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STUDIES ON SAMPLE PRECONCENTRATION IN ION CHROMATOGRAPHY

VII. REVIEW OF METHODOLOGY AND APPLICATIONS OF ANION PRECONCENTRATION

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SUMMARY

Factors which influence the success of sample preconcentration analysis of anions are reviewed. The recommended hardware consists of a programmable pump coupled to two high-pressure switching valves and a low pressure solvent selection valve, together with a conductivity or UV absorption detector. Desirable eluent characteristics are listed and the influences of sample loading parameters and concentrator column characteristics such as ion-exchange capacity and the chemical nature of the resin are discussed. Some practical applications involving both low and high ionic strength sample matrices are shown in order to illustrate the utility of preconcentration of real samples.

INTRODUCTION

The most commonly employed method for increasing the sensitivity of an ion chromatographic determination is to utilise some form of trace enrichment of the sample. This enrichment can be achieved in a number of ways; for example, the sample can be concentrated on the stationary phase itself through the use of large injection volumes[1,2], or alternatively enrichment prior to injection using Donnan dialysis can be employed[3,4]. However, the most widely used approach involves the use of a separate precolumn designed to trap trace levels of solutes from a large volume of sample[5]. The precolumn method is popular because it is simple and convenient to apply, is amenable to automation and offers high enrichment factors.

In precolumn sample enrichment, an accurately known volume of sample is pumped at a precise flow-rate through a small ion-exchange precolumn (or a reversed-phase precolumn coated with an anionic ion-interaction reagent[6]), called the concentrator column. Solute ions contained in the sample are selectively trapped on the concentrator column and are then eluted onto an ion-exchange analytical column for separation and quantitation. This procedure can be effective as an analytical method only if the processes of binding of solute ions on the concentrator column

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and their subsequent transfer to the analytical column are quantitative. We have undertaken an extensive study of the parameters which affect sample preconcentration methods[6–11] and in this paper we summarise the results of this study and show how these results may be applied to a range of samples.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used consisted of a Waters Assoc. (Milford, MA, U.S.A.) Model M590 programmable pump and events unit, a low pressure solvent selection valve, two pneumatically controlled six-port high-pressure switching valves, a Model M430 conductivity detector (or a model M481 UV–VIS absorbance detector) and a Model M730 data module. A Model U6K injector was incorporated into the liquid chromatograph when manual injection was required.

The analytical column used throughout this study was a Waters Assoc. IC PAK A (50 × 4.6 mm I.D.) methacrylate based anion-exchanger. Several different concentrator columns were employed and the specifications of these are listed in Table I. The concentrator columns were housed in a Waters Assoc. Guard Pak precolumn module, with the exception of column D. All concentrator columns used in this study were home-packed and some variation in packing densities occurred.

Reagents

All water was doubly distilled and passed through a Millipore (Bedford, MA, U.S.A.) Milli Q water purification system and when the water was to be used for the preparation of ultra-trace standard solutions, the in-line 0.22-μm filter was removed from the system in order to prevent contamination of the standard solutions with nitrate ion. Standard solutions (100 ppm) of the required anions were prepared by dissolving appropriate amounts of analytical grade sodium salts in pure water. These solutions were diluted daily with the aid of Gilson (Villiers, France) Pipetman au-

TABLE I

CONCENTRATOR COLUMNS USED IN THIS STUDY

Column	*Packing material*	*Column housing*	*Measured total ion-exchange capacity (μequiv.)*
A	Methacrylate resin, 25-μm particle size, 15 μequiv./ml	Guard Pak precolumn	2.15
B	Aminated poly(styrene–divinylbenzene) resin, 200 μequiv./g	Guard Pak precolumn	6.89
C	Methacrylate resin, 25-μm particle size, 15 μequiv./ml	Modified Guard Pak	12.42
D	Methacrylate resin, 25-μm particle size, 15 μequiv./ml	Steel precolumn	15.5

TABLE II

ELUENTS USED IN THIS STUDY

Eluent	*Composition*	*pH*
A	3.5 m*M* *p*-Toluenesulphonic acid	6.0
B	0.4 m*M* 2-Naphthylamine-1-sulphonic acid	6.0
C	1.0 m*M* Tetraborate, 4.2 m*M* boric acid, 1.0 m*M* gluconic acid	8.5
D	0.1 *M* Methanesulphonic acid	4.0
E	5 m*M* Heptanesulphonic acid	7.0
F	5 m*M* Phosphate	9.5

topipettes, using polypropylene volumetric ware which had been previously washed with clean water.

Several different eluents were used and the compositions of these are listed in Table II. Eluents were prepared by dissolving weighed amounts of analytical grade reagents in approximately 800 ml of water, after which the pH was adjusted to the desired value by the dropwise addition of 0.1 *M* lithium hydroxide and the solution diluted to 1 l. Each eluent was filtered through a 0.45-μm filter and degassed in an ultrasonic bath prior to use.

Procedures

The pump microprocessor was programmed to actuate the valves in a timed sequence. The general basis of this sequence is elaborated under Results and discussion, however the details were dependent on the type of sample analysed. An illustrative example of a pump program may be found in ref. 8.

New concentrator columns were conditioned prior to use by washing with 200 ml of acetonitrile–water (20:80), 200 ml of 1 m*M* phthalic acid (pH 6.0), 200 ml of pure water, and finally 200 ml of the eluent with which the column was to be used. The condition of the concentrator column was evaluated using a previously reported quality control procedure[10] in which the capacity factor for nitrate was measured using 0.5 m*M* sodium benzoate (pH 6.0) as eluent, with the concentrator column connected directly to the conductivity detector. When this capacity factor exceeded 8.0, the concentrator column was considered to be suitable for use with samples containing a total amount of anions of less than 0.15 μequiv.

RESULTS AND DISCUSSION

Hardware considerations

The simplest form of sample preconcentration device utilises a single, six-port, high pressure, switching valve which can be actuated either manually or automatically. Fig. 1 shows the plumbing arrangement and operation of this system. In the first step, the concentrator and analytical columns are equilibrated with eluent. The valve is then rotated and the concentrator column is removed from the flow-path whilst eluent continues to be pumped through the analytical column. A measured volume of sample is passed through the concentrator column using either a pump or a large volume syringe, with the effluent being directed to waste. At this stage, the

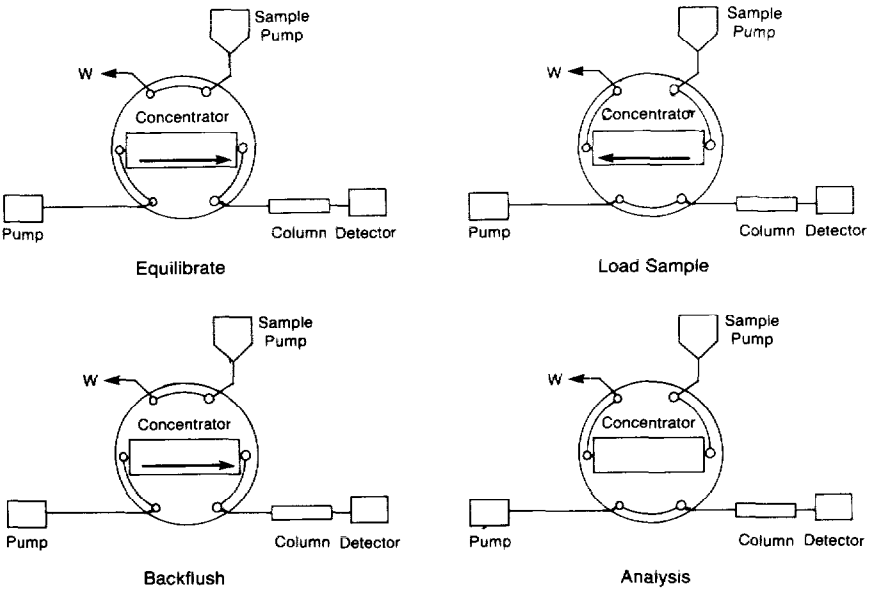


Fig. 1. Flow-paths used for preconcentration of a sample using a single-valve preconcentration system. W = Waste.

solute ions from the sample are assumed to be retained on the concentrator column and in the subsequent step, rotation of the valve permits eluent to be pumped in the reverse direction (*i.e.* the flow direction opposite to that used for sample loading) through the concentrator column. This operation is known as backflushing and is designed to transfer the solute ions to the analytical column in a small volume of eluent. In the final step, the valve is again rotated and eluent then carries the solute ions through the analytical column for separation and detection.

This system has the advantages of simplicity and ease of operation. The backflush volume is generally selected to be high enough to guarantee that all the ions are transferred. It should be noted here that the volume of eluent used to backflush the solute ions to the analytical column will necessarily have a lower concentration of eluent ions than that present in the bulk eluent. This is because some eluent ions are required to re-equilibrate the concentrator column which has been depleted of eluent ions during the passage of sample. The result of this is that severe baseline disturbances often occur in the final chromatogram when the detection method employed is sensitive to the background level of eluent ions in the mobile phase. Conductivity detection falls into this category and when this is used, the initial baseline disturbance in the chromatogram often masks early eluting solutes.

A more flexible preconcentration system is produced by the combination of a single, programmable pump with two high pressure switching valves and a low pressure solvent selection valve[8]. Here, the same pump can be used to deliver eluent and to load the sample onto the concentrator column. Fig. 2 shows the interconnections used for these valves and Fig. 3 illustrates the flow-paths achievable with this system. In Fig. 3a the pump tubing and interconnecting lines are flushed with sample solution and in Fig. 3b a measured volume of sample is loaded onto the concentrator column

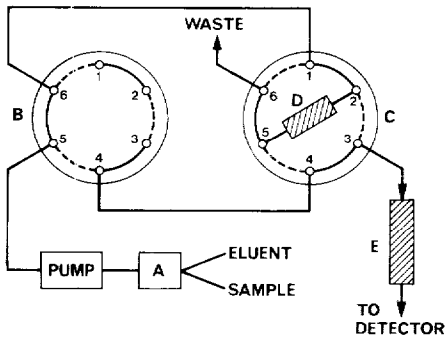


Fig. 2. Details of the interconnections used for an automated, multi-valve preconcentration system. A = Solvent selection valve; B, C = six-port high-pressure switching valves; D = concentrator column; E = analytical column. Reproduced from ref. 8.

at a precise flow-rate. The pump tubing and interconnecting lines are then flushed with eluent (Fig. 3c), after which a small, accurately known volume of eluent is pumped through the concentrator column in the same flow direction as that used for sample loading (Fig. 3d). This is termed a "wash" step and serves to partially re-equilibrate the concentrator column with eluent ions without loss of bound solute ions. Fig. 3e shows the sample stripping step in which the solute ions are backflushed from the concentrator column onto the analytical column using an accurately known volume of eluent. In the final step, the concentrator column is removed from the flow-path and the eluent is pumped directly to the analytical column (Fig. 3f).

Clearly, this multi-valve system is more complex than the single-valve approach and requires the use of a sophisticated pump. It does however offer the advantages of unlimited and precise control over the volumes of eluent used for the washing and stripping steps, and these may be readily manipulated to adapt to the requirements of a particular sample. In addition, the baseline produced in the final chromatogram is superior to that obtained with a single-valve preconcentration system. In our opinion, these advantages provide sufficient justification for the additional complexity and we have employed the system shown in Fig. 2 throughout our studies on sample preconcentration.

Choice of eluent

The most important realisation in selection of an appropriate eluent for a preconcentration method is that eluents which are perfectly suitable for direct injection ion chromatography may be quite inappropriate for use with preconcentration techniques. In the latter case, the eluent must perform three distinct functions: it must permit solute ions to bind onto the concentrator column during the sample loading step, it must transfer quantitatively these solute ions from the concentrator column to the analytical column during the stripping step, and it must provide adequate resolution of the sample components on the analytical column. Clearly these multiple requirements will limit the number of eluents which are suitable for preconcentration methods. The following desirable eluent characteristics may be enumerated for preconcentration using conductivity detection.

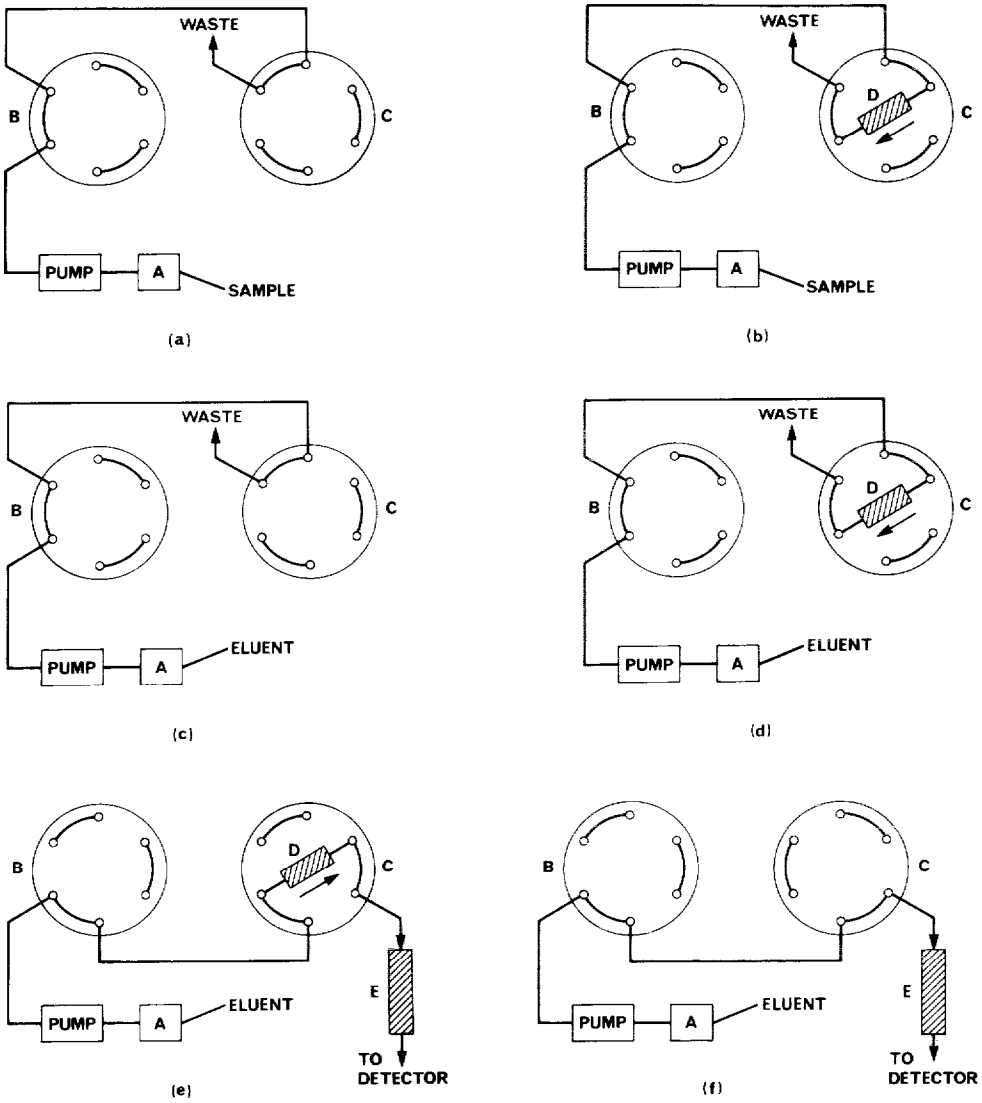


Fig. 3. Flow-paths used at various stages of sample preconcentration and elution, employing a multi-valve preconcentration system. (a) Sample flush mode, (b) sample load mode, (c) eluent flush mode, (d) concentrator wash mode, (e) sample strip mode, (f) analysis mode. A–E as in Fig. 2. Reproduced from ref. 8.

(1) Selectivity. The solute ions should elute within a range of capacity factors of 4–30. The lower limit is chosen to minimise interference of early eluting solutes from the relatively large solvent peak which invariably results in preconcentration chromatograms, whilst the upper limit ensures that excessive retention does not preclude reliable quantitation.

(2) Sensitivity. Since the purpose of sample preconcentration is to improve the sensitivity of an ion chromatographic method, the eluent should be chosen to

maximise the detectability of the solute ions. For this reason, an eluent anion with low limiting equivalent ionic conductance is preferred. In addition it is desirable that the eluent anion be singly charged in order to provide the greatest detector response to the elution of univalent solute anions.

(3) Eluent pH. Apart from the above considerations, the pH of the eluent exerts two additional important effects on the final chromatogram. In the first place, studies[12,13] have shown that the presence of neutral, protonated forms of the eluent can result in the appearance of system peaks due to elution of these neutral components under a reversed-phase mechanism. Secondly, bicarbonate ion is present in the majority of samples due to absorption of carbon dioxide from the atmosphere and if quantitation of this species is not required, the resultant large peak can represent a major interference to early eluting solutes. Both of these problems can be circumvented through the use of a fully ionised eluent operated at pH <6. Under these conditions, bicarbonate becomes fully protonated and elutes from the column with the solvent front.

We have surveyed a large number of aromatic carboxylic and sulphonic acids for use as eluents in preconcentration methods[9]. Aromatic monosulphonic acids such as *p*-toluenesulphonic acid and 2-naphthylamine-1-sulphonic acid have proved to be the most suitable for use with conductivity detection, whilst aliphatic sulphonic acids with methyl-, heptyl- or octyl-side chains are applicable when direct UV absorption detection is employed. In addition the longer chain aliphatic sulphonic acids can also be used with conductivity detection, provided that their surfactant properties are not intrusive.

Concentrator column characteristics

It is clear that sample preconcentration is not an open-ended technique and that practical limitations must exist on the amount of sample which can be loaded and recovered quantitatively. It is desirable that large sample volumes can be accommodated and that the sample be loaded at a high flow-rate in order to minimise the time required for the analysis. Studies with fixed-site anion-exchange concentrator columns[10] have shown that the maximum permissible flow-rates and sample volumes are dependent on the nature of the eluent used. As the ion-exchange affinity of the eluent increases, then binding of weakly retained solutes onto the concentrator column reduces markedly with larger sample volumes. However if the eluent conforms to the requirements listed above, sample volumes as high as 100 ml may be loaded at a flow-rate of 8 ml/min with quantitative binding of solute ions being maintained.

It might appear at first sight that the ion-exchange capacity of the concentrator column should be as high as possible in order to provide ample ion-exchange sites for the binding of solute ions. Certainly this situation does encourage quantitative binding, but as the ion-exchange capacity of the concentrator column increases, it becomes more difficult to transfer the bound ions onto the analytical column using a small volume of eluent. Attempts to use a high eluent strength for sample stripping and a lower strength for sample elution have not proved successful[8], thus the same eluent should be used for both purposes. In this case, the optimal ion-exchange capacity of the concentrator column is approximately 40% of that of the analytical column[11]. Increasing the ion-exchange capacity of the concentrator column beyond this value leads to the requirement for larger strip volumes, causing interference with early eluting solutes and band broadening effects.

The nature of the resin used to support the bonded ion-exchange functionalities can also exert a considerable effect on the preconcentration. Studies have shown[11] that concentrator columns packed with aminated methacrylate and aminated styrene–divinylbenzene resins of similar ion-exchange capacity gave markedly different performance in breakthrough experiments using a mixture of chloride, nitrate and sulphate. Both chloride and nitrate showed very poor retention on the styrene–divinylbenzene resin in comparison to that obtained using the methacrylate resin.

Finally, it is pertinent to comment on the possibility of replacing the fixed-site ion-exchanger in the concentrator column with a neutral, reversed-phase material which has been coated with a very hydrophobic reagent containing a charged functionality. In the case of anion preconcentration, this reagent could be cetylpyridinium ion or cetyltrimethylammonium ion, and is termed the "ion-interaction reagent". Provided that the ion-interaction reagent remains permanently bound to the stationary phase surface during sample loading, then an ion-exchange column may be produced. The main attractions of this approach are that the nature of the functional group may be varied by recoating the stationary phase with an alternative ion-interaction reagent, and the ion-exchange capacity of the concentrator column may be easily manipulated by altering the conditions under which the concentrator column is coated. We have shown[6] that concentrator columns prepared in this way do not show the degree of binding of solute ions expected from consideration of their ion-exchange capacities alone. A comparison of a fixed-site concentrator column with one prepared by the permanent coating method showed that equivalent retention of solute ions was obtained only when the ion-exchange capacity of the latter column was a factor of fifteen times higher than that of the former column.

Application to samples of low ionic strength

Most of the samples used for preconcentration methods consist of very dilute aqueous solutions. Examples include rain water, purified water and boiler feed water for power station generators. In each case the sample contains ppb levels of ionic species and is relatively free from organic impurities. Despite the low levels of ions present, accurate analysis may be essential in order to prevent damage to expensive plants such as steam turbines.

For such samples, a fixed-site ion-exchange concentrator used with a singly charged aromatic sulphonic acid eluent at pH 6 provides optimal results, especially when coupled with conductivity detection. A typical chromatogram obtained for the preconcentration of a standard mixture of anions is shown in Fig. 4, which illustrates the excellent separation obtained with this eluent. When applied to the analysis of deionised water containing low ppb levels of ions (Fig. 5), peaks were evident for chloride, nitrate and sulphate. The precision for quantitation of these solutes at the concentrations found were in the range 0.9–5.0% relative standard deviation (R.S.D.). It is noteworthy that the peak widths are similar for Figs. 4 and 5, despite the wide disparity in sample volumes used. An alternative eluent, 2-naphthylamine-1-sulphonic acid, has been applied in Fig. 6 to the determination of anions in water purified by reverse osmosis. The fluoride detected in the sample is a residue from that originally present in the fluorinated feed water used for the reverse osmosis plant.

The analysis of samples of the above type is relatively straightforward and

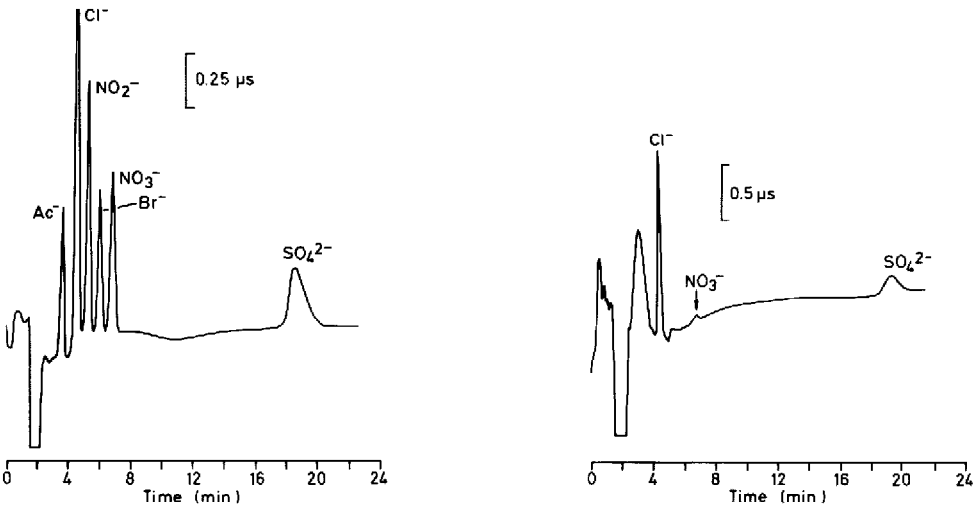


Fig. 4. Preconcentration of a standard anion mixture. Conditions: Waters IC Pak A analytical column; concentrator column A (see Table I); eluent A (see Table II); wash volume, 100 µl; strip volume, 500 µl; sample, 10 ml (loaded at 1.0 ml/min) of a mixture containing 100 ppb of each of the indicated anions, except for acetate which was 500 ppb; detection, conductivity.

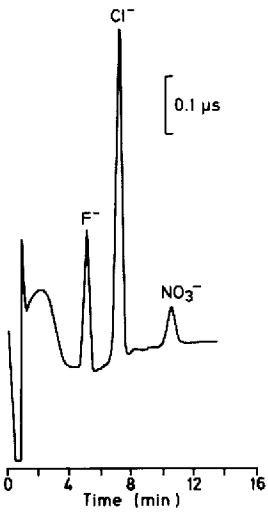
Fig. 5. Preconcentration of anions in deionised water. Conditions: Waters IC Pak A analytical column; concentrator column A (see Table I); eluent A (see Table II); wash volume, 100 µl; strip volume, 500 µl; sample, 100 ml (loaded at 4.0 ml/min); solute concentrations: 4 ppb chloride, 0.5 ppb nitrate and 3 ppb sulphate; detection, conductivity.

provided that the correct eluent is chosen, the condition of the concentrator column is periodically monitored, and the performance of the system is routinely assessed using recovery experiments, then a high level of confidence can be assigned to the result.

Application to samples of high ionic strength

It is a challenging prospect to attempt preconcentration analysis of trace components in samples which contain high levels of other ionic species. In such cases it is likely that binding of the trace components to the concentrator column will be adversely affected by mass-action influences of the bulk constituents. Indeed, successful preconcentration can be anticipated only after sample cleanup or when the ions of interest have a much higher affinity for the ion-exchange resin than do the matrix components.

An example of the latter case is the determination of phosphate, sulphate and oxalate in a leaf litter extract taken from coastal vegetation. Preliminary analysis of the extract showed high levels of chloride and nitrate. Two strategies were therefore employed to successfully analyse this sample. First, a high eluent pH was selected in order to convert the phosphate to HPO_4^{2-} and so increase its affinity for the ion-exchange resin in the concentrator column. Second, a styrene–divinylbenzene based ion-exchange material was used in the concentrator column because this resin has been shown to have poor affinity for chloride and nitrate. The final chromatogram



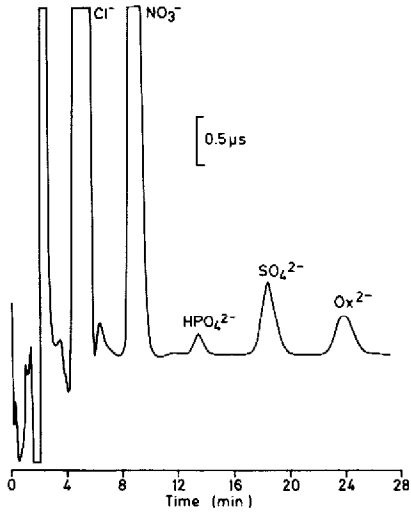


Fig. 6. Preconcentration of anions in water purified by reverse osmosis. Conditions: Waters IC Pak A analytical column; concentrator column A (see Table I); eluent B (see Table II); wash volume, 100 µl; strip volume, 500 µl; sample, 6 ml (loaded at 0.6 ml/min); solute concentrations: 5 ppb fluoride, 20 ppb chloride and 3 ppb nitrate; detection, conductivity.

Fig. 7. Preconcentration of an aqueous extract of coastal vegetation leaf litter. Conditions: Waters IC Pak A analytical column; concentrator column B (see Table I); eluent C (see Table II); wash volume, 200 µl; strip volume, 650 µl; sample: 4 ml (loaded at 0.4 ml/min); solute concentrations: 50 ppb phosphate, 150 ppb sulphate and 200 ppb oxalate; detection, conductivity.

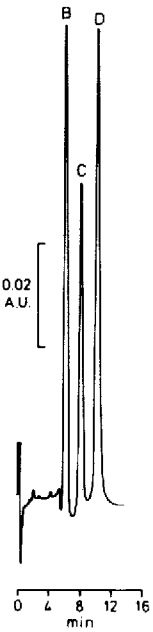
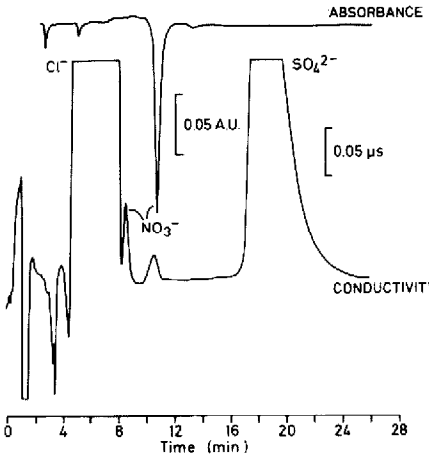


Fig. 8. Preconcentration of a trace mixture of chloride, nitrite (B), bromide (C) and nitrate (D). Conditions: Hamilton PRP-X100 analytical column; concentrator column A (see Table I); eluent D (see Table II); sample, 15 ml (loaded at 1.0 ml/min); solute concentrations, 40 ppb of each ion; detection, UV absorption at 205 nm. Reproduced from ref. 8.



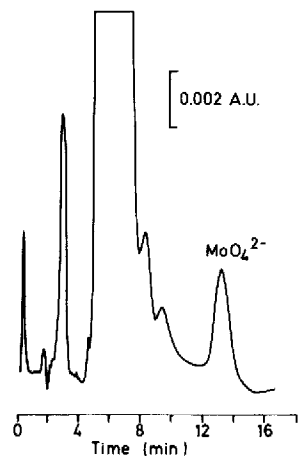


Fig. 9. Determination of nitrate in river water using simultaneous conductivity and direct UV absorption detection. Conditions: Waters IC Pak A analytical column; concentrator column C (see Table I); eluent E (see Table II); wash volume, 100 μl; strip volume, 750 μl; sample, 15 ml (loaded at 1.5 ml/min); solute concentration, 40 ppb nitrate; detection, conductivity and UV absorption at 210 nm.

Fig. 10. Determination of molybdate in a hydroponic plant nutrient solution. Conditions: Water IC Pak A analytical column; concentrator column D (see Table I); eluent F (see Table II); wash volume, 200 μl; strip volume, 800 μl; sample, 4 ml (loaded at 0.4 ml/min); solute concentration, 30 ppb molybdate; detection, UV absorption at 245 nm.

obtained using gluconate–borate buffer at pH 8.5 as eluent with concentrator column B (see Table I) is shown in Fig. 7.

In many cases it is beneficial to couple selective detection with sample preconcentration to achieve the desired sensitivity. An example of this approach is the determination of anions using direct UV absorption detection in the wavelength range 200–220 nm[14]. Only a relatively small number of anions show absorbance under these conditions and if a suitable UV-transparent eluent is chosen, then selective analysis is possible. Fig. 8 shows a chromatogram obtained for a mixture of chloride, nitrite, bromide and nitrate using UV absorption detection at 205 nm. No peak for chloride is evident. The determination of nitrate in river water using this approach is illustrated in Fig. 9, which also shows the chromatogram obtained with conductivity detection. Fig. 10 provides a further example of selective detection; in this case, the determination of molybdate in a hydroponic plant nutrient solution. In the latter case, a styrene–divinylbenzene ion-exchange resin was used in the concentrator column to minimise retention of high levels of monovalent anions. The large peak eluting at a retention time of 6 min was due to nitrate ion.

CONCLUSIONS

Sample preconcentration is a complex procedure and should not be considered to be a simple extension of direct injection ion chromatography. Care must be applied to the selection of the eluent and the concentrator column to ensure quantitative retention on the concentrator column of the solute ions of interest, and their sub-

sequent quantitative transfer onto the analytical column. The ability to optimise the wash and strip volumes of eluent for individual samples provides a high level of flexibility in attaining these goals. The techniques described in this paper can be applied to very dilute aqueous samples of low ionic strength and to more complex samples containing high levels of interfering ionic species.

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