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THE PREPARATION, PROPERTIES AND SOME APPLICATIONS OF BONDED ION-EXCHANGE PACKINGS BASED ON MICROPARTICULATE SILICA GEL FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The preparation of high-efficiency ion-exchange column packings based on silica gel of 5-μm particle size was investigated. Strong cation, strong anion and weak cation exchangers having efficiencies of up to 38,000 plates per metre have been synthesized. This paper describes the preparation of these materials, the effects of the variation of operating parameters on the separation of nucleosides using the strong cation exchanger, and the use of the ion-exchange materials in a number of chromatographic applications of interest to this laboratory.

INTRODUCTION

During the past 30 years numerous biochemical and pharmaceutical mixtures have been successfully separated by ion-exchange chromatography[1]. Until recently, the majority of such separations has been performed on column packings based on cross-linked polystyrene resins. The major disadvantage of such materials for use in high-performance chromatography is that the beads change volume with changes in solvent or ionic strength owing to the uptake of the solvent by an osmosis-like effect arising from the solvation of the ionic groups within them. This can have two results: an increase in bead volume which can lead to the development of an unacceptably high resistance to flow of the mobile phase in a tightly packed steel column and the formation of large solvent-filled pores within the beads. This latter effect leads to poor mass transfer characteristics between the moving eluent and the resin which in turn deleteriously affects the observed column efficiency.

As an attempt to overcome the problems arising from the use of polystyrene beads, the pellicular ion-exchange column packings were developed[2]. These consist of a thin coating of the resin on a non-porous core. Whilst they exhibit higher efficiencies than those based on polystyrene beads, these column packings have much lower ion-exchange capacities (5–50 μequiv./g in contrast to the 1–10 mequiv. per g of resin beads). Further improvements in pellicular ion-exchange packings have resulted

from the permanent bonding of a substituted silicone polymer to porous layer particles[3]. Such packings, however, are susceptible to the same mass transfer problems (owing to slow diffusion through the silicone polymer) as have been observed with similar bonded-phase partition materials[4].

During the past two years, three publications have appeared that approach a solution to the general problem of the preparation of high-efficiency, pressure-stable, non-swelling ion-exchange packings. The first of these[5] described the preparation of monomolecular layers of permanently bonded stationary phases by the reactions between chlorinated silica gel and substituted amines. A stationary phase with an ethanesulphonic acid group attached was thus prepared; unfortunately no details of its use were described. A later publication[6] concerned the use of other bonded ion-exchange column packings based on silicon–carbon linkages using silica gel of both 30–40 μm and, for a strong cation-exchange material only, 7–11 μm particle size. Heights equivalent to a theoretical plate (HETP) of 0.5 mm were quoted for a column packed with the latter material. The most recent publication[7] that described the preparation of strong cation-exchange materials based on silica gel detailed their preparation by the gas-phase sulphochlorination of bromobutyl bonded-phase packings. The best of these materials had an ion-exchange capacity of 0.25 mequiv./g and an HETP of 0.7 mm.

The work reported in this paper was carried out in order to prepare ion-exchange column packings having efficiencies equal to those currently observed for modern microparticulate adsorption and partition column packings.

EXPERIMENTAL

Chromatographic apparatus

The work was performed using instruments assembled in this laboratory[8,9] and a Varian 4200 liquid chromatograph.

Materials

Merckosorb (LiChrosorb) SI-60 of 5 μm average particle size (BDH, Poole, Great Britain) and Spherisorb S5W (Phase Separations, Queensferry, Great Britain) silica gels were used. Organotrichlorosilanes were used as purchased (Pfaltz and Bauer, via Phase Separations). Dioxane, analytical-reagent grade, (Fisons, Loughborough, Great Britain) was dried over molecular sieve 3A before use.

Pre-treatment of silica gel

Silica gel was heated under reflux with 2 *M* hydrochloric acid for 4 h, filtered and washed thoroughly with water and acetone. The pre-treated silica was dried overnight at 200°.

Preparation of strong cation-exchange material

Pre-treated silica gel (5 g) was heated under reflux with 2-phenylethyltrichlorosilane (2 ml) in dry dioxane (100 ml) for 4 h. The silica was filtered, washed thoroughly with dioxane and acetone and dried. This product was heated under reflux with 20% (v/v) chlorosulphonic acid in chloroform (100 ml) for 3 h and was filtered, washed and dried.

Preparation of strong anion-exchange material

Pre-treated silica gel (5 g) was heated under reflux with 3-chloropropyltrichlorosilane (1.5 ml) in dioxane (50 ml) for 4 h. The silica was filtered, washed and dried and was then heated under reflux with benzyldimethylamine (25 ml) in dioxane (25 ml). The product was filtered, washed thoroughly and dried.

Preparation of weak cation-exchange material

3-Chloropropylsilica (5 g, prepared as above) was refluxed with β-alanine (1.5 g) in 50% aqueous ethanol for 4 h. The product was filtered, washed and dried.

Determination of ion-exchange capacities

Strong cation exchange. The packing (0.5 g) was suspended in 0.5 *M* sodium chloride and was titrated with 0.001 *M* sodium hydroxide.

Strong anion exchange. The packing (0.5 g) was washed with 0.001 *M* hydrochloric acid and was then washed thoroughly with water; the chloride ions were liberated from the anion exchanger by dilute nitric acid, and were determined by Volhard's method[10], which involves the addition of 0.01 *M* silver nitrate (5 ml) and back-titration with 0.01 *M* potassium thiocyanate.

Column packing procedure

The columns used in this work were all 15 cm × 6.3 mm O.D. (4.6 mm I.D.), made from stainless-steel tubing. A disc (6.3-mm diameter) cut from stainless-steel wire mesh (8-μm nominal pore size; Sankey Green Wire Weaving, Warrington, Great Britain) was placed in a low-dead-volume Swagelok 1/4 to 1/16 in. column end fitting which had been drilled such that the column–detector connection tubing penetrated it as far as the disc.

The disc was trapped in the end fitting by tightening the column in the coupling. A precolumn (50 cm × 4.6 mm) was attached to the top of the column, and the assembly was filled with a suspension of the column packing (dry volume, 6 ml; *ca.* 1.5–2 g) in acetone (10 ml). The top of the precolumn was connected to the outlet of a Haskel liquid chromatography pump containing acetone pressurised to 3,500 p.s.i. Pressure was maintained until 400 ml of acetone had passed through the column. The packed column was removed and a disc (4.6-mm diameter) of the 8-μm porosity wire mesh was pressed firmly on top of the column bed. Silanised glass wool was packed tightly into the column above the disc. Samples were injected onto the top of the wire mesh using a 10-μl syringe.

RESULTS AND DISCUSSION

Column packing procedure

The column packing procedure described in Experimental has been used very successfully in this laboratory for some time, and was arrived at after considerable experience with the use of the more usual balanced-density slurries as well as the so-called viscous slurries[11]. Because of the toxic nature of the majority of solvents used in balanced-density slurries, several solvents of decreasing density were tried for use in packing columns. It was found that 2,2,4-trimethylpentane gave columns as good as those obtained with the balanced-density slurries. Acetone was the final solvent

of choice predominantly because its low viscosity allowed a higher flow of solvent during the packing procedure using constant pressure pumps. A further consideration in favour of this solvent is that it is miscible with both hydrocarbon and aqueous solvents. This is important in that the same procedure can be conveniently employed for the packing of both adsorption and reversed-phase materials, thus removing another of the problems met with halogenated balanced-density solvents.

The use of the wire mesh disc at the top of the column is also the result of considerable experience in attempting to obtain consistently reproducible injections, using many different sample introduction systems. Initially, as other workers have recently reported[12,13], direct injection into the top of the column packing gave chromatographic peaks of high efficiency. Unfortunately this efficiency decreased as the number of injections increased, presumably because the column packing around the point of injection became increasingly disturbed. Injection into a porous PTFE disc on top of the column bed was slightly more successful. A recent report describes the efficient use of such a system[13]. After a number of configurations had been tried, it was found that highly efficient sample injections could be carried out by use of the wire mesh discs described above. The major advantage of this system is that efficient and reproducible injections may be performed by persons having little or no experience of liquid chromatography. Its sole disadvantage is that the glass wool has to be replaced occasionally: the need for this is seen by a sudden deterioration in peak shape. Attempts to solve this problem by use of glass beads above the wire mesh in place of the silanised glass wool have resulted in less efficient chromatographic peaks.

Bonding reactions: general considerations

Several procedures have been used for the permanent bonding of stationary phases to column packings for high-performance liquid chromatography. One of the simplest involves the esterification of the surface silanol groups of silica gel with an alcohol[14]. Such reactions give rise to silicon–oxygen–carbon linkages which are readily solvolysed by water or alcohols and hence would be unsuitable for the preparation of ion-exchange column packings. Other types of bonding can be used which are more stable to the conditions likely to be encountered for ion exchange. The silicon–nitrogen–carbon bonded phases reported by Brust *et al.*[5] are stable between pH 3 and 8. This does, however, place some constraints upon the operation of such columns, and these phases have not been investigated in this work.

There are two basic procedures for the bonding of stationary phases that result in a direct carbon–silicon bond. Such phases are stable to a wider range of pH, and are generally resistant to aqueous reagents between pH 1 and 9. Probably the lesser used of these methods involves the reaction of a Grignard reagent with chlorinated silica[15]. The other procedure has been used extensively for the preparation of bonded-phase column packings in this laboratory and utilises the reaction of the silanol groups of the silica gel surface with organotrichlorosilanes. Widely differing results have been reported for this reaction: the original work[16] described the preparation of permanently bonded stationary phases by this procedure, whilst Aue and Hastings[17] found that they achieved permanent bonding only by the polymerisation of the organotrichlorosilanes by the addition of water to the reaction. When silica gel is pre-treated using the procedure described in Experimental, reproducible quantities of bonded stationary phase are achieved by simply refluxing the silica and silane together in an inert

TABLE I

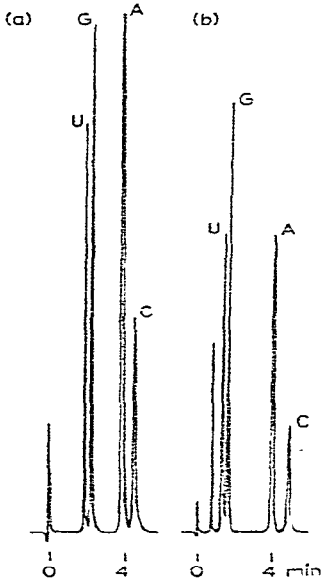
QUANTITIES OF STATIONARY PHASES BONDED TO MERCKOSORB SI-60

Trichlorosilane	*Quantity (mmoles/g)*
Allyl	0.71
Octadecyl	0.66
Phenyl	0.61
3-(Heptafluoroisopropoxy)propyl	0.72

solvent. Typical values for a number of stationary phases on Merckosorb SI-60 are shown in Table I. The ion exchangers produced by this bonding mechanism showed no change in volume with changes in solvent.

Strong cation exchange

The functional group most frequently employed as a cation exchange site is the sulphonic acid moiety. Given the original choice of bonding reaction, the most obvious procedure that could be employed in order to introduce such a group was that of the sulphonation of an aromatic nucleus that had previously been bonded to the packing material. This procedure was originally investigated using phenyltrichlorosilane to introduce a group susceptible to attack by chlorosulphonic acid. After sul-



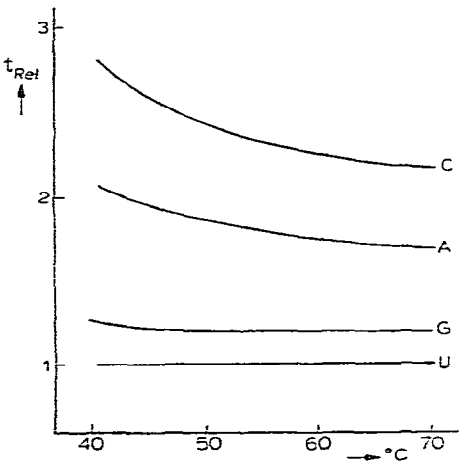


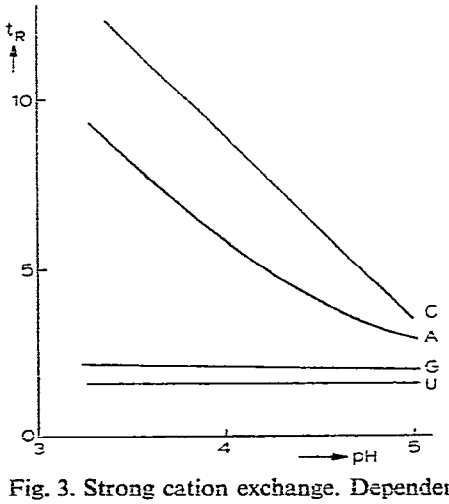
Fig. 1. Strong cation exchange. Separation of uridine (U), guanosine (G), adenosine (A) and cytidine (C). (a) Column, Merckosorb SI-60-SCX (5 μm), 15 cm × 4.6 mm; eluent, 0.05 *M* ammonium formate in 10% ethanol–water (pH 4.8); flow-rate, 1 ml/min; temperature, 50°. (b) Column, Spherisorb S5W-SCX; eluent, 0.05 *M* ammonium formate in 3% ethanol–water (pH 3.9); other conditions as in (a).

Fig. 2. Strong cation exchange. Dependence of relative retention of nucleosides with temperature. Other conditions as in Fig. 1a.

phonation, the packing material had an ion-exchange capacity of approximately 0.2 mequiv./g, corresponding to 30% reaction. A column prepared from this packing material exhibited low efficiencies, and in consequence studies were continued to find a more suitable bonded group. An analogous packing material prepared from 2-phenylethyltrichlorosilane had an ion-exchange capacity of 0.6 mequiv./g, a figure which agreed with that obtained for this material by thermal analysis.

The separation of the four commonly occurring nucleosides (adenosine, cytidine, guanosine and uridine) on columns packed with cation exchangers prepared from 2-phenylethyl-substituted Merckosorb SI 60 and Spherisorb S5W is shown in Fig. 1. Under the conditions described, the HETP for cytidine on the Merckosorb-based column was 27 μm: its capacity factor (k') was 1.4. Whilst the Spherisorb-based column gave rise to slightly more symmetrical peaks, the HETP for cytidine ($k' = 2.5$) was 37 μm. These HETP values compare favourably with those attained with microparticulate adsorption and partition columns[13,18] and represent a considerable advance on those reported for columns packed with other microparticulate cation-exchange materials[6,7].

The effects on the separation of nucleosides of the variation of temperature, pH and ethanol concentration were studied. Increases in temperature reduced the retention times; the relationships involved in this not unexpected result are illustrated in Fig. 2. An increase in pH caused a marked decrease in the retention times of adenosine and cytidine, the effect on guanosine and uridine was marginal (Fig. 3). Of more interest is the effect of ethanol concentration as illustrated in Fig. 4. When no ethanol is present, adenosine and cytidine are not separated. As the ethanol concentration is increased, the resolution between these components increases whilst their retention time decreases. A similar phenomenon is observed in the chromatography of lignocaine. With no ethanol added, the peak has a long retention time, is broad and



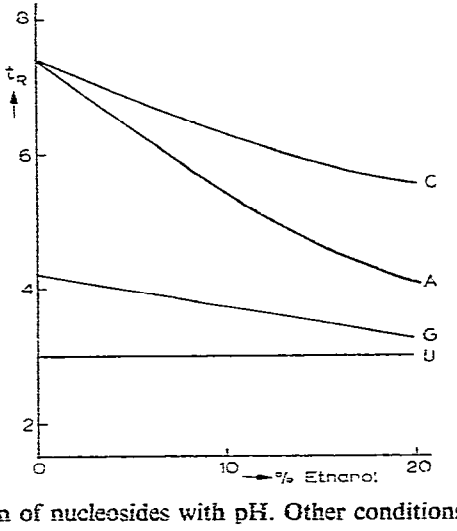


Fig. 3. Strong cation exchange. Dependence of retention of nucleosides with pH. Other conditions as in Fig. 1a.

Fig. 4. Strong cation exchange. Dependence of retention of nucleosides with ethanol concentration. Other conditions as in Fig. 1a.

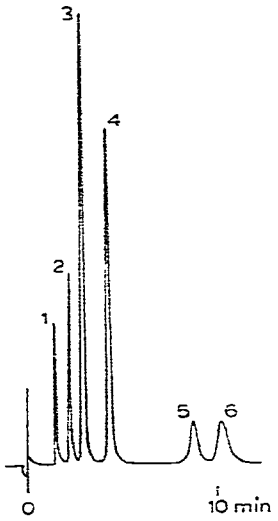


Fig. 5. Strong cation exchange. Separation of pharmaceuticals. 1 = Aspirin, 2 = paracetamol, 3 = phenacetin, 4 = caffeine, 5 = phenylephrine, 6 = salbutamol. Conditions as in Fig. 1a.

tails badly. On the addition of 10% ethanol to the solvent, however, the peak elutes rapidly and is both sharp and reasonably symmetrical; the column used was Merckosorb SI-60-SCX, the eluent 0.2 *M* potassium dihydrogen phosphate.

It was first thought that the effect of ethanol concentration could be ascribed to a problem in the wetting of the organosilane-substituted column packing by the aqueous solvent. The continuous variation in retention and resolution with increase in this parameter, however, suggest that an additional partition mechanism is also operating within the column. In practical terms, this variation in selectivity with ethanol concentration is of use in choosing optimal conditions for an analysis.

Another application of the strong cation-exchange column is the separation of a number of pharmaceuticals (Fig. 5). The first four components, *viz.*, aspirin, paracetamol, phenacetin and caffeine are of importance in the analysis of analgesic preparations whilst the other two compounds, phenylephrine and salbutamol, are of interest in cross-contamination studies.

One problem encountered in the analysis of pharmaceutical products is the determination of morphine in proprietary medicines, for example chloroform and morphine tincture. This sample was diluted five times with methanol in order to reduce its viscosity sufficiently to render it capable of being measured and injected with a microsyringe and 2-μl aliquots were analysed. The separation of morphine from this particularly complex sample was complete in 14 min, and as such constitutes a rapid method of analysis of such preparations; the column used was Merckosorb SI-60-SCX, the eluent 0.1 *M* ammonium formate in 10% ethanol–water, pH 4.0.

Strong anion exchange

The group most commonly employed in strong anion-exchange column packings is the quaternary ammonium ion. A synthetic route towards such a group bonded to silica gel was developed. This involved the reaction of 3-chloropropyltrichlorosilane with silica gel and treatment of the product with a tertiary amine. In the first instance,

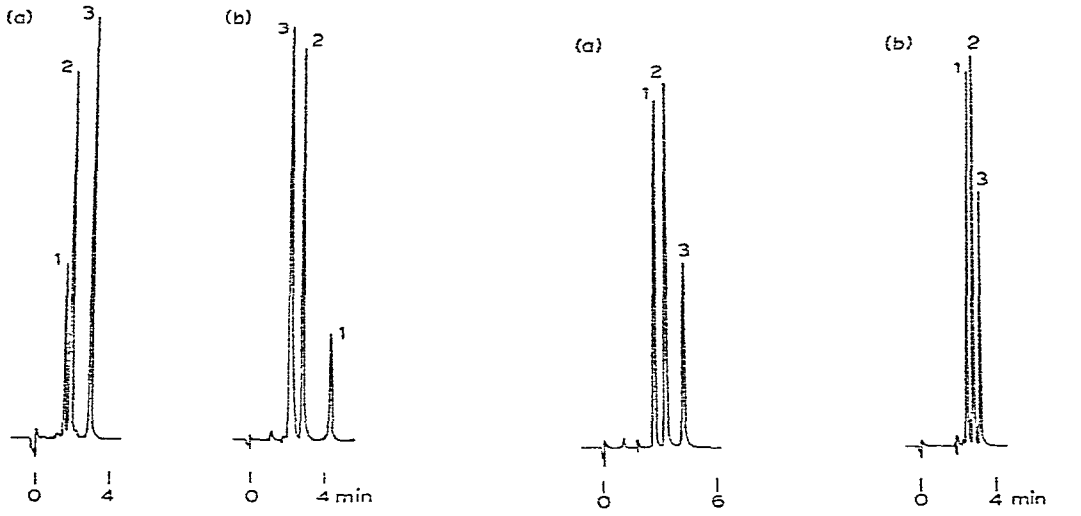


Fig. 6. Strong anion exchange. Separation of aspirin (1), caffeine (2) and salicylamide (3). Column Merckosorb SI-60-SAX, 15 cm × 4.6 mm. Eluent, (a) borate buffer (pH 9.2), flow-rate 1.1 ml/min; (b) 0.005 *M* sodium nitrate (pH 2.5), flow-rate 1 ml/min.

Fig. 7. Strong anion exchange. Separation of benzoic acid (1), toluic acid (2) and aspirin (3). (a) Conditions as in Fig. 6b. (b) pH 2.1; other conditions as in Fig. 6b.

triethylamine was used but this resulted in a product having a very low anion-exchange capacity. When benzyldimethylamine was employed for the secondary reaction, an anion-exchange material having a capacity of 0.6 mequiv./g was obtained. An alternative procedure employing chlorophenyltrichlorosilane was also tried, but this resulted in materials with ion-exchange capacities considerably lower than those obtained using chloropropyltrichlorosilane.

Separations achieved using columns packed with anion-exchange materials based on both Merckosorb SI-60 and Spherisorb are shown in Figs. 6 and 7. Fig. 6a illustrates the separation of aspirin, salicylamide and caffeine at pH 9.2. The elution order was reversed when the pH was changed to 2.5 and the mobile phase to aqueous sodium nitrate (Fig. 6b). Fig. 7a shows the separation of benzoic acid, toluic acid and aspirin under the latter conditions: reduction of the pH to 2.1 causes a marked reduction in retention times as seen in Fig. 7b.

Details of the application of this anion-exchange packing in the determination of uric acid in animal feeds will be published in due course. This analysis was performed on packings based on both Merckosorb SI-60 and Spherisorb S5W; the HETP values for uric acid in these columns were 29 μm and 36 μm, respectively. These values are remarkably close to those obtained using the strong cation-exchange columns based on the same silica gels (see above). It is also worthy of note that it was not necessary to add ethanol to the mobile phase in order to achieve successful separations on the anion-exchange columns.

Weak cation exchange

In the course of the development of a method for the analysis of coccidiostats in poultry feeds, the need for a weak cation-exchange column became apparent. The

problem involved the separation of amprolium, ethopabate, pyrimethamine and sulphaquinoxaline. Because of the wide differences in structure, it was not possible to achieve a useful separation using the strong cation-exchange column or any of the usual partition or adsorption systems.

The problem of the introduction of a carboxyl function that would act as a weak cation-exchange group into a bonded-phase column packing was solved by a modification of the procedure used for the preparation of the strong anion-exchange material. Silica gel (Merckosorb) that had been treated with 3-chloropropyltrichlorosilane was heated under reflux with a solution of β-alanine in 50% aqueous ethanol. Infrared and mass spectrometric evidence indicated that the product contained the β-alanyl function, whilst thermal analysis indicated that, assuming a 100% secondary reaction, 0.45 mequiv./g of stationary phase was present. The ion-exchange capacity was not checked by titration because of the possibility of obtaining erroneous results owing to zwitterion formation within the bonded phase.

Fig. 8 shows a chromatogram of the compounds of interest. For this column packing, as for the strong cation-exchange material, a low concentration of ethanol

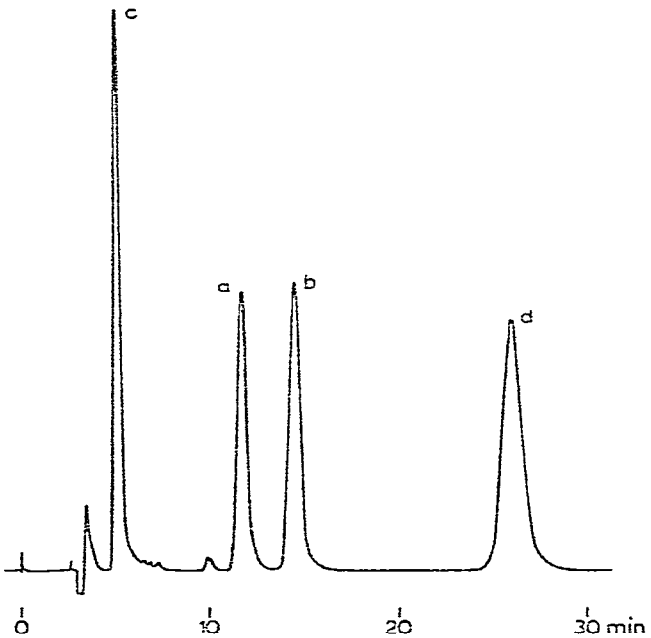


Fig. 8. Weak cation exchange. Separation of coccidiostats; a = pyrimethamine, b = ethopabate, c = amprolium, d = sulphaquinoxaline. Column, Merckosorb SI-60-WCX, 15 cm × 4.6 mm; eluent 0.3 *M* potassium dihydrogen phosphate in 10% ethanol–water (pH 3.0); flow-rate, 1 ml/min; detector wavelength, 220 nm.

in the mobile phase was essential in order to achieve acceptable peak shapes for all components eluted.

Work on other applications of these column packing materials is in progress.

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