile-phase flow and not onto the head of the chromatographic column. The latter system has greater proven efficiency.

Some of the disadvantages of septum injection may be overcome by the use of stop-flow technique. Here the pump is switched off or the solvent flow diverted and the column head pressure allowed to fall. A sealing cap is then removed, the syringe needle inserted, and injection carried out in the optimal position. The cap is then replaced and the flow resumed. The negligible diffusion of liquids means that there is very little loss of efficiency because of the disruption to the eluant flow. The speed of injection can be critical, however, since over-rapid injection may cause turbulence. This technique is not recommended for accurate quantitative analysis or the production of accurate retention time data because it generally lacks reproducibility owing to the finite time taken for the mobile-phase flow to restabilise. With careful standardisation of technique reproducible results can be achieved by a particular operator, however. When applying syringe injection techniques it is important to consider in detail the processes and events which occur during the charging and discharging of the syringe barrel. Unlike GC where the sample contained in the needle of the syringe is generally expelled when it enters the hot zone of the injector and is vapourised, in HPLC the sample contained in the needle remains trapped there. In order therefore to obtain accurate injection volumes the needle and entire syringe barrel up to the graduation mark required should be filled with sample, ie there should be no air space between the meniscus and tip of the syringe plunger. Such an air space would be compressed, when the needle tip penetrated the septum, by the column head pressure and an aliquot of elution solvent would enter the needle. An alternative method is to draw in a small volume of pure solvent ahead of the sample sufficiently large to discharge all of the sample from the needle. A small air gap is usually drawn into the syringe between solvent and sample to act as a buffer and the exact volume of sample injected is obtained by drawing the whole volume into the barrel of the syringe and recording scale readings at both ends of the sample plug.

3.2.2 Loop Injections

Accurate and reproducible injection of samples from a syringe requires a certain degree of skill by the operator. Accurate and reproducible loop injections, however, may be carried out by unskilled personnel although some loss of performance is unavoidable. In this system, which is shown schematically in Fig 4, a fixed-volume loop is filled with the sample and, by turning the valve, then switched into the solvent stream. The valve switching may occur at column inlet pressures of up to approximately 500 atmos.

3.2.3 Precolumn Methods

A special kind of sample introduction technique of particular value in the water pollution analysis field, is that of pre-column enrichment of aqueous samples for integrated extraction and determination of organic compounds in the water. This technique is specially suitable for operation with reversed-phase partition chromatography which allows sampling onto the pre-column in the aqueous phase. The pre-column is then connected to the analytical column and entrapped material separated by applying a reversed-phase solvent gradient. Particular advantages gained by employing this system are the integration of sampling and analysis, the fewer possibilities for introduction of artifacts, and the achievement of high concentration factors during sampling (depending upon capacity of the pre-column). The technique has potentially wide application both as a screening technique and for the quantitative determination of known pollutants, but a potential weakness is the limited polarity range of organic compounds normally separable on a reversed-phase partition column. The technique is discussed in greater detail in Section 4.5.

3.2.4 Preparative Scale Methods

Unlike analytical-scale liquid chromatography, where highest efficiency is achieved by addition of sample from a point source, it is at present generally accepted that the requirement for preparative-scale work is to inject the sample as a layer at the head of the column. This avoids the worst conditions of column overload which may cause bad peak tailing. The most convenient method, and the one most generally adopted, is that of sample loop injection. It is often necessary to employ special sample distribution heads at the inlet of the chromatographic column in order to ensure even sample application. This technique, as well as others, has been discussed in more detail by various authors [8,10-12]. Automated repetitive injection techniques have also been applied on conventional analytical columns for preparative work [13].

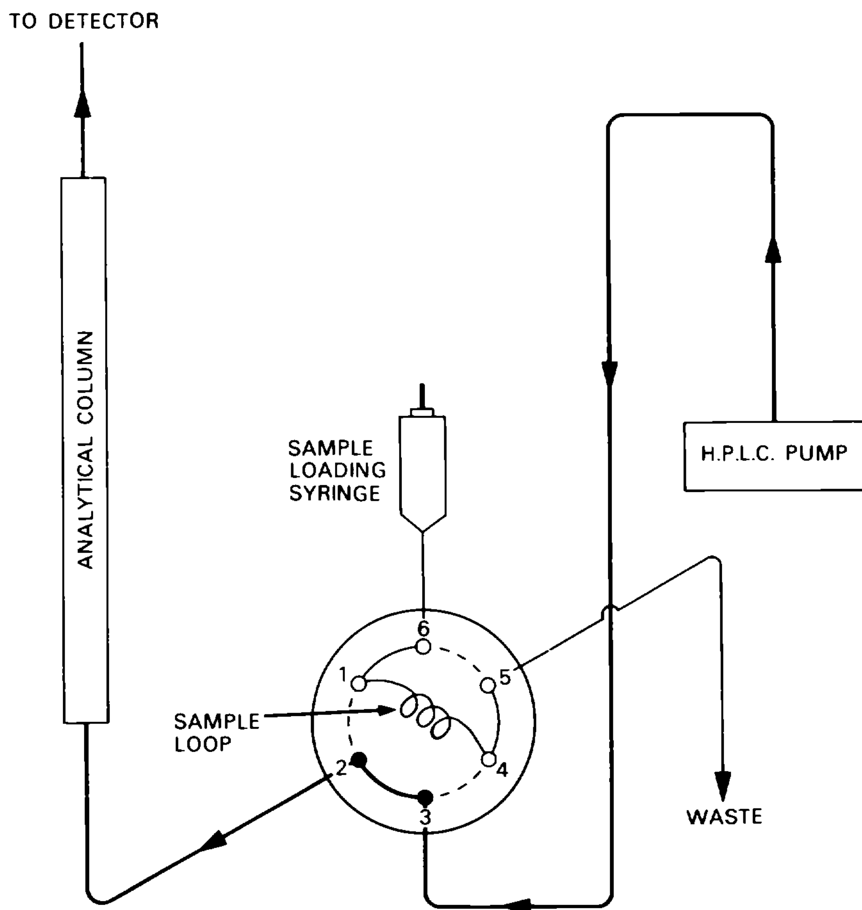


FIG 4 LOOP INJECTION

Work to compare the various methods of sample introduction led to the conclusion that syringe injection through a PTFE septum with continuous flow onto stainless-steel mesh produced the greatest column efficiency in a system incorporating split flow. This system of Webber and McKerrell[14] involves the by-pass of a fraction of the solvent flow around the point of injection, thus combining the control and flexibility of syringe injection with the reproducibility of sample loop injection. These findings have been confirmed by Kirkland *et al*[15] and Simpson[8]. A schematic diagram of the split-stream injection system designed by Simpson, which gives control over the rate of sample introduction, is shown in Fig 5.

Simpson has pointed out the poor results which are obtained when, for whatever reason, there is dead volume at the column head. The work of Majors[16] has established that the highest efficiences are obtained by syringe injection, preferably into the chromatographic bed. There are two main reasons for this, namely that a better injection profile is obtained by injecting directly onto the packing and also back diffusion effects are avoided. Also, at the point of injection, the entire cross-section of the chromatographic bed should receive a parallel stream of diluting solvent, thus producing more favourable chromatographic conditions. In effect, a point source of injection is produced, giving rise to a column of infinite diameter and thus avoiding the wall effects which detract from efficient separation. Some further advantages and disadvantages of this system have been discussed by Simpson[8].

3.3 Solvent Delivery Systems

The most important requirements of an efficient solvent delivery system are that it should deliver a stable flow, free from pulsations in order to minimise detector noise, a wide range of flow rates in order to utilise the various modes of operation of liquid chromatography including preparative scale work, a constant flow-rate delivery in order to facilitate interpretation of results in both quantitative and qualitative analyses, sufficient pressure to allow adequate flow rates to be maintained through all the various types of column likely to be employed, and suitability for use with solvent gradient generating systems.

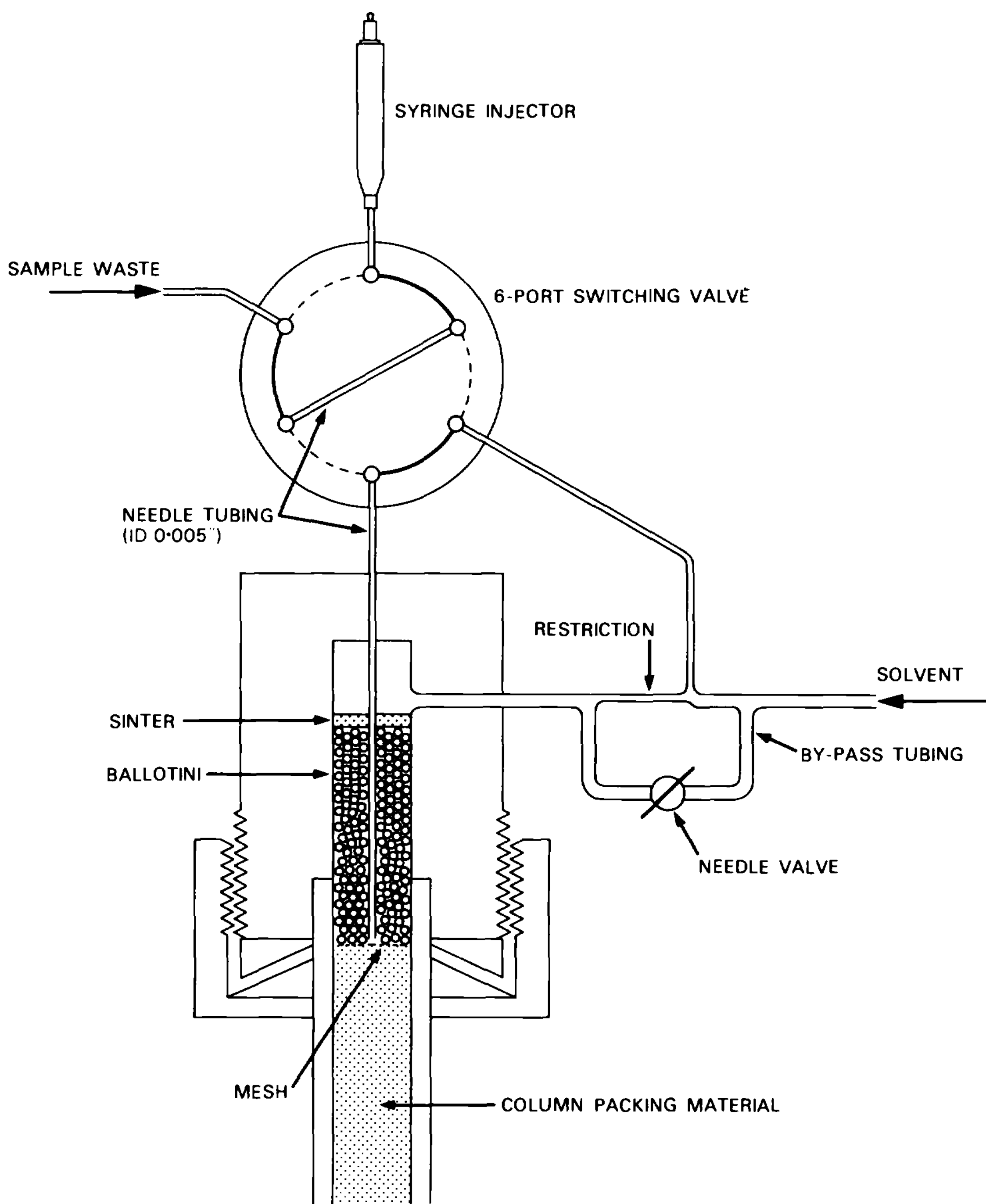


FIG 5 SPLIT STREAM INJECTION

There are several types of HPLC pump available on the commercial market, all with their own advantages and disadvantages. These are mentioned individually below. The subject has been more thoroughly reviewed in a book edited by Huber[C].

3.3.1 Gas Pressurisation Pumps

This type of pump provides a relatively cheap and reliable type of solvent delivery system although certain hazards do exist and it is advisable to adopt interlocking valve configurations for its control. The eluting solvent is usually retained in a stainless-steel holding coil which is pressurised by gas from a cylinder. Pressures in excess of approximately 100 atmos. cannot be employed because of the problem of solution of the pressurising gas in the eluting solvent causing degassing on the chromatographic column. This problem can be overcome by discarding a proportion of the total change of eluting solvent at the end of the coil. In some systems a non-permeable membrane is used to separate the solvent and driving gas.

3.3.2 Pneumatic Amplifier Pumps

This type of pump generates high pressure from relatively low-pressure (less than 15 atmos.) cylinders. The low-pressure gas is in contact with a large surface-area piston which is coupled to a small-area piston in contact with the mobile phase. The surface-area ratio of the gas piston to the solvent piston is the amplification factor of the gas pressure. Some designs allow rapid filling of the pump reservoir with minimal disruption of detector baseline. Solvent delivery is generated at a constant column head pressure and is pulse free. The advantages of this type of pump have been discussed by Henry[17]. A disadvantage is the expense of replacing damaged pistons which may be caused by particles in the pump.

3.3.3 Reciprocating Piston Pumps

In this type of pump a piston in direct contact with the eluting solvent is employed. Using a system of ball valves, solvent is pulled in from the reservoir on the intake stroke and forced out on the return stroke. Solvent delivery takes the form of a series of regular pulsations of flow and, in order to achieve noise-free detection, these have to be smoothed out by use of a suitable pulse-dampening system. This can be achieved either with the help of Bourdon tubes or with multi-head pumping systems in which the solvent delivery is offset. Problems include the relatively large dead-volumes of pump and pulse-dampening chambers, constant wear and tear on valve seatings, and sticking valves caused by particles in the liquid.

3.3.4 Syringe Pumps

In this system single-stroke large-capacity piston pumps are employed. They are generally operated by a screw gear which displaces a plunger through the solvent reservoir. Although they are expensive they generally produce pulseless noise-free and essentially constant flow solvent delivery. However, cleaning the system for operation with a new solvent is time-consuming and expensive on solvents, unless a rapid-fill pump design is employed.

The response from an HPLC detector is directly proportional to the concentration of solute in the eluting solvent. The peak area of any response is, therefore, inversely proportional to the rate of flow of the solvent through the column. Reproducibility in both qualitative and quantitative analyses can therefore clearly be seriously affected by inadequacies in the solvent delivery system's ability to produce constant and reproducible flowrates. None of the pumps described can, in fact, unaided produce constant flow under all conditions. Depending upon the design, their ability is more or less affected by variations in temperature, viscosity of the solvent, compressibility of the solvent, etc. The susceptibility of each type of pump to changes in these factors has been discussed in greater detail by Schrenker[18].

More recently the application of microprocessors to the control of pumping systems has led to the development of liquid chromatographs in which precision qualitative and quantitative analyses can be carried out with ease. The principles of various designs of equipment for this purpose have been described by Schrenker[18].

3.4 Detection Systems

3.4.1 Introduction

The purpose of any detection system is to afford a measurement of the concentration of all solutes eluted from the chromatographic column and produce an electrical response proportional to the sample concentration. The response of the detector should ideally be linearly proportional to the concentration of the sample being analysed in order that the chromatograms obtained may be used for quantitative estimations. Many detectors, however, have linear response only over a limited concentration range and, if quantitative results are required, it should be ensured that the detector is operating within this range.

There are basically two types of HPLC detection system. To date the most successful type is that which measures some specific characteristic of the eluting components. This type includes potentiometric, polarographic, spectroscopic, and radiochemical detection systems. The second type is that which measures some bulk property of the mobile phase

and is capable of detecting slight changes in this property when solute molecules pass through the flow cell.

To be ideally suitable for carrying out detection of micro-scale quantities of solutes with maintenance of the high resolution obtained on the analytical column, a detection system must possess certain basic characteristics. These are high sensitivity to the solute molecules, no response to the solvent molecules, no band-broadening effects on eluted components, and, finally, no destructive effect on the eluted component. Detectors which depend on non-specific monitoring methods, ie measurement of a bulk property of the mobile phase, are generally extremely sensitive to the eluting solvents and to all intents and purposes may not be used with gradient elution operations. Fluctuations in flow rate and temperature can also considerably disturb the baselines. Detectors which depend upon specific monitoring methods can generally provide very high sensitivity but do not necessarily respond to all of the sample constituents, unlike bulk property detectors. The latter type have the advantage that they respond to most if not all of the sample components. However, they are normally low-sensitivity devices.

Various aspects of detection in HPLC have been reviewed in more detail in works by Schomburg *et al*[19], Simpson[D] and Huber[C]. In particular the classification of detectors has been considered, with a distinction between those that are mass-dependent and those that are concentration-dependent. The sensitivity, rectilinearity and time response, the cell volume and shape, and the effects of temperature and pressure on the operation of the detectors have been discussed.

This review considers chiefly those detectors which have been successfully applied in the field of water pollution analysis or which show promise in that area, and are commercially available. Some mention is also made of new detection systems which show promise.

3.4.2 Detectors which Measure Specific Properties

3.4.2.1 Ultra-violet Photometric Detectors

Particularly in the UV region, photometric detectors are the most frequently used at the present time because they provide some degree of selectivity and high sensitivity. They are also non-destructive and not particularly temperature-sensitive. Differential signal monitoring in double-beam instruments means that errors caused by fluctuation in the emission of the source and changes in the optical densities of the solvents are minimised. Stop-flow techniques may be utilised to provide complete spectra with scanning instruments. Detectors which give "instant" full spectral analysis of chromatographic eluate should shortly be readily available through the application of electronic devices such as Vidicon (TV camera tubes) and linear diode arrays. This approach will be particularly useful for the identification of unknown organic compounds in water samples because of the existence of large libraries of recorded spectra.

Single-wavelength UV photometers[20] are the most popular type of HPLC detector in use at present. The most common source of illumination is the low-pressure mercury lamp emitting at 254 nm. Major advantages are that flow-cell capacities are generally less than 10μl, the detector is relatively cheap, sensitive (10^{-8} to 10^{-9} g), insensitive to normal flow and temperature fluctuations, and can be usefully used in gradient elution operations. The major disadvantage is the fixed wavelength operation which may not correspond to the wavelength of maximum absorption of the organic compounds of interest. Other single-wavelength detection systems operating at more useful lower wavelength, eg 214 nm using a zinc lamp[21], are now generally available.

More versatile detectors based on the medium-pressure mercury lamp, which offer a wider range of wavelengths, are also commercially available. With the use of appropriate filters, lines of various intensities at 254, 280, 312, 365, 436 and 456 nm can be utilised.

Also available are a competitive range of variable-wavelength detectors built around the deuterium lamp which can provide energy throughout the range 190 to 380 nm[22]. By using a monochromator the instrument can be operated over this entire wavelength range, thus offering the advantages that the wavelength can be adjusted to that of the maximum absorbance of the eluted compound in order to provide maximum sensitivity and that the detector can in some applications provide useful selectivity.

3.4.2.2 Visible Photometric Detectors

Most organic compounds do not absorb in the visible region of the spectrum. However,

by mixing column eluate with a suitable reagent, specific colour-developing reactions can be utilised to provide a highly specific and sensitive method of detection. Commercial modules exist which will automatically add reagent, mix, heat, dilute, and continuously monitor absorption at the appropriate wavelength. Particular examples include the monitoring of amino acids by using ninhydrin reagent to develop purple colours at 570 nm and the monitoring of carboxylic acids at 424 nm by reaction with potassium dichromate. The technique is, of course, not limited to visible spectrophotometric determination of reaction products. With suitable reagents UV-absorbing or fluorescing derivatives may be produced and the appropriate detector applied. The theoretical aspects and application of post-column derivatisation techniques have been considered by Frei and co-workers[23-25] and by Snyder[26]. Many pre-column derivatisation techniques have been reviewed by Ross[27].

3.4.2.3 Fluorimetric Detectors

Fluorescence detectors are gaining in popularity because of their obvious advantages [28-30]. These are their high sensitivity (10^{-11} g or low parts per million), high selectivity (both excitation and emission wavelengths can be selected to pinpoint relevant compounds), less selectivity if required (by use of wide-range excitation and emission filters), insensitivity to flow rate variations and small temperature fluctuations, and finally the fact that they can be used with UV-absorbing solvents.

Both fixed-wavelength and scanning fluorescence detectors are available from several manufacturers. These types can be particularly useful in the field of water pollution analysis where complex sample matrices are encountered. The high selectivity of the detection system tends to minimise the number of elaborate sample pre-treatment stages required.

A limited range of organic compounds exhibit natural fluorescence and so, as with colorimetric detection systems, pre- and post-column derivatisation techniques are frequently employed to increase the scope of this detector[31]. A variety of reagents are now sold commercially which are specific to certain functional groups, thus primary and secondary aliphatic alcohols, phenols, fatty acids, and primary and secondary aliphatic and aromatic amines can be specifically detected by choice of the appropriate reagent. Post-column derivatisation techniques generally require reagents capable of relatively rapid reaction. An alternative is to produce fluorescent species by more drastic redox reactions. An example of the latter is the reduction of cerium IV to produce the fluorescing cerium III species in the cerate oxidation and fluorescence monitor developed at Oak Ridge National Laboratory[32]. This detector is capable in theory of monitoring all oxidisable organic compounds.

The performance of fixed and variable wavelength UV and fluorescence detectors for environmental analysis has been evaluated[33].

3.4.2.4 Infra-red Detectors

Because of the broad and intense absorbance of the eluting solvents, this type of detector has not received as much attention as those based on other spectroscopic techniques. However, with the correct choice of solvent system, useful work can be carried out at wavelengths of strong infra-red absorbance characteristic of the functional group being monitored[34,35]. The advent of rapid scanning instruments where "instant" full spectral analysis is achieved makes their application in the field of water pollution analysis very attractive.

3.4.2.5 Mass Spectrometric Detectors

Although HPLC — mass spectrometer interfaces are still far from becoming routine tools for detection of non-volatile organic compounds, rapid progress is being made in their development and application. The subject of combined HPLC and mass spectrometry has been reviewed[36,37]. Systems which incorporate total and partial direct introduction of HPLC eluate, direct introduction with enrichment, and mechanical transfer, are considered.

There are two fundamental characteristics of HPLC which make the technique basically incompatible for direct combination with mass spectrometry. These are the inability of the mass spectrometer to cope with the flow and type of many of the solvent systems at present utilised in HPLC, and the inability of the mass spectrometer to ionise a wide range of the non-volatile organic compounds of interest. The development of microscale

HPLC[38], employing micro-bore HPLC columns requiring low solvent flow rates for elution, and the application of other methods of ionisation may eventually help to surmount these problems. However an LC MS system that is capable of handling the full range of compounds studied by HPLC, and all the types of solvent system utilised, is not likely to be with us for some years, although increasingly complex and improved systems are being continually developed[39]. In a recent review[40] on the subject of mass spectrometric identification of non-volatile organic compounds, the authors concluded that the most promising separatory method at the present time is off-line HPLC in a preparative mode. The subject of current applications of combined HPLC-MS has been reviewed recently[41] and a semi-automated method of collection of samples for manual introduction to a mass spectrometer has also been reported[42].

3.4.2.6 Radiochemical Detectors

The application of this type of detection system depends upon the separation of already radioactive compounds, or those that have been rendered so by use of a radioactive reagent. The most popular technique, using the principles of scintillation counting, depends upon the measurement of beta radiation which has relatively low ionisation power and is not likely to damage living material. Earlier techniques in which discrete sample collection was required are now giving way to devices which monitor continuously. This detector should find increasing application in studies on the fate of organic compounds in the environment and during treatment processes. Trace concentrations of relevant compounds may be spiked into process or environmental waters and degradation or reaction products monitored by their radioactivity. Thus Jolley and his co-workers[E] were able to determine some of the effects of chlorination on the organic constituents in natural and process waters by using ^{36}Cl in the chlorination reaction, followed by HPLC and radioactivity monitoring to detect tagged compounds.

3.4.2.7 Electron Capture Detector

The electron capture detector (ECD), which has been used widely as a highly selective and sensitive detection system for GC, has now been successfully developed and applied to HPLC detection[43]. Column eluate is passed through a stainless-steel furnace tube, and both solute and solvent are vaporised and swept into the ECD in a stream of nitrogen. The detector outlet is attached to a condenser system so that fractions of the mobile phase can be collected. The detector is severely limited in its application, giving useful response only to those solvent systems which exhibit a minimum of electron affinity, eg hexane or iso-octane. However, by the use of amplifiers with wide-range back-off controls, low concentrations of polar modifiers may be incorporated into the solvent system, eg 3% methanol and up to 10% dioxan, depending upon the flow rate. It is not possible to use the system with aqueous solvents and it is therefore not compatible with ion-exchange, reversed-phase partition or gel-permeation chromatography. Also care must be taken to remove oxygen and other electron-capturing impurities from the solvent employed.

In spite of the severe limitations, the high selectivity and sensitivity of the ECD detector for many organic compounds of environmental significance means that it should find many important applications, eg for the monitoring of compounds containing halogens, phosphorus, sulphur, lead, and many others. A separate phosphorus specific detector based on flame photometry has however already been developed[44].

3.4.2.8 Atomic Absorption

Metal ions or metal-containing compounds that have either been separated by HPLC[45] or formed in a post-column reaction[46] can be detected sensitively and selectively by atomic absorption spectroscopy. It is thought that with controlled flow of column eluate into the nebuliser, ng quantities of a compound carrying a metal label may be detected and that the method, if adapted to use with graphite furnace atomic absorption, will allow detection limits in the pg range to be achieved.

3.4.2.9 Electrochemical Detectors

This type of detection system depends upon the presence of a working electrode in the detector flow cell. The three critical functions which it performs are to apply a working potential to the solution passing through the flow cell, to provide a surface at which the electrochemical reaction can take place, and to provide a relay for the current to be measured. In order to function efficiently the electrode should be stable in the working solution over a wide potential range, have high surface activity, be resistant to poisoning, and have a low electrical resistance. Among the many materials tried, the most successful

to date has been glassy carbon, which is a highly impervious form of carbon which can be polished to a mirror-like finish.

By careful choice of the working potential and selection of suitable electrolytes and pH conditions, electrochemical detection can be made to be fairly specific. However, in most situstions, electrochemical detection is used to give a universal response to as many organic compounds as possible. The optimum point at which universal detection takes place in any particular system can be found only by experimentation. Only molecules which exhibit electroactivity can be detected but derivatisation reactions may be employed to impart this property. Other restraints placed on the chromatographic system by the use of this detector are that it can be employed only with an eluting solvent which is electrically conductive, the pumping system must be completely pulse-free, and there should be no requirement for sample collection since the detector is destructive. Its general application to HPLC has been reviewed(47).

3.4.3 Detectors which Measure Bulk Properties

3.4.3.1 Transport Ionisation Detectors

This type of detection system depends upon the transport of a part or all of the column eluate to a flame ionisation detector (FID) widely used in GLC. In order to achieve this, column eluate is coated onto a moving wire, chain, belt, or disc (48-50) and passed through a relatively low-temperature furnace which is purged with nitrogen and held at a temperature sufficient to drive off the chromatographic solvent. The less-volatile solutes remain on the transport system and are carried to the FID either directly or following pyrolysis in a high-temperature furnace. The pyrolysis products are entrained, mixed with hydrogen, and passed through a hydrogen flame. Particular advantages of this system are its lack of response to the mobile phase, its insensitivity to temperature, flow rate and other environmental factors, and the relatively small contribution it makes to solute band broadening. The major disadvantage is lack of sensitivity, since in most versions only a very small proportion of the column eluate is coated onto the transport system. Some workers have attempted to improve this feature by precoating various porous supports onto the moving-wire system(51).

3.4.3.2 Refractometric Detectors

This type of detection system monitors the difference in refractive index between pure mobile phase and the column eluate containing the separated components from the high performance liquid chromatograph. Both reflection(52) and interferometric(53) type instruments have been developed. One of the major advantages of this type of system is its universality of response, since the solution of any organic compound in a pure solvent will almost invariably deflect the refractive index. Detection limits are set by the concentration of the solute and by the change in the refractive index caused by its passage. Commercial refractive index detectors are capable of measuring down to 10^{-7} to 10^{-8} refractive index units, and under advantageous conditions are therefore capable of detecting sub-ug amounts.

The main disadvantages of this type of detector are its high sensitivity to small fluctuations in temperature and its inability to cope with gradient elution operations. The temperature coefficient of refractive index for many organic substances is of the order of 10^{-4} per degree centigrade so that, in order to achieve the potential sensitivity of commercial refractometers, temperature control to $\pm$ 0.001°C is required. In order to employ gradient elution, a close balance in the composition of analytical and reference flows would be required. Under practical conditions this is virtually impossible.

3.4.3.3 Miscellaneous Detectors

Modifications to various types of total organic-carbon analysers have resulted in the development and application of useful HPLC detectors. The whole or a part of the column eluate is fed into an oxidation furnace and the carbon dioxide generated either measured directly, using infra-red techniques(54), or catalytically reduced, ammonia, moisture etc removed, and the resulting methane measured quantitatively by FID(54). Universal response to all organic carbon compounds with good sensitivity is achieved although the system can be used only with aqueous media.

A relatively new detector which could find widespread application in the field of water pollution analysis is the so-called mass detector(55). It provides universal and reasonably sensitive response (down to $\pm$0.1μg) for non-volatile solutes in most solvent systems. The

solvent stream containing the solute is nebulised and carried by an air-stream through a heated column. The temperature is adjusted so that the solvent is evaporated, leaving a fine mist of solute particles which pass through a light beam. Scattered light is measured by a photomultiplier placed at a fixed angle to the light beam. Volatile solutes of low molecular weight are not detected.

A new system, which has great potential as a sensitive universal detector, is the flame aerosol detector[56], which is somewhat like an electron capture detector in its action. As shown in Fig 6 it consists of a chamber at reduced pressure containing a collector, polarising electrode system, and a turbulent air/hydrogen flame into which the mobile phase is nebulised. With water as mobile phase, a standing current of approximately 10^{-7} amps is generated owing to the collection of charged droplets. Organic compounds or inorganic salts eluted from the chromatographic column produce ionised species in the flame so that the solvent droplets acquire additional charge which results in an increase in the breakup of the droplets and ultimately in a reduction in the number of more massively charged droplets reaching the collector chamber. The decrease in response is amplified and measured.

3.5 Gradient Elution Systems

Arguments over the particular usefulness of gradient elution in HPLC continue. While it must still be true that isocratic elution produces more reproducible results, particularly where exact quantification is required, modern liquid chromatographs with microprocessor control over the operating parameters provide sufficient reproducibility for gradient elution to be carried out with confidence in all possible aspects of the work. This is particularly beneficial in the field of environmental pollution analysis where sample extracts containing mixtures of wide polarity range are frequently encountered. For effective separation, or the production of detailed characterisation profiles, long polarity gradients may be required.

There are many types and designs of gradient elution systems. The most popular type is of the low-pressure variety situated on the inlet side of the HPLC pump as shown in Fig 3. It consists of two programmable solenoid valves operated by a pulse generator so that increments of two solvents can be drawn in turn from a primary and secondary solvent reservoir. The solvent increments are mixed efficiently and passed on through the HPLC pump and column[F].

An alternative, although usually more expensive system, involves the use of two HPLC pumps (syringe type) which are themselves programmed so that the pump strokes blend together the required composition of a binary solvent mixture[F].

A variation on the solenoid valve system involves the use of a holding coil filled with the secondary solvent on the high-pressure side of the HPLC pump (pump outlet) as shown in Fig 7. Primary solvent is supplied directly or used to push secondary solvent from the holding coil into the mixing chamber by switching two solenoid valves. Although only one pump is required, the solenoid valves must be capable of withstanding high pressure and are therefore more prone to wear and leakage[F].

There are many relatively cheap devices (invariably of the low-pressure type) built from simple laboratory equipment and materials and which are described in the literature[57-59]. These are generally quite effective and, with a certain amount of care, reasonably reproducible. There is to date no known commercial system suitable for use with more than three solvents which can be used for the production of multiple solvent gradients of the type required for efficient elution of wide-polarity mixtures from adsorption media. Suitable systems as shown in Fig 8, which are built around a programmable multiple port valve, have however, been described and used[60-62].

Publications which consider the theoretical aspects of the various methods of solvent gradient production and their application with optimum efficiency are available[59,63,64].

3.6 Miscellaneous Ancillary Equipment

3.6.1 Solvent Reservoirs

Some equipment, especially older types, have built-in reservoirs in which solvents can be automatically degassed prior to use. Lengthy flushing and cleaning processes are,

however, required on solvent change-over. It is therefore much more convenient to use as a reservoir the Winchester quart bottles in which the solvent is originally provided. Plastic caps can usually be drilled to allow access for outlet tubes. Degassing problems if they occur may be overcome by using helium sparges or immersing the solvent in an ultrasonic bath. These methods have largely superseded early approaches which involved heating solvents under vacuum in reservoirs incorporated into the liquid chromatograph.

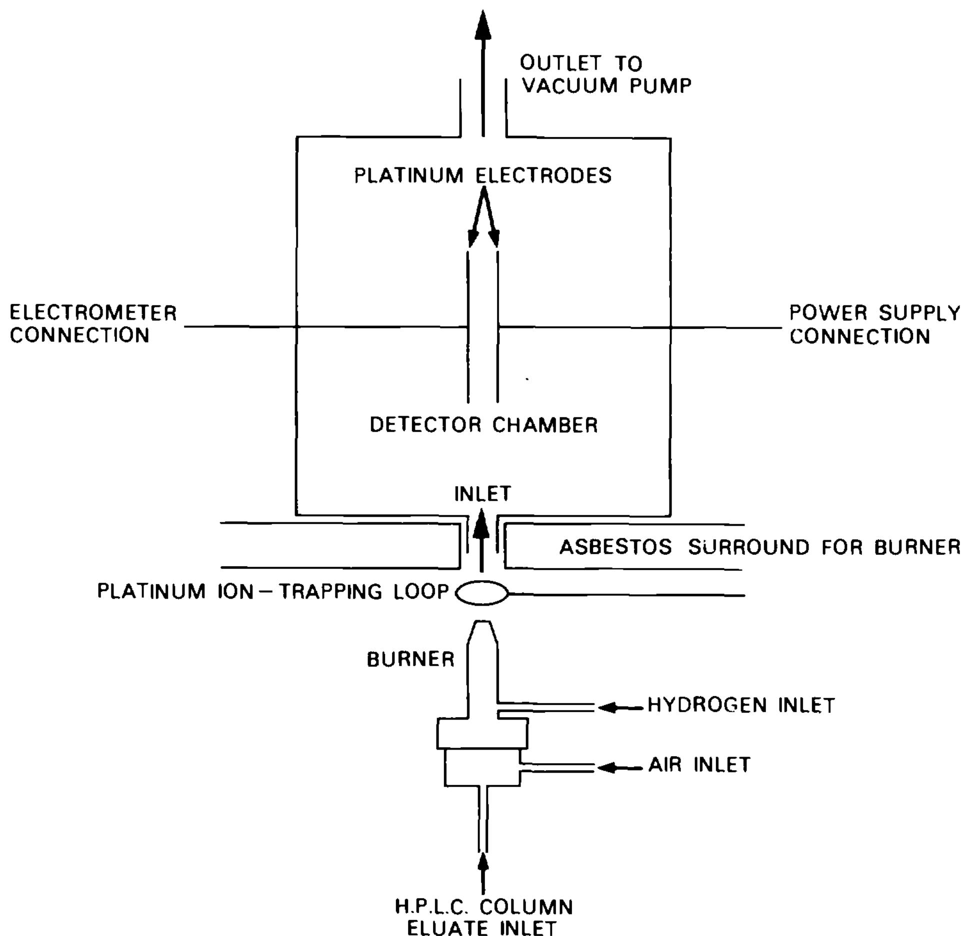


FIG 6 THE FLAME AEROSOL DETECTOR

It is a wise precaution to pre-filter all solvents through a glass sinter or 'Millipore' filter and to incorporate a sintered stainless-steel frit on the solvent inlet in order to remove dust particles which would otherwise slowly block pipes and valves within the chromatographic system.

3.6.2 Pulse Dampers

Reciprocating pumps produce pulsating flow which can cause two main problems. Firstly, certain types of detection systems are pressure-dependent so that surges in the flow cause regular baseline noise. Secondly, pressure surges within the chromatograph column can cause 'unpacking' of the column media unless the correct precautions are taken. Some method of suppression of the pulsations is therefore required. Various methods are available and have been reviewed[B]. One of the simplest and most effective is the use of a Bourdon tube gauge although frequently, with columns of microparticulate media, pulse dampening, is not required at all since the column itself provides a high resistance and, therefore, itself suppresses the pulsations.

3.6.3 Columns

The most common column-diameter employed is $\frac{1}{4}$ inch for analytical work, although $\frac{1}{8}$ and $\frac{3}{8}$ inch columns are also available. For preparative-scale work $\frac{1}{2}$-inch and 1-inch columns are used. The material of construction is most popularly stainless steel with internally polished surfaces although there is some controversy concerning the necessity

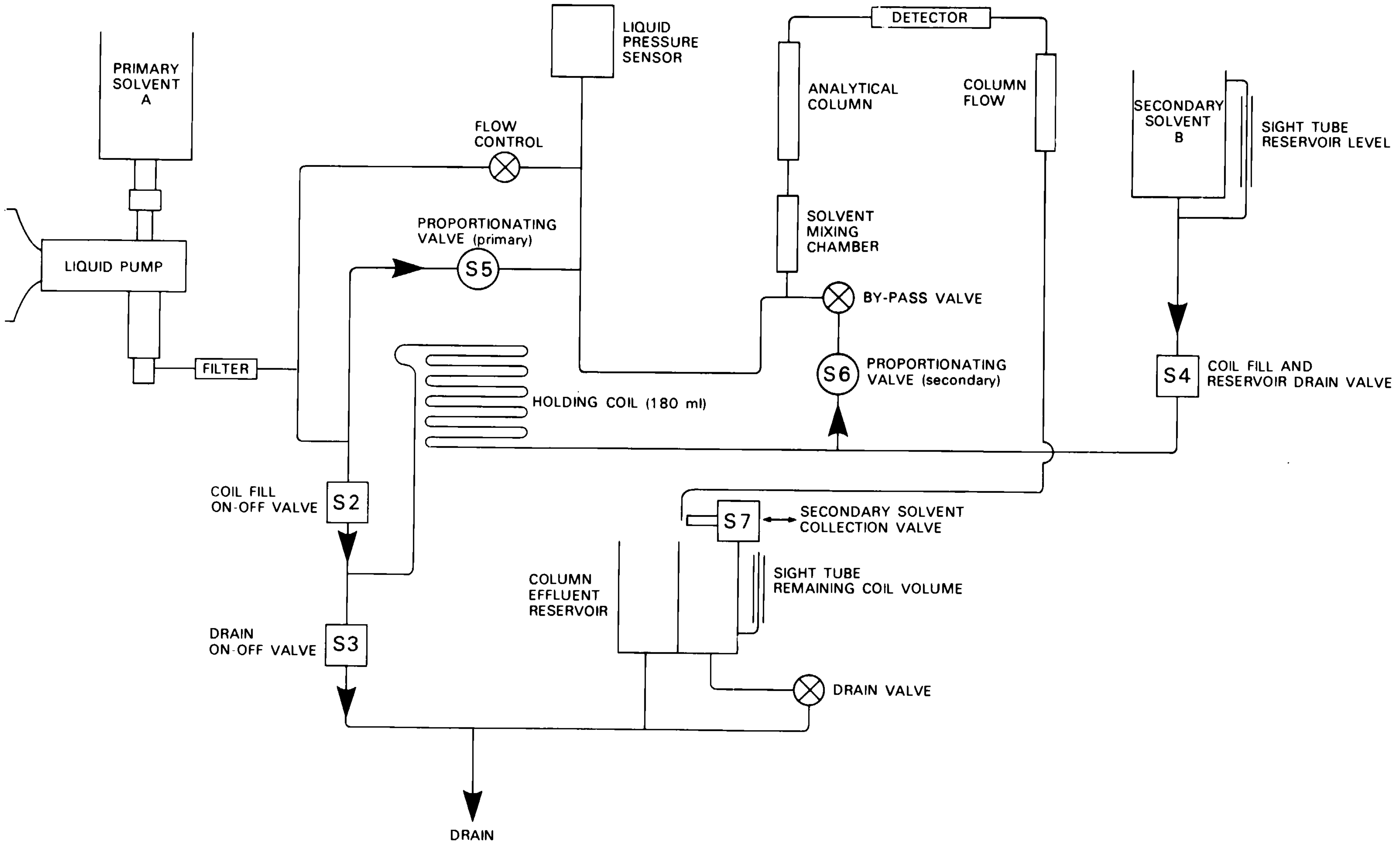


FIG 7 HIGH PRESSURE GRADIENT SYSTEM, (DUPONT CO LTD)

for this. It is claimed that glass columns (maximum pressure rating 500 psi) give significant improvement in performance because of the reduction of wall effects[65]. The use of glass-lined stainless-steel columns increases pressure ratings considerably. The most common column-lengths now employed are 10 cm (for microparticulate media) to up to 50 cm (for pellicular column media). It is now felt that, in a longer colunm, wall effects offset the increased efficiency obtained from the greater number of theoretical plates when efficient point-sources injection techniques are employed. Column media are usually retained by use of sintered stainless-steel or porous Teflon frits. Trends in column design have recently been reviewed [66].

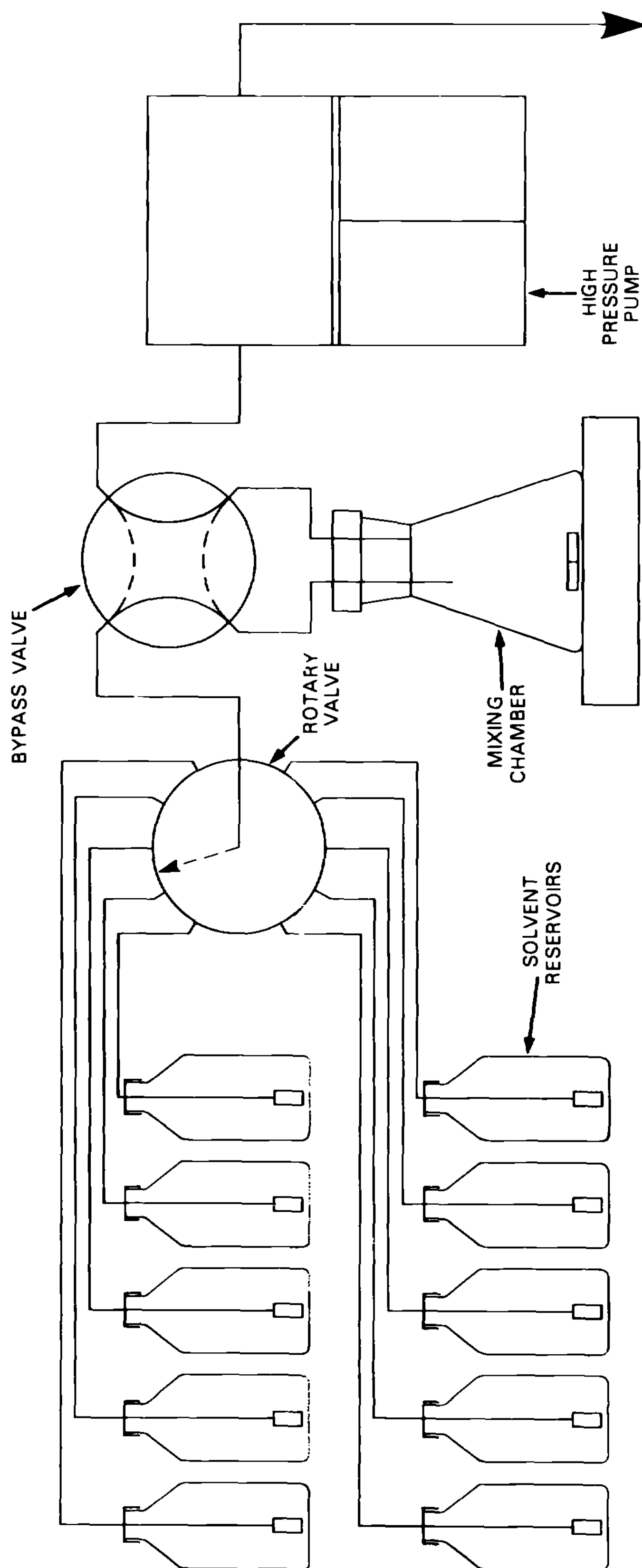


FIG 8 SYSTEM FOR MULTIPLE SOLVENT GRADIENT ELUTION IN HPLC

3.6.4 Compression Fittings

There is unfortunately a very wide range of compression fittings on the commercial market — almost as many as there are manufacturers of equipment. This can give rise to considerable problems when a modular HPLC system is assembled using pumps, columns or detectors, etc from different manufacturers since a much greater range of spares must be stocked. The situation is aggravated by the availability of certain types of compression fittings in both American and metric sizes.

If at all possible, it is best to standardise on one or, at most, two types.

3.6.5 Temperature Control

Most liquid chromatographic separation can be carried out efficiently at ambient temperature and there is little to be gained by operating at elevated temperatures. Some improvement can be affected, however, when viscous solvents are employed, in terms of both increased speed and efficiency of separation, and some equipment is commercially available which offers the option for operation at elevated temperatures.

Frequently of greater concern are small temperature fluctuations which can cause detector instability particularly at sensitive gain settings on the amplifier. In these situations it is usually sufficient to surround the column by lagging or a water jacket. Alternatively the entire column can be placed in a waterbath.

3.6.6 Fraction Collectors and Samplers

A very wide range of suitable equipment is available on the commercial market. Fully automated systems for sampling and analysis of an effluent stream on a semi-continuous basis[67] and for collecting fractions[68] have been described.

3.7 The Mobile Phase

In liquid chromatography, the mechanism by which separation is achieved depends upon the relative selective affinities of the components of the mixture for the stationary and the mobile phases. Thus, unlike GC, the mobile phase plays an important role in the separation process. The solutes must firstly be soluble in the mobile phase, which in turn must provide the correct degree of interaction, in conjunction with the stationary phase, on the solute molecules.

A further most important consideration in choosing the mobile phase is that it should have minimum effect on the detection system. The solvent is present in such excess that even small responses may be magnified and fall outside the dynamic range of the detector. A typical example is detection in the ultra-violet wavelengths where solvents with the correct cut-off, below the monitoring wavelength, must be chosen.

The most important property of a solvent in its effect on the separation process is that of polarity. Non-polar solvents such as hexane are those which contain no localised areas of high or low electron concentration in their molecular structure. Polar solvents such as the alcohols exert a dipole moment because they contain functional groups which cause localised electron concentrations. It is common practice to classify the solvents used in HPLC according to their polarity. In situations where precise polarity is required, this may be achieved by careful blending of suitable solvents.

Other important physical properties of solvents which can affect chromatographic separations are viscosity, compressibility, refractive index, vapour pressure, flash point, and threshold limit value. These factors have been comprehensively considered in many review articles [69-71].

3.8 The Stationary Phase

There are generally five modes in which HPLC may be operated. Each depends upon a different mechanism which provides a means of selective interaction between solute and stationary phase. They are adsorption, partition, ion exchange, ion pair and gel permeation chromatography. This variety of operational modes provides greater flexibility and allows the separation of a wider range of structural types than is possible by GC.

One of the major causes of the great advances made recently in the development and application of HPLC has been the continuous improvement in the efficiency of column packing materials, and this subject has recently been reviewed[66].

3.8.1 Adsorption Chromatography

Classical column chromatography as practiced for many years made use of porous packing materials (silica or alumina) comprised of particles of relatively large and irregular diameter (greater than 100 μm). Such media are not suitable for use in HPLC because the inherently slow mass transfers involved lead to significant band broadening, particularly at high solvent flow rates. This effect is caused by the deep cavities within the media and the interstitial spaces between the particles. However, the high surface area of such porous media (200 to 400 m^2/g gives them high sample capacity which is particularly useful for preparative chromatography or for situations where detectors of low sensitivity are employed, requiring the application of larger samples. In an attempt to overcome slow mass transfer effects, media of controlled surface porosity (pellicular beads) were developed[72]. These consist of solid non-porous cores (usually of glass and approximately 40 μm in diameter) surrounded by a thin porous outer shell (approximately 1 to 2 μm in diameter). Because there is only a thin layer of active porous material available, the rates of mass transfer of solute from mobile to stationary phase and vice versa are improved, with the result that higher solvent flow rates may be employed with little loss in efficiency. However, at the same time the absorptive capacity of the stationary phase is drastically reduced and for this reason pellicular media are now considerably less popular.

The adsorptive capacity of a column may be improved without loss of efficiency by, in effect, removing the inert core within the particle. More recently therefore, microparticulate porous particles with no inert core and diameters down to 3 μm have been developed[73]. Usually sold in pre-packed columns but also sometimes available in the free form, they give high efficiency with large sample capacity.

In Table 2 the newer types of silica available for high performance liquid chromatography are listed, together with some of their more important properties. A third type, not previously mentioned, has been included. This is the porous irregular type of particle, usually between 20 and 40 μm in diameter. It is generally manufactured by sieving and sizing of the classical types of liquid chromatography adsorbants and provides a cheap and relatively efficient packing material where larger amounts are required, eg in preparative-scale chromatography.

Recently porous irregular particles have been made available which are of narrow size distribution in the 5–10 micron range and which, it is claimed, are as easily packed as spherical particles. There is some debate as to whether irregular particles give as high efficiencies as spherical particles but in any case difference are likely to be small.

The operating pressures quoted in Table 2 are approximate values required to achieve reasonable flow rate (1-3 ml/min) through an average length (25 to 30 cm) column of acceptable diameter (2 to 4 mm). Because of continued improvements in the design and performance of high-pressure pumps, the resistance to flow of chromatography columns is not now as important as in the past; however, low-pressure pumps are cheaper and the practical problems are less severe when working with colums of low flow resistance.

In adsorption chromatography, separation is effected because of the different adsorption affinities of the stationary phase for the solute molecules. Affinity increases with polarity of the solute and so compounds are eluted from the chromatographic column in order of increasing polarity. Increasing or decreasing the polarity of the mobile phase can also either speed up or retard, respectively, the elution of the various compounds. These factors are demonstrated by the chromatograms shown in Fig 9.

There are several important papers and reviews available which discuss the importance of recent developments in stationary-phase technology[74,75].

3.8.2 Partition Chromatography

When two immiscible phases are in contact, solute molecules will partition between them in a ratio known as the partition coefficient. This is also the mechanism at work in partition chromatography in which a liquid is coated onto an inert support and an immiscible mobile phase percolated through it. Separation of a mixture depends upon the

components having sufficient differences in their partition coefficients. Normal phase partition chromatography involves the application of non-polar solvents across polar stationary phases, and in reverse phase partition chromatography polar solvents across non-polar phases. The order of elution of the components of a mixture separated by the two methods is therefore reversed.

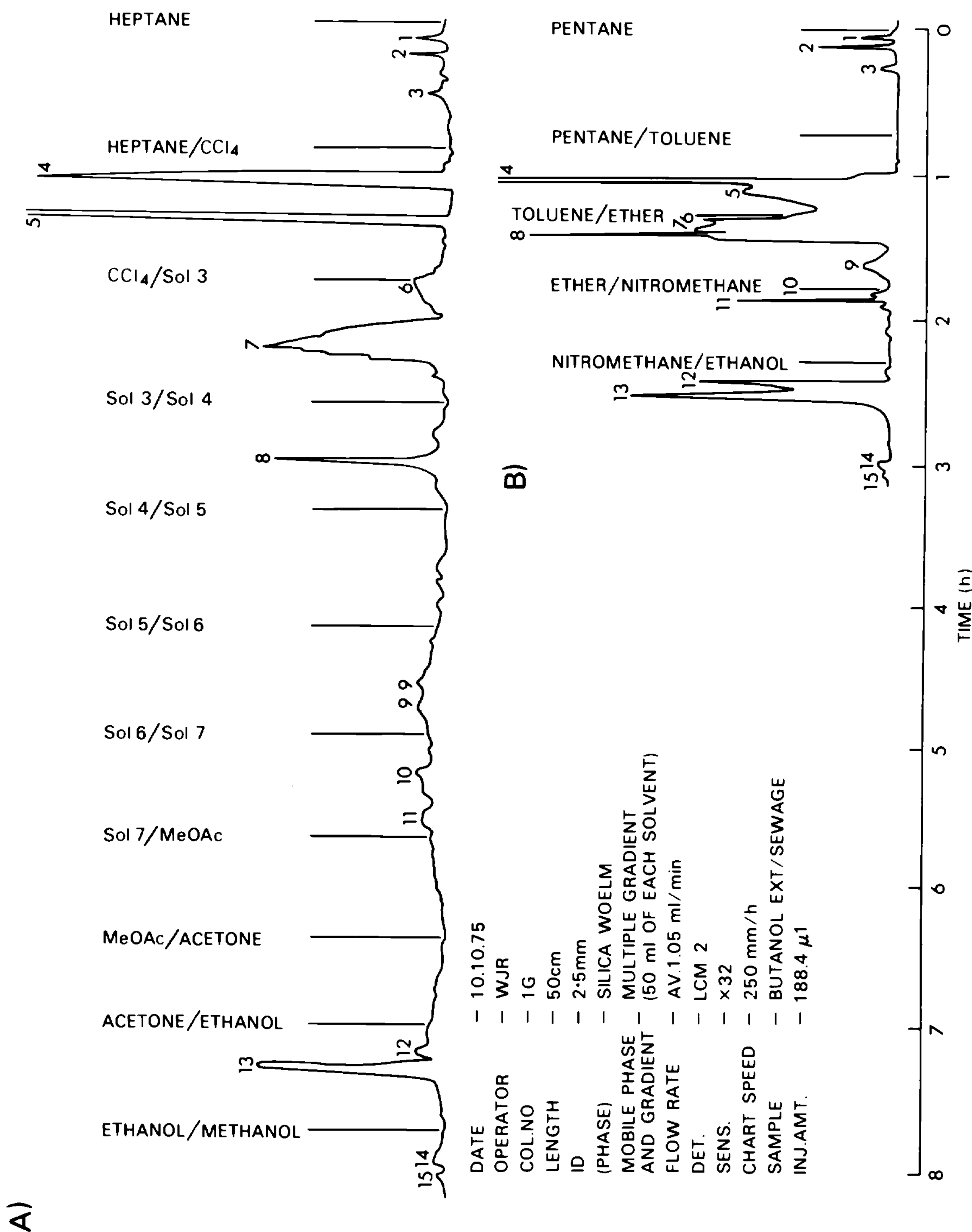


FIG 9 SEPARATION OF A BUTANOL EXTRACT OF SETTLED SEWAGE ON A SILICA ADSORBENT USING GRADIENT ELUTION AND EMPLOYING THE SOLVENT SERIES OF A) SCOTT AND KUCERA (1979). B) SNYDER (1967)

Original work in the field of partition chromatography made use of stationary phases which were merely physically adsorbed on the surface of the inert support. However, although the two phases are to all intents and purposes immiscible, a certain degree of mutual solubility inevitably exists which can lead gradually to stripping of the stationary phase. For chromatographic repeatability it is important that the relative proportion of stationary phase should remain constant. Usually therefore, the mobile phase was pre-saturated with the stationary phase before passage onto the column. Unfortunately, however, solubility is temperature-dependent and so it was important to maintain uniformity of temperature within the saturation device and also on the column. In practical terms this was difficult to carry out.

Table 2. Types of silica for high- performance liquid chromatography

Type	Pellicular (porous-layer on solid bead)	Porous	Porous
Shape	Spherical, regular	Irregular	Spherical, regular
Average particle size	40 μm (layer 1 um)	20–40 μm	<20 μm
Particle-size distribution	Narrow	Wide	Very narrow
Packing ability	Easy (dry pack)	Difficult (slurry method)	Easier (slurry method or dry pack)
Operating pressure	⩽1000 psi (70 bar)	⩾2000 psi (140 bar)	⩽250 psi (17 bar)
Capacity	0.1 mg/g	5 mg/g	5 mg/g
Efficiency (H)	0.2–0.3 mm	0.05–0.4 mm	0.01–0.03 mm
Cost	Expensive	Cheap	Expensive

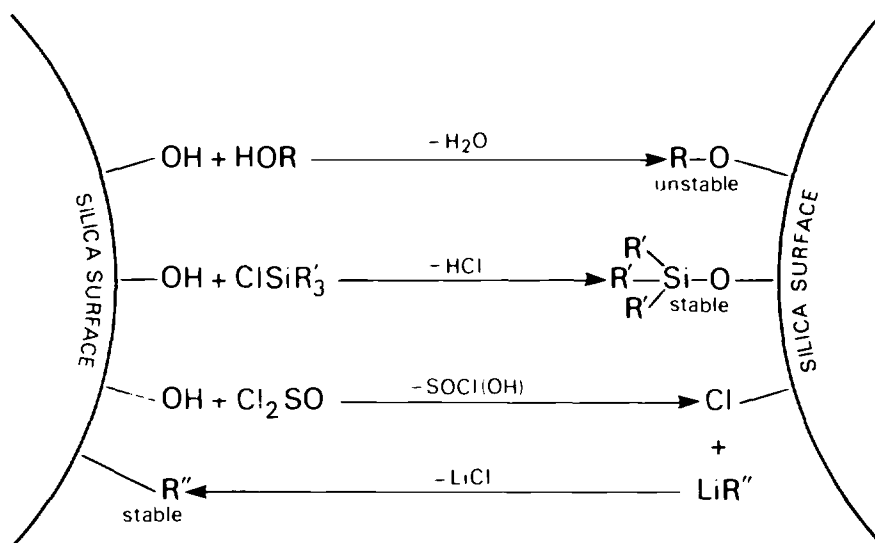


FIG 10 REACTIONS EMPLOYED IN PRODUCTION OF PARTITION PHASES

These problems have been overcome by the development of chemically-bonded media in which the stationary phase is linked permanently to the support by a chemical linkage(76). Various useful reactions by means of which this may be achieved are shown in Fig 10. With the exception of the R-O bond, the chemical linkages are reasonably stable within the pH range of 2 to 8 but are liable to hydrolysis outside this range. By using this type of reaction, monolayers of stationary phase may be attached having various structures and therefore providing different chromatographic properties. Phases currently available include octadecyl, phenyl, alkyl nitrate, propyl cyanide, ether, alkyl amine, trimethylsilyl, and silane diols. These types provide a complete range from non-polar to highly polar phases. Techniques for preparing bonded phases within columns already packed with adsorbents have been described(77). Papers giving more detail about the development and application of bonded-phase media are available(75,76,78,191).

In recent years partition chromatography has become so popular that it is adopted as the mode of separation in approximately 70% of all reported methods. Its popularity is due to its relatively wide applicability, the speed at which columns may be re-equilibrated, and the relatively simple choice of mobile phases. In fact the most difficult aspect of this mode of chromatography is choice of the correct stationary phase since the nature of the silica support and the technique used to produce the bonded phase can cause significant differences in the properties of similarly labelled commercial columns. The silica support material may differ in its particle shape and surface area and in its particle size and distribution as well as pore size and distribution. The silylating reagents used to coat the silica particles with a bonded mono-layer may differ in their alkyl chain length, the end functional group, eg methyl, cyanide, primary amide, phenyl etc, as well as the degree of crowning or capping of unreacted silanol groups. Unless reaction conditions are closely defined and controlled, considerable differences in properties of bonded phase partition

columns may be unwittingly produced. This is generally so in the case of columns of similarly coated phases from different manufacturers. All such columns should be chromatographically characterised before use.

3.8.3 Ion Exchange Chromatography

Ion-exchange resins depend upon ionic interactions for their powers of separation. Polar functional groups attached to an insoluble resin matrix competitively interact with charged solute molecules, and the order of elution is dependent upon the strength of the ionic bond. In ion exchange chromatography, retention time of a solute can be dependent upon several factors. These are the size and charge of the solute ion, the pH of the mobile phase, the concentration and nature of salts dissolved in the mobile phase, the presence of organic modifiers in the mobile phase, and finally the temperature of the column.

Classical ion-exchange resins based on divinyl benzene-polystyrene structures have a limited application in high-pressure work because they lack rigidity and can be seriously compressed at pressures greater than 1000 psi. More efficient and rigid media have been obtained by attaching the appropriate functional groups of various organic substances by chemically bonding them to the stationary phase. As with adsorption media, coating can be carried out with both pellicular[75,76] and microparticulate media[75,76,79]. The "state of the art" of ion-exchange chromatography has recently been reviewed[80].

3.8.4 Ion Pair Chromatography

This form of chromatography relies on the supposed neutralisation of polar functional groups in the solute molecules by a suitable ionic species so that they may be separated by partition chromatography as neutral species rather than the more normal ion exchange chromatography. The former is now rather more convenient to employ than the latter since it is not always easy to predict the behaviour of ion-exchange columns in certain separations. This is due to spurious adsorption and partition effects which may play some part in the separation process. A suitable paired-ion chromatography (PIC) reagent, which does not interfere with the detection system employed, must, however, be available. For compounds with both acid and basic functions, a suitable procedure frequently adopted is to employ a cationic PIC reagent (tetrabutyl ammonium chloride) to neutralise, say, strongly acidic sulphonic acid groups. In order to suppress the ionisation of the basic function, the PIC reagent is dissolved in a slightly basic mixed phosphate buffer solution. The nearly neutral species thereby produced will probably have a significant retention time on a reverse-phase partition column, whereas the parent molecule would undoubtedly be washed through with the solvent peak. For simpler acidic or basic species, quaternary amines and alkyl sulphonates, respectively, are suitable counter-ions for formation of neutral ion pairs.

A full theoretical discussion on the mechanism of action of PIC reagents in HPLC is available[81]. This is a subject about which there remains some uncertainty and controversy[82].

3.8.5 Gel Permeation Chromatography

Gel permeation or steric exclusion chromatography is a technique which allows molecules to be separated according to their molecular size and shape. Columns are packed with porous media which contain capillaries of controlled diameters which act somewhat like a molecular sieve. When a sample of a mixture is introduced and eluted through the column with a solvent, the smaller solute molecules are able to penetrate pores in the stationary phase and are hence held back while large molecules continue through the column. Thus, the largest molecules emerge from the column first followed by progressively smaller molecules. The mechanism operating is therefore entirely one of reversible diffusion into and out of capillaries, and it is the differentials in volume which are created by the presence of the capillaries which are available to solute molecules of different sizes which lead to effective separation. Ideally no other interaction of mobile and stationary phase should occur, ie adsorption or ionic effects.

After a sample has been injected onto a column, the volume of eluting solvent collected between injection and the point at which unretained molecules appears is known as the "void volume" and is equivalent to the intersitial volume, ie the volume between the stationary-phase particles packed into the column. The very large unretained solute molecules are followed by progressively smaller molecules and the elution volume over

which molecular separation takes place is equivalent to the pore volume of the stationary phase. A medium with a specific pore size will allow the separation of components over a specific and limited molecular size range. It is often necessary therefore to couple together in series columns containing media of different pore sizes in order to increase the separation range. Gel permeation columns may be calibrated so that elution volume can be approximately related to molecular weight. This is best achieved using standards of a similar nature to the compounds under investigation and plotting their elution volume against the log of their molecular weight, in the form of a calibration curve.

The technique has frequently been applied to the separation or "molecular weight profiling" of the organic matter in environmental waters but the media employed have generally been of the non-rigid gel type and so the work cannot be truly classed as a high performance separation. The 'state of the art' has recently been reviewed[83].

4. Methods

4.1 Gradient Elution

In situations where it is required to separate organic compounds containing a wide range of polarities, isocratic elution with a single solvent system may not elute all of the solute components. Thus a second solvent system with greater affinity for the solute molecules is required to complete the elution. The two solvent systems may be applied discreetly in two stages (stepwise elution) but a better procedure is gradually to increase the concentration of the stronger solvent in the weaker (gradient elution). The process is analogous to temperature programming in GC. The increased efficiency of separation is apparent from Fig 11 which compares isocratic elution for a sample mixture with gradient elution. The technique may be applied in adsorption, ion exchange, and partition chromatography.

In adsorption chromatography, the work of Scott and Kucera[84] has shown that for satisfactory gradient elution development it is necessary to employ a large number of solvents having small differences in polarities (Table 3) and not to attempt a separation

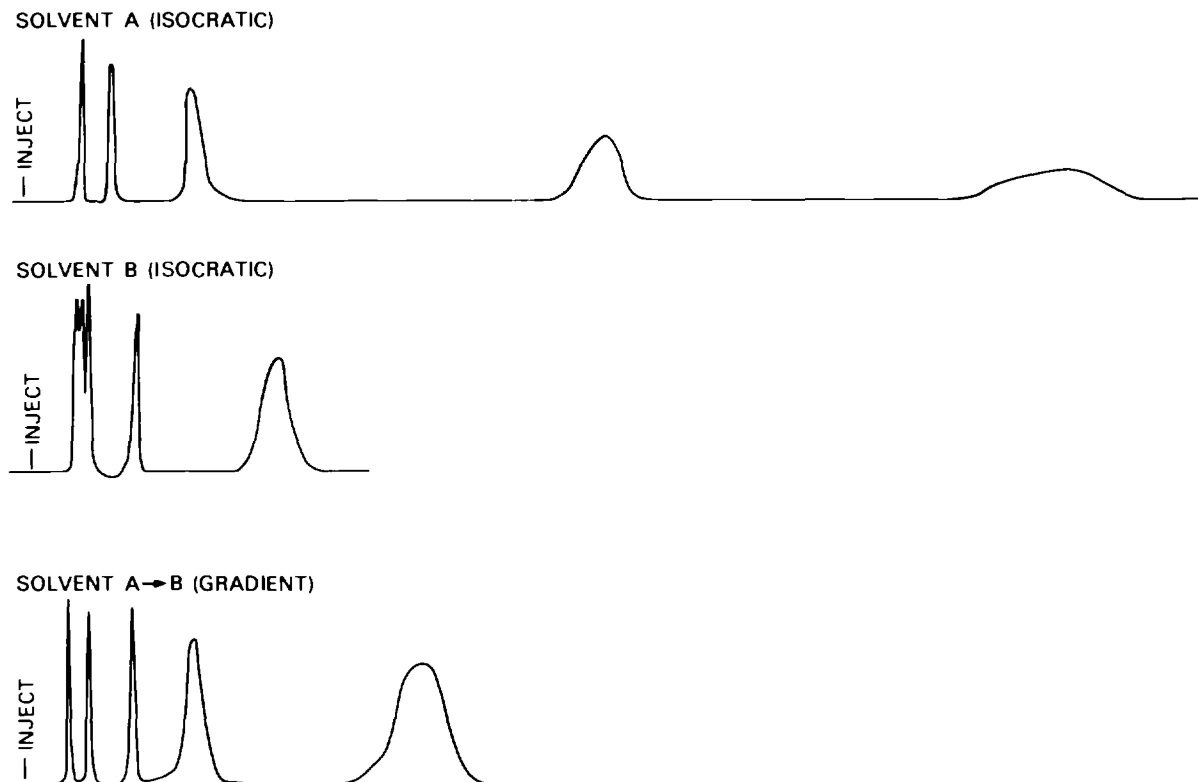


FIG 11 COMPARISON OF ISOCRATIC WITH GRADIENT ELUTION

using two or three solvents having large differences in polarity. Snyder[70] agrees with these findings but is somewhat critical of certain aspects of the solvent species proposed by Scott and Kucera, and proposes a simpler sequence (Table 3). In order to understand the basic reasons behind this approach, the processes that occur during gradient elution must be considered. Fig 11 depicts diagrammatically the development processes resulting from gradient elution by the method of mixing. It represents the effect of a linear gradient using three solvents, a non-polar solvent 1 (eg heptane), a semi-polar solvent 2 (eg butyl acetate), and a strongly polar solvent 3 (eg methanol). After injection of the sample, solute A emerges close to the dead volume of the column since it has greater forces exerted upon it by the mobile phase than by the stationary phase. No further solutes are eluted during the period of this constant-composition elution, since all the components constituting the rest of the injected sample have stronger forces exerted upon them by the adsorbent than by the mobile phase. However, when the semi-polar solvent 2 is introduced into the mobile phase the non-polar solvent 1 is immediately displaced from the adsorbent, resulting in its partial deactivation. Thus the solutes become much less strongly bound to the adsorbent which becomes saturated with the new solvent 2 when its concentration in the mobile phase is still relatively small, and therefore during a period corresponding to the passage of a relatively small volume of solvent from the time of commencement of gradient development. This is demonstrated by the fact that, when applying a heptane-isopropanol gradient on silica gel, the silica is found to be virtually saturated with isopropanol when its concentration in the heptane is still only about 2 per cent. Thus, as shown in Fig 12, two solutes, B and C, are eluted very close together. This is due to the rapid deactivation of the adsorbent during the short period that the mixed solvents are passing through the bed. This effect is known as the displacement effect of the gradient elution since B and C have in fact been displaced from the adsorbent by the solvent 2 together with the first solvent 1. During the subsequent period of gradient elution, solutes D and E are eluted discreetly and well resolved. This is because the relative forces between the solutes on the two phases are changing gradually and during the passage of a relatively large volume of solvent through the column. The second displacement and elution effect moving from the semi-polar to the polar solvent are entirely analogous.

Table 3. Rational series of solvents

Solvent	Scott and Kucera[79]	$\frac{k'(n)}{k'(n+1)}$ (Av, values)
1. n-Heptane		2.34
2. Carbon tetrachloride (CCl_4)		3.22
3. CCl_4 (57.8%) + chloroform ($CHCl_3$) (42.2%)		3.34
4. CCl_4 (36.1%) + $CHCl_3$ (26.3%) + ethylene dichloride (EDC) (37.6%)		2.44
5. CCl_4 (19.9%) + $CHCl_3$ (14.5%) + EDC (20.7%) + 2-nitropropane (2–NP) (44.9%)		2.06
6. CCl_4 (14.4%) + $CHCl_3$ (10.5%) + EDC (14.4%) + 2–NP (32.4%) + nitromethane (27.8%)		2.06
7. Nitromethane (36.3%) + propyl acetate (63.7%)		1.72
8. Methyl acetate		1.56
9. Acetone		1.96
10. Ethanol		2.48
11. Methanol		2.48
12. Water		2.48

Snyder[67]

Solvent	Adsorption energy (ϵ_o)	Viscosity (cP, 20°C)	UV cut-off (nm)
1. Pentane	0.00	0.23	210
2. 2-Chloropropane	0.29	0.33	225
3. Diethyl ether	0.38	0.23	220
4. Acetonitrile	0.65	0.37	210
5. Ethanol	0.88	1.20	210
6. Water	v. large	1.00	—

In order to separate an unknown mixture of solutes of wide polarity range it is fairly obvious from these arguments that the polarity of the mobile phase must be changed gradually using an extensive range of solvents, for if a limited range is used (eg 3 or 4 solvents) then the displacement effect at each solvent change can result in poor resolution or no separation at all.

As discussed in Section 3.7, there are certain practical limitations which must be taken ito account before considering the chromatographic properties of the various possible solvent choices. The solvents used should be readily available, either pure or easily purified, inexpensive, and have low viscosities for increased rates of mass transfer. They should also be compatible with the HPLC detector used. It is unfortunate that all suitable solvents having a medium polarity appear to absorb strongly in the UV frequencies. This would tend to restrict the use of the UV detection system for this type of work.

This problem has largely been overcome by using blends of isopropanol and heptane of the correct polarity as well as other non-adsorbing solvent mixtures, in place of the combinations shown in Table 3[85].

The work of Scott and Kucera[84] established a simple rule for choosing a rational series of solvents in order to restrict the number to a practical limit. The work has shown that the corrected retention volume of any solute chromatographed in solvent n in the series should be between 2 and 3 times that when it is chromatographed in solvent n + 1 in the series. This expression is shown in Table 3 in terms of partition coefficients (k'), together with average values obtained by chromatographing a solute or the solvent next in the polarity series finally chosen. In practice, three values for each solvent were usually obtained because the following three solvents in the series could usually be eluted when injected on column, or a particular solute could be eluted by three consecutive solvents in the series.

Snyder[70] has also listed the general requirements of the solvent programme for gradient elution. His work suggests that far fewer than the 12 solvents of Scott and Kucera are required to span the solvent strength range between heptane and water without encountering "solvent demixing" (which gives rise to displacement effects) as a problem. He suggests a reduced list of solvents chosen so that a UV detector at wavelengths as low as 230 nm may be operated. The effect of a twelve and six-gradient multiple programme of the Scott and Kucera and Snyder types on the separation of a complex extract is shown in Fig 9.

In situations where mixtures of organic compounds of relatively small polarity differences require separation, partition chromatography is now the preferred method. Because of the advent of bonded-phase media such systems are much simpler to use and also since only 2 solvents are generally employed and re-equilibration can be carried out more reliably, they are ultimately more reproducible.

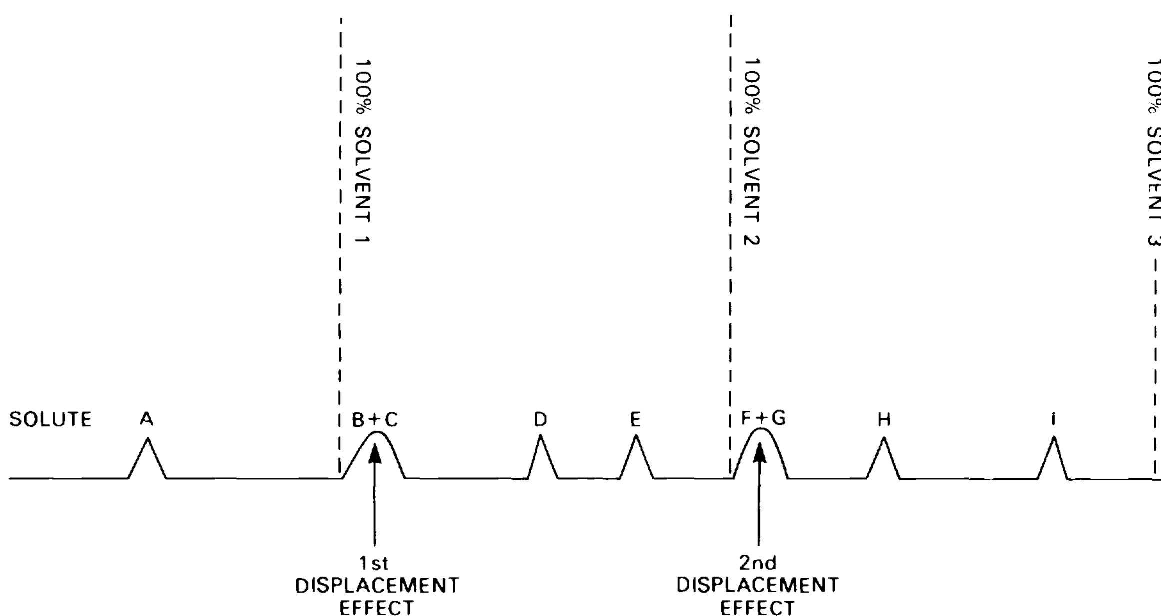


FIG 12 DIAGRAMMATIC REPRESENTATION OF THE EFFECT OF GRADIENTS, USING SOLVENTS OF WIDELY DIFFERENT POLARITIES

4.2 Choice of Stationary Phase

Presented with a particular problem in developing a separation technique based on HPLC, the analyst relies mainly on experience in his selection of a column most likely to yield results. However, the inexperienced chromatographer can apply certain basic guidelines in making his preliminary choice. Assuming a certain amount of basic knowledge on the nature of the sample and its solubility, a guide to the type of column which can best be employed may be determined by reference to the scheme shown in Fig 13. From thereon, however, choice of particular phases is much more difficult. For example the selectivity differences between octyl, octadecyl, and propyl cyanide stationary phases used in reversed-phase chromatography are very difficult to predict. Also there is no guarantee that the same phases produced by different manufacturers will yield similar results. For example, in the case of partition phases, the degree of coating and the extent of capping of unreacted silanol groups on the inert support can significantly affect chromatographic properties.

A compilation of all the major chromatographic support materials is published by United Trade Press Limited[86].

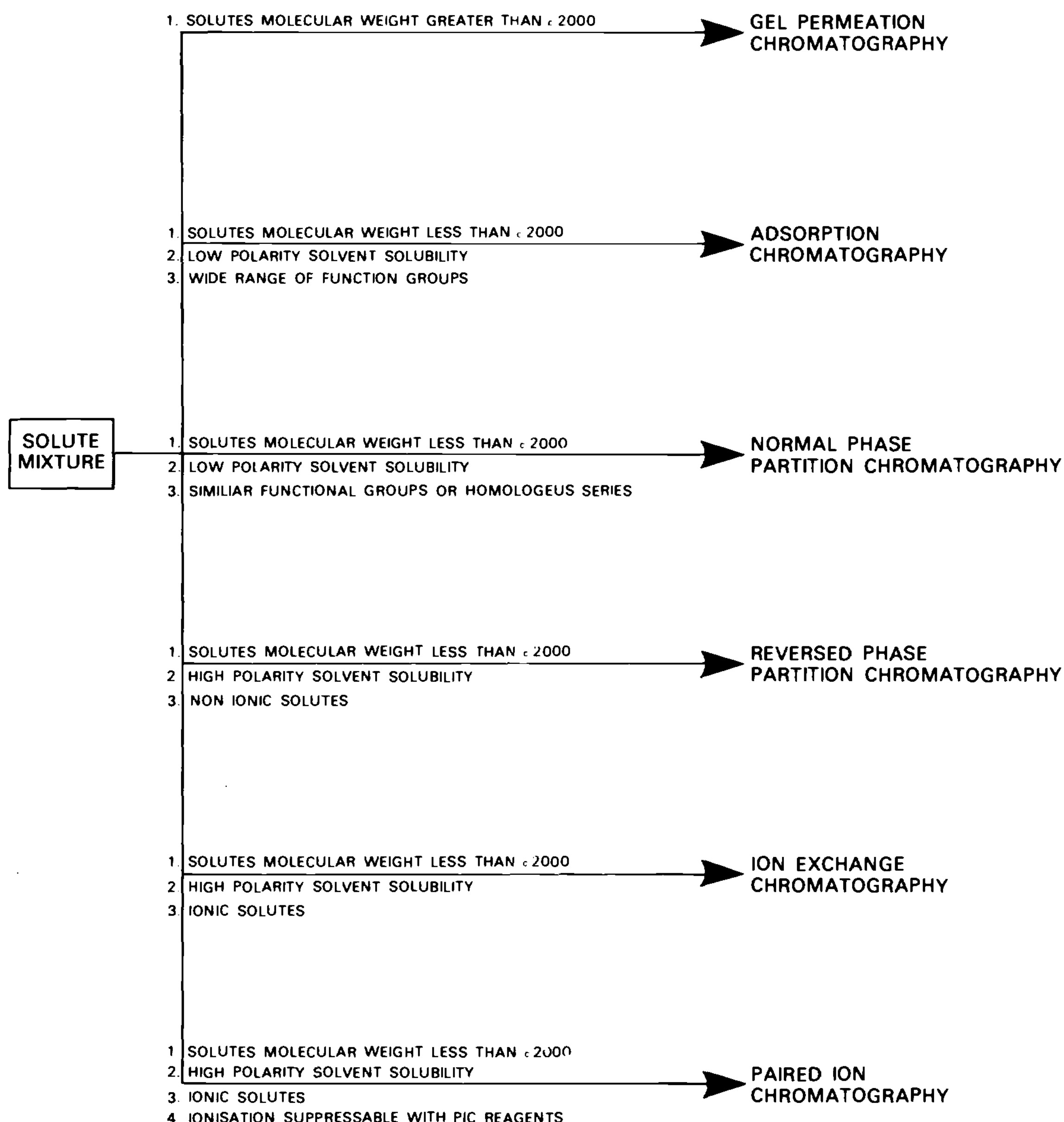


FIG 13 GUIDE TO CHOICE OF THE BEST STATIONARY PHASE

4.3 Column Packing Techniques

4.3.1 Introduction

When applying HPLC in the field of water pollution analysis, the ability to prepare one's own columns may well be of paramount importance. Prepacked columns obtained directly from the manufacturers are unduly expensive (£150–£400 sterling each at 1981 prices) and, with the occasional introduction of a sample extract which irreversibly contaminates the stationary phase, the effective working lifetime of the column may be significantly decreased and drastically increase the expense of the analysis. By itself the stationary phase is very much less expensive (£4–£81/g at 1981 prices) and significant savings can be made if column packing facilities are available. Since the procedures generally adopted are not difficult to follow, this course of action is recommended.

In every case the aim of a column packing procedure is to produce a regular distribution of the particle sizes within the bed, ie a homogeneous structure, since it is this factor which determines the ultimate efficiency of the column. There are basically two distinct methods of packing, ie wet and dry packing techniques. The choice of the appropriate method depends largely on the type of stationary phase. There is still much controversy about the techniques and arts of column packing and there are generally as many methods applied as there are operators in the field. However these are mainly variation on the two general themes described below.

4.3.2 Dry Packing Techniques

In this approach the chromatographic column is closed at one end and incremental additions of stationary phase introduced at the open end. Between each addition the column may be vibrated, bounced, rotated around its own axis and lightly tapped, with variations and combinations of all four processes either independently or simultaneously. When completely full a column is closed up.

4.3.3 Wet Packing Techniques

Wet packing techniques may be sub-divided into those which employ solvents of high viscosity, in which the stationary phase is slurried[14], and in which the particles of stationary phase sink very slowly if at all, and those employing a balanced density slurry[16] in which a mixture of solvents is employed, the relative quantities of each being adjusted until the density of the mixture is exactly the same as that of the stationary phase, so that particles neither sink nor rise. More recently a third variation has been introduced, in which mechanical agitation of the slurry is carried out during the packing procedure in order to keep it homogeneously mixed[87,88]. The solvent may therefore be chosen with factors such as the prevention of agglomeration of particles of the stationary phase and the future conditioning of the column in mind.

Other factors of considerable importance in carrying out wet packing techniques are the degassing of the slurry, the prevention of agglomeration by the addition of small quantities of a hydrophobic solvent, eg methanol (or, in the case of silica, the application of heat to remove surface water), the exclusion of air during the connection of the various components of the packing apparatus, and finally the application of a higher pressure during the packing procedure than will be subsequently used in the course of chromatographic analysis.

The various techniques of column packing have been reviewed[89].

4.3.4 Checking the Packing Efficiency

It is very important to check immediately the efficiency of a particular packing procedure in terms of the column's efficiency to separate various standard components (see Section 2), its reproducibility, and its permeability.

The chromatographic performance of the column may be tested by measuring the plate height or plate count of a routine test mixture of substances normally used to determine the decline in efficiency of the column during routine operation. It is beneficial to compare the results with those obtained from a manufacturer's prepacked column containing the same stationary phase.

In addition, by measuring the permeability of the column in terms of the linear velocity of a standard solvent at a certain column head pressure, it is possible to assess the packing quality and hence the reproducibility of the packing of different columns for the particular packing material used.

4.3.5 Summary

The best packing technique to adopt in terms of simplicity of operation and optimal efficiency is not easy to predict. However, there are several general rules which may be applied when considering the problems involved. These are:

i. Regular spherical media with narrow particle-size distribution and a particle diameter greater than approximately 20μm are relatively easy to pack and dry packing techniques may be generally employed.

ii. Porous irregular media with wide particle-size distribution are relatively difficult to pack and slurry methods, particularly balanced density slurry techniques, give best results. Very high efficiencies can still be achieved with this type of particle if great care is taken to obtain a homogenous distribution.

iii. Porous regular media with narrow particle size distribution and average particle size greater than approximately 20μm can still be fairly efficiently dry-packed although wet packing methods will generally give greater efficiency.

iv. Porous irregular media and regular spherical media with particle size less than approximately 20μm, even with narrow particle-size distribution, are packed with poor efficiency by dry packing procedures even though great care is taken. Better results are obtained with wet packing techniques.

In adopting a particular packing technique, the overall requirement of the analytical separation should be borne in mind. A packing technique which falls far short of achieving the optimum efficiency attainable by the stationary phase used may still be adequate in terms of the separation efficiency required from the analytical procedure. Such a column would therefore still allow the objectives of the analytical method to be achieved.

4.4 Sample Preparation

Various techniques of concentration, extraction and fractionation of organic compounds which may be applied to water samples as methods of sample preparation before chromatographic analysis have been reviewed[G]. It is, therefore, not necessary to describe these procedures here in detail. However, some techniques are more useful than others for the preparation of samples for HPLC separation and analysis. Those which isolate volatile organic fractions, eg head-space analysis and steam distillation, are generally not employed, since these fractions may be separated more efficiently by GC. The same is partially true for techniques such as liquid-liquid and synthetic-resin extraction. However, these can also isolate a high-molecular-weight fraction not amenable to GC analysis and therefore they may be usefully investigated also by HPLC procedures.

Some techniques are inherently capable of isolating or concentrating more non-volatile ranges of organic compounds. In this category are freeze drying followed by liquid-solid extraction, dialysis, ultrafiltration, and evaporative processes.

On the assumption that GC will be applied to the analysis of organic water pollutants which are sufficiently volatile and that HPLC will be applied to the remaining range of non-volatile compounds, some of the techniques mentioned which inherently are able to isolate non-volatile fractions, eg ultra filtration and dialysis, assume greater importance. A branch of liquid chromatography, namely gel permeation chromatography using soft gels, can also be applied to this end. Chromatographic profiles obtained from appropriate fractions are likely to be much simplified, non-volatile components being removed.

An alternative method of limiting HPLC to the separation and determination of truly non-volatile organic compounds is by choice of a suitable detection system. In the transport ionisation and mass detectors, volatile organic compounds are not detected, being volatilised in the course of a preliminary treatment stage for removal of the eluting solvent. Thus, only the relatively non-volatile organic compounds are detected.

A promising variation on synthetic resin concentration is its application in integrated extraction and separation techniques in which short pre-columns are used for direct

concentration of the organic material in water samples. These are then directly connected to an analytical column containing the same or similar medium and the entrapped organics are back-flushed onto the analytical column by application of a suitable solvent gradient. This technique is described in the following section.

As in GC, column switching techniques have been successfully applied to HPLC for organic trace analysis[90], in order to eliminate major interfering components, to isolate poorly resolved components for further resolution on a second stationary phase, and to aid separation of mixtures containing wide polarity or molecular weight ranges. Using appropriate columns, the method can effectively by-pass fractionation stages frequently separately applied in this work to obtain suitable extracts for analysis.

4.5 Enrichment Techniques

The application of HPLC for the determination of organic compounds in water samples is frequently limited by inadequacies of available extraction techniques. This limitation can be overcome by taking advantage of the order of elution imposed by reversed-phase partition chromatography. This allows the extraction and concentration of dissolved organic compounds from the water sample onto the media itself. This operation is usually carried out using a small precolumn incorporated into the system. When sampling is completed the precolumn is connected to the analytical column and entrapped material separated by applying a reversed-phase solvent gradient. Particular advantages gained by employing this system are the potential integration of sampling and analysis, fewer possibilities for introduction of artefacts, and the achievement of high concentration factors during sampling (depending upon capacity of the precolumn). The technique has potentially wide application as a screening technique and for the quantitative determination of known pollutants, but a potential weakness is the limited polarity range of organic compounds normally separable on a reversed-phase partition column.

Small columns for extraction of trace components from liquids, by displacement column liquid chromatography, have been frequently used. However the technique has not been systematically investigated theoretically or experimentally as an enrichment procedure until quite recently[91]. Many workers have established useful analytical techniques for determination of trace concentrations of water pollutants using the concentration of non-polar compounds which occurs on the head of a reversed-phase partition column when relatively large amounts of aqueous sample are introduced. This procedure was first discussed by Kirkland in 1974[92] and shortly after, Little and Fallick[93], using C18 reversed-phase columns, demonstrated the practicality of injecting very large volumes of up to 200 ml for preconcentration of relatively non-polar compounds without serious band broadening. Similar on-column enrichment techniques have been employed for determination of phthalate esters[94], and tetrachloroethylene[95], and for monitoring general pollution levels in various types of natural and process waters[96,97]. Schauwecker *et al*[98] and Frei[99] have discussed quantitative aspects and the influence of very large injection volumes on peak broadening, retention period, and base-line shift. Frei[94] also considers the problems and potential of the technique in relation to environmental analysis.

When applying the method to environmental water samples, the enrichment stage is carried out using the analytical column itself, a serious problem can occur, ie the gradual irreversible contamination of the stationary phase by components of the sample, with a resulting decline in efficiency of the column. If this eventually necessitates replacement of the column, use of the technique could become prohibitively expensive. It is better, therefore, to employ short replaceable precolumns or 'guard' columns for the concentration stage and subsequently to connect these to the analytical column. Methods for the determination of phthalate esters [94, 100, 101] and polynuclear aromatic hydrocarbons[102] applicable to water pollution analysis have been developed using separate stages of sampling and analysis. A similar method has also been used for the determination of sea-water-soluble fractions of oil[103]. The precolumn is loaded with sample either locally or at a remote site before it is introduced into the chromatographic system. Methods for the determination of polynuclear aromatic hydrocarbons[104,105], phthalate esters[100], photographic chemicals[106], optical brighteners[106], and acid blue dyes[106] using an integrated enrichment column have also been developed. As can be seen from the system shown in Fig 14[106], which also allows direct monitoring of the pre-concentration process, the technique requires a second pump and suitable high-pressure low dead volume switching valves. It also has the advantage that it does not require the routine and extensive replumbing of the systems required by other equivalent techniques.

Initial results employing PIC reagents suggest that they may be used to extend the scope of the technique to include polar organic compounds not normally retained on an octadecyl silyl (ODS) phase[106].

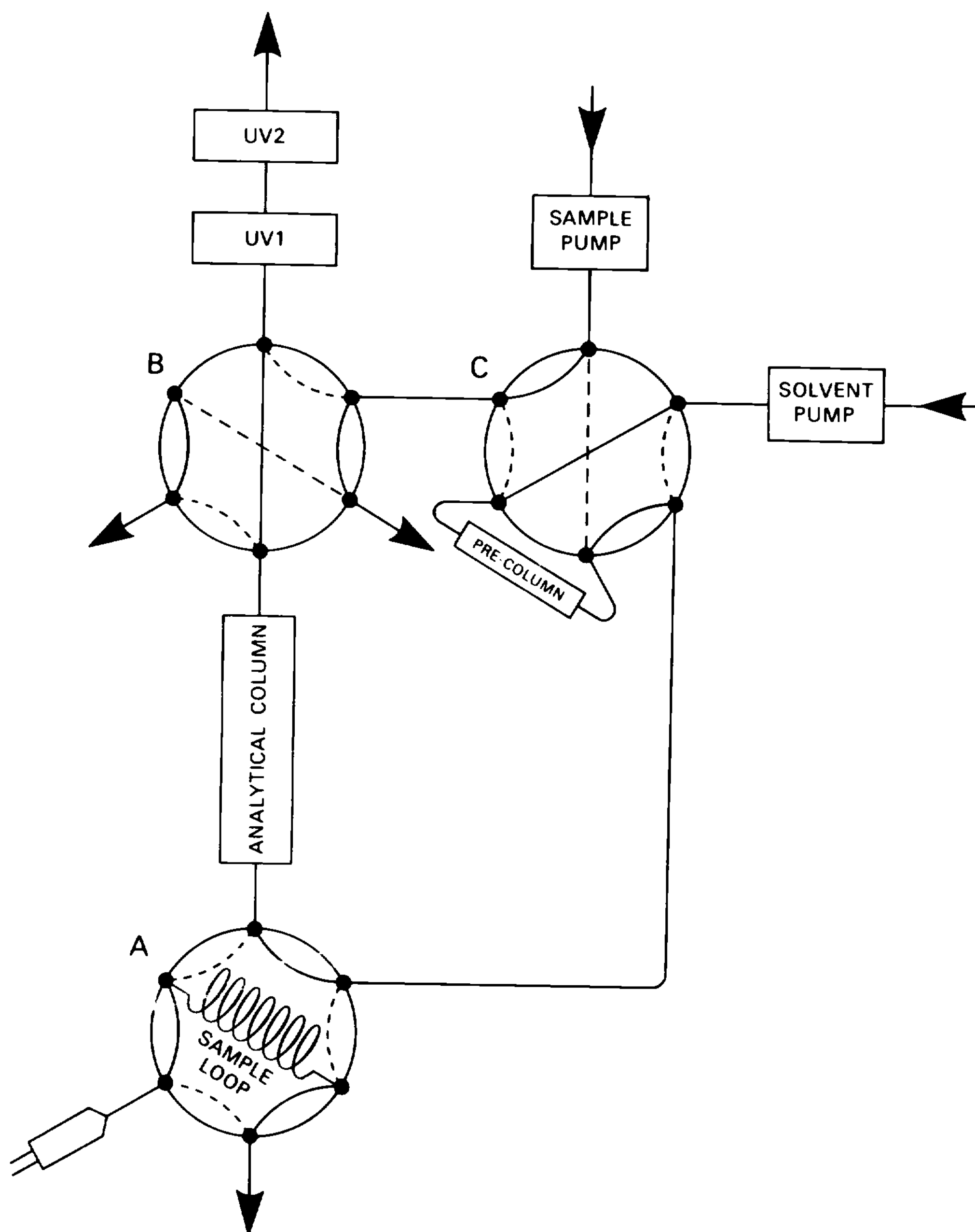


FIG 14 INTEGRATED SAMPLE CONCENTRATION AND SEPARATION HPLC SYSTEM: PRECOLUMN BACKFLUSH AND ANALYSIS POSITION SHOWN

5. Applications

5.1 Introduction

The early development of modern HPLC was mainly under the aegis of the pharmaceutical industry where it offered the possibility of the very rapid separation of relatively simple mixtures in drug formulations containing non-volatile and, at times, heat-labile organic substances. Instrumental design, therefore, reflected this requirement, with the result that it was not particularly suitable for application in the field of water pollution analysis where greater flexibility is generally required. The scope and flexibility of modern equipment is such, however, that there is no longer any restriction on the application of the technique to this field. This is also true in the area of development of new and improved stationary phases for HPLC. Early high-efficiency media tended to be of the pellicular variety and therefore of relatively low capacity, and unsuitable for the separation of trace amounts of organic pollutants in complex environmental extracts.

Modern media, however, are generally of the micro-particulate variety with greater sample capacity and therefore much more suitable for the analysis of environmental extracts.

A particular benefit has also been the development of a wide range of more sensitive detection systems, with types which give a highly selective response or a universal response now being available. In cases where highly selective detectors are available this can frequently mean a greater simplication in the sample preparation and purification stages. The development of sensitive universal detectors is of importance in accomplishing broad spectrum or fingerprinting analysis.

These developments have led to increasing application of HPLC to analysis in the field of water pollution and only such applications will be considered in this section.

5.2 Specific Methods

5.2.1 Polynuclear Aromatic Hydrocarbons

As a group, polynuclear aromatic hydrocarbons (PAH) are eminently suitable for separation by HPLC. Although with complex samples resolution can still be a problem, the application of available highly selective spectrophotometric detectors (particularly fluorescence detectors either singly[104,105,107] or several in series can help to overcome this[108]. HPLC has an advantage over GC particularly for the more non-volatile PAH. High temperatures are required to elute PAH of molecular weight similar to and beyond that of coronene, giving rising to problems of column bleed and poor peak shape.

The main approach in the analysis has almost invariably been to carry out a liquid-liquid extraction into a non-polar solvent (cyclohexane, hexane, etc), Kuderna-Danish evaporation, at times an alumina or floricil column clean-up, and finally separation by reversed-phase partition chromatography on an ODS-bonded stationary phase[109]. Hagenmaier and his co-workers, however, have used an octadecane bonded phase[104,105,110]. The technique has been widely applied to the analysis of environmental samples including river waters[109,111], finished and distributed waters[104,105,108,109], as well as municipal and industrial waste waters[107], landfill leachate[109] and bottom sediments[112]. UV detection generally allows limits of detection in the lower ng/l region, and with fluorescence detection, factors of ten to hundred lower than this have been achieved.

Hunt and his co-workers have studied the efficiencies of many column packing materials for the separation of PAH, including some suitable for normal phase partition separations. Using a specially prepared phthalimidopropyltrichlorosilane-coated microparticulate silica, they have separated the six WHO PAH standards[113].

5.2.2 Pesticides and Herbicides

HPLC has been extensively and fruitfully applied to pesticide and herbicide residues analysis. Although GC has been successfully applied in this area for many years, HPLC offers certain advantages and in some respects offers a complementary technique. An example of this occurs in the case of the urea herbicides which under normal GC operating conditions are unstable, being heat-labile. Additional derivatisation stages are, therefore, required in the analysis, whereas liquid chromatographic separation may be achieved using the underivatised compounds at ambient temperature and simpler analytical procedures thereby evolved.

The approach almost invariably adopted has been to use reversed-phase partition chromatography on ODS-coated phases with variable or fixed wavelength UV detection systems to monitor column eluate. This lacks the sensitivity of electron-capture detection frequently employed with GC analysis. However, pre-[114] or post-column[115] fluorogenic labelling techniques have been established for HPLC analysis of pesticides and herbicides which will yield comparable limits of detection. The whole field of application of chemical derivatisation techniques for pesticide analysis has been reviewed[116]. There is also an excellent review available on the subject of the application of HPLC to pesticide and herbicide analysis which gives chromatographic data for 166 pesticides, including retention data on various columns under specific operating conditions[117].

Examples of actual applications of HPLC separations of pesticides and herbicides to environmental water extracts are too numerous to relate in detail. However papers

relating to determinations of organophosphorus[118], triazine[119-121], phenylurea[122-127], carbamate[124,125], thiocarbamate[124,128], pyridizone[129], and chlorophenoxy acid and ester[130] types in various surface waters, including river[122,124,129], stream[127], and lake waters[123], and agricultural run-off[118,119,131] are available. Of particular note is the work of Schulten and his co-workers[124] in which separated pesticide residues were identified by field desorption mass spectrometric techniques.

5.2.3 Phenols

Although the separation of phenolic compounds by HPLC has been extensively studied, application of the technique to analysis of phenols in environmental samples has not been great, the favoured method being gas chromatographic analysis with or without derivatisation and using sensitive FID and ECD detection systems. HPLC analysis can, however, offer certain advantages at times, particularly in simplication of techniques with certain selective and sensitive detectors. Thus, Armentrout and co-workers[132] have used selective electrochemical detection with a carbon-polyethylene tubular anode (limit of detection 1 ng/l) for the analysis of industrial effluent discharges, drainage water, and tap water. Also Wolkoff[133] has applied fluorescence spectroscopy in a reaction detector whereby cerium IV sulphate is allowed to react with phenols in the column eluate and the fluorescent cerium III thus produced is detected and measured. Limits of detection in environmental samples of 0.4 ng/l are reached. The system has been applied to the separation of phenols reported to have been found in pulp mill effluent.

Most of the reported work has again made use of reversed-phase partition chromatography on ODS-coated microparticulate silica phases. Realini[134] has developed a method for the separation and determination of trace levels of the phenols required to be analysed by the Environmental Protection Agency in wastewaters and drinking waters. Complete separation of all 11 phenols is accomplished in less than 25 min. Detection is by fixed-wavelength UV photometry at 280 nm, which is near the adsorption maximum for most phenols. The method has been applied to the analysis of a canal water containing a high proportion of industrial effluent and was capable of detecting down to 1 ng absolute amounts of injected phenols. Similar techniques have been applied to the analysis of waste waters and polluted surface waters[135,136].

Of particular interest has been the analysis of pentachlorophenol which is relatively difficult to determine by GC techniques. Methods for its determination in wastewaters[131,137] by HPLC have been developed and applied.

5.2.4 Surfactants

Methods for the determination and characterisation of surfactants and surfactant metabolities in water have in the past posed many problems. Application of techniques involving GC are limited because of volatility problems, while non-specific colorimetric procedures lack discrimination. HPLC offers considerable promise for increasing the scope of surfactant analysis and has already been fairly extensively studied.

Krejci and his co-workers[138] have separated non-ionic surfactants of the alkylphenol type in the presence of mineral oil by means of HPLC using partition chromatography. An integrated precolumn enrichment technique was successfully employed. Separation was effected according to the number of ethylene-oxide units in the surfactant molecule. Column eluate was monitored by a UV detector at 254 and 285 nm and by a transport ionisation detector (moving-wire type).

Cassidy and his co-workers[139] have also determined non-ionic detergent residues and other decomposition products in industrial process waters, but this time using gel permeation chromatography on Styragel (Waters Associates Ltd). Detection limits of approximately 0.09 to 0.1 mg/l were achieved. Gel chromatography has also been used to determine surfactants of the alkyl benzene sulphonate type in river water following clean-up on a cation-exchange resin[140]. Gloor and his co-workers[141] have also separated linear alkyl benzene surfactants present in waste water, using reversed-phase paired ionpartition chromatography. Studies were carried out on the removal of surfactant during conventional sewage-works treatment. Other workers have carried out similar studies on the biodegradability of surfactants using adsorption chromatography[142].

5.2.5 Phthalate Esters

Although phthalate esters can generally be relatively easily separated using GC, the

higher isomers are not easily eluted because of the high temperatures required. It may therefore be more appropriate to employ HPLC.

Mori[143] has determined phthalate ester in river waters using 3 separate separation modes with an ultra-violet absorption detector of variable wavelength. This has provided the basis of a very sensitive method (absolute detection limit of 2 ng for dioctyl phthalate at 224 nm). Gel permeation, and normal and reversed-phase partition chromatography were also examined.

Otsuki[94] has determined phthalate esters in water samples using reversed-phase adsorption chromatography. Large injection volumes from a loop were applied and gradient elution techniques were used for separation of the phthalate esters.

Van Vliet and co-workers[101] have used phthalate esters as model compounds to demonstrate the benefits of on-line trace enrichment in HPLC using a precolumn. The application of the technique to the determination of 2 esters in tap, distilled, mineral, and river waters, as well as in soft drinks, is reported.

5.2.6 Amino Acids

Liquid chromatographic separation of amino acids is carried out routinely in clinical chemistry and pathology laboratories, and commercial amino acid analysers have been available for many years for this purpose. These systems are generally based on cation-exchange chromatography using citrate buffers for elution and ninhydrin reagent for post-column reagent detection. More recently, reagents producing strongly fluorescent reaction products, eg fluescin and fluorescamine, have been employed with sensitive fluorimeters, allowing limits of detection to be lowered. Conventional separations of this type generally take 4 h or longer for completion.

Advances have been made in terms of speed of analysis by applying HPLC in the reversed-phase partition mode using derivatisation techniques or PIC reagents in buffered solutions. Of particular note is the use of phenylthiohydantion derivatisation in which the reaction can be carried out in aqueous media making it particularly useful for water analysis[144,145]. These techniques have not yet been applied to the analysis of environmental samples but the more classical technique has been applied to the analysis of wastewaters[109,146], ground water[109], river water[109], drinking water[109] and saline water[147,148].

5.2.7 Carbohydrates

As with amino acids, routine liquid chromatographic analysis for carbohydrates in physiological fluids has been carried out in pathology laboratories using commercially available equipment. Katz and his co-workers[149,150] have successfully applied such equipment to the analysis of sewage and sewage effluent concentrated by low-temperature distillation.

The above method is based upon classical separation of carbohydrates using ion-exchange resins in the presence of borate buffer. Josefsson[151] has determined soluble carbohydrates in sea water by partition chromatography on an ion-exchange column using the method of Samuelson *et al*, which employs ethanol/water mixtures as column eluent.

5.2.8 Nitro Compounds

Separation and determination of nitro compounds by HPLC has chiefly been carried out in connection with pollution abatement studies of munition wastes. Methods for the determination of trinitrotoluene and tetryl and the 6 isomeric dinitrotoluenes have been developed. A method suitable for the determination of tetryl in the presence of HMX, RDX, and TNT has also been described[152,153].

Chandler and his co-workers[154] have developed a method for the determination of nitroglycerine and by-products in wastewaters. It has been applied to the study of the effectiveness of their removal by treatment of the wastewaters with sodium sulphide or ozone.

5.2.9 Fatty Acids

Although fatty-acid analysis has been traditionally carried out using methods based on GC, in certain respects HPLC can offer some advantages. One real problem is the difficulty of detecting compounds of this type with the required degree of sensitivity with the well-tried and tested commercially available systems. Some Japanese workers have overcome this problem by using a thermal detector [155,156], the results from which are, however, difficult to interpret, because of its unusual response characteristics. This is especially true with unresolved peaks. Cassidy and Niro [139] in their studies on the decomposition products in industrial process waters have compromised by using isocratic elution with a refractive index monitor.

In studies of the free and bound fatty acids in river water, Hullett and Eisenreich [157] have achieved relatively efficient separation and detection by employing gradient-elution reversed-phase partition chromatography on the phenacyl ester derivatives. Although an additional derivatisation stage is required, this has meant that UV detection could be employed. Comparison of the results of such separations with those from GC analysis of the same river-water extracts has shown greater resolution of unsaturated and branch-chain fatty acids by HPLC. This resolution is important in determining the source of the organic carbon associated with the fatty acids.

The other methods mentioned above employ direct analysis of the acids without derivatisation. Horikawa [156] employs an on-column concentration technique on anion-exchange chromatographic column subsequently employed in the analytical separation.

5.2.10 Optical Brighteners, Dyestuffs and Pigments

Methods of separation of optical brighteners of the *p*-aminostilbene type using HPLC have been studied at the Water Research Centre [106]. These compounds are highly polar, containing sulphonic-acid groups as well as basic functions. This, together with their high molecular weight, makes HPLC an obvious method to adopt. Cation- or anion-exchange chromatography is an obvious choice of separation mode and the fact that many systems employed gave poor or partial resolution reflects the closely similar structure and properties of these compounds [106].

As a result of work carried out to date [106], optical brighteners are separated isocratically mainly by two solvent systems, the more polar structures by 45% methanol containing tetrabutyl ammonium chloride as PIC reagent (0.005 molar) and phosphate buffer (0.05 molar) at pH 7.5, and the less polar structures by 70% methanol — 30% water containing 0.01 molar sodium dihydrogen phosphate. The stationary phase employed is an ODS-coated microparticulate silica. Separation methods for optical brighteners are required in connection with studies concerned with water quality catchment control in a river basin where considerable indirect re-use of water is practised.

In order to monitor levels of acid blue dyes in natural waters, a method of separation and analysis has been developed at the Water Research Centre based on HPLC [85,106]. The method uses reversed-phase partition on an ODS-phase and employs paired ion chromatography. The dyes of the sulphonated triphenyl methane type were paired with tetrabutyl ammonium hydroxide. This reagent was also used to condition a small pre-column which was then used for direct enrichment of aqueous samples.

Rushing and co-workers [158] have also successfully separated basic dyes of the triphenyl methane type using normal reversed-phase HPLC on ODS phases. Of particular interest was gentian violet which was determined in samples of waste water.

Some excellent separations of phytoplankton pigments from their degradation products and carotenoids in phytoplankton cultures and marine particulate matter have been achieved by Abaychi and Riley [159] using adsorption chromatography and spectrophotometric detection at 440 nm. All the chlorophyll pigments are efficiently separated by this method.

5.2.11 Chelating Agents

The discharge of synthetic chelating agents into the aqueous environment is of concern because of their ability to mobilise heavy metals. Because of their lack of volatility and high polarity, methods of analysis based on GC can be quite arduous, if not impossible, for those of highest molecular weight. HPLC has therefore been explored as a possible

alternative. Longbottom[160] has successfully applied anion-exchange chromatography for the separation of nitrilotriacetic acid (NTA) from other amino-acid chelators. The method was applied to the determinaton of NTA in sewage. Possible interferences from metal ions were eliminated by converting all metal chelates to the analysable ferric chelate.

Jones and his co-workers[45] have also separated the copper chelates of various synthetic complexing agents using anion-exchange chromatography, but instead of UV detection as applied by Longbottom, the liquid chromatograph was interfaced to an atomic absorption spectrophotometer set up to detect copper. The system was applied to the analysis of various types of waste water.

5.2.12 Miscellaneous Applications

As well as those already mentioned, HPLC has been applied to the separation and determination of a wide range of organic compounds in natural and waste waters. Among these are the determination of aromatic amines in waste waters[85,161], chlorinated organic compounds in drinking waters following disinfection with chlorine[162], acrylic acid momomer[163] and acrylamide[164] in natural and waste waters, tetrachloroethylene in natural waters[95], 2-mercaptobenzothiazole in waste dump effulent[165] organophosphorus compounds released by algae into natural waters[166], methylmercury in natural water[167], and organoselenium compounds in drinking, surface and waste water[168].

5.3 Non-specific Methods

In order to provide a truly non-specific method for separation and determination of unknown organic compounds, HPLC requires the development of a universal detection system comparable to the flame ionisation detector of gas-liquid chromatography. As yet such a system does not exist since those detectors which are available, and are capable of giving a response to all organic compounds, have some other inherent limitation such as poor limits of detection, incompatibility with operation under gradient elution conditions, sensitivity to a limited volatility range, and incompatibility with important solvent systems. Thus, although HPLC has been frequently applied to broad screening or fingerprinting analysis, it has rarely been used with truly universal detection systems. Application of the best available detectors has meant, in practical terms, some degree of selectivity in the total response. Thus, ultra-violet detection at various wavelengths has been used to detect aromatic and conjugated organic compounds in sewage[149,150,169-171], sewage effluents[97,149,150,169,171], trade waste waters[169,172], surface waters[96,97,173,174], process waters[96], well waters[96], and tap waters[97,175].

A cerium oxidation monitor utilising a fluorescence photometer to detect reduced cerium III has been employed to characterise the oxidisable organic compounds in a wide range of effluents and natural waters[157,171,173]. Radiochemical detection has also been used in research to determine the effects of disinfection of sewage effluents and tap waters with chlorine employing the chlorine-36 isotope[D,176,177]. Other detectors which have been employed to characterise the organic compounds in sewage and sewage effluents in connection with studies on the effects of sewage-treatment processes are a transport ionisation detector (moving wire detector)[62] and a total organic carbon monitor[54].

In applying HPLC to non-specific analysis of the organics in water, all four major modes of operation have been used, ie adsorption chromatography[175], ion-exchange chromatography[96,149,150,160-173], gel permeation chromatography[178], and partition chromatography in both normal and reversed phase operations[85,96,131]. All four modes have various strengths and weaknesses. Separations across a wide polarity range have been achieved using adsorption chromatography, but analyses are time-consuming, lasting up to several hours because of the large number of solvent combinations which must be used in gradient elution to overcome displacement effects. Ion-exchange chromatography offers a wide range of well-tried and proved methods for separating polar organic compounds, but analyses tend to be lengthy and relatively more difficult to carry out. Gel permeation chromatography can provide useful profiles of molecular-weight ranges of organic compounds in a particular sample. However problems of column instability, reproducibility of results, and molecular-weight calibration are either difficult or remain to be overcome.

The technique which holds most promise at present is partition chromatography. Chemically-bonded high-efficiency media are now available for both normal and

reversed-phase work. Displacement effects encountered in adsorption chromatography are no longer a problem with the more favourable partition mechanism. Thus fewer solvent combinations are required to cover a wide polarity range. The application of PIC reagents to reversed-phase partition work also increases the polarity range of organic compounds which can be separated with this phase.

Characterisation using non-specific HPLC techniques has been used to examine virtually all types of water. Among the particularly noteworthy applications have been water-quality monitoring of drinking[175] and process waters[97], an examination of the effects of the various stages of sewage treatment[149,150,171], and to investigate the production of potentially toxic organic compounds as a result of disinfection using chlorination processes[D,169,176].

6. Quantitative Analysis

6.1 Introduction

Before considering the application of HPLC to quantitative analysis, it is important to define the terms used to express the efficiency of the method. These terms, accuracy, precision, and reproducibility, are often misleadingly used. The accuracy of a method is its ability to give the true value. Therefore in comparing the efficiencies of various techniques, a standard of known composition and concentration should be employed. The precision of a method measures its ability to give the same value for replicate determinations, ie determinations carried out in parallel at the same time. Thus, a particular method may be highly precise (giving results within a narrow range) without being accurate. The reproducibility of a method measures its ability to produce consistent results in independent operations. This could apply to the same analyst using the same equipment on different days or different analysts using different equipment in different laboratories. When employing the term these conditions should be defined.

The ability to achieve good quantitative results by applying HPLC to routine analysis was until fairly recently something of a problem unless the more expensive ranges of equipment were available. With the help of microprocessor control[3-5], cheaper equipment is now available which allows close control of operating parameters. Thus, it is in the area of the precision of results that improvements can be made. The accuracy of a method remains almost totally dependent upon the analyst's ability to calibrate against suitable standards, and its reproducibility upon his ability to define and control the operating conditions. Thus, if one piece of equipment set up to deliver 2 ml/min of solvent actually delivers 1.9 ml/min, while a second piece of equipment set up for the same analysis delivers 2.1 ml/min, it is method reproducibility which is affected. Such deviations in operating conditions can, however, be overcome by the correct choice of calibration technique. In this respect detailed attention must be given to all stages of the analytical procedure including original sampling. There is obviously no point in carrying out accurate and precise analysis on a non-representative sample.

6.2 Sources of Error

In developing an analytical procedure based on any separation technique, recognition of the possible sources of error is of fundamental importance in minimising their effect. In applying HPLC to the analysis of water samples, it is of primary importance first of all to obtain a representative sample. This involves an understanding of conditions such as currents, flow patterns, stratification effects, distribution of solid particles, and temporal effects[179].

The next stage in any analytical procedure is the application of a suitable concentration technique, usually to produce an extract suitable for injection into the liquid chromatograph. Whatever the technique chosen, the conditions of each application must be standardised and recovery values measured for each specific compound of concern. It is of vital importance that the extract produced must be a true solution. Extracts containing precipitated solids or colloids, when injected into a liquid chromatograph, produced a multitude of adverse effects including lack of reproducibility and early column failure. For this reason it is always safest either to centrifuge or filter sample extracts before injection. A syringe with an attachment suitable for filtering small volumes of liquid is manufactured under the name "Swinnex Filter" from Millipore Ltd. Both aqueous and organic-phase filters are available.

It is also important that extracted components from the water sample be introduced into the liquid chromatograph in an organic solvent which is compatible with the eluting solvent. Ideally they should be the same, since this completely eliminates solvent effects and, in particular, large solvent peaks which may obscure the eluting components. An incorrect choice of solvent can lead to precipitation of dissolved substances on the head of the column with subsequent disruption to the chromatographic separation.

In situations where solvents of greater solubilising power than the eluting solvent are required to dissolve all the components of the sample extract, the solvent should be chosen with great care. It should be completely miscible with the eluting solvent, not give rise to serious baseline disruption, not de-activate or affect the equilibrium of the stationary phase, and not cause serious displacement of the retention times of eluted components.

The chromatogram in Fig 15 illustrates the serious effect that the injection solvent can have on the subsequent chromatographic profile produced. In this particular case, 188μl of a standard containing 5 organic compounds has been loop-injected onto a silica column, using first of all pure hexane and then an ethanol-hexane mixture as the solvent for sample introduction. The serious effect on the retention times of the five components is apparent. The shortened retention times in the case where a hexane-ethanol mixture was employed is probably due to a combination of de-activation of the silica and partial elution by the ethanol. In these circumstances it becomes extremely important to standardise the injection volumes.

The method of injection introduces an important source of error affecting the precision of quantitative data. In practice, valve injection from a loop gives greater precision than syringe/injection because it allows exact volumes from a fixed cavity to be introduced into the chromatographic system. The cavity is loaded at low pressure, eliminating the possibility of leakage which is a significant source of error in syringe injection, particularly with older syringes having worn plungers and barrels. The actual operation of sample introduction is also smoother and under greater control than in syringe injection where reproducibility depends more upon the skill of the operator.

Sources of error arising from the chromatographic separation itself are generally not so severe once the column has reached equilibrium with the mobile phase. Keeping errors to a minimum depends on not upsetting this equilibrium, particularly in maintaining constant flow rate, constant temperature, and choosing a suitable solvent for sample injection.

The application of solvent gradient elution for quantitative analysis in HPLC is more difficult than for isocratic elution, although the problems are not so severe that they may not be overcome if required. Practical problems stem mainly from spurious peaks which may be generated by adverse mixing properties of certain solvent combinations, the increased analytical time required because of re-equilibration periods which are required between analyses, and wandering baseline effects caused by different responses of the primary and secondary solvents. Detectors of the transport ionisation and fluorescence type do not give rise to this problem since they are not influenced by the solvent. For the purposes of quantitative analysis an ideal detection system should have wide linear response, be stable, and have low noise characteristics. Environmental factors which affect the long-term stability of the detector should also be recognised and counteracted.

Further errors can creep into analytical results during the actual process of quantification from data obtained from either peak-height or peak-area measurements. Both measurements have their respective advantages and disadvantages but for a particular analysis the choice is generally clear as to which one will minimise errors. In general, however, greater precision is achieved by measuring peak areas since this reduces minor variations caused by operator technique, eg injection method or instrumental control settings. However, with equipment which has difficulty in providing truly constant flow rates, greater precision is achieved by measuring peak heights, since the width of a peak measured in units of time is very dependent on the velocity of the mobile phase through the chromatographic column. Thus, the area of the peak becomes much more dependent on the flow rate than the peak height.

A similar situation arises in the case of overlapping peaks, particularly where one is much larger than the other. This is a frequent situation in water pollution analysis where trace components must be determined amongst large overlapping peaks. The effect on a small

peak of being sited on the tailing edge of a large peak is to cause significant broadening. The effect of this on the precision and accuracy of quantitative measurements has been studied by Kirkland[92] who showed that peak area measurements in such situations are more seriously affected than peak height measurements when both calculations are made on the basis of an imaginary baseline. In fact while greater precision is achieved from peak area measurements, greater accuracy is achieved from a peak height measurement. In trace analysis the latter factor is of much greater importance.

6.3 Calibration and Measurement

The choice of the best possible calibration technique is perhaps the single most important consideration in the development of an analytical technique, since, if correctly applied, it can eliminate many of the sources of error described in the preceding sections.

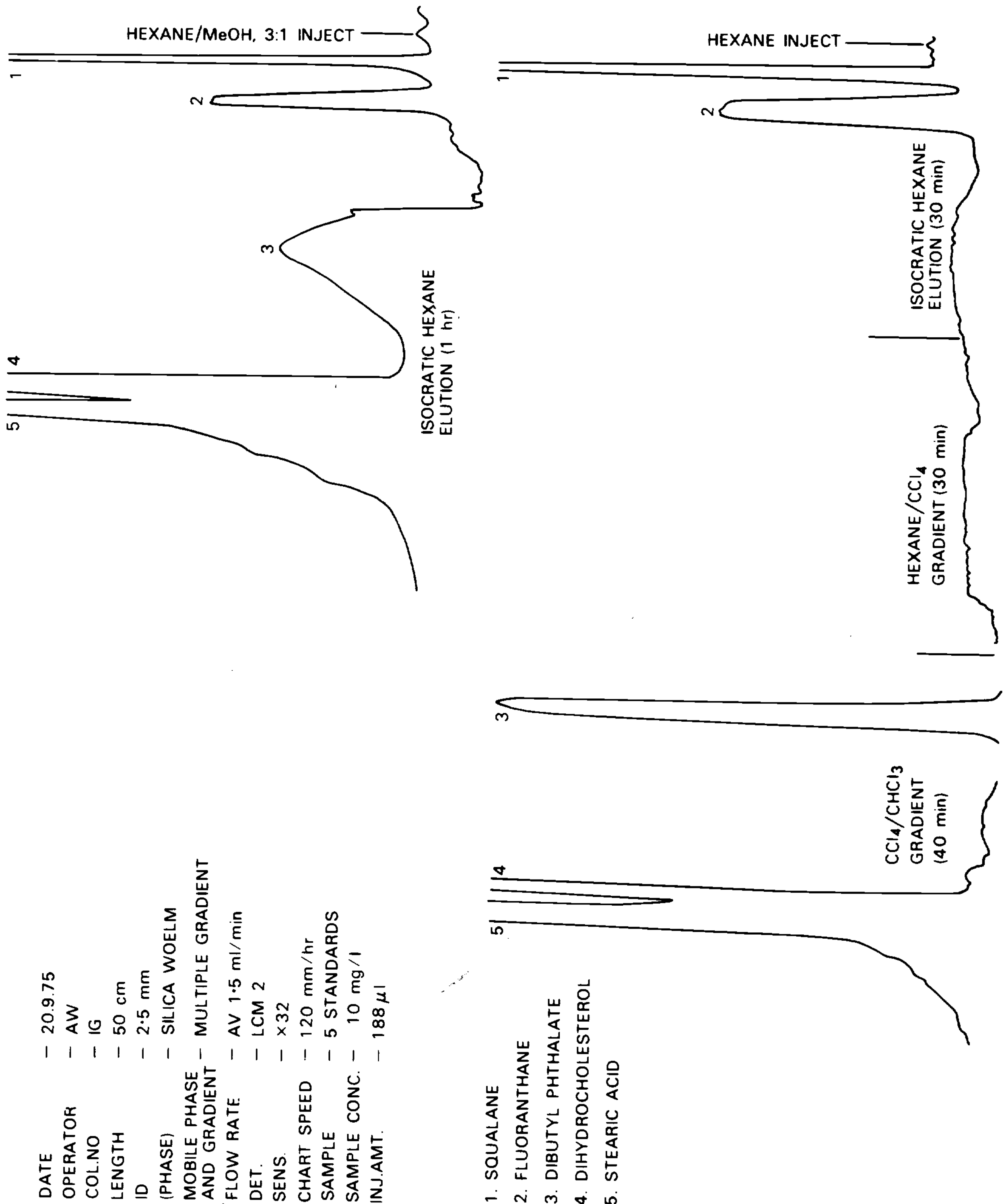


FIG 15 EFFECT OF SAMPLE SOLVENT

6.3.1 External Standards

Many quantitative analyses can be satisfactorily carried out by injecting a series of standards containing different known amounts of the materials to be determined between each unknown sample. Calibration curves of mass or concentration of unknown against peak areas may be drawn, and concentrations of unknown substances thereby obtained by interpolation from these graphs. Provided that interpolation rather than extrapolation is used, even slight deviations from linearity may be accommodated.

In acquiring the data for the preparation of such graphs it is often worth considering injecting different volumes of the same standard solution in order to minimise errors caused by dilution. A calibration graph is shown in Fig 16 with a fairly typical deviation from linearity and a negative intercept frequency caused by a systematic error created by the volume of a syringe needle. This sort of error can be significantly reduced by using valve injection from a loop which virtually eliminates sample losses.

The main disadvantage of external calibration techniques is that each component to be determined requires a separate calibration. The method is therefore extremely tedious and is only really applicable where a limited number of components are of interest.

6.3.2 Internal Standards

The addition of an internal standard eliminates the main cause of error of the external standard technique, ie that due to injection. The method depends upon finding a suitable standard which will give a separate isolated peak in the chromatographic profile. Constant and accurate amounts are then added to the sample before injection and relative responses of unknown to standard measured. Thus, the absolute amount injected onto the column is no longer of importance. Calibration curves may also be drawn for routine analysis by adding the internal standards to each of the standard mixtures covering the concentration range required. Calibration graphs are drawn of the ratio of the peak areas of the internal standards and the peak areas of the normal standards against the concentration of the normal standard.

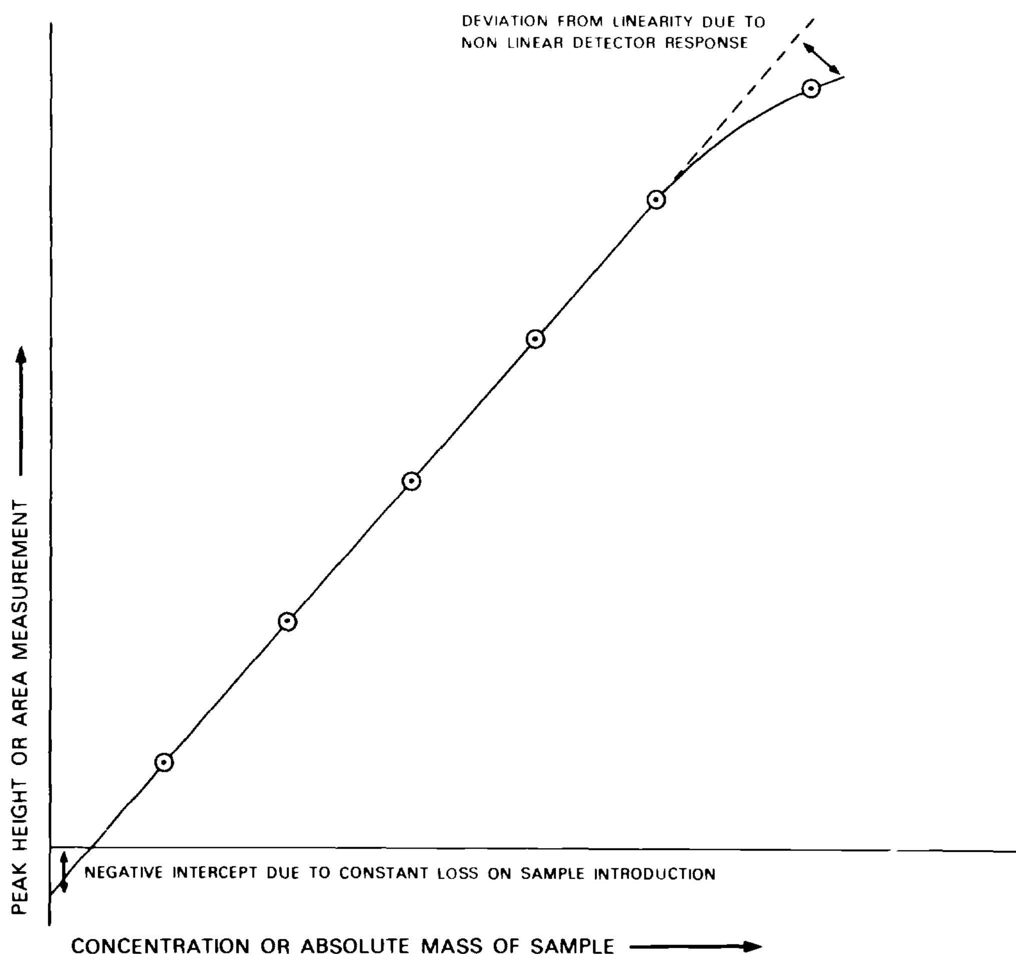


FIG 16 USUAL TYPE OF CALIBRATION CURVE OBTAINED FROM A HPLC DETECTOR AND NORMAL INJECTION TECHNIQUES

In the analysis of water, further benefit may accrue if the internal standard can be added to the water sample before concentration and extraction since this will also eliminate errors associated with the concentration stage of the procedure. It is always advisable, however, to carry out calibration procedures of this type using waters of a similar nature to those to be analysed in order to eliminate sample effects, eg association with other dissolved material. These effects can have a bearing on extraction efficiency. For the same reason it is important to try and obtain an internal standard with structure and properties similar to the unknown components of interest.

The subject of calibration techniques in the analytical chemistry of water samples has been discussed in more detail by Wilson and Cheeseman[180].

7. Practical Problems and Their Solution

7. Practical Problems and Their Solution

Any of the major components of a liquid chromatograph can cause problems which effect the efficient functioning of the instrument. The same symptoms can frequently be generated from different faults and so it is important to adopt a systematic approach in order to trace the source. Most instrument manuals provide a section for "trouble-shooting" and this is also available in a chapter of the book by Walker *et al*[H]. As well as providing a rapid means of pin-pointing the source of any malfunction, these guides should ideally provide the solution to each problem.

A thorough and comprehensive trouble-shooting guide such as the one provided by Walker *et al*[H], can help to overcome lack of training or experience of the operator. Its provision can, therefore, mean a considerable saving in instrument down-time and the costs of obtaining the services of an engineer. The general format of such a guide is generally a description of symptoms, causes, and corrective action for each major section of the liquid chromatographic system. These are solvent transport system, sample injection system, columns, detectors, and recorders.

Problem areas with solvent transport systems may be associated with solvent degassing, pump malfunctions, and leaks and blockages in associated transfer lines. Problems frequently encountered with injection systems are associated with the leaching of organic material from the septa, the leaking of septa and loop injectors, and blockage by particulate matter particularly with valve-type loop injectors. The chromatography column itself can often be the source of problems associated with column bleed, leaking fittings, inefficient packing, and plugging. Symptoms of problems with detectors are often seen as low sensitivity, non-linearity, noise, and erratic response. Many of these malfunctions are diagnosed from the traces of the chart pen recorder. However, it is always important to establish that it is not the recorder itself which is giving rise to the problems which can be, typically, mechanical faults such as slipping gears or sticking slide mechanisms and electrical faults such as poor connections and background noise.

One of the major causes of swift decline in the efficiency of HPLC systems is lack of care in the use and maintenance of the column. This subject will therefore be dealt with in greater detail.

7.1 Problems with the Mobile Phase

Problems frequently arise because of lack of purity of eluting solvents. Solvent impurities can over a period of time build up on a column altering its separation characteristics or, particularly in gradient elution operations, cause the appearance of spurious peaks. Thus purification of solvents becomes an important aspect of work with HPLC although for many purposes chromatographic grade solvents are already commercially available. The book by Riddick and Bunger[I]— 'Organic Solvents' — is an authoritive treatise on the subject. This problem is specially pertinent to the use of water in reversed phase partition chromatography. It has been overcome by passing water through special carbon cartridge systems which are available commercially, by pre-passage of the water through bonded ODS columns, by distillation from alkaline permanganate and acid dichromate, or even purchase of LC grade water.

Other solvents also give rise to specific problems. Thus acetonitrile which is particularly useful because of its low UV cut-off and viscosity requires complicated procedures to

remove its many impurities. Also when ethers are employed, peroxides and peroxide inhibitors must be removed, and care must be taken with chlorinated solvents which are susceptible to oxidative breakdown and which thereby may be harmful to the stainless steel components of an HPLC system.

Filtration of the mobile phase before its use is an absolute necessity. The intention is to remove fine dust and other particulate matter which in the long term will gradually block the HPLC system at points of restriction. Sintered glass, glass fibre, or millipore filters of the correct grade (~0.2μm) are suitable. The latter type should be first of all washed with a portion of the solvent to remove binders and other impurities.

A problem which repeatedly catches the unwary and is worthy of a special mention is that of volume contraction which occurs when strongly hydrogen bonded solvents are mixed. Such mixtures are most usually made up in a volume: volume ratio and it is therefore very important to measure out respective volumes in separate graduated vessels. An example of this is the case where 50 ml of methanol mixed with 50 ml of water gives approximately only 96 ml of a 1:1 mixture. Thus if an attempt were made to make up this mixture in a single one-litre graduated cylinder by pouring one solvent into the other, a ratio other than 1:1 would be obtained. Other important points to take into consideration are the use of a more accurate measuring device, eg a volumetric pipette, when measuring out components which make up less than 10% of the total mixture and in all cases to make up as large a volume as is practically possible in order to minimize errors in measuring out small volumes.

Frequently in the course of reversed-phase partition chromatography where water to methanol solvent gradients are applied, problems arise because of the formation of bubbles during the early stages of gradient development. This is caused by the evolution of heat due to the initial breakdown of the hydrogen bonding of the water with the introduction of methanol. The exothermic nature of this reaction causes a fraction of the methanol to volatilise. This problem can be overcome to some extent by using a high pressure type of gradient generating device. A more appropriate solution however is to employ 5% methanol in water as the primary solvent. Initial mixing problems are thereby overcome. Degassing operations may also change the composition of the eluting solvent mixture if a volatile component is used. Even one percent changes in composition caused by evacuation or sparging processes can effect separation and retention times. Degassing of the components solvents separately can frequently overcome this unless further problems arise because of the generation of heat during the mixing.

Some of the problems mentioned also occur when elution is performed using buffered solutions or PIC reagents. The introduction of particulates with salts of this type is a more common problem although artefact peaks may also be generated. A further problem which does not appear to have been widely recognised is the recovery of columns used with salt solutions in the mobile phase to their original selectivity. For example it is very important to establish that a column required for operation in the reversed-phase partition mode which has previously been employed with PIC reagents can recover its original selectivity properties. Recent work at the Water Research Centre suggests that this is not possible and that columns should be kept for one type of operation. Other precautions which should be taken when using salt solutions include the prevention of bacterial growth in phosphate solutions, and pacifying treatment of the chromatographic system with 20–50% nitric acid solution if halide salts and acids are employed in the mobile phase.

A final very important point when employing solvent gradient elution with sàlt solutions is to establish that the mixing of primary and secondary solvent will not cause precipitation to occur, ie to ensure that the salt will be completely soluble in the organic/water ratio to which the gradient is programmed. The inadvertent precipitation of salt may totally destroy the column since flow may not be capable of restoration once it has been stopped, and the stationary phase may be irrevesibly damaged.

7.2 Problems with Operating Conditions

Some polar solvents have significant solubilizing powers for silica and when such solvents are used as the mobile phase in HPLC, solubilizing power has been found to increase with the rate of flow. Thus column lifetime can be prolonged by reducing column flow rate as much as possible which according to the general trend of the van Deemter equation also

increases the efficiency of separation. If separation time consequently becomes unduly long it is frequently better to increase the eluting power of the mobile phase or reduce the length of the column.

In general the column bed is extremely sensitive to physical changes and can be badly disturbed when such changes occur too rapidly. These include temperature, pressure and solvent composition as well as sudden physical shocks. Thus the column should be handled carefully at all times and any change in the required operating conditions made gradually. Thus pressure surges which may occur during the normal operation of some pumps are to be avoided particularly with microparticulate stationary phases. It is also less of a strain on the column bed if the head pressure can be applied and removed gradually particularly during conditioning operations when higher flow rates are generally applied. During the conditioning process itself care must be exercised so that sudden changes in solvent composition are avoided. For example after application of a solvent gradient it is safer to apply a reverse gradient albeit more rapidly to regain the initial composition rather than an immediate and sudden change.

Similar considerations apply to operation at elevated temperature which may be of benefit in speeding up separations particularly where relatively viscous solvents are used. It is also important to maintain the solvent flow during gradual temperature increases or decreases.

If attention is paid to these details of operating technique the life-time of chromatographic columns will be significantly extended. Also short-term losses in the efficiency of separation and resolution of the column due to disturbance of the stationary phase will be avoided.

7.3 Problems caused by the Column System

Modern liquid chromatographs are designed with columns both vertically and horizontally mounted. When the column is new the position of mounting is immaterial since the column packing completely fills the column cavity and is hard against the retaining frit. However with an older column where the bed has been worn down and disturbed, a vertical mounting position is preferred since in the horizontal position under the effects of gravity packing would tend to fall to the bottom wall of the column.

A significant problem in operating an HPLC system is the gradual blockage of column frits. Modern columns are usually manufactured with frits as part of the end fitting and these can be fairly easily replaced. Inlet frits of approximately 2μm size appear to be best. Although smaller porosity frits are available these tend to clog more rapidly. Although not advisable, attempts to clean blocked frits may be made by using a strong acid wash in an ultrasonic bath. Porous PTFE frits are not recommended since they may contract under pressure.

It cannot be assumed that the 326 grade stainless steel used in the manufacture of HPLC columns has an indefinite life, particularly if corrosive mobile phases, eg halide salt solutions are employed. It is always advisable to wash out salt-containing mobile phases at the end of a working day and even more important that columns should be stored after washing thoroughly by the passage of distilled water or pure organic solvent.

Keeping the system leak-free can also be a problem particularly to the inexperienced operator. With use, compression fittings can become flared out or scratched and eventually it is impossible to maintain a leak-free seal. Each time the fitting is released it must be tightened a little further to achieve a seal and there is therefore a practical limit to the number of times this may be accomplished. Spare compression fittings should therefore always be available and both male and female components as well as ferrules replaced when the occasion demands since a damaged ferrule will quickly ruin a new one. Ferrules can often only be removed by cutting them from the tubing and, since it is frequently small-bore tubing which is involved, it is important not to collapse the bore by crushing it with a cutting tool or saw. The tubing must be stress broken by scoring it and bending it away from and towards the score mark using pliers.

When fitting a new ferrule it is very important to establish the ferrule locking position on the inlet or outlet tubing. This can vary with different types of compression fittings and even to a small degree with the same type of fitting. Failure to pay attention to this detail will invariably mean a leaking fixture.

7.4 Problems encountered with the Sample and Sample Injection

Particularly with complex samples it is important to establish that all components are completely dissolved in the injection solvent and completely soluble in the mobile phase, and that the injection solvent and mobile phase are completely miscible. Failure to observe these details will give rise to extraneous peaks, lead to precipitation of sample components, and produce non-reproducible chromatograms. These considerations also apply to pH differences between the sample and the eluting solvent.

In order to maintain most efficient column performance it is usually best to dissolve the sample in a solvent with just sufficient solubilizing power. This maintains best conditions of solute selectivity between mobile and stationary phase. If the injection solvent is too polar and the volume introduced sufficiently large the equilibrium conditions achieved on the column may be upset.

Sample injection techniques which have been discussed in Section 3.2 can give rise to many chromatographic problems since they can drastically effect the final separation. It has been established that point injection at the top and centre of the bed gives most efficient results[14,15]. However this method quickly causes bed disruption with resultant loss in efficiency. Loop injection whilst not so efficient affords more reproducible results and does not cause these problems.

8. Future Trends and Developments

As a separation technique, HPLC has come a long way in a short time. In just over 10 years it has developed from a classical technique of gravity-fed large-scale columns and manual collection of samples to a fully automated instrumental technique with close control over all operating parameters. In many respects it is generally felt that the optimum in terms of performance has already been reached. For example, in terms of optimum particle sizes of column stationary phases.

It has become increasingly apparent that the search for columns of higher and higher efficiency cannot be attained by reducing particle sizes indefinitely. There comes a time when the pressures required to produce usable flow rates through such columns are not realistic. Pumps designed to operate at 50,000 psi would create immense problems, not the least being the design of the pump itself. At these pressures liquids are appreciably compressed and their thermodynamic properties may differ in unpredictable ways from those at normal pressures. Also some of the advantage gained from high-pressure operation is offset by increases in viscosity. Resolution may also be seriously affected since temperature gradients can develop across the column because of the energy generated by the frictional forces of the flow of fluid. Because of these considerations, the recent tendency has been to settle for columns of relatively short length containing stationary phases with particle diameters of 5 or 10 μm.

A particular area in which there is considerable activity at present is the development of a wide variety and type of stationary phases for all modes of HPLC. This is especially true for chemically bonded phases for application to normal and reversed-phase HPLC. This trend may be expected to continue.

There is also a lot of activity in the area of the development of detectors. The best detector at present available is still the tunable-wavelength UV photometer, and a sensitive detector which will respond to compounds having no UV absorption is urgently required. It is to be hoped that a solvent transport ionisation type of detector with these properties will ultimately be developed, but this is likely to be expensive to manufacture. Fluorescence detectors have the obvious advantage of high sensitivity and zero response to the eluting solvent. However they have a limited range of application although there is potential to increase this by the development of miniaturised reagent reaction systems so that solutes containing a specific functional group may be converted into fluorescent derivatives. This approach has already been exploited to a certain extent[31].

There appears to be generally little potential in further development of detectors based on bulk property measurement, since in liquids these are very little affected by small changes in composition. The limits of detection achievable by such devices inherently cannot exceed approximately one part in 10^7. A better prospect will be to develop detectors based on broad specific properties which may allow detection limits of up to one part in 10^{11}.

There appears to be considerable potential in the recent development of detectors based on derivative and multi-channel operation employing diode arrays or silicon vidicon detection devices. Although this approach is still limited, rapid scanning spectrometers based on these devices can offer a more flexible and powerful detection method in HPLC particularly when the response data is manipulated by a computer system. Among other advantages the possibilities of enhanced resolution are presented.

It is to be expected that aspects of the application and development of preparative-scale HPLC will continue to develop. It is now generally recognised that with currently available highly refined stationary phases, columns of very wide diameter can be prepared and used provided that problems associated with sample injection can be overcome. Modern columns can be used with loads as high as 100 $\mu g/mm^2$ cross-section without serious overloading and it may be calculated therefore that a 10-cm diameter column should be able to handle approximately 1 g of material.

Very recently there has been a significant development in HPLC in the advent of microcolumn chromatography[38,181,182]. The columns which are usually fabricated of PTFE tubing are 0.5 mm in diameter and a few cm long. In another approach, silica stationary phases are packed into capillary columns which are then drawn out to lengths typically 15 m long and 0.07 mm ID. The ratio of column diameter to particle diameter can be as small as 2:1. The silica phase can be chemically treated *in situ* to produce phases for partition chromatography. Fused silica open tubular columns with bonded stationary phases are also beginning to be used[183].

Several papers discuss various aspects of the application of small-scale columns of this type[184–188]. In order to take advantage of the potential improvement in performance which may be achieved by such column (plate heights only 3 times the particle diameter are possible), the pumps, injectors, and detectors used in conjunction must also be miniaturised. Further benefits may accrue if the volume of sample injected is not proportionally decreased since dilution within a column of these dimensions is reduced leading to a theoretical increase in sensitivity. Also the consumption of solvent is relatively small and the pressures required to generate optimum flow rates are relatively low. It is claimed that, because of this, several columns may be connected in series, allowing plate height number of up to 750,000 to be reached.

The biggest advantage of this development is the increased scope for coupling with a mass spectrometer. The column outlet may be directly led in to the mass spectrometer, or a single-stage jet separator can be used since there is relatively little solvent vapour to be pumped away[189,190]. Such systems may also eventually provide means of both highly selective and non-selective as well as sensitive detection which is still a main requirement for the further development of this technique.

9. Inorganic Analysis by HPLC.

(contributed by other authors)

Except for occasional brief mention, this review has dealt almost exclusively with organic impurity analysis. However, provided a suitable column and detector system is available, HPLC is equally effective for inorganic analysis (both ionic and non ionic). It is often used in conjunction with ion chromatography described in the next review; but other columns than ion exchange resins may sometimes be more suitable, and columns have been developed which rely on the selective formation of co-ordination complexes between a substance and the active groups in the resin or other column filling. The ability of a substance to fit onto these co-ordination sites or into a cavity in the solid phase may be important, and cavity size in the solid phase has been used for separating dissolved gases. Gels containing liquid phases other than water are also used as a means of varying the polarity of the absorbant. A very large range of organic substances can be used as the liquid phase including hydrocarbons, aromatic derivatives and polyalcohols. This latter technique is sometimes called Reverse Phase Chromatography. The solid phases used include silica, alumina, and various plastics. Some solid phases with controlled size cavities are known as molecular sieves, some, but not all of which, are aluminosilicate zeolites.

The main detector systems used, include ultra violet and visible absorbance, fluorescence, and polarographic and electrical conductivity measurements. Atomic Absorption and direct flame emission are also used. Radiation measurements are occasionally possible. Infra red absorption is limited to the window in the absorption bands of water; subsequent addition of a colour-forming reagent extends the usefulness of visible

absorption, whilst elution with an ultra violet absorbing substance such as potassium hydrogen phthalate followed by monitoring for a reduction in the absorption when other substances are eluting (reversed absorption) may be of use when no direct detector is possible. Similarly refractive index changes may be used. One problem with the use of conductivity as a means of detection is the large cation signal from most elutants, but this can be avoided if, prior to analysis, the cations are replaced by hydrogen ion using a suitable ion exchange resin.

Care is essential and, if time is available it is recommended that both instrument makers and the literature be consulted before a hitherto untried separation is attempted; and that tests be made as to whether the substances present can be eluted from the column after application. Thus while many compounds such as nitrate, cyanide, thiocyanate, thiosulphate, sulphite and sulphide can be separated easily and measured at quite low concentrations, similar substances such as polythionates, mercaptans and many other polythio and organothio anions cannot be eluted from ion exchange resins but can be separated using some of the reverse phase columns now available. Other substances such as borates sulphate, the halides and the noble gases are easy to separate, but require special detection techniques at low concentrations.

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Ion Chromatography in the Analysis of Water Samples 1983

An Essay Review by G. H. Mansfield and A. Westwell

1. Introduction

Mikhail Tswett (1903)[1] is usually credited with being the first to use a chromatographic technique. He separated leaf pigments by passing an extract containing the pigments through a column of chalk and he was the first to call this type of separation procedure 'chromatography'. His work remained largely unnoticed until the 1930s when the technique was further developed by biochemists studying carotenoid pigments. A major advance was made by Martin and Synge in 1941 [7] who put chromatography on a firm theoretical basis and predicted the separation potential of the main types of chromatographic system, including gas chromatography, which developed rapidly from about 1950 onwards. More recently separation of organic compounds using a finely divided solid and a liquid mobile phase under high pressure has been termed 'high performance liquid chromatography' (HPLC). An extension of HPLC using specially prepared ion-exchange resins now affords a means of separating ions in solution, called 'ion-chromatography' (IC) and is the subject of this review.

Whilst ion exchange resins have been known to have different affinities for different ions [2] they have been developed in the past to have high ionic capacities and, in consequence, the degree of separation of different types of ions loaded on to them was small. In 1975 Small, Stevens and Bauman [3] achieve a good separation of lithium, potassium, rubidium and caesium from a stationary phase of a finely divided cationic exchange resin which had a very low ionic capacity contained in a surface layer of each resin bead. When this new type of sulphonic acid resin was treated with an aqueous suspension of very finely ground strong base anion exchange resin a good separation of anions could be achieved. The separation of anions was increased by using various salt solutions as the eluent. Removal of the oppositely charged ions to those being measured has enabled the eluting peaks to be measured using a conductivity cell.

By employing other types of selective ion exchange systems and changing the mode of operation the range of analytical uses of IC has been markedly increased.

IC is a technique which can be used to analyse ions in water in an analogous way to the use of gas liquid chromatography in measuring volatile organic compounds in mixtures. However, as IC has only been available for a relatively short time further developments are to be expected.

2. Theory

2.1 Basis of the Method

An aqueous solution of a salt, known as the eluent, is continuously pumped through a column exchange material (see Fig 17) a discrete sample for analysis is injected into the flowing eluent stream. The different types of either cations or anions present in it are separated from each other as they pass through the column as they have different affinities for the type of ion exchange resin used. The eluted ions are monitored by a suitable detection system ie conductimetric, electrometric or photometric. The simplest detection method is based on measurement of electrical conductivity, but the measurement directly after separation is difficult because of the high background conductivity of the common eluents eg sodium carbonate/bicarbonate for anions.

However, in one system by removing the ions of opposite charge to those being measured using a column of conventional ion exchange resin, called a "suppressor column", and the choice of the ion remaining in the eluent solution, the separated ions can be measured by conductivity and compared with the response of ions of the same type at known concentration. For example, in anion analysis a separator column of anion resin separates

the anions from a discrete sample injected into a flowing stream of 0.0035M sodium carbonate solution. The suppressor column removes all the cations by replacing them with hydrogen ions leaving the free acids corresponding to the anions to be measured in carbonic acid solution which has a very low background conductivity.

2.2 Separation of Ions

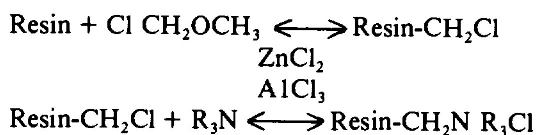
In one type of ion chromatography a gel polystyrene resin of particle size in the range 10 to 30 microns having a 1 to 3.5% divinyl benzene cross-linkage is to be preferred, as the basis for the separator column resin[5]. The 'gel' polystyrene beads are prepared by emulsion polymerisation and some details of this method are given in Helffrick[4]. Such polymers have no visible pores even at magnification of up to 20,000 times in contrast to the honeycombed structure of macroporous resins. As the gel resins are prepared by emulsion polymerisation a Gaussian distribution of bead particle size is produced and a selection of size has to be carried out to remove fine and coarse materials. This is usually done by sieving and fluidised bed techniques.

Sulphonic acid groups are incorporated on to the selected polystyrene beads by reaction with concentrated sulphuric acid:

$$\text{Resin} + H_2SO_4 = \text{Resin } SO_3H + H_2O$$

For ion chromatography the active group must only be on the surface of the beads so the resin has a very low ionic capacity. Surface sulphonation is carried out by careful choice of the sulphonation conditions. Thus, for example, a quantity of polystyrene beads of a narrow particle size range having a 2% divinyl benzene cross-linked structure was treated with excess concentrated sulphuric acid at 100°C for three minutes. The precise conditions can only be worked out experimentally for a given type of polystyrene bead. There are too many variables involved to allow an easy definition of reaction conditions for a given cation or anion separation. Indeed a resin which gives a good cation separation of lithium, sodium and potassium may not provide a good resin base on which to make an anion separating resin. However, for a given "batch" of resin of narrow size range the sulphonation conditions can be defined experimentally to provide a ± 10% variation in retention time for both cation and anion analysis.

Conventional anion exchange resins are made by reacting the cross-linked polystyrene bead with chlorodimethyl ether using a Friedel Crafts reaction. The chloromethylated resin is then reacted with trimethylamine or dimethylmethanolamine.



$$\text{Resin} + Cl\ CH_2OCH_3 \xleftrightarrow[ZnCl_2]{} \text{Resin-}CH_2Cl$$

$$AlCl_3$$

$$\text{Resin-}CH_2Cl + R_3N \longleftrightarrow \text{Resin-}CH_2N\ R_3Cl$$

where R_3 is $(CH_3)_3$ or $(CH_3)_2\ CH_2CH_2OH$

However, it is very much more difficult to obtain a surface coating of anion groups than the sulphonic acid groups of cation resin. For this reason conventional anion exchange resin is ground up and suspended in demineralised water so that only colloidal particles remain in suspension and this colloidal suspension is then used to treat the surface sulphonated cation resin prepared as outlined above. Whilst there is little published information, either "gel" or "macroporous" resin of type I or type II strong base character can be used for preparing the colloidal suspension. The definition of the colloidal suspension remains to be published. There is some indication, that some manufacturers prefer to use a "latex" for anion resin separation columns in the literature.

Other types of ion exchange materials based on a surface coated silaceous substrate are also used to pack the I.C. columns[21,22]. Reactions involving ion exchange resins and particularly those in ion chromatography are equilibrium reactions. For example for cation separation we have:

$$R.SO_3A + B^+ \longleftrightarrow R.SO_3B + A^+$$

The type and concentration of species in the eluent will determine the form of the resin. Thus where hydrochloric acid is used as eluent at equilibrium, B in the equation will be the hydrogen ion whilst with m-phenylenediamine as eluent, B will be m-pheny-lenediamine. The size and charge of ions in the sample will determine the affinity of the ions for the resin. Generally as the ion size and the ionic charge of the species increases so the affinity of the ion for the resin increases. For conventional ion exchange system Helfferick [2] has given the following list of increasing affinities for the alkali metal cations Li^+ Na^+ K_+ Rb^+ Cs^+. Trivalent ions have a stronger affinity than divalent ions and divalent ions have a stronger affinity than monovalent ions for the ion exchange resin.

As well as the basic structure, the active groups on the ion exchange resin and the eluent used in the ion chromatographic process, the size and the size distribution of the ion exchange particles and the packing of the column will affect the separation achieved. Generally the smaller the particle the greater the efficiency of the separation, but the greater the pressure required for a given flowrate through a column. The larger the size distribution of ion exchange particles present the more difficult it is to separate ions. Where particles allow a channelling effect of the eluent through the column separation is adversely affected. Thus, when a column is of a narrow size distribution and well packed it should not be subjected to large physical or chemical shocks.

Even from the superficial view of factors affecting separation given here it can be seen that the degree of separation cannot be calculated, but can only be determined practically for a given species. However, it is useful to classify columns so that separation efficiences can be compared. The time taken for a species to go through the separation column is called the retention time. The retention time is a function of many variables. The greater the flowrate the smaller the retention time. To a first approximation, retention time is inversely proportional to flowrate. The production of the flowrate and retention time is the retention volume. Often the retention volumes are compared relative to a reference substance called an internal standard. The partition coefficient is defined as the ratio of the retention time to the time for unretained liquid to pass through the void volume of the column (see (a) in Figure 18). The selectivity of a column for one ion relative to another is defined as the ratio of their partition coefficients (see (b) in Figure 18). The selectivity is a function of the chemical nature of the components, the ion exchange resin used and the eluent. The width of a peak is a function of the column design, ion exchange resin used and manner of operation. The separation of two species into peaks is dependent on the selectivity and the width of the peak. The resolution of two peaks has been defined by a IUPAC Committee [6] by the equation:—

$$R = \frac{2(V_2 - V_1)}{W_2 + W_1}.$$

V are the peak retention times and W are the peak widths of the two peaks, (see (c) in Figure 18). A comparison of the separation achieved by one column relative to another is best carried out by comparing the number of theoretical plates. The concept of theoretical plate, first introduced by Martin and Synge [7] imagines a column divided along its length into a number of zones each of which is such a length as achieving complete equilibration between the eluent system and the stationary phase. Littlewood [8] describes the theory as applied to gas chromatography and it has been extended to ion chromatography. Practically it is most easily measured by drawing the tangents to the points of inflexion, measuring the peak width and retention time and calculating the number of theoretical plates as shown in (d) in Figure 18). It should be recognised that the number of theoretical plates so calculated is about the square of the number required in distillation theory for the same degree of separation because of the considerable differences in the techniques.

2.3 Removal of Complementary Ions (2 column technique).

The conductivity of strong acids and bases is very much greater than their salts (see Section 2.4). Thus the measurement of ions in the free acid or base form in a pure water stream is an ideal. The sensitivity of IC measurement can be enhanced by removing ions having an opposite charge to those being measured on the "suppressor column" and choosing an eluent such that the residual ion gives a low conductivity. However, the quantity of ions to be removed by the suppressor column and the suppressor column size will determine the regeneration frequency. The ions to be removed by the suppressor column and the suppressor column size will determine the regeneration frequency. The

ions to be removed by the suppressor column arise from the eluent and the samples injected. The type of eluent, its concentration and the flowrate used give one part of the ionic loading for the suppressor column. For a particular separation column the eluent system determines the separation which can be achieved for particular ions. The smaller the ionic concentration of the eluent and the slower the flow-rate the longer the period between regenerations of the suppressor column. Usually the concentration of eluent is in the range 0.001 to 0.05 molar. The lower the flowrate the longer the retention time of a particular species. A flowrate in the range 1 to 7 ml min^{-1} is normally used. The concentration of ions present in the sample and the size of the sample injected gives the second part of the ionic load for the suppressor column.

Usually a sample size of 100 to 300 μl is used and the concentration of ions present in the sample is a total about 300 mg l^{-1} at a maximum, but a precise definition cannot be given as much must depend on the separation which is required. As the sample size is usually determined by a fixed injection coil, or loop, and this would need to be changed to vary the size if the sample is too concentrated, it is common practice to dilute the sample before injection where the need arises. The size and design of the suppressor column and the resin used in it can adversely affect the peak shape and measurement sensitivity as well as the frequency of regeneration.

The void volume in the suppressor column must be kept as low as possible to avoid mixing giving rise to peak spread. The capacity of resin used in the suppressor column should be as high as possible. Total capacity of the resin is usually 2 to 5 m equiv. g^{-1}. In practice the suppressor column is between 1 and 5 times the size of the separation column and a period between regenerations of 8 to 24 hours is achieved.

Small [23] has defined the maximum number of samples that can be analysed before the suppressor column must be re-generated by the following empirical equation:

$$N = \frac{V_B \cdot C_B}{V_A \cdot C_A K}$$

where N is the maximum number of samples between re-generations.

V_A is the volume of the separation resin

C_A is the capacity of the separation resin

V_B is the volume of the suppression resin

C_B is the capacity of the suppression resin

K is the largest selectivity coefficient of ion in samples to be analysed using a particular eluent.

The regeneration of the suppressor column usually takes 45 to 90 minutes including washing regenerant free.

Later developments have replaced the suppressor columns by a "fibre" suppressor based on a coil of semi permeable material through which the eluent passes. The coil is also in contact with strong acid to form an equilibrium with the exchanged cations. This system requires no regeneration.

2.4 Peak Detection

a) Conductimetric.

A detector is required which will indicate the amount of determinand in the effluent from the suppressor column. Whilst refractive index, ultra-violet and infra red absorbance, and atomic absorption have been used for this purpose in high performance liquid chromatography, electrical conductivity is an obvious choice for detecting the presence of ions in aqueous solution. It is easy to do, a small cell volume can be used, it has a fast response and does not alter the sample. Electrical conductivity of a liquid is a bulk property and is a function of the distance between two electrodes, 1, the area of the electrodes, a, and the specific conductivty of the substance, K. It can be defined by the expression conductivity = Ka/1. As the ratio of "a" to "1" is constant for a particular cell,

it is known as the cell constant. The volume and the shape of the cell determines the size of the peak which can be measured so it should be as small as possible and flow through it should be unrestricted.

The electrical measurement of conductivity of a liquid using a small alternating current to avoid polarisation is well established [9].

Practically the specific conductivity of ions whilst being directly proportional to concentration varies from one ion to another and is dependent on the temperature. The hydrated proton has a very much higher conductivity than the metal ions so that where an acid is completely dissociated a higher sensitivity is achieved for anion analysis in ion chromatography using a suppressor column. Some temperature effects are given in Figure 19.

b) Electrometric.

The sensitivity for some important species such as cyanide and sulphide is limited by their low conductivity when compared with halides etc. This can be overcome by using electrometric detection by means of a pair of platinum electrodes set to the correct potential [24]. Phenols and carbohydrates can also be detected with this technique.

c) Spectrophotometric.

By means of a small volume flow through cell the optical properties of the eluting ions can be monitored in the visible or ultra-violet region of the spectrum. For species having no direct optical properties it is possible to provide a "post column reactor" (PCR) which bleeds a colorimetric reagent into the eluent to form a coloured complex which can be detected at the appropriate absorbing wavelength. This procedure can be used when carrying out the analysis of transitional metals by means of pyridylazo (PAR)[25].

d) Fluorimetric.

This is similar to spectrophotometric detection except that a reagent reacting with the eluting ions to form a fluorescing complex is used.

e) Indirect U.V.

This is probably the best system for adaption to conventional HPLC equipment involving the use of a U.V. absorbing eluent such as 10^{-3}M potassium hydrogen phthalate and mainly applicable to the detection of anions [26]. The anions elute and are detected by a U.V. detector cell as a series of negative going peaks. Simple reversal recorder polarity and a backing off facility will produce the usual chromatographic display.

The advantages of the non conductometric based detectors are that no suppressor column is necessary and that the essential sensitivity of IC is preserved.

2.5 Other Ion Separation Techniques

The advent of alternative detection systems and the availability of the equipment being marketed for carrying out high pressure liquid chromatography has brought other manufacturers into the field. The separation of ionic species can now be carried out by mechanisms other than direct ion exchange [28]. The column packings can be made from suitable particle size ion exchange resins, styrene — divinylbenzene based resins, or silaceous supports which are surface treated to confer ion selectivity properties.

a) Polystyrene - Divinyl Benzene Based Resins

(i) Ion Exclusion Chromatography (ICE). Conventional ion chromatography as described in 2.2 does not adequately resolve all the ions present in systems such as mixtures of organic acids and other hydrophobic species. In ICE the resin bead surface acts as a semi-permeable membrane where Donnan exclusion prevents highly ionised molecules, such as strong acids (HCl, HNO_3 etc), from entering the pores in the resin and as a result they pass rapidly out from the column. The uncharged and weakly charged species diffuse through the resin pores and are eluted in a finite time dependent on factors such as pKa of the eluent, temperature, concentration etc. Some rules governing elution order can be formulated, but as the hydrophobic portion of the

molecule dominates by Van der Waals attractions the simple rules are progressively overcome. An example of this technique is shown in Figure 38.

(ii) Coupled Ion Exclusion Chromatography/ion Chromatography (ICE/IC). The rapid elution of strongly ionised species through the ICE column facilitates the analysis of solutions containing both weak and strong ions such as organic acids and mineral acids [27]. The ions passing rapidly through the ICE separator can be retained on a short column of IC separating packing for later elution, or switched directly onto a conventional IC column. ICE/IC facilitates the measurement of traces of hydrophobic species in large concentrations of mineral acids.

(iii) Mobile Phase Ion Chromatography (MPIC) — This separation technique is carried out on columns packed with beads of neutral cross linked polystyrene resin which permit resolution of complex mixtures of organic hydrophobic molecules such as aliphatic and aromatic amines/sulphonates with no pH limitations on the eluent. This particular type of ion chromatography will probably develop to be a major application for industrial analysis of both hydrophilic and hydrophobic molecules. It has been shown recently by Iskundarani and Pietrzyk[29] that the retention enhancement of an organic anionic molecule on a non polar S-DBV resin from an eluent containing a quaternary ammonium salt, it's co anion and a mixed solvent follows a double layer not an ion pair system. In this two major parameters affect the separation. The first is the retention of the quaternary species on the bead surface forming a double layer. The second equilibrium defines the selectivity due to the secondary surface layer.

An example of the potential of this MPIC technique is shown in Figure 39.

b) Silica Based Supports for Ion Chromatography

The present patent situation excludes the sale of S-DVB resin beads to manufacturers of HPLC equipment. As a result alternative packings are being offered such as those marketed by the Separations Group (Hesperia, California, USA) under the "Vydac" Trade name. These are pellicular silica in which the functional ion exchange groups are attached by the established silane type reaction. When used for I.C. they perform satisfactorily, but display different selectivities to the resin based packings. This can be advantageous for ions such as SCN^- and I^- which are strongly retained on resin beads. The major disadvantage of the "Vydac" type materials is in the use of eluents above pH7 with their adverse effect on the silica backbone and the stability of the silyl bond.

Separations of strong ionic species, similar to those found using ion exchange based IC are observed with phthalate based eluents possible using these bonded phases. They also possess the ability to carry out ICE separation.

2.6 Concentration

Whilst the direct methods outlined in previous sections can be used to measure ions in favourable cases down to 0.1mg l^{-1} a small column of "separator" type ion exchange resin can be used to collect the ions from a sample of several hundred millilitres prior to elution and analysis [10]. The quantity which can be accumulated depends on the ion exchange resins, the size of the column and the ions present. As some separation will occur on the concentrator column prior to elution it is preferable to carry out pilot experiments with known quantities of determinands in the same relative proportions as in the sample to be analysed.

2.7 Chemical Reaction

By including reaction columns in the system one or more ions can be removed. Thus, for example, a cation resin which has been previously converted to the silver form using silver nitrate can be used for removing halide ions from the sample in anion analysis[12].

3. Instrumentation

A number of ion chromatographs are available commercially. They fall into two types:

a) Dual column systems using "suppression" of the eluent ions.

b) Single column based equipment.

Type a) appears to have widest acceptance amongst users; with the Dionex Corporation

of Sunnyvale, California, being the sole manufacturer of both hardware and ion exchange resins. The design of this system is shown in Figure 20.

Type b) can be purchased complete, or built into an existing HPLC apparatus using purchased columns and existing detection devices such as conductometric or UV/visible spectrophotometric.

3.1 General

The following is a description of the components of a dual column ion chromatographic system using conductometric detection.

3.1.1 The Eluent System

A high pressure liquid chromatography pump is required to pump eluent solution. This pump should be capable of handling corrosive eluents, usually at low concentrations, have an adjustable delivery rate of 1 to 15 ml min^{-1} and be capable of pumping at pressures up to 100 bar. Usually the working parts are fabricated of stainless steel and the pump is a ram injection type. This type of pump should be fitted with a pressure relief valve or pressure trip switch so that protection is available in the event of blockage on the outlet side.

It is common practice to contain the eluent in a thin-walled collapsible plastic bag which is an advantage when the system is to be operated under anaerobic conditions.

3.1.2 Sample Injection

This can be done manually using a syringe equipped with a fine bore needle and septum cap, but repeatability is not as good as when a mechanical injection device is used. The sample size is dependent on the analysis to be carried out and the concentration of the determinands. Using a six port injection valve, shown in Figure 20, the size of the sample loop can be varied but for many uses a 0.3 ml sample is satisfactory. The "dead" volumes in the system must be minimised if contamination is to be avoided. The injection loop can be filled manually or using a pumped system. In automated units, samples can be loaded into a suitable turntable using a rinse cycle between each injection to avoid the risk of cross contamination.

3.1.3 Separator and Suppressor Columns

The size and packing of the columns have been considered previously The earlier low pressure instruments used glass or plastic columns with plastic connectors and fine bore PTFE tubing connections. The use of these was limited to about 500psi using 30 micron resins. However, with the advent of the high efficiency columns using 10 micron material the operating pressures have been in the region of 500psi requiring the columns and connections to be fabricated from stainless steel.

3.1.4 Conductivity Measurement

The outline of conductivity measurement has been given in section 2.4 where the importance of cell volume and shape has been stressed. Whilst a crude cell shown in Figure 21A can be used, for work at maximum sensitivity the cell should be based on a tubular system as shown in Figure 21B. There is some advantage to using a conductivity cell system which has been calibrated in absolute units and has a switch to enable a range change to be made. A suitable set of ranges in 0 to 0.3, to 1, to 3, to 10, to 30, to 100, to 300 and to 1000 microsiemens cm^{-1}. As conductivity is a linear response for a particular determinand the range change can be used to attenuate the signal measured.

3.1.5 Ion Exclusion Columns (HPICE)

The size and packing of ion exclusion columns is similar to that of the separator and suppressor system but as has been shown in Table 4 very much longer columns are used. Whilst only one column is used the length is more easily achieved using stainless steel tubing than with borosilicate glass columns.

3.1.6 Signal Measurement

The output from the conductivity cell is fed to a "bridge system" linked to a suitably

ranged potentiometric recorder and either peak height or peak area measured, and compared with that of measurement from a calibration graph for the appropriate ionic species; where a large number of samples need to be measured there is an advantage to using an electronic integrator which avoids the need for laborious manual measurement and calculation. With a computing digital integrator the precision can be better than that using peak height measurement, but much depends on the integrator being used and the retention time of the peak. As a "rule of thumb" peak area becomes superior to peak height measurement when the retention time is greater than 5 minutes and a "good" integration system is being used. The measurement of baseline and the way in which a peak is defined and measured vary from one make of integrator to another so it is not easy to define a "good" integrator system without the practical resource of comparing manually measured peaks with integrator measured peaks on a series of samples or standards. An example of the results of doing this for a cation analysis is shown in Figure 22 [13].

3.1.7 Valves

One of the key items of equipment in an ion chromatograph is the design and operation of the multi-port switching valves. Ideally they should have a small void volume, be fast acting, able to operate with corrosive liquids and be maintenance free. The Dionex instruments are equipped with slide valves made of nylon and polytetrafluorethylene. They are pneumatically operated and a diagrammatic outline is shown in Figure 23A. They operate well up to pressures of 30 to 50 bar but at higher pressures tend to develop leaks. They have small sample hold-up volumes and are simple and easy to maintain. Valve corrosion does not appear to be a problem with the system. Later high pressure systems require special valves to prevent leakage. Figures 23B shows the design of a multi-port stainless steel valve which is easy to use in a manual mode.

3.2 Automatic Operation

Whilst a fully manually operated instrument can be made from conventional high pressure liquid chromatographic equipment, automation can be used on all stages. The later Dionex range of instruments (see section 3.3) have been designed with automation in view and all valves are actuated by electrical switches operating magnetic valves which in turn control the flow of compressed air used to position slide valves. Moreover in this range of instruments the suppressor column is continuously regenerated.

A microprocessing unit can be used to automate the steps in IC analysis as follows:

(i) Sample selection and injection
(ii) Sample analysis
(iii) Suppressor column regeneration where necessary.
(iv) Calculation and output of results.

The key to the sequence is the second stage, sample analysis. This has to be defined in terms of the eluent used and the time required for the analysis to be completed. A simple sequence for the low pressure column suppressed Dionex equipment would be:

(i) Sample injection
(ii) Selection of conductivity range $0.3 \mu S\ cm^{-1}$
(iii) Change to conductivity range $1 \mu S\ cm^{-1}$ at 3 minutes
(iv) Change to conductivity range $0.3 \mu S\ cm^{-1}$ at 6 minutes
(v) Analysis complete at 30 minutes
(vi) Repeat stages (i) to (v) 15 times
(vii) Suppressor column to be regenerated after 15 samples have been analysed. This may mean 45 to 90 minutes when no analysis results are available or where two suppressor columns are installed, two activities going on simultaneously. This later possibility saves 9 to 18% of instrument time if the instrument is being operated 24 hours a day.

Samples can be loaded into cups on a turntable which is coupled to the IC electrically such that when each sample has been analysed the sample probe is moved to the next sample. The sample is transferred to the injection loop on the analyser using a peristaltic pump. Care must be taken to ensure the sample lines are short, have a small volume and they are

adequately washed out between samples with deionised water to minimise cross-contamination. As several different makes of turntable system are available and each operates in a different way they will not be considered in detail here.

The conductivity peaks produced from the IC analyser can be measured manually from the trace produced on a strip chart recorder or taken into an electronic integrator, calculated, and the results printed on a paper tape as discussed in section 3.1.6 above. On some integrators a sequence of calibration standards can be included to enable a frequent cross-check to be made. However, care must be exercised when this is done as a small reduction in retention time, will take place slowly and changes in the "time window" will be required. (see Section 5.5). When gross contamination of the separator column occurs a marked change will be found.

For an instrument required for general analysis it is desirable to be able to change the program in a simple way by erasing a stage from the computer memory and writing in a new stage. With the Dionex Model 12 the position of valves is shown by indicator lights so that the program written into the microprocessing unit can be checked line by line. Some typical programs designed for the Model 12 Dionex instrument as shown in Figure 24.

3.3 Commercial Equipment Available

Developments in I.C. have progressed to the point where the description of a particular model of chromatograph is pointless. All manufacturers supply adequate literature on their product which can be consulted for details.

4 Analysis

4.1 Sample Preparation

It is essential that the sample for analysis by ion chromatography should be single phase. Any particulate matter must be removed prior to injection as otherwise the first column in the system will become blocked. The life of the separator column can be enhanced by using a small "pre" column containing "separator resin" prior to the separator column.

4.2 Eluent Preparation

In the preparation of the eluent it is essential that good quality deionised water (conductivity less than 5 μ siemens cm^{-1}), is used and that all apparatus is cleaned to the highest standards. The composition of some typical eluent solutions are shown in Table 4.

4.3 Methods of Measurement

A series of three or preferably four solutions of the determinand are prepared in deionised water, (conductivity less than 5 S cm^{-1}), such that their concentrations are evenly spaced over the range of interest. Thus, for example, the range of concentration might be 0 to 50 mg l^{-1} and the standard solutions would contain 0, 10, 20, 30, 50 mg l^{-1}. The peak height or area is then measured on the recorder chart for each solution and the results are subjected to linear regression analysis, for a plot of peak height, or area, against determinand concentration (see Fig 25). Where the line is not linear a quadratic regression analysis can be used. This gives a mean line and 95% confidence limits of the position of the line. Using the 95% confidence limits of this line a useful control graph is provided which allows a single point check on a day to day basis to be used. The full calibration is then only checked about twice a week for 24 hour a day operation or after 150 samples unless there are deviations from the single point checks on the control graph. Alternatively to this procedure the best estimate of the line of peak height, or area, against concentration may be drawn graphically, but this can only provide a guide to errors involved.

Whilst the calibration curve should pass through the origin it is sometimes marginally positive or negative at zero concentration. If positive deviation is found this may be a result of contamination of the standards probably resulting from the dilution process used to prepare them and if negative it may be due to leaks or again errors in preparing the

standards (see Figure 26). Occasionally a non-linear curve is found due to strong absorption of the determinand on to the ion exchange system (see Figure 26).

A calibration curve of response against determinand concentration can be made using the method of "standard additions". That is by adding 50, 100 and 150% of the determinand, to the sample and measuring the response. Regression analysis, usually linear regression, can again be used to estimate the response — concentration curve as shown in Figure 27. However, the method of standard additions does not overcome errors arising from a species showing the same retention time as the determinand (see Section 4.6).

Table 4. Typical Eluents used in I.C.

	Analysis	Separator Column (mm)		Eluent	
		bore	length	Strength (M)	Quantities required for 41
	Anions				
1.	General to and including sulphate	3	500	0.003M $NaHCO_3$ 0.0024M Na_2CO_3	1.00 8g $NaHCO_3$ 1.0176g Na_2CO_3
2.	Weak eluent for F^- Cl^- and NO^-_2	3	500	0.0015M $NaHCO_3$	0.5040g $NaHCO_3$
3.	For measurement to NO^-_3 eliminating $PO^{4\equiv}$ interlerence	3	500	0.001M NaOH 0.002M Na_2CO_3	0.1600g NaOH 0.848g Na_2CO_3
4.	Estimation of Weak carboxylic acids	3	500	0.005M $Na_2B_4O_7$	0.76284g $Na_2B_4O_7$ $1OH_2O$
	Cations				
1.	Alkali Metals	3	500	0.005M HCl	20 mls M. HCl
2.	Mg^{++} and Ca^{++}	3	250	0.0015M m-phenylenediamine	1.0865gm $C_6H_4(NH_2)_2$ HCl

4.4 Standard Solution

4.4.1 Mixed Anion Standard

It is useful to check the resolution and sensitivity for anion analysis using a mixed standard of fluoride, chloride, nitrite, phosphate, bromide, nitrate and sulphate. This can be prepared using the quantities and dilutions shown in Table 5. A typical recorder chart trace obtained using this mixed standard is shown in Figure 28.

Table 5. Mixed Standard for Anion Analysis

Anion	Weight Required in 1.1 of solution for 1000 mg 1^{-1} (A)	mls of solution (A) required for 1 1. mls	mg 1^{-1} in standard
Fluoride	2.2100g NaF	3	3
Chloride	1.6484g NaCl	4	4
Nitrite	1.4998g $NaNO_2$	10	10
Phosphate	3.7710g $Na_2HPO_412H_2O$	50	50
Bromide	1.2877g NaBr	10	10
Nitrate	1.3707g $NaNO_3$	30	30
Sulphate	1.8142g K_2SO_4	50	50

4.4.2 Mixed Cation Standard

Two cation mixed standards have been found to be useful. One containing lithium, sodium and potassium and the other sodium, calcium and magnesium. These can be prepared using the quantities and dilutions shown in Table 6. Two typical recorder chart traces obtained using these standards are shown in Figures 29 and 30.

Table 6. Mixed Standards for Cation Analysis

Cations	Weight Required in 1 l. of solution for 1000 mg l^{-1} (C)	mls of solution (C) required for 1 l. mls	mg l^{-1} in standard
1. Alkali Metals			
Lithium	6.1091g LiCl	5	5
Sodium	2.5420g NaCl	20	20
Potassium	1.9067g KCl	50	50
2. Alkali & Alkaline Earth Metals			
Sodium	2.5420g NaCl	15	15
Calcium	3.6691g $CaCl_2 2H_2O$	50	50
Magnesium	8.3632g $MgCl_2 6H_2O$	10	10

4.5 Suppressor Column Regeneration (where necessary)

4.5.1 Anion Analysis Suppressor Column

The eluent is switched off and demineralised water is pumped through the suppressor column from the bottom to the top at 2.5 ml min^{-1} for 10 minutes. 0.5M sulphuric acid is then pumped from the bottom to the top of the column at 2.5 ml min^{-1} for 15 minutes. Following the acid, deionised water is pumped through the column from the bottom to the top of the column at 2.5 ml min^{-1} for 45 minutes. The eluent flow is reinstated from the top of the column to the bottom at the required flowrate until the base line is again steady which usually takes 15 minutes. The regeneration of the column in a direction which is "counter current" to the mode of operation gives the most efficient method.

4.5.2 Cation Analysis Suppressor Column

It is carried out similarly to the regeneration of the anion suppressor column except that 0.5M sodium hydroxide is used in place of 0.5M sulphuric acid.

Note Dionex Ion Chromatographs are also fitted with fibre suppression systems where the suppressor is continuously regenerated so that the equipment may be used continuously, if required. This type of suppressor has the advantage that the size of the nitrate ion peak does not vary with the condition of the suppressor column as found in the earlier Dionex chromatographs and that there is no loss in separation efficiency.

4.6 Interference

Common to any chromatographic procedure several determinands may have the same retention times and this can lead to a gross mis-interpretation of the results. A guide to anions and cations which can have similar retention times is given in tables 8 and 9, respectively. Reduction of the particle size of the ion exchange resin has, however, resulted in greater column efficiency providing separation of some species having close retention times. There will still be some ions which cannot be separated.

A negative peak is often seen just after the sample is injected and interference can result with ions having a small retention time (see tables 6 and 7). This is caused by the sample having a low ionic strength relative to the eluent as the discrete slug of sample solvent passes through the conductivity cell. This can be corrected by addition of the appropriate concentration of salt used in the eluent to the sample.

Where there is a large content of a particular determinand relative to others a section of the trace may be blanketed. Thus, for example, chloride in sea water can blanket an appreciable section of the chromatogram. This can only be overcome by chemically removing the offending determinand. For example in the case of sea water by removing halides by passing the sample through a cation resin in the silver form.

5 Uses in Water Analysis

5.1 Anion Analysis

IC is a very useful method for the analysis of cations and anions in aqueous solution. It is particularly useful for the analysis of anions when a high sensitivity is required, quick results are needed, only a small sample is available and there are several different ions involved. An illustration is provided in the analysis of a rain water sample using a 0.1 ml sample and measurement of 4.1 mg l^{-1} of nitrate and 3.6 mg l^{-1} of sulphate (17) (see Figure 31). Measurement of anion present in boiler drum water, glycollate, chloride, sulphite, sulphate and phosphate is shown in Figure 32. Table 7 shows the analysis of typical ground and drinking water from six locations on the Bay Area at San Francisco:

Table 7. Anion Content of Ground Water Samples (14)

SAMPLE	mg l^{-1}			
	Fluoride	Chloride	Nitrate	Sulphate
Sunnyvale tap water	0.065	47	1.61	31.32
Nancy Zellhoefer's Well	0.45	82	22.3	12.2
Crystal Springs Reservoir	0.027	3.6	0	4.2
San Francisquito Creek	0.22	161	4.5	528
Stevens Creek Reservoir	0.164	44	0	117.7
Lake Vasona	0.26	28.5	0.86	213

As the retention times can be used as an aid to identification it is useful to list some relative values as shown in Table 8 (13).

5.2 Cations

Whilst the same general usefulness listed for anions might be expected for cations, atomic absorption techniques are well developed and there is in consequence not such a great need for another method. Moreover, some determinands can be precipitated as the hydroxide on the strongly basic suppressor column unless hydrochloric or nitric acid eluent is used.

However, some retention times have been reported for cations using m-phenylenediamine as the eluent as shown in Table 9 (10) where total precipitation has been avoided by the eluent system used.

5.3 Analysis of Weak Acid Anions

As outlined in section 2.5 a cation exchange column can be used to separate strong and weak acids using a pure water eluent. A suitable flow system for carrying out this type of analysis is shown in Figure 33 and this can be done using the Dionex model 16 instrument (see section 3.3). Table 8 gives the relative retention times and responses of some organic acids (13). IC does not give a good method for measuring phenol as shown by the poor response given in Table 10.

Table 8.

A 300μ 1 sample injection was used; solvent system 0.003M $NaHCO_3$ and 0.0024M Na_2CO_3 at 138 mls hour^{-1} using a 250 mm long 4.5mm bore column of separation resin; detector range 0 – 3μS cm^{-1}. (Resin used was prepared from 30μm size polystyrene beads with 4% divinyl benzene cross linked, sulphonated, (see section2.2), and treated with an emulsion of ground Rohm and Haas IRA 402 resin).

Anion	Retention Time (Mins)	Peak Height Per mg l^{-1} (a) (mm)
Fluoride	2.5	63
Chloride	4.0	25
Nitrite	4.5	5
Lactate	4.5	6
Glycollate	4.8	8
Formate	4.8	15
Acetate	5.0	6
Phosphate	6.0	9
Propionate	6.0	16
Butyrate	6.8	6
Bromide	8.0	9
Nitrate	9.0	10
Benzoate	12.0	8
Succinate	15.3	3
Glutarate	15.3	2
Adipate	17.0	2
Malonate	17.8	4
Sulphite	18.5	5
Sulphate	19.0	7
Oxalate	22.0	2

(a) corresponds to 0.3μg determinand injected.

Table 9.

A 300μ 1 sample injection was used; solvent system 0.002M m-phenylenediamine hydrochloride at 3.5 mls min^{-1} using a 250 mm long 4.5 mm bore column of separation resin; detector range 0 – 3μS cm^{-1} (resin used was prepared from 30μm size polystyrene beads with 4% divinyl benzene cross linkage, sulphonated (see section2.2)).

	Retention Time (mins)	Conc (mg l^{-1})	Peak Height (mm)	Extrapolated Peak Height for 20 mg l^{-1} (a) (mm)
Lithium	1	2	180	1800
Sodium	1	2	55	550
Potassium	1	2	40	400
Ammonium	1.5	10	62	126
Vanadous	1.5	20	50	50
Titanic	2.0	200	14	1.4
Rubidium	2.5	2	14	140
Caesium	4.5	2	12	120
Magnesium	4.5	10	55	110
Ferrous	4.5	50	3	1.2
Ferric	4.5	50	3	1.2
Nickelous	5.5	10	4	8
Cobaltous	6.0	10	9	18
Calcium	8.5	20	111	111
Strontium	12.0	20	16	16
Zinc	15.5	10	6	16
Barium	35.0	20	8	8

Table 10.

Weak Acid Separation. A 300μl sample injection was used; solvent system, water at 60 ml/hour using a 1900 mm long 4.5 mm bore column packed with Dowex 50W × 4 200 - 400 US mesh cation strong acid resin.

Weak Acid	Retention Time (Mins)	Peak Height mm per mg l^{-1} (a)
Benzoic	10.0	6.2
Malonic	11.0	5.6
Phthalic	11.5	2.9
Oxalic	12.0	4.2
Phenolic	12.0	$<$0.0001
Formic	12.0	24
Lactic	14.5	5.9
Glycollic	14.5	6.1
Succinic	15.1	5.3
Glutaric	19.3	4.0
Acetic	20.0	5.7
Adipic	20.8	3.2
Propionic	25.0	0.9
Carbonic	25.5	0.9
Butyric	29.5	6.3

(a) corresponding to 0.3μg determinand injected

Table 11.

Added ion Content ($\mu g.l^{-1}$)			Volume used (mls)	Peak height (mm)			Calculated Peak height(a) for 100 mls (mm)		
Chloride	Nitrate	Sulphate		Chloride	Nitrate	Sulphate	Chloride	Nitrate	Sulphate
-	-	-	150	34, 32	Nil, Nil	20, 8			
1	10	10	100	33, 35	8, 9	10, 10	11, 13	8, 9	0.7, 0.7
1	10	10	200	75, 76	12, 14	18, 20	15.5, 16	6, 7	- , 0.7
1	10	10	300	117	18, 27	29, 38	17	6, 9	0.4, 3.0
1	10	10	400	138, 113	38, 38	48, 44	17.5, 6.25	9.5, 9.5	2.3, 1.3
				Mean			13.75	8.0	1.3
				Standard Deviation			4.0	1.5	0.98

(a) $\left\{ \frac{\text{peak height (mm)}}{\text{Volume (mls)}} - \frac{\text{blank peak height (mm)}}{\text{Volume (mls)}} \right\} \times 100$

5.4 Analysis of Ultra Pure Water

The concentration of anions in a small pre-column and subsequent elution and measurement of anions from high purity water has been the subject of several papers [10,19]. A flow system for achieving this is shown in Figure 34. Separation column ion exchange resin is used for the concentration column. Some separation of ions takes place during the concentration stage such that the maximum loading is dependent on the relative concentration and relative retention times of the species involved. As the rate of passing the sample through the concentration column is increased the retention time on eluting the sample on to the separation column tends to decrease. The change in eluent concentration produced by the water in the void volume in the concentrator gives an immediate negative deflection as shown in Figure 35 and this can cause peaks eluting close to this position to have a greater error. With the method developed, chloride

measurements of $15 \times 10^{-11} \pm 15 \times 10^{-11}$ gm^{-1} have been achieved as can be calculated from the results shown in Figure 36. The method is generally applicable to other anions as can be seen from results shown in Table 11 for nitrate and sulphate.

Cations can be concentrated using cation separation resin and eluted on to a cation separator column in a similar way to that used for the anion system.

5.5 On-Line Analysis

Ion analysis provides a means of on-line analysis for plant control purposes where measurement of several cations or anions is required in a sample. It is particularly useful where the frequency of change in concentration is not greater than once an hour. Thus it provides a useful method for river water analysis where there is a short water course and variable rainfall and a demineralisation plant is being operated. However, the life of the separator resin does present a restriction in this at present as the retention time decreases with time. The reason for this is not known, but it is not entirely a function of the eluent system. The rate of decrease in retention time for a phenol eluent system used in anion analysis is shown in Figure 37[10]. This lifetime represents a possible analysis load, incuding standards of about 1500 samples. It has been shown that cation separation columns have a similar limited life.

Whilst there has been no published information of on-line IC systems the author is aware of units which have operated for at least two years. A commercial instrument has yet to be made.

6 Comparison with other Methods

6.1 Anion Analysis

It is possible to inject on to an anion separation column samples in the range 0.1 to 1.0 ml and so IC provides a method for measuring a range of different anions in an aqueous sample at a sensitivity in the 1 to 50 mg l^{-1} range of concentration in less than half an hour. It provides one of the most sensitive methods for the measurement of many anions in aqueous solution particularly when a concentration procedure is used (see section 2.6). The results obtained with IC are the same as those obtained by classical methods of analysis where each is free from interference [15,16]. IC is particularly useful for carrying out a scan to see which anions may be present in an aqueous sample [10]. The measurement of sulphate [17], the organic carboxylic acids by ion exclusion [11] and anions in highly coloured aqueous solutions [18] are particular cases where the ease of measurement of IC is often very much superior to existing methods.

Where a given type of analysis is required on a large number of samples automated colorimetric analysis can provide a better system than IC. Thus, for example, the analysis of 40 samples for chloride can be carried out on a Technicon Auto Analyser channel in an hour [16] C provides a useful method of analysis of anions where two analyses per hour are required for several different anions.

6.2 Cation Analysis

IC can be used to measure the alkali and alkaline earth metals, but as yet the measurement of sodium, potassium calcium and magnesium cannot be easily achieved with a single eluent. Whilst some of the transition metals have been measured, the sensitivity is often poor [10]. With atomic absorption and emission measurement of cations easily carried out at low concentrations and now well established, it seems unlikely that IC will find an overall use in the laboratory for cation measurements. However, there will be small areas where cation analysis will be useful, for example in amine analysis. IC may also be useful for measurement of cations in on-line measurements when a suitable on-line instrument is developed.

7. Fault finding

Whilst the following details some of the faults and their response in IC, a diagnostic system has yet to be drawn up which will enable a systematic survey to be undertaken. The following is arbitrarily divided into two sections concerned with Operating Problems and

Instrumental Faults. At this stage of development the list should not be taken as comprehensive, but is meant to include the main problems likely to be encountered in sample analysis systems.

7.1 Problems Arising From Measurement of Determinand

Problem	Possible Reason	Corrective Action
Retention times too short	Resin has lost selectivity	Replace resin or try cleaning
	Eluent flowrate too high	Check flowrate and reduce if necessary
	Eluent too strong	Check eluent concentration and reduce if necessary
	Wrong eluent	Check and replace if necessary
Two peaks together	Resolution cannot be achieved	Remove one peak chemically
	Conductivity meter on incorrect range	Check and change range
Poor sensitivity (peaks too small)	Sample too small	Increase sample concentration or size
	Too little ion in sample	Use concentration process
Negative Peak	Water diluting eluent from sample injected	Add eluent to sample where practical
Unstable base line	Suppressor column not at equilibrium	Re-equilibrate suppressor column
	Particles in eluent	Make fresh eluent
	Liquid Leaks	Check and remake joints where necessary
	Air voids in system	By-pass column and pump at high flowrate for short period
	Contamination of column with poor quality demineralised water	Check conductivity of demineralised water and replace if $> 5 \mu S\ cm^{-1}$
	Conductivity meter on too sensitive a scale	Use higher range of conductivity meter
Poor Repeatability	Ambient temperature fluctuations	In extreme cases insulate reservoirs and columns
	Flowrate irregular	Check pump operation and examine for leaks
	Fouled suppressor column	Replace suppressor column
	Valve fault	Check valve operation
	Not sufficient flush of injection point between samples	Increase flush period for injection loop
Ghost Peaks	Lack of time between sample injections	Increase the time between sample analysis
High conductivity reading after regeneration of suppressor column	Poor rinse down of suppressor column	Equilibrate the suppressor column with eluent
	Wrong regenerant used	Check regenerant composition
	No regeneration carried out	Check that the sequence of operations is carried out correctly

7.2 Problems from Instrument Faults

Problem	Possible Reason	Corrective Action
Valves not Operating	Slide component sticking	Check valve operation; dismantle clean or replace as necessary
	Compressed air pressure too low	Check pressure
	Fault in magnetic valve operator	Check operation; replace as necessary
	Electrical switch fault	Check circuit
	Fault in control circuit	Check circuit
Too high a pressure	Blockage of line	Locate source of choke by disconnecting lines and pumping
	Blockage of column	Prove which column is choked by disconnecting and pumping. Inversion of a column can sometimes clear a choke. Otherwise empty and repack after washing or renew column.
Liquid leaks	Faulty joints	Locate leak and remake joint
	Faulty valves	Dismantle and clean valve Replace where necessary
Resin Leakage from column	Column Support Failure	Replace column support. If a mixture of resin has taken place replace all columns involved.
No conductivity reading	Fault in cell	Check cell with 0.001 M potassium chloride solution which should have a conductivity of $147 \pm 2 \mu S\ cm^{-1}$ at 25°C or $98 \pm \mu S\ cm^{-1}$ at 18°C
	Fault in electronic circuit	Check if possible and replace

8. Future Developments

8.1 Separator Columns

Developments in this field seem likely to give resins with a better separating efficiency than those available at present. Whether they will have a longer life cannot be forecast as the life of anion resins has presented problems since they were first used. It seems likely that more selectiveion exchange resins will be developed for particular analyses.

8.2 Instrumentation

Whilst the commercial instruments are already at the forefront of the field it seems likely that development will continue at a rapid pace and will make use of more sophisticated microprocessing units to aid its progress. It seems likely that only the start of development in flow switching systems has yet been seen.

The technique seems to be suitable for on-line measurement and the development of a more robust system for use on the plant seems likely. This would aid continuous measurement of water quality where relatively rapid changes occur. In this case the microprocessor might also be used to control small plant sections simultaneously

There could be development by changing eluent composition during the course of analysis which would be similar to solvent gradient elution in high performance liquid chromatography [20].

8.3 Detectors.

Although conductometric detection is simple and sensitive, it falls short on specificity and general applicability to weakly conducting species. Spectrophotometric, amperometric and fluometric detectors are available and no doubt, in the future, other types will be produced. The ability to react the ionic species eluting from the separator column with chemical reagents will broaden the field of application to cover the selective measurement of many ions.

Further development in more specific detection systems seem probable.

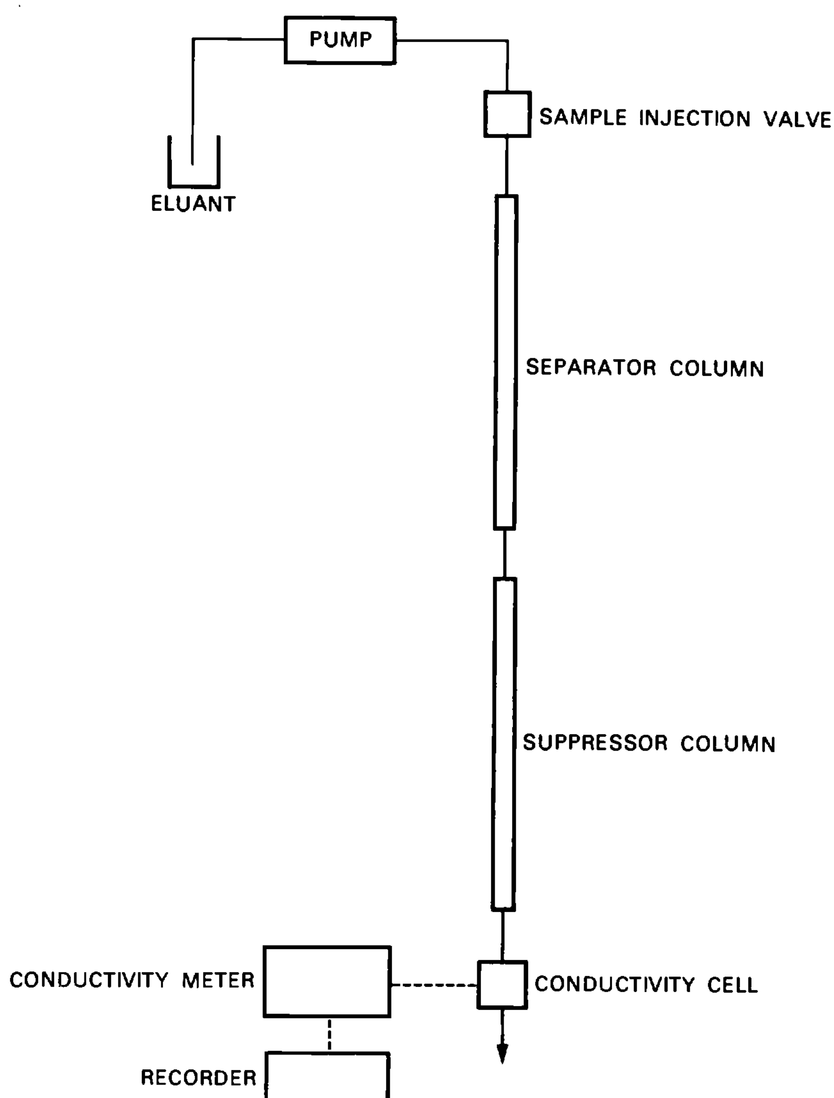
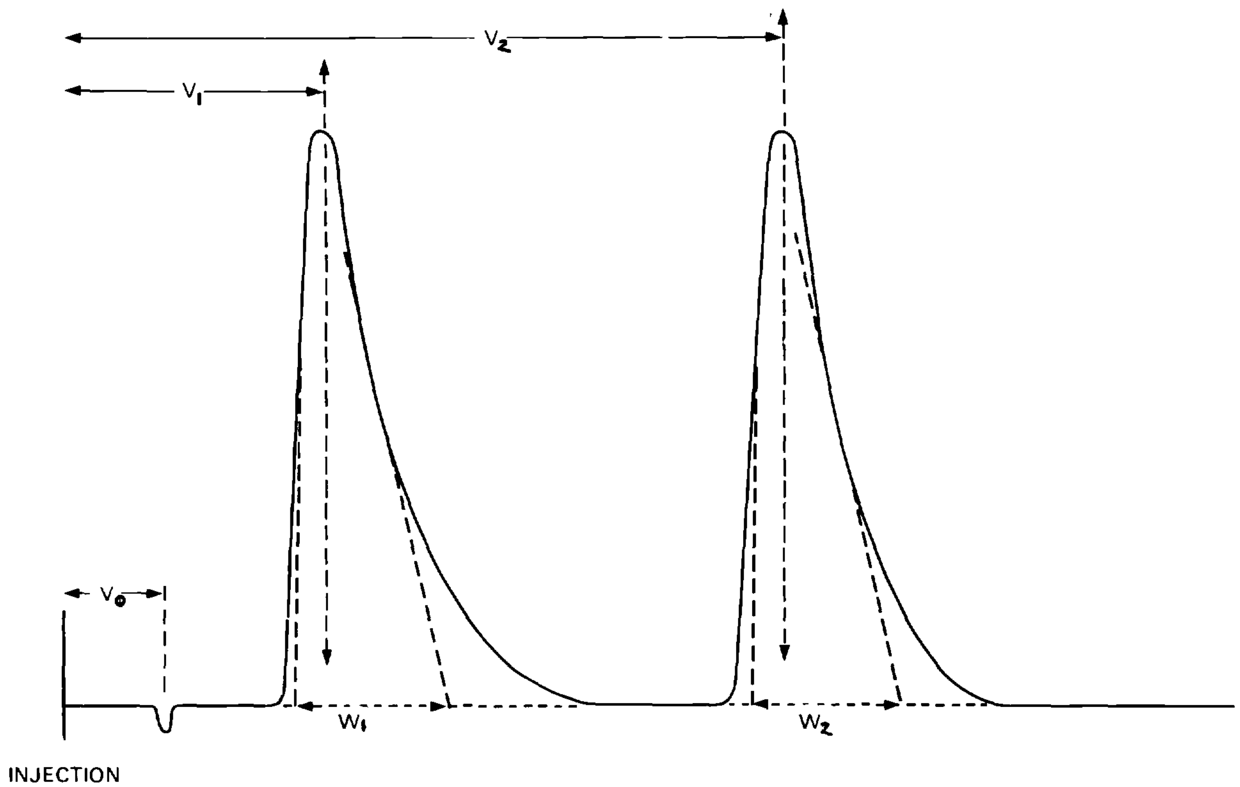


FIGURE 17 DIAGRAMATIC ION CHROMATOGRAPHIC ANALYSER



(a) PARTITION COEFFICIENT $K = \frac{V - V_o}{V_o}$

(b) SELECTIVITY $= \frac{K_2}{K_1}$

(c) RESOLUTION $R = \frac{2(V_2 - V_1)}{(W_2 + W_1)}$

(d) NUMBER OF THEORETICAL PLATES $N = 16\left(\frac{V}{W}\right)^2$

FIGURE 18

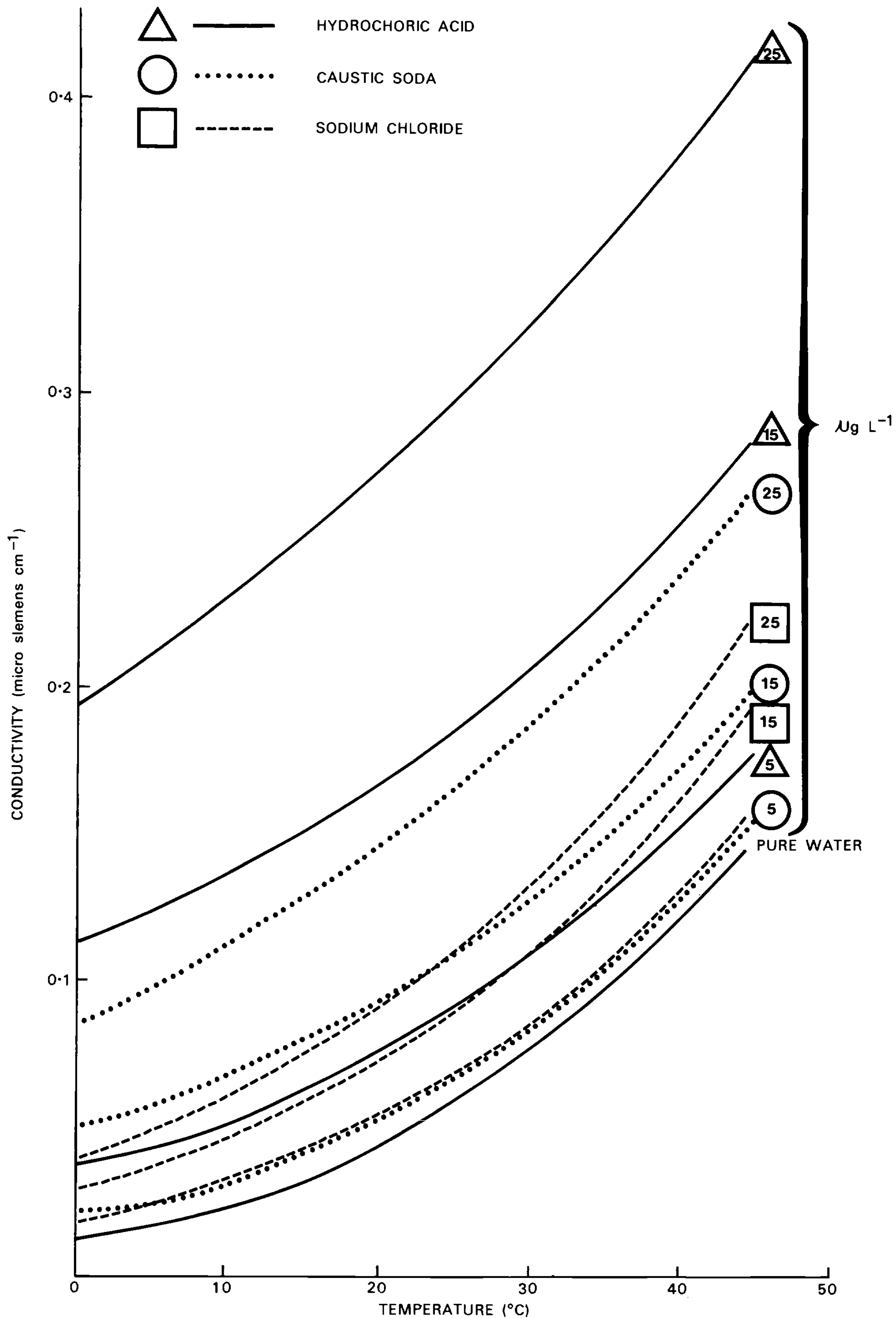


FIGURE 19

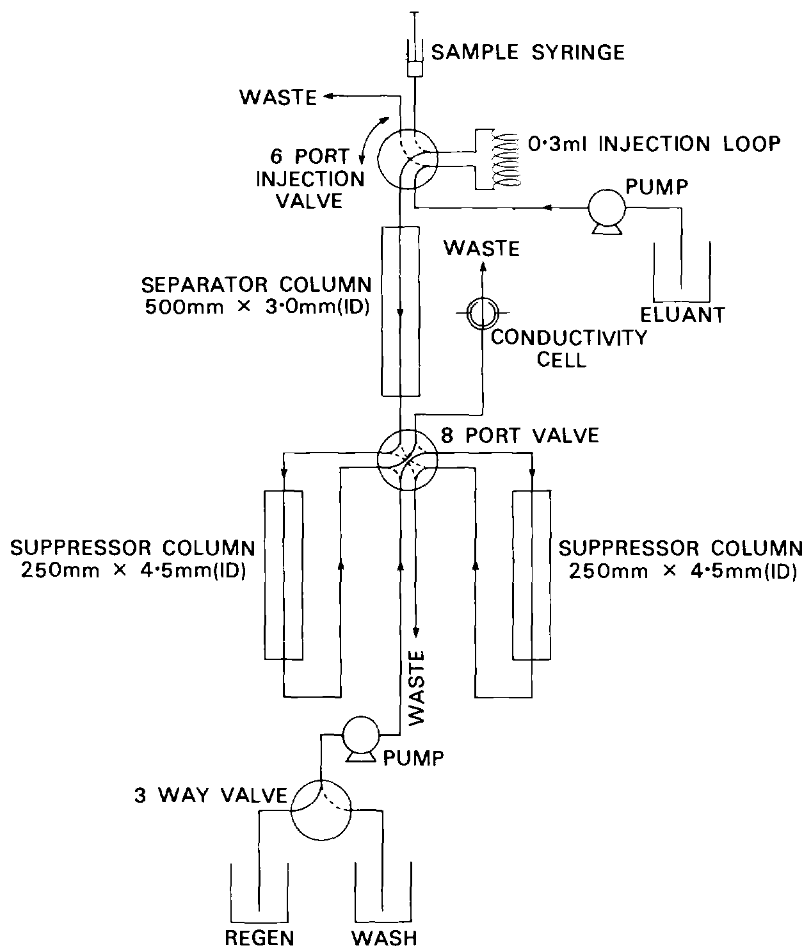


FIGURE 20

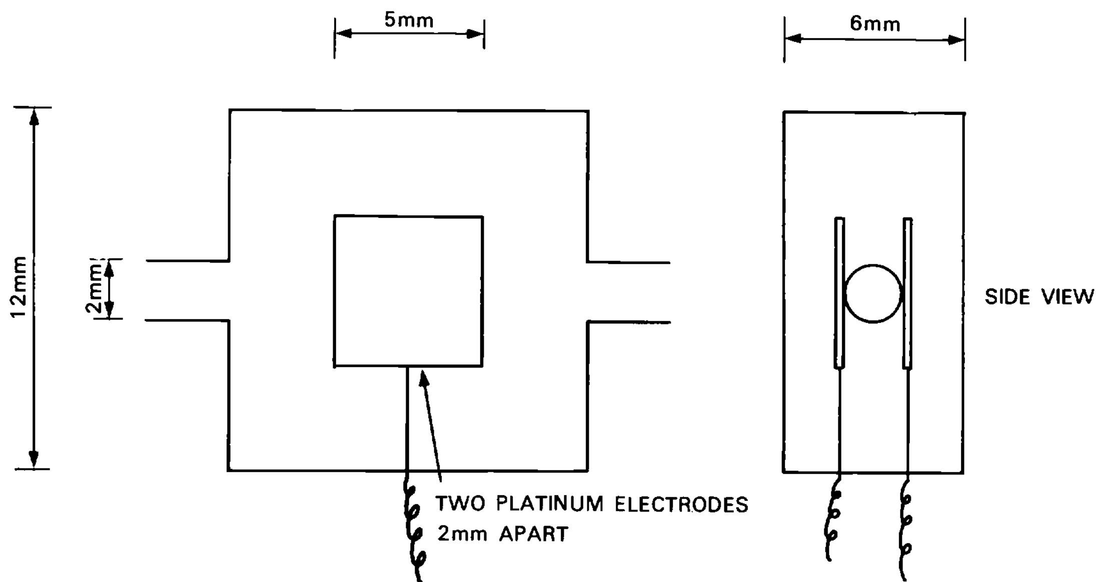


FIGURE 21A GLASS FLOW CELL CELL VOLUME ≃ 0·5ml K=0·1

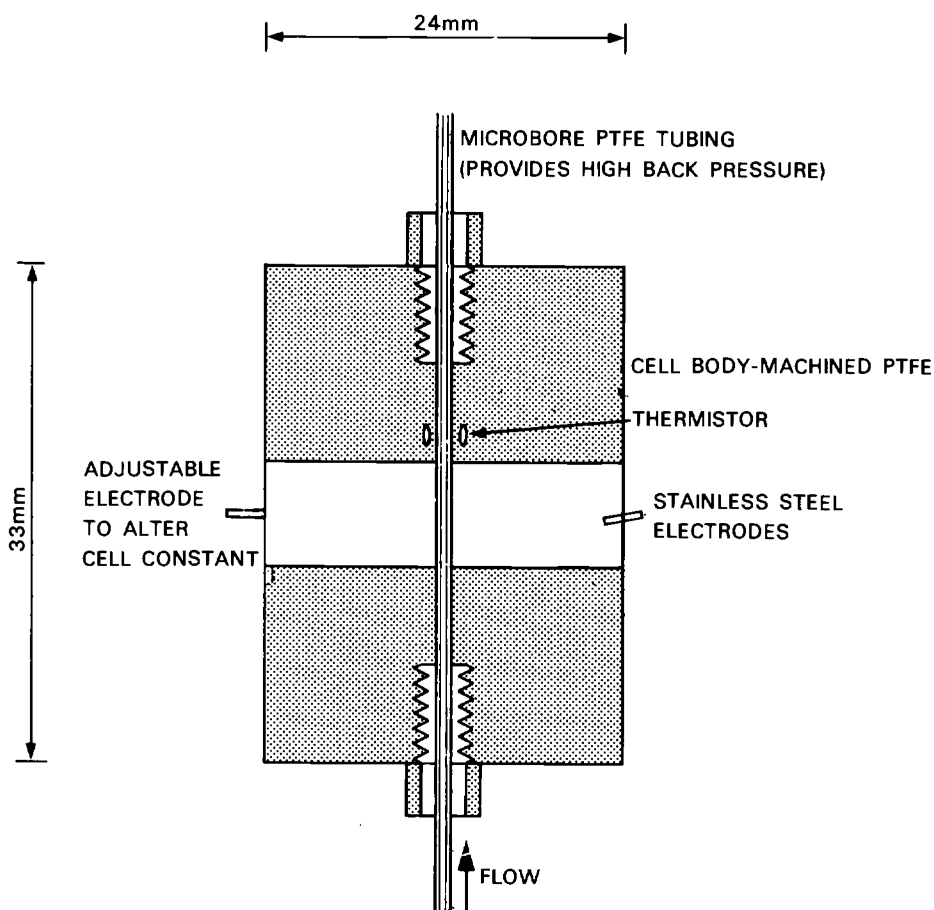


FIGURE 21B SMALL BORE FLOW CELL CELL VOLUME ≃ 6μl

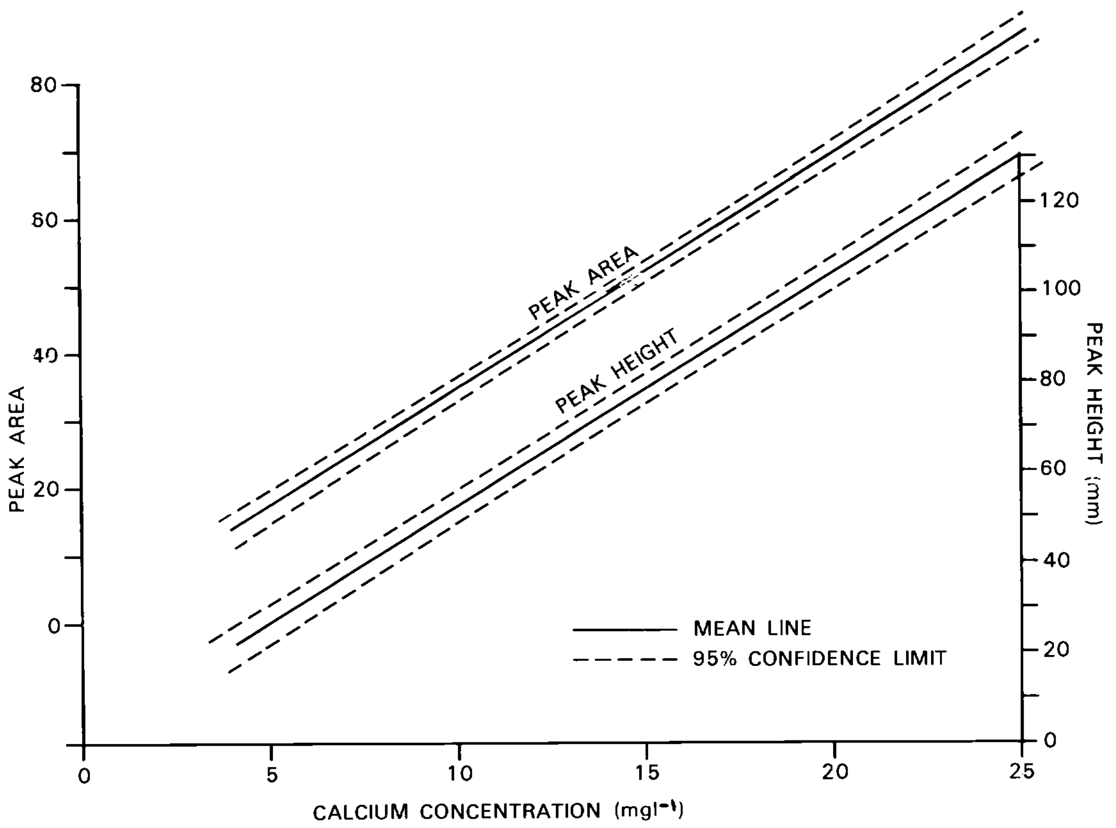


FIGURE 22

FIGURE 23A 4 PORT SAMPLE INJECTION VALVE (MANUAL)

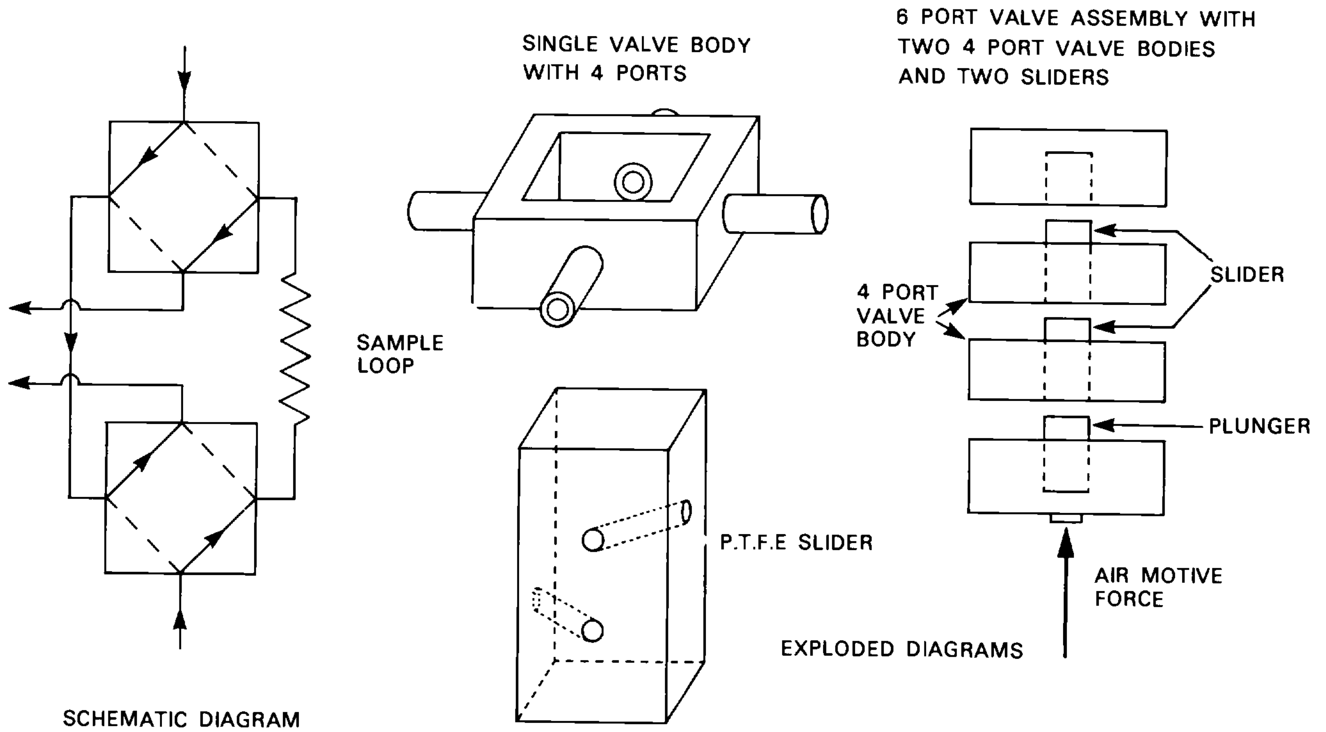
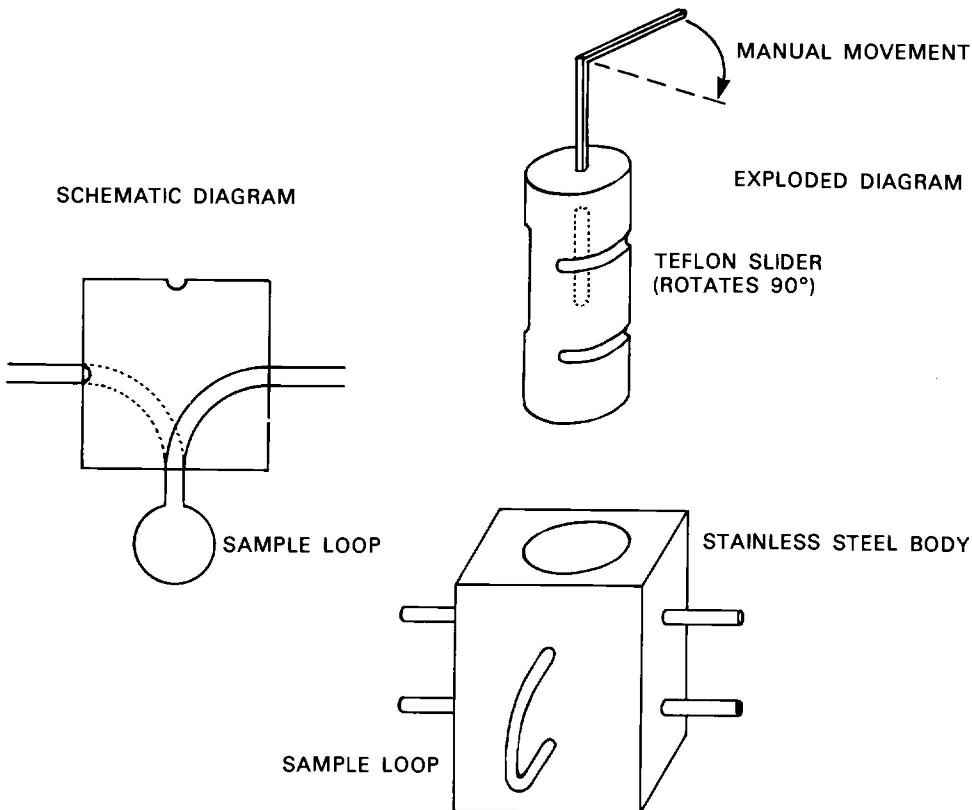


FIGURE 23B 6 PORT SAMPLE INJECTION VALVE (MANUAL)



PROGRAMMING FORM

PROGRAM | TYPE ANALYSIS | ANION ANALYSIS & REGENERATION | DATE

STEP	TIME (MIN)	DETFCTOR RANGE (a)	H_2O/RGN	ELUANT	PUMP		LOAD/INJ	SEP	SUP	AUXILIARY FUNCTIONS		
					RGN	ELU				1 RECORDER	2 INTEGRATOR	3 SAMPLER
						ANION ANALYSIS						
0	0·1	4	H_2O	E_1	OFF	ON	LOAD	ON	ON	ON	OFF	OFF
1	1·5	4	"	"	"	"	"	"	"	"	"	ON
2	0·1	3	"	"	"	"	INJECT	"	"	"	"	OFF
3	2·5	4	"	"	"	"	LOAD	"	"	"	"	"
4	0·1	4	"	"	"	"	"	"	"	"	"	"
			SUB PROGRAMME FOR REGENERATION OF SUPPRESSOR COLUMN									
0	0·1	8	H_2O	E_4	OFF	ON	LOAD	ON	ON	OFF	OFF	OFF
1	10	"	"	"	"	"	"	"	"	"	"	"
2	15	"	RGN	"	ON	"	"	"	OFF	"	"	"
3	45	"	H_2O	"	"	"	"	"	"	"	"	"
4	0·1	"	"	"	OFF	"	"	"	ON	"	"	"

(a) DETECTOR RANGE 3 = 0 to 10; 4 = 0 to 30; 8 = 0 to 10,000
RGN = REGENERANT; ELU = ELUANT; SEP = SEPARATOR COLUMN
SUP = SUPPRESSOR COLUMN; ELUANT E_1 = 0·003M $NaHCO_3$ 0·0024M Na_2CO_3;
E_4 = DEMINERALIZED WATER

FIGURE 24

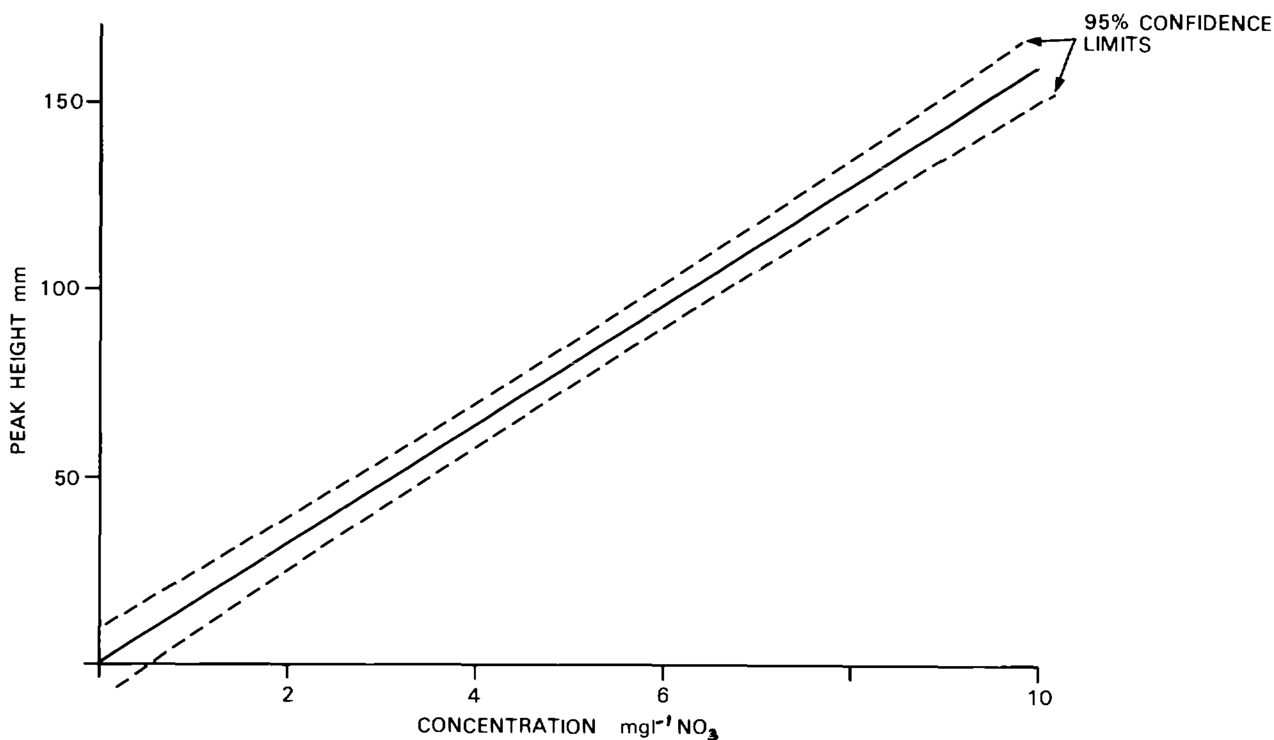


FIGURE 25 NITRATE CALIBRATION

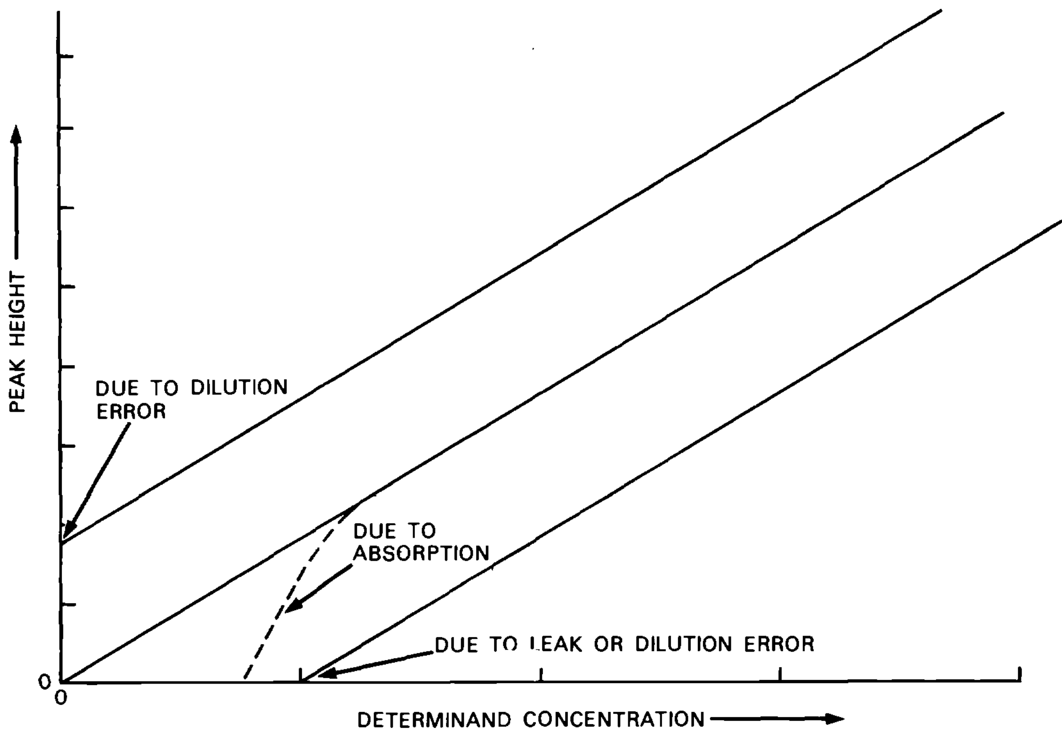


FIGURE 26

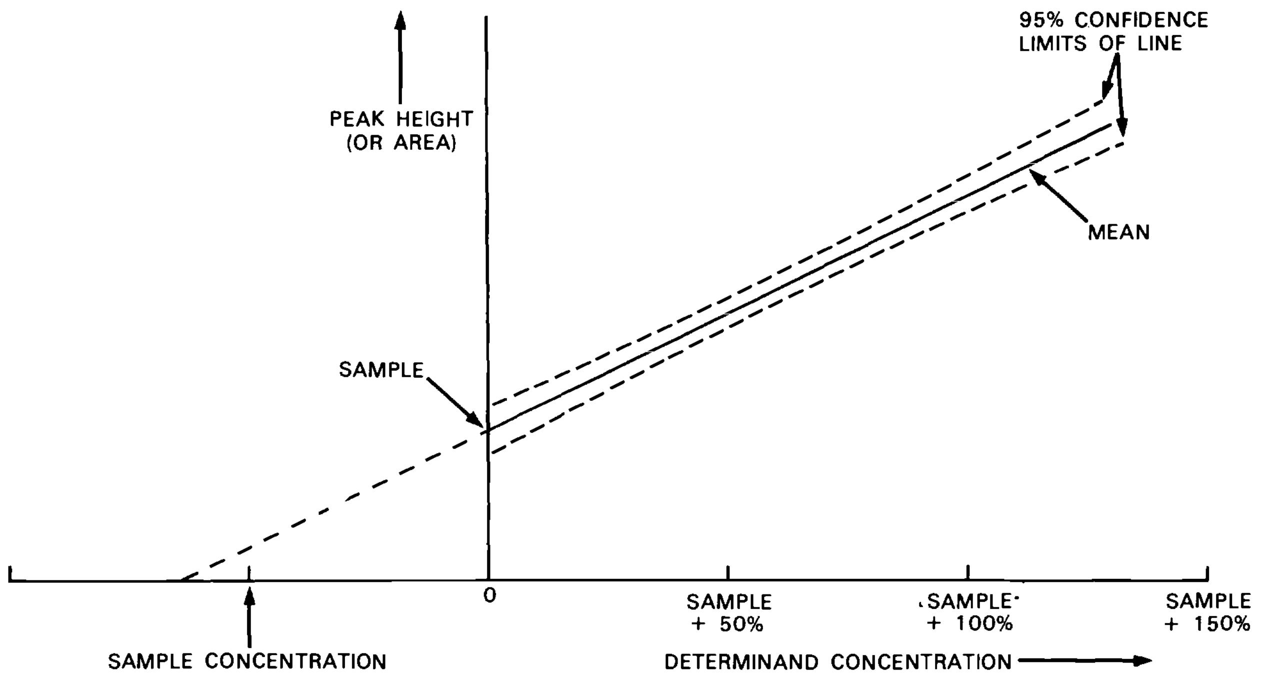


FIGURE 27

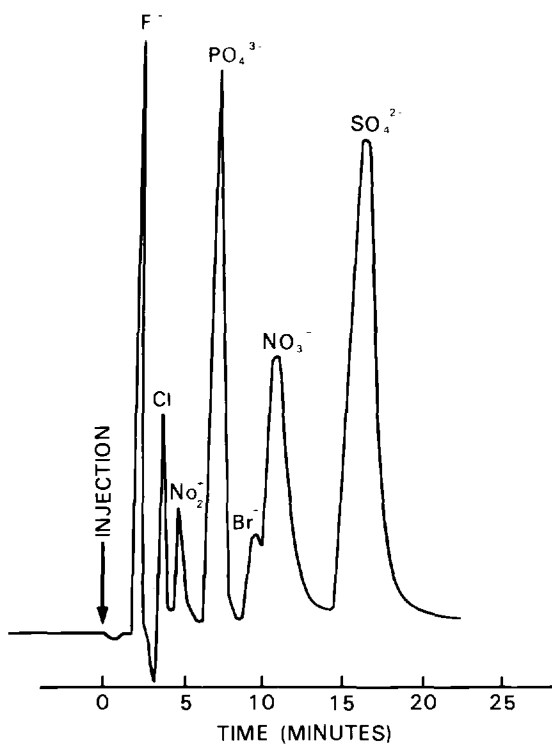


FIGURE 28 CHART OUTPUT FOR MIXED ANION STANDARD (ACTUAL SIZE)

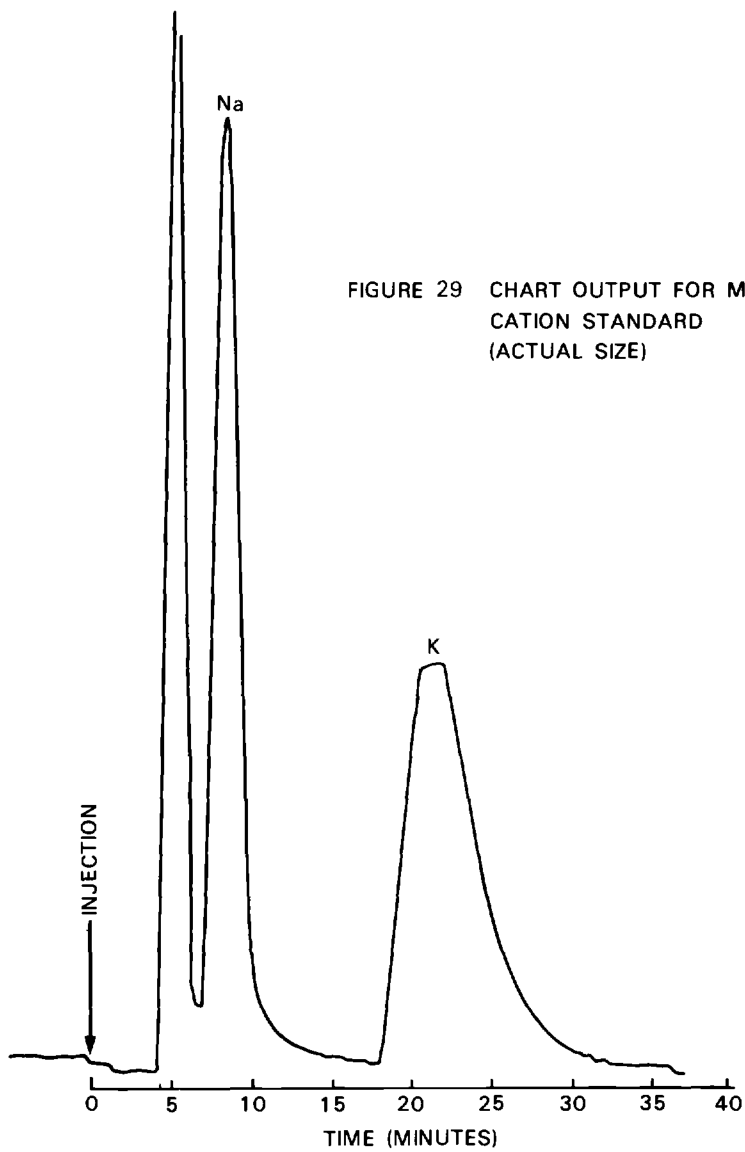


FIGURE 29 CHART OUTPUT FOR MIXED CATION STANDARD (ACTUAL SIZE)

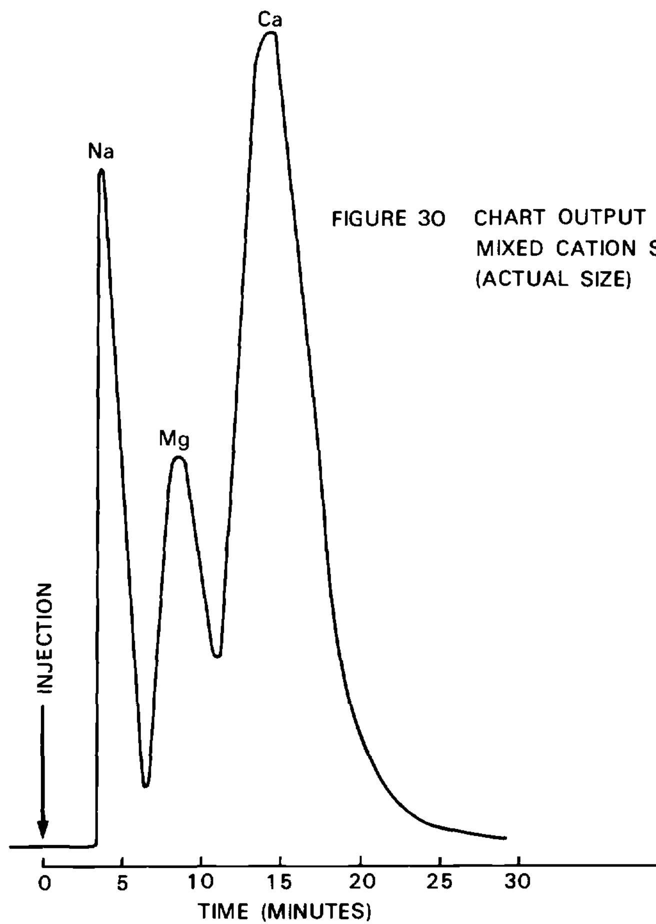


FIGURE 30 CHART OUTPUT FOR MIXED CATION STANDARD (ACTUAL SIZE)

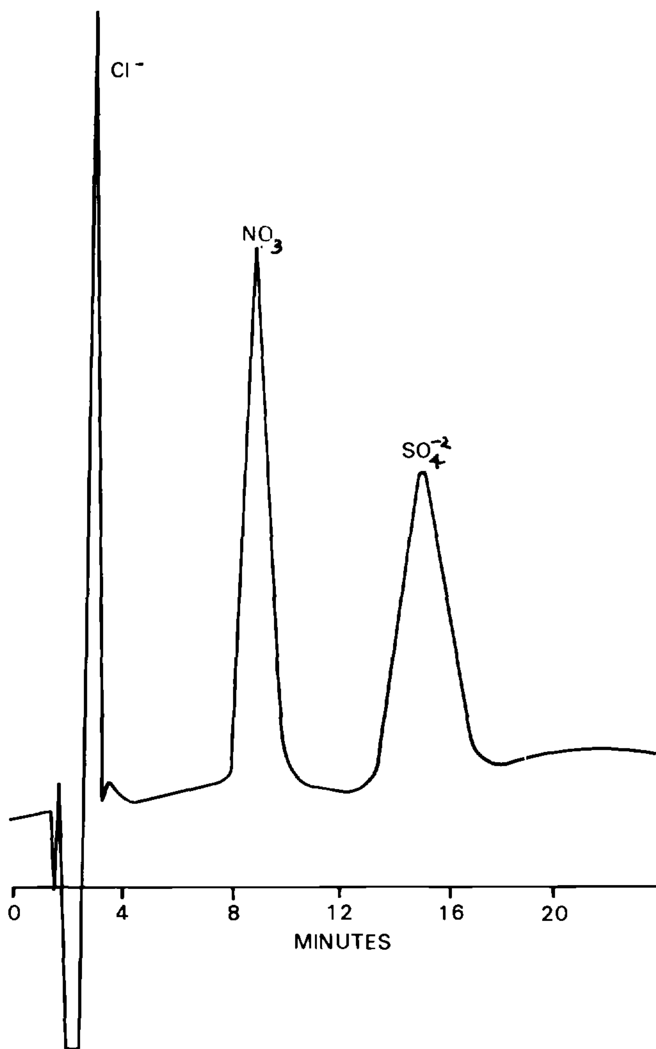


FIGURE 31 ION CHROMATOGRAM OF A RAIN WATER

2·8mm × 500mm SEPARATOR COLUMN; 2·8mm × 250mm SUPPRESSOR COLUMN; ELUANT 0·003M $NaHCO_3$/ 0·0024M Na_2CO_3; FLOWRATE 2·7mls min ; 100 µl SAMPLE; 10 µSIEMENS cm^{-1} FULL SCALE

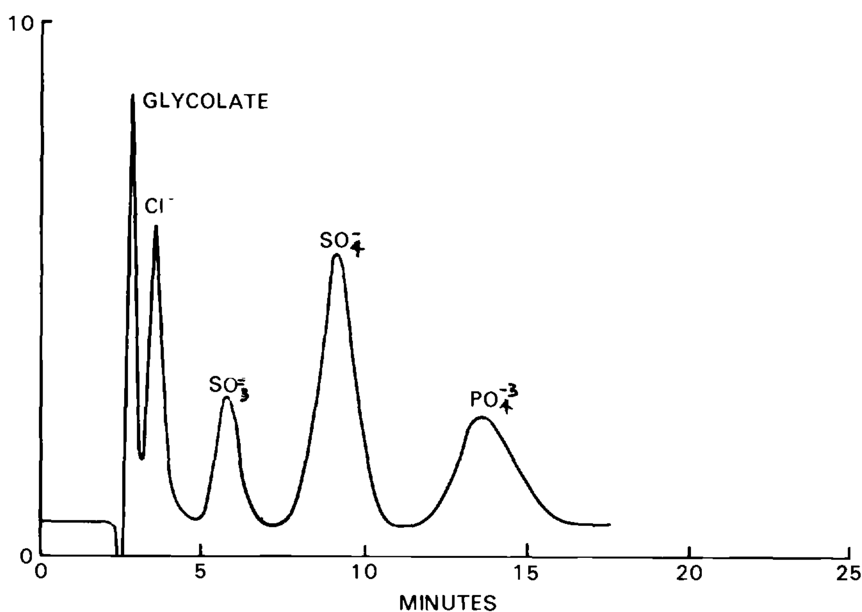


FIGURE 32 ION CHROMATOGRAM OF BOILER BLOW-DOWN WATER

2·8mm × 1000mm SEPARATOR COLUMN; 2·8mm × 300mm SUPPRESSOR COLUMN; ELUANT 0·005M Na_2CO_3/0·004M NaOH; FLOWRATE 1·9mls min 500 ul SAMPLE· 10 µSIEMENS cm^{-1} FULLSCALE

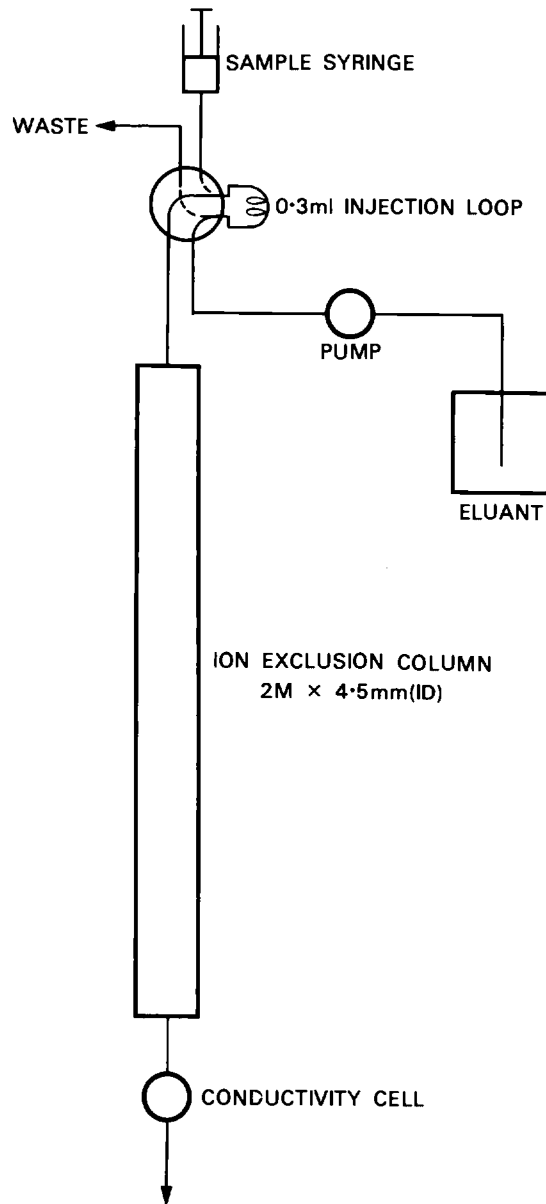


FIGURE 33

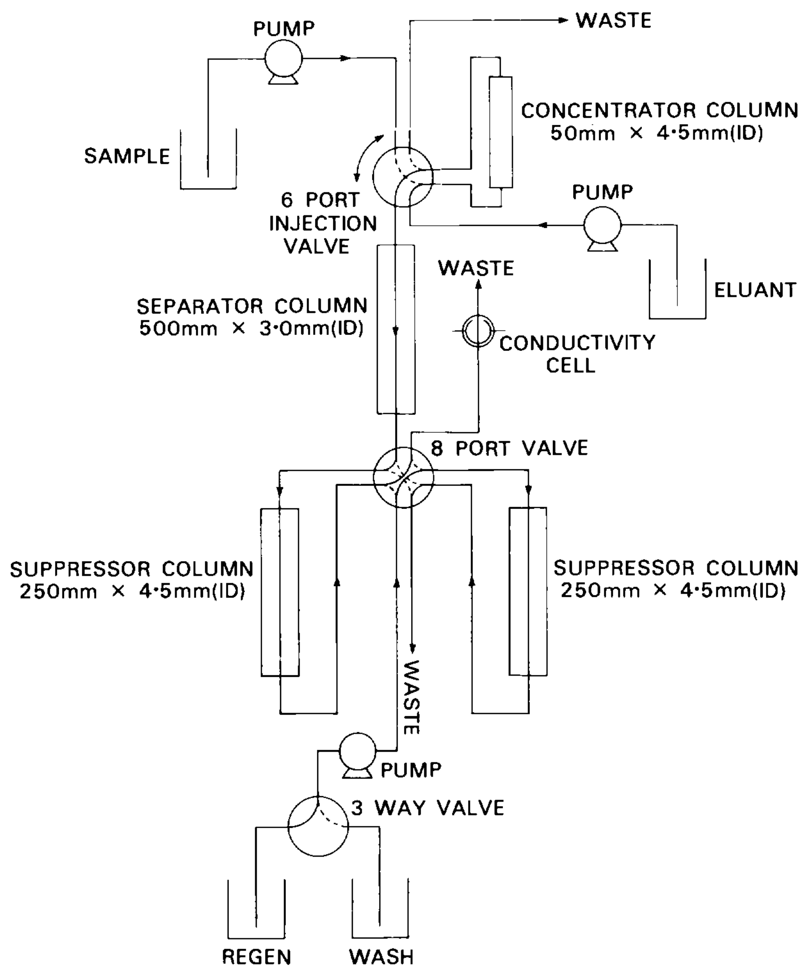


FIGURE 34

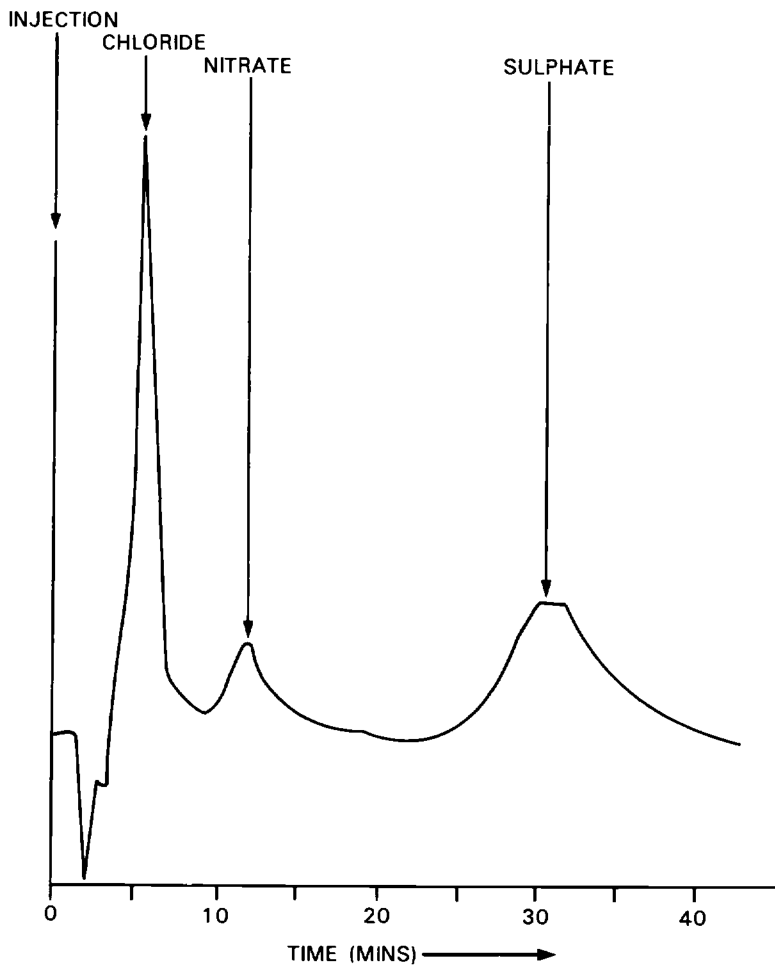


FIGURE 35 TYPICAL ION CHROMATOGRAM FROM ELUTION OF CONCENTRATOR COLUMN

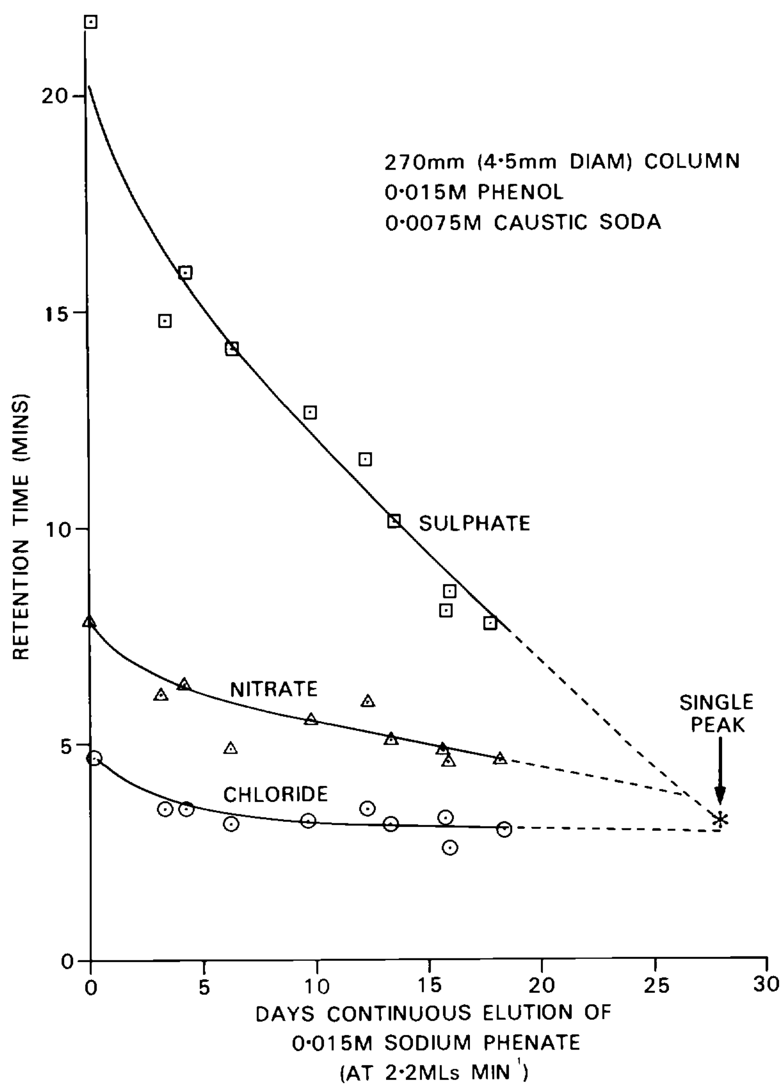


FIGURE 36 ANION SEPARATOR STABILITY

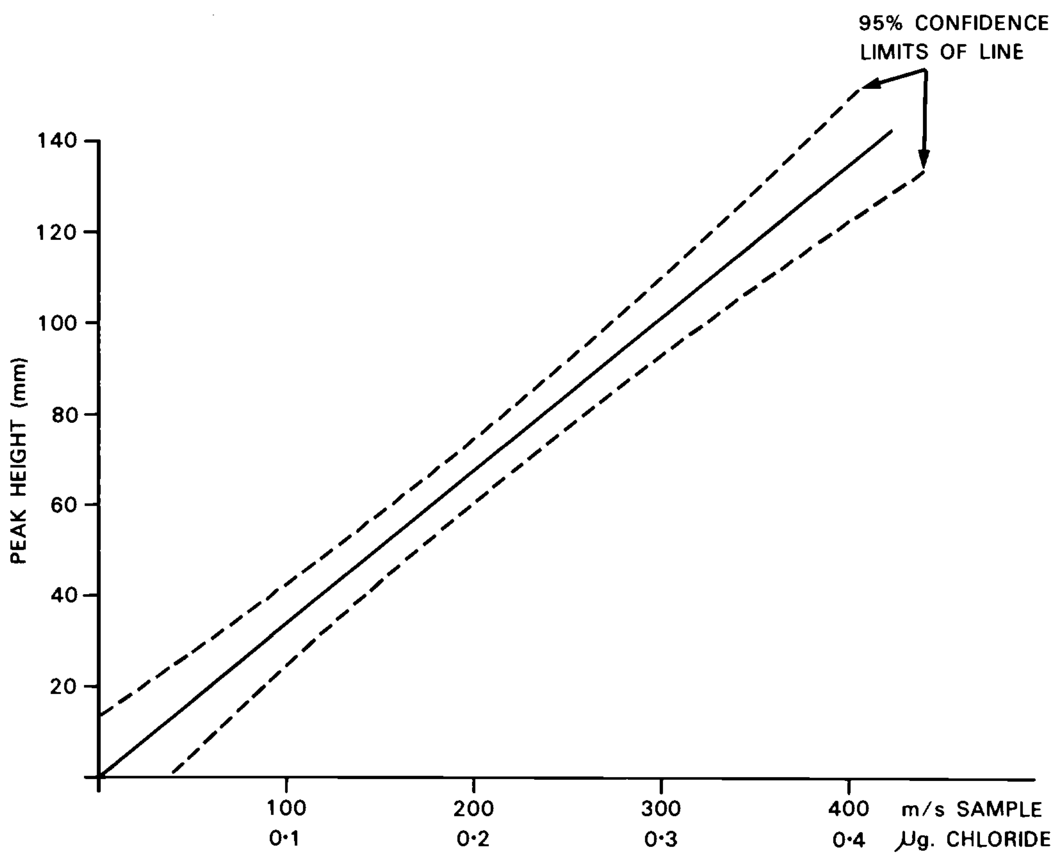


FIGURE 37 ION CHROMATOGRAPHIC RESPONSE AFTER CONCENTRATION OF SOLUTION CONTAINING 1 $\mu g.l^{-1}$ ADDED CHLORIDE ION

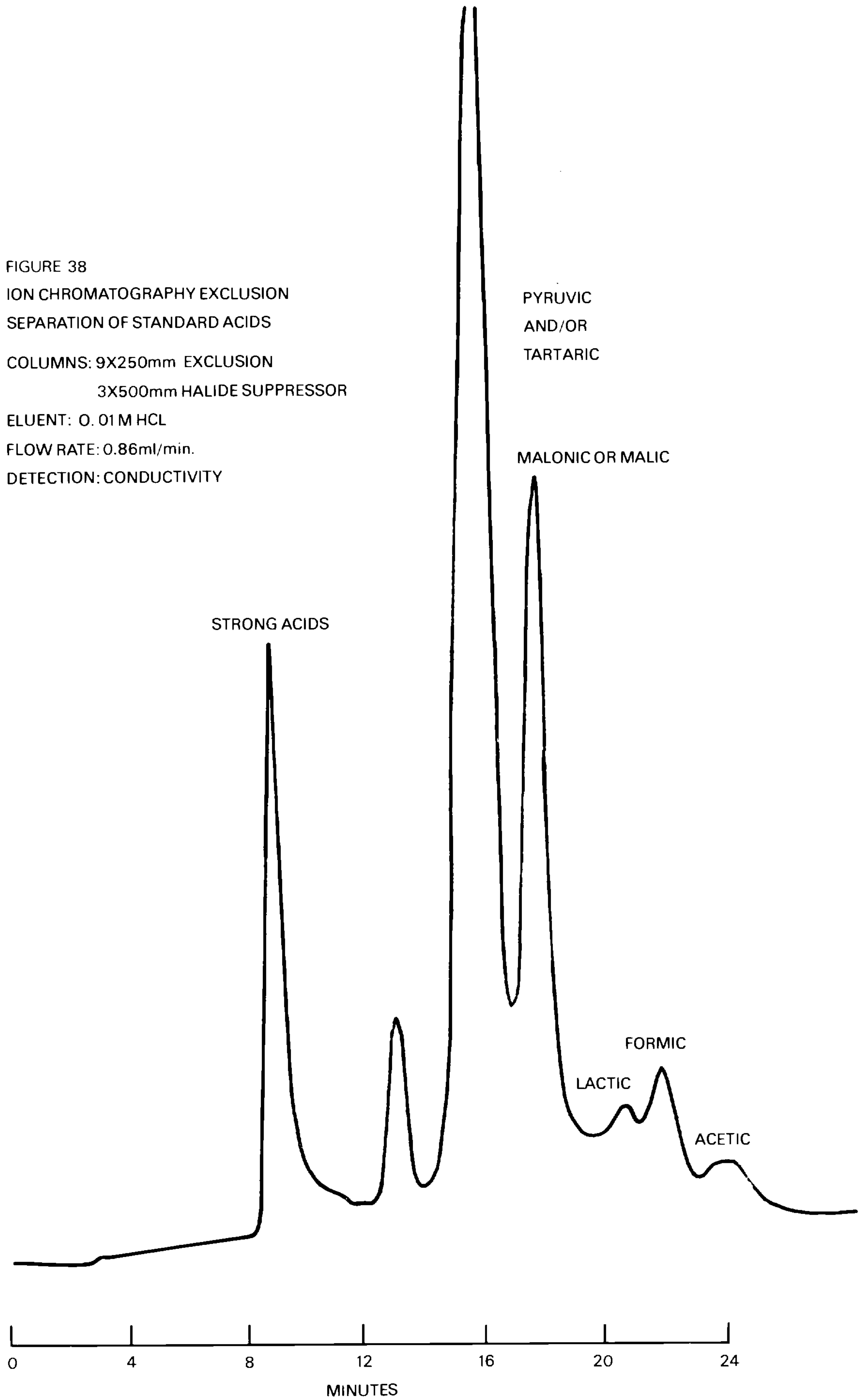


FIGURE 38
ION CHROMATOGRAPHY EXCLUSION
SEPARATION OF STANDARD ACIDS
COLUMNS: 9X250mm EXCLUSION
3X500mm HALIDE SUPPRESSOR
ELUENT: O. 01 M HCL
FLOW RATE: 0.86ml/min.
DETECTION: CONDUCTIVITY
PYRUVIC
AND/OR
TARTARIC
MALONIC OR MALIC
STRONG ACIDS
FORMIC
LACTIC
ACETIC
0
4
8
12
16
20
24
MINUTES

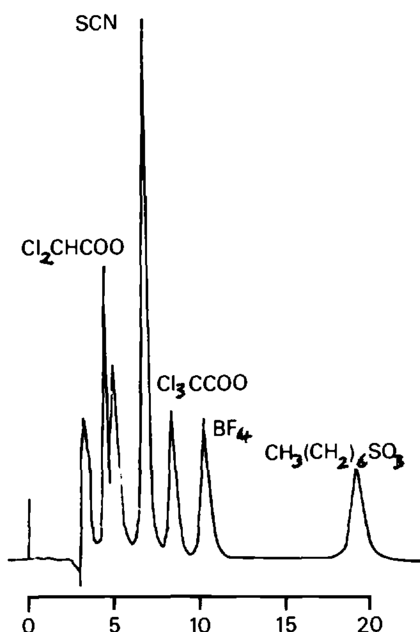


FIGURE 39 SEPARATION OF HYDROPHOBIC ANIONS BY MPIC

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A Brief Review of Column, Paper and Thin Layer Chromatography and Related Techniques

Chromatography is a method of separating substances by selective absorption or adsorption on to solids or suspended liquids which may or may not involve changes of bond formation in the process; but there are many different techniques. Separations are achieved by so choosing the stationary and mobile phases and conditions that the components of the sample are separated during the flow along the stationary medium and are either eluted in succession, or remain at the end of the separation located on different parts of the solid medium.

The stationary phase may be solid, or liquid supported either in or on a solid. This supporting solid may be a pure support or may itself have a contributing effect to the separation. The degree of chemical similarity between the stationary phase, the eluent and the substances being separated is quite important, separation being on differences in solubility, volatility, polarity, basicity, coordination, or a combination of these effects. Thus typical stationary phases are silica or alumina gels with varying water contents and residual ion contents, calcium carbonate (calcite), zirconyl phosphate, sucrose (castor sugar), cellulose — especially paper, waxes and hydrocarbons, and even glass, metals, clays, and diatoms. In addition, polymeric resins of varying basicity and coordinating ability due to substituent groups such as carboxylate, sulphonate, amino, quarternary ammonium, or phosphorus or halo-derivatives are also used.

In addition to the three techniques specifically covered by reviews in this series, the basic techniques are:—

Simple column chromatography, which may be either downward or upward flow on the columns;

Paper chromatography in which the eluent either flows slowly down the paper from a trough or drip feed above, or diffuses up from a trough below, or flows outwards from a central point (in this latter case usually, but not always, separation occurs during diffusion of the initial sample); and

Thin layer chromatography in which the eluent diffuses along a thin layer of the solid phase supported on a glass slide or similar support.

With column chromatography, the fractions are usually eluted off the column in succession and collected with a sample fraction collector or analysed directly by an automatic analyser. In some instances, fractions are absorbed into the column and eluted in succession by changing the eluent, or sample fractions may be transferred to a second column for further separation. Occasionally, the column is pushed out of the tube and cut up into sections and those fractions remaining on the support identified by some physical technique such as fluorescence, or chemically by streaking with a test reagent.

The ratio of the distance travelled by the sample fraction front to that travelled by the solvent front (known as R_f) can be used for identification or the sample fraction may be identified by further analysis. The column technique is useful when relatively large amounts of the various fractions are needed for further analysis. It is also used for preparative large scale purifications.

With paper chromatography, the sample fractions are usually not eluted off the paper, but located as spots on the paper, usually either by fluorescence, or by spraying with a test reagent or similar means. Fractions are usually identified by R_f and or by means of test reagents, but differences in fluorescence or colour formation are also used. Elution can be made in succession with two different eluents used at right angles, the paper being rotated 90° between elutions.

Thin Layer Chromatograms are usually analysed by measuring the R_f; but occasionally, the fraction is carefully scraped off for subsequent further study. The main advantage of TLC over paper chromatography is that other solid phases than paper may be used.

Ion exchange can be part of any of these while HPLC can be regarded as a special case of column chromatography.

In addition, variations in the speed with which ions migrate under applied electric potential differences can also be used to separate ions in liquids either along packed columns, gels or soaked paper. The two phenomena may even be combined. For the separation of ionizable substances such as dyes, and some drugs, the sample may be chromatographed vertically down the paper while being simultaneously separated horizontally by electrophoresis. Sample fractions are eluted into separate tubes by means of separations along the bottom of the paper. [1]

Occasionally, applied thermal effects have also been used, as in gas chromatography, and trace impurities can sometimes be concentrated or removed by a similar liquid-solid phase separation technique in which packed tubes of solid sample are passed through a heating-cooling cycle so that a thin molten zone moves along the tube. Impurities often tend to collect in the melt and move with it. Repeated cycling is usually necessary.

Although the majority of Chromatographic separations reported date from after World War II, the earliest known paper on the analysis of flower pigments dates from 1903. Chromatographic separations of almost every type mentioned occur in methods in this series, and sucrose columns were used for natural product analysis in the 1930s. Paper chromatography dates from 1944 and some of the first papers on ion exchange chromatography also date from the 1940s. Thin Layer Chromatography came later. Separation of ions by electrophoresis using horizontal tubes filled with conducting gels dates from before World War II.

Semi-quantitative results are occasionally obtained with paper and Thin Layer Chromatography by comparative measurement of the fluorescence or reflected light from sample and standard spots; but column techniques either with measurement of the signal from some form of detector or fraction collection for subsequent analysis are used for most quantitative work.

The following texts have been recommended for further reading:

A Manual for Paper and Thin Layer Chromatography and Electrophoresis, Ivor Smith, J G Feinberg, 2nd Edition, with amendments and corrections 1977 — reprinted 1982. Shandon Southern Products Ltd., Runcorn, Cheshire.

Methods in Zone Electrophoresis, John R Sargent and Stephen G George, 3rd Edition. 1975. BDH Chemicals Ltd., Poole, England.

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