Journal of Chromatography, 209 (1981) 191–201
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,638

HYDROPHOBIC CHROMATOGRAPHY WITH DYNAMICALLY COATED STATIONARY PHASES

IV. ANIONIC SURFACTANT EFFECTS ON ALUMINA

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(First received December 3rd, 1980; revised manuscript received January 13th, 1981)

SUMMARY

The effects of addition to aqueous methanolic eluents of two alkyl sulphate surfactants on the chromatographic properties of aluminium(III) oxide gels have been studied. It was found that the basic active sites on the alumina surface interacted with anionic surfactants in a fashion analogous to that previously observed with acidic active centres on silica and cationic surfactants.

INTRODUCTION

Knox and Laird[1] reported that aromatic sulphonic acids could be retained and separated on columns packed with porous silica gel particles when a cationic surfactant was added (in low concentration) to the aqueous–organic eluting solvent system. These findings were amplified and extended to non-polar eluites separated on columns packed with cerium(IV) oxide, silicon(IV) oxide, and zirconium(IV) oxide in the studies of Gilbert and Wall[2] and Ghaemi and Wall[3].

Since dynamic coating of cationic surfactants clearly gave analytically useful hydrophobic column packing materials from hydrophilic porous acidic oxide gels, clarification of the nature of the interaction which produced the hydrophobic surface became necessary. Extension of the surfactant range to non-ionic and anionic types appeared to be an obvious experimental route. A report on the first of these two types by Ghaemi and Wall[4] apparently confirmed the initial hypothesis[3] that strong interactions between the polar oxide surface and the polar "end" of the surfactant provided the means of binding a partial monolayer of hydrophobic alkyl chains to that surface. However, as mentioned in a further study of "dynamic soap chromatography" of peptides by Wall[5], *anionic* detergents do not so react with acidic oxide surfaces in the polar aqueous eluents used.

0021-9673/81/0000–0000/$02.50

Accordingly, this present study describes the effects of addition of anionic (and non-ionic) surfactants to aqueous methanolic eluents of columns packed with aluminium(III) oxide gel particles. These commercially available γ-alumina packings are known to possess basic as well as acidic adsorption sites, and may be shown to adsorb and retain small amounts of anionic surfactants from external aqueous methanolic solutions. Furthermore, as has been previously demonstrated with cationic and non-ionic detergents, retention of both charged and uncharged eluites increases with increasing alkyl carbon chain length of anionic detergent. Note, however, that in aqueous "micellar" solutions of high surfactant concentration, non-polar analytes were shown by Armstrong and Terrill[6] to be less strongly retained on the alumina–surfactant interface.

EXPERIMENTAL

Instrumentation

Chromatographic systems were assembled from components as required as outlined in an earlier report[4].

Column packings and reagents

The porous alumina used in the majority of these experiments was Spherisorb (Phase Separations, Queensferry, Great Britain; A1OY grade, $d_p \approx 10\ \mu m$, $S_{BET} \approx 93\ m^2\ g^{-1}$ and A2OY grade, $d_p \approx 20\ \mu m$, $S_{BET} \approx 93\ m^2\ g^{-1}$), although LiChrosorb Alox T (E. Merck, Darmstadt, G.F.R.; $d_p \approx 10\ \mu m$, $S_{BET} \approx 70\ m^2\ g^{-1}$) was used in some confirmatory studies. Until the alumina particles were subjected to calcination in air (24 h at 600°C), it proved difficult to get fully reproducible retention results from column to column.

Columns were packed by the "upward slurry" techniques described by Bristow *et al.*[7] at 300–500 bar constant pressure, using methanol both for packing and suspension of the alumina particles. Solvent methanol was either AnalaR grade (BDH, Poole, Great Britain) or HPLC grade (Rathburn Chemicals, Walkerburn, Great Britain) as required. Sodium laurylsulfate (SDS), "puriss", and Tergitol 7, sodium "heptadecyl" sulfate were purchased from Fluka, Buchs, Switzerland. Tween 40, stated to be "industrial quality" was obtained from Sigma, London, Great Britain. All other solvents and reagents used were of Reagent grade and were used as received from the suppliers. Water was distilled from glass.

RESULTS AND DISCUSSION

Fig. 1 shows that the amount of Tergitol 7 sorbed on the alumina surface is a (non-linear) function of detergent concentration in the contacting solution, as has been shown by Rupprecht[8] and Scott and Kucera[9]. Surface coverage was measured both by a column breakthrough technique (*cf.* Knox and Laird[1]) and by a procedure of constant withdrawal and addition (of an aliquot of concentrated surfactant) to a suspension of alumina in the 1:1 aqueous methanol eluent base. However, since the Tergitol 7 active principle is anionic, a cationic dye must be used for the colorimetric[1] estimation of detergent concentration. Methylene blue was so used in these experiments, although the relationship of surfactant–dyestuff ion-pair concentration to

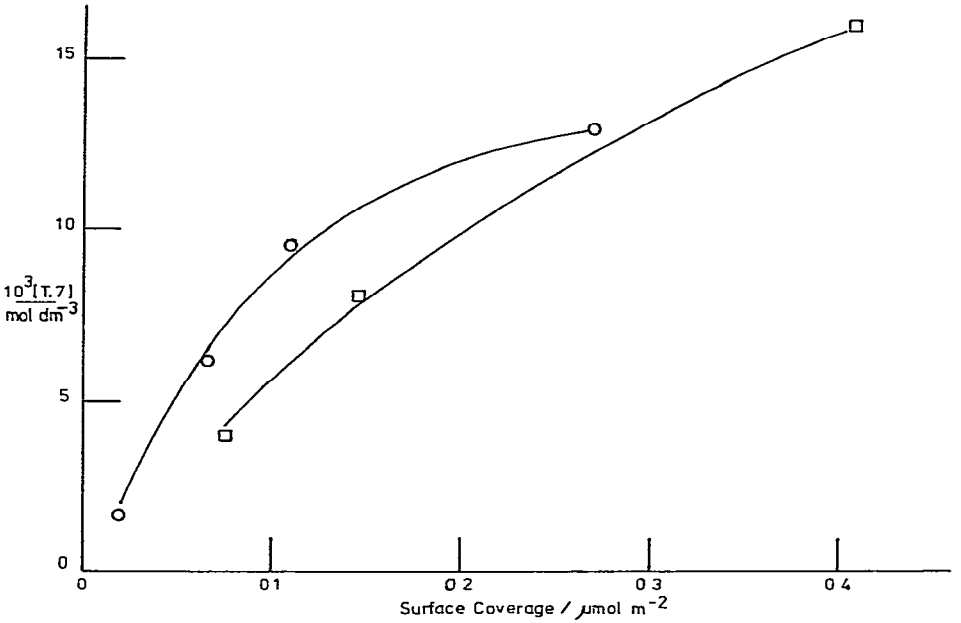

Fig. 1. Surface coverage of alumina by Tergitol 7 (T. 7) ("C_{17}" alkyl sulphate). ○, LiChrosorb Alox T; □, Spherisorb AlOY.

absorbance at 656 nm was distinctly non-linear. The highest degree of sorption measured (at 3.2×10^{-2} *M* Tergitol 7) showed that there were *ca.* 1.1 μmoles of detergent on each square metre of the alumina surface. The best coverage of silica by covalently bonded monolayer alkyl chains of equivalent length was shown by Roumeliotis and Unger[10] to be roughly three times as dense as that above.

Surfactant–retention relationships

A 1:1 (v/v) solution of methanol and water was used as the eluent base in all the present experiments. Solid surfactant (SDS) or a concentrated solution of liquid surfactant (Tergitol 7 and Tween 40) in the 1:1 aqueous methanol was added as appropriate to this base. All separations were done at ambient temperature (15–23°C) with an eluent flow-rate of 1 cm^3 min^{-1}. Quite large volumes (200–500 cm^3) of the least concentrated solutions of surfactants used in this study had to be passed through the packed columns before constant retention values could be observed. In all these surfactant–oxide gel systems there is apparently a very slow equilibration process occurring after the initially rapid uptake of sorbed detergent. Accordingly, if a column is to be prepared for a *practical* analytical separation, it is possible to save working time by establishment of partial equilibrium with a concentrated solution before equilibrating with the final, usually more dilute eluent.

Fig. 2 demonstrates the dependence of the capacity factor, $k' = (t'_{\text{retention}} - t_{\text{unretained}})/t_{\text{unretained}}$, of some non-polar aromatic compounds on the SDS-modified alumina column as a function of surfactant concentration. The form of this relationship, rising through a maximum (at [SDS] $\approx 3 \times 10^{-2}$ mol dm^{-3}) and declining thereafter with increasing solvating power (surfactant concentration), closely resem-

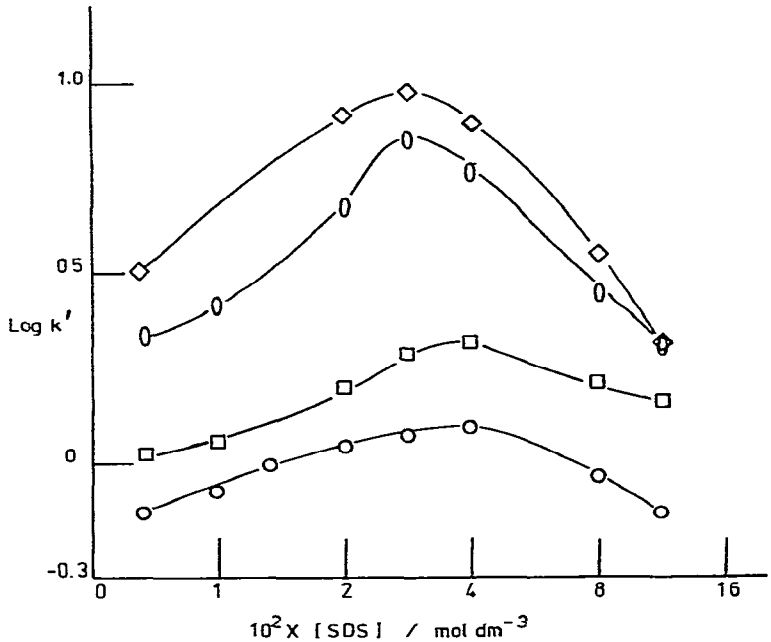

Fig. 2. Variation in retention with change in SDS concentration. ○, 9-Fluorenone; □, naphthalene; ◊, anthracene; and □, pyrene.

bles the earlier data with the same eluites on cationic[3] and non-ionic[4] surfactant-modified silicas. Furthermore, both the order of elution and the relative retention of these analytes are also similar to those observed in these earlier studies and on columns of alkyl bonded silica, suggesting strongly that there is a common mechanism of retention, *i.e.*, hydrophobic interaction.

This conclusion is reinforced by the data presented in Fig. 3, in which the same k' vs. [surfactant] relationship is displayed for a Tergitol 7–alumina system. Since Tergitol 7 is an alkyl sulphate differing only in chain length from SDS ("C_{17}" as opposed to C_{12}), the evident increase in maximum retention, achieved at lower Tergitol 7 concentration accords well with the work of Hemetsberger *et al.*[11] on the effects of alteration of (covalently bonded) alkyl chain length in hydrophobic chromatography, and the extended discussion by Horváth *et al.*[12] of (alkyl sulphate) ion-pair chromatography on alkyl bonded silicas.

The same picture of retention rising to a maximum and then falling with increasing surfactant concentration is also shown in Fig. 4, which represents the effects of a *non-ionic* surfactant, Tween 40, on the chromatographic properties of the alumina surface. Alumina adsorbents are usually considered to have a more reactive, polar surface than those based on silica gel and perhaps residual surface polarity reduces the tendency of hydrophobic analytes to be retained at the Tween 40–alumina interface. Certainly this range of eluites (and several others not shown) yields k' values about half of those measured in earlier studies[4] on silica–Tween 40 column systems. However, the surfactant concentration at which maximal retention is observed is essentially the same as that found earlier in the silica column study. This

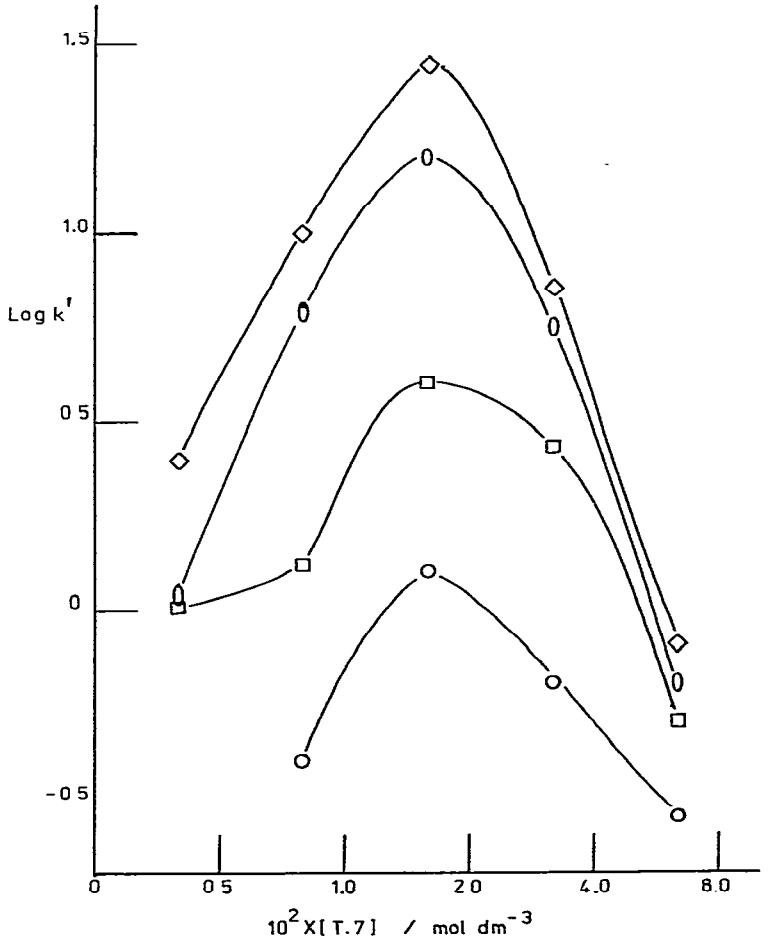

Fig. 3. Variation in retention with Tergitol 7 (T. 7) concentration. Analytes as in Fig. 2.

latter finding suggests that the dominant feature governing maximum sample retention is the solvating power of the eluent rather than the density of surface coverage by sorbed surfactant molecules. Further work will be necessary to clarify this point.

Mechanism of retention of cationic eluites

The above data support the hypothesis that the mode of retention and separation of *non-polar* analytes on alumina–surfactant column systems is qualitatively similar to that observed with silica–surfactant and alkyl-bonded silica column systems. However, when cationic samples were examined on alumina–SDS (and Tergitol 7) columns, puzzling results were obtained. The mixed non-ionic–anionic surfactant separations of peptides reported earlier[5] were shown to have at least some of the pH and counter-ion dependence to be expected if a cation-exchange process were operating. As pointed out in this earlier work, k' for a particular cationic substance should be inversely proportional to the concentration of added (counter) cation if retention is

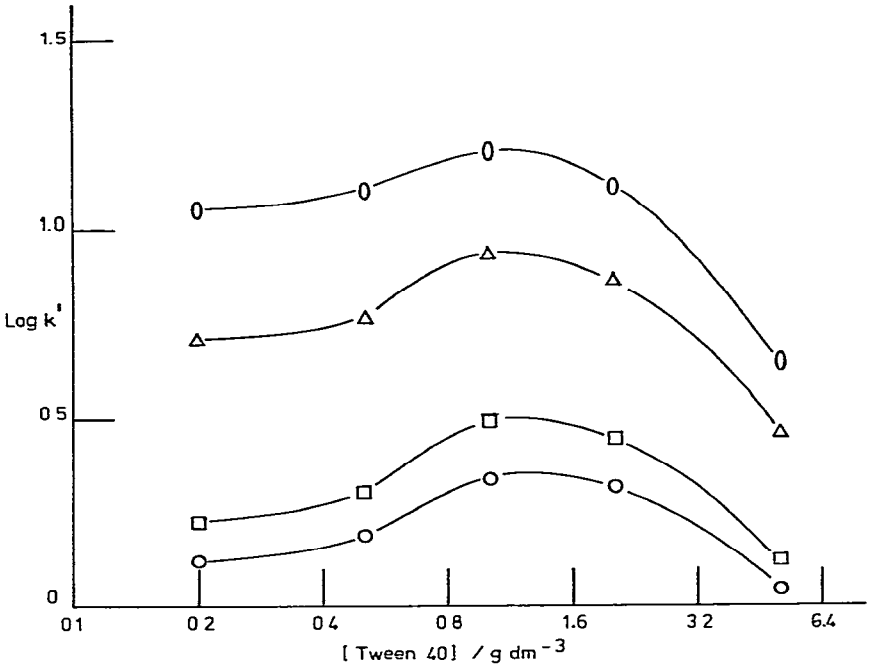

Fig. 4. Variation in retention as a function of non-ionic surfactant (Tween 40) concentration. Analytes as in Fig. 2, except △, *meso*-benzanthrone.

due primarily to ion-exchange processes.

Fig. 5 shows that whatever the mechanism for retention of aralkylammonium ions may be, it is apparently not a simple ionic exchange between mobile and stationary phases in the column. The relationship demonstrated between k' and $1/[Na^+]$ has a negative slope over most of the concentration range tested but reverses to a positive slope at higher salt levels. Apparently these relatively hydrophobic ammonium ions (or their ion pairs with alkyl sulphate anions) are retained primarily by hydrophobic interaction with the hydrocarbon side chains of surfactant molecules sorbed on the alumina surface. This conclusion is borne out by comparison of the data given in Fig. 6 with the similar plot given as Fig. 4 of the earlier study[5]. Clearly the changes in retention in this present study with increasing salt are similar for both non-polar and ammonium ion analytes up to *ca.* 5×10^{-2} *M* $[Na^+]$, but the large decrease in retentive power at even higher salt concentration was not observed in the earlier studies on the silica–non-ionic–anionic surfactant system. However, these two sets of data are not strictly comparable, since the oxide–surfactant systems and contacting liquids are not the same.

Because the effects of added salt on retention of cationic analytes did not support a cation-exchange mechanism, it seemed of interest to examine the effects of changes in acidity of eluents. The variation of pH was achieved by addition of mineral acid, H_3PO_4, to a solvent system in which methanol, surfactant, and sodium ion concentrations were held constant, so that all variables except $[H^+]$ and ionic strength were controlled. Similar types of experiments were shown by Hamilton *et al.*[13,14] to

lead to a steady decrease in retention of cationic analytes (amino acids) on polystyrenesulphonic acid exchangers with increasing pH over the range from 2–6. Fig. 7 shows just such a relationship for cationic analytes on an alumina–Tergitol 7 column system.

Since the ammonium ion analytes of this present study are at least 99% in the ionised form over the whole of the observed pH range, no simple explanation seems possible. However, this behaviour is typical of the pattern observed with classical ion exchangers, and retention in the present system is accordingly best described as a balance of ion exchange and hydrophobic bonding. If the density of alkyl chains on the alumina surface was proportional to $[H^+]$, that could account for the results described by Fig. 7. No data on the pH sensitivity of anionic surfactant binding to alumina are presently available, so the question must be left open.

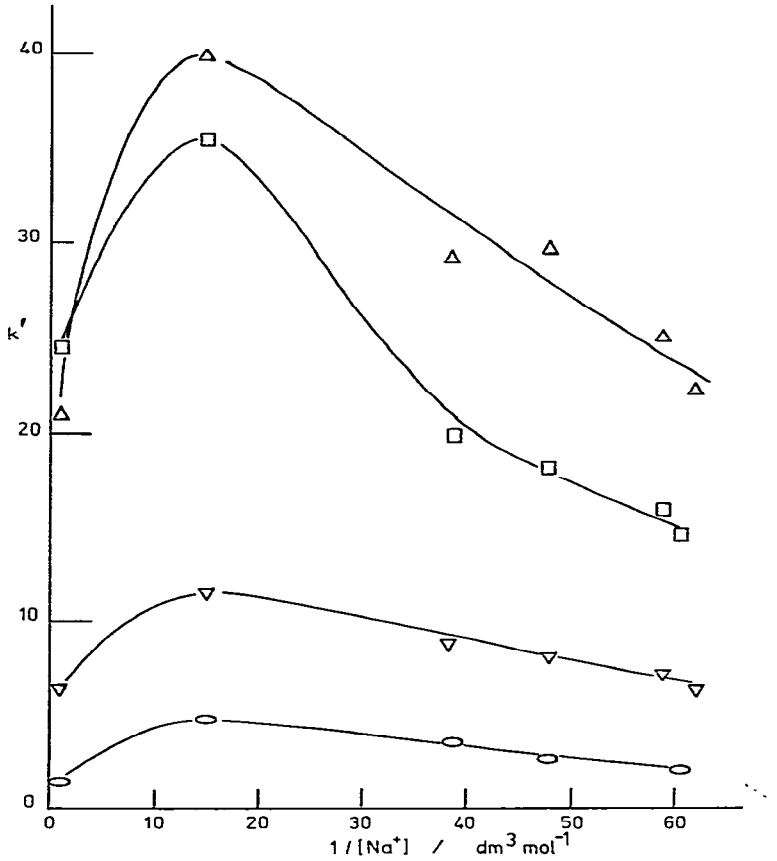

Fig. 5. Effects of change in counter-ion (sodium) concentration on retention at constant (1.6×10^{-2} mol/dm^3) concentration of Tergitol 7. ○, Tyrosine methyl ester; △, 1-amino-1-phenylethane; □, naphthalene; and △, 1-amino-1-(1-naphthyl)ethane.

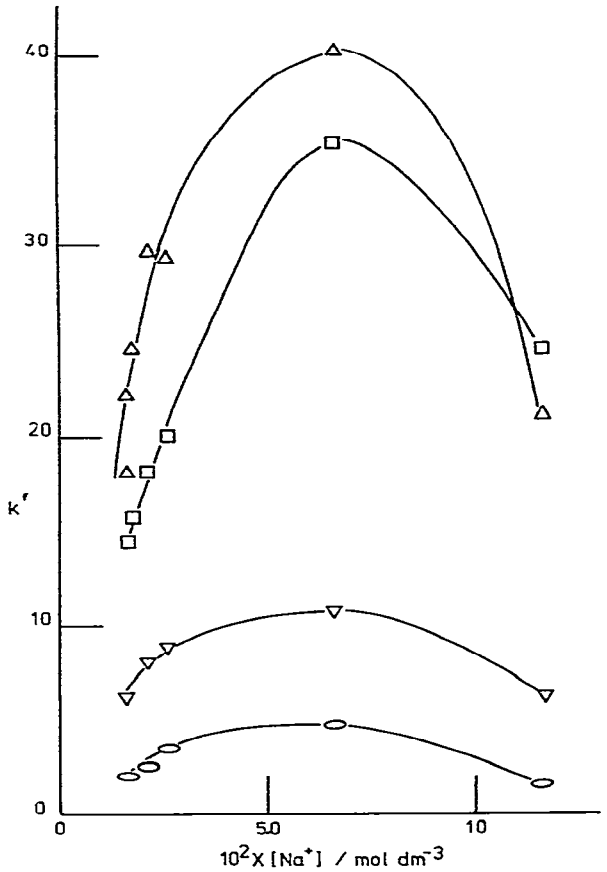

Fig. 6. Same data as in Fig. 5 but plotted as a linear function of counter-ion concentration.

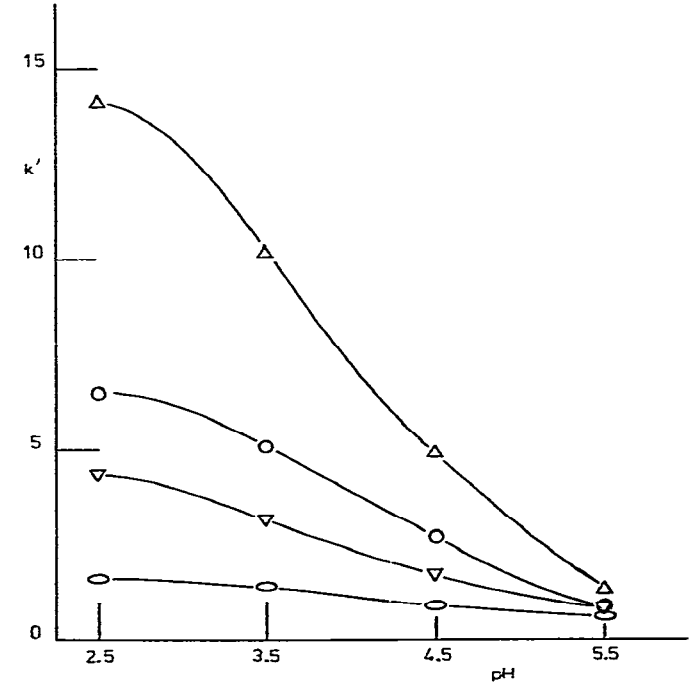

Fig. 7. Effect of change of pH on retention at constant counter-ion and Tergitol 7 concentrations. Analy as in Fig. 5, except O, tryptophan methyl ester.

Application

This new technique extends the sampling range amenable to analysis by oxide gel–surfactant chromatography in that cationic solutes will be well retained (under control by variation of pH, surfactant concentration, counter-cation concentration, and organic modifier concentration of the eluent system) in operating conditions such that acidic, zwitterionic and neutral analytes will also be retained (according to their hydrophobic binding capacities). The more complex eluting solvent systems discussed in the earlier study[5] on "dynamic ion exchange" of tyrosinyl peptides would generate the same analytical capabilities, but as shown above, the retention mechanisms are different in these two procedures, hence their selectivities for a given analysis will also differ.

Fig. 8 is a record of a specimen separation of four neutral analytes on an alumina–SDS column. The efficiency of this separation was tested at eluent flow-rates of 1.0, 0.8, 0.6, 0.4, and 0.2 $cm^3\ min^{-1}$ and was shown to increase steadily (the number of theoretical plates increased from 3000 to 4000 over this eluent flow range for the unretained acetone peak and from 750 to 3000 for the fluorenone peak) with decreasing speed of elution. Estimation of diffusion constants by the Wilke–Chang[15] approximation and calculation of reduced parameters as outlined by Knox[16] suggested that reduction of eluent flow-rate to 0.1 $cm^3\ min^{-1}$ would have been necessary to achieve optimal column efficiency for separation of 9-fluorenone. However, the above data show that this newest mode of oxide gel–surfactant liquid chromatography is capable of giving separation efficiencies comparable to those obtained with more conventional techniques.

This assertion is reinforced by examination of Fig. 9, which is a record of the separation of three fully protonated amines with the same column–eluent system used to produce Fig. 8. In the absence of surfactant the aqueous methanol eluent base would have eluted all the above analytes at or near the solvent front, and indeed the

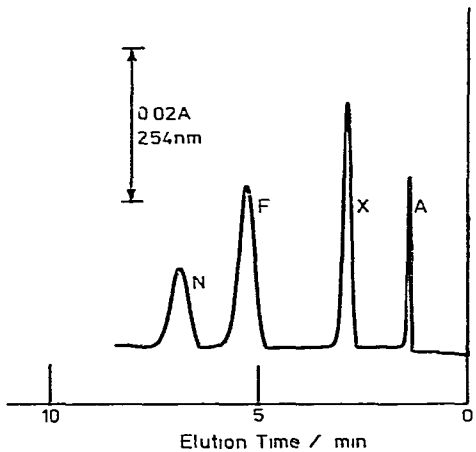

Fig. 8. Specimen separation of a mixture of neutral, unionised analytes. Column packed with Spherisorb AlOY, 125 × 4.6 mm; flow-rate 1 cm^3/min of 1:1 methanol–water containing 3.2×10^{-2} mol/dm^3 SDS (pH adjusted to 3.0 by addition of phosphoric acid). Analytes in order of elution are acetone, 2,3-xylenol, 9-fluorenone, and naphthalene.

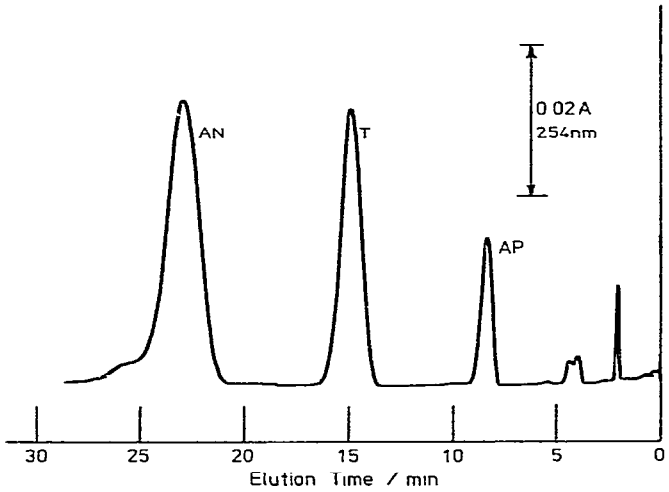

Fig. 9. Specimen separation of cationic analytes on same column as in Fig. 8. Eluent as in Fig. 8, but analytes in order of elution are: three impurities, 1-amino-1-phenylethane, tryptophan methyl ester, and 1-amino-1-(1-naphthyl)ethane.

three cationic eluites would be little retained on columns of *alkyl-bonded* silica from this mobile phase at pH 3. The observation of pronounced retention of *quaternary* ammonium analytes as well as of aralkyl ammonium ions suggests that although a purely ion-exchange mechanism cannot be supported (*cf.* Fig. 5) for these ionic eluites, there must be a substantial element of ion exchange or ion pair partition in the retentive processes.

CONCLUSIONS

The present investigation confirms the potential and versatility of a new approach to hydrophobic and ion-exchange chromatography based on dynamic generation of a retaining surface on porous oxide gel column packing materials. Moreover, it has shown that the surface layer(s) generated by direct (electrostatic?) interaction of an anionic surfactant with a basic aluminium(III) oxide differs significantly from the product of an acidic silicon(IV) oxide and mixed non-ionic–anionic surfactants.

Chromatographic systems based on those described above are readily set up, and are clearly versatile, since analyte retention is controlled (primarily) by eluent composition variables, *i.e.*, pH, ionic strength, organic modifier type and concentration, and surfactant type and concentration. Since the mechanism of retention of cationic analytes appears to have both hydrophobic and ion-exchange components, variation of operating temperature should alter selectivity of separation as well. Further study is required before a greater understanding of the detailed mechanism(s) of analyte retention in this new "dynamic" mode of liquid chromatography can be attained, but its value as an additional tool in the analytical workshop is apparent.

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