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HYDROPHOBIC CHROMATOGRAPHY WITH DYNAMICALLY COATED STATIONARY PHASES

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SUMMARY

A novel form of hydrophobic ("reversed-phase") high-performance liquid chromatography is described. Column packings are conventional porous oxide (silica and zirconia) gels with relatively hydrophilic surfaces whose polarity is drastically altered by reaction with quaternary ammonium cationic surfactant dissolved in the eluting solvent. Retention and separation of mixtures of aromatic hydrocarbons, an aromatic ketone and aryl sulphonic acids is shown and is tabulated as a function of mixed solvent (methanol–water) composition and also as a function of surfactant concentration.

Column resolving power in this mode of separation is unchanged from that obtained with the same column packings used in the adsorbent mode with non-polar solvents.

INTRODUCTION

The usage of "reversed-phase" liquid chromatography has developed rapidly since the introduction of well-graded microparticle ($d_p \approx 5\,\mu$m) materials based on the virtually complete coverage of the reactive silanol groups of silica gels by covalently attached hydrocarbon chains[1,2]. Many authors have pointed out the potential of a high-performance liquid chromatographic (HPLC) mode which may encompass virtually all types of analyte from the hydrophobic polycyclic aromatic hydrocarbons to such polar molecules as amino acids and sulphonic acids. This versatility is achieved by the control over selectivity inherent in partially aqueous solvent systems with which a wide variation in hydrophobicity (content of organic modifiers, *e.g.*, methanol), pH and ionic strength may be easily accommodated.

Separations of the most polar analytes are usually achieved by some variation of the ion-pairing techniques originated by Horváth and Lipsky[3] and Eksborg and Schill[4], in which a relatively low concentration of a charged solute (*e.g.*, alkyl sulphonic acid or alkyl quaternary ammonium salt) in the solvent allows formation of hydrophobic ion pairs with the analyte. Hydrophobic interaction of these ion pairs with the covalently bonded alkyl chains on the packing surface then provides the mechanism for separation of the polar analytes.

In some experiments which preceded this current study Gilbert and Wall[5] found that porous ceria column packings gave retention data similar to those reported by Knox and Laird[6] in their studies of "soap" chromatography on short-chain alkylated silica. The conclusion of this earlier study[5] was that "soap" chromatography provided a useful probe of the relative hydrophobicity of the surface of the column packing. Subsequently, Horváth *et al.*[7] demonstrated that the diagnostic sample retention–surfactant concentration relationship is not necessarily a function solely of the column-packing surface hydrophobicity but also (and primarily) is derived from the hydrophobic character of the added surfactant.

Accordingly, at the beginning of this present work in which a porous zirconia gel was tested as an HPLC column packing, the interaction of conventional porous silica gel packing with quaternary ammonium salt surfactants was re-examined. It soon became obvious that the dissolved surfactant reacts with native oxide surfaces to produce a new packing–solvent interface which behaves very much as do "bonded phase" column packings. The properties of this "solvent-generated" hydrophobic surface were used for HPLC separations of a number of non-polar and polar analytes. Apparently, many analyses which have been done on surface-modified column packings could have been accomplished with equal efficiency using conventional oxide gel packing materials without prior chemical modification.

EXPERIMENTAL

The chromatographic measurements were carried out on a variety of equipment, including Altex Model 110 and Waters M6000A pumps, Cecil Model 212 and Applied Chromatography Systems Model 710 ultraviolet flow photometric detectors and SGE microsyringes in association with laboratory-constructed septum injector–column systems.

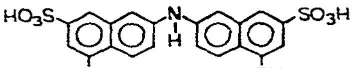
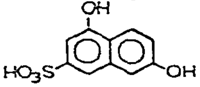
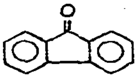
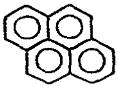
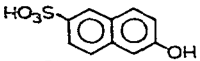
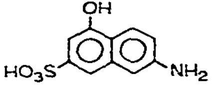
Two silicaceous column packing materials were used: Hypersil (Shandon, London, Great Britain), a spherical silica gel, $d_p = 5\,\mu$m, surface area ≈ 200 m^2 g^{-1}; and ODS (octadecylsilyl)-Hypersil (Shandon), in which the adsorbent activity due to residual silanol groups has been reduced to a minimum. The zirconium oxide gel was very kindly supplied by Magnesium Elektron (Manchester, Great Britain) and was their highest purity, spherical microparticulate *S* grade. This zirconia was modified by hydrothermal treatment to increase effective pore size and had a surface area of 9 m^2 g^{-1}; a fraction ($d_p = 10 \pm 3\,\mu$m) was isolated by hydraulic elutriation and was used in subsequent experiments.

Columns were made from Apollo grade (Accles and Pollock, Oldbury Warley, Great Britain) polished (4.6 mm diameter) bore AISI 316 grade stainless-steel tubing in lengths of 100 or 125 mm. They were packed by the "upward-flow" slurry-packing technique described by Bristow[8] at *ca.* 300 bar liquid pressure; both silica and zirconia were suspended and packed in methanol.

The sulphonic acid solutes were kindly gifted by I.C.I. Dyestuffs Division (Macclesfield, Great Britain) and are listed under their common names and structural formulae in Table I. Hydrocarbon and ketonic solutes are also listed in Table I and were obtained from the usual commercial sources. Purity of the methanol solvent used in these experiments proved to be critical for reproducible measurements; attempts to use reagent grade methanol from several suppliers gave

TABLE I

ANALYTES

Name	*Formula*	*Elution order (2.7 × 10^{-2} M cetrimide)*
A Anthracene		3
B Di-J-acid		
C Dioxy-J-acid		
D Fluorenone		1
E Naphthalene		2
F Pyrene		
G Schäffer's acid		
H J-acid		

variations in solute retention over a four-fold range. Consistent solvent action was obtained from AR grade methanol after distillation from magnesium methoxide solution or by use of HPLC grade methanol obtained from Rathburn Chemicals (Walkerburn, Great Britain).

Hexadecyltrimethylammonium bromide, puriss ("cetrimide"), was purchased from Fluka (Buchs, Switzerland).

RESULTS AND DISCUSSION

Separations on silica gel

An equal volume (1:1) mixture of methanol and water was used as eluent in all measurements quoted in Fig. 1, with the addition of sufficient cetrimide to cover the concentration range from 1.7 × 10^{-3} to 5.5 × 10^{-2} *M*. All the data used to develop the relationship between log *k'* and cetrimide concentration were collected on columns which had previously been exposed only to solvent containing the measured or lower concentrations of the same added surfactant. This precaution was thought to be necessary since it was observed that attempts to convert a silica–cetrimide column to one in equilibrium with a different quaternary ammonium salt (benzyltrimethylammonium chloride and propyltrimethylammonium iodide were used in preliminary experiments) gave retention data different from those of silica columns which had been in contact with these other salts only. Moreover, several attempts to regenerate a "clean" silica surface by acid washing (dilute acetic, formic and nitric acids were examined) to remove "bound" surfactant did not generate a surface capable of interacting to the original extent with added surfactant. Although these "acid-washed" columns did not retain their surfactant binding properties, the washing process did apparently give a silica surface similar to the original "as packed" condition, since measurements of retention of some standard solutes in the adsorption LC mode with 1% methanol in hexane as solvent were essentially identical before and after surfactant–acid treatment.

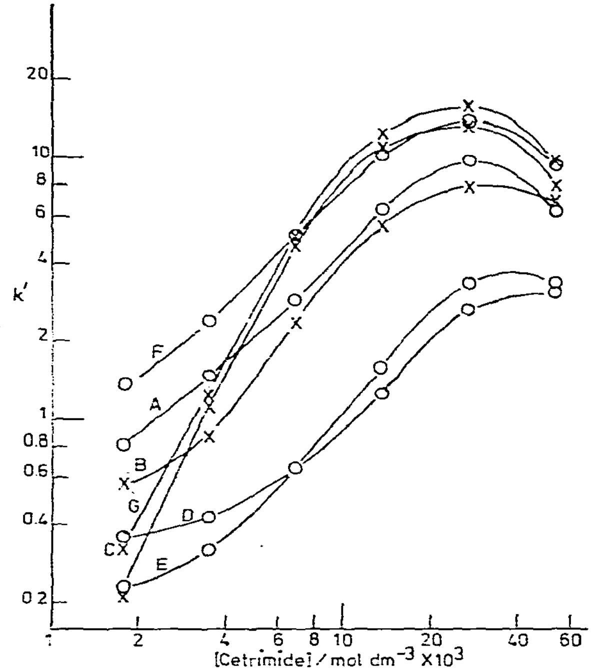


Fig. 1. Relationship of sample retention (k') to concentration of added surfactant. Eluent: methanol–water (50:50, v/v). Analytes coded as in Table I. Column packing: Hypersil.

In spite of this clear evidence of a surface which could not be readily regenerated on the column packing, it was found that retention data from several columns which were equilibrated in the same way with the same surfactant were consistent. Furthermore, the retentive characteristics of fully equilibrated cetrimide–silica columns were constant over periods of several weeks.

The relationship of log k' (of a range of analytes) with cetrimide concentration is shown in Fig. 1; there was no retention of these solutes on silica which had not been exposed to cetrimide with the same solvent in the absence of added surfactant. Although the shapes of the curves describing the variation of k' with the cetrimide concentration are all similar, there are several changes in the order of elution of solutes evident as crossover points of the various curves. These changes in selectivity probably reflect the differences in chemical reactivity of such a diverse collection of analytes (mono- and disulphonic acids, hydrocarbons and a ketone), and are an advantage to the practising chromatographer, since they imply that some degree of control over relative as well as absolute retention may be obtained by varying the concentration of added surfactant. This control may be extended by variation of the solvent composition as well, although changes in selectivity arising from change of water content are small compared to cetrimide-induced differences.

Our results do not precisely parallel those of Knox and Laird[6], which were obtained on silica and SAS-silica packings, in that virtually all our measured k' *vs.* cetrimide concentration curves pass through a maximum in k' at *ca.* 2.7×10^{-2} *M* cetrimide. However, our results were obtained with silica of lower surface area ($200 \text{ m}^2 \text{ g}^{-1}$ as opposed to $500 \text{ m}^2 \text{ g}^{-1}$) and with a different solvent system. According to Horváth *et al.*[7] the shape of these curves is a function of a balance of interaction between the packing surface and the solvent. The 1:1 (v/v) methanol–water mixture used in these experiments is more polar than the 3:1 (v/v) propanol–water mixture used by Knox and Laird[6]. This difference in solvent polarity is reflected in the retention measurements: $k' = 0.15$ for Schäffer's acid in the propanol–water solvent and $k' = 13$ for the same solute in the methanol–water solvent when both eluents contain the same (2.7×10^{-2} *M*) amount of cetrimide.

The amount of cetrimide adsorbed on the silica surface was measured by the colorimetric method of Knox and Laird[6], and the polarity difference in the solvents employed was again reflected in the coverage data. At 1.4×10^{-2} *M* cetrimide it was found that 64 mg of surfactant were sorbed on 1 g of silica, which infers an area coverage of 0.5 molecules per nm² of silica surface, roughly equivalent to one sixth of the maximum possible coverage if sorption proceeds by $-\overset{+}{N}(CH_3)_3$ reaction with the surface. The highest reported[9] silica surface coverage by covalently attached $-Si(CH_3)_3$ groups is *ca.* 2.3 groups per nm², so the solvent-generated hydrophobic surface is clearly less well covered than the best commercial "bonded-phase" packings.

Table II indicates that the effective differences between a solvent-generated hydrophobic packing and an ODS–TMS bonded-phase packing are less than might have been estimated from the surface coverage data. Analyte retention was measured with the same solvent system on both columns. Selectivity calculations ($\alpha_{1-2} = k'2/k'1$) reinforce the apparent similarity in the chromatographic behaviour of these two different column packings.

Lack of suitable samples of alkyltrimethylammonium halides prevented a

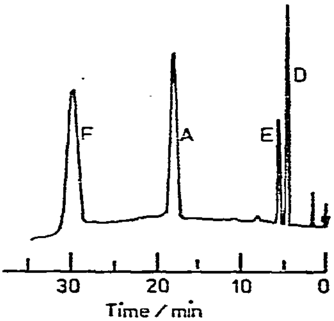
TABLE II

COMPARATIVE RETENTION DATA (1.4×10^{-2} *M* CETRIMIDE)

Datum	*Hypersil*	*ODS-Hypersil*	*Zirconia*
k' Fluorenone (1)	2.3	3.7	0.38
k' Naphthalene (2)	2.7	5.0	0.63
k' Anthracene (3)	10.7	17.3	2.5
k' Pyrene (4)	18.5	28.3	4.1
$\alpha_{1-2} = k'(2)/k'(1)$	1.14	1.37	1.66
$\alpha_{2-3} = k'(3)/k'(2)$	4.00	3.47	4.00
$\alpha_{3-4} = k'(4)/k'(3)$	1.73	1.63	1.65

systematic investigation of the effect of alkyl chain length on solute retention, but preliminary investigations with propyltrimethylammonium ($CH_3CH_2CH_2$–$\overset{+}{N}[CH_3]_3$) and benzyltrimethylammonium ($C_6H_5CH_2$–$\overset{+}{N}[CH_3]_3$) halides showed that k' does increase with chain carbon number. Hemetsberger *et al.*[10] showed that k' was a linear function of percentage carbon content on a series of alkylmethylsilyl "bonded-phase" packings, and if the retention mechanism is as closely related to that of the alkyl bonded phases as these results suggest, a similar dependence on k' upon alkyl chain length of the added surfactant would be predicted for the solvent-generated system.

Fig. 2 is a representative chromatogram of simple mixtures using methanol–water–cetrimide solvent systems with spherical silica column packing. Fig. 3 is a chromatogram of the same mixture with the same eluent used in Fig. 2, but on the octadecyl "bonded phase" ODS-Hypersil. As suggested by the data of Table II, the two separations are very similar.



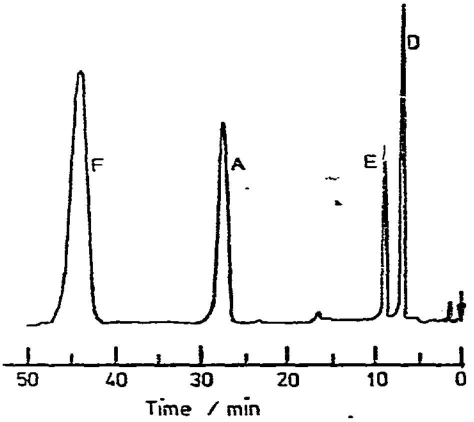


Fig. 2. Separation of four non-polar aromatic compounds (*cf.* Table I for code) on a column (125 × 5 mm) of Hypersil. Flow-rate: 1 cm^3 min^{-1}. Eluent: methanol–water (50:50, v/v) solution of cetrimide (1.4×10^{-1} mol dm^{-3}).

Fig. 3. Separation of four non-polar aromatic compounds on a column 125 × 5 mm) of ODS-Hypersil. Conditions as in Fig. 2, except for the eluent: methanol–water (60:40, v/v).

Separations on porous zirconia

The data shown in Fig. 4 were obtained on porous zirconia columns by development with the same solvent system used in the experiments which gave the information recorded in Fig. 1 and Table II. A lesser retentive power would be predicted for this zirconia (9 m^2 g^{-1}, calculated pore = 36 nm) than for the silica (200 m^2 g^{-1}, calculated pore = 12 nm) described above, but the disparity in "available" surface is not as great as it appears, since the packing surface area in a typical 125-mm long column would be about 240 m^2 for the silica (bulk density *ca.* 0.5 g cm^{-3}) and 43 m^2 for the zirconia (bulk density *ca.* 4 g cm^{-3}). In fact, k' (max.) for pyrene was *ca.* 18.5 on silica (at 1.4×10^{-2} M cetrimide) and 4.1 on zirconia (at 1.4×10^{2} M cetrimide), so retention is slightly higher on the latter packing than a simple surface area ratio would suggest.

The structure of the pores in this zirconia is clearly not ideal for HPLC usage, since even a well-fractionated sample (according to particle diameter) did not

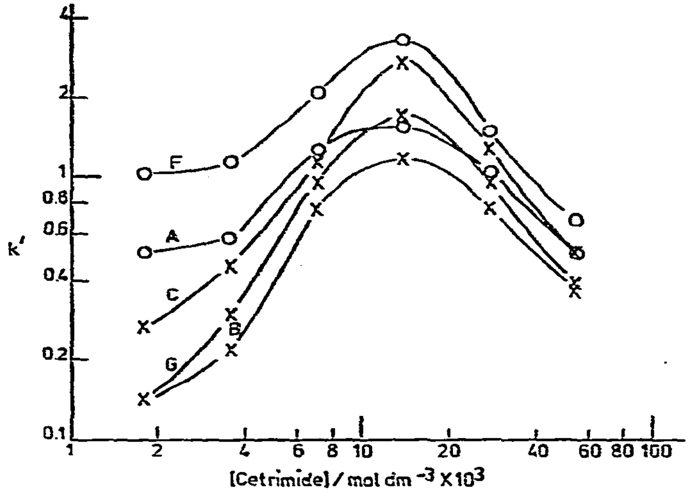
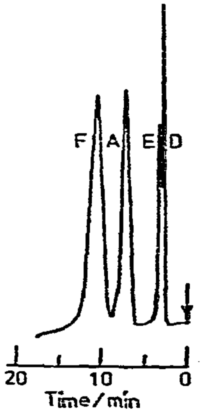


Fig. 4. Relationship of sample retention (k') to concentration of cetrimide. Eluent as in Fig. 1; analyte coding as in Table I. Column packing: hydrothermally modified zirconia, $d_p \approx 10\,\mu m$.

exhibit plate heights smaller than 10–12 particle diameters at optimum flow-rates as is evident from Fig. 5 and 6. However, column efficiency was adequate to the purpose and it was felt that the surface characteristics of this zirconia gel would be comparable to those of a gel with the smaller pores (pore ≈ 6–12 nm) characteristic of high-efficiency LC packings.



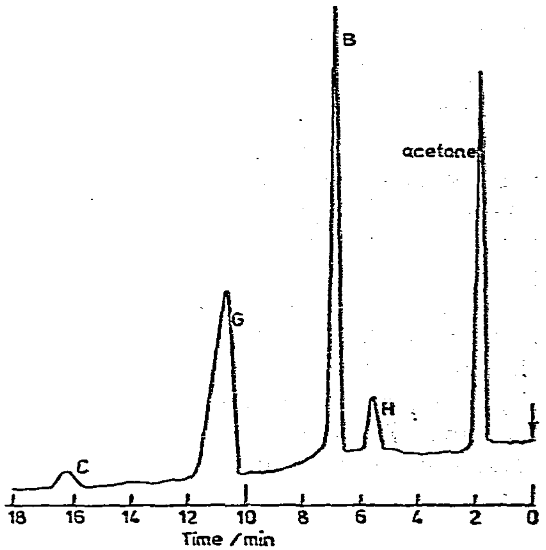


Fig. 5. Separation of four non-polar aromatic compounds as in Fig. 2 and 3. Operating conditions as in Fig. 2 except flow-rate = 0.6 cm^3 min^{-1}. Column packing: hydrothermally modified zirconia, $d_p \approx 10\,\mu m$.

Fig. 6. Separation of four sulphonic acids as cetrimide ion pairs (*cf.* Table I for code) on the zirconia column of Fig. 5. Operating conditions as in Fig. 2.

Adsorption LC with this zirconia suggested that its surface polarity (density of strong adsorption sites) is between that of silica and that of the ceria of the earlier study[5]. The position of the retention maxima with respect to cetrimide concentration on Fig. 4 supports this idea, since this maximum lies between 3×10^{-3} and 7×10^{-3} mol dm^{-3} for ceria (ref. 5, Fig. 3) and for SAS-silica (ref. 7, Fig. 1): the maximum for zirconia packings lies slightly higher than these at 1.4×10^{-2} *M*: highest of all are the *k'* maxima of Fig. 1 at 2.7×10^{-2} *M* cetrimide on silica. Unfortunately, the data from these three studies are not strictly comparable, since different solvent systems were used; however, a short series of experiments confirmed that maximum retention on zirconia with the same solvent used in the earlier work on ceria separations was also at 1.4×10^{-2} *M* cetrimide concentration.

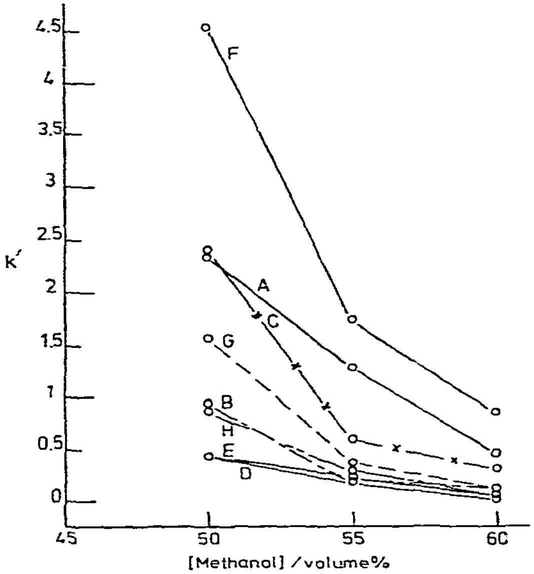


Fig. 7. Relationship of sample retention (*k'*) to volume % methanol in eluent at constant cetrimide concentration (7×10^{-3} mol dm^{-3}). Column packing: hydrothermally modified zirconia. Analyte coding as in Table I.

If the mechanism of separation on the oxide gel–cetrimide packings is similar to that of alkyl-bonded silicas as suggested earlier, then the retention of analyte should vary with solvent composition[10,11]. That this is so on zirconia is evident from Fig. 7, in which variation of *k'* with percentage (by volume) of methanol in the methanol–water eluent (containing 0.7×10^{-2} *M* cetrimide) is plotted for a range of solutes. Note that selectivity also varies with solvent composition, although this latter effect is rather less pronounced than solvent-induced changes in retention.

CONCLUSIONS

Data from this study and that from previous investigations[5,6] on the interaction between cationic surfactant dissolved in the eluent phase and LC column packings prepared from porous gels of cerium(IV) oxide, silicon(IV) oxide and zirconium-

(IV) oxide may be interpreted as arising from the *in situ* generation of a strongly (probably electrostically) bonded alkyl hydrocarbon surface phase. Horvath *et al.* (ref. 7, Fig. 12) have shown that the relationship between concentration of surfactant and k' for a given analyte is a function of both the alkyl chain length of the surfactant (*i.e.*, hydrophobicity) and the polarity (*i.e.*, water content) of the eluent. So, as hydrophobic character of the stationary phase is increased with a constant composition solvent, a corresponding increase in the surfactant concentration at maximum solute retention would be predicted. Such an increase is observed with the silica, zirconia and ceria stationary phases used in this and the preceding study[5]. When this observation is combined with the fact that the relative retention of hydrocarbon solutes in the cetrimide–silica system is very similar to that observed on octadecylsilica, it seems clear that an eluent-generated hydrophobic surface is the main retentive agent in the surfactant–oxide gel systems.

The results reported here suggest that many "reversed-phase" LC separations at present carried out on alkyl-bonded silica gel packings could be done with equal facility on unmodified porous oxide gel packing materials by addition of a suitable surfactant to the typical aqueous–organic eluents of the technique. Both absolute and relative retention of polar and non-polar analytes are shown to be controlled by solvent composition both with respect to the bulk organic components (*i.e.*, methanol, propanol, etc.) and the trace surfactant. Additional control of the parameters affecting a given separation may be obtained by use of oxide gels with differing surface characteristics, as in classical adsorption chromatographic practice. This extension to a wider range of analytes (and a novel zirconia column packing) of the "soap" chromatographic systems described in a study by Knox and Laird[6] of separations of polar sulphonic acids as ion pairs on an alkyl-bonded silica and on silica column packings would appear to be a useful additional to the LC separation modes in current use.

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