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## HYDROPHOBIC CHROMATOGRAPHY WITH DYNAMICALLY COATED STATIONARY PHASES

### III. NON-IONIC SURFACTANT EFFECTS

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#### SUMMARY

The effects of addition to aqueous-organic eluents of four members of a group of chemically similar non-ionic surfactants (Tweens) on the chromatographic properties of silicon(IV) oxide gels has been investigated. It was found that small amounts of Tween added to eluent altered the silica surface so that hydrocarbon, phenolic and ketonic elutes were retained to give separations very similar to those obtained with an alkyl (C<sub>18</sub>) covalently bonded silica.

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#### INTRODUCTION

Knox and co-workers<sup>1-3</sup> demonstrated that retention of charged analytes on columns of alkyl-bonded silica gels could be increased by inclusion in the mobile phase of suitably hydrophobic "counter-ions". This technique is often referred to as "soap" or "reversed-phase ion-pair" chromatography. These adducts to the mobile phase in this technique are often charged surfactants such as alkyl sulphonates and sulphates or tetraalkylammonium salts. However, little information is available on the possible effects of addition of non-ionic detergents to these separation systems.

Gilbert and Wall<sup>4</sup> found that porous ceria column packings gave elute retention from aqueous methanolic eluents containing hexadecyl trimethylammonium bromide similar to that reported by Knox and Laird<sup>1</sup> on SAS-silica, an alkyl-bonded high-performance liquid chromatography (HPLC) column packing. Ghaemi and Wall<sup>5</sup> reported separations of some aromatic hydrocarbons, ketones and sulphonic acids on columns of silica and zirconia modified by dynamic interaction with quaternary ammonium cationic surfactants dissolved in aqueous-organic eluents, and also separations<sup>6</sup> of peptides on columns of silica modified by reaction with solutions of mixtures of non-ionic and anionic detergents.

In the present study the effects of addition of some non-ionic surfactants to aqueous methanolic eluents of silica columns are described. This class of surface-active compound is also shown to be deposited onto the silica gel surface. Furthermore, data presented here show that as the hydrophobicity of the surfactant increases

(i.e., as the hydrophobic carbon chain length increases), retention of aromatic hydrocarbons and ketones also increases.

## EXPERIMENTAL

### *Instrumentation*

Chromatographic systems were assembled as required from M6000A (Waters Assoc., Milford, MA, U.S.A.) or 110A (Beckman, Irvine, CA, U.S.A.) pumps; CE212 (Cecil Instruments, Cambridge, Great Britain) or SF650 (Applied Chromatography Systems, Luton, Great Britain) ultraviolet absorption detectors; septum injectors and columns (125 × 4.6 mm I.D.) made in the laboratory; and guided plunger microsyringes (Scientific Glass Engineering, Melbourne, Australia).

### *Column packings and reagents*

The silica gels used in these experiments were Hypersil (Shandon Southern Products, Runcorn, Great Britain;  $d_p = 5 \mu\text{m}$ ,  $S_{\text{BET}} \approx 200 \text{ m}^2 \text{ g}^{-1}$ ) and GA 43, an experimental spherical material ( $d_p = 9 \mu\text{m}$ ,  $S_{\text{BET}} \approx 170 \text{ m}^2 \text{ g}^{-1}$ ).

Columns were packed by the "upward slurry" technique described by Bristow *et al.*<sup>7</sup> at 300 bar. The silica packing materials were suspended and packed in methanol. Solvent methanol was either AnalaR grade (BDH, Poole, Great Britain) or HPLC grade (Rathburn Chemicals, Walkerburn, Great Britain). Tweens 20, 40, 60, and 80 were purchased from Sigma (London, Great Britain) and were all described as of industrial quality. No manufacturers' batch numbers were quoted by the vendors.

### *Measurement of "adsorbed" surfactant*

Samples (ca. 1 g) of silica gel (GA 43) were weighed into tared, stoppered flasks and dried *in vacuo* for 30 min at 120°C. Flask and contents were cooled in a desiccator and reweighed to determine the dry weight of the silica. Methanol-water (1:1, 200 cm<sup>3</sup>) containing a known concentration of surfactant was mixed with the dried silica for 5 min in an ultrasonic cleaning bath to ensure complete dispersion of the particles. The mixture was filtered by suction through a sintered glass funnel and the excess solvent carefully sucked from the retained silica. The "filter-dry" silica was weighed and then dried *in vacuo* at 120°C, cooled in a desiccator and weighed again to obtain both the dry weight and the "wet" weight. In no case was the amount of detergent adhering to "filter-dry" silica as excess solution greater than a few per cent of the amount bound via surface interaction.

The above procedure was repeated for each determination with a fresh 200 cm<sup>3</sup> sample of surfactant solution to ensure complete equilibration.

## RESULTS AND DISCUSSION

### *Adsorption of Tweens*

Table I shows that the amount of non-ionic surfactant sorbed on the silica surface is a function of detergent concentration in the contacting solution, as has been shown by Rupprecht<sup>8</sup>. A general tendency to decreasing adsorption with increasing hydrophobicity was also observed, which will be discussed later in greater detail. The highest degree of sorption shown in Table I (197 mg of Tween 20 per gram of

TABLE I  
SORPTION OF TWEEN ON GA 43 SILICA

	$10^3[\text{Tween}]/\text{kg dm}^{-3}$	Tween adsorbed (mg per g $\text{SiO}_2$ )	Hydrophobic alkyl chain
Tween 20	2.0	123	$(\text{CH}_2)_{11}\text{CH}_3$
	3.0	197	
Tween 40	2.1	65	$(\text{CH}_2)_{15}\text{CH}_3$
	4.3	150	
Tween 60	2.2	52	$(\text{CH}_2)_{17}\text{CH}_3$
Tween 80	5.0	85	$(\text{CH}_2)_8\text{CH}=\text{CH}(\text{CH}_2)_7\text{CH}_3$

$\text{SiO}_2$ ) corresponds to a surface coverage of *ca.*  $1.5 \mu\text{mol m}^{-2}$ , which is about half the value reported by Roumelotis and Unger<sup>9</sup> for  $\text{C}_8$  and  $\text{C}_{18}$  alkyl-bonded silicas.

#### Separations on silica gel

Methanol-water (1:1) was used as the eluent base in all experiments reported here, with addition of the appropriate Tween to give concentrations between 0.5 and

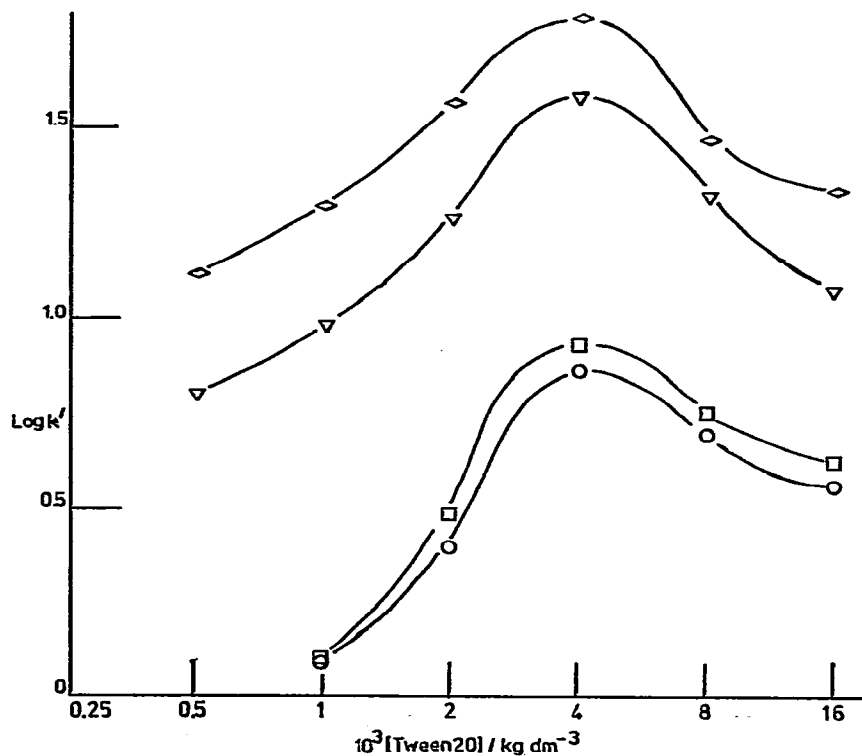


Fig. 1. Relationship of  $\log k'$  to concentration of Tween 20 in methanol-water (1:1) eluent. Column packed with Hypersil ( $d_p \approx 5 \mu\text{m}$ ). Flow-rate  $1 \text{ cm}^3 \text{ min}^{-1}$  at ambient temperature. Elutes: ○ = 9-fluorenone; □ = naphthalene; ▽ = anthracene; ◇ = pyrene.

$16 \times 10^{-3} \text{ kg dm}^{-3}$ . All separations were carried out at ambient temperature ( $15\text{--}23^\circ\text{C}$ ) with an eluent flow-rate of  $1 \text{ cm}^3 \text{ min}^{-1}$ .

Passage of between 100 and  $150 \text{ cm}^3$  of solvent containing Tween 20 was necessary to completely equilibrate a column ( $125 \times 4.6 \text{ mm I.D.}$ ) packed with Hypersil. Fig. 1 shows the dependence of the capacity factor,  $k'$ , of some aromatic hydrocarbons and ketones on the Tween 20 modified silica gel columns as a function of detergent concentration. Fig. 2 shows the same retention/[surfactant] relationship for Tween 80, which has an unsaturated carbon chain.

The composite graph, Fig. 3, shows that the Tween concentration at which maximum retention is observed for two elutes is a function of surfactant hydrophobicity. As would be expected by analogy with alkyl-bonded silicas<sup>10</sup>, the largest values of  $k'$  are observed with the most hydrophobic Tween, and, moreover, the surfactant concentration needed for maximum retention is progressively *reduced* as the carbon chain length is increased. Hemetsberger *et al.*<sup>10</sup> showed that  $k'$  was a linear

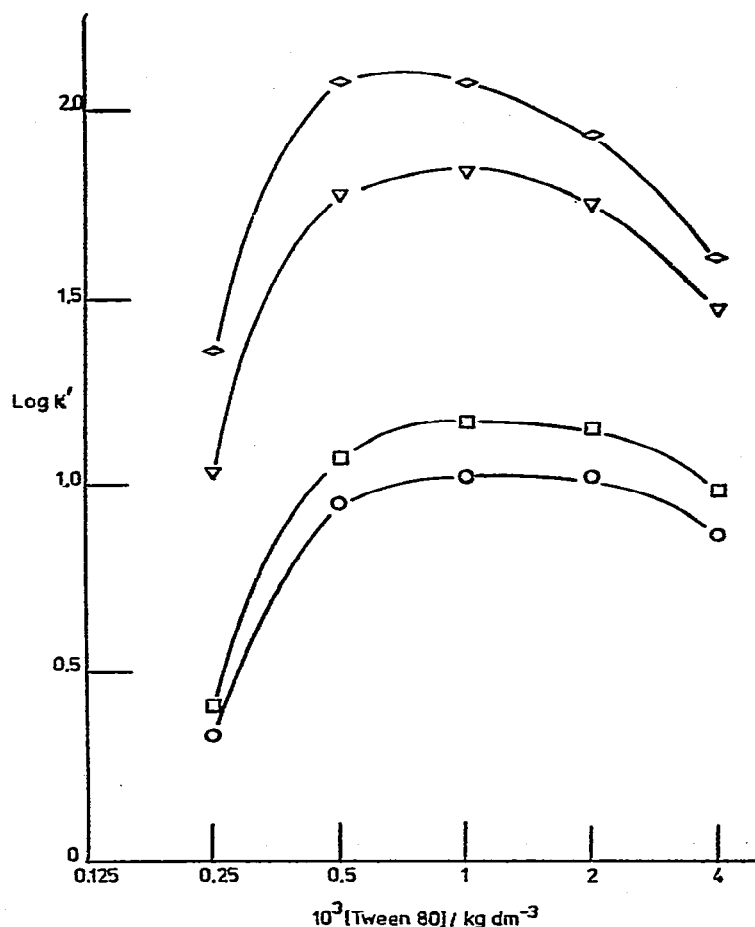


Fig. 2. Relationship of  $\log k'$  to concentration of Tween 80 in eluent. Chromatographic conditions and elutes as in Fig. 1.

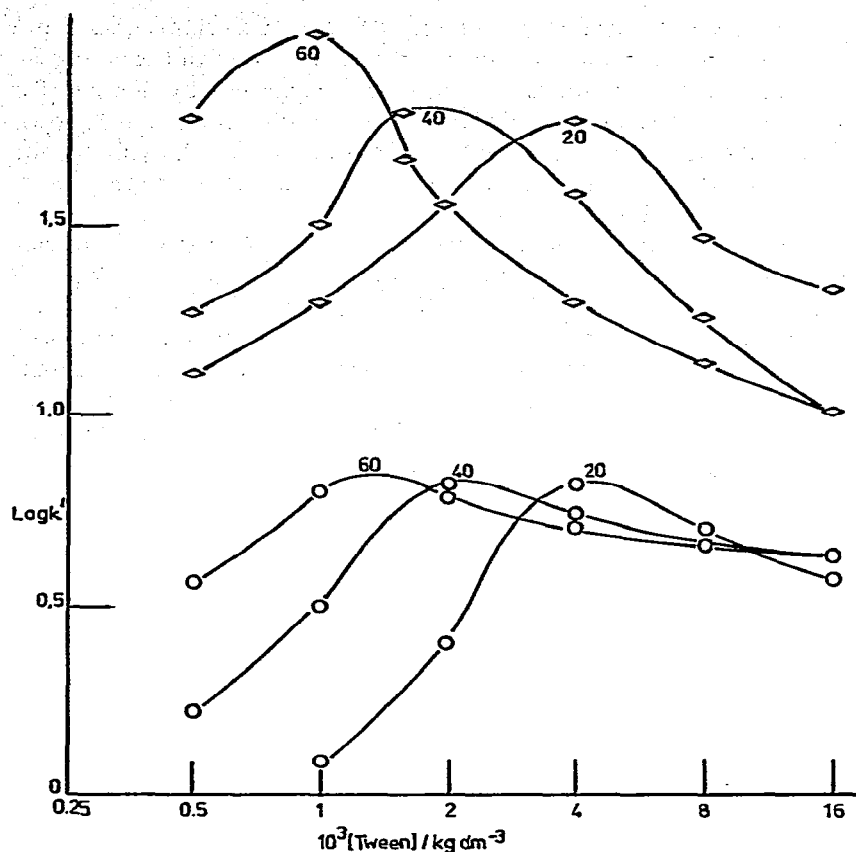


Fig. 3. Relationship of  $\log k'$  to concentration of Tweens 20, 40, and 60 in the eluent. Samples and conditions as in Fig. 1.

function of percentage carbon content on a series of alkyl methyl silyl "bonded-phase" packings, and a similar trend is observed with these dynamically coated silica surfaces.

In view of the observations by Hemetsberger<sup>10</sup> on the relationship of elute retention to chain length of *bonded* alkyl residue, one would expect  $k'$  values measured in those surfactant solutions giving maximum retention induced by that surfactant to increase by a relatively large factor for an increase of two or four methylene groups in the alkyl chain length of the detergent molecule. However, it can be seen from Table I that surface sorption of Tweens varies *inversely* as chain length. So the greater hydrophobicity of the longer chains is counterbalanced by lower surface coverage of the hydrophilic silica. Fig. 3 demonstrates that either no increase in  $k'$  (fluorenone) or a relatively minor increase in  $k'$  (pyrene) is observed with increasing hydrophobicity of the surfactant. It is noteworthy that introduction of a single double bond into the 18-carbon chain of Tween 60 to produce Tween 80 (compare Figs. 2 and 3) significantly increased retention of all elutes shown, but did not apparently change the detergent concentration at which maximum  $k'$  was observed.

Figs. 4 (Fig. 4a is without surfactant and Fig. 4b with surfactant) and 5 are representative chromatograms of aromatic hydrocarbons, phenols and ketones on silica. Separations of one of the standard test mixtures suggested by Knox<sup>11</sup> for determination of the efficiency of columns of alkyl-bonded silica columns are shown in Figs. 6 and 7. The order of elution and relative retention of the analytes on the ODS-Hypersil column (Fig. 6) is very similar to that observed in aqueous methanolic Tween 40 elution from a silica column (Fig. 7).

The similarity in elution order and relative retention of the various analytes shown in Figs. 6 and 7 suggests a similarity in separation mechanism. As suggested in earlier studies in this series, the result of interaction between silica and the polyol terminus of the Tween molecule is probably a modified surface bearing "brushes" of surfactant molecules. As the concentration of surfactant in the eluent is increased, two competing processes occur. Firstly, coverage of the silica by "brushes" increases and, secondly, association of "bound" and free surfactant molecules (probably by hydrophobic bonding of alkyl chains) also increases.

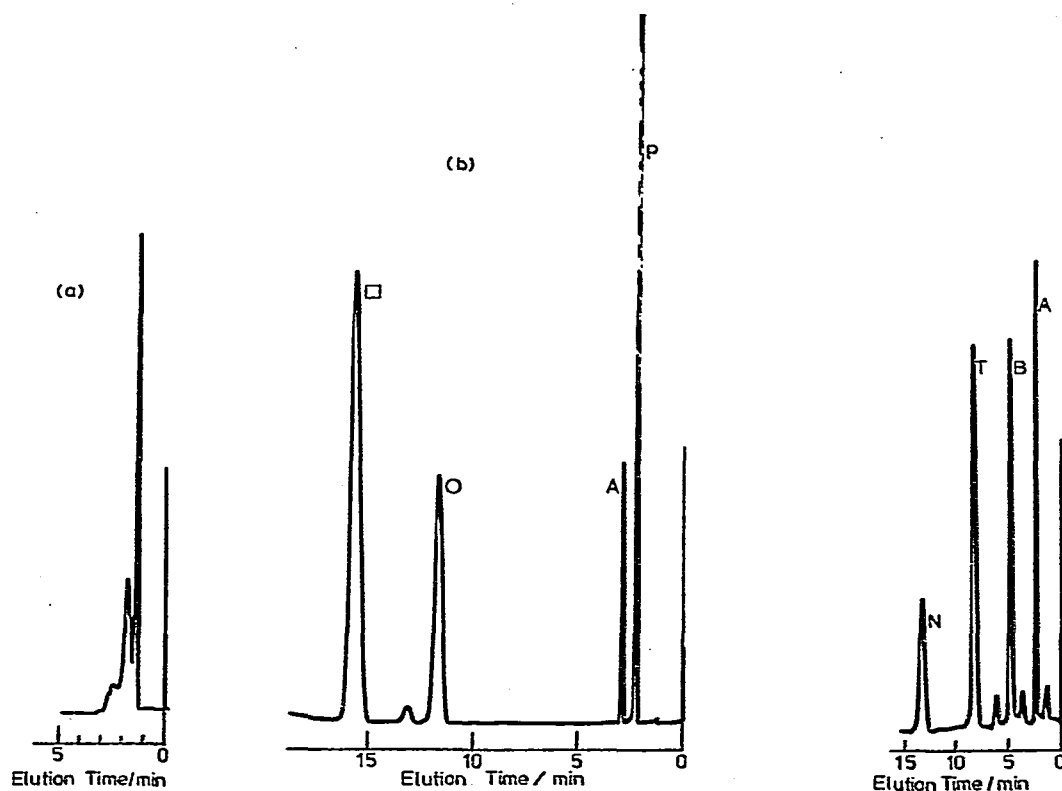


Fig. 4. (a) Demonstration of minimal retention of sample mixture of Fig. 1 on Hypersil from methanol-water (1:1) solvent system in absence of surfactant. (b) Separation of fluorenone (O) and naphthalene (□) on column of Fig. 4a in equilibrium with a solution of Tween 40 ( $2 \cdot 10^{-3} \text{ kg dm}^{-3}$ ) in methanol-water (1:1). Less retained eluities are acetophenone (A) and phenol (P), respectively.

Fig. 5. Separation of acetophenone (A), benzene (B), toluene (T), and nitrobenzene (N). Column, eluent, and conditions as in Fig. 4b.

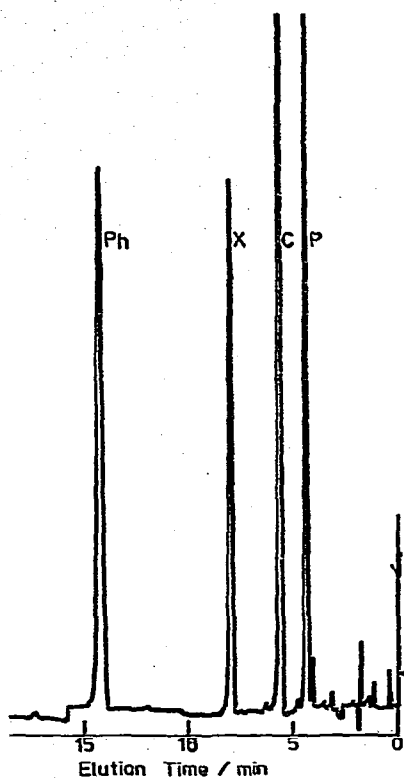


Fig. 6. Separation of phenol (P), *para*-cresol (C), 3,5-xylene (X), and phenetole (Ph) on a column (125 mm  $\times$  4.6 mm I.D.) of ODS-Hypersil ( $d_p \approx 5 \mu\text{m}$ ). Methanol-water (6:4) was passed through the column at ambient temperature at  $1 \text{ cm}^3 \text{ min}^{-1}$ .

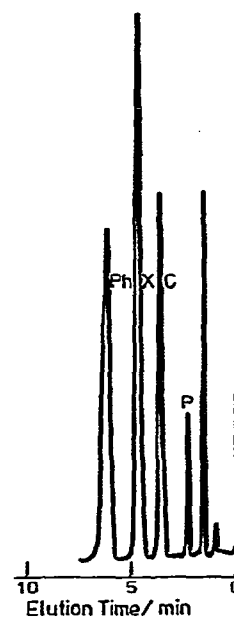


Fig. 7. Separation of sample mixture as shown in Fig. 7 with column and solvent system as in Fig. 4b.

The consequence of the first process would be an increase in retention of hydrophobic solutes with increasing brush density, but the second process would lead to an increasingly *polar* surface, studded with brushes with polyol termini instead of aliphatic hydrocarbon chains. Horváth *et al.*<sup>12</sup> have shown that the elution order of solutes from columns of alkyl-bonded silica packing materials is largely a function of the balance of surface and solute hydrophobicities. Accordingly, since essentially the same elution order is observed from the Tween-silica system as from octadecyl silica packings, retention in the former system should be largely due to the same properties of solute and surface.

If this hypothesis does accurately reflect the retention mechanism, then a steady increase in retentive power for chemically similar analytes should be observed with increasing brush density. This increase in retentive character will be counter-balanced by a decline in brush hydrophobicity which would ultimately lead to a decline in  $k'$  as surfactant concentration is increased. This behaviour has been observed with all single surfactant systems examined so far (*cf.* Figs. 1, 2 and 3 and refs. 1, 4 and 5), but may not be the case when ionic and non-ionic surfactants are used in combination<sup>6</sup>.

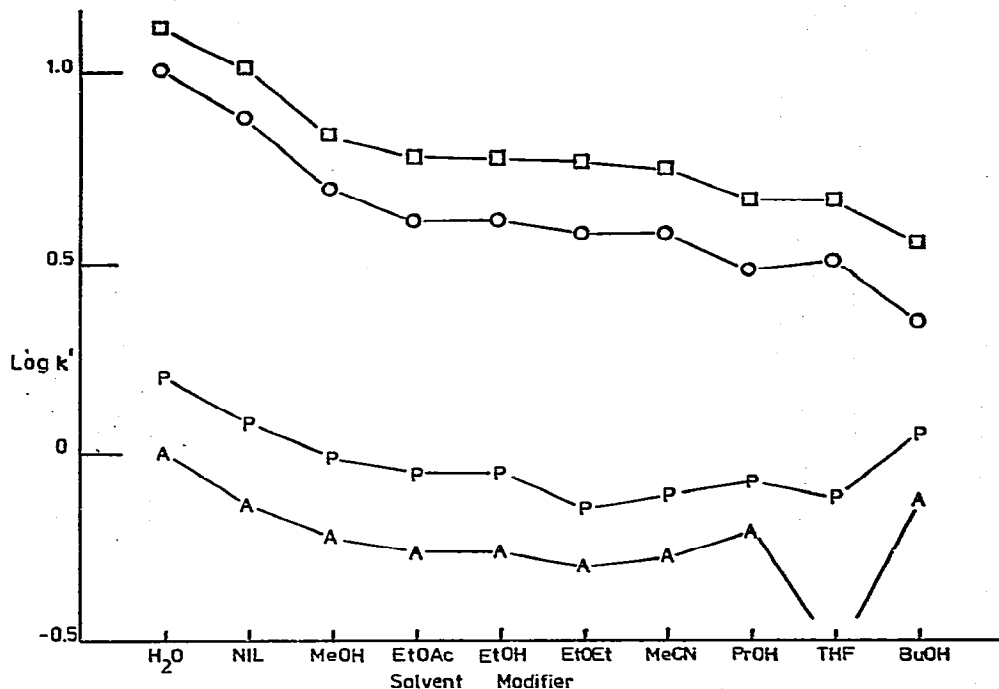


Fig. 8. Relationship of  $\log k'$  to 10% (v/v) addition of a range of solvent modifiers. Conditions as in Fig. 4b, except solvent now 10% additive plus 90% [methanol-water (1:1) containing Tween 40 ( $2 \cdot 10^{-3} \text{ kg dm}^{-3}$ )]. NIL = No addition; MeOH = methanol; EtOAc = ethyl acetate; EtOH = ethanol; EtOEt = diethyl ether; MeCN = acetonitrile; PrOH = propan-1-ol; THF = tetrahydrofuran; BuOH = butan-1-ol.

Control of both absolute and relative retention of several different elutes is usually maintained by choice of organic modifier and its concentration in "reversed-phase" chromatography. Tanaka *et al.*<sup>13</sup> and Bakalyar *et al.*<sup>14</sup> have clearly shown that selectivity in this LC mode is critically dependent on the nature and proportion of organic modifier in the eluent system. Fig. 8 is a composite diagram showing the effects on retention of four elutes of small changes in eluent composition at constant surfactant concentration. It shows that, as an added third solvent component decreases in polarity, there is a general trend to more ready elution. However, as would be expected by analogy with alkyl-bonded silica separations, the effects of solvent change are by no means equal on all four solutes. Clearly, control of organic modifier nature and proportion allows considerable control of selectivity in any separation attempted in this new HPLC mode.

#### CONCLUSIONS

Previous investigation<sup>1,4,5</sup> of the interaction between cationic surfactant and/or mixed non-ionic-anionic surfactants<sup>6</sup> dissolved in the eluent phase and LC column packings prepared from porous silicon(IV) and zirconium(IV) oxides and the present data on similar interactions of non-ionic surfactants with silicon(IV) oxide have



shown that this new mode of HPLC may be used to separate a wide range of analytes varying from sulphonic acids through neutral aromatic hydrocarbons and ketones to basic amines, amino acid esters, and peptides.

Selectivity in these separations may be enhanced by control of pH, solvent composition, ionic strength, chemical nature of the oxide surface, and selection of the appropriate surfactant. In the present work, Tweens (alkyl sorbitan polyethoxy polyols) were shown to be strongly bonded to silica gel surfaces; indeed, they proved to be impossible to wash off the column packing with several litres of water or aqueous alcoholic solvents. This technique of separation is rapidly and easily set up, and many of the GPLC separations presently done by "reversed-phase" chromatography on alkyl-bonded silicas could be carried out with equal efficiency using this new oxide-surfactant approach.

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