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HYDROPHOBIC CHROMATOGRAPHY WITH DYNAMICALLY COATED STATIONARY PHASES

V. "DUPLEX SOAP CHROMATOGRAPHY"

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SUMMARY

The retention of a wide range of ionised and non-ionised analytes on silica gel liquid chromatographic columns modified by dynamic interaction with solutions of mixtures of ionic and nonionic surfactants has been examined systematically using both aqueous and aqueous methanolic eluent systems. It is shown that efficient separations of mixtures of several classes of organic compounds can be achieved by these "duplex soap chromatography" procedures.

INTRODUCTION

Knox and Laird[1], Gilbert and Wall[2], and the present authors[3–5] have shown that aqueous alcoholic solutions of surfactants interact with oxide gel liquid chromatographic column packing materials to generate a liquid–solid interface that facilitates separations of charged and uncharged solutes by a combination of ion-exchange and solvophobic processes. An essentially similar technique was used by Armstrong and Terrill[6] for separations by chromatography on thin alumina layers without examination of the mechanism of separation. It was clear from the study[7] of effects of addition of anionic surfactants (which do not interact in aqueous or aqueous alcoholic solutions with acidic oxides like silica) to retentive silica–non-ionic surfactant systems that this "duplex soap chromatography" was of considerable potential interest.

The present study describes the effects of additions of either a quaternary ammonium salt soap, or an ammonium salt soap, or alkyl sulphate salt soaps to chromatographic column eluents containing polyoxyethylene sorbitan ester (Tween®) non-ionic surfactants. Retentions of several groups of analytes were studied as functions of non-ionic soap concentration and (independently) of ionic soap concentration.

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EXPERIMENTAL

Instrumentation

Chromatographic systems were assembled from components as outlined in earlier reports in this series[3–5,7]. Columns were packed by the "upward slurry" techniques described by Bristow *et al.*[8] at 300–500 bar constant pressure, using methanol both for packing and suspension of the spherical silica gel (Hypersil, $d_p \approx 5$ μm, $S_{BET} \approx 170$ m^2 g^{-1}, Shandon Southern Instruments, Runcorn, Great Britain) used in all the reported experiments.

Solvents and reagents

Solvent methanol was HPLC grade (Rathburn Chemicals, Walkerburn, Great Britain) and water was doubly distilled from glass and stored in glass containers. Care with preparation and storage of solvent water largely eliminated the problems of column contamination observed in the earlier study[7] on peptide separations by these techniques.

Hexadecyltrimethyl ammonium bromide (CTAB), sodium lauryl sulphate (SDS), puriss. grade, and Tergitol 7 (T.7) were purchased from Fluka, Buchs, Switzerland. Tweens 20 and 40, stated to be of "industrial quality" were obtained from Sigma (London) (Poole, Great Britain). All other reagents used were of reagent grade and were used as received from the various suppliers.

Procedures

In all experiments except the aqueous solution series, the measurement of retention with a given set of surfactants in the eluent was carried out on columns packed with silica which had been in contact with only those unique soaps. The columns were brought to equilibrium by washing with at least 0.5 l of Tween solution (2–5 g dm^{-3}) before initiation of duplex soap experiments, and in all cases columns were washed with the chosen eluent until constant retention was observed for the test substances.

In practice, this equilibration procedure (carried out at ambient temperature, 15–21°C) usually consisted of passage of some 0.2 l of eluent through a column before any measurement of retentive power. More often than not work timetabling led to partial equilibration on one working day followed by data collection on the following or later days. No particular significance attached to this timing when aqueous organic eluents were used, since achievement of a constant retaining surface appeared to be solely a function of passage of sufficient eluent through the column. However, when purely aqueous eluent systems were examined carefully, it was observed that retention of neutral analytes often increased after overnight storage of a column already in apparent equilibrium with the eluent. Fortunately, this very slow reorganisation of the retaining surface molecular architecture was found to be complete after 16–20 h storage. All aqueous soap chromatographic data reported below were collected only after an overnight storage period following passage of at least 0.2 l of eluent through the column. Studies on the time and temperature dependence of the retentive surface characteristics are now in hand.

RESULTS AND DISCUSSION

Dynamic anion-exchange systems

It was clearly of interest to see whether or not the interactions between non-ionic and anionic surfactants found earlier[7] to be so useful could also be demonstrated when a *cationic* soap was used in place of the SDS or T.7 of the peptide cation-exchange experiments. Fig. 1 shows that mixed cationic–non-ionic soap systems exhibit properties different from those of single surfactant–oxide gel chromatographic procedures. This figure outlines the large variations in selectivity attainable by changing the concentration of the ionic soap component of the eluent. Comparison of the present Fig. 1 with the data expressed as Fig. 1 of the earlier study on CTAB–silica interactions[3] shows clearly that a slightly lower maximum retention of charged sulphonic acid analytes is obtained in the duplex soap system, and that this maximum k' [$k' = (t_{\text{sample retention}} - t_{\text{void volume clearance}})/t_{\text{void volume clearance}}$] is achieved at much lower ionic surfactant concentrations than in the single surfactant system ($k' \approx 20$ at $2 \cdot 10^{-2}$ $\overline{M}$ [CTAB] *versus* $k' \approx 13$ at $6 \cdot 10^{-3}$ $\overline{M}$ [CTAB].

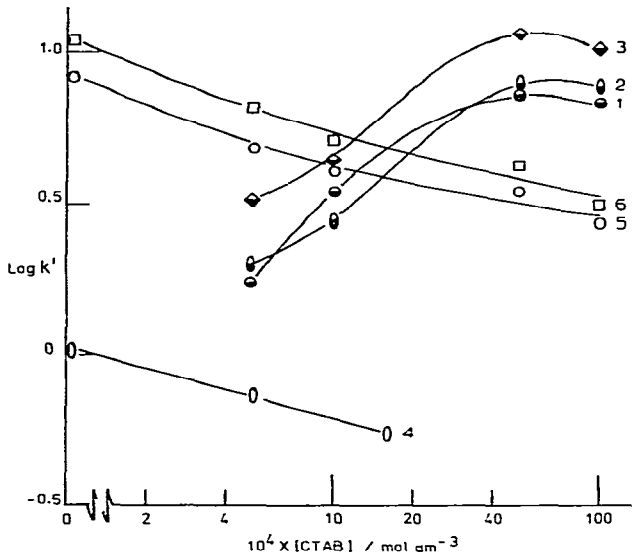

Fig. 1. Effect of variation of quaternary ammonium salt concentration on sample retention. Water–methanol (1:1, v/v) solution of Tween 40 (2 g dm^{-3}), pH 5 (with H_3PO_4). Analytes: 1 = di-J-acid; 2 = Schäffer's acid; 3 = dioxy-J-acid; 4 = acetophenone; 5 = fluorenone; 6 = naphthalene. For structures of sulphonic acids *cf.* Knox and Laird[1].

A second, potentially useful difference between the single soap and duplex soap eluents is apparent in the very different relationship of retention to CTAB concentration for unionised analytes. There is a steady increase to maximum k' for all analytes in the single soap system, whereas retention of non-polar eluites is steadily *reduced* by addition of CTAB to the Tween–SiO_2 column system. That this difference, which is probably an exaggerated case of the phenomena detailed by Graham and Rogers[9] in their investigation of surfactant effects on retention of neutral analytes on a column of octadecyl silica, is general will be shown later in this present work.

Apparently the analogy used in the peptide study[7] of a "brush" type of induced ion-exchanging surface might also account for the observed chromatographic properties of the duplex cationic–non-ionic soap-modified silica surface. As the ionic soap concentration increases, so also retention of anionic eluites increases through a maximum and thereafter decreases as the solvating power of the surfactant in the mobile phase begins to dominate the distribution of solute between stationary and mobile phases.

Determination of the uptake of CTAB from a water–methanol (1:1, v/v) solution containing Tween 40 (2 g dm^{-3}) showed $0.06 \cdot 10^{-6}$ mol m^{-2} at [CTAB] = $5 \cdot 10^{-3}$ mol dm^{-3}. This coverage of the silica surface should be compared to the binding capacity for alkylbenzyldimethyl ammonium salts on the ODS-Spherosil surface, which was reported by Tomlinson *et al.*[10] to be $0.13 \cdot 10^{-6}$ mol m^{-2} from a 10^{-3} mol dm^{-3} solution of the surfactant in water–methanol (1:1, v/v).

The apparent success of the above experiments in generation of what might be described as a "strong" anion exchanger led to attempts to combine the effects of a non-ionic surfactant and the surface active dodecylammonium ion. Fig. 2 shows that the retention *vs.* [dodecylammonium] relationship is similar in form to that observed above with the fully quaternised CTAB. The eluent systems were buffered to pH 5.5 in this latter series of experiments to ensure protonation of the amine soap as well as (*ca.* 80% complete) ionisation of the carboxylic acid analytes.

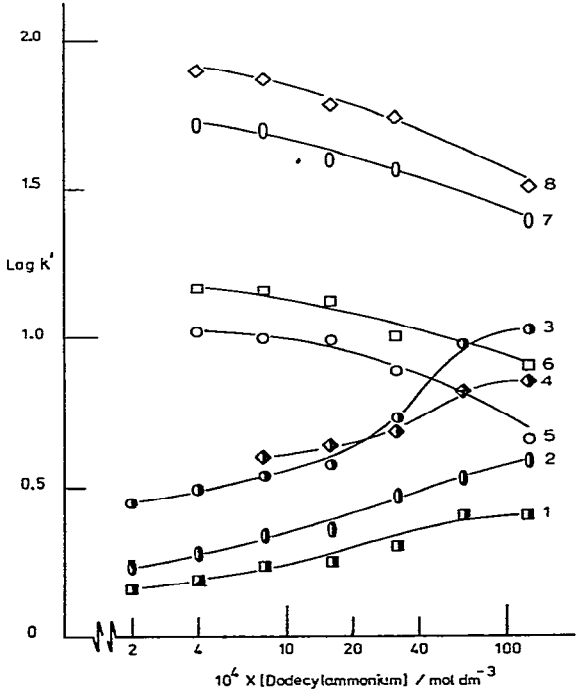

Fig. 2. Changes in eluite retention with variation in dodecylammonium ion concentration. Water–methanol (1:1, v/v) solution of Tween 40 (2 g dm^{-3}), pH 5.5 (with H_3PO_4). Analytes: 1 = 3-phenylbutanoic acid; 2 = *trans*-2-phenylcyclopropane-1-carboxylic acid; 3 = 3,3-diphenylpropenoic acid; 4 = 1-naphthyl acetic acid; 5 = fluorenone; 6 = naphthalene; 7 = anthracene; 8 = pyrene.

It can be seen that maximum retention of the carboxylate anions occurs at significantly higher cationic soap concentrations than did the corresponding maximum retention of arylsulphonate anions on the Tween 40–CTAB–silica columns. This finding is consistent with the greater surfactant activity of CTAB as opposed to dodecylammonium —the critical micelle concentration (CMC) of the quaternary ion is slightly less than a tenth that of the primary ammonium ion in water[11]. Interestingly, retention of non-polar analytes appears to be significantly greater (at equimolar cationic soap concentrations) in the dodecylammonium system than in the CTAB eluent, possibly reflecting a greater induced polarity of the surface from the quaternary salt.

Dynamic cation-exchange systems

A more complete investigation of the relationship of anionic surfactant (T.7) concentration to ionised and non-polar analyte retention on a silica column equilibrated with Tween 40 (2 g dm^{-3} in water–methanol, 1:1) than was illustrated in the early study[7] of this system is shown in Fig. 3. Comparison of the data represented in Fig. 3 with those showing the equivalent relationship for the shorter chain alkyl sulphate, SDS, reveals that retention in the duplex system does not appear to be as direct a function of surfactant chain length as with the single soap eluent–oxide column (*cf.* Fig. 3 of ref. 4 and Figs. 2 and 3 of ref. 5). It would appear that the lipophilicity of the dynamically generated stationary phase in duplex soap systems is

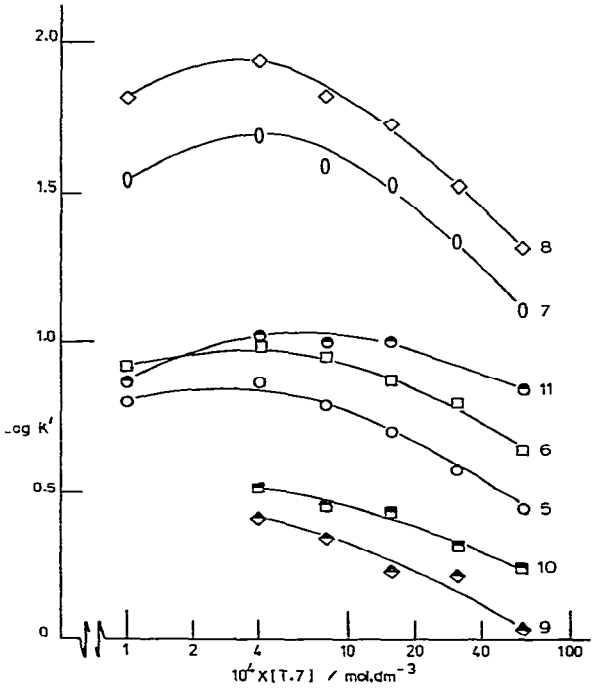

Fig. 3. Effect of variation of Tergitol 7 ("C_{17}" alkyl sulphate) concentration on sample retention. Water–methanol (1:1, v/v) solution of Tween 40 (2 g dm^{-3}), pH 4.0 (with H_3PO_4). Analytes 5–8 as in Fig. 2, and: 9 = 2-nitroaniline; 10 = 1-amino-1-phenyl ethane; 11 = 1-amino-1-(1-naphthyl)ethane.

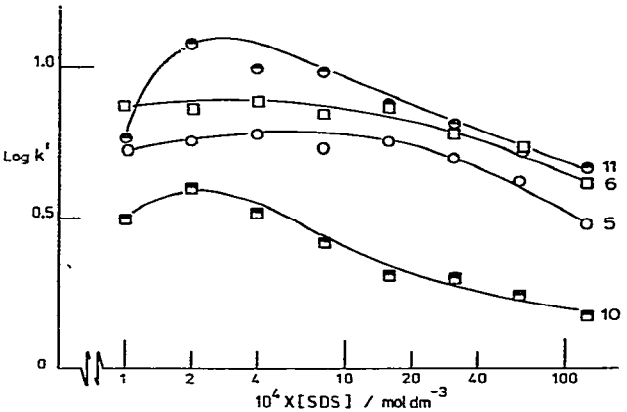

Fig. 4. As Fig. 3 except anionic surfactant was sodium lauryl sulphate (SDS).

governed primarily by the nature of the *non-ionic* surfactant. This latter assertion is supported by further comparisons of Figs. 1, 3, 4 and 5 from this report with Fig. 1 of ref. 3, Fig. 3 of ref. 4 and Figs. 2 and 4 of ref. 5, since maximum retention of charged analytes in the single cationic and anionic systems occurs at much higher concentrations of the appropriate surfactant, whereas maximum retentions of uncharged (and charged, *cf.* Fig. 5) analytes were observed at approximately the same concen-

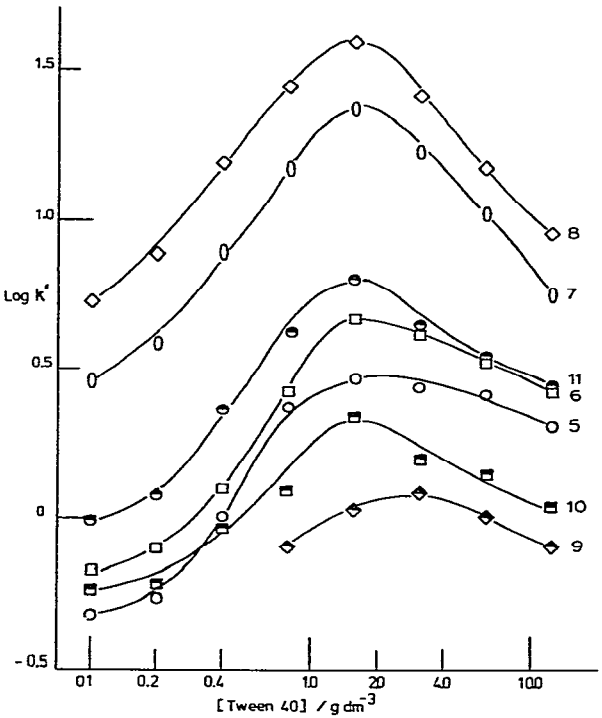

Fig. 5. Effect of variation of concentration of non-ionic (Tween 40) soap on analyte retention. Water–methanol (1:1, v/v) solution of SDS (10^{-2} mol dm^{-3}), pH 4.0 (with H_3PO_4). Analytes as in Figs. 2 and 3.

tration (*ca.* 2 g dm^{-3}) of the non-ionic surface active eluent component in *both* single and duplex soap systems.

The other feature of duplex surfactant liquid chromatographic systems which is shown very clearly in Figs. 1 and 2 and less obviously in Figs. 3 and 4 is the very different response of charged and uncharged analytes to variations in the ionic soap constituent of the eluent. The change in retaining surface charge density with increasing ionic soap content gives a steady rise in retentiveness for ionised analytes (of charge opposite to that of the soap) through a maximum shared by analytes of the same charge. The effect of the increasing surface charge density on *uncharged* analytes is simply to reduce their retention from the maximum which is observed in the absence of the ionic soap eluent component. So in many cases the order of elution (*i.e.*, selectivity) of a given set of charged and uncharged eluites may well alter drastically with changes in ionic surfactant concentration (at constant non-ionic concentration).

The more complete data collections of the present work confirm the tendencies to selective retention observed in Part II[7] of this extended study, and suggest that gradient elution systems in which either counter ion or ionic surfactant concentrations were varied might prove to be useful separation procedures.

Dynamic coating with aqueous surfactant solutions

A possible deterrent to wider use of the oxide gel–surfactant liquid chromatographic systems described so far in this study is the relatively low lipophilicity of these dynamically coated surfaces compared to current alkylbonded silica surfaces. Retention of aromatic ketones and phenols on duplex or simplex (non-ionic) soap coated silicas is apparently of the same order as found on the least hydrophobic bonded silicas. However, Fig. 8 of Part III[4] (and unpublished results associated with ref. 7) of this series shows that alteration of the organic components of eluents containing non-ionic surfactants leads to the expected large changes in retention of neutral analytes.

Taking these latter observations to their logical conclusion, especially in view of the "micellar chromatography" studies of Armstrong and Terrill[6] on separations on alumina thin layers with aqueous SDS eluents, it seemed appropriate to determine whether aqueous non-ionic surfactant solutions could also be used to generate a controlled retentive surface on silica gel. In the event, such interactions did take place and gave the very large increase in retention expected by analogy with the effects of similar eluent changes in alkyl-bonded silica chromatography.

As can be seen in Fig. 6, particularly by comparison with Fig. 8 of ref. 4, k' for acetophenone (and several other analytes not shown) in aqueous eluents is about ten times that found with water–methanol (1:1) eluents. Further inspection of Fig. 6 reveals two other differences between the aqueous and organic-modified aqueous chromatographic systems. First, and most important, the fairly sharp maximum in the retention *vs.* non-ionic detergent concentration curve so characteristic of aqueous methanol eluents (*cf.* Fig. 5) does not appear in purely aqueous systems. Instead, retention rises slowly to a limiting value defined by interaction of pure water eluent and pre-loaded (with 0.5 g dm^{-3} [Tween 20]) silica gel. The k' measurements on the [Tween 20] = 0 axis were obtained after passage of approx. 0.8 l of water through the pre-loaded column. Secondly, the order of elution of the second and third eluites is reversed from that previously observed on columns of alkyl-bonded silica or dynamically coated silica eluted with aqueous organic eluents (*cf.* Fig. 11 of ref. 7), which

suggests considerable involvement of surface silanols in the retention mechanism[12] of these fairly polar eluites. Note also that there does not appear to be any pronounced change in the chromatographic characteristics of the single non-ionic surfactant eluent–gel system as might be expected near the CMC[11] of this detergent.

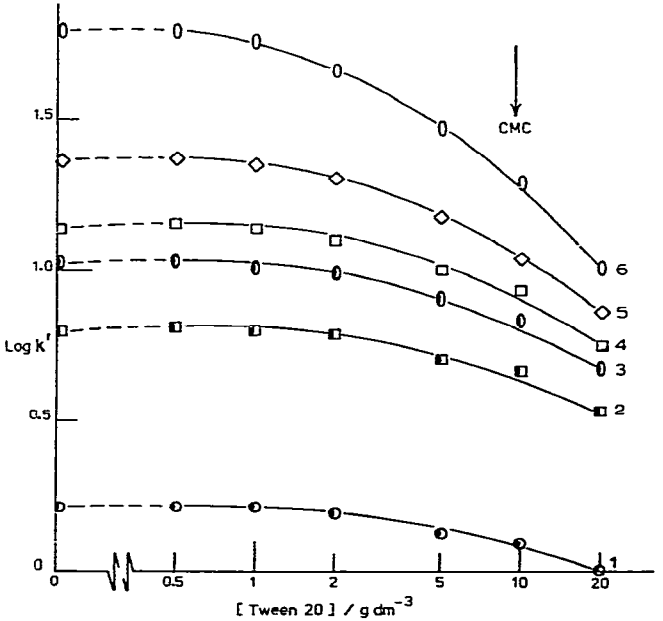

Fig. 6. Effect of variation of concentration of Tween 20 on aqueous single soap chromatographic retention of uncharged samples. Analytes: 1 = 4-methylpentan-2-one; 2 = acetophenone; 3 = 4-methoxyacetophenone; 4 = salicylamide; 5 = 4-methylphenol; 6 = 2,3-xylenol.

Fig. 7 is a simple demonstration that column efficiency with this aqueous non-ionic soap eluent is comparable to that obtained in conventional "reversed-phase" alkyl-bonded silica separation systems. Note the unusual order of elution of acetophenone, 4-hydroxyacetophenone and 4-methoxyacetophenone.

In line with the general theme of the present work, aqueous duplex soap eluents were examined with the same pre-loaded (after collection of the water elution data) silica column used for the studies described by Fig. 6. The retention *vs.* [SDS] data shown in Fig. 8 were measured in a series of eluents of *decreasing* anionic soap concentration, following the same methodology used with the aqueous single soap eluents. A very large disturbance in baseline detector signal resulted from the application of the most concentrated (*ca.* 2 × CMC of SDS, ref. 11) duplex soap eluent tested. Moreover, measurement of the column void volume by injection of pure water or aqueous potassium nitrate solution demonstrated a considerable increase over the values recorded in the absence of the anionic detergent. Apparently the high solubilising power of aqueous SDS at concentrations at or above the CMC removes most of the dynamically deposited non-ionic surfactant and hence leads to minimal retentive power for all analytes examined.

Note, however, that as the concentration of the ionic soap falls below the

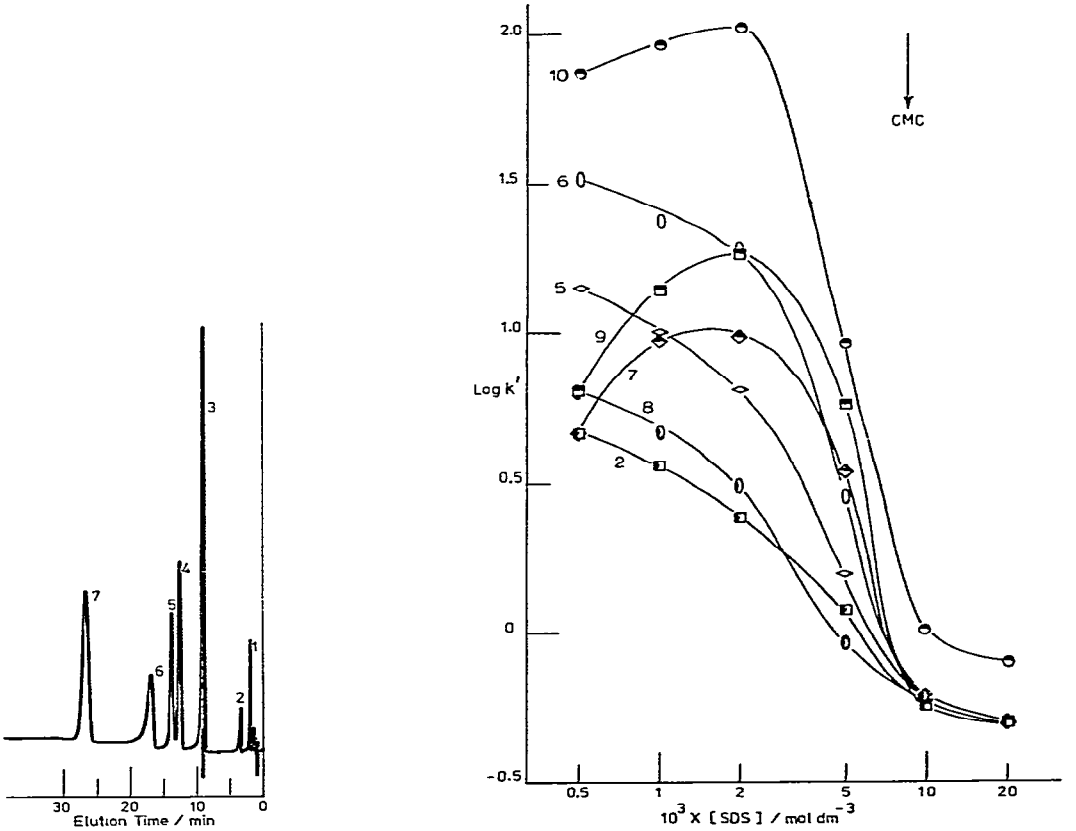

Fig. 7. Sample separation of some uncharged analytes by aqueous single non-ionic soap chromatography. Column of Hypersil, 135 × 4.6 mm I.D., eluted by aqueous solution of Tween 20 (2 g dm^{-3}) at 1 cm^3 min^{-1}. Detection by ultraviolet absorption at 254 nm. Analytes: 1 = butan-2-one; 2 = 4-methylpentan-2-one; 3 = acetophenone; 4 = 4-hydroxyacetophenone; 5 = 4-methoxyacetophenone; 6 = salicylamide; 7 = 4-methylphenol.

Fig. 8. Effect of variation of concentration of sodium lauryl sulphate on aqueous duplex soap chromatographic retention of cationic and neutral samples. [Tween 20] = 1 g dm^{-3}, pH adjusted to 3.0 with phosphoric acid after addition of Na_2HPO_4 to give $[Na^+]$ = 0.020 mol dm^{-3}. Analytes 2, 5 and 6 as in Fig. 6, and: 7 = glycyltyrosine; 8 = 4-hydroxyacetophenone; 9 = tyrosine methyl ester; 10 = 1-amino-1-(1-naphthyl)ethane.

CMC, there is a rapid generation of retentive character for both cationic and neutral analytes. The behaviour shown in Fig. 8 is distinctly different from that observed with the aqueous single soap eluent exemplified in Fig. 6 above. Concomitant with the restoration of retention at sub-CMC SDS concentrations there was the expected baseline detector perturbation and a measureable decrease in the void volume as if non-ionic Tween was again deposited on the silica surface.

Note that the relationship of retention of uncharged analytes to surfactant concentration in the duplex soap system resembles that in the single soap system, although Fig. 8 makes clear that (at moderate ionic strength) neutral eluites are not bound quite so firmly to the SDS–Tween surface as to the Tween surface. However,

the retaining power of the duplex surface for cationic analytes passes through a well defined maximum as anionic soap concentration is reduced. That this difference in selectivity for charged as opposed to uncharged eluites is a general property of all the duplex soap systems examined to date may be confirmed by examination of Figs. 1–4 and 8 and Figs. 1 and 4 of ref. 7.

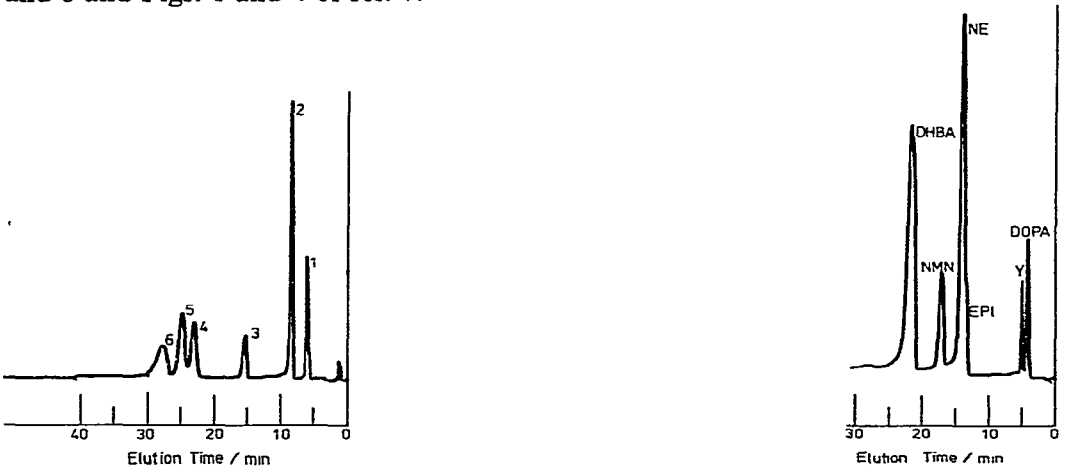

Fig. 9. Sample separation of some analytes of Fig. 8. Same column, detector setting and eluent flow-rate as Fig. 7. Eluent: aqueous solution of Tween 20 (1 g dm^{-3}) and SDS ($2 \cdot 10^{-3}$ mol dm^{-3}) containing Na_2HPO_4 ($9 \cdot 10^{-3}$ mol dm^{-3}), pH adjusted to 3.01 by addition of H_3PO_4. Analytes: 1 = 4-hydroxyacetophenone; 2 = 4-methoxyacetophenone; 3 = glycyltyrosine; 4 = 3,5-xylenol; 5 = 2,3-xylenol; 6 = tyrosine methyl ester.

Fig. 10. Sample separation of tyrosine and some of its metabolites. Column, eluent, and operating conditions as in Fig. 9 except detection at 272 nm. Analytes: DOPA = 3,4-dihydroxyphenylalanine; Y = tyrosine; EPI = epinephrine; NE = norepinephrine; NMN = normetanephrine; DHBA = 3,5-dihydroxybenzylamine (internal standard for catecholamine analyses).

A sample separation of some of the test substances used in the experiments with aqueous single and duplex soap eluents is shown in Fig. 9. Although there is evidence of increased peak asymmetry resultant from extended usage of the column, the perceived separating power has remained acceptable. Since it was clear that relatively polar analytes could be well retained with aqueous soap eluents, it seemed appropriate to examine possible separations of the biologically interesting catecholamines and their precursor amino acids. Fig. 10 shows such a trial separation. Although this model mixture does not include all the biologically significant tyrosine metabolites, the analytical possibilities inherent in this non-optimised separation appear to warrant further study, particularly since the elution order of these amines is distinctly different from that observed in aqueous or aqueous–organic buffer elution from anionic surfactant-modified alkyl-bonded silica columns[13–15].

CONCLUSIONS

Equilibration of porous silica gel with an aqueous methanolic solution of a ("duplex soap") mixture of a cationic and a non-ionic surfactant may be used to produce a liquid chromatographic column packing material which will separate neu-

tral analytes by solvophobic interactions and anionic analytes by a combination of anion-exchange and solvophobic distribution processes. Retention of charged and uncharged analytes on such dynamically coated silica is a function of the concentrations of *both* surface-active agents in the eluting solvent system, although greatest selectivity in control of relative retention is achieved by variation of the concentration of the cationic rather than the non-ionic soap.

Duplex cationic soap chromatographic systems qualitatively resemble single cationic soap–acidic oxide gel separation systems. However, maximum analyte retentions occur at much lower ionic soap concentrations on duplex soap modified oxide gel column packings than on single soap modified materials.

Replacement of the cationic soap in the above duplex soap chromatography procedure by an anionic surfactant produces a separation system which discriminates between analytes by a combination of cation-exchange and solvophobic interactions. These discriminative properties are preserved with both aqueous and aqueous–organic eluting solvents.

Although analyte retention in all single and duplex soap chromatography systems examined to date has been found to be a non-linear function of surfactant concentration in the mobile phase, very rapid changes in the retentive properties of the dynamically modified stationary phase associated with micellisation have been observed only with an aqueous duplex soap chromatography procedure as a consequence of variation of the ionic surfactant concentration in the eluent.

Each of the single and duplex "soap chromatography" procedures studied in this and earlier reports[1–7] has been found to give efficient separations of a wide variety of organic compounds. Many questions remain to be answered about these separation systems, but what has been found so far clarifies the potential value of (dynamic) single *and* duplex soap chromatography methods.

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