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INVESTIGATION OF THE CHROMATOGRAPHIC PROPERTIES OF POROUS CERIA MICROSPHERES

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SUMMARY

Preliminary investigation of a batch of porous ceria microspheres indicated that the surface had minimal adsorptive properties. Observation of the retention of polycyclic aromatic hydrocarbons from a relatively polar eluent (methanol–water, 50:50) suggested that the particles were hydrophobic.

Porous ceria was found to be a useful inert support for ion-pair partition or “soap column chromatography”. Representative sulphonic acids were separated using a mobile phase consisting of methanol–water containing a small amount of hexadecyltrimethylammonium bromide.

INTRODUCTION

There are many possible applications of modern high-performance liquid chromatography (HPLC) which are restricted by the stability of commercially available alumina, silica or silica-based packing materials at high pH. It would therefore be of considerable interest to obtain an alkali-stable material with suitable chromatographic characteristics, and an investigation of the chromatographic properties of ceric oxide gels was therefore initiated.

EXPERIMENTAL

Porous ceria microspheres were supplied by the Atomic Energy Research Establishment (A.E.R.E., Harwell, Oxon., Great Britain). Initial experiments were performed on material that had been fired at 500–600°. Subsequently, unfired material was supplied and batches of this were heated at 350° or 450° for 20 h before examination. No significant differences were noted between batches. The material had an average particle diameter of 20 μm but a rather wide size distribution (5–40 μm). The physical properties of the porous ceria microspheres are summarized in Table I.

The liquid chromatograph was constructed in the laboratory and consisted of a home-made pneumatic pressure intensifier and a CE212 variable-wavelength monitor (Cecil, Cambridge, Great Britain) fitted with an 8- μl flow-cell. Both glass and stainless-steel columns (125 \times 5 mm I.D.) were used. The columns were packed by

TABLE I

PHYSICAL PROPERTIES OF POROUS CERIA MICROSPHERES FIRED AT 450° FOR 20 h

<i>Property</i>	<i>Value</i>
Particle size distribution	5–40 μm
Average particle size	20 μm
Bulk density	6.4 $\text{g}\cdot\text{cm}^{-3}$
Surface area	1 $\text{m}^2\cdot\text{g}^{-1}$
Pore volume	0.02 $\text{cm}^3\cdot\text{g}^{-1}$

the rotate, bounce-and-tap method. Samples were loaded with a microsyringe (SGE, Melbourne, Australia) on to the column via a septum injector designed in the laboratory.

During the experiments on ion-pair partition chromatography, a stainless-steel pre-column (200 \times 8 mm I.D.) packed with diatomaceous earth which was coated with stationary phase was used to maintain equilibrium conditions in the analytical column. Both the pre-column and the analytical column were heated to $35 \pm 0.5^\circ$ with circulated water-jackets. In all other experiments the columns were operated at ambient temperature. The picrate buffer used as the stationary phase was prepared by addition of sodium hydroxide to a picric acid solution to give the required pH and then adjustment to the desired concentration with distilled water. The mobile and stationary phases were equilibrated with each other prior to use. The quaternary ammonium sample cations were injected as picrate ion pairs.

The sulphonic acids used in the "soap chromatographic" study were a gift from ICI Dyestuffs Division (Macclesfield, Great Britain). They are listed under their common names and formulae in Table II. Samples of these acids were dissolved in methanol before injection on to the ceria columns.

Hexadecyltrimethylammonium bromide (cetrimide) was obtained from ICI (Pharmaceuticals) Ltd. (Macclesfield, Great Britain).

RESULTS AND DISCUSSION

Preliminary experiments with the ceria microspheres using a mobile phase consisting of 1% methanol in *n*-hexane and various solutes indicated, as shown in Table III (a), that the surface had minimal adsorptive properties.

Subsequently, several polycyclic aromatic hydrocarbons were found to be retained on the ceria surface in equilibrium with a polar mobile phase [Table III (b)]. Similar "reversed-phase" properties were previously noted in experiments on natural diamond¹. However, a very polar mobile phase (water-methanol, 80:20) was required in order to obtain any reasonable retention of the hydrocarbons and the resultant peaks were broad and badly "tailed" for components with a capacity factor greater than 1.

The data in Table I clearly indicate that this product has a low surface area and pore volume, which probably contribute to the low retention capacity observed in these experiments.

The minimal adsorptive properties of the ceria suggested that it might prove to be a suitable support material for ion-pair partition chromatography, and further experiments were carried out in order to confirm this suggestion.

TABLE II
SULPHONIC ACIDS AND DYESTUFF INTERMEDIATES EXAMINED BY "SOAP CHROMATOGRAPHY"

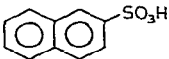
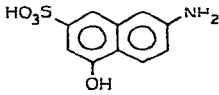
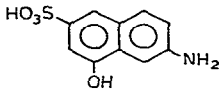
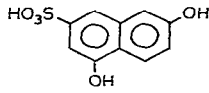
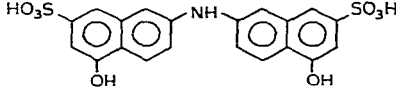
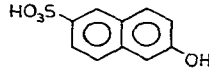
No.	Name	Formula
1	2-Naphthalenesulphonic acid	
2	J-acid	
3	γ -acid	
4	Dioxy-J-acid	
5	Di-J-acid	
6	Schäffer's acid	

TABLE III
CAPACITY FACTORS (k') ON CERIA MICROSPHERES

Chromatography	Eluent	Solute	k'
(a) Adsorption	1% Methanol- <i>n</i> -hexane	Benzaldehyde	Unretained
		Methyl benzoate	Unretained
		Nitrobenzene	Unretained
		Benzyl alcohol	Unretained
		α -Phenylethylamine	Unretained
		Ephedrine	Unretained
(b) Reversed-phase	Methanol-water (50:50)	Naphthalene	Unretained
		Phenanthrene	Unretained
		Anthracene	Unretained
		11,12-Benzfluoranthrene	0.44
		1,2,5,6-Dibenzofluorene	0.66
	Water-methanol (80:20)	Perylene	0.61
		3-Phenylphenanthrene	0.44
		Naphthalene	0.2
		Phenanthrene	2.0
		Anthracene	4.6

Ion-pair partition chromatography

Ion-pair partition chromatography can be applied to virtually all types of ionizable substances, both cationic and anionic. The technique was pioneered by

Eksborg and co-workers^{2,3} and many successful applications have been achieved with both normal-phase⁴⁻⁹ and reversed-phase¹⁰⁻¹² systems. The basic principles of the technique have been described by Eksborg and Schill.²

In the present investigation, an aqueous solution (picrate buffer, pH 6.7) was the stationary phase and an organic mobile phase (2% pentanol-chloroform) was used. Quaternary ammonium ions were separated as their picrate ion pairs.

The stationary phase of 0.06 *M* picrate buffer was coated on the ceria packing by two different procedures. In the first method, designated as de-loading, the column was initially washed with the mobile phase (50 cm³, 2% pentanol-chloroform). Picrate buffer solution (6 cm³) was then passed through the column at 0.4 cm³·min⁻¹ followed by another wash of mobile phase saturated with stationary phase (50 cm³). The column was then connected to the pre-column and equilibrated with the mobile phase at 2 cm³·min⁻¹ at 35°. When the system had reached equilibrium, the quaternary ammonium compounds were separated as ion pairs. The capacity factors (*k'*) obtained are given in Table IV.

TABLE IV

CAPACITY FACTORS FOR ALKYLAMMONIUM COMPOUNDS CHROMATOGRAPHED AS PICRATE ION PAIRS

Stationary phase, 0.06 *M* picrate buffer, pH 6.7; mobile phase, 2% pentanol-chloroform.

<i>De-loading</i>		<i>Loading</i>	
<i>Solute</i>	<i>k'</i>	<i>Solute</i>	<i>k'</i>
Tetrabutylammonium (TBA)	0.27	TBA	0.5
Tetrapropylammonium (TPrA)	0.32	TPrA	0.5
Trimethylpropylammonium (TMPPrA)	1.05	TEMA	1.0
Triethylpropylammonium (TEPrA)	0.64	TMEA	2.38
Triethylmethylammonium (TEMA)	1.13	TEA	4.0
Trimethylethylammonium (TMEA)	2.2		
Tetraethylammonium (TEA)	3.2		
Tetramethylammonium (TMA)	3.5		

In the second procedure (loading), the column was connected to the pre-column and washed with mobile phase saturated with stationary phase at 1 cm³·min⁻¹ at 35° until equilibrium was reached. Equilibration of the system was followed by measurement of the *k'* values of the alkylammonium ions at intervals until constant values were obtained. As shown in Fig. 1, the *k'* values of tetrabutylammonium (TBA) and tetrapropylammonium (TPrA) initially increased with the volume of eluent passed through the column. However, maximal values were reached after about 400 cm³ had been passed through the column. The capacity factors then gradually decreased and reached final equilibrium values close to those obtained by the first coating procedure (Table IV). The *k'* values for the other quaternary ammonium ions followed a similar pattern.

The capacity factor, *k'*, by ion-pair chromatography is given by²

$$k' = V_s (V_m E q_x^* C_x)^{-1}$$

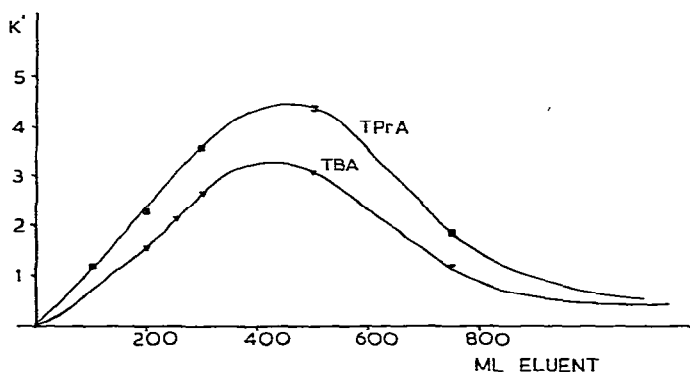


Fig. 1. Variation of k' of alkylammonium ions with volume of mobile phase passed through the column during the loading procedure.

where V_m is the interstitial volume, V_s the volume of the stationary phase, Eq_x^* the extraction constant and C_x the concentration of the pairing ion in the aqueous phase.

This expression shows that the capacity factor of the sample will be influenced by the concentration of the pairing ion in the aqueous phase. The unusual equilibration pattern observed in these experiments can be rationalized by an initial extraction of picrate ions from the mobile phase on to the ceria surface, resulting in a concentrated picrate layer as the stationary phase. Once the surface has become saturated, a re-equilibration¹³ takes place between the concentrated picrate stationary phase and the aqueous picrate component of the mobile phase, resulting in a decrease in the k' values of the solutes until equilibrium is reached.

Examples of the separations obtained with this system are illustrated in Fig. 2. The efficiencies were not very high (maximal number of plates for TBA = 800, equivalent to $H = 0.15$ mm), but as the average particle size was $20 \mu\text{m}$ this corresponds to a reduced plate height ($h = H/d_p$) of 7.5. However, the particle size distribution was rather large ($5\text{--}40 \mu\text{m}$) and the efficiencies might well be improved by closer fractionation of the material.

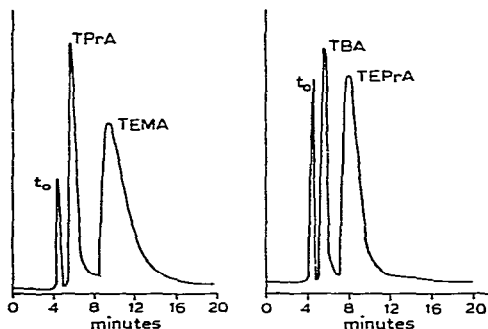


Fig. 2. Separation of quaternary alkylammonium compounds as picrate ion pairs. Stationary phase, $0.06 M$ picrate buffer, pH 6.7. Mobile phase, 2% pentanol-chloroform. Column, 125×5 mm I.D. glass, packed with ceria microspheres (average particle size *ca.* $20 \mu\text{m}$).

"Soap chromatography"

The concept of "soap chromatography" was introduced by Knox and Laird in 1976¹⁴ as an extension of ion-pair chromatography, whereby a small amount of a suitable surface-active compound is added to the mobile phase to permit the separation of components that are more or less unretained in the absence of the "soap". In order to examine the potential of ceria as a support for "soap chromatography", the analysis of sulphonic acid dyestuff intermediates was investigated. Water-methanol (60:40, v/v), containing less than 1% of cetrimide, was found to be a suitable eluent. The dependence of $\log k'$ cetrimide concentration for three of the acids examined is shown in Fig. 3. These curves more closely resemble those obtained by Knox and Laird¹⁴ on SAS-silica (a hydrophobic bonded-phase packing material) than those obtained on Partisil 10. However, the curves drop much more sharply on ceria, and at cetrimide concentrations greater than 1% the sulphonic acids were virtually unretained.

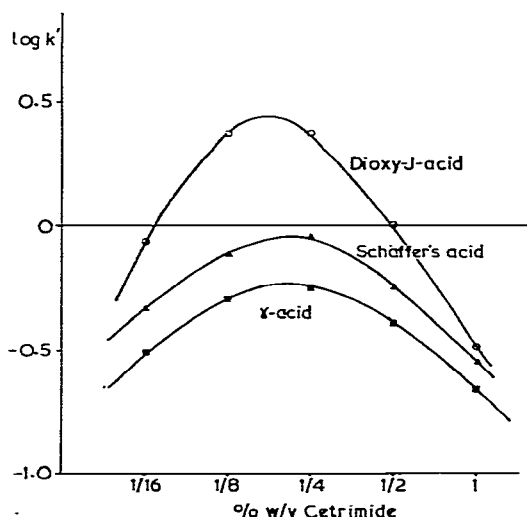


Fig. 3. Dependence of $\log k'$ on cetrimide concentration (grams per 100 ml) for three sulphonic acids. Eluent, water-methanol (60:40, v/v); column packing, ceria microspheres (ca. 20 μ m).

Representative separations are illustrated in Figs. 4 and 5. The separation of sulphonic acids shown in Fig. 4 was obtained under the conditions described above while working near the maximum on the curve (0.25% cetrimide). For the separation illustrated in Fig. 5, the mobile phase was buffered at pH 6.8 using a sodium phosphate buffer and the methanol content of the mobile phase was reduced to 25%. Under these conditions, partial resolution of the isomeric aminosulphonic acids J-acid (2) and γ -acid (3) was obtained.

Examination of the effects of pH on the retention of several phenols, carboxylic acids and naphtholsulphonic acids in the "soap chromatographic" mode was also undertaken. As shown in Fig. 6, the capacity factors of the sulphonic acids and carboxylic acids decrease as the pH increases, the effect being more pronounced for the sulphonic acid. The dramatic rise in k' between pH 6 and 7 is obviously due to the change from sodium citrate buffer to tris-sulphuric acid buffer. As the anion concen-

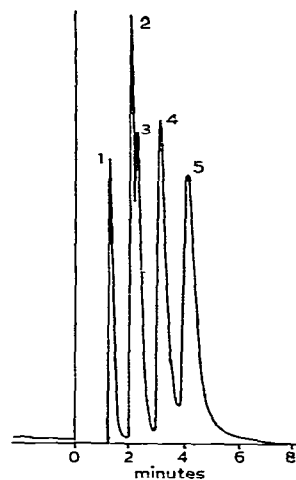
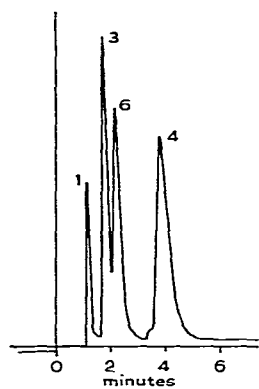


Fig. 4. Separation of sulphonic acids by "soap chromatography". Column, 125 × 5 mm I.D., stainless steel; packing, ceria microspheres (ca. 20 μm); eluent, water-methanol (60:40) containing 0.25% (w/v) of cetrimide. Peaks: 1 = unretained peak; 3,4 and 6 as in Table II.

Fig. 5. Separation of sulphonic acids by "soap chromatography". Column, 125 × 5 mm I.D., stainless steel; packing, ceria microspheres (ca. 20 μm); eluent, sodium phosphate buffer (pH 6.8)-methanol (3:1) containing 0.16% (w/v) of cetrimide. Peaks: 1 = unretained peak; 2-5 as in Table II.

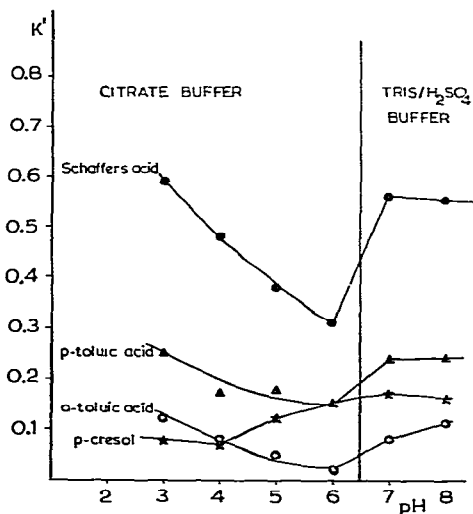


Fig. 6. Variation of capacity factor with pH of the eluent for representative sulphonic acids, carboxylic acids and phenols.

tration in the buffer solution was kept constant throughout, the reduction in k' could not be explained on the basis of competition from the citrate ions for the cetrimide. It seems more likely that this is an ionic strength effect.

For *p*-cresol, k' remained fairly steady until pH 4 and then gradually increased as the pH was increased further. Such behaviour might be expected as the phenol becomes more ionized, permitting the formation of ion pairs with cetrimide.

CONCLUSIONS

Ceria appears to be a useful and essentially inert support for either normal-phase or reversed-phase ion-pair partition chromatography. The material used for this study has a fairly low sample capacity but it should be possible to produce particles with a larger surface area. Ceric oxide gels should be chemically and physically stable at high pH and accordingly should prove to be useful for chromatographic separations carried out with alkaline eluents.

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