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SOAP CHROMATOGRAPHY—A NEW HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC TECHNIQUE FOR SEPARATION OF IONIZABLE MATERIALS

DYESTUFF INTERMEDIATES

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SUMMARY

A new form of high-performance ion-pair chromatography is described in which a detergent (cetyltrimethylammonium bromide) is added at around the 1% level to a propanol-water eluent. The column packing may either be a reversed-phase material (*e.g.*, SAS silica) or a silica gel (*e.g.*, Partisil). The method allows high-resolution separations of a wide range of sulphonic acids and derived dyestuffs containing one to three $-SO_3H$ groups. The degree of retention can be varied by changing cetrimide concentration, acidity of eluent, concentrations, and nature of additives.

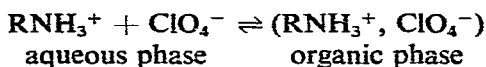
In the reversed-phase system it is probable that cetrimide-sulphonate ion pairs are extracted from the water-rich eluent into an adsorbed layer rich in propanol and cetrimide. In the silica gel system ion pairs are probably present in the eluent phase and are adsorbed onto the silica gel surface with propanol acting as a protective agent for both ion pairs and surface. Column performance in terms of plate height is equivalent to that obtainable in normal adsorption chromatography. Specific separations are shown of naphthylaminesulphonic acid isomers, including J-acid and γ -acid, and of sunset yellow from impurities.

INTRODUCTION

The chromatography of ionizable substances such as amines and organic acids often presents problems in respect of retention, plate efficiency, and peak symmetry. In order to separate such compounds by adsorption chromatography it is necessary to use polar eluents, which reveal the inhomogeneity of the adsorbent surface and produce tailed peaks. This is particularly liable to occur if the substance contains several readily ionizable or highly polar functional groups. It is also a general experience that buffering of an ionizable material either with acid or base is necessary since changes in the degree of ionization throughout any chromatographic band due to change in concentration over the band will cause peak asymmetry. In such cases it

is often better to employ ion-exchange chromatography. Buffering is again required to maintain the optimum balance between ionized and neutral forms but most ion-exchangers give relatively poor performance compared to adsorbents⁴.

Recently ion-pair chromatography²⁻⁷ has been shown to be valuable for high-performance liquid chromatography (HPLC) of ionizable compounds and to possess great versatility. In "normal-phase" ion-pair chromatography of say amines, RNH_2 , an aqueous acid, such as 0.1 *M* HClO_4 , might be used as stationary phase, the eluent being, say, a halocarbon containing a few per cent of a higher alcohol. The amine is then present in the stationary phase as a cation and is partitioned into the eluent as an ion pair according to the following equation



Schill, Persson and co-workers²⁻⁴ have pioneered this technique and it has recently been applied successfully by Karger and co-workers^{5,6} to the separation of biogenic amines, sulfonamides and other pharmacologically active material. Knox and Jurand⁷ have applied the technique to tricyclic tranquilizers.

"Reversed-phase" ion-pair chromatography can also be carried out where the stationary phase is organic and the eluent is an aqueous acid or base. Some separations on bonded supports may be of this type.

While ion-pair partition systems have great flexibility², it is always difficult to maintain the stability of two-phase partition systems over long periods and, of course, gradient elution is not possible except with reversed-phase bonded supports. In some cases the complexities of ion-pair equilibria may again result in tailed peaks, but this can usually be avoided.

In the present study of the separation of various naphthalenesulphonic acid derivatives, we were unable to solve the problem of tailing either by conventional adsorption or ion-pair partition methods using tetraalkylammonium counter ions in an aqueous stationary phase and we ascribed this to the very strong polarity of the $-\text{SO}_3^-$ group, which is probably able to displace water from silanol sites on the silica surface even when a strongly aqueous mobile or stationary phase is present. It occurred to us that this problem might be solved by employing an ion-pairing substance which might be expected to form highly stable ion pairs which would not dissociate in the presence of strongly hydrogen-bonding surface groups. We therefore examined the effect of adding the quaternary ammonium detergent cetyltrimethylammonium bromide, "cetrimide", to the aqueous alcoholic eluent for the chromatography of sulphonic acids. Initial experiments were unexpectedly successful and led to the more detailed study reported below. For conciseness we have termed this new technique "soap chromatography".

It was found in the initial experiments that while the addition of about 1% of "cetrimide" greatly improved the adsorption chromatography of a range of mono-, di- and trisulphonic acids on silica gel, the dependence of retention upon cetrimide concentration and other operating parameters was difficult to interpret. Accordingly, we turned to a reversed-phase support, Wolfson SAS silica, in which the surface of silica is covered by short alkylsilyl groups bonded to the surface by Si-C bonds⁸.

With this material the detergent was expected to be directly adsorbed by the support and we anticipated that the dependence of retention upon cetrimide concentration would be more readily interpretable.

Subsequently to this work it was pointed out to us that the effect of detergents on the paper chromatography of dyestuff intermediates *inter alia* had previously been studied by Farulla *et al.*⁹. They found that while addition of up to 1% cetrimide to the eluent increased R_F values (and so reduced k' values), pretreatment of the paper with cetrimide, or its addition to the solute sample had undesirable effects producing tailing and double spots in many cases. These results, for a rather different chromatographic system, contrast sharply with our own where cetrimide addition decreased R_F values (increased k') and brought about remarkable improvements in resolution, retention, peak symmetry and peak sharpness.

In regard to the chromatography of sulphonic acids, previous separations have been carried out by gas chromatography after derivatization^{10,11}, and by various classical liquid chromatographic methods such as column¹², thin-layer¹³, and paper chromatography^{9,14}, but results have generally been unsatisfactory. This inadequacy of conventional methods was confirmed by our initial experiments using Spherisorb silica as adsorbent and water-methylene chloride-methanol as eluent. These showed that while toluene could be eluted with a column efficiency of 1700 theoretical plates, the peak for sulphanic acid corresponded to only 130 plates.

Results previously obtained for HPLC of sulphonic acids were equally disappointing. Schmit and Henry¹⁵ in 1970 separated some naphthalenesulphonic acids on Zipax[®] SAX but the column efficiency was again poor with plate numbers around 100 on a column which for ideal solutes would have been expected to give 1000-2000 plates.

EXPERIMENTAL

The chromatographic equipment comprised an Orlita DMP 1515 reciprocating pump, an air-filled pulse damper (a 300-mm \times 5-mm-bore stainless-steel tube capped at one end), a pressure gauge, an injector and column as described elsewhere¹⁶, and a DuPont Type 410 single-wavelength (254 nm) UV detector.

Two column packing materials were used. For normal-phase chromatography Partisil 10 (Whatman Biochemicals, Springfield Mill, Great Britain) was used. Some experiments were carried out using a pre-production batch of characteristics slightly different from the later commercial material. For the high-performance chromatograms and plate height measurements we used a 6.5- μ m fraction obtained from Partisil 10 by fractionation in water. The surface area of Partisil is ~ 400 m² g⁻¹. For the reversed-phase experiments we used 7- μ m SAS silica with a surface area of ~ 200 m² g⁻¹, prepared by the Wolfson Liquid Chromatography Unit (Department of Chemistry, University of Edinburgh)⁸. This material is a spherical silica gel having short-chain hydrocarbon groups bonded to the surface. The material has virtually no adsorptive activity attributable to residual silanol groups.

Columns were of 5-mm-bore polished stainless steel and 100 or 120 mm in length. They were packed by pumping a slurry of the packing material (roughly 2 g for a column in 25 ml of supporting solvent) into the column with a constant pressure pump set at 2500 p.s.i. Methanol was used as the supporting liquid for Partisil and methyl iodide for SAS silica.

Column efficiencies were assessed by determining the reduced plate height, h , at various reduced velocities, v (for further discussion of the method see ref. 1) and comparing with previous data obtained with normal-¹⁷ and reversed-phase materials⁸.

The solutes used in the study were kindly gifted by I.C.I. Dyestuffs Division (Macclesfield, Great Britain) and are listed under their common names and formulae in Table I.

Sample solutions in water generally contained $\sim 0.1\%$ of each solute and injections were normally in the range of 1–5 μ l.

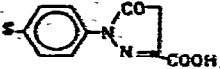


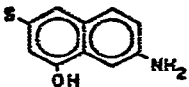
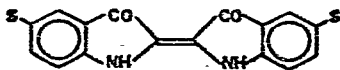

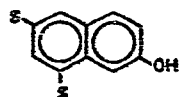
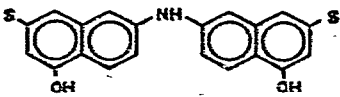
Cetrimide was obtained from BDH (Poole, Great Britain).

TABLE I
SULPHONIC ACIDS AND DYESTUFFS

In the formulae the $-\text{SO}_3\text{H}$ group is represented by S. Bracketted numbers give the elution order on Partisil for the six components also studied by the reversed-phase method.

Name	Formula	Elution order	
		Reversed phase	Silica gel
<i>A. Sulphonic acids</i>			
Monosulphonic acids			
5-Sulphoisatin			1 =
Sulphanilic acid		1	5 (2)
Naphthionic acid			4
Schäffer's acid		2	1 = (1)
Nevile and Winthers acid (NW-acid)			3
5-Sulphoanthranilic acid			12

TABLE I (continued)

Name	Formula	Elution order	
		Reversed phase	Silica gel
I-(4'-Sulphophenyl)-3-carboxy-5-pyrazalone			15
J-acid			
Dioxy-J-acid			
γ-Acid			
Disulphonic acids			
Blue-X			6
R-acid			9
G-acid			7
Di-J-acid			

(Continued on p. 22)

TABLE I (continued)

Name	Formula	Elution order	
		Reversed phase	Silica gel
<i>B. Azo dyes</i>			
Disulphonic acids			
Sunset yellow		3	8 (3)
Triazine			
Carmosine			10 =
Tartrazine		4 =	14 (5)
Ponceau MX		4 =	12 (4)
Trisulphonic acids			
Amaranth			10 =
Ponceau 4R		6	16 (6)

RESULTS AND DISCUSSION

A. Separations on SAS silica (Wolfson Unit reversed-phase material)

A 5:2 mixture by volume of water and propanol was used as eluent with the addition of between 0.031 and 2 g of cetrimide per 100 ml of eluent (denoted below by the % concentration of cetrimide) giving cetrimide concentrations from 8.6×10^{-4} to $5.5 \times 10^{-2} M$. The dependence of $\log k'$ upon cetrimide concentration is shown in Fig. 1. With no cetrimide present all solutes were unretained. The elution order is scarcely affected by the concentration of cetrimide. The curves for a variety of sulphonamic acids all show an increase of k' up to a concentration of about 0.25% ($7 \times 10^{-3} M$) cetrimide followed by a gradual decline as the concentration rises to 2%.

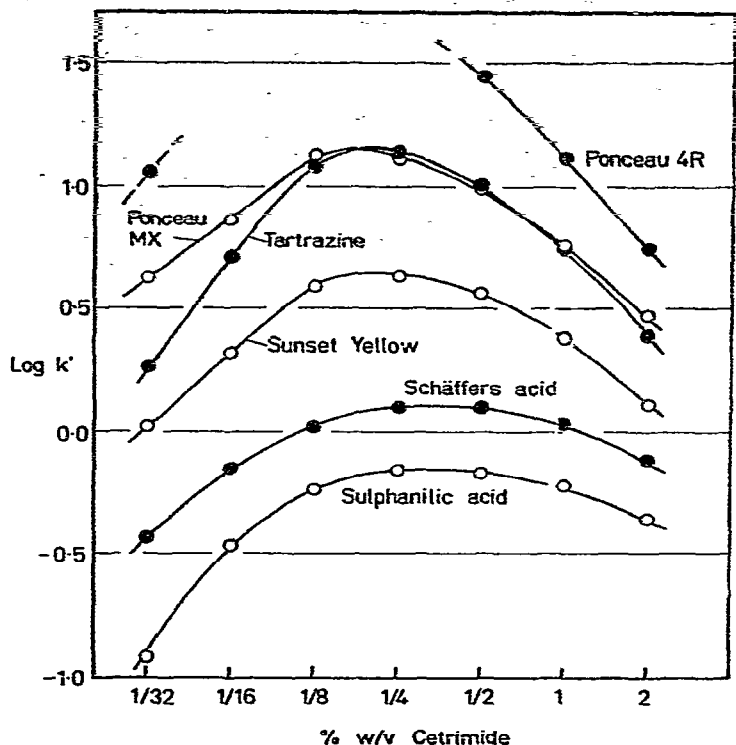


Fig. 1. Dependence of $\log k'$ upon w/v cetrimide concentration (g/100 ml eluent) for six dyestuffs and intermediates. Eluent, water-propanol (5:2); column packing, 7- μ m SAS silica (Wolfson Unit).

The ratio of water to propanol has no effect on elution order but k' increases with increase in water content as might be expected in a reversed-phase system. Finally k' in general increases as the number of sulphonic acid groups in the solute molecules increases.

The results are most simply explained if it is supposed that with the dilute solutions of cetrimide the detergent and sulphonic acid exist in the ionised form in the eluent phase, while cetrimide-sulphonate ion pairs are sorbed into the hydrophobic stationary phase, which is probably associated with a tightly held layer of propanol, for it is well known that organic solvents containing propanol are good solvents for cetrimide ion pairs⁴. It may also be anticipated that the surface of the reversed-phase packing will adsorb cetrimide cations which will form a molecular layer at the interface between the stationary and mobile phases. The overall situation may be represented by the interlinked equilibria shown in scheme A, where the subscripts aq and ads refer to the eluent and interface phases, respectively, and where underlined species are those thought to be present in high relative concentrations at low cetrimide concentrations.

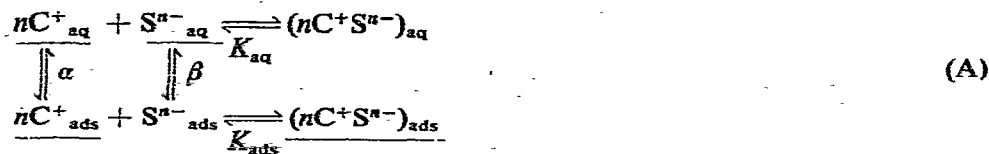


Fig. 2 shows that cetrimide is indeed adsorbed by the support in the concentration range of interest. The adsorption was measured in the following way: a column, packed with SAS silica, was equilibrated by passing water-propanol (5:2). The eluent was then switched to a mixture containing a known concentration (C) of cetrimide. A cetrimide concentration front thus moved down the column. If cetrimide was adsorbed by the packing material the break-through volume of the front V_R would exceed the void volume of the column V_0 . The amount of cetrimide adsorbed would then be $(V_R - V_0)C$. The break-through volume, V_R , was determined by collecting 0.2-ml aliquots of eluent in sample tubes containing a dilute solution of sunset yellow in a two-phase mixture made from equal volumes of water, methylene chloride and propanol. Initially the dye was partitioned strongly into the aqueous phase but where cetrimide was present in any aliquot the dye was extracted as the (C^+S^-) ion pair into the organic layer.

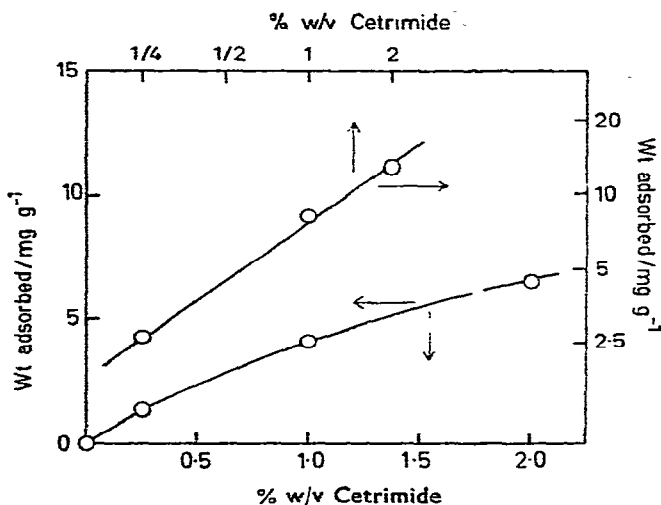


Fig. 2. Adsorption of cetrimide by SAS silica (Wolfson Unit) as a function of cetrimide concentration in eluent.

The adsorption isotherm shown in Fig. 2 is significantly curved and within experimental error obeys the simple Freundlich-type equation

$$[C^+_{ads}] = \alpha [C^+_{aq}]^{0.5} \quad (1)$$

For a 2% cetrimide concentration, 13.0 mg of cetrimide is adsorbed per gram of support. With a support surface area of about $200 \text{ m}^2\text{g}^{-1}$ this implies an area per molecule of about 1000 \AA^2 . Since the molecular volume of the cetrimide cation is about 500 \AA^3 , the degree of surface coverage by cetrimide is between 5 and 15%, depending upon the "depth" assigned to the cetrimide chain on the surface. This relatively low surface coverage suggests that the chain lies flat on the surface and that a considerable part of the surface must be covered by adsorbed propanol, the less polar component of the eluent phase.

If we now assume that the sorption of the sulphonic acid ions, S^{n-} , onto the surface is linear with respect to concentration in the eluent phase (which is reasonable since the S^{n-} concentration is very low), and that the equilibria in the eluent and surface phase obey the normal laws, we can write the following equilibrium conditions in addition to eqn. 1

$$[S^{n-}_{ads}] = \beta [S^{n-}_{aq}] \quad (2)$$

$$[(nC^+ S^{n-})_{aq}] = K_{aq} [(C^+_{aq})^n (S^{n-}_{aq})] \quad (3)$$

$$[(nC^+ S^{n-})_{ads}] = K_{ads} [(C^+_{ads})^n (S^{n-}_{ads})] \quad (4)$$

Assuming that the concentration of S^{n-}_{ads} is negligible compared to that of the sulphonate ion pair, we obtain for the distribution coefficient D between the surface and eluent phase

$$D = \frac{\alpha^n \beta K_{ads} [C^+_{aq}]^{0.8n}}{1 + K_{aq} [C^+_{aq}]^n} \quad (5)$$

This expression shows that at low C^+ concentrations, k' , which is proportional to D , will rise with $[C^+_{aq}]$. The exponent of $[C^+_{aq}]$, being $0.8n$, should increase with the number of sulphonic acid groups which are paired with cetrimide cations. In Fig. 1 the gradients at the lowest cetrimide concentrations are between 0.9 and 1.5, and therefore in the correct region. That for sulphanilic acid is high, but this could be due to experimental error since measurement of the lowest value of k' , viz. 0.12, is sensitive to the elution time of the solute assumed to be unretained. The gradient for Schäffer's acid is about 0.9, in fair agreement with the predicted value of 0.8 for a monosulphonic acid. The values for the three disulphonic acids are generally somewhat higher than unity but the correlation with the number of $-SO_3H$ groups is poor.

A rather better correlation exists between gradient and number of sulphonic acid groups for the decline of k' with increasing concentration of cetrimide in the 0.5–2% concentration range. The negative gradients for the mono-acids are about 0.5, for the di-acids 1.0 to 1.1, and for the tri-acid about 1.3. It is clear from eqn. 4 that if K_{aq} or $[C^+]$ are very large so that virtually all S^{n-} in the eluent phase are in the form of ion pairs at the highest cetrimide concentration, the largest negative gradient which can be allowed is $0.2n$, whereas the experimental data show negative gradients closer to $0.5n$. The likely explanation is the formation of micelles or at least of cetrimide ion clusters which can solubilize the sulphonate ions in the eluent phase. Formally this can be accommodated by writing the equilibrium in the eluent phase as



Eqn. 5 then becomes

$$D = \frac{\alpha^n \beta K_{ads} [C^+_{aq}]^{0.5n}}{1 + K'_{aq} [C^+_{aq}]^m} \quad (5')$$

where K'_{aq} is the equilibrium constant for reaction B. Evidently, when $m > n$, the negative gradient can be large. The data are explained if we set $m \sim 1.3$ with K'_{aq}

relatively large but not so large that the second term in the denominator dominates at low concentrations of cetrimide.

In pure water the critical micelle concentration (CMC)⁷ of cetrimide is $9.8 \times 10^{-4} M$, corresponding to 0.036% cetrimide¹⁸. The addition of propanol is known to lower the CMCs of long-chain quaternary ammonium salts but larger quantities may raise them again: for example Emerson and Holtzer¹⁹ found that in 2.0 *M* propanol [water-propanol (approx. 11:2)] the CMC of dodecyltrimethylammonium bromide was reduced from about 0.017 *M* to about 0.01 *M*. But they also found that by this concentration it was becoming extremely difficult to detect the onset of micelle formation by the conductivity method employed. It is therefore probable that at the concentration used in the present work, equivalent to about 3.7 *M*, there is no precise concentration of cetrimide at which micelle formation could be said to occur. It is probably more realistic to suppose that with increase in detergent concentration there is an increasing tendency for cetrimide cations to associate into micelle-like clusters of a range of sizes, the mean size increasing with concentration. Within this range of concentration increasing solubilization of the sulphonic acid groups in the eluent phase would be expected as the cetrimide concentration increased. From the present data it appears that each $-SO_3H$ group is on the average associated with about 1.3 cetrimide cations when the cetrimide concentration is between 1 and 2%. Further experiments at higher cetrimide concentrations would clearly be of interest.

The excellent chromatography which can be achieved in the reversed-phases system is illustrated by two representative chromatograms shown in Figs. 3 and 4. In the former the high selectivity of soap chromatography for isomeric naphthylamine-sulphonic acids is demonstrated, while in the latter the difficult separation of γ - and

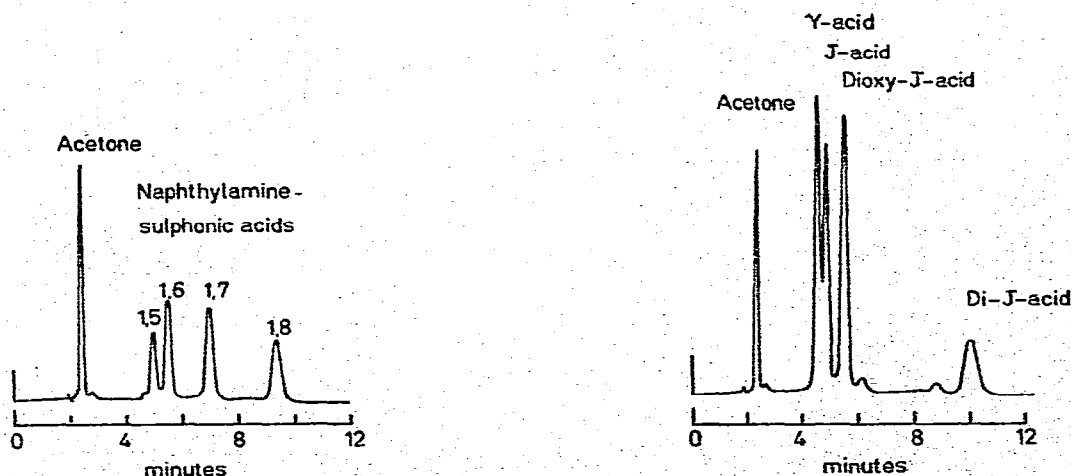


Fig. 3. Separation of naphthylaminesulphonic acid isomers by soap chromatography. Column, 120 mm \times 5 mm stainless steel; packing, 7- μ m SAS silica (Wolfson Unit); eluent, water-propanol (5:2) containing 1% w/v cetrimide. Peak elution order: acetone followed by 1.5, 1.6, 1.7, and 1.8 isomers of naphthylaminesulphonic acid.

Fig. 4. Separation of dye intermediates. Conditions, as for Fig. 3. Elution order: acetone, γ -acid, J-acid, dioxy-J-acid, di-J-acid.

J-acids is shown. The plate height for the final peak in Fig. 3 is $32 \mu\text{m}$, giving a reduced plate height of $h = 4.5$ at a reduced velocity of about 25 [based upon the Wilke-Chang equation²⁰ (see also ref. 21) with an eluent viscosity of 2.2 cP]. This result implies a very high efficiency for solutes of exceedingly high polarity.

B. Separations on silica gel (Partisil)

Whereas with cetrimide present a 5:2 water-propanol mixture was required for reasonable retention of solutes by SAS silica, a much less hydrophilic 1:3 mixture was necessary for adequate retention on silica gel.

Fig. 5 shows the dependence of the capacity ratio, k' , on cetrimide concentration over a relatively small concentration range for sixteen sulphonic acids using 1:3 water-propanol mixtures. The data were obtained with the pre-production batch of Partisil. In the absence of cetrimide all solutes were unretained. Fig. 6 shows the dependence of k' over a wider range of cetrimide concentrations for the more restricted group of acids examined under reversed-phase conditions. These data were obtained on a commercial sample of Partisil; the absolute values of k' are somewhat different with these two samples although the elution order is unchanged. Under the propanol-rich conditions used the formation of micelles is unlikely and the cetrimide is probably present as ion pairs solubilised by propanol²². The surface of the silica gel is likely to be covered by a layer containing water and propanol in a ratio considerably higher than 1:3 due to the strong adsorption of water by silanol groups.

The broad trends are clear. The degree of retention increases continually as the cetrimide concentration increases, and there is no indication of the maximum found with the reversed-phase system, although there is a flattening out of the plots

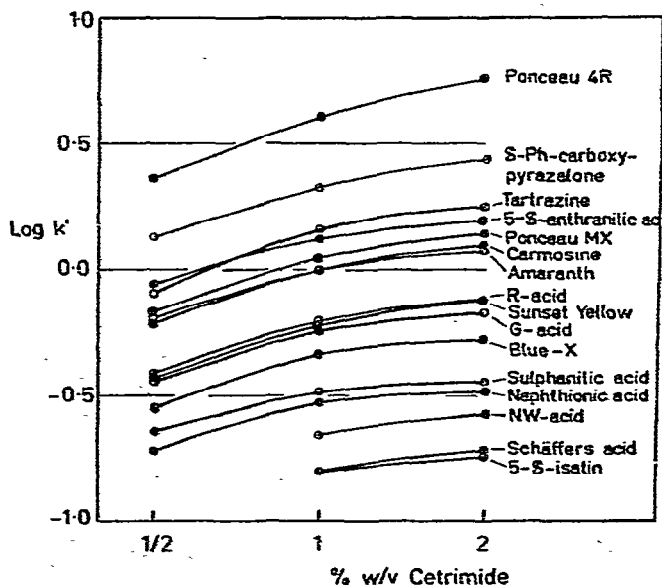


Fig. 5. Dependence of k' upon cetrimide concentration for sixteen dyestuffs and intermediates. Eluent, water-propanol (1:3); packing, Partisil 10. The $-\text{SO}_3\text{H}$ group is represented by $\$$.

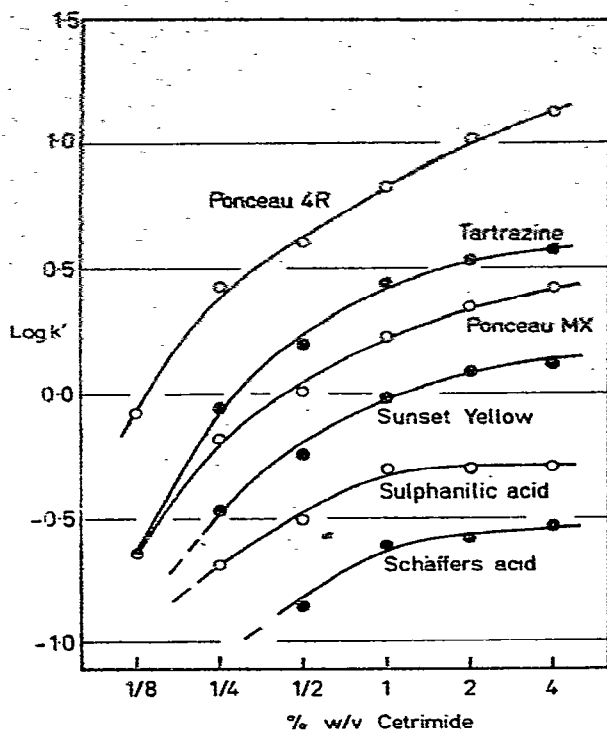


Fig. 6. As Fig. 5 but for six compounds over a wider range of cetrimide concentrations.

at high concentrations of cetrimide. The order of elution is essentially unaffected by cetrimide concentrations and is much the same as that observed with the reversed-phase packing. Although retention generally increases with the number of sulphonic acid groups in the molecules, there are notable exceptions in that 5-sulphoanthranilic acid and 1-(4'-sulphophenyl)-3-carboxy-5-pyrazalone, both monosulphonic acids containing carboxyl groups, are eluted towards the end of the disulphonic acid group. The carboxyl group in these and in tartrazine apparently increases retention substantially.

Fig. 7 shows that k' is reduced by increasing the acidity of the eluent and that the change from neutral to 0.025 *M* sulphuric acid produces roughly a fourfold decrease in k' . The gradients of the $\log k'$ vs. pH plots at low pH approach unity for all solutes irrespective of the number of sulphonic acid groups in the molecules. Tartrazine, a disulphonic acid containing a carboxyl group, shows a rather steeper gradient at higher pH than the other acids due probably to ionization of the carboxyl group but approximately the same gradient at low pH when the carboxyl group will be fully protonated.

Fig. 8 shows the effects on k' of diluting the standard 1:3 water-propanol mixture containing 1% cetrimide by adding 20% v/v of a number of additives. The addition of water reduced k' , while the addition of propanol and other alcohols increased k' . The addition of relatively large polar molecules produced effects similar to those produced by the addition of alcohols, while the addition of a non-polar

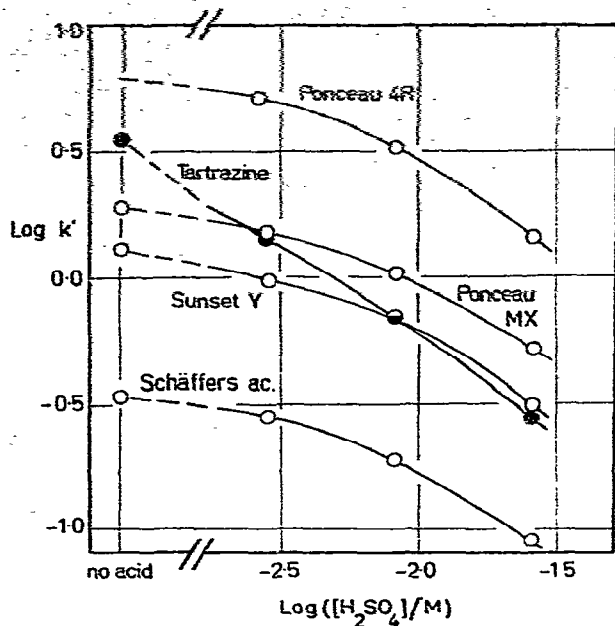


Fig. 7. Dependence of k' upon acid concentration for elution of five dyestuffs and intermediates. Standard eluent, water-propanol (1:3), containing 1% w/v cetrimide; packing, Partisil 10.

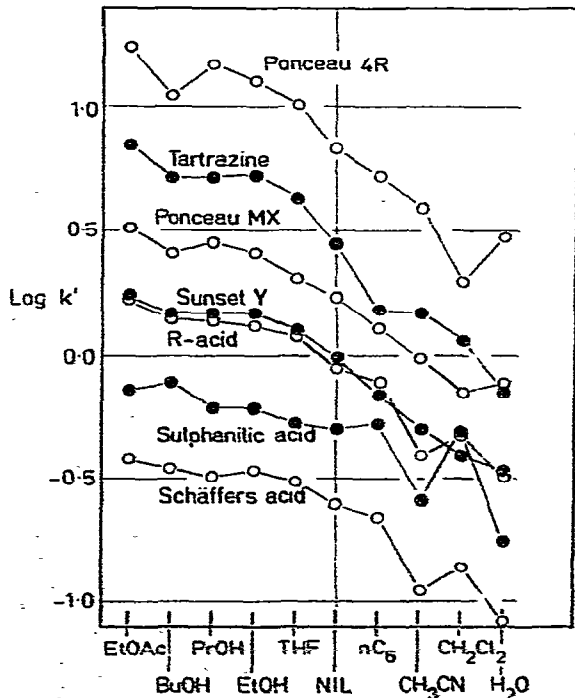


Fig. 8. Effects upon k' of addition of 20% v/v of a number of additives to the standard eluent for seven dyestuffs and intermediates. For other details, see Fig. 7.

modifier such as hexane had little effect on retention except for tartrazine. The effects of methylene chloride and acetonitrile were variable but in general were to reduce k' . Evidently, additives which tend to solvate ion pairs increase retention, while those which fail to solvate ion pairs decrease retention. From the practical point of view it can be seen that the addition of non-solvating modifiers can significantly alter the retention order and this may be useful in improving the separation of certain components. Thus, for example, γ - and J-acids cannot be separated by the standard 1:3 water-propanol mixture but, as shown by Fig. 9, are well resolved when methylene chloride is present.

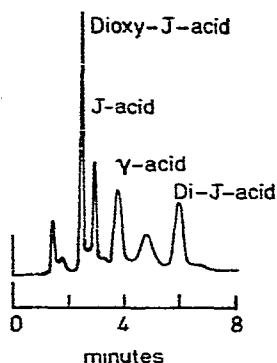
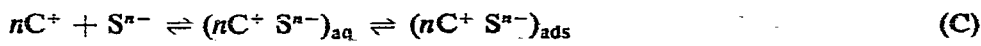


Fig. 9. Separation of dye intermediates. Column, 100 mm \times 5 mm stainless steel; packing, 6.5- μ m Partisil; eluent, propanol-methylene chloride-water (70:40:12) containing 2% w/v cetrimide. Elution order: dioxy-J-acid, J-acid, γ -acid, di-J-acid.

The data presented in Figs. 5-8 reflect a complex mechanism of retention which we cannot claim to have elucidated completely. The fact that the elution order is similar to that found for the reversed-phase system suggests that adsorption of ion pairs must be the basis of retention. However, we have established by direct measurement (using the same technique as for the reversed-phase system) that cetrimide is not adsorbed by the silica gel to any extent and therefore we are not dealing here with the extraction of ion pairs from a solution where the cetrimide and sulphonic acids are predominantly present as ionized single molecule forms. More probably cetrimide-sulphonate ion pairs are a predominant form of the sulphonic acids in the eluent phase, which is much richer in propanol than the eluent used in Part A. As the cetrimide concentration is increased a higher and higher proportion of the sulphonate groups will be paired with cetrimide. Since in the absence of cetrimide, the acids are essentially unretained, it seems that the increasing retention with increasing cetrimide concentration reflects the state of the equilibrium C, where again the predominant species are underlined.



Increasing the concentration of H_2SO_4 may have two general effects. The first is to reverse the equilibrium D by increase in (H^+)



The second is to decrease the availability of cetrinide for formation of ion pairs by reaction E



If the effect of H_2SO_4 is largely due to the former, then it would be necessary for n to equal unity in the general scheme C or that the ionization of only one of the $-\text{SO}_3^-$ groups in di- and trisulphonic acids could be suppressed. Since neither of these seem probable, it is most likely that the addition of H_2SO_4 reduces retention mainly by competing with the sulphonic acids in the formation of ion pairs and effectually reducing the cetrinide concentration available for this purpose. In this connection it is notable that the cetrinide concentration was around $2.7 \times 10^{-2} M$, while that of the H_2SO_4 when k' is significantly reduced was between 10^{-2} and $3 \times 10^{-2} M$.

The effect of water in reducing retention and the reverse effect of alcohols are readily interpreted in terms of their effect on the composition of the layer in contact with the adsorbent surface. Retention of the ion pairs which themselves will tend to adsorb propanol rather than water molecules will be greater the higher the proportion of the solvating molecules which the adsorbent surface can accommodate.

Replacement of water on the surface by propanol will make the surface layer more lipophilic, so will tend to increase retention, whereas the reverse will be true if propanol is replaced by water. Ethyl acetate and tetrahydrofuran apparently behave in much the same way as alcohols. Acetonitrile, on the other hand, will be rather strongly adsorbed by a silanol surface yet will not solvate ion pairs so well as propanol. It will therefore reduce retention. Hexane will be excluded from the surface layer and should therefore have little effect on retention as is observed. Methylene chloride has an intermediate effect, sometimes behaving like acetonitrile and sometimes like hexane.

We conclude that the most satisfactory model for the retention on silica gel is one wherein cetrinide sulphonate ion pairs are present in the eluent and are solvated predominantly by propanol molecules. These solvated ion pairs are then adsorbed most readily onto a surface which can itself be heavily solvated by propanol: the propanol molecules thus act as a binder to hold the lipophilic ion pairs onto the hydrophilic silanol surface. Any additive which interferes with this binding function, say, by displacing propanol from the surface of either the adsorbent or the ion pair or any additive which prevents the formation of ion pairs in the eluent (*e.g.*, a strong acid), will reduce retention since neither cetrinide nor the sulphonic acids are individually retained to a significant extent with water-propanol (1:3) as eluent. By contrast to the situation with a reversed-phase packing, where a predominantly aqueous eluent was used, micelles or detergent clusters are not involved.

The variation of retention with temperature is in the direction expected, that is, increase of temperature decreases k' . The usual Van 't Hoff type of plot indicates heats of adsorption in the range of 20–30 kJ mol^{-1} .

Column performance was assessed by determining the reduced plate height

vs. reduced velocity curves for two standard solutes in normal adsorption chromatography (anisole and nitrobenzene) and for three sulphonic acids in soap chromatography. The results are shown as reduced plate height vs. reduced velocity plots in Fig. 10. Diffusion coefficients required for the calculation of the reduced velocity were calculated by the Wilke-Chang equation²⁰, which gave D_m (anisole) = $3.3 \times 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$ and D_m (cetrimide-sulphanilic acid ion pair) = $1.5 \times 10^{-6} \text{ cm}^2 \text{ sec}^{-1}$. The second value was based upon a directly determined viscosity of 2.8 cP for water-propanol (1:3) containing 1% cetrimide. The presence of cetrimide increased the viscosity of water-propanol (1:3) by about 5%. It is clear that there is little if any difference in performance between the two systems and that a minimum plate height of around two particle diameters is attainable in both. The full performance of HPLC which we are accustomed to expect in adsorption chromatography^{1,8,17} can therefore be obtained in soap chromatography. In practice, eluents for soap chromatography are likely to be several times more viscous than for normal adsorption chromatography and the absolute mass transfer rate correspondingly lower. This means that analyses of equivalent plate performance can be performed on columns of the same dimensions operated at the same pressure drops, but that the time taken will be significantly longer. This disadvantage can be removed by working at a higher temperature where the viscosity of the eluent is reduced, and mass transfer rates proportionately increased, or by using higher pressures.

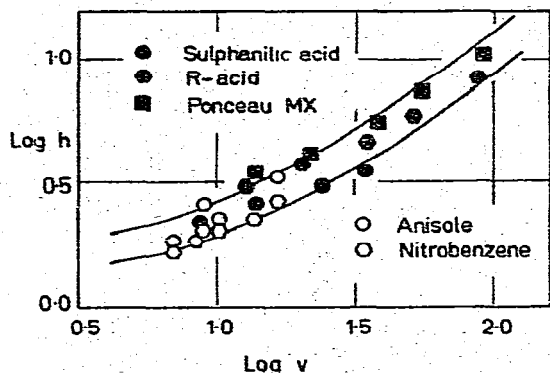


Fig. 10. Logarithmic plot of reduced plate height, h , against reduced velocity, v , for the elution of standards and dyestuff intermediates. Packing, 6.5- μm Partisil. Eluents: hexane for adsorption chromatography of anisole and nitrobenzene; standard eluent (see Fig. 7) for soap chromatography of sulphanilic acid, R-acid, and Ponceau MX.

Two separations carried out using the ion pair adsorption system are shown in Figs. 9 and 11. Fig. 9 again illustrates the excellent resolution of γ - and J-acids which can now be achieved, but in the reverse order to that obtained using SAS silica. Fig. 11 shows the analysis of the dye sunset yellow for impurities including residual uncoupled sulphanilic and Schäffer's acids. A small proportion of triazine, a known impurity in sunset yellow, has been added to the mixture.

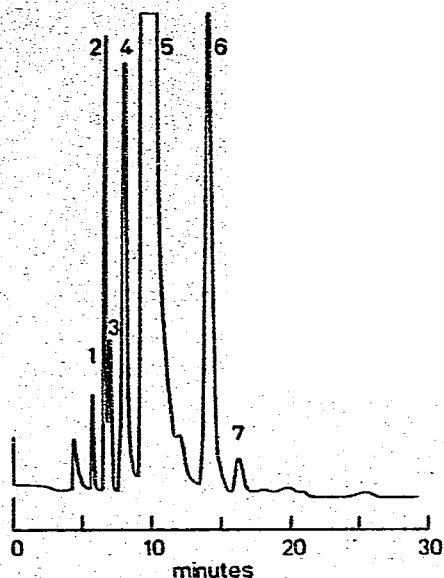


Fig. 11. Separation of impurities in sunset yellow. Conditions, as for Fig. 9. Injection, $5 \mu\text{l}$ of an 0.2% solution of dyestuff. 1 = Schäfer's acid; 2 = unknown; 3 = sulphanilic acid; 4 = triazine (added); 5 = sunset yellow; 6 and 7 = unknown.

CONCLUSIONS

The technique of "soap chromatography" has proved to be remarkably effective for the resolution of sulphonic acids of interest in the dyestuff industry. The technique employs a long-chain cationic detergent, in the present work cetyltrimethylammonium bromide, which forms ion pairs with the sulphonate groups.

Separations can be carried out either on bonded reversed-phase materials or on adsorbents. The chromatographic efficiency is similar in both cases and equivalent in terms of plate height to that obtainable in high-performance adsorption chromatography.

The technique can equally well be applied to amines if an anionic detergent is used.

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REFERENCES

- 1 J. N. Done, J. H. Knox and J. Loheac, *Applications of High-Speed Liquid Chromatography*, Wiley, New York, 1974.
- 2 S. Eksborg and G. Schill, *Anal. Chem.*, 45 (1973) 2092.
- 3 B.-A. Persson, *Acta Pharm. Suecica*, 5 (1968) 343.

- 4 G. Schill, R. Modin and B.-A. Persson, *Acta Pharm. Suecica*, 2 (1965) 119.
- 5 B. L. Karger, S. C. Su, S. Marchese and B.-A. Persson, *J. Chromatogr. Sci.*, 12 (1974) 678.
- 6 B. L. Karger and B.-A. Persson, *J. Chromatogr. Sci.*, 12 (1974) 521.
- 7 J. H. Knox and J. Jurand, *J. Chromatogr.*, 103 (1975) 311.
- 8 J. H. Knox and A. Pryde, *J. Chromatogr.*, 112 (1975) 171.
- 9 E. Farulla, C. Iacobelli-Turi, M. Lederer and F. Salvetti, *J. Chromatogr.*, 12 (1963) 255.
- 10 J. S. Parsons, *J. Chromatogr. Sci.*, 11 (1973) 659.
- 11 J. J. Kirkland, *Anal. Chem.*, 32 (1960) 1388.
- 12 D. M. Marmion, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 723.
- 13 H. de Clercq and D. L. Massart, *J. Chromatogr.*, 93 (1974) 243.
- 14 J. C. Brown, *J. Soc. Dyers Colour.*, 85 (1969) 137.
- 15 J. A. Schmit and R. A. Henry, *Chromatographia*, 3 (1970) 497.
- 16 J. H. Knox, *Chem. Ind. (London)*, (1975) 29.
- 17 J. H. Knox, J. Jurand and G. R. Laird, *Proc. Soc. Anal. Chem.*, (1974) 310.
- 18 P. Mukerjee and K. J. Mysels, *Critical Micelle Concentrations of Aqueous Surfactant Systems*, National Bureau of Standards, Washington, Publication No. NRDRS-NBS-36.
- 19 M. F. Emerson and A. Holtzer, *J. Phys. Chem.*, 71 (1967) 3320.
- 20 C. R. Wilke and P. Chang, *Amer. Inst. Chem. Eng. J.*, 1 (1955) 264.
- 21 J. H. Knox and M. J. Saleem, *J. Chromatogr. Sci.*, 7 (1969) 614.
- 22 A. W. Ralston and C. W. Hoerr, *J. Amer. Chem. Soc.*, 68 (1946) 851.