Journal of Chromatography, 389 (1987) 65–74
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 19 220

STUDIES ON SAMPLE PRECONCENTRATION IN ION CHROMATOGRAPHY

V. EFFECT OF THE ION-EXCHANGE CAPACITY OF THE CONCENTRATOR COLUMN

P. E. JACKSON and P. R. HADDAD*
Department of Analytical Chemistry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033 (Australia)
(Received November 7th, 1986)

SUMMARY

The effect of the ion-exchange capacity of the concentrator column on the success of the preconcentration process is discussed for the case of anion analysis using non-suppressed ion chromatography. Monovalent anions such as chloride and nitrite are shown to be subject to self-elution during the sample loading process and losses of these ions occurs at levels of loaded sample well below the total ion-exchange capacity of the concentrator column. It is therefore the effective ion-exchange capacity which is of importance, rather than the total ion-exchange capacity.

The ion-exchange capacity of the concentrator column is determined largely by the ion-exchange capacity of the analytical column used and in this work, optimal results were achieved when the capacity of the concentrator column was approximately 40% of that of the analytical column. The type of resin used in the concentrator column and the geometry of the column housing are also shown to exert significant effects.

INTRODUCTION

Preconcentration of trace levels of inorganic anions prior to ion chromatographic analysis is most commonly achieved through the use of a small ion-exchange pre-column (generally referred to as the concentrator column) which is mounted before the analytical column[1]. Solute ions are trapped on the concentrator column by passage of a measured volume of sample through this column, and these ions are then eluted onto the analytical column for separation and quantitation in the usual manner.

The ion-exchange capacity of the concentrator column has obvious importance in that it must be sufficiently high to ensure quantitative capture of the solute ions during the sample loading process. At the same time, this capacity must also be sufficiently low to permit elution of the bound solute ions from the concentrator

0021-9673/87/$03.50

column as a compact band. Failure to do this will result in diffuse peaks and poor resolution even when the solute ions are eluted from the concentrator column in the reverse direction to which they are loaded; that is, in a "backflush" mode[2,3].

In previous papers of this series, we have considered a number of factors which influence the preconcentration process, including the design of the chromatographic system[4,5], criteria for selection of an appropriate eluent[6] and the effect of sample loading parameters[7]. In this contribution, we address the question of the ion-exchange capacity of the concentrator column, as well as related aspects such as resin selectivity and packing configuration of the concentrator column.

EXPERIMENTAL

Instrumentation

The liquid chromatograph used in this work 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 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, and a Model M45 pump was used to pass the salt solutions through the concentrator columns in the preparation of breakthrough curves.

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.

Concentrator columns

A variety of concentrator columns and housings was used in this work. Breakthrough studies were performed using a Waters Assoc. IC concentrator column (5.0 × 6.0 mm I.D.), packed with methacrylate anion-exchange resin of particle size 25 μm and ion-exchange capacity of 15 equiv./ml. This column was housed in a Waters Assoc. Guard Pak pre-column module. Other concentrator columns and their packing materials and column housings are listed in Table I. The resins used in these columns were obtained by unpacking commercial ion chromatography columns: Waters Assoc. IC Pak-A resin was used for concentrator columns B, E, F and G (see Table I); Hamilton (Reno, NV, U.S.A.) PRP-X100 resin was used for concentrator column C; column D was custom packed with aminated styrene–divinylbenzene copolymer by Waters Assoc.

Columns were packed by slurrying an appropriate weight of the resin in ethylene glycol–water (50:50, v/v) and pumping the slurry into the column housing at a flow-rate of 0.3 ml/min. The packed column was then washed with 100 ml of deionised water and 200 ml of the eluent prior to use in preconcentration experiments.

Reagents

All water was doubly distilled and passed through a Millipore (Bedford, MA, U.S.A.) Milli-Q water purification system. When the water was to be used for the preparation of ultra-trace standard solutions, the 0.22 μm in-line filter was removed from the Milli-Q apparatus in order to prevent contamination with nitrate ion[8]. Standard solutions (100 ppm) of acetate, chloride, nitrite, nitrate and sulphate were

TABLE I

CONCENTRATOR COLUMNS USED IN THIS STUDY

Column	*Packing material*	*Column housing*	*Measured total ion-exchange capacity of the column (μequiv.)*
A	Methacrylate resin, 25-μm particle size, 15 μequiv./ml	Guard Pak pre-column (5.0 × 6.0 mm I.D.)	2.15
B	Methacrylate resin, 10-μm particle size, 30 μequiv./ml	As above	5.03
C	Aminated polystyrene–divinyl benzene resin, 10-μm particle size, 200 μequiv./g	As above	6.16
D	Aminated polystyrene–divinyl benzene resin, 10-μm particle size, 160 μequiv./g	As above	6.89
E	As for B above	As above	8.24
F	As for B above	As above	12.42
G	As for B above	Steel column (20 × 3.5 mm I.D.)	12.56

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 the required trace solutions. Polypropylene volumetric flasks which had previously been rinsed five times with water were used for the preparation of trace solutions and for the collection of fractions in the breakthrough experiments.

All preconcentration studies were performed using 4.5 m*M* *p*-toluene sulphonic acid at pH 6.0 as eluent, operated at a flow-rate of 1.0 ml/min. Benzoic acid (0.5 m*M*, pH 6.0) was used as the eluent in the measurement of the chromatographic efficiencies of the concentrator columns[7]. Eluents were diluted daily from concentrated stock solutions which were prepared by dissolving weighed amounts of analytical grade reagents in approximately 800 ml of water, after which the pH was adjusted 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 membrane filter and degassed in an ultrasonic bath before use.

Procedures

The pump microprocessor was programmed to actuate the valves in a timed sequence, the details of which are given elsewhere[5].

The total ion-exchange capacities of the concentrator columns were measured in the following manner. The column was first washed with 200 ml of 5 m*M* sodium nitrate and then 5 ml of 1 m*M* sodium nitrate, after which the interstitial nitrate ion

was removed by washing the column with 400 μl of deionised water. The bound nitrate was then eluted from the column with 20 ml of 8 m*M* sodium sulphate and the eluate collected in a 25 ml volumetric flask and the solution diluted to the mark. The nitrate concentration in this final solution was then determined by ion chromatography using 4.5 m*M* *p*-toluene sulphonic acid at pH 6.0 as eluent.

The effective ion-exchange capacities of the concentrator columns were measured using breakthrough techniques as follows. The column was first equilibrated with 100 ml of the *p*-toluene sulphonic acid eluent and then a solution containing the test anion(s) at a concentration of 500 ppb* was passed through the column at a flow-rate of 2.0 ml/min. Fractions (20 ml) of the effluent were collected and then analysed for their anion content by preconcentrating 2 ml portions on a Waters Assoc. IC Pak anion concentrator column.

RESULTS AND DISCUSSION

Characterisation of the concentrator columns

Prior to commencement of studies on the effect of the ion-exchange capacity of the concentrator column on the preconcentration process, the concentrator columns were characterised in terms of their retention behaviour and their ion-exchange capacity. The ability of each column to retain anions was assessed using a previously reported performance criterion[7] in which the capacity factor for nitrate ion was required to exceed a value of 8.0 when direct injections of 100 μl of a 5-ppm mixture of chloride and nitrate were made onto the concentrator column and 0.5 m*M* sodium benzoate at pH 6.0 was used as eluent. Attention was also paid to the peak shape in these chromatograms so that any voids present in the columns would be detected.

The ion-exchange capacity of each concentrator column was measured both under equilibrium and dynamic conditions. In the former case, the column was equilibrated with excess nitrate ion and the bound nitrate was then eluted quantitatively with sulphate. Determination of the eluted nitrate provided a measure of the total ion-exchange capacity achievable under equilibrium conditions. The results obtained for the columns under study are given in Table I which shows that the ion-exchange capacities varied from 2.15 to 12.56 μequiv.

Under operating conditions, it is unlikely that all ion-exchange sites will be available for solute binding. For this reason, the effective ion-exchange capacity was assessed by monitoring the breakthrough of an ionic solution from a concentrator column which had been equilibrated with eluent. Chloride, nitrite and sulphate were used as individual test solutes and the aim of this determination was to assess the ability of the column to retain solutes under typical operating conditions. Breakthrough curves were plotted as the amount of ionic solute present in the column effluent, expressed as a percentage of that originally in the initial sample solution, *versus* the volume of solute which had been passed through the column. Three critical points may be identified as a means of reporting the shapes of these breakthrough curves: these are when the concentration of solute in the effluent reaches 5, 50 and 95% of the original concentration. That is, the points at which breakthrough first occurs, is half-complete and is fully complete. Fig. 1 shows a typical breakthrough

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

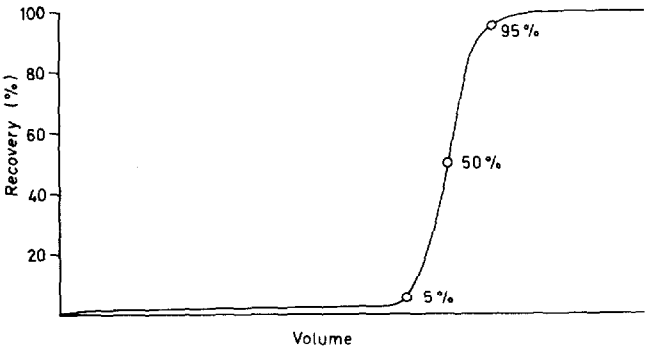

Fig. 1. Typical breakthrough curve illustrating the three critical points corresponding to 5, 50 and 95% recovery.

curve with these critical points labelled, and Table II lists the values obtained for the seven concentrator columns studied.

Table II shows the expected trend that sulphate ion was more strongly bound

TABLE II

AMOUNTS OF CHLORIDE, NITRITE AND SULPHATE REQUIRED TO REACH CRITICAL BREAKTHROUGH POINTS (SEE FIG. 1) WITH DIFFERENT CONCENTRATOR COLUMNS

Column	*Ion*	*μequiv. of solute required to reach indicated percentage breakthrough*		
		5%	*50%*	*95%*
A	Chloride	0.33	1.13	2.53
	Nitrite	0.39	1.30	3.96
	Sulphate	1.87	2.16	2.58
B	Chloride	0.73	2.59	5.64
	Nitrite	1.04	2.70	6.09
	Sulphate	4.66	5.33	5.70
C	Chloride	0.06	0.28	2.71
	Nitrite	0.09	0.30	2.39
	Sulphate	4.71	5.25	5.79
D	Chloride	0.06	0.68	3.10
	Nitrite	0.09	0.83	3.26
	Sulphate	5.08	5.87	6.70
E	Chloride	1.41	4.01	10.16
	Nitrite	2.52	5.04	11.87
	Sulphate	8.03	8.64	9.56
F	Chloride	2.51	5.72	14.86
	Nitrite	3.58	7.67	16.84
	Sulphate	11.28	12.91	14.78
G	Chloride	1.97	4.80	11.70
	Nitrite	2.61	4.96	13.69
	Sulphate	10.53	11.12	11.61

than the monovalent ions, and exhibited a steep breakthrough curve in which the 5 and 95% breakthrough points were relatively close together. This indicated that sulphate was retained as a compact band which, with the passage of sample, progressively increased in width until breakthrough occurred. The amount of sulphate required to achieve 5% breakthrough was generally only slightly less than the total ion-exchange capacity of the column measured under equilibrium conditions. On the other hand, the monovalent species chloride and nitrite showed different breakthrough behaviour. Here, 5% breakthrough occurred for amounts of sample which were very much less than the total ion-exchange capacity of the column, suggesting that these species were retained as a diffuse band on the concentrator column and were subject to self-elution during the loading process. This effect occurred with all columns tested and the onset of breakthrough was dependent both on the total ion-exchange capacity and the nature of the resin used in the concentrator column.

The results given in Table II show that even on the concentrator column having the lowest total ion-exchange capacity, large amounts of solute could be retained quantitatively from a sample solution. This can be exemplified by reference to sulphate, for which the onset of breakthrough with column A occurred at 1.87 μequiv. (that is, the approximate amount of solute contained in 10 ml of a 9 ppm solution)

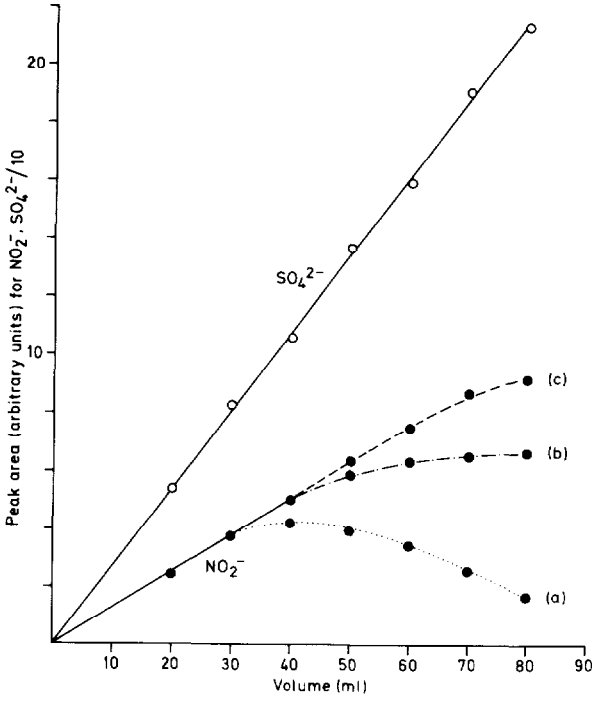

Fig. 2. Calibration curves obtained when preconcentrating a solution containing 50 ppb nitrite and 1000 ppb sulphate using columns A (a) and B (b,c). The peak area for sulphate is divided by ten to permit plotting on the same scale as for nitrite. Both columns gave the same calibration curve for sulphate. Conditions: analytical column, Waters Assoc. IC Pak-A; eluent, 4.5 m*M* *p*-toluenesulphonic acid at pH 6.0; flow-rate, 1.0 ml/min; sample loading rate, 1.0 ml/min; strip volume (see text), 500 μl for curves a and b, 1000 μl for curve c.

which is well beyond the concentration range for which preconcentration methods would normally be used. However, of more concern is that incomplete sample recovery can be expected to occur for some ions well before the amount of sample loaded exceeds the total ion-exchange capacity of the concentrator column. This effect is likely to be exacerbated when the sample contains a mixture of solute ions.

Effect of total ion-exchange capacity on preconcentration

For the purposes of this study, a test sample solution comprising 50 ppb nitrate and 1000 ppb sulphate was used. Calibration curves were constructed by loading increasing amounts of this solution onto column A using a preconcentration system which has been optimised to give maximal recoveries. The details of this system are given elsewhere[5], however for the present discussion it should be noted that the design incorporates the ability to wash interstitial sample from the concentrator column and to then strip the bound solute from the concentrator column in the backflush direction. The volume of eluent used for this purpose is called the "strip volume" and this technique has the advantage that the concentrator column can be removed from the eluent flow-path after the sample ions have been eluted. This leads to improved baselines in the final chromatogram obtained from the analytical column. The strip volume is the minimum volume of eluent required to quantitatively remove sample ions from the concentrator column.

Fig. 2 shows the calibration curves obtained for nitrite and sulphate. With concentrator column A, the nitrite curve exhibits a departure from linearity when the sample volume exceeded 32 ml (Fig. 2, curve a), corresponding to 0.035 μequiv. of solute, which is much less than the 5% breakthrough point given in Table II for this solute in the absence of sulphate. On the other hand, the sulphate curve remained linear for all sample volumes considered. Also shown in Fig. 2 is the calibration curve obtained with concentrator column B, which has a total ion-exchange capacity of more than double that of column A. As expected, the calibration curve for column B has a greater linear range of sample loading (Fig. 2, curve b), although this increased range is not in proportion to the increased total ion-exchange capacity.

One possible explanation for this behaviour was that the strip volume used for column B was insufficient for quantitative removal of bound nitrite, since the nitrite band can be expected to reside further down the column due to displacement by the more strongly bound sulphate. To investigate this, the strip volume was increased from 500 μl to 1000 μl, with the result (Fig. 2, curve c) that the linear portion of the calibration curve was extended.

Whilst this improvement was significant, two further problems emerged with this approach. The first was a marked increase in the width of the nitrite peak when the strip volume exceeded 1 ml, which was attributed to increased band broadening resulting from the requirement to backflush the nitrite band through an appreciable part of the relatively inefficient concentrator column. The second problem was an increase in the size of the solvent peak which introduced the possibility of interference with early eluting solutes if the strip volume was further increased. Since the strip volume was related directly to the total ion-exchange capacity of the concentrator column, the above problems would be expected to increase in importance for the higher capacity columns. Results obtained with these columns supported this prediction.

In an effort to overcome these effects, a reduced volume (500 μl) of a more concentrated (9.0 mM) eluent was used to strip the bound ions from the concentrator column, whilst at the same time retaining the original eluent for the separation step on the analytical column. This approach was quite unsuccessful since the baseline perturbation produced when the band of more concentrated eluent passed through the conductivity detector was so great that quantitation of solute peaks was impossible.

The results obtained show that in a preconcentration system where the same eluent is used both to strip bound solute ions from the concentrator column and to effect their separation on the analytical column, the optimal total ion-exchange capacity of the concentrator column is dictated by the ion-exchange capacity of the analytical column.

Effect of resin selectivity

Two different resin types were used as packing materials for the concentrator columns used in this study: aminated methacrylate (columns A, B, E, F and G) and aminated styrene–divinylbenzene copolymer (columns C and D). It is interesting to compare the behaviour of columns B and C, which have similar total ion-exchange capacities and functional groups, but different resin substrates. Fig. 3 shows breakthrough curves for chloride, nitrite and sulphate obtained using these columns. It can be seen that breakthrough of the monovalent species occurred at lower sample volumes on the styrene–divinylbenzene column and this was attributed to the selectivity behaviour of this particular material. This contention was supported by our experience with the Hamilton PRP-X100 column (from which the packing material for concentrator column C was derived), where we have found that monovalent ions are less retained than with other column packing materials[9]. Column D gave very similar results to those shown in Fig. 3 for column C, and with both of these columns, the calibration curve for increasing volumes of the 50 ppb nitrite/1000 ppb sulphate test sample showed only a very small linear range of sample loading. Here, departure from linearity occurred when less than 10 ml of sample had been passed through the

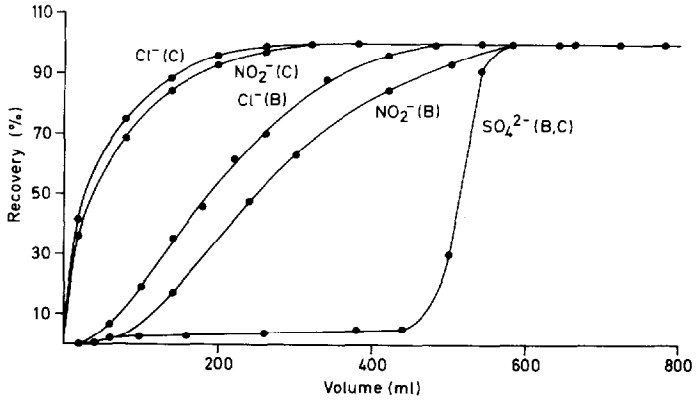

Fig. 3. Breakthrough curves for chloride, nitrite and sulphate using columns B and C. Conditions: the sample was a 500-ppb solution of the indicated ion, pumped through the column at a flow-rate of 0.4 ml/min. Fractions (20 ml) of the effluent were collected for analysis.

concentrator column. This value should be compared with that of 40 ml obtained for column B under the same operating conditions (see Fig. 2, curve b).

These results suggest that the selectivity of the resin material in the concentrator column plays a major role in the ability of the column to achieve quantitative retention of solute ions during the loading step.

Effect of concentrator column geometry

Columns F and G provide an opportunity for assessment of the effect of the geometry of a concentrator column on its performance. These two columns were packed with the same material and had almost identical total ion-exchange capacities, but column F had a larger internal diameter and shorter length than column G (see Table I). When tested using the performance criterion described earlier[7], both columns showed adequate retention characteristics, however column G exhibited superior efficiency (42 and 382 theoretical plates for columns F and G, respectively).

The breakthrough studies conducted with these columns (Table II) show that column F had a higher effective ion-exchange capacity for all ions tested than did column G. This effect may be attributable to poorer radial dispersion of the solute in column G during sample loading, leading to less efficient binding of the solute. It should be pointed out that both columns had high total ion-exchange capacities in comparison to the other columns used in this study and both performed well as concentrator columns. The effect of their differing geometries was only evident in the breakthrough studies. Nevertheless, the results obtained suggest that the preferred configuration is a short concentrator column with large internal diameter. The chromatographic efficiency of the concentrator column appears to have little effect on its performance.

CONCLUSIONS

The total ion-exchange capacity of a concentrator column is governed largely by the ion-exchange capacity of the analytical column, since the latter determines the eluent concentration. This work has shown that monovalent anions are retained as a diffuse band on the concentrator column and are subject to self-elution in the loading process. Losses of these ions can therefore be expected to occur at levels of loaded sample well below the total ion-exchange capacity of the concentrator column.

In this work, optimal results were obtained when the total ion-exchange capacity of the concentrator column was approximately 40% of that of the analytical column. Increasing the ion-exchange capacity of the concentrator column beyond this value improved the retention of solute ions but necessitated the use of larger strip volumes to quantitatively remove solute ions from the concentrator column. This, in turn, led to large solvent peaks in the final chromatogram and concomittant interference with early eluting solutes, as well as band-broadening effects. The above relationship between the magnitudes of the ion-exchange capacities of the concentrator and analytical columns is expected to apply to other anion-exchange preconcentration systems where the same eluent is used to strip sample ions from the concentrator column and to effect separation of these ions on the analytical column.

In addition to the total ion-exchange capacity of the concentrator column, the selectivity of the resin used as packing material must also be considered. The optimal geometry appears to be short concentrator columns with a large internal diameter.

ACKNOWLEDGEMENT

We thank Waters Associates (Australia) for the generous loan of equipment.

REFERENCES

1 R. A. Wetzel, C. L. Anderson, H. Schleicher and G. D. Crook, *Anal. Chem.*, 51 (1979) 1532.
2 K. M. Roberts, D. T. Gjerde and J. S. Fritz, *Anal. Chem.*, 53 (1981) 1691.
3 T. Okada and T. Kuwamoto, *J. Chromatogr.*, 350 (1985) 317.
4 P. R. Haddad and A. L. Heckenberg, *J. Chromatogr.*, 318 (1985) 279.
5 A. L. Heckenberg and P. R. Haddad, *J. Chromatogr.*, 330 (1985) 95.
6 P. E. Jackson and P. R. Haddad, *J. Chromatogr.*, 355 (1986) 87.
7 P. R. Haddad and P. E. Jackson, *J. Chromatogr.*, 367 (1986) 301.
8 R. Bagchi and P. R. Haddad, *J. Chromatogr.*, 351 (1986) 541.
9 P. R. Haddad, P. E. Jackson and A. L. Heckenberg, *J. Chromatogr.*, 346 (1985) 139.