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STUDIES ON SAMPLE PRECONCENTRATION IN ION CHROMATO-GRAPHY

IV. EFFECT OF SAMPLE LOADING PARAMETERS ON THE PRECONCENTRATION PROCESS

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SUMMARY

The effects of variation of the flow-rate used to load a sample and the sample volume employed in a preconcentration procedure using a concentrator column are discussed. It is shown that the recoveries of solute anions in preconcentration methods, calculated by comparison of peak areas with those obtained with a manual injection of the same amount of solute, were independent of the flow-rate used to load the sample, up to a maximum flow-rate of 8 ml/min. The sample volume used was varied over the range 2–100 ml and it was found that the recoveries of solute anions were dependent on the eluent used to equilibrate the concentrator column prior to commencement of the preconcentration procedure. A singly ionised eluent (p-toluenesulphonate at pH 6.0) gave essentially quantitative recoveries at all sample volumes, whereas a doubly ionised eluent (phthalate at pH 6.0) showed marked decreases in recovery as the sample volume was increased beyond 2 ml. Singly ionised eluents are therefore recommended for use with preconcentration methods.

A performance criterion is suggested for evaluation of the condition of a concentrator column and this criterion is based on the degree of retention on the concentrator column of nitrate ion when a weak eluent such as 0.5 mM sodium benzoate at pH 6.0 is used.

INTRODUCTION

The determination of anions at the trace level by ion chromatography may be achieved using large injection volumes[1–3] or by sample preconcentration techniques[4,5]. The latter method most commonly involves the use of a small ion-exchange precolumn to trap the solute ions from a relatively large volume of sample, with subsequent elution of these trapped solute ions onto an analytical column where they are separated and quantitated in the usual manner. Recently, Cox and Tanaka[6] have shown that preconcentration of anions is also possible by Donnan dialysis using a

carbonate–bicarbonate receiver solution: enrichment factors of up to sixteen were achieved for chloride ion, however the procedure used was applicable only to suppressed ion chromatographic methods in which a carbonate–bicarbonate buffer was used as the eluent. Of the abovementioned preconcentration methods, the use of a precolumn (or "concentrator column") is the most widely applicable and flexible and also provides the greatest enrichment factors.

The amount of sample loaded onto the concentrator column can be varied by changing the flow-rate at which the sample is passed through the concentrator column, or alternatively by changing the volume of sample used. The success of the preconcentration process is dependent on the quantitative and reproducible binding of solute ions on the concentrator column and it has generally been assumed that this is independent of the manner in which the sample is loaded[4,7]. That is, the total volume of sample used or the flow-rate at which it is loaded has no effect, provided that the ion-exchange capacity of the concentrator column is not exceeded. A rather different assessment has been made by Roberts et al.[5] who have noted that peak heights for chloride were not linear when sample volumes greater than 20 ml were loaded onto a concentrator column equilibrated with a phthalate eluent.

In previous papers we have described the design and operation of an automated, single pump preconcentration system suitable for UV absorption[8] or conductivity[9] detection and we have also established criteria for the selection of eluents suitable for preconcentration methods[10]. In this paper, an investigation into the parameters which govern the sample loading process is reported, together with comments on the lifetimes and efficiencies of concentrator columns.

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 and two pneumatically controlled high-pressure switching valves, a Model M430 conductivity detector and a Model M730 data module. A Model U6K injector was incorporated into the liquid chromatograph when manual injection was required.

A Waters Assoc. IC Pak A (50 × 4.6 mm I.D.) methacrylate based anion-exchange column with an ion-exchange capacity of 30 μequiv./ml was used as the analytical column, and a Waters Assoc. IC anion concentrator column (5.0 × 6.0 mm I.D.) was used as the preconcentration column. This latter column was packed with a methacrylate resin similar to that used in the analytical column but with a larger particle size (25 μm) and lower ion-exchange capacity (15 μequiv./ml). The concentrator column was housed in a Waters Assoc. Guard Pak precolumn module.

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[11]. Standard solutions (100 ppm) of chloride, nitrite, nitrate and sulphate

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 autopipettes to give trace solutions which were made up in polypropylene volumetric flasks which had been previously rinsed with pure water.

The eluent used were phthalic, benzoic and p-toluenesulphonic acid solutions. All eluents were diluted daily from concentrated stock solutions to ensure that no variations in eluent strength occurred. The stock eluent solutions were prepared by dissolving weighed amounts of analytical grade reagents in approximately 800 ml of water, after which the pH was adjusted where necessary by dropwise addition of 1.0 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. Details of actual mobile phase conditions are provided in the figure captions.

Procedures

The pump microprocessor was programmed to actuate the valves in a timed sequence, the details of which are given elsewhere[9]. For sample preconcentration, a wash volume of 200 μl and a strip volume of 500 μl were used (see ref. 9 for definitions of these terms).

Before use, new concentrator columns were washed with 200 ml of acetonitrile–water (20:80), 200 ml of 1 mM phthalic acid at 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 used was evaluated by coupling the concentrator column directly to the conductivity detector and injecting 100 μl of a 5-ppm solution of chloride and nitrate, using 0.5 mM sodium benzoate (pH 6.0) as eluent at a flow-rate of 1.1 ml/min. The capacity factor for nitrate ion was then measured and if this value exceeded 8.0, the concentrator column was considered suitable for use with samples which contained a total amount of anions of 0.15 μequiv. or less.

RESULTS AND DISCUSSION

Assessment of concentrator column performance

At the commencement of this study, it was noticed that newly purchased concentrator columns did not provide the expected degree of binding of solute ions. Treatment with acetonitrile–water (20:80), phthalic acid at pH 6.0 and water (as described in the Experimental section) was found to rectify this problem, which was probably due to strongly bound surfactant present as a residue from the manufacturing process.

In a study of the sample loading parameters in preconcentration, it was imperative that the results obtained could be interpreted in terms of the loading parameters themselves and were not influenced by changes in the performance of the concentrator column. To achieve this aim, an evaluation procedure was devised as a means of qualitatively assessing the performance of a particular concentrator column. This procedure involved injection of a 5-ppm mixture of chloride and nitrate onto the concentrator column using 0.5 mM sodium benzoate (pH 6.0) as eluent at a flow-rate of 1.1 ml/min. The analytical column was removed from the flow path and the effluent from the concentrator column directed to the conductivity detector. Chromatograms obtained with this procedure for three different concentrator col-
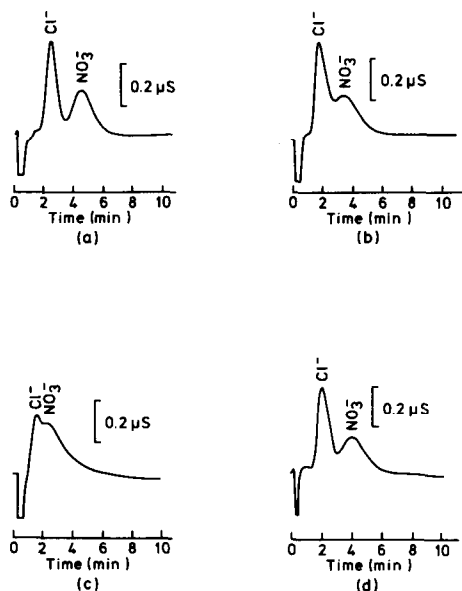
Fig. 1. Separation of chloride and nitrate on different concentrator columns. Conditions: columns, Waters Assoc. anion concentrator columns (see text for details of the previous histories of these columns); eluent, 0.5 mM sodium benzoate at pH 6.0; flow-rate, 1.1 ml/min; sample, 100 μl of a solution containing 5 ppm each of chloride and nitrate; sensitivity, 2.5 μS f.s.

umns are shown in Fig. 1. The first concentrator column (Fig. 1a) was newly purchased and had been conditioned according to the procedure described above; chloride and nitrate were well retained (capacity factors 4.8 and 10.0, respectively) and also well resolved. The second concentrator column (Fig. 1b) had been used for more than 300 preconcentrated samples and it can be seen that retention was reduced

TABLE I

RECOVERIES OF CHLORIDE, NITRITE, NITRATE AND SULPHATE FROM THREE DIFFERENT CONCENTRATOR COLUMNS, USING PHTHALATE AND p-TOLUENESULPHONATE ELUENTS

Columns A, B and C were as used in Fig. 1a, b and c, respectively. See text for details of the previous histories of these columns. The figures shown in brackets are the percentage relative standard deviations for four replicate measurements.

Anion	Recoveries (%)					
	Eluent 1 (4.5 mM p-toluenesulphonate at pH 6.0)			Eluent 2 (0.6 mM phthalate at pH 6.0)		
	Column A	Column B	Column C	Column A	Column B	Column C
Chloride	92.1 (0.4)	90.8 (3.2)	84.5 (1.0)	39.8 (0.5)	35.9 (0.7)	36.4 (0.6)
Nitrite	93.9 (4.6)	93.3 (5.0)	84.5 (0.6)	43.3 (1.6)	40.2 (0.5)	41.0 (2.6)
Nitrate	96.8 (1.1)	94.8 (2.5)	91.9 (3.5)	46.4 (0.2)	45.7 (3.0)	41.3 (1.4)
Sulphate	95.4 (1.9)	102.1 (2.4)	92.7 (1.1)	95.7 (1.3)	94.3 (0.6)	91.7 (2.3)

(capacity factors 3.3 and 7.3 for chloride and nitrate, respectively) in comparison to the new column. Fig. 1c was obtained with a concentrator column which had been employed as a pre-column in the injection of contaminated samples such as treated sewerage effluent, to the extent that the resin was visibly discoloured. With this column retention of chloride and nitrate (capacity factors 2.5 and 4.8, respectively) was lower than with any other column tested. The final chromatogram (Fig. 1d) was obtained with the same column as used in Fig. 1a, but after completion of this study which involved the preconcentration of approximately 250 samples: the capacity factors for chloride and nitrate were 4.3 and 9.3, respectively.

    Recovery experiments were also conducted with the same three columns used in Fig. 1a–c, using phthalate and p-toluenesulphonate eluents. In each case, peak areas obtained with a manual injection of 10 μl of a mixture containing 100 ppm each of chloride, nitrite, nitrate and sulphate were compared with those from a preconcentration run of 10 ml of a sample containing 100 ppb* each of the same ions. The results are given in Table I, which shows that whilst recoveries differed markedly between the two eluents (in accordance with our previous observations[10]), the three concentrator columns gave remarkably similar recoveries, considering their different degrees of retention of solute ions evident from Fig. 1. The poor recoveries with the phthalate eluent were attributed to the fact that at the eluent pH used, phthalate existed primarily in the doubly ionised form and the sample ions (with the exception of sulphate) were unable to effectively displace these phthalate ions during the binding process. It is noteworthy that the amounts of solutes used in the recovery experiments (0.028, 0.022, 0.016 and 0.042 μequiv. for chloride, nitrite, nitrate and sulphate, respectively) were in total only a small fraction (approximately 4%) of the total ion-exchange capacity of the concentrator column, which has previously been calculated to be 2.5 μequiv.[10]. As long as this situation applied (for example, in trace analysis applications), the results suggested that the condition of the concentrator columns did not strongly influence the recoveries obtained in preconcentration runs. We have adopted a performance criterion for concentrator columns in that they must provide a capacity factor of at least 8.0 for nitrate under the test conditions used in Fig. 1 in order to be suitable for preconcentration applications. Fig. 1 shows that the column used in this study exceeded this performance criterion for the entire duration of the study.

Effect of sample loading parameters

    Having established that the concentrator column to be used in the study was performing suitably and that minor changes in performance would not have a significant effect on the recoveries of loaded anions, it was then necessary to devise a procedure which would enable loading parameters to be investigated. The flow-rate used for sample loading and the sample volume were to be varied, whilst keeping constant the total amount of sample ions to be loaded at a level approximately 4% of the ion-exchange capacity of the concentrator column. In this way, the ion-exchange capacity of the concentrator column would not be an important factor in the interpretation of the results. The experimental ranges selected were 0.5–10 ml/min. for the flow-rate and 2–100 ml for the sample volume, with the loaded amount being

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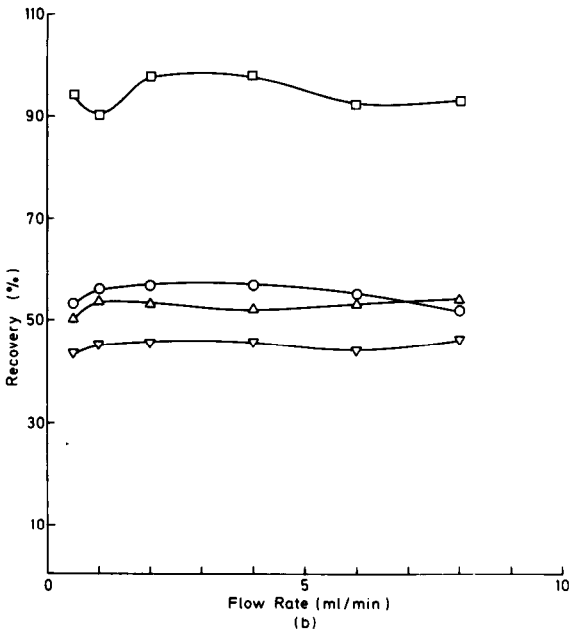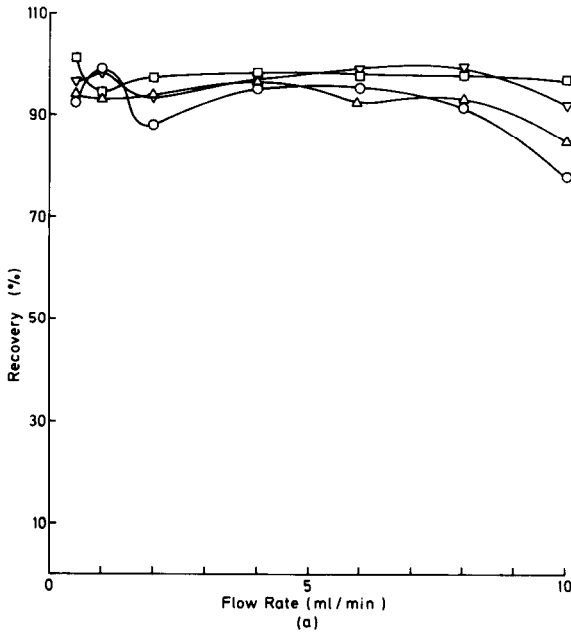* Throughout this article the American billion (10^9) is meant.

Fig. 2. Effect of the flow-rate used for sample loading on the recoveries of preconcentrated samples using
p-toluenesulphonate (a) and phthalate (b) eluents. Conditions: columns, Waters Assoc. IC Pak A and
anion concentrator columns; eluents, 4.5 mM p-toluenesulphonate at pH 6.0 (a) and 0.6 mM phthalate
at pH 6.0 (b); flow-rate, 1.0 ml/min; sample, 10 ml of a solution containing 100 ppb each of chloride,
nitrite, nitrate and sulphate; detector sensitivity: 2.5 μS f.s. (∇) Cl$^-$, ($\triangle$) NO$_2^-$, ($\bigcirc$) NO$_3^-$, ($\square$) SO$_4^{2-}$.
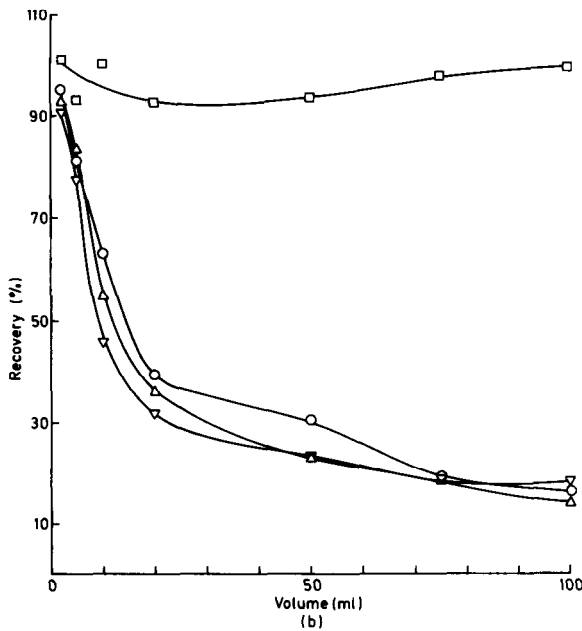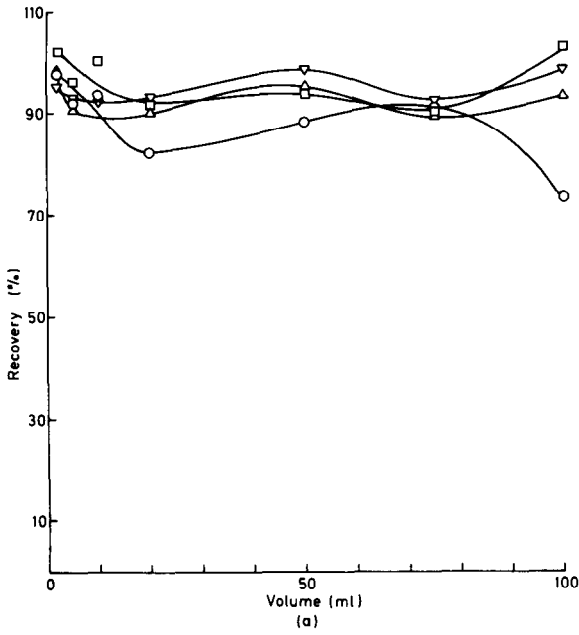
Fig. 3. Effect of the sample volume on the recoveries of preconcentrated samples obtained using p-tolu-enesulphonate (a) and phthalate (b) eluents. Conditions: as for Fig. 2 except that the flow-rate used for sample loading was 2.0 ml/min. Key as in Fig. 2.

equivalent to a 10-ml sample containing 100 ppb of each anion. Two eluents were chosen, the first being singly ionised (*p*-toluenesulphonic acid at pH 6.0) and the second being doubly ionised (phthalate at pH 6.0).

With each eluent, duplicate estimates of the recoveries resulting at various flow-rates were obtained by comparison of peak areas from manual injections and preconcentration runs, after subtraction of the peak areas obtained from blank runs. The results given in Fig. 2a for the *p*-toluenesulphonate eluent and Fig. 2b for the phthalate eluent show that the recoveries were essentially independent of the sample loading flow-rate, even for flow-rates as high as 8 ml/min. At a flow-rate of 10 ml/min, a slight decrease in recoveries was observed for the *p*-toluenesulphonate eluent, presumably due to channelling effects in the column resulting from the high liquid velocity. Although Fig. 2 was prepared for a fixed sample volume (10 ml), other volumes were also examined and the results obtained were similar to Fig. 2.

The effect of varying the sample volume was studied by loading samples with volumes in the range 2–100 ml at a flow-rate of 2.0 ml/min, using the same two eluents described above. Sample concentrations were adjusted so that the total amount of solute ions in each sample was the same. The results are given in Fig. 3a for the *p*-toluenesulphonate eluent and Fig. 3b for the phthalate eluent. Recoveries were essentially quantitative and independent of sample volume for the *p*-toluenesulphonate eluent, but showed strong dependence on sample volume for the phthalate eluent. The somewhat erratic nature of the recovery values shown in Fig. 3a is attributable more to the occurrence of comparatively high and variable blank levels due to the large sample volumes used than to any variance inherent in the preconcentration process itself. The loss of univalent solute anions from the concentrator column equilibrated with the phthalate eluent, particularly at high sample volumes, was indicative that these ions were not retained as a compact band on the column during sample loading, but instead eluted continuously from the column.

A major problem encountered in the preconcentration of large sample volumes (in excess of 20 ml) was the purity of the water used to make up the samples. Despite the precautions taken in purification of the water and the use of polypropylene volumetric ware, detectable amounts of chloride, nitrate and sulphate were found to be present in blank samples prepared in the same manner as the standard solutions. Chloride was particularly ubiquitous, with levels of up to 2 ppb being detected in the blanks and this suggests that the lower concentration limits of the working range of preconcentration methods are defined by the purity of the water available and the degree of confidence in accurately preparing standard solutions at the sub parts per billion level.

CONCLUSIONS

In the sample preconcentration process, the rate at which the sample was loaded onto the concentrator column did not influence the recoveries of the solutes being concentrated, within the range 0.5–8.0 ml/min. On the other hand, the recoveries obtained when the sample volume was varied were dependent on the type of eluent used to condition the concentrator column. When a singly ionised eluent such as *p*-toluenesulphonate was used, recoveries were essentially quantitative at all sample loading volumes up to 100 ml: with doubly ionised eluents such as phthalate, recov-

eries decreased sharply when the sample volume was increased above 5 ml. These results suggest that variable recoveries can be expected with eluents comprising a mixture of a singly and doubly ionised species, such as carbonate/bicarbonate buffers.

The results of this study further endorse our previous conclusions[10] that singly ionised eluents are the most suitable for preconcentration methods in which the same eluent is used to equilibrate the concentrator column, to strip the trapped solute ions from the concentrator column and to separate these ions on the analytical column.

ACKNOWLEDGEMENT

REFERENCES

1 A. L. Heckenberg and P. R. Haddad, *J. Chromatogr.*, 299 (1984) 301.
2 A. E. Bucholz, C. I. Verplough and J. L. Smith, *J. Chromatogr. Sci.*, 20 (1982) 499.
3 T. Okada and T. Kuwamoto, *J. Chromatogr.*, 350 (1985) 317.
4 R. A. Wetzel, C. L. Anderson, H. Schleicher and G. D. Crook, *Anal. Chem.*, 51 (1979) 1532.
5 K. M. Roberts, D. T. Gjerde and J. S. Fritz, *Anal. Chem.*, 53 (1981) 1691.
6 J. A. Cox and N. Tanaka, *Anal. Chem.*, 57 (1985) 2370.
7 M. A. Fulmer, J. Penkron and R. J. Nadalin, in E. Sawicki and J. D. Mulik (Editors), *Ion Chromatographic Analysis of Environmental Pollutants*, Vol. II, Ann Arbor Publ., Ann Arbor, MI, 1979, p. 381.
8 P. R. Haddad and A. L. Heckenberg, *J. Chromatogr.*, 318 (1985) 279.
9 A. L. Heckenberg and P. R. Haddad, *J. Chromatogr.*, 330 (1985) 95.
10 P. E. Jackson and P. R. Haddad, *J. Chromatogr.*, 355 (1986) 87.
11 R. Bagchi and P. R. Haddad, *J. Chromatogr.*, 351 (1986) 541.