STATIONARY PHASE EFFECTS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

H. COLIN*, A. KRSTULOVIĆ and G. GUIOCHON

Laboratoire de Chimie Analytique Physique, École Polytechnique, Route de Saclay, 91128 Palaiseau Cedex (France)
and
Z. YUN
University of Peking, Department of Chemistry, Peking (China)

SUMMARY

In addition to dispersive and non-specific interactions at the surface of the adsorbent in reversed-phase liquid chromatography, specific effects also exist. The latter are mainly due to unreacted silanol groups and to the extracted layer of organic solvent. These specific effects are investigated by comparing capacity factors and liquid–liquid extraction constants, K_{LLE}. When chemically identical LLE and chromatographic systems are compared, plots of log k' vs. log K_{LLE} allow one to estimate specific stationary phase effects. The rôle of the solute and the solvent composition is discussed.

INTRODUCTION

It is generally accepted that the mobile phase interactions are of paramount importance in reversed-phase liquid chromatography (RPLC). The solvophobic effect itself is associated with the mobile phase, and the stationary phase plays, *a priori*, only a passive rôle as the solute acceptor. Strong specific interactions exist in the mobile phase (Keesom, Debye, hydrogen bonding, etc.), whereas only weak dispersive forces are associated with the stationary phase, if all "side effects" which may occur in this phase are ignored. Although the dispersive forces have a non-negligible contribution to the free energy change accompanying the transfer of the solute from one phase to the other[1], it is justified, at least to a first approximation, to describe the principal properties of reversed-phase systems in terms of the solvent interactions. This approach has been used recently for the derivation of a retention model based on interaction indices[2].

In the simplest case, the stationary phase can be described as a "fur" of alkyl groups. Depending on the method used to prepare the phase, this fur can resemble a liquid film or a brush (polymeric or monomeric packings). In the latter case —the most frequent among the present generation of phases— the bonded moieties can stand straight up on the surface of the silica, but they can also be intertwined to form

bundles, or they can even be bent[3,4]. The surface topology depends on the mobile phase composition, but also on the length of the alkyl moieties. In theory, the only interactions that should exist with reversed-phase materials are London-type dispersive forces. The associated energies are undoubtedly weaker than those of the mobile phase interactions.

This description is, in fact, a simplistic one, and several additional phenomena can occur in the stationary phase. The first is related to unreacted silanol groups which result not only from the geometrical impossibility to react all the OH groups of the silica surface, but also from the bonding procedure which does not necessarily yield the maximum coverage possible. The effects of these unreacted groups have often been reported (e.g., ref. 5). They are particularly important in the case of polar solutes, especially the basic ones, because of the acidic character of the silica surface. The second specific effect is inherent to the chromatographic process itself: when the mobile phase is not a pure liquid, one or several of its components are preferably extracted by the support which is thus covered by a layer of adsorbed liquid whose composition is different from that of the mobile phase[6-8]. The adsorbed solutes can interact more or less strongly with this layer[9,10], and the associated energy depends on the solute, as well as the mobile phase composition. This effect occurs also in liquid–liquid partition chromatography because of the usually large area of the interface between the two liquids.

The least specific effect of the stationary phase is related to its conformation. Due to the more or less ordered structure of this phase, the solute–ligand association may be accompanied by a decrease in entropy. This effect can occur only with solute molecules which are susceptible to rotations resulting in conformational changes. In contrast to the other effects, this one decreases the retention. It must also be pointed out that the entropy of $n\text{-}C_{18}$ bonded to silica is different from that of the free $n\text{-}C_{18}$ molecule. This results in a particular contribution to the free energy of transfer in RPLC whose affect on capacity factors (k') can be important in some cases[11].

A comparison of liquid–liquid extraction (LLE) constants and chromatographic capacity ratios offers a convenient way to estimate the specific stationary phase effects in RPLC, provided that an appropriate LLE system is adequately chosen. Indeed, if one compares a reversed-phase system with a chemically identical LLE one, composed of the same polar phase (the mobile phase of the chromatographic system) and an alkane of the same chain length as the chromatographic stationary phase, the differences between the behaviour of the two systems result only from the effects associated with the presence of the chromatographic support in RPLC. Thus such comparisons for various types of solutes in different solvents can enable a better understanding, and control, of the rôle of the stationary phase in RPLC.

The differences between LLE and RPLC can easily be estimated from a graph of $\log k'$ vs. $\log K_{\text{LLE}}$ (where K_{LLE} is the LLE equilibrium constant) for various solutes in a given solvent. If the interactions between the solutes and the non-polar phase are the same in LLE and in RPLC, K_{RPLC} equals K_{LLE}, where K_{RPLC} is the thermodynamic equilibrium constant. It is thus possible to write

$$\log k' = \log \varphi + \log K_{\text{RPLC}}$$

and: (1)

$$\log k' = \log \varphi + \log K_{LLE}$$

Eqn. 1 shows that, in the absence of specific effects, there is a linear relationship between $\log k'$ and $\log K_{LLE}$. The slope and intercept of the corresponding line in the $\log k'$ vs. $\log K_{LLE}$ plot are 1 and $\log \varphi$, respectively. The use of this method for the evaluation of $\log \varphi$ will be discussed in a forthcoming publication[12].

In this paper we describe an investigation of the rôle of the stationary phase and the specific interactions between the unreacted silanol groups and the adsorbed solvent layer, using an analogy between RPLC and LLE. It must be pointed out that this approach to the study of specific stationary phase effects is not new. Löchmuller and Wilder[3] have already used it to study the conformation of bonded phases.

EXPERIMENTAL

Chromatography

The equipment consisted of a Waters 6000A pumping system (Waters Assoc., Milford, MA, U.S.A.), a Model 440A UV photometer (Waters), an R 401 refractive index detector (Waters) and a Rheodyne 7125 sampling system (Rheodyne, Berkeley, CA, U.S.A.). The columns were home-packed with LiChrosorb RP-18, RP-8 and RP-2,5 μm (E. Merck, Darmstadt, G.F.R.), and Spherisorb C_6 (Phase Separations, Queensferry, Great Britain). They were made from stainless-steel tubing (15 cm × 4 mm I.D. × 1/4 in. O.D.). The C_{14} column was a gift from Professor De Galan (Delft, The Netherlands).

The experiments were carried out at 21.0 ± 0.5°C using a Haake D3 thermostatting system (Haake, Karlsruhe, G.F.R.). Peak areas and heights were measured using an HP Model 3390A integrator (Hewlett-Packard, Avondale, PA, U.S.A.). The dead time was measured using ^2H_2O (ref. 6). Although this method may not provide the true value of the dead volume it is, however, a good estimate[12].

Liquid–liquid extraction

For the sake of convenience, experiments were carried out using n-hexadecane rather than n-octadecane as the non-polar solvent. However, it will be seen below that the chain length of the alkane has only a very small effect on the value of the extraction constants. The extraction system consisted of 1 ml of each phase in a 3-ml vial closed with a PTFE-lined septum. The area of the liquid–liquid interface was about 1 cm^2, thus limiting drastically the extent of adsorption at the interface. The samples were dissolved in pure actonitrile, and very small volumes (5–10 μl) of the solution were introduced into the extraction system. Such sample volumes resulted in little change in the volume of the polar phase and avoided the possibility of saturation of each phase. For the sake of accuracy, the volume ratio of the phases was modified when necessary.

After the sample was introduced, the extraction vial was thermostatted for 24 h with periodic shaking.

The quantitative analysis was performed by reversed-phase liquid chromatography using an internal standard. In some cases (mostly with the alkanes as solutes),

gas chromatography (GC) (with electron capture detection) was used instead of liquid chromatography. The quantification was based on duplicate analyses using peak heights and peak areas. The precision was always better than 5 %.

In cases where the K_{LLE} values were large (more than 95 % of the solute in the alkane phase), only the polar phase was analyzed. Conversely, only the alkane phase was analyzed when the K_{LLE} values were small. In the intermediate range (between 5 to 95 % of the solute in the aqueous phase) both phases were analyzed.

For the analysis of the alkane phase, an additional extraction step with pure acetonitrile was necessary, since it is preferable not to inject large volumes of almost pure alkane (10–100 μl) into the reversed-phase chromatographic system. In order to ensure that the K_{LLE} values were independent of the solute concentration, the extraction constants were measured twice using different amounts of solute, m_0 and 10 m_0, introduced into the liquid–liquid system. If the agreement between both measurements was not satisfactory due to an experimental error or a dependence of K_{LLE} on the concentration of the solute (in, at least, one of the phases), the measurement was carried out with one tenth of the original mass of solute. This procedure was repeated until the agreement between two determinations of K_{LLE} with different masses of solute was within 5 %. In cases where it was not possible to obtain this agreement, the corresponding solutes were not included in the comparison between RPLC and LLE.

The polar phase was prepared by pipetting doubly distilled water and LiChrosolv-grade solvents from Merck. For both RPLC and LLE, the composition of the polar phase is given in volume per cent.

An important point to consider is the solubility of *n*-hexadecane in the polar phase. This was measured in methanol and acetonitrile, pure or mixed with water. The measurements were carried out by RPLC using a LiChrosorb RP-18 column and water as eluent. The values obtained were 0.0195 and 0.0098 g/g, respectively, at 30.0 $\pm$ 0.5°C. With both solvents mixed with water (22.2 %, v/v), the corresponding values are about $6 \cdot 10^{-4}$ and $3 \cdot 10^{-4}$ g/g. This indicates that, if in pure methanol and acetonitrile the solubility of *n*-hexadecane may not be neglected, it becomes very small upon addition of water, and actually close to the concentration of the solutes introduced in the system. It is difficult to determine whether the interactions of any solute with methanol or acetonitrile are modified by the presence of 1–2 % of *n*-hexadecane. The following experiment was made in order to answer the question.

The extraction constants of naphthalene and *n*-heptane were measured in the system methanol–*n*-hexadecane, using either pure methanol or methanol previously saturated with *n*-tetradecane. The results indicated that the values of K_{LLE} were the same within experimental errors. It thus seems to be reasonable to ignore the solubility of the alkane. A mobile phase saturated with *n*-hexadecane could have been used in chromatography, but under such conditions there would have been a strong extraction of the alkane by the stationary phase, yielding a modified adsorbent covered by one or several layers of alkane. Thus, it would have been impossible to evaluate the specific effects of the stationary phase. The saturation of the solvent with a long chain alkane is actually a very efficient way to "deactivate" a reversed-phase support. Results will be reported elsewhere[12].

In conclusion, we will ignore the solubility of the *n*-hexadecane in the polar phase in LLE. This also means that it was assumed that there was no change in the phase ratio after shaking.

RESULTS AND DISCUSSION

Rôle of the polar phase

As explained above, the comparison between LLE and RPLC data enables a simple estimation of the specific stationary phase effects in chromatography. The evaluation of the rôle of the mobile phase in RPLC necessitates, however, knowledge of the variation of the phase ratio with the composition of the eluent. This point is critical, and although it may be possible to determine this ratio[12], the accuracy of the method becomes very questionable when the solvent contains more than 30–40% water.

In order to solve this problem, it is preferable to use the relative retention and extraction constants, rather than the absolute ones. Thus, it is important to chose an appropriate reference compound. Such a compound should ideally be non-polar or have a low polarity in order to avoid as much as possible the specific effects of the unreacted silanol groups and those of the adsorbed layer. Moreover, its capacity factor and extraction constant must not be too high when the polar phase contains large amounts of water, so that measurements can be carried out over a wide range of composition. Benzene, which is UV detectable, is a convenient reference for solvents containing up to 80% water.

It is difficult to make an absolute study of the solvent effect since it is not easy to dissociate its rôle from that of the solute. In order to be as general as possible, the experiments were carried out with three pairs of compounds, one of which was always benzene. The first pair (I) was composed of acetophenone and benzene, and characterized the non-polar–polar selectivity for molecules of similar sizes. The second pair (II) consisted of 1,2,4-trimethylbenzene and benzene and permitted an evaluation of the non-polar–non-polar selectivity of molecules of different sizes with a rigid conformation. The last pair (III: biphenyl and benzene) characterized also the non-polar–non-polar selectivity of molecules of different sizes, one of them being more polarizable (biphenyl). The rôle of the solute *per se* will be investigated in the following section.

The experiments were performed with two solvent systems (water–methanol and water–acetonitrile) over a large range of composition (0–80% water). Fig. 1A and B show the variations of log α_{RPLC} and log α_{LLE} with the water content for the three pairs of solutes investigated. α is the ratio of the capacity factors or the extraction constants for each pair. The negative values of log α observed for pair I result from a greater retention of the reference compound (benzene) than the other compound of the pair. In order to conform with the usual chromatographic meaning of selectivity ($\alpha > 1$) the inverse ratios, $k'_{\text{benzene}}/k'_{\text{acetophenone}}$, should have been calculated. For the sake of uniformity, however, it was preferred to use the retention of each solute relative to benzene.

It is clear that the specific stationary phase effects are largest for pair I (Fig. 1C) which contained a polar solute. The greater selectivity in RPLC than in LLE was due to the fact that, relative to benzene, acetophenone was retained "abnormally" strongly in RPLC because of the polar interactions at the surface of the packing. With both polar phase systems, water–methanol and water–acetonitrile, log α_{LLE} for acetophenone decreases slightly and almost linearly with increasing water content, whereas log α_{RPLC} is relatively constant. The variation of log α_{LLE} with composition can readily be explained in terms of increasing polar specific interactions for ac-
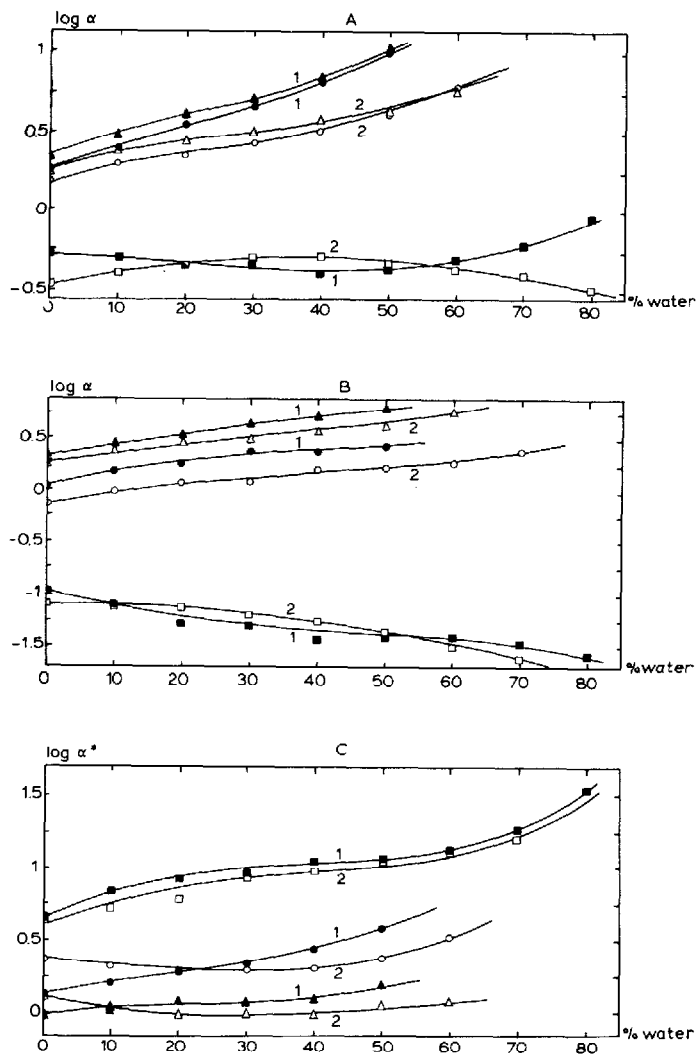
Fig. 1. Variation of the selectivity (relative to benzene) with the polar phase composition. A, Liquid chromatography on LiChrosorb RP-18 (log α_{RPLC}); B, liquid–liquid extraction using *n*-hexadecane (log α_{LLE}); C, log $\alpha^* = \log \alpha_{RPLC} - \log \alpha_{LLE}$. Solutes: □ and ■, acetophenone; △ and ▲, 1,2,4-trimethylbenzene; ○ and ●, biphenyl. Mobile phases: 1, methanol–water mixture (closed symbols); 2, acetonitrile–water mixture (open symbols).

etophenone in the aqueous phase with increasing water content, which partly compensate for the increasing solvophobic effect. This compensation does not hold with benzene whose extraction constant increases more rapidly than that of acetophenone. The consequence is a decreased log α value.

The more or less constant α value in RPLC suggests that the increasing solvation of the polar group of acetophenone with increasing water concentration is compensated for by enhanced stationary phase interactions. This means that the polarity of the stationary phase increases with increasing water content. Analogous results

were also obtained with other pairs of compounds (containing a polar solute and benzene). It is difficult to explain this observation without knowing the composition of the adsorbed layer. This may indicate that the water content of this layer increases with increasing water concentration in the mobile phase, and that the adsorbed layer is not composed of pure organic solvent. This is in agreement with some results[7] but in disagreement with others[6]. The increased polarity of the surface in RPLC appears clearly in Fig. 1C which shows the variations of the relative selectivity ($\log \alpha^* = \log \alpha_{RPLC} - \log \alpha_{LLE}$) with the water content. If all support effects were really negligible, $\log \alpha^*$ should be zero. The results indicate that, for the compounds investigated, the difference between the behaviour of methanol and acetonitrile is not significant, and that the water content is the most important parameter.

For pairs II and III, the polar–polar interactions do not have to be taken into account since the solutes are non-polar (although they are polarizable). For both pairs, $\log \alpha_{LLE}$ and α_{RPLC} increase with increasing water content in the two solvent systems. This results from the larger molecular size of 1,2,4-trimethylbenzene and biphenyl compared to benzene. This enhances the solvophobic effect. Fig. 1 indicates that $\log \alpha_{LLE}$ and $\log \alpha_{RPLC}$ are almost identical for the pair 1,2,4-trimethylbenzene–benzene although $\log \alpha_{RPLC}$ increases slightly more rapidly than $\log \alpha_{LLE}$ with water content, particularly in the water–methanol system. However, in both solvent systems, the difference between LLE and RPLC is very small. This is not surprising since it was predictable that the specific interactions with the stationary phase would not be very different for 1,2,4-trimethylbenzene and benzene. *However, this does not mean that these interactions do not exist*, as shown below. It just indicates that the methyl groups do not contribute significantly to the specific interactions with the stationary phase.

The results obtained with the last pair show that $\log \alpha_{RPLC}$ is larger than $\log \alpha_{LLE}$, and that the difference $\log \alpha_{RPLC} - \log \alpha_{LLE}$ increases with water content, almost identically for both solvent systems. Two effects can contribute to this situation. First, because the molecule of biphenyl has an additional aromatic ring compared to that of benzene, it is expected to interact more strongly with the stationary phase in RPLC, resulting in enhanced retention (see next section). Secondly, there may be an entropic effect because, in the biphenyl molecule, the free rotation of one ring relative to the other can be blocked when the molecule is in (or on) the stationary phase, due to the more or less rigid structure of this phase. As indicated above, this effect should decrease the retention of biphenyl. The larger value of $\log \alpha_{RPLC}$ compared to $\log \alpha_{LLE}$ indicates that the interactions of the second ring with the stationary phase more than compensate for the entropic effect. This problem will be discussed further in the next section.

From the results obtained with the three pairs of solutes, it can be concluded that the specific effects associated with the stationary phase in chromatography vary with the solvent composition for polar compounds. For non-polar ones, the effects are weaker and less dependent on the solvent composition. Similar results were obtained with seven other pairs of solutes.

Rôle of the solute

It would be possible to study the rôle of the solute in an absolute way by comparing $\log K_{RPLC}$ and $\log K_{LLE}$, if, as indicated earlier, the phase ratios were known. A relative evaluation of the rôle of the solute is possible, however, by study-

ing the plots of log k'_{RPLC} *vs.* log K_{LLE}. This approach was chosen using various families of compounds and four different mobile phase compositions: pure methanol; pure acetonitrile; acetonitrile–water (90:10 and 60:40). The various solutes investigated can be grouped into the families:

homologous series
polar compounds having a rigid or flexible conformation
non-polar compounds having a rigid or flexible conformation
polyaromatic hydrocarbons

The results obtained with different solvents are shown in Figs. 2 and 3. In the absence of specific interactions, eqn. 1 indicates that the points corresponding to *all* solutes must fall along the line of slope unity and intercept log φ. Except in the case of the entropic effect, the contribution of specific interactions tend to increase the retention and thus to displace the solutes above this line. The farther the solute is from the line, the more intense are the specific effects.

(a) Homologous series. The results obtained with the pair 1,2,4-trimethylbenzene–benzene in the previous section have indicated that the specific effects associated with the methyl groups are not significant. This must hold true also for the methylene group and, consequently, the classical plots of log k' *vs.* carbon number and log K_{LLE} *vs.* carbon number should have the same slope. In other words, a homologous series must yield a straight line of slope unity for the plot log k' *vs.* log K_{LLE}. As can be seen from Figs. 2 and 3, this was indeed obtained for the series: *n*-alkanes, *n*-alkylbenzenes, 1-chloro-*n*-alkanes, *n*-methyl esters and *n*-alcohols. In some cases only one alcohol could be studied and the dotted line corresponding to the whole homologous series was thus estimated from the four other series. Since it can be assumed that there are no specific interactions in the case of the alkanes, this series can be used to estimate the phase ratio[14]. The relative position of the series provides some interesting information.

In pure methanol, the line for alkylbenzenes is very close to that for the alkanes, whereas in all solvents containing acetonitrile the former series lies above the latter. The higher the water content, the greater is the distance between the lines, at least in the range 0–40 % water. This indicates that there are specific interactions between the stationary phase and the benzene ring which depend on the nature of the organic solvent, and probably to a much lesser extent on the unreacted OH groups whose reactivity diminishes with inreasing water content. Stronger interactions with acetonitrile than with methanol can be explained on the basis of the higher electron density of the CN group compared to OH. The CN group can develop much stronger π–π interactions than OH. Similar results have been reported previously[10].

The line corresponding to the chloroalkane series is very close to that for the alkylbenzenes, regardless of the solvent composition. This indicates that the interactions of the stationary phase with the Cl atom and the phenyl group are of the same magnitude. Consequently, the affinity of chlorine for acetonitrile is greater than for methanol. This is in agreement with the value of the extraction constant of the Cl atom which can be obtained by extrapolating the graph of log K_{LLE} *vs.* carbon number to zero carbon in the two solvents. The corresponding values are 0.69 and 0.38 in methanol and acetonitrile, respectively.
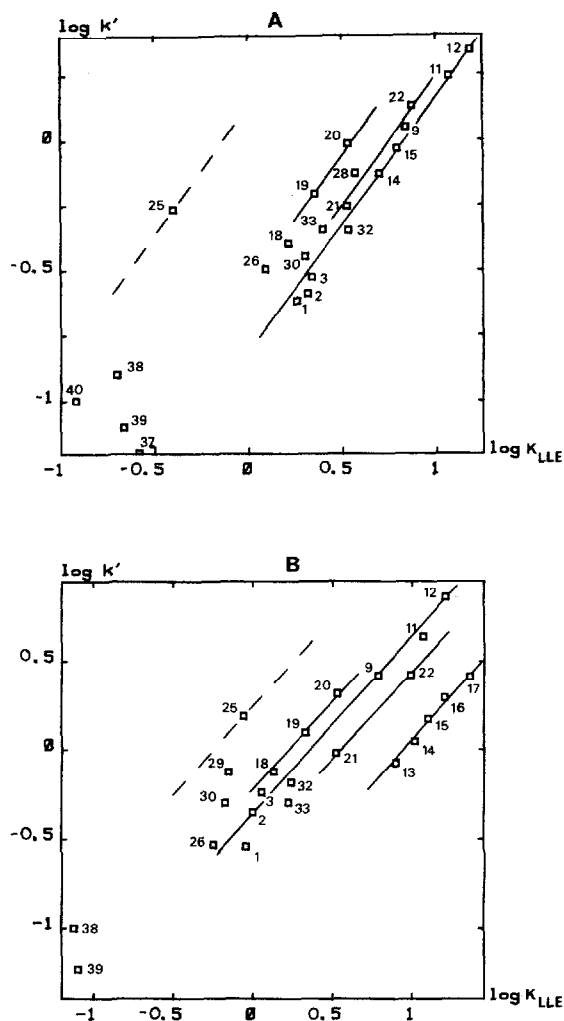
Fig. 2. Comparison of liquid chromatography (LiChrosorb RP-18) and liquid–liquid extraction (n-hexadecane). Polar phase: A = methanol; B = acetonitrile. Solutes: 1 = benzene; 2 = toluene; 3 = ethylbenzene; 4 = propylbenzene; 5 = butylbenzene; 6 = pentylbenzene; 7 = hexylbenzene; 8 = heptylbenzene; 9 = octylbenzene; 10 = nonylbenzene; 11 = decylbenzene; 12 = undecylbenzene; 13 = n-hexane; 14 = n-heptane; 15 = n-octane; 16 = n-nonane; 17 = n-decane; 18 = nonyl methanoate; 19 = undecyl methanoate; 20 = tridecyl methanoate; 21 = 1-chloro-n-octane; 22 = 1-chloro-n-dodecane; 23 = n-octanol; 24 = n-nonanol; 25 = n-dodecanol; 26 = naphthalene; 27 = anthracene; 28 = 1,3-diphenylbenzene; 29 = 1,3,5-triphenylbenzene; 30 = biphenyl; 31 = diphenylmethane; 32 = 1,2,4-trimethylbenzene; 33 = 1,2,4-trichlorobenzene; 34 = benzyl alcohol; 35 = benzonitrile; 36 = quinoline; 37 = 3,4,5-trimethylphenol; 38 = acetophenone; 39 = nitrobenzene; 40 = diphenyl phthalate.

The lines corresponding to the methyl esters and alcohols are above the alkylbenzenes. This is due to the relatively high polarity of the functional groups in the first two series. The most polar group is OH and the most polar series is the alcohols. It should be noted that, *relative to the other series*, the specific effects associated with the n-alcohols are larger in methanol- than in acetonitrile-containing solvents. This is
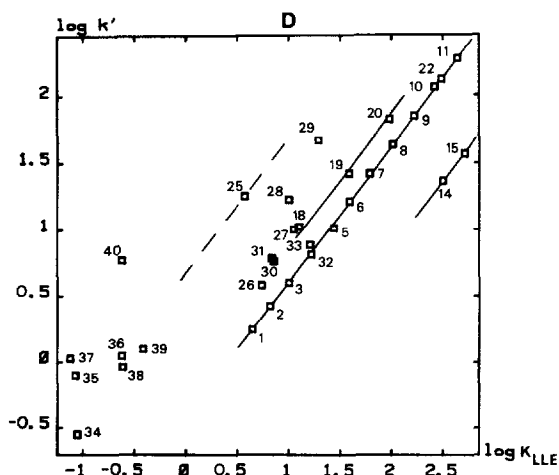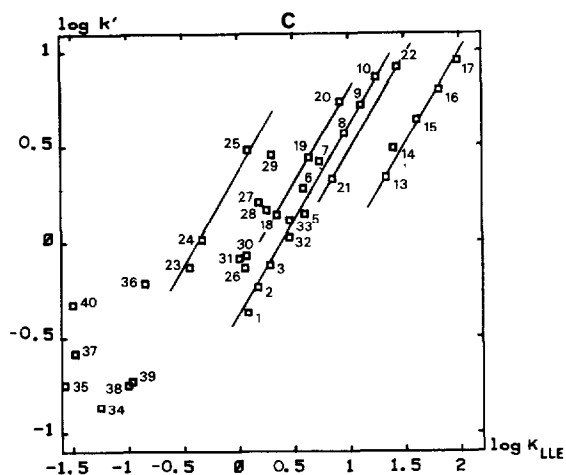
Fig. 3. As Fig. 2 except polar phases: C = acetonitrile–water (90:10); D = acetonitrile–water (60:40).

probably due to the existence of strong interactions such as hydrogen bonding between the OH group of the adsorbed methanol and the OH group of the *n*-alcohols.

(b) Polar solutes. The polar compounds include diphenyl phthalate, nitrobenzene, acetophenone, benzyl alcohol, benzonitrile, 3,4,5-trimethylphenol and quinoline. Because of the low values of the extraction constants, it was not possible to investigate the behaviour of some of these compounds in pure methanol and acetonitrile.

All solutes used (polar and non-polar) contained at least one aromatic ring. Thus, they should be found in the region above the line corresponding to the alkylbenzenes in the log k' *vs.* log K_{LLE} plots. This was indeed observed.

It is clear from Figs. 2 and 3 that the specific effects associated with the polar

solutes are generally strong, particularly in the case of diphenyl phthalate. For all polar compounds investigated, these effects increase slightly with increasing water content.

It is interesting to compare the results obtained with 3,4,5-trimethylphenol, benzyl alcohol and the *n*-alcohols (same functional group). It can be seen in Fig. 3 that benzyl alcohol falls almost on the same line as the *n*-alcohols, although the benzene ring of the aromatic alcohol is expected to enhance retention. It must be noted, however, that the OH group of this compound is less polar than that of the *n*-alcohols, thus giving a smaller specific effect. It appears that there is a compensation between these two effects. As far as 3,4,5-trimethylphenyl and benzyl alcohol are concerned, the position of the phenol above the alcohol is in agreement with the retention data in normal phase chromatography where the phenol is more strongly retained than the alcohol. It is thus probable that the larger specific effects for the phenol stem partly from the unreacted OH groups. This is, however, in disagreement with the fact that, with increasing water content, 3,4,5-trimethylphenol elutes closer to benzyl alcohol.

For all other polar solutes, the specific effects are strong. It is difficult to explain all the results individually, but relative positions give interesting information (Figs. 2 and 3). For instance, the results indicate that for acetophenone, nitrobenzene and quinoline, the values of the extraction constant are almost the same. However, in the mixture acetonitrile–water (90:10), the retention of quinoline is much greater than that of the two other solutes (which are similarly retained), whereas the three compounds are eluted together in the 60:40 mixture. The relative decrease of the specific effects for quinoline with increasing water content is due to the smaller accessibility of free silanol groups. It is indeed a common observation that such basic compounds are very sensitive to acidic OH groups, resulting in poorly shaped peaks. Increasing the water content of the eluent or adding small amounts of organic bases such as triethylamine generally eliminates these problems[13].

The comparison of Figs. 2 and 3 reveals that the smallest specific effects are observed in pure methanol and that they increase in pure acetonitrile and water–acetonitrile mixtures. This is not contradictory to the observations made in the previous section where *relative* effects were studied. Although methanol can give qualitatively stronger effects than acetonitrile because of its higher polarity, it is well known that acetonitrile is extracted more than methanol[6,7], which overcompensates for the polarity effect. It should also be noted that, in pure methanol, there is no compound above the line of the *n*-alcohols, not even diphenyl phthalate. This indicates clearly that the interactions between the *n*-alcohols and the adsorbed methanol are extensive.

(c) Non-polar solutes. Although these compounds are not polar, they are nevertheless polarizable. They can be divided into three groups: polynuclear aromatics (naphthalene, anthracene), polycyclics (diphenylmethane, di- and triphenylbenzene and biphenyl) and substituted benzenes (1,2,4-trimethyl- and 1,2,4-trichlorobenzene).

The last two series of compounds are located almost on the line corresponding to the alkylbenzenes at all mobile phase compositions. This is not at all surprising for the trimethylbenzene, since there are no effects associated with the methyl group. The result obtained with the trichlorobenzene was not expected at first, based on the similarity between the chloro and the phenyl groups in terms of specific interactions (see above), at least when located on an alkyl chain. Thus it can be concluded that the

effect of a group is different when it is located on a ring or on an alkyl chain. It should be noted that the extraction constants for trimethylbenzene and trichlorobenzene are very similar, which indicates that the behaviours of a chlorine atom and a methyl group *on a benzene ring* are similar. This explains the absence of a specific stationary phase contribution for trichlorobenzene compared to trimethylbenzene.

All other solutes are relatively clustered around a line whose slope is much larger than unity. The slope increases slightly with the water content of the solvent, and it is close to 2 in the mixture acetonitrile–water (60:40). For a given number of cycles, conjugated polynuclear hydrocarbons are located below the non-conjugated ones. The addition of aromatic rings strongly enhances the specific effects. This can be seen with 1,3,5-triphenylbenzene, for instance, which lies close to the line corresponding to the *n*-alcohols. The specific effects associated with the benzene rings are quantitatively and qualitatively larger in acetonitrile-containing solvents than in methanol mixtures. This result is in agreement with the observations made in the previous section.

Rôle of the non-polar phase

The rôle of the chain length of the stationary phase in RPLC is a topic of considerable interest and has been discussed in numerous papers. More or less contradictory results have appeared in the literature on the influence of the chain length on selectivity and on absolute retention[4]. This is a difficult problem since many parameters such as the method used to prepare the phase, as well as the characteristics of the silica gel, the solvent, the solute, etc., may play a critical rôle. As far as absolute retention is concerned, the change of the phase ratio with the chain length is obviously very critical, and does not seem to have received the attention it deserves.

The results presented in this section (Tables I and II, Fig. 4) are preliminary. They deal with the effects of the chain length of the stationary phase on log K_{LLE} and log k'_{RPLC}, as well as the corresponding selectivities for four compounds (acetophenone, nitrobenzene, naphthalene and diphenyl phthalate), in acetonitrile–water (50:50). In order to avoid the problem of the phase ratio, LLE and RPLC will be compared in terms of selectivity.

The data presented in Table I indicate that log K_{LLE} varies only slightly with alkane chain length. This is an important point which confirms the assumption that dispersive interactions in the non-polar phase play only a secondary rôle in the free energy of transfer from one phase to the other. For the non-polar compound naphthalene, K_{LLE} is remarkably constant (10.52 $\pm$ 0.8 %) between C_6 and C_{16}. In the case of the other polar solutes, K_{LLE} decreases slightly with increasing chain length: 2 to 3 % per carbon atom. This is probably the result of decreased solubility of a polar compound in alkanes of increasing molecular weight. The variations of the selectivities (relative to naphthalene) are shown in Fig. 4A. log α_{LLE} decreases almost linearly with the carbon number.

The chromatographic results are given in Table II. Since both the silica and the methodology used to prepare the phases are different, the absolute log k' values are not very meaningful. However, there is a general trend: log k' increases steadily with increasing chain length. This result is the opposite of that obtained in LLE, but it must not be concluded that log K_{RPLC} increases with increasing chain length since the phase ratio is undoubtedly larger for the C_{18} packing than for the C_1. (LiChrosorb
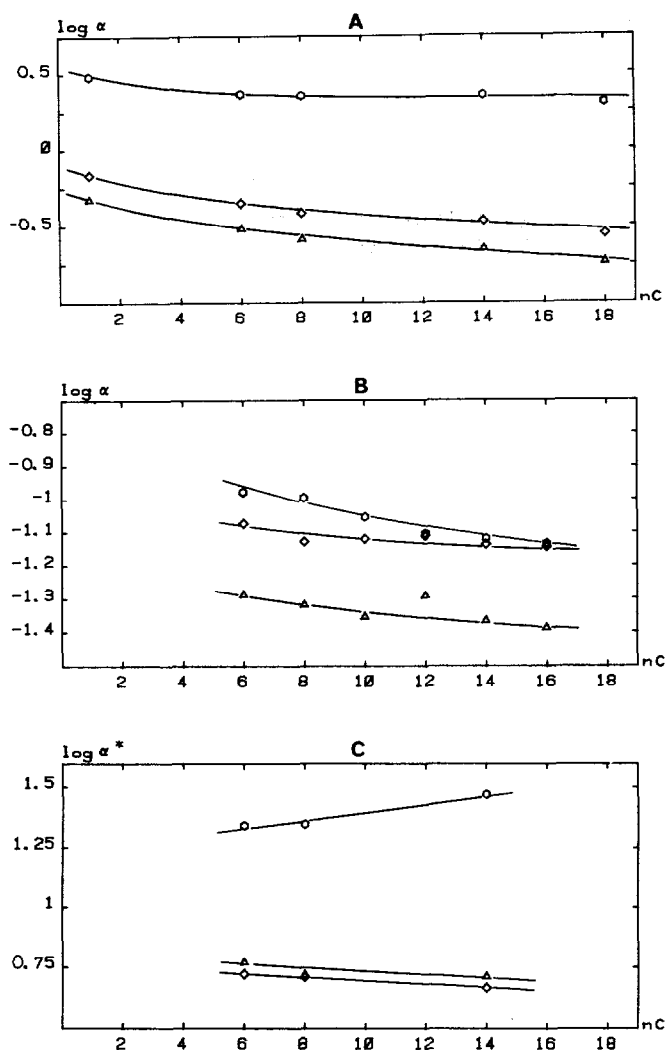
Fig. 4. Effect of the chain length of the non-polar phase on selectivity (relative to naphthalene). Polar phase = acetonitrile–water (50:50). A, Reversed-phase chromatography (log α_{RPLC}); B, liquid–liquid extraction (log α_{LLE}); C, log $\alpha^* = \log \alpha_{RPLC} - \log \alpha_{LLE}$. Solutes: $\bigcirc$, diphenyl phthalate–naphthalene; $\diamondsuit$, nitrobenzene–naphthalene; $\triangle$, acetophenone–naphthalene.

RP-2 is actually a C_1 type phase since it is dimethyl- and not ethyl-bonded.) The selectivity changes are shown in Fig. 4B (relative to naphthalene). It is clear that the general trend is the same as in LLE. Except for the C_1 phase, log α_{RPLC} decreases almost linearly with the chain length, at least in the range of carbon numbers investigated.

The LLE and RPLC systems can be compared conveniently in terms of log $\alpha^* = \log_{RPLC} - \log \alpha_{LLE}$. The corresponding results shown in Fig. 4C indicate that, for the two pairs nitrobenzene–naphthalene and acetophenone–naphthalene, log α^* is almost independent of the chain length. This means that the specific effects for these pairs do not depend on the chain length, or more exactly, they vary in a similar

TABLE I

LOG K_{LLE} VALUES FOR *n*-ALKANES IN ACETONITRILE–WATER (50:50)

Solute	*n*-Alkane					
	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}
Naphthalene	1.027	1.023	1.035	1.018	1.017	1.011
Acetophenone	−0.261	−0.295	−0.319	−0.275	−0.350	−0.377
Nitrobenzene	−0.046	−0.105	−0.085	−0.094	−0.121	−0.134
Diphenyl phthalate	0.050	0.028	−0.018	−0.087	−0.103	−0.124

TABLE II

LOG k' VALUES USING REVERSED-PHASE PACKINGS OF VARIOUS CHAIN LENGTHS AND ACETONITRILE–WATER (50:50)

Solute	Packing				
	LiChrosorb RP-1	Spherisorb C_6	LiChrosorb RP-8	LiChrosorb RP-14	LiChrosorb RP-18
Naphthalene	0.274	0.631	0.751	0.820	0.944
Acetophenone	−0.052	0.113	0.162	0.165	0.202
Nitrobenzene	0.110	0.280	0.333	0.347	0.384
Diphenyl phthalate	0.757	0.995	1.103	1.170	1.244

fashion for both solutes of the pair, so they cancel each other. Thus, it can be concluded that the specific effects for the solutes in question are not related to unreacted OH groups, but depend on the adsorbed liquid phase. It would be interesting to carry out similar experiments with solutes covering a larger range of polarity, using a non-polar reference such as an alkane. It is difficult, however, to measure accurately the extraction constant for alkanes in water-rich solvents. Preconcentration techniques would probably be necessary.

For the diphenyl phthalate–naphthalene pair, log α^* increases significantly with the chain length (Fig. 4C). This suggests that the specific effects increase more rapidly for diphenyl phthalate than for naphthalene, which is again in favour of the interactions with the adsorbed layer.

CONCLUSIONS

The comparison of liquid–liquid extraction and reversed-phase liquid chromatography makes possible the evaluation of the rôle of the stationary phase and the specific interactions which result from the existence of unreacted silanol groups and an adsorbed layer whose composition is different from that of the mobile phase.

The preliminary results indicate that the specific effects of the stationary phase are sometimes very important, particularly for polar compounds and molecules containing several aromatic rings. In acetonitrile the effects are generally larger than in methanol, most probably because of the larger volume of the extracted layer in the former solvent. The results presented indicate that it is *a priori* difficult to predict

retention in liquid chromatography only on the basis of mobile phase interactions. Although these interactions (governed by the solvophobic effect) undoubtedly represent the major contribution to retention, the secondary effects of the stationary phase can often largely influence the selectivity. This suggests that the prediction of retention in chromatography using liquid–liquid partition data (such as with the water–octanol system) is questionable, except for solutes belonging to the same family where one can assume that the secondary effects are more or less similar for different compounds.

Finally, liquid–liquid extraction seems to be a powerful method for studying the interactions in the liquid phase in chromatography.

REFERENCES

1 Cs. Horváth, W. Melander and I. Molnár, *J. Chromatogr.*, 125 (1976) 129.
2 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
3 C. H. Löchmuller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
4 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *High Performance Liquid Chromatography —Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980.
5 W. Melander, J. Stoveken and Cs. Horváth, *J. Chromatogr.*, 199 (1980) 35.
6 R. M. McCormick and B. L. Karger, *Anal. Chem.*, 53 (1980) 1050.
7 E. H. Slaats, W. Markovsky, J. Fekete and H. Poppe, *J. Chromatogr.*, 207 (1981) 299.
8 F. Riedo and E. sz. Kovátz, *J. Chromatogr.*, 239 (1982) 1.
9 R. M. McCormick and B. L. Karger, *J. Chromatogr.*, 199 (1980) 259.
10 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1980) 327.
11 D. Martire, personal communication.
12 A. Krstulovic, H. Colin and G. Guiochon, *Anal. Chem.*, in press.
13 H. Colin, J. M. Schmitter and G. Guiochon, *Anal. Chem.*, 53 (1981) 625–631.
14 A. Krstulović and H. Colin, unpublished results.