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LIQUID MODIFIERS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY WITH GRAPHITIZED CARBON BLACK ADSORBENTS

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SUMMARY

Based on the solvophobic theory, two qualitative models have been derived for describing the role played by the liquid modifier when graphitized carbon black adsorbents are used as stationary phases for reversed-phase liquid chromatography. The accuracy of these models has been tested by measuring the effect of water–methanol and tetraethylenepentamine–methanol solutions on the retention of several eluates of practical interest. These results, combined with measurements of the asymmetry factor of the chromatographic peaks, suggest that liquid modifiers affecting the surface properties of the adsorbent are suitable for modulating the carbon retention in reversed-phase liquid chromatography. Rapid elution without tailing of many polar and non-polar compounds can be obtained without significant losses in the carbon selectivity and this improves the sensitivity and efficiency of the chromatographic system.

INTRODUCTION

Although reversed-phase liquid chromatography (RPLC) is one of the most powerful and widely used techniques for the separation of complex organic mixtures, its present success almost entirely relies on the performance achievable with high-performance liquid chromatography (HPLC) columns packed with chemically bonded phases (CBP)[1]. As the reproducibility and the efficiency of these columns can be maintained as long as the mobile phase is kept at pH < 7[2], increasing attention has been devoted to the development of non-polar adsorbents exhibiting an improved pH stability over the alkyl-bonded silica materials. This research has lead to a renewed interest in carbon-based phases in general and particularly in graphitized carbon black (GCB) adsorbents which are known in gas chromatography (GC) as highly homogeneous and inexpensive stationary phases characterized by a high selectivity for geometrical isomers and a high thermal and chemical stability[3]. HPLC experiments carried out using columns packed with pyrolytically hardened carbon blacks[4,5], GCB[6] and, more recently, porous glassy carbon covered with a thin graph-

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ite layer[7] have led to the conclusion that graphitic carbons are sufficiently inert to be used in RPLC.

The strong adsorbtive nature of the flat and rigid carbon surface[5] represents, however, a limiting factor on the widespread use of these stationary phases in RPLC as the so-called hydrophobic expulsion from aqueous solutions is often unsuitable for achieving the optimum chromatographic conditions necessary for many practical separations. With pure methanol, the use of HPLC columns packed with GCB particles is limited to the separation of relatively low-boiling isomeric compounds[4–7] whereas the elution of large molecules, such as polyaromatic hydrocarbons, is possible only when non-polar eluents of high eluotropic strength are used as mobile phases[4,5,7].

In order to find out whether the addition of liquid modifiers to the eluent can provide a suitable means of circumventing these limitations, the effect of binary solvent mixtures on the carbon retention has been analysed in the light of the solvophobic theory[8]. By using a variant of the equation developed by Horváth *et al.*[2] for CBP, two qualitative models have been derived. One, similar to that usually adopted for CBP, considers the case in which the modifier affects the retention by changing the solubility of the eluite in the mobile phase, while the other describes the case in which the modifier affects mainly the surface properties of the carbon and the solute–mobile phase interactions occurring in the adsorbed phase. Experiments carried out with dilute water–methanol and tetraethylenepentamine (TEPA)–methanol solutions confirm the different effects predicted by the two models. A comparison between the results suggests that the role of modifiers preferentially adsorbed on the carbon is analogous to that played by the mobile phase in gas–liquid–solid chromatography (GLSC)[9,10]. Small additions of TEPA to methanol are proven to be particularly effective for changing the capacity ratio of polar and non-polar solutes as well as the symmetry of the chromatographic peaks. As a result, rapid elution of the sample without tailing is achieved by simply adjusting the concentration of the modifier in the mobile phase. The sensitivity and efficiency of the chromatographic system is improved without any significant loss in the carbon selectivity. The possibilities and limitations associated with the use of liquid modifiers affecting the surface properties of the adsorbent are critically discussed.

THEORETICAL

Retention model for carbonaceous adsorbents in RPLC

Among the various theoretical models developed for predicting the effect of the mobile phase in RPLC[2,11], the solvophobic one appears to be the most accurate as it provides a comprehensive picture of the solute–mobile phase interactions in solution and takes into account the specific nature of the adsorbent used[2,12].

In the solvophobic treatment, the adsorption of a solute S on the surface C is viewed as a reversible association process occurring in the presence of the eluent E, *i.e.* $S + C \rightleftharpoons SC$. This equilibrium can be conceptually split into two processes which take place simultaneously but which are in competition with each other. One is the reversible adsorption of the solute occurring in the gas phase, while the other is the solution process necessary for transferring the eluite from the gas phase into the

liquid solvent. The free energy change for the adsorption process taking place in liquid chromatography (LC) (ΔG^{ads}_{liquid}) can be thus written as

$$\Delta G^{ads}_{liquid} = \Delta G^{ads}_{gas} - \Delta G^{net}_{solvent} \quad (1)$$

where ΔG^{ads}_{gas} is the free energy for the adsorption process of S from the gas phase and $\Delta G^{net}_{solvent}$ is the free-energy change relative to the solution (or partition) equilibrium of S in E. ΔG^{ads}_{liquid} can be evaluated directly from HPLC chromatographic measurements as

$$-\Delta G^{ads}_{liquid}/RT = \ln k'_{LC} + \ln \varphi_{LC} \quad (2)$$

where k'_{LC} is the capacity ratio of S and φ_{LC} is the phase ratio of the column. The energy term associated with the net solvent effect can be evaluated from solubility data or calculated theoretically by using the treatment developed by Sinanoglu[8]. Theoretically, the dissolution process of S in E is assumed to occur in two steps: first, a suitable cavity has to be made in the solvent to accommodate the solute into solution, and second, after the solute is placed into the cavity, it interacts with the surrounding solvent molecules. The energy of interaction comprises van der Waals as well as electrostatic interactions. As transferring a solute from the gas phase into the solvent is accompanied by a change in entropy, a free-volume reduction term is necessary to balance the energetics of the solution process. Each one of the terms in which the solvent effect is split can be calculated from a knowledge of the physicochemical properties of each species (E, S, C, SC) using detailed expressions from the literature[2,12,13].

While the close dependence of $\log k'_{LC}$ on the measurable quantities which enter into $\Delta G^{net}_{solvent}$ (surface tention of the mobile phase, molecular surface area of the solute, *etc.*) has been shown unambiguously by the elegant experiments reported by Horváth and co-workers[2,13] on CBP, very little is known about the dependence of $\log k'_{LC}$ on the surface potential energy term, ΔG^{ads}_{gas}. In order to account for surface effects, we have recently proposed a variant of eqn. 1 in which ΔG^{ads}_{gas} is expressed in terms of the capacity ratio of *S* measured in GLSC when the solid surface is covered with one monomolecular layer of eluent molecules ($\vartheta_E \approx 1$). This value of the surface coverage has been selected since it is known from GLSC[9,10] experiments that further additions of liquid phase beyond the first monolayer only slightly affect the GCB retention. If an identical column is used, the values of V_S (the volume of eluent corresponding to the monolayer) and V_0 (the column dead volume) are the same in LC and GLSC. The phase ratios, φ_{LC} and φ_{GLSC} cancel each other out and eqn. 1 becomes

$$b \log k'_{LC} = b \log k'_{GLSC} - \Delta G^{net}_{solvent} + C \quad (3)$$

where $b = -2.3RT$, k'_{GLSC} is the capacity ratio of S measured in GLSC at $\vartheta_E \approx 1$ and *C* is a constant correction term for the free-volume reduction, eqn. 3 has been tested by comparing the values of $\Delta G^{net}_{solvent}$ obtained from chromatographic measurements, solubility data and theoretical calculations[12]. Although the absolute values obtained from the various methods are somewhat different, a fair agreement is found

in the measure of the eluotropic strength of some pure eluents, and the elution sequence expected for some isomeric eluites is correctly predicted. The way in which eqn. 3 is expressed is particularly useful for discussing the effect of the liquid modifier, as the surface potential-energy term and the solute–eluent interactions in the adsorbed phase can be predicted from GLSC data previously collected for GCB[9,10].

Influence of the liquid modifier on the GCB retention

When a modifier M is added dynamically to the eluent E, a competition or displacement process between M and E can take place on the first monolayer coating the adsorbent surface[11,14]. The new equilibrium established within the column can be written as

$$M + nEC \rightleftharpoons MC + nE \tag{4}$$

where a molecule of M in the mobile phase displaces some number (n) of preadsorbed molecules of eluent EC to yield an adsorbed modifier molecule MC and n molecules of desorbed eluent E.

The relative concentration of the various species present in the mobile as well in the adsorbed phase will depend upon the normalized free-energy change of the process ($\Delta G^0/2.3RT = \Delta G_{ME}$). According to eqn. 3, this energy can be expressed by the following relationship:

$$\Delta G_{ME} = (\Delta G_M^{gas} - \Delta G_M^{sol}) - n(\Delta G_E^{gas} - \Delta G_E^{sol}) \tag{5}$$

where ΔG_M^{gas} and ΔG_E^{gas} are, respectively, the free-energy changes for the adsorption process of M and E measured in the gas phase and ΔG_M^{sol} and ΔG_E^{sol} are the free-energy changes relative to the solution process of M and E from the gas into the mobile phase.

Depending upon the physico-chemical properties of M and E and their relative concentrations in the mobile phase, the equilibrium of eqn. 3 can be moved toward the preferential adsorption of M or E. The change in retention of the solute S observed in these two limiting situations can be a useful approach for differentiating the working mechanism of a given liquid modifier as well characterized effects can be observed.

Mechanism I

Let us consider the case where the following condition is verified:

$$n(\Delta G_E^{gas} - \Delta G_E^{sol}) \ll (\Delta G_M^{gas} - \Delta G_M^{sol}) \tag{6}$$

The equilibrium of eqn. 4 leads to the formation of EC and the eluent is preferentially adsorbed with respect to the liquid modifier.

For dilute solutions of M in E, we can assume that M is practically unretained and its molar fraction present on the first monolayer can be neglected, *i.e.* $\vartheta_M \approx 0$. Under these conditions, the surface potential energy of carbon and the solute–mobile phase interactions in the adsorbed phase are unaffected by the addition of M and

$\log k'_{GLSC}$ is constant. The change in retention of the solute S injected into the column becomes dependent solely upon the interaction between M and S in the mobile phase. From eqn. 3 we can write

$$\frac{\delta \log k'_{LC}}{\delta N_M} = \frac{\delta \Delta G^{net}_{solvent}}{\delta N_M} \tag{7}$$

where N_M is the molar fraction of M measured at the column inlet.

The shape of the curves obtained by plotting $\log k'_{LC}$ *vs.* N_M is dependent upon how the various free-energy terms in which the net solvent effect is split varies with the mobile-phase composition. From the data obtained by Melander and Horváth[13] on CBP, a monotonic dependence of $\log k'_{LC}$ on N_M can be expected when dilute solutions of M are used and undissociated solutes are analysed. For instance, with water–methanol solutions, the trend of the curves is influenced most by the free-energy term relating to the cavity formation. The slope of the plots obtained with various solutes is a linear function of the surface tension (γ) of the mobile phase and depends on the hydrocarbonaceous surface area (HSA) of the solute. Plots of $\log k'_{LC}$ *vs.* increasing amount of water are not linear since the surface tension correction which enters into the cavity term is non-linear with the mobile phase composition[11].

Mechanism II

Let us now consider the other situation where

$$(\Delta G^{gas}_M - \Delta G^{sol}_M) \ll n(\Delta G^{gas}_E - \Delta G^{sol}_E) \tag{8}$$

The equilibrium of eqn. 4 is moved towards the formation of MC and, even for dilute solutions of M in E, we can assume that a substantial displacement of the eluent molecules adsorbed onto the first monolayer takes place.

In this case, the surface potential-energy term becomes a function of the molar fraction of M present in the first monolayer (ϑ_M) and the value of k'_{GLSC} is dependent on the mobile-phase composition. Provided that no chemical reactions between M and S are taking place and very dilute solutions (< 1% v/v) are used as the mobile phase, the change in the interaction in solution can be neglected and the retention of S assumed to be dependent solely upon the surface potential-energy term. According to eqn. 3 we can write that

$$\frac{\delta \log k'_{LC}}{\delta N_M} = \frac{\delta \log k'_{GLSC}}{\delta \vartheta_M} \tag{9}$$

Probably, the conditions under which eqn. 8 describes the effect of the liquid modifier are satisfied when M is selected from among the polar stationary phases commonly used in GLSC (temperatures above 100°C are commonly used without desorption of the liquid phase) and E is a low-molecular-weight eluent (methanol, acetonitrile, diethyl ether).

The retention of S can be adjusted until the first monolayer of M is reached

and the dependence of $\log k'_{LC}$ from N_M is limited within a well defined range of concentrations which can be estimated by the following equation:

$$\Delta \log k'_{LC} = \log [k'_{GLSC}]_{\vartheta_M=0} - \log [k'_{GLSC}]_{\vartheta_M=1} \quad (10)$$

Plots of $\log k'_{LC}$ *vs.* N_M will be similar to those observed in liquid adsorption chromatography[14] and will be characterized by an exponential trend followed by a plateau region where further additions of M are uneffective in changing the retention of S. While the exponential portion occurs between $0 < \vartheta_M < 1$, the plateau region covers the range of concentrations where more than one monolayer of M covers the carbon surface. Similar to what happens in GLSC, the inflection point corresponding to the completion of the first monolayer should be largely independent of the chemical nature of the solute.

Strictly related to the change of k'_{GLSC}[9,10] is the effect of the modifier concentration on the symmetry of the chromatographic peak. Although the interaction of an organic molecule with carbon is essentially non-specific, $2 \cdot 10^{-2}$ active sites are present on 100 Å^2 of the GCB surface[10]. Whenever the polar moiety of a solute molecule interacts with these sites, the adsorption proceeds partly via a hydrogen bond and an asymmetric distribution of the chromatographic peak is observed. If the addition of the liquid modifier prevents such an interaction, M acts as a "tail reducer". With liquid modifiers following mechanism II, a substantial linearization of the adsorption isotherm can be observed because the modifier molecule is virtually bonded to the active site (strong localized interaction) and not displaced by the incoming solute molecule. Experiments carried out in GLSC with GCB[9,10] indicate that the deactivation of the carbon surface is so effective that the linearization of the adsorption isotherm can be used as diagnostic means of identifying liquid modifiers following mechanism II.

When the adsorption and partition of M and E are comparable, the typical features of the two mechanisms described above may be observed simultaneously. Since, in this case, it becomes difficult to distinguish surface from solubility effects, this kind of mechanism is not considered here.

EXPERIMENTAL

Reagents and materials

To illustrate the different effects described in the previous section, water and TEPA were selected as liquid modifiers. The former compound was chosen because is the most widely used modifier in RPLC and its effects have been extensively studied. The latter compound was preferred not only because its GLSC properties are known[10] but also because it is highly soluble in methanol and transparent to UV radiation down to 220 nm.

Water and methanol (HPLC grade) were supplied by Carlo Erba, Milan, Italy. Binary mixtures ranging from 5 to 15% (v/v) were obtained directly at the column inlet using the pumping system of the liquid chromatograph.

With TEPA, the various methanol solutions (0.01, 0.05, 0.1, 0.2 and 0.5%, v/v) were prepared in separate bottles prior to analysis. Measured volumes of TEPA

[GC grade (Carlo Erba)] were added slowly to methanol under continuous stirring. The reservoir of the chromatograph was filled with fresh solutions and the mobile phase was passed through the column using the isocratic mode. Injections of the sample were made when a dynamic equilibrium within the column was established. The achievement of the steady-state conditions was detected by monitoring the UV signal of TEPA at the column outlet. Columns were regularly washed with methanol before and after use of the TEPA as liquid modifier.

Various compounds, representative of the different chemical classes usually found in biological, pharmaceutical and environmental samples, were selected to test the effect of water and TEPA. Phenethylamine, phenol, *o*-cresol, benzoic acid and biphenyl were supplied by Carlo Erba. Dimethyltoluidine, 4-aminodiphenyl, benzidine, *o*-toluidine, α-naphthylamine, adenine, cytidine and guanosine were supplied by Fluka, Buchs, Switzerland. Tryptophan and adenosine were from Eastman-Kodak, Rochester, NY, U.S.A., whereas caffeine, glysolamide and tetracaine were supplied by various sources. Solutions containing 10, 20 and 80 ng/μl were prepared for each compound tested. The solvents for the standard solutions were selected for each individual case, taking into account the solubility of the specific eluite. Measurements of k'_{LC} were carried out by injecting the individual compounds into the column; analyses were repeated five times and the final result obtained by averaging the values measured at various concentrations of the eluite.

Apparatus and columns

Chromatographic data were collected on a Varian (Walnut Creek, CA, U.S.A.) Model 5000 liquid chromatograph equipped with a Valco (Houston, TX, U.S.A.) sampling valve. The injection loop was 10 μl. The column effluent was monitored at 254 nm with a Variscan UV absorbance detector supplied by Varian.

Home-made glass columns (250 × 2 mm I.D.) were used in all cases. These were filled with Carbopack B particles having a mean diameter of 20–25 μm. The original material, supplied by Supelco (Bellefonte, PA, U.S.A.), was ground and sieved according to the procedure described elsewhere[6]. Home-made fittings were built to connect the columns to the liquid chromatograph; polyamide ferrules (6 mm I.D.), able to stand an inlet pressure of 100 kg/cm^2, were used to make a tight connection between the glass tube and the metal fittings. The dead volume of the tube connecting the column to the valve was less than 3 μl.

RESULTS AND DISCUSSION

Retention data

To determine whether the mechanisms proposed in the Theoretical section are suitable for describing the effect of the liquid modifier, the capacity ratios (k'_{LC}) of different solutes were measured in HPLC using water–methanol and TEPA–methanol solutions as mobile phases. The values of the function log k'_{LC} obtained at various mobile-phase compositions were plotted against increasing amount of modifier present in the eluent. Figs. 1 and 2 show the curves obtained with TEPA and water respectively. To distinguish better the trend followed by the various solutes, both figures are split into two (a and b).

As can be seen, the curves obtained with TEPA are quite different from those

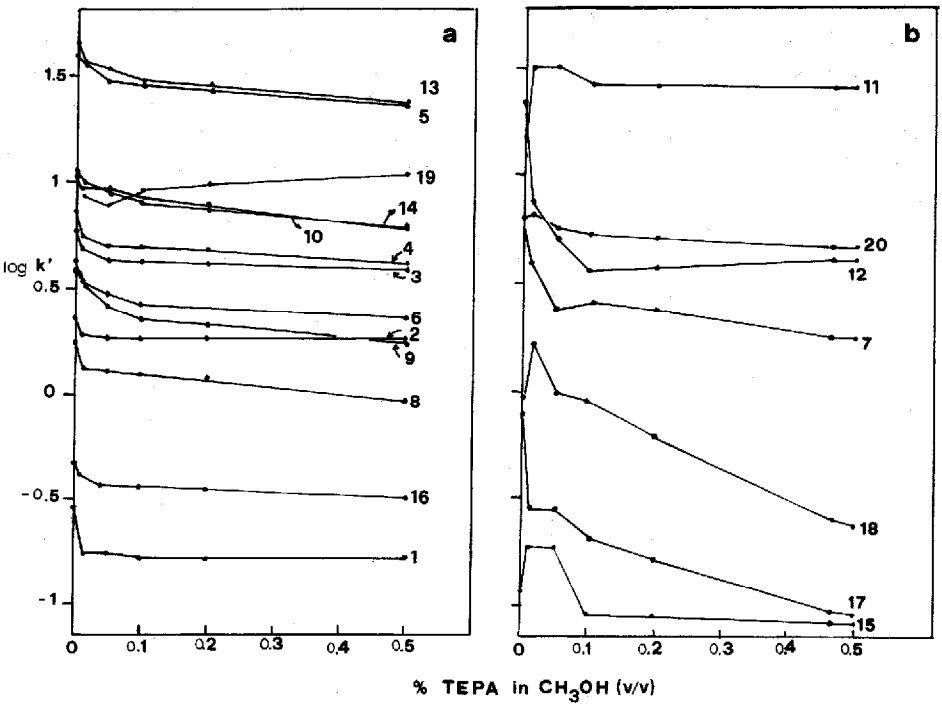

Fig. 1. Plots of log k'_{LC} *vs.* increasing amounts of TEPA added to methanol. Measurements were carried out on Carbopack B columns. The numbers reported in a and b are referred respectively to the following compounds: 1 = phenethylamine; 2 = dimethyltoluidine; 3 = 4-aminodiphenyl; 4 = benzidine; 5 = toluidine; 6 = α-naphthylamine; 7 = adenine; 8 = cytidine; 9 = tryptophan; 10 = adenosine; 11 = caffeine; 12 = glysolamide; 13 = tetracaine; 14 = guanosine; 15 = phenol; 16 = *o*-cresol; 17 = benzoic acid; 18 = phthalic acid; 19 = pentachlorophenol; 20 = biphenyl.

observed with water and the results seem to be consistent with the fact that the former liquid modifier follows mainly mechanism II whereas the latter follows mechanism I.

The trend of the curves shown in Fig. 2 is similar to that observed with CBP[1,2]. The change in the function log k'_{LC} is negligible at low concentrations of water and only beyond a certain value (5%, v/v) does the retention become monotonically dependent upon the water content in the eluent. For many of the compounds tested, the retention increases with the addition of water, the function log k'_{LC} depends linearly on the surface tension of the mobile phase[1,12] and the slope of the curves is a function of the hydrocarbon surface area of the solute[12,13]. For this reason, 4-aminodiphenyl, α-naphthylamine and benzidine, which are characterized by different structure, reactivity and affinity for the GCB surface, show the same increase in retention.

The close dependence on the surface tension of the eluent suggests that solute–mobile phase interactions in general, and particularly the cavity-formation term, play a determining role in changing the chromatographic properties of carbon. The increase in retention can be attributed to the decreased solubility of the solute in the mobile phase whereas surface effects can be, as a first approximation, neglected

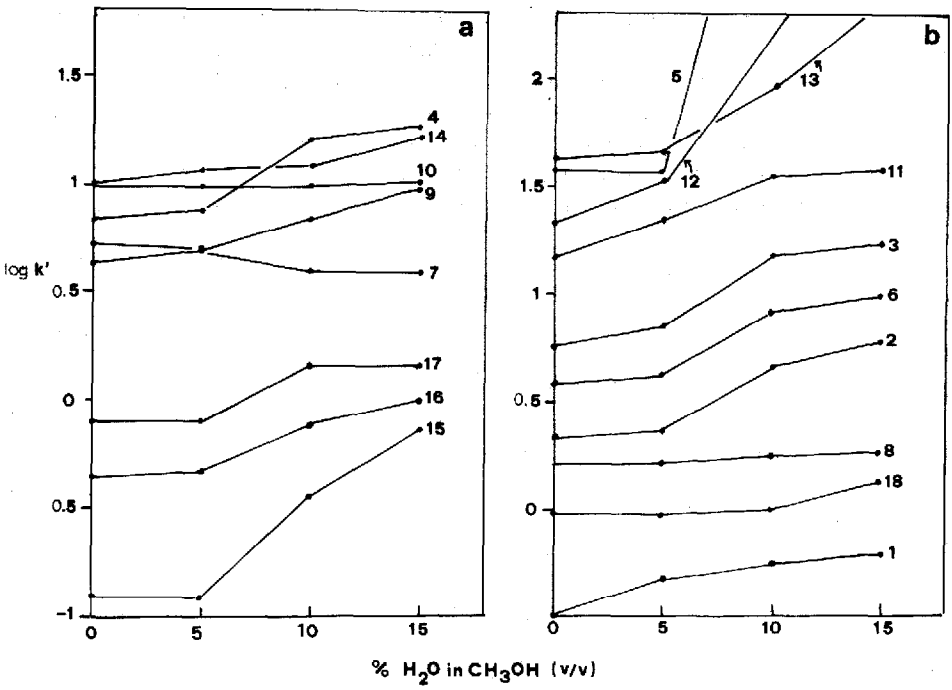

Fig. 2. Plots of log k'_{LC} *vs.* increasing amounts of water added to methanol. Measurements were carried out on the same column as used for Fig. 1. The numbers reported in a and b refer to the same compounds as in Fig. 1.

(*i.e.* k'_{GLSC} is constant). The different behaviour observed for adenine can be explained by considering that, in this case, attractive forces (electrostatic and Van der Waals) dominate over repulsion forces (cavity formation and free-volume reduction) and that an increase in solubility is observed with the addition of water.

Owing to the nature of the interactions involved in the process, the increase in retention is accompanied by an increase in selectivity (defined as $\alpha_{i,n} = \log([k'_{LC}]_i/[k'_{LC}]_n)$ where i and n are two compounds of the mixture) only when the differences in surface area between the solutes are sufficiently high to affect the cavity-formation term. In the other cases, the selectivity of the carbon is similar to, or even less than, that observed with pure methanol.

By contrast, all the curves of Fig. 1a, and many of those shown in Fig. 1b, fit well with eqns. 9 and 10 describing the behaviour of solvent mixtures following mechanism II. A drastic change in retention occurs in the region between the origin and a concentration of TEPA corresponding to 0.1% (v/v). Beyond this value, no substantial changes in the function log k'_{LC} are observed. The inflexion point of the curves is largely independent of both the chemical nature of the eluate as well as the way how the modifier affects the retention of the solute. The comparison between caffeine and the other compounds reported in Fig. 1a illustrates this point well. Although the plateau region starts at the same value of the mobile-phase composition, the retention of caffeine increases 100% with respect to pure methanol whereas, with the other compounds, it drops to a value which is, on average, 50% less than that measured with pure methanol.

Since the retention of TEPA can be adjusted until the concentration of the modifier reaches a value of 0.1% (v/v), it is likely that this point of the curve corresponds (or is near) to the completion of the first monolayer of TEPA. By assuming that the amount of TEPA necessary for covering the carbon surface is the same as that measured in GLSC, 68 μmole per g of organic modifier are present in the adsorbed phase when the concentration in solution is 5 μmole/ml and this is a reasonable value of the adsorption capacity for a compound strongly interacting with carbon.

The only curves not following mechanism II are those relating to the three acidic compounds shown at the botton of Fig. 1b. Here the dependence on the concentration is extended far above the value corresponding to the monolayer and no plateau regions are observed. It has to be noted, however, that association reactions between the acidic solute and the basic modifier can explain this trend. These reactions have been evidenced by the simultaneous presence of two peaks in the chromatogram when phthalic or benzoic acids were injected into the column. The unreacted solute and the product were observed only when the concentration of TEPA was in the range 0.05–0.1% (v/v) and the relative concentration of the product was increasing with the concentration of the liquid modifier. This suggests that the lower part of these curves ($<0.05\%$, v/v) represents the change in retention of the unreacted solute, whereas the upper part ($>0.1\%$, v/v) relates to the change in retention of the association reaction product. At low TEPA concentrations the unreacted solute behaves like caffeine, whereas beyond a value of 0.1% (v/v) of the modifier the product appears to follow mechanism I. This could explain the occurrence of a maximum in the curves relating to phthalic acid and phenol.

When no association reactions are taking place, the selectivity of the column is almost completely maintained, as the gap between many of the curves shown in Fig. 1 is constant with the addition of the modifier. This effect is in agreement with the observations made in GLSC because, if it is true that the monolayer of liquid phase greatly reduces the carbon retention, most of the column selectivity is still determined by the adsorbent[10].

Asymmetry factors

Additional information on the influence of the liquid modifier on the chromatographic process can be obtained by looking at the asymmetry factors (A_s) of the chromatographic peak. This function[15], not described in the Theoretical section, is defined as the ratio between the half-widths of the peak measured at 10% of its total height. Although empirical, the function A_s is somehow related to the adsorption isotherm of the solute, is simple to measure and sufficiently accurate to detect whether, and to what extent, the modifier acts as a "tail reducer".

In Figs. 3 and 4 the A_s values relating to some of the test compounds reported in the previous figures are plotted against increasing amounts of TEPA and water, respectively. Many of the compounds present in Figs. 1 and 2 but not shown in Figs. 3 and 4 are missing because the relative A_s values were already close to unity and did not change detectably with the addition of the modifier. In other cases, the curves are missing because the high retention made it difficult to measure accurately the half-widths at 10% height.

The results of Figs. 3 and 4 are in good agreement with the conclusions based

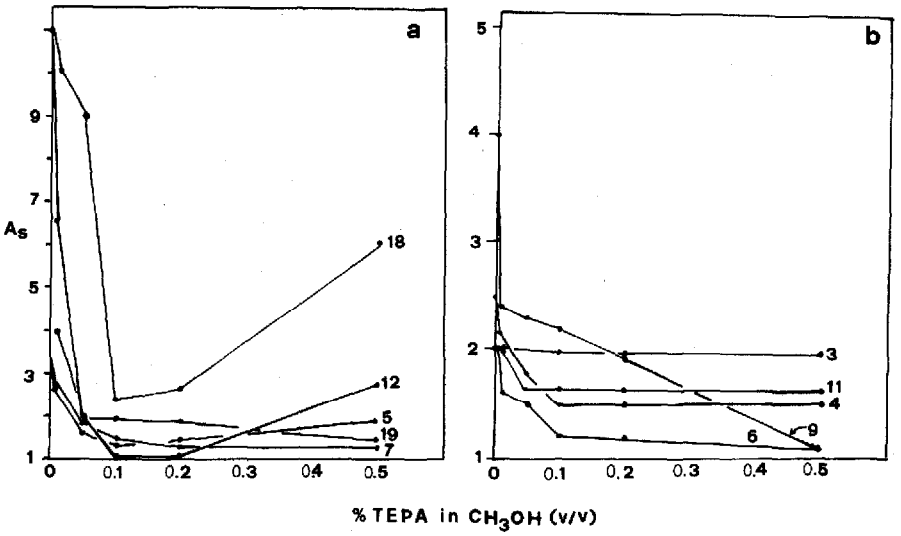

Fig. 3. Plots of the asymmetry factors (A_s) *vs.* increasing amounts of TEPA added to methanol. Measurements refer to some of the compounds listed in Fig. 1.

on the retention data as TEPA is more effective in reducing the peak tailing of polar solutes. Small additions of the modifier (0.01–0.1%, v/v) are sufficient to decrease dramatically the asymmetry factors of all the compounds reported in Fig. 3 and a substantial improvement in column performance is observed in the region where the completion of the first monolayer is reached. This effect occurs regardless of the way in which the modifier influence the retention and is observed also with caffeine. The

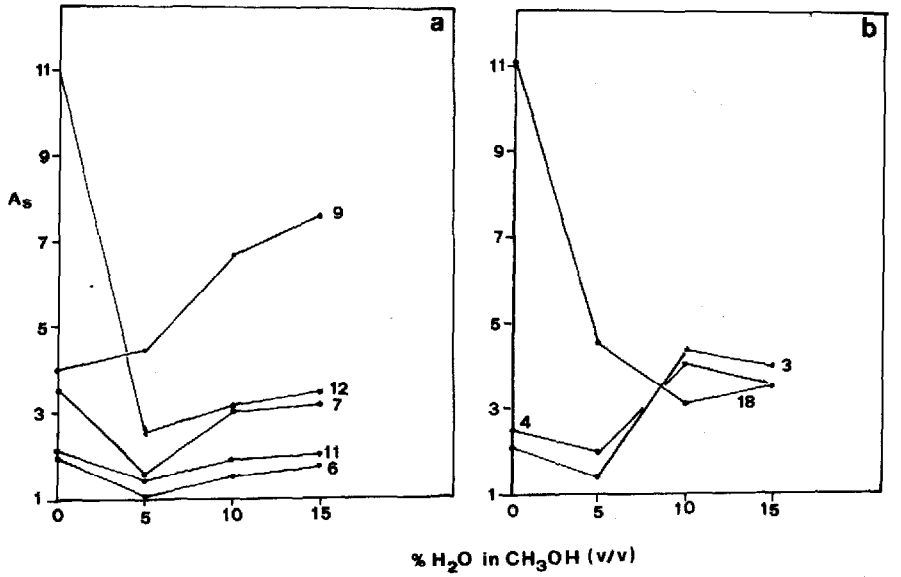

Fig. 4. Plots of the asymmetry factors (A_s) *vs.* increasing amounts of water added to methanol. Measurements refer to some of the compounds listed in Fig. 2.

analogy with the results obtained in GLSC[10] is striking and strongly supports the idea that displacement of the methanol molecules from the first monolayer and preferential adsorption of the modifier are responsible for the change in the chromatographic properties of the adsorbent.

By contrast, the curves of Fig. 4 cannot be explained in terms of surface effects because the values of A_s can either increase or decrease with respect to pure methanol. As the effect depends upon the chemical nature of the eluite, it is likely that water acts as a "tail reducer" only when the interactions in solution hinder the interaction between the polar moiety of the solute and the carbon surface[2].

CONCLUSIONS

The comparison between water and TEPA serves well to show the practical

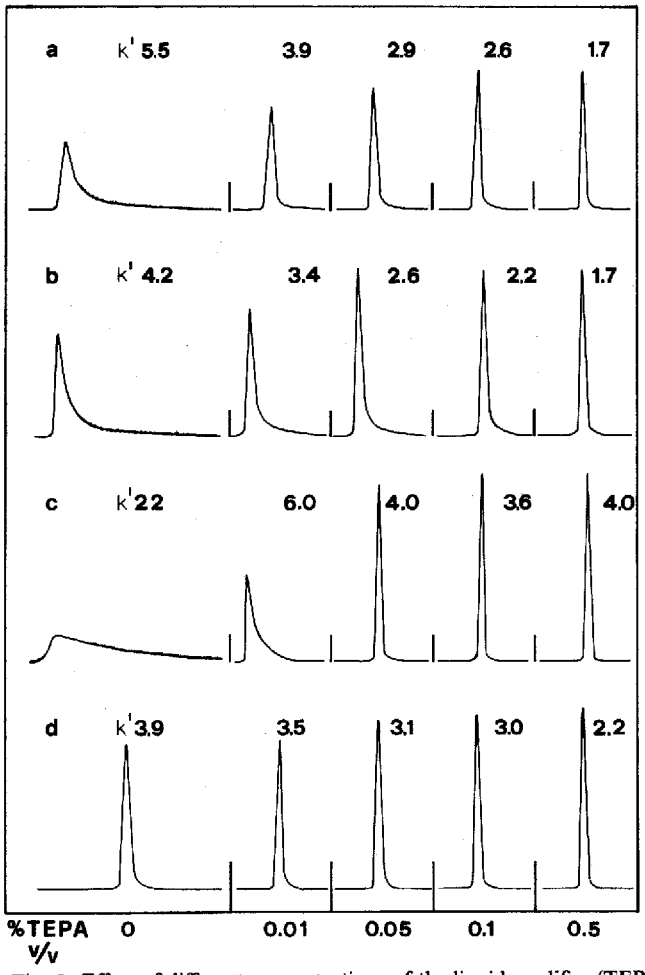

Fig. 5. Effect of different concentrations of the liquid modifier (TEPA) on the retention (k'_{LC}) and on the shape of some of the chromatographic peaks reported on Fig. 1: a, adenine; b, tryptophan; c, glysolamide; d, α-naphthylamine. All measurements were carried out on Carbopack B at a flow-rate of 0.5 ml/min.

advantages which can be gained when solvent systems which follow mechanism II are used in RPLC. Provided that the modifier and the eluent are properly chosen, it is possible to increase the analysis speed and to improve the sensitivity of the chromatographic system by keeping constant the selectivity of the adsorbent. The examples reported in Fig. 5 summarize the considerations made above. This figure shows the changes in the shape of the chromatographic peaks and capacity ratios observed with adenine, tryptophan, glysolamide and α-naphthylamine when different amounts of TEPA are added to the eluent. While, in the absence of modifier, the analysis of these compounds can be performed in 30 min and the quantitative determination of the former three compounds is difficult, at a TEPA concentration corresponding to half a monolayer (*ca.* 0.05%, v/v) the analysis time is reduced to 5 min, the resolution is sufficient to separate all the compounds and the sensitivity allows a fairly accurate quantitation of the components. Here also, a strong analogy exists with the data reported in GLSC[9] where the best compromise between time, resolution and capacity is obtained when the surface coverage corresponds to half a monolayer.

Although the very low concentrations of modifier and the long time required to equilibrate the column make it difficult to obtain reproducible chromatograms in gradient elution, this method is undoubtedly advantageous when strong adsorption is observed. Since with GCB the modifier can be selected from among acidic as well as basic organic compounds, a gamut of solvent effects wider than that obtained with CBP is available for the elution and separation of complex mixtures. Experiments carried out with different modifiers (aconitic acid, *n*-heptylamine and, recently, diethylamine) dissolved in methanol and acetonitrile have confirmed the results obtained with TEPA and proved the usefulness of this method. Moreover, the results reported by Gilbert *et al.*[7] on porous glassy carbon suggest that the use of liquid modifiers which follow mechanism II can be also extended to the case in which non-polar eluents need to be used. These authors have been able to achieve a rapid elution of polyaromatic hydrocarbons without tailing by using dilute solutions (0.1%, v/v) of 1-3 terphenyl in dichloromethane.

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