Journal of Chromatography, 603 (1992) 63–71
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 153

Solvent–stationary phase interactions in normal bonded phase high-performance liquid chromatographic columns

I. Investigation of system peaks in amino bonded phase columns

C. W. Hsu and W. T. Cooper

Department of Chemistry, Florida State University, Tallahassee, FL 32306-3006 (USA)

(First received November 5th, 1991; revised manuscript received February 25th, 1992)

ABSTRACT

System peaks in normal bonded phase high-performance liquid chromatography (NBP-LC) have been used to characterize solvent–stationary phase interactions in an aminopropyl (amino) NBP column. Mobile phases ("solvents") consisted of binary mixtures of hexane and a polar modifier. The term "system peak" is used to describe peaks arising from mobile phase components under isocratic conditions. These peaks can be either positive or negative, depending on the absorbance of the component relative to that of the bulk mobile phase. This study indicates that at high modifier concentrations the partition model seems to be more appropriate in describing retention in amino NBP columns than the adsorption–displacement model.

INTRODUCTION

The introduction of bonded phases has significantly broadened the utility of normal-phase high-performance liquid chromatography (HPLC) because of the distinct selectivities made possible by varying both mobile phase components as well as the polar functional groups of the stationary phase [1]. However, predicting solute retention in normal bonded phase liquid chromatography (NBP-LC) has proven to be more difficult than when solid adsorbents such as silica or alumina are used. This difficulty is due in large part to the complexity of associations possible between solvent molecules and the chemically and physically heterogeneous bonded phase surface. Unfortunately, it is this very complexity which gives rise to much of the suggested potential of NBP-LC and which must ultimately be incorporated into predictive retention models [2,3].

At this point there seems to be no generally accepted mechanism of retention in NBP-LC, unlike reversed-phase LC where solvophobic partitioning is the acknowledged starting point. A number of models of retention in NBP-LC have been proposed [5–10], but there still remain unsettled questions such as realistic modelling of adsorbent heterogeneity and the functional dependence of retention on mobile-phase composition in mixed-solvent systems. However, the most commonly accepted retention mechanism is Snyder's adsorption–displacement model [4–6], which assumes competitive adsorption between solutes and solvent molecules in a monolayer at the surface of a homogeneous surface. As we will discuss later, polar mobile phase modifier molecules tend to accumulate on (or in) the stationary phase in concentrations far beyond a monolayer, and thus the assumption of competitive monolayer

Correspondence to: Dr. W. T. Cooper, Department of Chemistry, Florida State University, Tallahassee, FL 32306-3006, USA.

adsorption on the stationary phase is probably not suitable at high modifier concentrations (*ca.* 60%). Under these conditions, the uptake of organic modifier increases the thickness of the stationary phase, which then behaves more like an amorphous bulk fluid than a homogeneous surface. Consequently, the driving force for retention will change as solute molecules can become fully embedded within the stationary phase; that is, partitioning rather than adsorption–displacement becomes the dominant retention process.

THEORY

System peaks [11–13] appear in liquid chromatography when the mobile phase contains more than one component. When a compound is injected into an LC system from a solution that does not precisely match the mobile phase composition, the sample that arrives at the head of the column is relatively vacant in one or both of the mobile phase components compared to the rest of the bulk mobile phase. This results in desorption of the depleted mobile phase component(s) from the stationary phase surface into the bulk, flowing mobile phase. Each of the desorbed components migrates through the column with a characteristic velocity dictated by its distribution coefficient, and they appear as peaks in the chromatogram. These peaks can be either in the negative or positive direction, relative to the detector base line, depending on the response of the particular component with regard to the bulk mobile phase. It has been shown [11,12] that system peaks are directly related to the adsorption of mobile phase components on the stationary phase surface and they can be utilized in the calculation of adsorption isotherms. Thus, by changing the modifier concentration and using one of the system peaks as a reference, it is possible to semi-quantitatively evaluate the amount of desorbed component in the vacant sample zone. This amount is related to the quantity adsorbed on the stationary phase prior to the injection, which in turn is related to the adsorption isotherm of the modifier.

In normal phase LC the hexane peak is normally used as the void volume marker. It is thus assumed that neither partitioning nor adsorption will occur for hexane molecules into (onto) the stationary phase. In order for the hexane molecules to experience partitioning, the stationary phase has to behave like an amorphous bulk fluid. However, due to the strong adsorption sites of the normal bonded phase at low modifier concentrations (<60%), the stationary phase is far from a homogeneous bulk fluid. While there is the possibility of hexane adsorption onto polar amino sites through weak dispersion forces, this should be minimal. Even if hexane is adsorbed to some extent, the resulting error in t_0 (dead time) will not change the *relative* shapes and positions of the isotherms for the three solvent modifiers. Comparison of the relative behavior of the three modifiers is really the goal of this work. We therefore believe that the use of hexane as the t_0 marker is suitable in this situation.

Chromatographic retention involves solute transfer from a mobile phase into or onto a stationary phase. Partitioning, adsorption, or both can be involved in the association of the solute with the stationary phase. The distinction between partitioning and adsorption is that "partitioning" implies the solute is approximately fully embedded within the stationary phase, whereas "adsorption" implies the solute is only in surface contact with the stationary phase and not fully embedded. In either case, transfer is characterized by an exchange of the environment at the surface of the solute molecule: solute is initially surrounded by neighboring mobile phase molecules and is finally surrounded, fully or partially, by neighboring molecules of the stationary phase. As proposed by Snyder and Poppe [4–6], in NBP-LC solute retention is assumed to involve a competition of solute and mobile phase molecules for a place on the stationary phase surface. In such a case the retention of a solute molecule S can be described by

$$S_m + nM_s \rightleftharpoons S_s + nM_m \quad (1)$$

where S refers to the solute, M to the solvent, m to the mobile phase and s to the stationary phase. The coefficient n is used to adjust the model for solutes and solvents of differing cross-sectional areas. From this model, the net energy of adsorption can be written as

$$\log K = E = (E_{S,s} - nE_{M,s}) + (nE_{M,m} - E_{S,m}) \quad (2)$$

(i) (ii)

were K is the equilibrium constant of eqn. 1. Solute

retention (which is proportional to the equilibrium constant K) is seen to be determined by partial molar solute free energies in each phase; term i corresponds to interactions in the stationary phase, and term ii groups similar interactions in the mobile phase. Thus, the compositions of each phase contribute to retention. From the thermodynamic point of view eqn. 2 does not involve any particular retention mechanism. However, it is often useful to assume that either term i or ii dominates solute retention as mobile phase composition is varied.

If term i is dominant, then eqn. 2 becomes

$$E \approx E_{S,s} - nE_{M,s} \quad (3)$$

which is Snyder's basic equation for NBP-LC. In the Snyder model, the adsorption surface is considered to be energetically homogeneous (no solute or solvent localization [4–6]) and solute-solvent interactions in the mobile phase are assumed to be cancelled by corresponding interactions in the adsorbed phase. That is, the acceptor cavity in the stationary phase is far more important than the donor cavity in the mobile phase. However, if term ii is more important then eqn. 2 becomes

$$E \approx nE_{M,m} - E_{S,m} \quad (4)$$

Eqn. 4 is in perfect agreement with the solvophobic [14–16] concept which postulates that the hydrophobic effect plays a fundamental role in reversed bonded phase liquid chromatography (RBP-LC). It also suggests that the main contribution to solute retention originates from the mobile phase ($E_{M.m}$). $E_{M.m}$ is directly related to the energy of cohesion between solvent molecules and represents the energy necessary to create a cavity in the solvent to accommodate for solute molecules. $E_{S,m}$ is associated with the specific interaction between solute and mobile phase. Obviously, the solvophobic theory is based on the premise that the only cavity which is relevant to retention is that in the mobile phase solvent; it neglects the acceptor cavity in the stationary phase. However, as popular as it might be in RBP-LC, the solvophobic theory is not an entirely satisfactory model for chromatographic retention processes because it requires a change of cavity size in only a single phase [17] (the mobile phase). For NBP-LC under certain circumstances, not only the mobile phase but also the acceptor cavity in the stationary phase should be involved in the retention process. In such cases, the partitioning model [17,18] will prevail, *i.e.*, the contributions from both stationary (term i) and mobile phases (term ii) are important in retention.

If the transfer process is dominated by partitioning rather than adsorption, the simplest model of retention is based on the assumption that the stationary phase is an amorphous bulk fluid and that retention resembles ordinary bulk-phase partitioning. In this case, the principal driving force for the transfer of solute is its relative chemical affinity for mobile and stationary phases; *i.e.* solute transfer involves (i) the creation of a solute-sized cavity in the stationary phase, (ii) the transfer process, and (iii) the closing of a solute-sized cavity in the mobile phase (see Fig. 1). As we will discuss later, it appears that the amino bonded exhibits dual retention characteristics: adsorption dominates at low modifier contents, with partitioning becoming important as the modifier reaches a mobile phase concentration that saturates the stationary phase.

EXPERIMENTAL

Equipment

All measurements were obtained with an HPLC system consisting of a IBM 9533 programmable ternary gradient liquid chromatographic system, Beckmen 506 Autosampler with 20-μl sample loop, Gilson Model 111 UV detector (254 nm), Nelson 900 Series interface and an IBM-compatible AT computer.

Analytical columns

The aminopropyl column (25 cm × 46 mm I.D., 5 μm packing) was purchased from E. M. Science (Cherry Hill, NJ, USA). The column was used as received.

Solutes and solvents

Hexane, methyl *tert.*-butyl ether (MTBE), chloroform and dichloroethane, each HPLC grade, were obtained from Fisher Scientific (Pittsburgh, PA, USA). Aromatic hydrocarbon solutes (phenanthrene, chrysene and perylene) and alkyl aryl ketone homologous series (propiophenone, butyphenone, hexanophenone, heptanophenone and octanophenone) were both obtained from Aldrich (Milwaukee, WI, USA). Phenol, nitrobenzene and aniline (re-

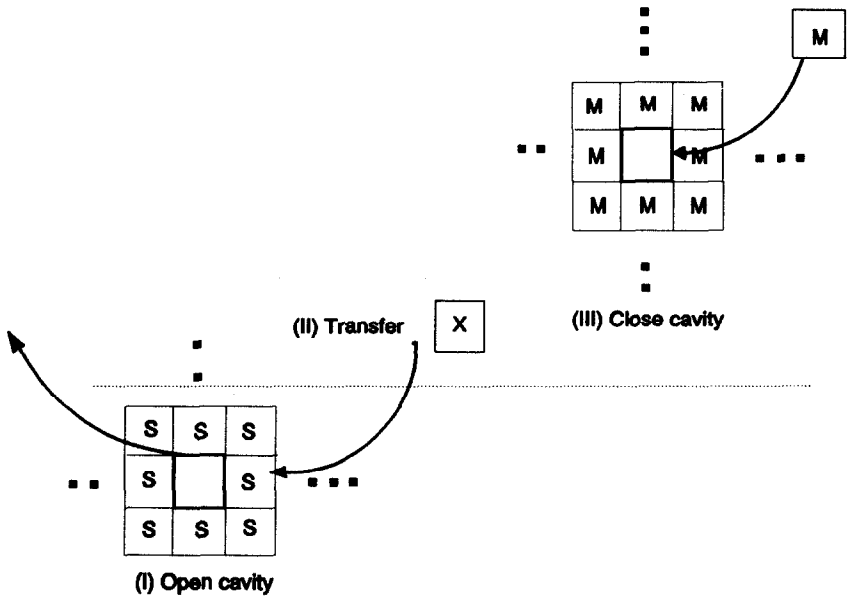

Fig. 1. Mechanics of molecule exchange in transfer processes such as partitioning or adsorption. The transfer of solute molecule X requires the opening of a cavity in stationary phase S and the closing of a cavity in mobile phase M.

agent grade) were obtained from Mallinckrodt (St. Louis, MO, USA). Aniline and nitrobenzene were purified by distillation, while phenol was used without further purification.

Procedures

Repeated injections of perylene and phenanthrene were used to measure the reproducibility of retention times. Reported t_0 values are the result of at least three replicate injections. The flow-rate was maintained at 0.5 ml/min during the course of these studies. No less than fifteen column volumes were allowed for column equilibration upon a change of mobile phase.

In the system peaks studies, pure hexane was injected as a solute. Hexane's negative system peak was also used as the t_0 marker.

RESULTS AND DISCUSSION

System peaks

The deflection (positive or negative) of the system peak, as mentioned before, depends on that component's absorbance relative to the bulk mobile phase at the detector wavelength. Fig. 2 is an illustration of the system peak in a real chromatogram, where the positive peak is due to chloroform, which absorbs significantly at 254 nm, while the negative peak is due to hexane.

Hexane is generally thought to be the least adsorbed mobile phase component available for NBP-LC. Thus, the retention volume of hexane is usually used to represent the mobile phase volume V_m (or void volume V_0). However, the total column volume must remain constant, and any decrease of the mobile phase volume must therefore result in a corresponding increase in the volume of the stationary phase. As can be seen in Fig. 2b, the retention of hexane decreased as the modifier concentration increased from 20% to 40%. It has been suggested by Scott [19] that mobile phase can accumulate near the polar surface of the stationary phase, forming a stagnant layer. The statistical mechanical theories of retention [19–22] also predict what has been referred to as "breathing" [22]. These predictions are supported by evidence of decreasing void volumes as the modifier concentration is increased. Uptake of the polar modifier in this way could affect the driving

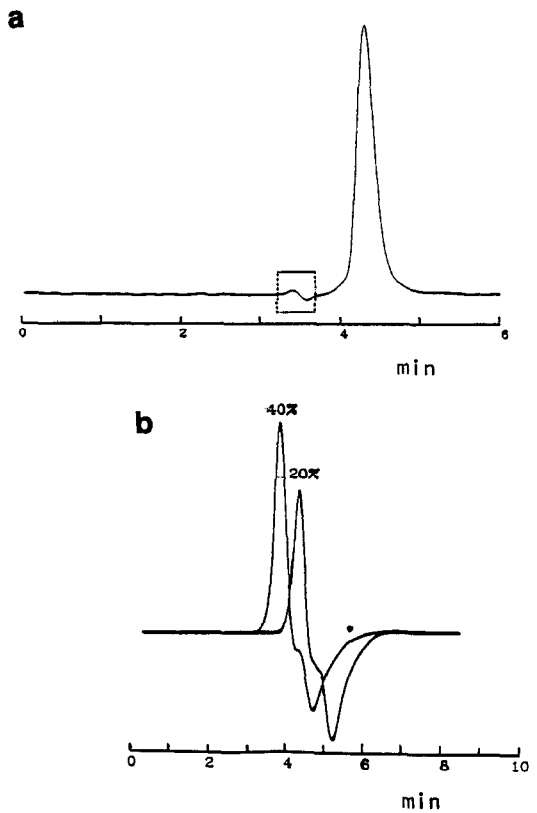

Fig. 2. (a) System peaks of a real chromatogram, with chloroform (20%, v/v) as the solvent modifier. The positive peak is due to chloroform, while the negative peak is due to hexane. (b) Enlargement of the system peak from (a). A system peak with chloroform at 40% is included for comparison. (see text for detail).

force for retention of solutes. Solutes could partition between this layer and the bulk mobile phase without directly interacting with the stationary phase.

Fig. 3 shows the trends in void volume decrease for three different modifiers. At about 60% polar modifier content, the void volume decreases reach a plateau. If the void volume at 0% modifier content is used as the reference point, the absolute value of the void volume differences can be interpreted as the degree of stationary phase accumulation under different modifier concentrations (Fig. 4). If the plateau in Fig. 4 is taken as the maximum coverage of stationary phase by modifier, then Fig. 4 can be replotted as the fractional coverage of the stationary phase (Fig. 5).

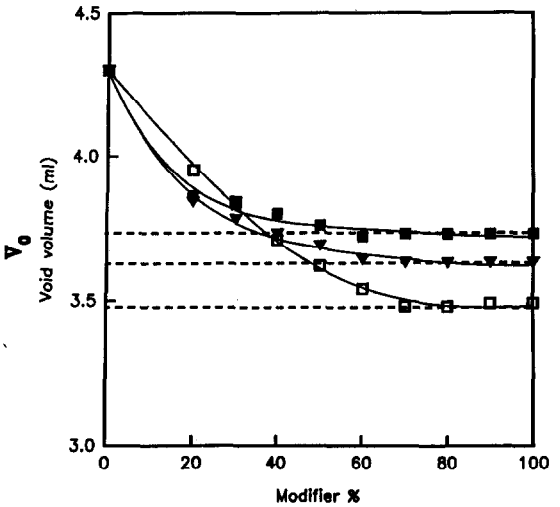

Fig. 3. Void volume changes *vs.* % modifier for three different solvent modifiers. □ = Chloroform; ▼ = dichloroethane; ■ = methyl *tert.*-butyl ether. Amino column.

While the fractional surface coverage plots of Fig. 5 are not in actuality adsorption isotherms, they are related to isotherms. It is not our intention here to use the data of Figs. 4 and 5 for quantitative

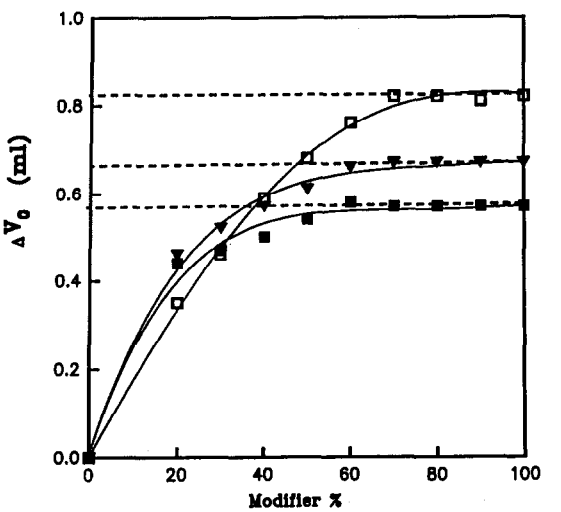

Fig. 4. If the void volume at 0% modifier content is used as the reference point, the absolute value of the void volume differences can be interpreted as the degree of modifier accumulation in the stationary phase. At about 60%, modifier ceases to accumulate in the stationary phase. Symbols as in Fig. 3. Amino column.

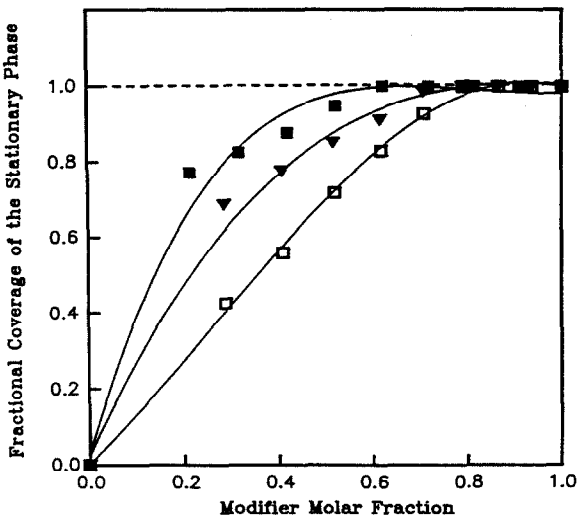

Fig. 5. Fractional coverage of the stationary phase. The plateau of Fig. 3 is taken as maximum surface coverage. Symbols as in Fig. 3.

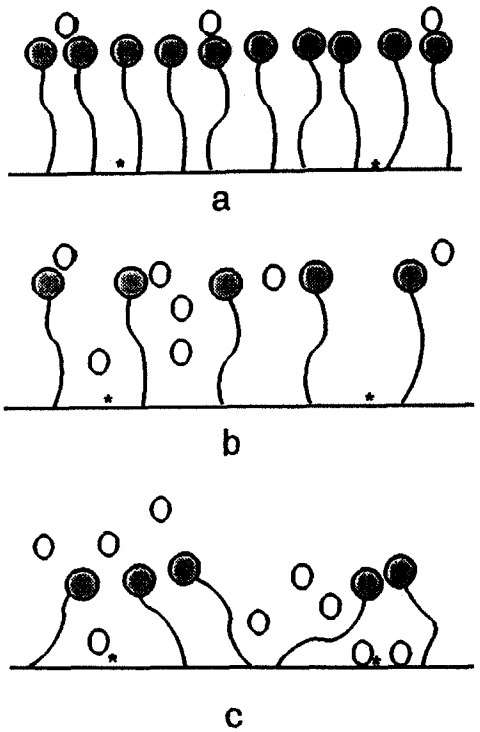

Fig. 6. Three different types of stationary phase configurations: (a) picket fence, (b) fur and (c) stack. In the "stack" structure, the grafted alkyl chains of NBP-LC stationary phases are not perpendicular to the surface, are in close contact with each other, and are less mobile than those in the "fur" configuration. Asterisks represent the strong adsorption sites (silanol groups).

isotherm calculations. Rather, we are interested in the *relative* affinities of the modifiers for the amino stationary phase. Clearly, chloroform shows both a higher stationary phase saturation concentration than the other two modifiers (greater V_0 in Fig. 4), and more rapid approach to this saturation concentration (Fig. 5). Dichloroethane exhibits an intermediate affinity for this bonded phase, while MTBE has the lowest. Chloroform is categorized as an acidic solvent. Thus, its interaction with a basic column (amino) is expected to be the largest among these three modifiers. The observed trends for dichloroethane (dipolar solvent) and MTBE (basic solvent) are also in agreement with expectations and previous characterizations of the amino phase [23]. These data are also consistent with pure solvent strength calculations (chloroform 0.14, dichloroethane 0.12 and MTBE 0.10).

Partition vs. adsorption

Models of the surface structure of bonded phases help in visualizing LC retention mechanisms. For example, the surface of monomerically derivatized silica particles of the type used in this study may be pictured as a forest of organic functional groups standing on end. Thus, they are sometimes referred to as "brushes". As will be discussed, not only the character of the polar functional groups but also the mobility of the alkyl chains (alkyl "spacers") affect the retention behavior of normal bonded phases.

A number of models describing the configuration of these surface molecular brushes have been proposed [17,18,24–26]. Fig. 6 illustrates schematically three possible configurations at the silica gel surface. If a sufficiently dense stationary phase structure existed, solute molecules could not fit between the brushes and would interact only with the tip of the three-dimensional "picket fence". The nature of polar functional groups at the stationary phase surface would thus be the dominant retention factor. Obviously, the adsorption–displacement model would be applicable for such a structure, and the chromatographic effect of silanol groups at the silica surface would be negligible. However, because of steric constraints, the "fur" configuration (Fig.

6b) appears to be more realistic. This model implies that the distance between the alkyl chains is sufficiently large for certain solute molecules to bind to the chains laterally. In this configuration, both the polar functional groups and the strong adsorption sites on silica (silanol groups) can be involved in the retention process. Thus, the adsorption-displacement model needs to be modified to account for these effects [3,4,27]. In the "stack" structure, which is illustrated in Fig. 6c, the alkyl chains are not perpendicular to the surface but are in close contact with each other. In this arrangement, the alkyl chains are less mobile than those in the "fur" configuration. Instead of a distinct mobile–stationary phase boundary, the polar bonded phase and adsorbed mobile phase components are forming a "stationary phase layer". In this fashion, an adsorbed solute molecule is not just in surface contact with the stationary phase anymore. Instead, it is more likely to partition between the three-dimensional stationary phase layer and the free-flowing, bulk mobile phase. Notice that here the alkyl chains of the bonded phase have a certain mobility, unlike the rigid rod approximation. It is the mobility of these polar functional groups which accounts for the dual behavior of this stationary phase.

Dill and Dorsey [17,18] have pointed out that in RBP-LC the "fur" models predict the grafted chains are fully exposed to the mobile-phase solvent. In light of the strength of the hydrophobic effect it should be prohibitively expensive in free energy terms for the chains to configure themselves to permit such a large degree of exposure. However, mobile phases in normal-phase LC are generally organic solutions. Thus, free energy prohibitions against "fur" and "stack" configuraations do not exist in NBP-LC. In addition, strong polar interactions between active functional groups of the stationary phase and polar modifiers only enhance solvent–bonded phase mixing.

The existence of a distinct, bulk stationary phase in a fur or stack arrangement would suggest partitioning rather than adsorption would be the dominant retention process. In the bulk-phase partitioning model, retention is described as a process of transfer between bulk media of solute S from a single-component mobile phase A. However, for real chromatographic retention processes, the mobile phase is not generally a single-component solvent; typical mobile phase solvents for the NBP-LC are mixtures of hexane with organic modifiers such as chloroform or dichloroethane. Thus, the partitioning model can be readily generalized to account for mobile phase mixtures of components A and B, with relative concentrations φ_A and φ_B, respectively, provided A and B are randomly dispersed. Thus, the partition model predicts [17,18] that there is a quadratic, not linear, relationship between ln k' and the volume composition of the modifiers (φ).

$$\ln k' = A - B\varphi + C\varphi^2 \tag{5}$$

or

$$(1/\varphi)\ln(k'/k'_0) = -B + C\varphi \tag{6}$$

where k' is the capacity factor, φ is modifier fraction and k'_0 is the value of k' when $\varphi = 0$; A, B and C are constants.

The A constant of eqn. 5 has been replaced in eqn. 6 by the logarithm of the capacity factor of the solute in a purely hexane mobile phase. This constant cannot normally be determined directly since, without organic modifier in the mobile phase and thus no modifier absorbed on the stationary phase, the nature of the stationary phase is considerably altered [28].

Interestingly, a similar relationship between the retention of acidic (phenol), basic (aniline), and dipolar (nitrobenzene) solutes and mobile composition has been observed for the amino column at high modifier contents (Fig. 7). The bulk phase partitioning model predicts that a plot of $(1/\varphi)\ln(k'/k'_0)$ *vs.* φ should be linear (eqn. 6), provided the random mixing approximation [20–22] holds. However, when the modifier concentration is below *ca.* 10%, dispersive intermolecular interactions of the polar stationary phase result in a reduction of the effective chromatographic surface area and reduced retention behavior [29]. Once these "brush-type" phases are in contact with a mobile phase containing *ca.* 10% of an organic modifier, they exhibit normal retention. From the data of Fig. 6, with modifier contents between 10 and 50%, eqn. 6 does not adequately describe retention. However, as the modifier concentration increases beyond 50% the linear prediction appears valid, implying that the partition model seems to be suitable under these conditions. These retention data correlate nicely

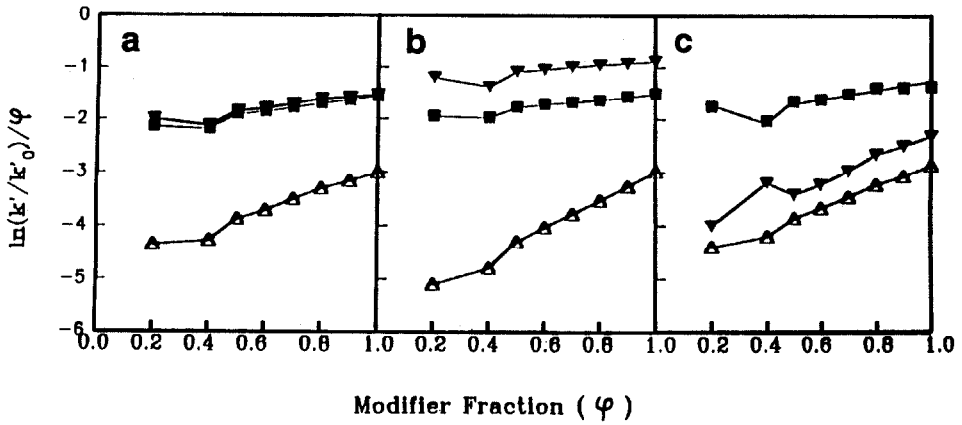

Fig. 7. Retention data for phenol (△), aniline (▼) and nitrobenzene (■) in three different solvent modifiers plotted according to eqn. 6. Amino column. (a) Chloroform; (b) methyl *tert.*-butyl ether; (c) dichloroethane.

with the data obtained from system peaks (Figs. 3–5), where the fractional coverage of the stationary phase reached 100% at about 60% modifier concentration. At low concentrations of modifier the bonded stationary phase is not well-solvated, and alkyl chains with their polar ends are largely ordered. As modifier content in the mobile phase increases, modifier molecules begin to penetrate the bonded phase, essentially solvating it. Apparently, at *ca.* 60% modifier content, the stationary phase can now be considered a randomly mixed bulk phase, and retention is best described as partitioning rather than adsorption.

There is another retention feature that is diagnostic of bulk-phase partitioning [17,18,30–32]: the logarithm of the capacity factor depends approximately linearly on the size of the solute molecule, as does the partition coefficient, since the volume(s) of the cavity(s) created in the mobile (and possibly stationary) phase(s) to accommodate the solute molecule is (are) a significant term in the overall free energy change (eqns. 2 and 4 and Fig. 1). This dependence of retention on molecular volume in partitioning systems has been widely observed [30–32]. In order to see if such a dependence was operating here, the retention of a series of describe (propiophenone, butyrophenone, hexaphenone, heptanophenone and octanophenone) was measured with MTBE as the polar modifier (Fig. 8). Below 60% modifier concentration, the log k' *vs.* the carbon number (n_c) plots seem to be somewhat random, although a distinct trend is observed. However, for modifier contents greater than 60%, a strong linear relatioship between log k' and n_c is obvious. We believe this data further supports the argument that at polar modifier concentrations above *ca.* 60%, partitioning rather than adsorption is the dominant retention mechanism. It should be noted that only MTBE data is shown here. The other two modifiers are too strong for retention of the alkyl aryl ketone homologues to be observed at concentrations above 60%. However, all data point to chloroform and dichloroethane behaving qualitatively exactly like MTBE.

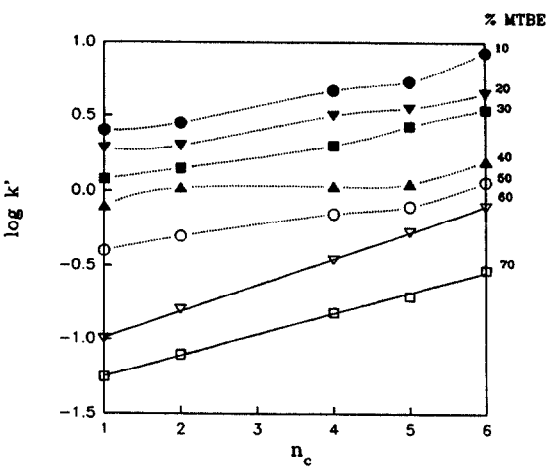

Fig. 8. log k' *vs.* solute size of alkyl aryl ketone homologues. Amino column.

CONCLUSIONS

We have concluded that two different retention mechanisms are possible in an aminopropyl NBP-LC column. At low modifier concentrations the adsorption–displacement model appears to work, provided certain modifications are included. However, when the concentration of modifier reaches 60%, a bulk partitioning process seems to result. These conclusions are based on three lines of evidence. First, hexane system peaks indicate that stationary phase volumes increase with increasing mobile phase modifier concentrations up to *ca.* 60%. Thus, the assumption of a monolayer of mobile phase at the surface of the stationary phase does not appear to be valid. Above 60%, however, where the volume expansion of stationary phase ceases, retention data indicate that the partition model is obeyed quite nicely. It is at this level of modifier that the stationary phase is completely solvated and the random mixing approximation holds. Finally, the retention data of the describe also suggest that the creation of the mobile phase cavity is important for modifier concentrations above 60%. It is the unique character of NBP-LC which accounts for the dual behavior of the stationary phase. For modifier concentrations $< 60\%$, adsorption dominates. However, as modifier kept accumulates in the stationary phase, a bulk stationary phase layer forms, with partitioning becoming the dominant retention process.

ACKNOWLEDGEMENT

The authors would like to acknowledge the support of the Environmental Protection Agency, Office of Research and Development, Grant R-814485-01-0.

REFERENCES

1 S. R. Abbott, *J. Chromatogr. Sci.*, 18 (1980) 540.
2 R. E. Majors, *J. Chromatogr. Sci.*, 18 (1980) 488.
3 W. T. Cooper and P. L. Smith, *J. Chromatogr.*, 249 (1987) 410.
4 L. R. Snyder and H. Poppe, *J. Chromatogr.*, 184 (1980) 363.
5 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
6 L. R. Snyder, *Anal. Chem.*, 46 (1974) 1384.
7 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
8 M. Jaroniec and J. A. Jaroniec, *J. Liq. Chromatogr.*, 4 (1976) 2121.
9 M. Jaroniec and J. Oscik, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 3.
10 L. R. Snyder, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography —Advances and Perspectives*, Vol. 3, Academic Press, New York, 1983, p. 157.
11 S. Levin and E. Grushka, *Anal. Chem.*, 57 (1985) 1830.
12 S. Levin and E. Grushka, *Anal. Chem.*, 58 (1986) 1602.
13 J. J. Stranahan and S. N. Deming, *Anal. Chem.*, 54 (1982) 1540.
14 P. Jandera, H. Colin and G. Guiochon, *Anal. Chem.*, 54 (1982) 435.
15 Cs. Horváth, W. Melander and I. Molnar, *J. Chromatogr.*, 129 (1976) 15.
16 Cs. Horváth and W. Melander, *J. Chromatogr. Sci.*, 15 (1977) 339.
17 K. A. Dill, *J. Phys. Chem.*, 91 (1987) 1980.
18 K. A. Dill and J. G. Dorsey, *J. Chem. Rev.*, 89 (1989) 337.
19 R. P. W. Scott, *J. Chromatogr. Sci.*, 18 (1980) 297.
20 D. E. Martire and R. E. Boehm, *J. Phys. Chem.*, 84 (1980) 3620.
21 D. E. Martire and R. E. Boehm, *J. Liq. Chromatogr.*, 3 (1980) 753.
22 D. E. Martire and R. E. Boehm, *J. Phys. Chem.*, 87 (1983) 1045.
23 W. T. Cooper and P. L. Smith, *J. Chromatogr.*, 355 (1986) 57.
24 W. R. Melander and Cs. Horváth, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography —Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980, p. 113.
25 L. C. Sander, J. B. Callis and L. R. Field, *Anal. Chem.*, 55 (1980) 1068.
26 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
27 L. R. Snyder, *J. Chromatogr.*, 255 (1983) 3.
28 L. B. Rogers and M. E. McNally, *J. Chromatogr.*, 17 (1985) 879.
29 R. P. W. Scott and C. F. Simpson, *J. Chromatogr.*, 197 (1980) 11.
30 H. Colin, A. M. Krstulovic, M. F. Gonnord, G. Guiochon and Z. Yun, *Chromatographia*, 17 (1983) 9.
31 G. E. Berendsen and L. de Galan, *J. Chromatogr.*, 196 (1980) 21.
32 B. A. Bidlingmeyer, S. N. Deming, W. P. Price, Jr., B. Sachok and M. Petrusek, *J. Chromatogr.*, 186 (1980) 419.