Journal of Chromatography, 482 (1989) 401–406
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 832

ALUMINA AS STATIONARY PHASE FOR ION CHROMATOGRAPHY AND COLUMN-COUPLING TECHNIQUES

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SUMMARY

Columns packed with alumina were combined with common anion-exchange columns and applied to the ion chromatographic determination of sulphate in brines and biological fluids and for the trace determination of iodide in mineral waters and fruit juice samples. Alumina was found to be a highly selective stationary phase for the preconcentration of sulphate from complex matrices. Further, owing to its selectivity, which is different from that of R_4N^+-type anion-exchange materials, it is well suited for on-line column-coupling techniques. In this way sample clean-up can be minimized, and the sensitivity of the chromatographic system allows determinations of iodide down to the low ppb range.

INTRODUCTION

The chromatographic separation of inorganic anions is carried out mainly with strongly basic anion exchangers of the tetraalkylammonium type (R_4N^+) or by ion interaction chromatography using ordinary C_{18} reversed-phase columns and hydrophobic ion-pairing reagents in the mobile phase. The selectivities of these systems are similar, but for some applications it might be advantageous to have a chromatographic system with different selectivity as an alternative.

Major changes in the elution order relative to R_4N^+-type ion exchangers have been reported for amine-modified silica[1,2] and for alumina[3,4]. Especially alumina might be the stationary phase of choice for sample preconcentration and clean-up and for column-coupling techniques in combination with conventional ion-exchange columns.

In this paper, the use of alumina-filled cartridge columns combined with a polymer-based R_4N^+-type ion-exchange column is described for the determination of trace amounts of sulphate in brine (containing about 20 g/l of sodium chloride) and of sulphate in complex biological matrices such as serum.

The advantages of coupling an alumina column with silica-based R_4N^+-type ion-exchange columns are demonstrated for the determination of iodide in mineral

0021-9673/89/$03.50

water and juice samples. Multi-column analysis is a well established method in high-performance liquid chromatography (HPLC). In ion chromatography, there have been few reports of coupling columns with different stationary phases, such as the combination of ion-exchange chromatography with ion-exclusion chromatography[5,6]. The whole range of benefits of column-switching techniques in ion chromatography seem not yet to have been fully exploited.

EXPERIMENTAL

Instrumentation and reagents

The chromatographic instrumentation consisted of Waters M510 HPLC pumps, Rheodyne 7010 injection valves with a 20-, 50- or 200-μl loop or a precolumn (40 × 4 mm I.D.) filled with a Vydac anion exchanger (30-μm particle size), a Waters M430 conductivity detector and a Waters M481 UV detector. The following separation columns and mobile phases were used: a Beckman XL column (70 × 4.6 mm I.D.) packed with 3-μm Ultraspher-ODS and 0.01 *M* octylamine (adjusted to pH 6.5 with phosphoric acid)–methanol (37:4, v/v) as the mobile phase; a Hamilton column (250 × 4.1 mm I.D.) packed with 10-μm PRP-X100 and 2m*M* potassium hydrogenphthalate as the mobile phase; a Vydac 302-IC column (250 × 4.6 mm I.D.) and 10 g/l of methanesulphonic acid (adjusted to pH 4 with sodium hydroxide) as the mobile phase; and a Stagroma column (125 × 4.6 mm I.D.) packed with 5-μm Spherisorb alumina and 1 g/l of methanesulphonic acid (adjusted to pH 4 with sodium hydroxide) as the mobile phase.

Samples for sulphate determination were preconcentrated or cleaned up with Waters Sep-Pak cartridges filled with neutral alumina. Before use these cartridges were washed with 1 ml of 1 *M* ammonia, 2 ml of water and 1 ml of 0.7% hydrochloric acid.

Determination of sulphate in brine samples

About 600 ml of the sample were passed through a glass column (150 × 15 mm I.D.) filled with a Dowex 50W-X-8 (H^+) cation exchanger (50–100 mesh). A 500-ml volume of the eluate was passed through a Sep-Pak alumina cartridge, followed by 1 ml of 0.7% hydrochloric acid and 1 ml of water, then sulphate was eluted with 4 ml of 1 *M* ammonia solution and 1 ml water. The eluate was passed through a cartridge (15 × 10 mm I.D.) filled with Dowex 50W-X-8 (H^+) and 20 μl were injected on to the Hamilton PRP-X100 column. Detection was effected by conductivity measurement.

Determination of sulphate in serum

Serum (5 ml) was diluted to about 50 ml with 0.7% hydrochloric acid and passed through a Sep-Pak alumina cartridge. The subsequent treatment was the same as for brine samples.

Determination of iodide in mineral water and fruit-juice samples

For screening purposes, 50 μl of mineral water were directly injected on to the RP-18 column in combination with UV detection at 227 nm. Samples that possibly contained more than 20 ppb of iodide were analysed a second time by injecting 200 μl on to the alumina column with UV detection at 227 nm. With complex matrices the

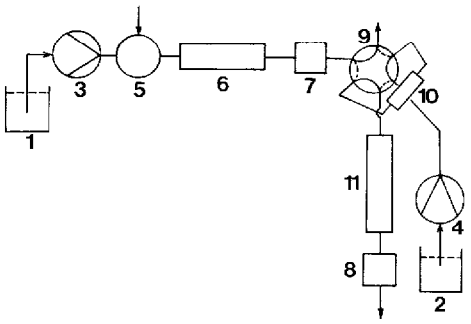

Fig. 1. Experimental set-up for column switching. 1, 2 = Reservoirs for mobile phases; 3, 4 = HPLC pumps; 5 = injection valve with 200-μl loop; 6 = separation column packed with alumina; 7, 8 = UV detectors; 9 = column-switching valve; 10 = precolumn packed with 30-μm Vydac anion exchanger; 11 = Vydac 302-IC separation column.

alumina column was coupled with the Vydac column (Fig. 1); the iodide-containing fraction was eluted on to the Vydac precolumn, which was then switched into the flow of the Vydac separation column. UV detection at 227 nm was used.

RESULTS AND DISCUSSION

Brines may be used for balneotherapeutic purposes[7], which makes the determination of all components of such samples necessary. In general, the ion chromatographic determination of trace amounts of sulphate at levels down to less than 1 mg/l in the presence of about 20 g/l of sodium chloride is not possible by direct injection. Dilution of the sample might be the method of choice if the sulphate-to-chloride ratio is not too low, but in our case dilution would prevent the detection of trace concentrations of sulphate. This problem can be circumvented either by elimination of chloride in the injected sample by on-line precipitation on a silver-loaded cation-exchange column coupled with the separation column[8] or by selective preconcentration of sulphate. The latter method was chosen for our investigations.

Fritz *et al.*[9] have already reported the use of alumina-filled columns for the separation of sulphate from other anions before titrimetric determination of sulphate. Under acidic conditions, sulphate is strongly retained, whereas chloride is not. The anion-exchange capacity of alumina is pH dependent and alumina will change from an anion exchanger to a cation exchanger on altering the pH from acidic to basic conditions[3]. Therefore, after the enrichment step sulphate can be eluted with a small volume of ammonia solution. Such an approach is practicable, because nowadays disposable cartridge columns filled with alumina are commercially available. The pH of the brine sample was lowered to an appropriate value by running it through a strongly acidic cation-exchange column. We believed this method to be better than the addition of an suitable acid, because in this way an increase in the ratio of the total concentration of anions to the concentration of sulphate could be avoided; otherwise, the recovery of sulphate might decrease. After preconcentration on alumina and subsequent elution, the eluate was neutralized by passing it through a cation-exchange cartridge.

A sodium chloride content of the brine sample of up to at least 20 g/l does not affect the recovery of the preconcentration procedure. The recovery is also independent of the volume of the brine sample up to at least 500 ml, which was checked by loading a constant amount of 0.55 mg of sulphate in different volumes of sodium chloride solution on to the cartridge. The recovery as a function of the sulphate concentration was 86.0% for 0.72 ppm, 89.9% for 1.1 ppm, 95.3% for 2.4 ppm and 97.7% for 4.8 ppm of sulphate (brine sample volume 500 ml). The lower recovery of sulphate at lower concentrations must be attributed to incomplete elution from the alumina cartridge by ammonia; to a small extent sulphate might be adsorbed on alumina by mechanisms different from ion exchange.

A typical chromatogram of a brine sample containing 0.49 mg/l of sulphate and 20.99 g/l of sodium chloride is shown in Fig. 2. The sensitivity of the chromatographic system would permit the determination of concentrations considerably lower than 500 ppb, but then the recovery would decrease to unacceptable levels. The relative standard deviation was 2.1% ($n = 4$) for a sample containing 1 ppm of sulphate.

Alumina is not only well suited for preconcentration but also for sample clean-up of complex biological matrices. This is shown for a serum sample in Fig. 2. The sample was diluted with 0.7% hydrochloric acid and passed through the alumina cartridge column. In this way a chromatogram free from any interferences can be obtained.

All the applications may be automated by commercially available sample preparation devices, so that, at least partially, the alumina-filled cartridges can be used on-line coupled with conventional anion-exchange separation columns.

The applications described above are based on the use of alumina-filled columns as a means of retaining or not retaining some ions. Nevertheless, the advantages of alumina in ion chromatography can only be fully exploited if alumina is used as stationary phase in a true separation column. Then the coupling with R_4N^+-type columns will result in a significantly increased separation power owing to the different selectivities of the stationary phases. Such a column-coupling technique was developed for the determination of trace amounts of iodide in mineral water and fruit-juice samples. Iodide is an essential micronutrient, but in some regions of Europe the alimentary iodide supply is insufficient[10]. Recently in Austria it has been reported

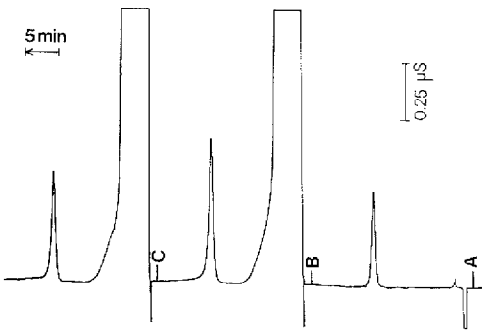

Fig. 2. Typical chromatograms for the determination of sulphate in brine and serum. Flow-rate, 2 ml/min. (A) Sulphate standard; (B) brine sample containing 0.49 ppm of sulphate, 100-fold preconcentrated; (C) human serum containing 31.4 mg/l of sulphate.

that mineral waters and several fruit juices can substantially contribute to a sufficient iodide supply[11]. Unfortunately, the data available on the iodide contents of mineral waters differ greatly (sometimes by more than one order of magnitude for the same sample), so that a reinvestigation of the iodide concentrations of such samples seemed necessary.

First we looked for a rapid screening method to differentiate between samples containing less than 20 ppb of iodide and those containing higher concentrations, because only the latter could contribute substantially to a sufficient iodide supply. For that purpose ion-interaction chromatography with RP-18 as the stationary phase, octylammoniumphosphate as the ion-pairing reagent and UV detection at 227 nm was used. Typical chromatograms for two mineral water samples are given in Fig. 3; while sample 1 unequivocally has a content of less than 20 ppb, the content of sample 2 might be higher, but an exact determination is difficult owing to some interferences. The sample was therefore injected a second time on to an alumina-filled separation column. This gave a clear chromatogram without interferences, as is shown in Fig. 3. An alumina column also proved advantageous as it can be loaded with an injection volume of 200 μl, whereas only up to 50 μl could be injected on to the RP-18 column without peak distortion. On the other hand, the disadvantage of the alumina column is that sulphate, which commonly occurs in mineral waters in high concentrations, is accumulated on the column, so that frequent flushing of the column with buffers of appropriate strength and pH might be necessary. To avoid this, we injected on to the alumina column only those samples in which elevated iodide levels could be expected from the initial screening procedure. The number of samples that can be processed without reconditioning the alumina column depends on the type of mineral water (*i.e.*, the concentration of sulphate). In general, the column was used for 1 day (about 20 injections of samples) and flushed overnight with an eluent containing 5 g/l of methanesulphonic acid adjusted to pH 6.

Serious interferences in the chromatograms were observed with fruit-juice samples. This could be circumvented by coupling the alumina column with a silica-based R_4N^+-type anion-exchange column, as shown in Fig. 1. Methanesulphonic acid,

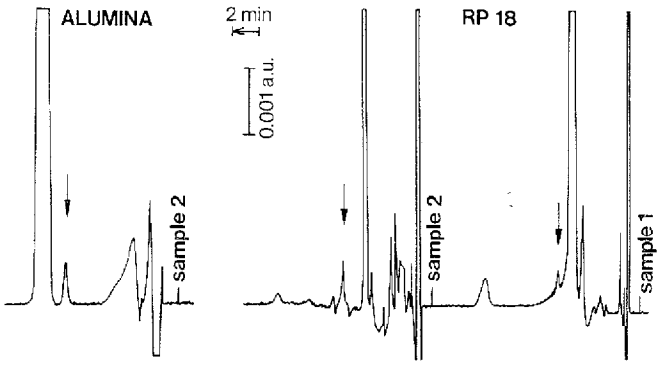

Fig. 3. Typical chromatograms for iodide in mineral waters using ion-pair chromatography on RP-18 or ion chromatography on alumina. Flow-rate, 0.8 ml/min. Sample 1 contains less than 20 ppb of iodide, sample 2 contains 25 ppb of iodide.

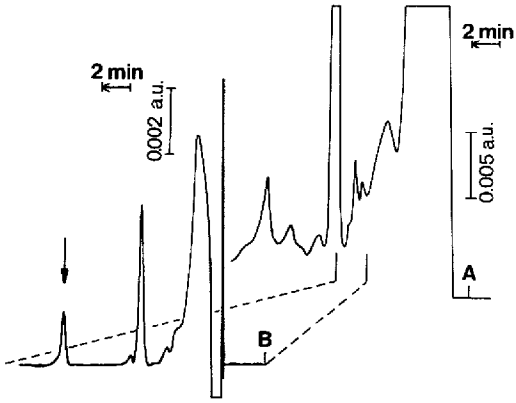

Fig. 4. Determination of iodide in a fruit-juice sample containing 72 μg/l of iodide. (A) Separation column packed with alumina (flow-rate, 0.8 ml/min); (B) column coupling with Vydac 302-IC separation column (flow-rate, 1 ml/min).

adjusted to pH 4, as the mobile phase is compatible with both columns. Its concentration for the alumina column needs to be only about one tenth of that necessary for the R_4N^+-type ion-exchange column. In this way a favourable peak compression could be obtained in the column-switching procedure. A typical chromatogram without and with column coupling is shown in Fig. 4.

The examples presented in this paper demonstrate some favourable aspects of alumina as a stationary phase in ion chromatography. It is unlikely that alumina will replace existing ion-exchange stationary phases, but it turned out as a valuable complement, which in some instances can facilitate the determination of ions in difficult matrices.

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