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RETENTION MECHANISMS IN CYANOPROPYL NORMAL BONDED PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS

W. T. COOPER* and P. L. SMITH
Chemistry Department, Florida State University, Tallahassee, FL 32306-3006 (U.S.A.)
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SUMMARY

The retention of polar solutes by a cyanopropyl bonded phase column using binary mixtures of hexane and polar solvents has been studied as a function of the selectivity of both solutes and solvents. Three solutes and three solvents were chosen to represent the apices of the selectivity triangle. This generated a 3 × 3 matrix of data and nine complete data sets. Normal phase retention in a cyanopropyl column can be adequately described by the adsorption–displacement model if localization and secondary solvent effects are taken into account. In order to incorporate all of the selectivity effects we have observed in a single description of a normal bonded phase column, we have developed a selectivity matrix which not only provides practical retention information, but also gives insight into the mechanisms responsible for the observed retention behavior. The selectivity matrix clearly identifies the important localization and solvent effects responsible for the unique selectivity of cyano-silica toward polar solutes, such as changes in stationary phase acidity–basicity owing to changes in mobile phase composition.

INTRODUCTION

The relatively recent introduction of bonded phases in normal phase liquid chromatography should significantly broaden the utility of this technique[1]. However, predicting solute retention in normal bonded phase high-performance liquid chromatography (NBP-HPLC) will also be more difficult, since stationary phase strength and selectivity must also be considered. Indeed, since solvent (mobile phase) molecules are themselves associated with the stationary phase, it is the combination of mobile and stationary phase strengths and selectivities which must be incorporated into predictive models of retention in NBP-HPLC.

The Snyder solvent selectivity triangle[2] has provided a foundation for the development of systematic mobile phase optimization strategies in HPLC[3]. However, the solvent selectivity approach has made a greater impact on reversed-phase than on normal phase HPLC, since solvent effects dominate retention processes in reversed-phase systems employing alkyl bonded phases. Solute–surface and solvent–

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surface interactions are clearly more important in normal phase HPLC when conventional solid adsorbents are used. Because of the limited number of solid phases available (primarily silica or alumina), the adsorption–displacement model[4,5] and empirical solvent strength parameters[6,7] have proved sufficient in describing and predicting solute retention in liquid–solid chromatography.

As we will demonstrate in this paper, single-valued solvent strength parameters are insufficient for describing retention of polar and hydrogen-bonding solutes in a cyanopropyl column, and it is necessary to consider specific solute–stationary phase, solvent–stationary phase, and solute–solvent interactions. Fortunately, the adsorption–displacement model and the selectivity triangle appear to provide an adequate framework for considering these specific interactions.

Snyder proposed that a solvent could be described completely by its proton donor, proton acceptor and dipole interaction tendencies[2]. Solvents could therefore be placed in a triangular coordinate system according to these tendencies, with the apices of the triangle represented by solvents capable primarily of proton donor, proton acceptor or dipole–dipole interactions. We refer to selectivity as the relative importance of these proton donor, proton acceptor and dipole characteristics of solvents and bonded phases. This is a somewhat more general definition of selectivity than that typically used in a chromatographic context, where selectivity usually refers to the ability of a particular solvent to increase the spacing of peaks of compounds with similar physical and/or chemical properties. These two definitions of selectivity are actually closely related since it has been demonstrated that maximum differences in chromatographic resolution occur with solvents from different regions of the triangle[2].

It is important to note that in the Snyder system, "proton donor characteristics" actually refers to a solvent's ability to interact with a proton acceptor (dioxane). It is not an actual measure of proton donating capability, and thus a solvent (or solute) can be classified as a proton donor even though it contains no protons. The same qualification applies to proton acceptors, which are classified as such based on an ability to interact with a proton donor (ethanol).

A number of studies of retention in amino[8–11] and cyano[12–16] NBP-HPLC columns have been published. Retention in these columns appears to behave according to the displacement model if delocalization[17,18] and secondary solvent effects[18] are included. Unfortunately, none of these studies specifically address the combined effects of stationary and mobile phase selectivities on solute retention; rather, single-valued solvent strengths have been used to characterize these columns.

In this paper we present the results of studies of retention of polar solutes in cyanopropyl NBP-HPLC columns with three binary solvent mixtures. Each solvent mixture consisted of variable amounts of hexane (non-selective) and a representative solvent from one apex of the selectivity triangle. Solutes used in these studies were also chosen to represent the triangle apices. These results clearly demonstrate that the selectivity of cyanopropyl NBP columns to polar solutes is a function of both stationary and mobile phase selectivity. Furthermore, cyano-silica does not appear to behave as a simple deactivated silica when polar solutes and solvents are involved. It has been suggested that retention of non-polar and moderately polar solutes on cyano-silica with hexane–dichloromethane binary solvent mixtures can be described by assuming the active sites of cyano-silica are unreacted silanols on the silica sup-

port[13]. However, our results support other studies[14,15] which indicate that the cyanopropyl bonded phase possesses unique properties when polar and hydrogen-bonding solutes are employed.

In order to describe the unique selectivity of all possible stationary–mobile phase pairs now available in NBP-HPLC, we have developed a selectivity matrix. The selectivity matrix not only provides quantitative retention information about acidic, basic and dipole solutes in all possible phase pairs, but also quantitates the impact of localization and secondary solvent effects on solute retention.

It is important to note that a bonded stationary phase includes not only the bonded organic phase, but also adsorbed solvent molecules and, in all probability, unreacted silanols on the surface of the silica support. The results presented here were obtained on a cyanopropyl column which had been endcapped with chloromethyl silanes to the maximum extent possible. We will show in a subsequent paper[19] that residual, unreacted silanols have a significant impact on retention in cyanopropyl columns.

THEORY

Extensive descriptions of the displacement model of retention in liquid-solid chromatography have been published elsewhere[1,18,20], and thus only a brief review of those aspects pertinent to the present study will be presented here. Snyder's original model[4] presumed that solvent and solute molecules formed an adsorbed monolayer in the stationary phase and that retention was controlled by the competition between solute and solvent molecules for positions in the adsorbed phase adjacent to the adsorbent surface. This competition is described by

$$X_m + nM_a \rightleftharpoons X_a + nM_m \tag{1}$$

where X refers to solute molecules, M solvent molecules, and the subscripts a and m designate molecules in the stationary (adsorbed) and mobile phases, respectively. The displacement model thus assumes that a solute molecule replaces n solvent molecules in the adsorbed monolayer, where n is given by the ratio of the adsorption cross-section of solute to that of the solvent.

Snyder's original model also assumed that the adsorbent surface was homogeneous (no localized, high-energy adsorption sites), and that interactions between solute and solvent in the mobile phase were effectively cancelled by similar interactions in the adsorbed phase. With these assumptions, Snyder derived an empirical expression which described variations in solute retention as a function of the solvent strength of mobile phases:

$$\log (k'_2/k'_1) = \alpha' A_s(\varepsilon_1 - \varepsilon_2) \tag{2}$$

In eqn. 2, k's represent capacity factors, α' an adsorbent activity coefficient, A_s the solute adsorption cross-sectional area, and ε's empirically determined solvent strengths of mobile phases. If one or both of the mobile phases are binary mixtures of a weak (A) and strong (B) solvent, then the solvent strength of the mixture can

be calculated from:

$$\varepsilon_{ab} = \varepsilon_a + \frac{\log (N_b 10^{\alpha' n_b(\varepsilon_b - \varepsilon_a)} + 1 - N_b)}{\alpha' n_b} \quad (3)$$

In eqn. 3, ε_a and ε_b refer to the pure solvent strength values of A and B, N_b the mole fraction of B, and n_b the adsorption cross section of B solvent molecules.

The displacement model thus allows calculation of changes in retention of a solute as a function of changes in mobile phase composition if the solute's and solvent's cross-sections are known or can be calculated and solvent strength parameters of the solvents are available. Failures of this model were quickly noted, however, particularly when using silica adsorbents. It was recognized that silica possessed strong, localized adsorption sites (surface silanols) which can interact selectively with polar molecules —a violation of the assumption of a homogeneous adsorbent surface. Localization effects can easily be incorporated into the displacement model, however, by using A_s or n_b values larger than the actual molecular cross sections. Once these corrected A_s or n_b values have been determined for a particular solute–solvent–stationary phase system, eqns. 2 and 3 can be used to predict solute retention as a function of solvent composition, even when significant solute and/or solvent localization occurs.

One localization effect particularly relevant to retention in NBP columns is "site-competition delocalization"[7]. When polar solvent molecules interact laterally with adsorption sites on which solute molecules are localized, solute localization is affected and there is an apparent increase in the A_s term of eqn. 2. Site-competition delocalization is predicted to occur on adsorbents with mobile active sites held in rigid positions at or above the surface (*e.g.* silanols, cyanopropyl groups), in contrast to restricted-access delocalization. Restricted-access delocalization is commonly observed with polar solvents and adsorbents with active sites at the surface, and is manifested by variations in pure solvent strength ε_b with mole fraction N_b.

One final adaptation of the displacement model which is particularly useful for determining the effects of solvent selectivity is the incorporation of a secondary solvent term. This term is needed because eqn. 2, even when corrected A_s or ε_b values are used, is not particularly effective in predicting changes in the relative retention of similar solutes (Snyder's definition of selectivity). Eqn. 2 now becomes[18]

$$\log (k'_2/k'_1) = \alpha' A_s(\varepsilon_1 - \varepsilon_2) + (\Delta_2 - \Delta_1) \quad (4)$$

where Δ_1 and Δ_2 are secondary solvent terms for solvents 1 and 2, respectively.

The need for a secondary solvent term arises because of a failure in an assumption of the original Snyder model that solute–solvent interactions in the mobile phase are cancelled by interactions in the stationary phase. For moderately polar to polar solutes and solvents, or those capable of hydrogen bonding, this assumption can break down. Since the more polar solvent of a binary mixture will predominate in the adsorbed layer, the concentration of this more polar solvent will be greater in the stationary phase than in the mobile phase. These considerations have led other workers to conclude that secondary solvent effects will be determined by solute–solvent interactions in the stationary phase (ref. 4, ch. 8).

In the present study, we have investigated retention in cyanopropyl NBP columns using mixtures of hexane and polar solvents from each apex of the selectivity triangle. Since hexane has been assigned a solvent strength of 0 and presumably induces no secondary solvent effects, eqn. 4 can be written

$$\log k_2 = -A_s\varepsilon_2 + \log k'_h + \Delta_2 \quad (5)$$

In eqn. 5, k'_2 represents the capacity factor of a solute in a mixture of hexane and chloroform, methyl *tert.*-butyl ether (MTBE) or dichloromethane; k'_h the corresponding capacity factor in pure hexane; ε_2 the solvent strength of the mixture calculated via eqn. 3; and Δ_2 the secondary solvent correction term. Note that we have arbitrarily assumed an activity coefficient of 1 for this column. Weiser *et al.*[13] assumed that cyano-silica behaved much like a deactivated silica toward non-polar and moderately polar solutes and solvents, and they estimated an activity coefficient of 0.2 by comparing group retention selectivities of a number of substituted aromatic compounds they determined on cyano-silica with those previously obtained on bare silica. Other studies[14,15], however, have shown that as the polarity of solutes and solvents increases, cyano-silica behaves less like silica. Our data with polar and hydrogen-bonding solutes and solvents support these latter conclusions and we have chosen to consider the cyanopropyl phase a surface of unique selectivity and have arbitrarily assigned an activity coefficient of 1 in these initial studies.

EXPERIMENTAL

Equipment

All chromatographic measurements were carried out with an isocratic HPLC system consisting of a Beckman 110A pump, Altex 210 sample injection valve with 20-μl sample loop, and Varian Vari-Chrom UV–VIS detector. Each solute was detected at the wavelength corresponding to its UV absorption maximum.

Columns

The cyanopropyl column used in these studies was a Hibar-RT, 5 μm LiChrosorb-CN (25 cm × 4.6 mm I.D.) manufactured by E. Merck (Darmstadt, F.R.G.) and purchased from E. M. Science (Cherry Hill, NJ, U.S.A.). Bonded phase surface coverage of the bare LiChrosorb (300 m^2/g) was 6.8%, or 3.1 μmole/m^2. The column was endcapped *in situ* using a commercially available reagent (trimethylchlorosilane) obtained from Alltech (Deerfield, IL, U.S.A.). The column was prepared for endcapping with 80 ml of dry toluene. Endcapping reagent (200 ml) was then pumped through the column at a flow-rate of 0.2 ml/min for 16 h.

Solvents and solutes

Chloroform and dichloromethane (ChromAr grade) were obtained from Mallinckrodt (St. Louis, MO, U.S.A.), while hexane and MTBE (pesticide grade) were obtained from Burdick & Jackson (Muskegon, MI, U.S.A.). All solvents were dried by equilibration with conditioned (200°C, 24 h) Union Carbide 3A sieves. In this fashion, water content was controlled to less than 1.5 ppm (gas chromatographic determination). Phenol, aniline and nitrobenzene solutes (reagent grade) were ob-

TABLE I

RETENTION DATA FOR NON-LOCALIZING AROMATIC HYDROCARBONS USED TO CALCULATE SOLVENT STRENGTHS

	Log k' and corresponding ε_{ab} value for indicated volume percent of modifier (ε_{ab} in parentheses)						*A_s (Å²)*		*Log k'_h (intercept)*	*Correlation coefficient*
	0%	*5%*	*10%*	*15%*	*20%*	*25%*	*Exp.*	*Calc.*		
(A) Hexane–chloroform										
	(0.000)	(0.013)	(0.028)	(0.040)	(0.048)					
Phenanthrene	−0.271	−0.406	−0.558	−0.707	−0.753		10.33	10.20	−0.272	0.998
Chrysene	−0.067	−0.243	−0.406	−0.572	−0.649		12.10	12.30	0.074	0.999
Perylene	0.002	−0.163	−0.342	−0.493	−0.643		13.08	12.80	0.010	0.997
(B) Hexane–MTBE										
	(0.000)	(0.017)	(0.023)	(0.027)	(0.032)	(0.035)				
Phenanthrene	−0.271	−0.447	−0.493	−0.544	−0.613		10.40	10.20	−0.268	0.997
Chrysene	−0.067	−0.271	−0.368	−0.406	−0.447		12.23	12.30	−0.070	0.997
Perylene	0.002	−0.217	−0.271	−0.346	−0.407	−0.459	12.98	12.80	0.007	0.998
(C) Hexane–dichloromethane										
	(0.000)	(0.020)	(0.036)	(0.046)						
Phenanthrene	−0.271	−0.470	−0.636	−0.748			10.34	10.20	−0.268	0.999
Chrysene	−0.067	−0.301	−0.544	−0.634			12.67	12.30	−0.063	0.997
Perylene	0.002	−0.256	−0.448	−0.573			12.46	12.80	0.001	0.999

tained from Mallinckrodt. Aniline and nitrobenzene were purified by distillation (b.p. range less than 1°C), while phenol was used without further purification. Aromatic hydrocarbons (phenanthrene, chrysene and perylene) were obtained from Aldrich (Milwaukee, WI, U.S.A.) and used as received.

Procedures

Column void volumes were determined by repeated injections of hexane with a chloroform mobile phase. The first baseline disturbance was taken as the void volume. The flow-rate was maintained constant at 1.0 ml/min. No less than fifteen column volumes was allowed for column equilibration upon a change of mobile phase. This was shown to be sufficient for the mobile phases and column used by monitoring the retention of the solutes as a function of the equilibration time. In general, retention times stabilized by ten column volumes.

Repeated injections of perylene and phenanthrene were used to measure the reproducibility of retention times and capacity factors. The relative standard deviation of these retention times was no greater than 3%.

RESULTS AND DISCUSSION

Pure solvent strengths

Binary solvent strength values (ε_{ab}) were calculated via eqn. 2 (ε_2 being the binary solvent strength) using non-localizing aromatic hydrocarbons as the solutes[13]. The adsorbent activity coefficient, α', was defined as one and ε_1 as zero[9]. Aromatic hydrocarbon cross-sectional areas[9] are given in Table I. Pure solvent strengths, ε_b, were then calculated from binary solvent strengths by rearranging eqn. 3:

$$\varepsilon_b = \varepsilon_a + \frac{\log\left(\frac{10^{\alpha' n_b(\varepsilon_{ab} - \varepsilon_a)} - 1 + N_b}{N_b}\right)}{\alpha' n_b} \tag{6}$$

where N_b is the mole fraction of the polar modifier, ε_a the pure solvent strength of hexane (equal to zero)[9] and n_b the modifier solvent cross-sectional area. Values of n_b were 5.0, 4.5 and 4.1 for chloroform, MTBE and dichloromethane, respectively[4,7].

Retention data for the aromatic hydrocarbons used to determine binary solvent strengths (and subsequently, pure solvent strengths) in this study are summarized in Table I. As a check of the applicability of using these solutes to calculate binary solvent strengths, we fit ε_{ab} versus log k' values for each of the nine PAH data sets of Table I to a straight line via a linear, least squares regression. Eqn. 2 predicts that these plots should be linear, and the slope of these lines (A_s,exp) should agree with calculated adsorption cross-sections (A_s,calc). The data of Table I indicate good agreement between experimental and calculated A_s values.

Table II contains pure solvent strengths calculated from eqn. 3 and the data of Table I. These values are plotted *vs.* volume percent of polar modifier in Fig. 1. The constant ε_b values obtained for chloroform and dichloromethane indicate that these are non-localizing solvents when used with a cyanopropyl NBP column. Fig. 1 also indicates, however, that at low concentrations (less than 25%), MTBE does

TABLE II

PURE SOLVENT STRENGTHS CALCULATED FROM AROMATIC HYDROCARBON DATA

ε_B values calculated from eqn. 3 for indicated volume percent of solvent

Solvent	*5%*	*10%*	*15%*	*20%*	*25%*	ε_B *(Average)*
Chloroform	0.097 ± 0.007	0.107 ± 0.002	0.112 ± 0.004	0.110 ± 0.004		0.106 ± 0.007
MTBE	0.147 ± 0.004	0.119 ± 0.004	0.106 ± 0.001	0.100 ± 0.004	0.094 ± 0.006	0.085*
Dichloromethane	0.120 ± 0.009	0.123 ± 0.007	0.118 ± 0.002			0.120 ± 0.007

* See text for description of how this value was calculated.

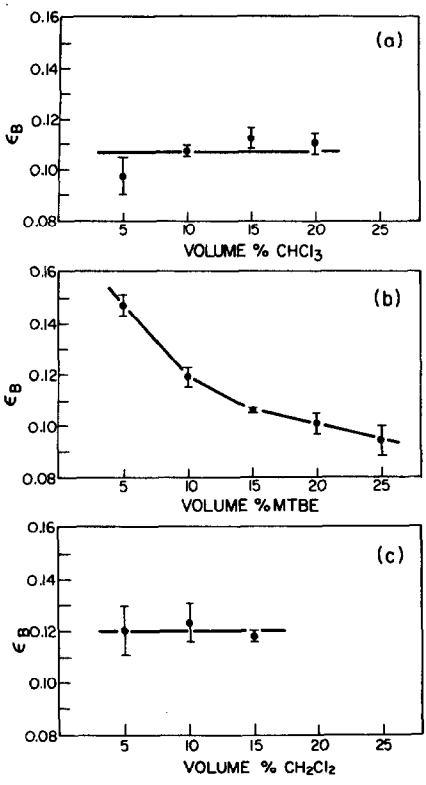

Fig. 1. Pure solvent strengths plotted *vs.* volume % of organic modifier. Indicated ranges represent one standard deviation.

localize. Furthermore, the nature of the MTBE curve suggests that restricted-access delocalization is occurring, an unanticipated result for a cyanopropyl bonded phase with mobile active sites above the adsorbent surface[17].

These localization effects observed for MTBE indicate that unreacted silanols on the silica support still play a significant role in retention in cyanopropyl BP columns, even when the column has been thoroughly endcapped. The conclusion that MTBE is localizing on silanol groups can be rationalized in a number of different ways. First, silanols are acidic (pK_a *ca.* 5), and localization occurs only with the basic solvent. This points out the importance of hydrogen bonding in an NBP system, and emphasizes the necessity of considering solvent selectivity, not just solvent polarity, when describing retention mechanisms. Secondly, ε_b values for MTBE asymptotically approach a constant value of 0.07 at 50 volume %. This is a much lower value than reported for bare silica[7], and indicates that once unreacted silanols are completely shielded by MTBE molecules, restricted-access delocalization no longer occurs. One practical consequence of this is that above 15 volume %, MTBE will not be as strong a solvent when used with cyano-silica as predicted from its solvent strength value determined on silica alone and Weiser's estimated activity of 0.2. Thus, improvements in band spacing due to selective localization effects in cyanopropyl columns may not

TABLE III

RETENTION DATA FOR SUBSTITUTED, LOCALIZING AROMATIC SOLUTES AS A FUNCTION OF VOLUME PERCENT OF ORGANIC MODIFIER

	Log k'								*A_s ($Å^2$)*		*Log k'_h + Δ_2*	*Correlation coefficient*
	5%	*10%*	*15%*	*20%*	*30%*	*40%*	*50%*	*60%*	*Exp.*	*Calc.*		
(A) Chloroform												
Nitrobenzene	−0.067	−0.217		−0.447					10.24	7.3	0.074	0.999
Phenol		0.992		0.726	0.561	0.404		0.155	14.43	6.4	1.408	0.999
Aniline		0.502	0.269	0.097	−0.076	−0.243		−0.447	16.47	6.6	0.929	0.995
(B) MTBE												
Nitrobenzene	−0.135		−0.257	−0.333					12.82	7.3	0.087	0.999
Phenol	0.366	0.084	−0.085	−0.217	−0.447	−0.544	−0.602		40.03	6.4	1.000	0.996
Aniline	0.434	0.269	0.133	0.015	−0.105	−0.192	−0.301	−0.426	30.40	6.6	0.966	0.996
(C) Dichloromethane												
Nitrobenzene	−0.125	−0.317	−0.406						10.01	7.3	0.954	0.999
Phenol		0.899		0.582	0.369	0.176		−0.105	15.06	6.4	1.438	0.999
Aniline		0.277		0	−0.168	−0.368		−0.602	13.24	6.6	0.755	0.998

be as obtainable as predicted from extrapolations of solvent selectivity in MTBE–silica systems[13].

The pure solvent strength for dichloromethane listed in Table II is almost twice that which we calculate from Weiser's data (0.120 *vs.* 0.07). This translates into an activity of 0.37 for our cyanopropyl column relative to activated silica based on a comparison of the pure solvent strengths of dichloromethane in the two columns (0.12 *vs.* 0.32). Weiser calculates an activity of 0.2 for his cyanopropyl column. We attribute this difference to quantitative and/or qualitative differences in endcapping of the two columns. We will show in a subsequent paper[19] that a great number of non-endcapped silanols are adjacent to cyanopropyl groups, suggesting that steric hinderance prevents chlorotrimethylsilane from interacting with nearby silanols in the column we used.

The fact that MTBE has the lowest solvent strength of the three solvents might seem surprising in light of its strong interactions with residual silanols. However, the solvent strength for MTBE listed in Table II was determined with observed and extrapolated retention times using volumes of MTBE between 15 and 50%. At these concentrations, most silanols have been effectively blocked and MTBE has little affinity for the remaining cyano groups of the stationary phase. Chloroform, however, has a higher solvent strength than MTBE because of its slight ability to hydrogen bond with cyano groups. Although dichloromethane has the higher solvent strength on cyano-silica as well as silica, the ratio of solvent strengths of dichloromethane and chloroform we determined on cyano-silica (1.15) is *ca.* 10% less than the same ratio determined on bare silica (1.25, ref. 4). Combined with results presented in the next section, we believe this indicates some basic character to the cyanopropyl bonded phase, and that the cyano group does contribute to retention in these columns. The importance of this slightly basic character will be seen in the next section, in which the retention of polar, localizing solutes is discussed.

Solvent selectivity

We have evaluated solvent selectivity in cyanopropyl NBP columns by measuring the retention of substituted benzene compounds containing acidic (OH), basic (NH_2) and dipole (NO_2) groups. The retention behavior of the three solute probes (phenol, aniline and nitrobenzene) was observed in all three solvents. This generated a 3 × 3 matrix of data and nine complete data sets. This data is summarized in Table III.

One of the most important features of the displacement model is that when retention data is fit to eqn. 5 and empirical binary solvent strength parameters are used, direct comparisons of solvent selectivity can be made. That is, differences in solute retention due to differences in solvent *selectivity*, not solvent *strength*, will be obvious since the displacement model and eqn. 5 normalize for differences in pure solvent strength.

These differences in solvent selectivity are illustrated in Fig. 2, in which log k's are plotted *vs.* binary solvent strengths. For example, Fig. 2a demonstrates the selective ability of MTBE to elute phenol from a cyano column relative to chloroform and dichloromethane. Minimal differences in selectivity occur for phenol in chloroform and dichloromethane, as indicated by the similar retention plots for these solvents. MTBE is obviously a much stronger solvent for phenol than predicted from

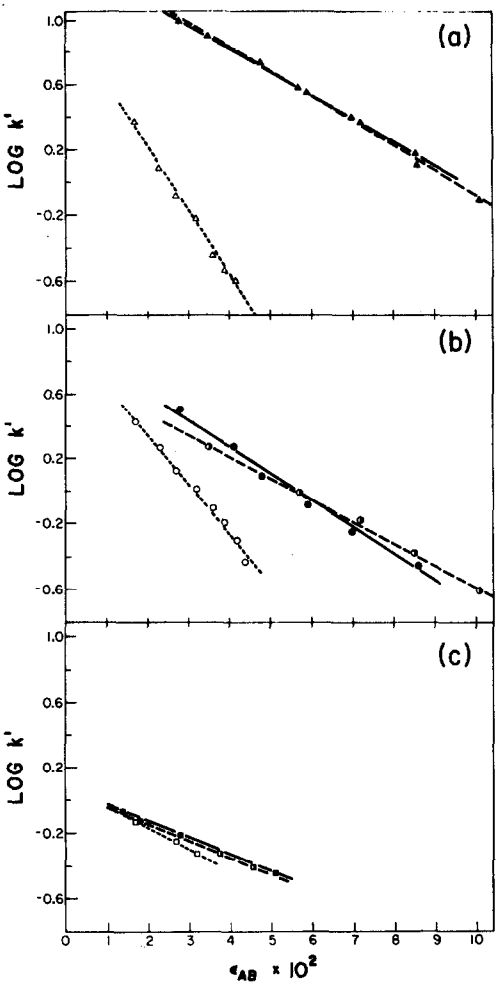

Fig. 2. Log k' plotted *vs.* binary solvent strength for (a) phenol [△], (b) aniline [○], (c) nitrobenzene [□]. Solid lines and solid symbols represent chloroform, large hatched lines and half-filled symbols dichloromethane, small hatched lines and open symbols MTBE.

pure solvent strength values alone. A similar, though not as dramatic, effect is observed with aniline and the three solvents. Again, MTBE is a stronger solvent for aniline than predicted, but the discrepancy is not as pronounced as with phenol.

Although Fig. 2a and b indicate MTBE is a stronger solvent than predicted for both phenol (acidic) and aniline (basic), the origins of this anomolous behavior appear to be somewhat different. The MTBE plot for phenol differs from those of phenol in chloroform and dichloromethane in both slope and intercept. The MTBE plot for aniline differs from the corresponding plots of chloroform and dichloromethane primarily in slope. Inspection of eqn. 5 and the discussion which accompanies it suggests that both delocalization and secondary solvent effects are responsible for decreased retention of phenol in hexane–MTBE binary mixtures, whereas

delocalization effects are primarily responsible for decreased retention of aniline in the same mixtures.

The nature of these secondary solvent effects are of interest because they decrease rather than increase retention. Also, the origin of the effects seem to depend on the acid–base properties of the solute. The decrease in retention of aniline can be explained as being due to the localization effect of MTBE on residual silanols, effectively blocking these acid sites from interacting with the weakly basic solute. The net result is a negative secondary solvent effect in the stationary phase.

The negative solvent effect observed for phenol is more difficult to explain since concentration of basic MTBE in the stationary phase should increase retention of acidic phenol. However, if MTBE molecules are strongly localized on silanol sites with their oxygen atoms oriented above these silanols, the bulky *tert.*-butyl groups may prevent phenol from interacting with either the basic portion of an adsorbed MTBE molecule or basic sites (oxygen atoms) on the adjacent silica surface. Furthermore, there may be a mobile phase effect as well, since phenol molecules can experience true acid–base interactions with MTBE molecules in the mobile phase.

An indication of the selectivity of the cyanopropyl stationary phase can be obtained from Fig. 3, in which the retention behavior of all three solutes are plotted together for each solvent. In the non-localizing solvents chloroform and dichloromethane, the acidic solute (phenol) is more strongly retained than the basic solute (aniline), whereas the reverse is true in the localizing, basic solvent MTBE where aniline is more strongly retained. This behavior is a dramatic illustration of the synergistic effects of stationary and mobile phase selectivities; here we are seeing an inversion of a column's relative acid–base properties owing to a change in solvent selectivity.

The selectivity matrix

The selectivity effects just described indicate to us that retention in NBP-HPLC can be adequately described only by including the selectivities of the stationary phase, mobile phase and solute. Single-valued solvent strength parameters will not be sufficient to accurately predict retention of polar compounds and to optimize polar solvent compositions in cyano-silica columns (or *any* NBP column, for that matter).

In order to include all of the selectivity effects we have observed in a single description of a NBP column, we have developed a selectivity matrix. This is a 3 × 3 matrix, with each element given by

$$r_{ij} = -a_{ij}\varepsilon_{ij} + \Delta_{ij} \tag{7}$$

In eqn. 7, r_{ij} is the selectivity matrix which describes the relative retention of solute i in a binary mixture of hexane and polar solvent j (note that rows correspond to solutes, columns to solvents). The selectivity matrix is generated via matrix multiplication of the localization matrix, a_{ij}, by the solvent strength matrix, ε_{ij}; the product matrix is then added to the secondary solvent matrix Δ_{ij}. Elements of the localization matrix a_{ij} represent the ratio of the i^{th} solute's adsorption cross section determined experimentally in solvent j $[(A_{ij})_{\text{exp}}]$ to that calculated from molecular dimensions $[(A_{ij})_{\text{calc.}}]$. The solvent strength matrix, although it contains only three elements, is not a simple column matrix, but rather a diagonalized 3 × 3 matrix with elements

defined only when $i = j$ (Fig. 4). This is the only form of the solvent strength matrix in which matrix multiplication of the localization and solvent strength matrices generates the correct 3 × 3 product matrix. This product matrix can be viewed as weighted solvent strength values and reflects not only a solvent's affinity for the stationary phase [ε_{ij}], but also its ability to selectively displace polar solute molecules from the surface [a_{ij}].

The secondary solvent matrix represents the retention of the i^{th} solute in pure hexane plus any secondary solvent effects which might be occurring in either the mobile or stationary phases. We have incorporated both of these parameters into a single matrix because it is not possible *a priori* to determine them individually. Extrapolation of a log k' *vs.* ε_{ab} plot gives an intercept value which is the sum of (log $k'_h + \Delta_2$). The magnitude of the Δ_2 term is best determined by comparison of Δ_{ij} elements for a solute in each mobile phase.

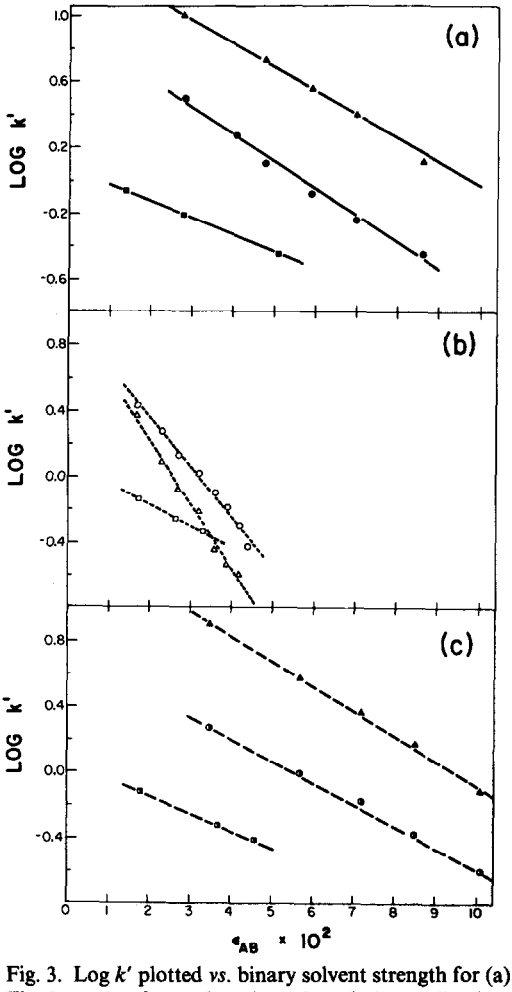

Fig. 3. Log k' plotted *vs.* binary solvent strength for (a) chloroform, (b) MTBE, (c) dichloromethane. See Fig. 2 caption for explanation of symbols and line hatching.

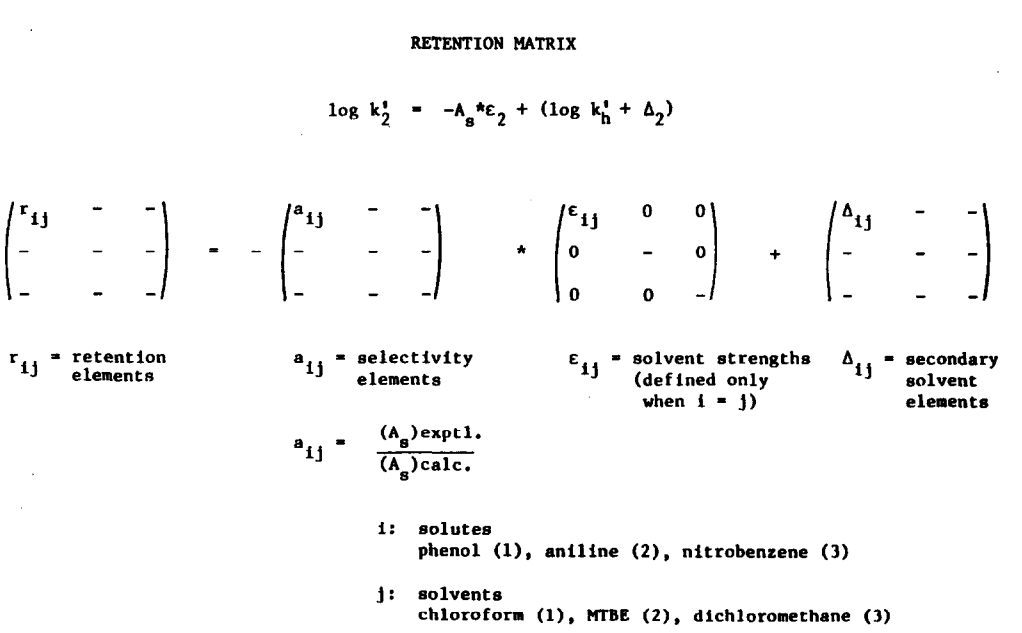

Fig. 4. Schematic representation of matrices used to calculate selectivity matrix.

Comparison of eqns. 5 and 7 reveal that eqn. 7 is the matrix analogue to the Snyder equation, with log k's represented by the selectivity matrix, A_s values represented by the relative cross-section elements of the localization matrix, and the zero-volume modifier intercept ($\log k'_h + \Delta_2$) represented by the secondary solvent matrix. The individual matrices involved in the calculation of a selectivity matrix and their analogous Snyder equation parameters are summarized in Fig. 4.

Results of our studies of selectivity in cyanopropyl NBP columns are presented in matrix form in Table IV. Elements of the localization matrix were obtained by plotting log k' *vs.* ε_{ab}, where ε_{ab} values were determined experimentally with non-localizing aromatic hydrocarbons. The slopes of these plots give $[(A_{ij})_{exp}]$ values when the activity coefficient is unity.

Inspection of the representative matrices of Table IV reveal much about the

TABLE IV

RETENTION MATRIX FOR CYANOPROPYL BPC

N = nitrobenzene, A = aniline, P = phenol.

$$\begin{pmatrix} 1.17 & 0.47 & 1.16 \\ 0.67 & 0.58 & 0.51 \\ -0.08 & -0.06 & -0.11 \end{pmatrix} = -\begin{pmatrix} 2.25 & 6.25 & 2.35 \\ 2.49 & 4.61 & 2.01 \\ 1.40 & 1.76 & 1.37 \end{pmatrix} * \begin{pmatrix} 0.106 & 0 & 0 \\ 0 & 0.085 & 0 \\ 0 & 0 & 0.120 \end{pmatrix} + \begin{pmatrix} 1.41 & 1.00 & 1.44 \\ 0.93 & 0.97 & 0.75 \\ 0.07 & 0.09 & 0.05 \end{pmatrix}$$

Observed retention order

Chloroform: N < A < P

MTBE: N < P < A

Dichloromethane: N < A < P

retention of polar solutes in a cyanopropyl column when using mobile phases which differ considerably in their selectivity. First, it is important to note that the selectivity matrix correctly predicts the observed retention order in all three solvents. For example, in chloroform the observed retention order (nitrobenzene < aniline < phenol) is reflected in the elements of the first column (the chloroform column) of the selectivity matrix [nitrobenzene (−0.08) < aniline (0.67) < phenol (1.17)]. In addition, the selectivity matrix indicates which polar solvents are able to selectively elute a particular solute; phenol is much more rapidly eluted in MTBE (r_{12} = 0.47) than either chloroform (r_{11} = 1.17) or dichloromethane (r_{13} = 1.16).

The selectivity matrix thus presents a clear, empirical picture of the retention behavior of polar solutes in a NBP column when using binary solvent mixtures representative of the apices of the selectivity triangle. It has a number of practical implications. The optimal binary mixtures for maximum resolution of solutes with different selectivities can be quickly determined. For example, proton donor and proton acceptor solutes appear to be best separated with dichloromethane (r_{23}/r_{13} = 2.28) than chloroform (r_{21}/r_{11} = 1.75), even though the absolute retention of both is greater in chloroform than dichloromethane. This behavior, clearly evident from the selectivity matrix, supports Snyder's long-standing contention that maximum resolution is best achieved through optimization of solvent selectivity, not through changes in solvent strength.

The selectivity matrix also provides a means by which the chromatographic behavior of solutes can be assessed. That is, the relative importance of a solute's proton donor, proton acceptor and dipole characteristics in a particular NBP column can be determined by generating its matrix row and comparing the elements of the row to those of phenol, aniline and nitrobenzene in the same column.

Another important feature of the selectivity matrix is that it indicates changes in the retention characteristics of a NBP column due to changes in mobile phase selectivity. Thus, the selectivity matrix can be used as a guide to changing the selectivity of a stationary phase through changes in mobile phase selectivity, thus in principle expanding the range of separations obtainable with a single column. This supports the conclusions presented in a recent publication[21] that a single stationary phase (cyano-silica) is sufficient to separate a wide variety of polar substances, separation depending on an optimized binary or ternary mixture of hexane and polar solvent(s). Cyanopropyl columns, for example, appear to have basic tendencies in chloroform (greater retention of phenol relative to aniline), but acidic tendencies in MTBE (reversal of phenol/aniline elution order).

One application of the selectivity matrix which we feel has significant potential is in the development of "stationary phase programming". As we have noted, cyanopropyl columns can have either acidic or basic tendencies, depending on the selectivity of the mobile phase. We have generated selectivity matrices on a number of other columns[22], and have noted similar effects. What is particularly interesting about these results is that the same solvent can produce opposite characteristics in different NBP columns depending on the nature of the stationary phase. As we have noted, the retention order in a cyanopropyl column when using chloroform is nitrobenzene < aniline < phenol while the retention order in a diol column is nitrobenzene < phenol < aniline. These observations suggest that dissimilar columns can be coupled in series and used in an isocratic mode to produce approximately two-di-

mensional separations. We refer to this technique as stationary phase programming. In a recent review article, Giddings[23] pointed out that true two-dimensional separations can be achieved only when the retention mechanisms of the two systems are different. Serial coupling of similar columns produce separations which approach only $2R_1$ (R_1 = resolution factor of the columns), whereas the coupling of dissimilar columns with unique retention characteristics can approach, in the best case, $R_1 \times R_2$. One of the most attractive features of this stationary phase programming approach is that, in contrast to solvent programming, reverse programming and mobile phase–column equilibration are not necessary, greatly reducing time and expense.

In the preceding paragraphs we have described the practical implications of the selectivity matrix. However, inspection of the other matrices which are generated and used to calculate the selectivity matrix (localization, solvent strength and secondary solvent matrices) reveals several features about the mechanisms responsible for a column's overall retention characteristics. As we noted earlier, secondary solvent effects and site-competition delocalization appear to be responsible for the inversion of the retention order of phenol and aniline in a cyanopropyl column when using MTBE instead of chloroform or dichloromethane. The localization and secondary solvent matrices for our cyanopropyl column (Table IV) quantitatively measure the importance of these effects. The second column of the secondary solvent matrix indicates that a negative solvent effect is responsible for a reduction in the retention of phenol, since the second column elements (MTBE elements) for these solutes are less than the corresponding elements for chloroform and dichloromethane. We attribute this to specific acid–base interactions in the stationary phase.

MTBE localization reduces the retention of both phenol and aniline, but the relative magnitude of this stationary phase solvent effect appears roughly equivalent for both solutes. The change in retention order of these solutes, then, must be due to site-competition delocalization, as indicated by the extremely large localization element for phenol in MTBE ($a_{12} = 6.25$) relative to aniline ($a_{22} = 4.61$). We believe this is again an indication of some basic character to the cyanopropyl bonded phase. Phenol (proton donor) can hydrogen bond with the cyano group and thus localizes on the mobile sites of the bonded phase. MTBE localizes on the fixed silanol sites above the silica support surface, thus interfering with phenol localization on adjacent bonded phase sites. We believe this is a classic example of a localizing solvent interacting laterally with mobile bonded phase sites and interrupting solute localization on those bonded phase sites; that is, site-competition delocalization.

The localization matrix indicates that all of these solutes localize to some extent on a cyanopropyl bonded phase, regardless of the solvent mixture. The magnitude of these localization effects is larger than those observed in other columns with less polar solutes. As a check on our results, we calculated a localization element for nitronaphthalene in dichloromethane from the data of Wieser *et al.*[13] and compared it with an analogous element determined on our column. The agreement, 1.66 calculated from Weiser's data *vs.* 1.64 from our's, is an indication of the accuracy of our localization matrix.

CONCLUSIONS

The retention of polar solutes by cyano-silica using binary mixtures of hexane

and polar solvents representative of the apices of the selectivity triangle has been studied as a function of the selectivity of the solvents and solutes. Our results confirm previous suggestions that, when used with polar solutes and/or solvents, cyano-silica possesses unique retention characteristics which distinguish it from deactivated silica.

We have developed a selectivity matrix in order to include all of the selectivity effects in a single description of a NBP-HPLC column. The selectivity matrix provides not only practical information about the relative retention of acidic, basic and dipolar solutes in different mobile phases, but also insight into the mechanisms responsible for the observed retention behavior. In our characterization of a cyanopropyl bonded phase column, we observed that the selectivity of the bonded phase varies due to changes in the selectivity of the mobile phase. The cyanopropyl phase appears to have basic tendencies when used with hexane–chloroform mobile phases but acidic tendencies with hexane–MTBE. The selectivity matrix also identified a pronounced negative secondary solvent effect occurring in the stationary phase due to localization of basic MTBE molecules on residual silanol sites of the silica support.

Unique localization effects have also been noted with this bonded phase. These effects are responsible for the distinct difference in character between cyano-silica and deactivated silica. In particular, acidic solutes appear to localize on cyano groups of the bonded phase, and this localization can be affected by lateral interactions between mobile cyano groups and basic solvent molecules which themselves are localized on adjacent residual silanols. The localization and secondary solvent effects just described can be utilized to extend the selectivity, and thus applicability, of cyano-silica NBP columns. We believe these results for cyano-silica, and analogous results soon to be published for other NBP columns[22], can lead to the development of stationary phase programming using coupled NBP columns and isocratic mobile phases.

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