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SEPARATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY ON DYE-COATED SILICA GEL RP-18

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SUMMARY

RP-18 was coated with methylene blue, methyl green and crystal violet to obtain anion-exchange columns with adsorbed quaternary ammonium groups. The retention behaviour of fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate anions was investigated on these columns using an aqueous solution of *p*-hydroxybenzoic acid as the mobile phase. Using crystal violet for coating, a permanently coated ion-exchange column was obtained, which allowed efficient separations of the seven anions without adding any organic modifier or dyestuff to the mobile phase.

INTRODUCTION

Since the introduction of ion chromatography in 1975[1], a number of alternative chromatographic approaches have been investigated[2]. One of these techniques is based on the effect of ion-pair formation and is called ion-pair or ion-interaction chromatography, developed by Eksborg *et al.*[2,3]. Other terms used for this method were listed by Bidlingmeyer *et al.*[4]. Ion-interaction columns are generally produced by sorption of an organic, hydrophobic and ionic molecule on the surface of a stationary phase. Silica-based reversed-phase columns[5–9], neutral styrene–divinylbenzene copolymer columns[6,10–13] and cyano columns[14] have been used as supports for the ion-interaction reagent. A large number of ion-interaction reagents, mainly quaternary ammonium salts, have been investigated. For dynamic coating tetramethyl-[8], tetraethyl-[12], tetrabutyl-[6,12], tetrapentyl-[10] and cetyltrimethylammonium salts[14] have been used to condition the columns. For permanent coating, more hydrophobic ion-interaction reagents such as trioctylmethyl-, tetraoctyl- and tridodecylmethylammonium[12] and cetylpyridinium salts[6,11] have been employed.

Instead of these reagents, it is also possible to use dyestuff molecules as counter ions in ion-pair chromatography. DiNunzio and Freiser[15] used brilliant green as a counter ion for the separation of aliphatic acids, and Gnanasambandan and Freiser[16] separated a series of aliphatic alcohols on an ODS column loaded with methylene blue. Schmuckler *et al.*[17] achieved an improved separation of inorganic anions on a

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RP-18 column with a tetrabutylammonium salicylate eluent by conditioning the column with methyl green. Golombek and Schwedt[13] developed a chromatographic system for the separation of inorganic anions on a methyl green-loaded styrene–divinylbenzene copolymer column using aqueous solutions of 2,4-dihydroxybenzoic acid or 4-hydroxybenzoic acid with potassium hydroxide and small amounts of dyestuff as eluents.

The purpose of this work was to develop a permanently coated reversed-phase column that possesses a performance comparable to that of a chemically bonded ion-exchange column.

EXPERIMENTAL

Apparatus

A modular high-performance liquid chromatograph consisting of a Waters pump (M501), a Model 7125 injector (Rheodyne), a conductivity detector (Waters 430) and a computing integrator (Waters 740 Data Module) was used.

Reagents

p-Hydroxybenzoic acid was of analytical-reagent grade (Janssen Chimica). Potassium hydroxide and the sodium or potassium salts of the analytes were of analytical-reagent grade and were obtained from Merck. Methylene blue (Merck), methyl green (Fluka) and crystal violet (Merck), all for microscopy, were used as received. Distilled water was degassed in an ultrasonic bath.

Columns

Columns of 125 mm × 4.5 mm I.D. slurry packed with LiChrosorb RP-18 (Merck), particle size 7 μm, were used. After coating, the crystal violet-coated column was protected with a 40 mm × 4.5 mm I.D. precolumn containing LiChrosorb RP-18 (Merck), particle size 7 μm.

Column coating

Aqueous methylene blue solution (1.0 m*M*) was adjusted to pH 9 using potassium hydroxide. This solution was pumped through the column at a flow-rate of 1 ml/min until the dyestuff appeared at the column exit. Then methylene blue (1.0 m*M*) was dissolved in an aqueous solution of *p*-hydroxybenzoic acid (3 m*M*) and the pH was adjusted to 9 with potassium hydroxide. The solution obtained was pumped through the column at a flow-rate of 1 ml/min until the dyestuff appeared at the column exit again. Similar procedures were performed with either methyl green or crystal violet.

For coating the column with methyl green, first at 1 m*M* solution of the dye, adjusted to pH 9, and then a 0.7 m*M* solution of the dye in an aqueous solution of *p*-hydroxybenzoic acid (3 m*M*) was pumped through the column.

For the coating procedure with crystal violet, first a 0.6 m*M* aqueous solution (pH 9) and then a 0.05 m*M* solution of the dye in a 3 m*M* aqueous solution of *p*-hydroxybenzoic acid were used.

After column coating, the chromatographic equipment (except the columns) was washed with water, then with methanol and finally with the mobile phase. The columns were then equilibrated with the eluent.

Mobile phase

Mobile phases were prepared by adding the appropriate amount of *p*-hydroxybenzoic acid to water and dissolving it by adding potassium hydroxide until the required pH was reached.

RESULTS AND DISCUSSION

In a first series of experiments, silica gel RP-18 was allowed to adsorb several organic dyes in order to test their suitability and their retention behaviour. This was achieved by pumping solutions of the dyes in water and additionally in a 3 m*M* aqueous solution of *p*-hydroxybenzoic acid (*p*-HBA) through the columns filled with 1.2 g of RP-18 (column size 125 × 4.5 mm I.D.). The test substances were methylene blue, methyl green and crystal violet (Fig. 1). It was found necessary to use alkaline dye solutions because otherwise the adsorption rate decreased owing to the reduced hydrophobicity of the dyes. The two-stage coating procedure was chosen in order to complete and accelerate the column coating. Addition of *p*-HBA to the dye solutions resulted in higher specific adsorptions, but it also limited the pH values and the dyestuff concentrations by reducing the solubility of the dyes. Thus first aqueous solutions of the dyes, adjusted to pH 9 with potassium hydroxyde, were used for column coating. This resulted in a specific adsorption of 19 mg of methylene blue, 38 mg of methyl green and 70 mg of crystal violet per gram of RP-18. A second coating procedure with solutions of the dyes in a 3 m*M* aqueous solution of *p*-HBA increased the specific adsorption of the dyes. With methylene blue, a specific adsorption of 47 mg of dye per gram of RP-18 was determined, which means a specific exchanging capacity of 0.14 mequiv./g. The total adsorption of methyl green was 84 mg/g RP-18 (= 0.34 mequiv./g) and that of crystal violet was 97 mg/g RP-18 (= 0.21 mequiv./g).

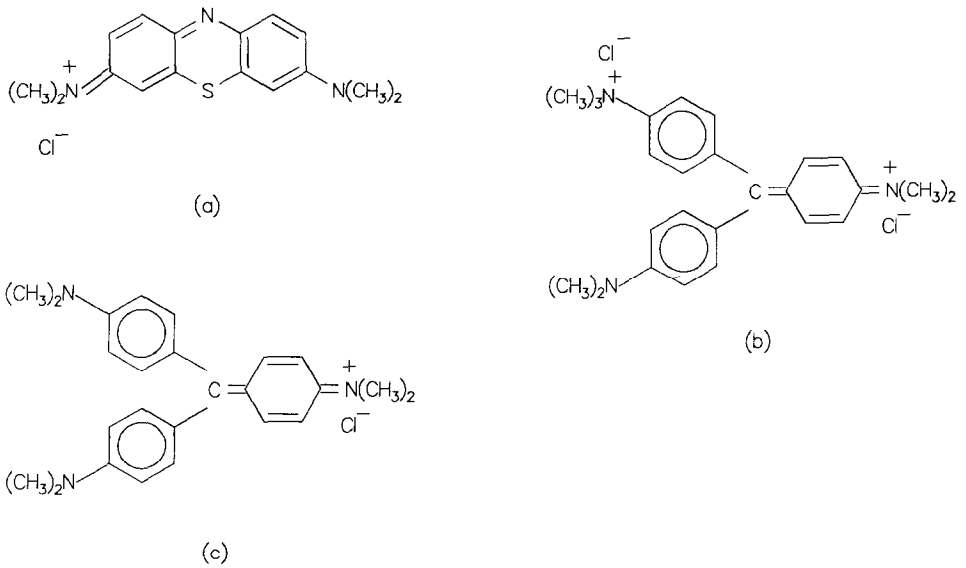

Fig. 1. Structures of (a) methylene blue, (b) methyl green and (c) crystal violet.

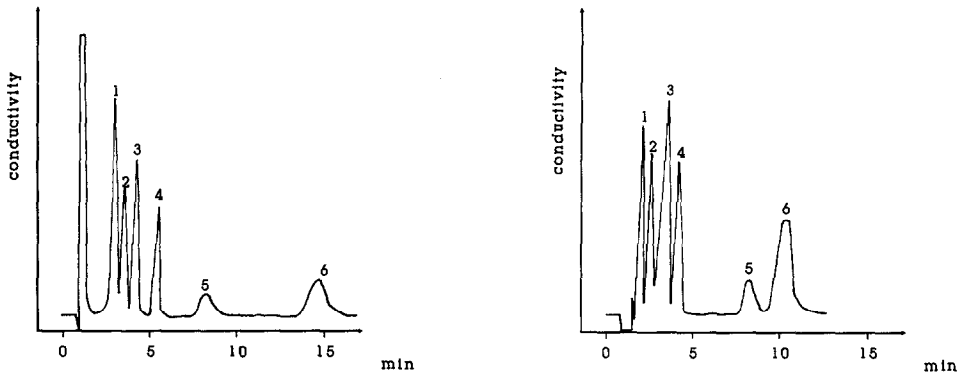

Fig. 2. Anion-exchange chromatography on methylene blue-coated sillica gel RP-18. Column: 125 × 4.5 mm I.D., packed with 1.2 g of LiChrosorb RP-18 (7 μm) and coated with 47 mg of methylene blue per gram of RP-18. Eluent: 3 m*M* *p*-HBA, pH 6.0; flow-rate, 1 ml/min. Sample size: 60 μl of standard anion mixture (Cl^- 16.7 mg/l, all other anions 47.7 mg/l). Conductivity detection. Peaks: 1 = F^-; 2 = Cl^-; 3 = NO_2^-; 4 = NO_3^-; 5 = PO_4^{3-}; 6 = SO_4^{2-}.

Fig. 3. Anion-exchange chromatography on methyl green-coated silica gel RP-18. Conditions as in Fig. 2, except 84 mg of methyl green per gram of RP-18 and eluent pH 8.0. Peaks as in Fig. 2.

In order to test the anion-exchanging properties, 60 μl of a standard mixture of fluoride, chloride, nitrite, nitrate, phosphate and sulphate (Cl^- 16.7 mg/l; all other anions 41.7 mg/l) were applied to the coated columns and then eluted with a 3 m*M* solution of *p*-HBA. Fig. 2 shows the chromatogram achieved with the methylene blue-coated column and Fig. 3 that of the methyl green-coated column. The separations obtained are similar to that achieved by Golombek and Schwedt[13] on a methyl green-coated PRP-1 column, but in both instances the dyes were consecutively

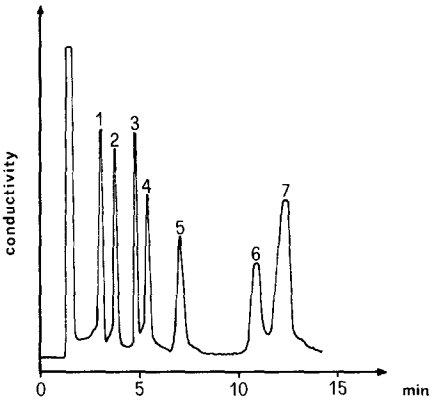

Fig. 4. Anion-exchange chromatography on crystal violet-coated silica gel RP-18. Conditions as in Fig. 2, except 97 mg of crystal violet per gram of RP-18, eluent pH 8.0 and standard anion mixture (Cl^- 14.3 mg/l, all other anions 37.7 mg/l). Peaks: 1 = F^-; 2 = Cl^-; 3 = NO_2^-; 4 = Br^-; 5 = NO_3^-; 6 = PO_4^{3-}; 7 = SO_4^{2-}.

TABLE I

CAPACITY FACTORS (k') AND DETECTION LIMITS FOR THE SEPARATION OF STANDARD ANIONS ON CRYSTAL VIOLET-COATED SILICA GEL RP-18

For conditions, see Fig. 1.

Anion	k'	*Detection limit (μg/l)*
F^-	1.5	100
Cl^-	2.1	50
NO_2^-	2.9	100
Br^-	3.4	100
NO_3^-	4.7	200
PO_4^{3-}	7.9	500
SO_4^{2-}	9.1	300

washed out of the columns, so that it was necessary to dissolve small amounts of dyestuff in the mobile phase in order to keep the elution times constant. On the other hand, the combination of crystal violet with RP-18 proved to be stable under comparable chromatographic conditions.

The chromatogram of a standard mixture of 14.3 mg/l of chloride and 37.7 mg/l each of fluoride, nitrite, bromide, nitrate, phosphate and sulphate is shown in Fig. 4. Each of the seven anions is eluted in a single well resolved peak, the order of its appearance being the same as found by Lee[18] on a chemically bonded anion exchanger. The present system has a long total elution time, but a more efficient separation of fluoride from the injection peak and a baseline separation of nitrate is

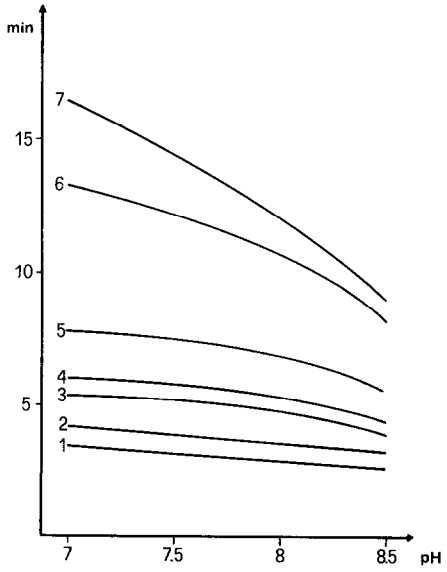

Fig. 5. Dependence of the elution times on pH of the eluent during anion chromatography on crystal violet-coated RP-18. Conditions and peaks as in Fig. 4.

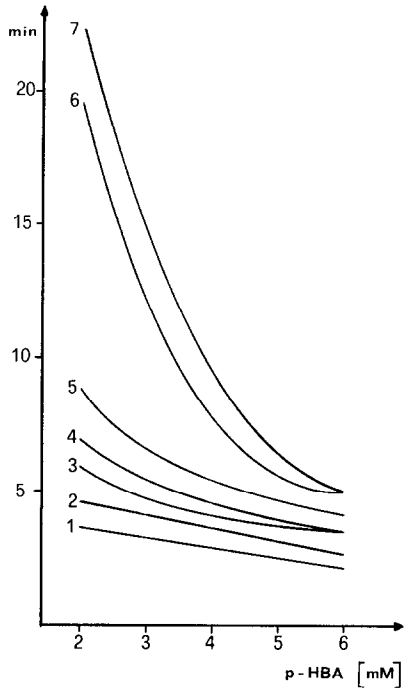

Fig. 6. Dependence of the elution times on the concentration of *p*-HBA during anion chromatography on crystal violet-coated RP-18. Conditions and peaks as in Fig. 4.

achieved. The corresponding detection limits, based on twice the baseline noise[19], and the capacity factors[20] are listed in Table I.

The influence of the pH on the elution times of the anions is illustrated in Fig. 5. The optimum range using a 3 m*M* aqueous solution of *p*-HBA was found to be between pH 7.5 and 8. At lower pH the elution times become too long, whereas at higher pH the peak resolution deteriorates and the baseline noise increases.

The influence of the *p*-HBA concentration on the separation is shown in Fig. 6. Higher concentrations of the eluting agent generally result in shorter elution times and in sharper peaks. The best separations were obtained with a 3 m*M* aqueous solution of *p*-HBA of pH 8. Higher concentrations give insufficient resolution.

The lifetime of the coated column was tested. It was found that the column can be run for about 65 h without any change in the quality of separation. After this time, however, the separation deteriorates very quickly, and it was impossible to regenerate the column again. This might be a consequence of the sensitivity of silica-based columns towards alkaline pH conditions.

Preliminary studies with other supports showed that polar materials such as silica gel or aluminium oxide are unsuitable for the described method[21]. Even if adsorption rates comparable to that on RP-18 were obtained, it was impossible to achieve an efficient separation, which is probably a consequence of the different adsorption mechanisms between the polar materials and the organic dyes. This work demonstrates that efficient separations of inorganic anions can be performed with

RP-18 coated with anion-active organic dyes without any dyestuff or organic modifier in the eluent.

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