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COMPARISON OF STATIONARY PHASE FORMATION IN RP-FOR METHANOL–WATER SYSTEMS

CLEMENT R. YONKER, THOMAS A. ZWIER* and MICHAEL F. BURKE*
Department of Chemistry, University of Arizona, Tucson, AZ 85721 (U.S.A.)
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SUMMARY

A proposed model for a ternary stationary phase system for RP-18 and RP-8 is evaluated. The stationary phase is a combination of bonded organic moiety, silica substrate and associated solvent molecules. The volume and composition of the stationary phase were found to vary under changing mobile phase conditions. Stationary phase formation by solvation of the bonded organic moiety is different but related for RP-18 and RP-8. Selectivity (α) was also seen to plateau for RP-18 and RP-8 at different values. The overall stationary phase formation was found to be dependent on the chain length of the bonded organic moiety and residual silanol activity for RP-18 and RP-8.

INTRODUCTION

Since the development and implementation of chemically bonded phases for chromatographic separations[1], the structure, composition and volume of the stationary phase have been shrouded in uncertainty. The structure of the stationary phase is dependent on the initial silane starting material, *e.g.*, trichlorosilanes, polymerization and cross-linking; dichlorosilanes, polymerization; and monochlorosilanes, no polymerization. The composition is defined by the organic moiety attached to the silica surface through the silane, be it an octydecyl, octyl or propylamine group. The volume of the stationary phase (V_S) is of fundamental importance in chromatography, as seen in the equation

$$V_R = V_m + KV_s \tag{1}$$

where V_R is the retention volume of the solute and V_m is the volume of mobile phase in the column. The need for an actual value of V_s can be circumvented by using the retention factor (k') (ref. 2), which is defined by the equation

$$k' = \frac{t_r - t_0}{t_0} \tag{2}$$

* Present address: The Upjohn Company, 7000 Portage Road, Kalamazoo, MI 49001, U.S.A.

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where t_0 is the breakthrough time for a non-retained species and t_r is the retention time of a solute species. The relationship between K and k' is

$$k' = K \cdot \frac{V_s}{V_m} \tag{3}$$

Therefore, the use of k' assumes that the phase ratio is constant, which is not proved to be a valid assumption[3]. It is therefore of considerable interest to know the actual volume of the stationary phase as a function of the mobile phase composition and the bonded material.

The measurement of V_s has been undertaken by many workers[3–7]. Melander *et al.*[3] estimated the relative magnitude of the phase ratio between columns showing homoenergetic retention by plots of $\ln k'_A$ *versus* $\ln k'_B$, where k' is the retention factor for a solute under similar conditions for columns A and B. Sander and Field[4] estimated the phase ratio using a geometric model of the silica surface based on manufacturer's information on silanol surface coverage and percentage of carbon bonded to the surface. From a knowledge of the phase ratio, which could be calculated by one of the above-mentioned methods, V_s can be determined for any column once V_m is accurately known. Berendsen *et al.*[5] studied various methods for the determination of t_0, the elution time of a non-retained component, by using salts, deuterated mobile phase components and linearization of a homologous series. They demonstrated that the most satisfactory method for determination of t_0 and thus V_m was by linearization of a homologous series. They also showed that t_0 changed for a column on varying the percentage of organic modifier in the mobile phase. McCormick and Karger[6] and Slaats *et al.*[7] measured the distribution isotherms of methanol, acetonitrile and tetrahydrofuran (THF) between the bonded organic moiety and the mobile phase for varying ratios of non-aqueous modifier in the mobile phase. These investigators showed an enrichment of the stationary phase by the non-aqueous modifier. Ruckert and Samuelson[8] first studied the distribution of both water and organic modifiers between aqueous mobile phases and in ion-exchange resins. Tilly-Melin *et al.*[9] measured both the change in V_m and the enrichment of the stationary phase by the non-aqueous modifier. They related the two together for RP-8 with acetonitrile as the non-aqueous modifier from 10 to 60% (v/v).

As the work by the above investigators demonstrates, the formation of the stationary phase is a dynamic process under control of the mobile phase. In the debate concerning the underlying mechanism of the separation process in reversed-phase chromatography (RPC), the mechanisms of a solvophobic effect and partitioning have been proposed. The hydrophobic effect consideres the bonded phase as a passive receptor for the solute. The solvation of the bonded moiety serves no role in retention except to present a barrier between the organic moiety and the solute. Scott and Kucera[10] investigated if the solute displaces the solvent layer absorbed to the stationary phase, as would occur in a hydrophobic mechanism, and found no solvent displacement.

All the above-mentioned work supports the view of the stationary phase being a ternary combination of bonded organic moiety, absorbed solvent molecules and residual silanols on the silica surface. The solvation of the bonded organic moeity by the non-aqueous modifier should be viewed as a dynamic process dependent on the

moiety present and the substrate to which the moiety is bonded. This dynamic process must be considered in the overall separations mechanism in RPC. In this work a study was undertaken to compare the enrichment of the bonded organic moeity by methanol for RP-8 and RP-18, and the possible role solvation plays in the selectivity of the separations process.

EXPERIMENTAL

By linearization of a homologous series of alcohols, following the procedure set forth by Berendsen *et al.*[5], one obtains an equation for a line:

$$t_{rN+1} = \alpha t_{rN} - t_0 (\alpha - 1) \tag{4}$$

Plotting t_{rN-1} (retention time of the N + 1-carbon homologue *versus* t_{rN} (retention time of the N-carbon homologue), t_0 can be determined from the slope of the line α (relative retention) and the intercept. The elution time of a non-retained solute, t_0, can be expressed as V_m, the volume of mobile phase in the column by multiplying t_0 by the flow-rate through the column. The change in t_0 (Fig. 3) is inversely related to the change in volume of the stationary phase, V_s. Thus, the measurement of V_m will be sensitive to volume changes in V_s owing to solvation of the bonded moiety and or substrate by the non-aqueous modifier.

The alcohols in this study were obtained from Aldrich (Milwaukee, WI, U.S.A.), except for methanol (Fisher Scientific, Fair Lawn, NJ, U.S.A.), and were used without further purification. The alcohols included methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, 1-decanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol and 1-octadecanol. Depending on the mobile phase composition, a selected series of alcohols were used as solutes, *e.g.* methanol–water (20:80) for RP-18; the alcohols used as solutes were ethanol ($\bar{t}_r$ = 114.5 sec), *n*-propanol ($\bar{t}_r$ = 181.4 sec), *n*-butanol ($\bar{t}_r$ = 401.7 sec) and *n*-pentanol ($\bar{t}_r$ = 1176.3 sec). The solutes were dissolved in the mobile phase before being injected on to the column. The columns employed were slurry packed in the laboratory [100 × 4.6 mm I.D. LiChrosorb RP-18 and RP-8, 10 μm particle size (MCB, Cincinnati, OH, U.S.A.)]. The solvent delivery system was an Altex Model 420 with 110a pumps (Beckman Instruments, Irvine, CA, U.S.A.). The detector was a Waters R403 differential refractometer with a 10-μl flow cell (Waters Assoc., Milford, MA, U.S.A.). The solvents were methanol and doubly distilled water, which were filtered, mixed by volume and sonicated for 15 min prior to use. The column and detector were both thermostated at 25°C in a water-jacket. Prior to making flow-rate measurements, the column was equilibrated with approximately 200 column volumes of the appropriate solvent. After equilibration was complete, the probes were injected (concentrations were *ca.* 2.5 μl/ml) and their retention times were measured by a System I computing integrator (Spectra-Physics, San Jose, CA, U.S.A.) to 0.1 sec. The average of five replicate injections was used in calculating t_0. The extra column volume was determined by injecting methanol–water (8:2) into 100% methanol with the column removed.

The total amount of methanol extracted from the column was determined using a Varian Model 1700 gas chromatograph with a flame-ionization detector. The

column was a 1:1 (w/w) mixture of Poropak Q and R in a 6 ft. × 1/4 in. I.D. copper column at 185°C, with nitrogen as the carrier gas at a flow-rate of 25 ml/min. The detector and injector temperatures were 200°C, respectively. Water was determined using a Gow-Mac Model 550 GC with a thermal conductivity detector. A 4 ft. × 1/4 in. I.D. copper column of Poropak R was used at 140°C with helium as the carrier gas at a flow-rate of 25 ml/min. The detector bridge current was 200 mA at 200°C and the injector temperature was 180°C. After measuring V_m, the column was flushed with approximately 48.75 ml of dioxane (distilled from potassium hydroxide) at 2 ml/min into a 50-ml volumetric flask containing 1.00 ml of isopropanol as internal standard. The contents of the flask were brought to volume with dioxane. An injection volume of 3 μl was used. Peak areas were measured with a Spectra-Physics Autolab Minigrator and the appropriate area ratios from at least three injections were averaged for the determination of the amount of solvent extracted. The volume of solvent extracted was determined from a calibration graph relating a known amount of methanol to the peak-area ratio of methanol to isopropanol. Subtracting the percentage of methanol in V_m and extra-column volume from the gas chromatographic (GC) measurement, the amount of modifier in the solvation layer can be determined by the equation

$$V_T = \%MV_m + \%MV_{ec} + V_s \tag{5}$$

where V_T is the total amount of modifier measured by GC, V_m is the volume of mobile phase in the column measured by eqn. 2, $\%M$ is the volume percentage of modifier in the mobile phase, V_{ec} is the extra-column dead volume and V_s is the volume of the solvation layer of stationary phase. The amount of water extracted was determined in the same manner. The validity of these measurements are borne out by those of Westerlund and Theodorsen[11], who used RP-8 with methanol–buffer (40:60) as the mobile phase and determine 0.18 ml/g of methanol in the stationary phase. This value is within the experimental error of our own measured value using methanol–water (40:60) with RP-8 (see Table II).

RESULTS AND DISCUSSION

The model of the stationary phase, as proposed, is a combination of silica substrate, bonded organic moiety and solvation layer consisting of mobile phase components. The interaction of the mobile phase with the silica substrate and bonded moiety controls the composition and volume of the solvation layer. The influence of the mobile phase on the formation of the stationary phase for RP-18 and RP-8 can be seen in Tables I and II and Figs. 1 and 2. In general, during solvation the organic moeity can selectively enrich the stationary phase in lipophilic non-aqueous modifiers through dispersion interactions, while residual silanols also influence the uptake of aqueous and non-aqueous modifiers with hydrogen bonding capabilities such as those normally used in RPC, *e.g.*, water, methanol, acetonitrile and THF.

The data in Tables I and II have been normalized by the surface area of the packing material. This normalization by surface area [RP-8 250 m^2/g, RP-18 150 m^2/g (see ref. 12)] allows one to compare the two packing materials with respect to the effect of chain length and residual silanols. Surface coverage and degree of derivatization are

TABLE I

INFLUENCE OF MOBILE PHASE ON FORMATION OF STATIONARY PHASE FOR RP-18

RP-18: surface area 150 m^2/g; pore size 150 Å; length of chain C_{18}; carbon content 19.8%; functional group bonded dimethyloctyldecylchlorosilane; calculated degree of derivatization 42%; surface coverage 5.5 μmol C/m^2; weight in column 0.8948 g.

Parameter	*Methanol–water composition of mobile phase*					
	0:100	*20:80*	*40:60*	*60:40*	*80:20*	*100:0*
t_0 (sec)	67.18	61.95	54.85	49.72	49.68	46.91
V_m (ml)	1.12	1.03	0.914	0.829	0.828	0.782
V_{CH_3OH} in stationary phase (ml/g) (±0.03)	0.00	0.04	0.16	0.25	0.30	0.37
V_{CH_3OH} in stationary phase (μmol/m^2) (±5.60)	0.00	7.24	25.7	40.5	49.1	61.4
V_{H_2O} in stationary phase (ml/g) (±0.03)	0.27	0.04	0.08	0.09	0.03	0.00
V_{H_2O} in stationary phase (μmol/m^2) (±11.1)	101	16.3	31.1	34.8	10.4	0.00
V_s total volume in stationary phase (ml/g) (±0.04)	0.27	0.09	0.24	0.34	0.33	0.373
V_s, total volume in stationary phase (μmol/m^2) (±12.4)	101	23.5	56.8	75.3	59.5	61.4
α	4.06	3.48	2.59	1.90	1.54	1.46
Methanol in stationary phase (%, v/v, total)	0	50	65	72	91	≈100

TABLE II

INFLUENCE OF MOBILE PHASE ON FORMATION OF STATIONARY PHASE FOR RP-8

RP-8: surface area 250 m^2/g; pore size 100 Å; length of chain C_8; carbon content 12.2%; functional group bonded dimethyloctylchlorosilane; calculated degree of derivatization 30%; surface coverage 4.0 μmol C/m^2; weight in column 0.7344 g.

Parameter	*Methanol–water composition of mobile phase*					
	0:100	*20:80*	*40:60*	*60:40*	*80:20*	*100:0*
t_0 (sec)	77.61	67.94	62.61	57.29	45.3	
V_m (ml)	1.29	1.13	1.04	0.955	0.755	
V_{CH_3OH} in stationary phase (ml/g) (±0.04)	0.00	0.07	0.18	0.37	0.72	
V_{CH_3OH} in stationary phase (μmol/m^2) (±4.10)	0.00	7.25	17.9	37.0	71.8	
V_{H_2O} in stationary phase (ml/g) (±0.04)	0.25	0.05	0.08	0.13	0.19	
V_{H_2O} in stationary phase (μmol/m^2) (±9.10)	54.6	10.9	18.2	29.3	42.0	
V_s total volume in stationary phase (ml/g) (±0.06)	0.25	0.12	0.26	0.50	0.91	
V_s, total volume in stationary phase (μmol/m^2) (±10.0)	54.6	18.2	36.1	66.3	114	
α	3.62	3.18	2.40	1.75	1.71	
Methanol in stationary phase (%, v/v, total)	0	60	69	74	79	

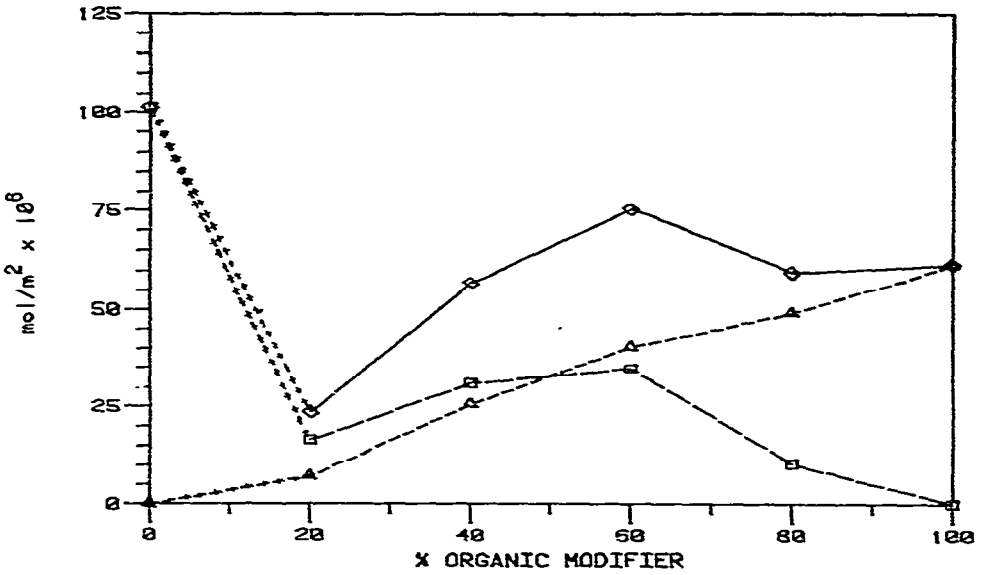

Fig. 1. Concentration of solvent absorbed into the stationary phase *versus* percentage of organic modifier in the mobile phase for RP-18. Individual points are connected for clarity. △, Methanol; □, water; ◇, total.

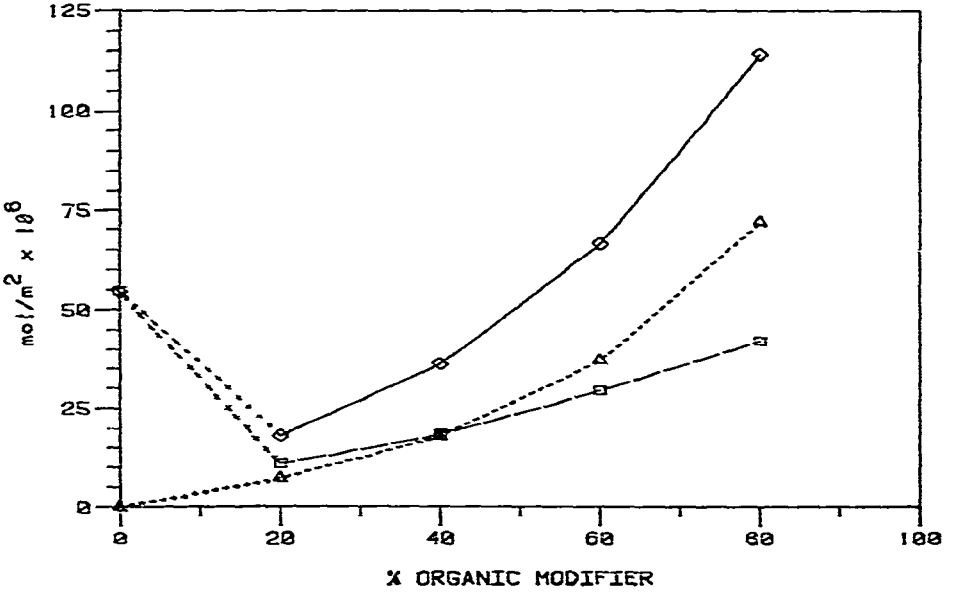

Fig. 2. Concentration of solvent absorbed into the stationary phase *versus* percentage of organic modifier in the mobile phase for RP-8. Individual points are connected for clarity. Symbols as in Fig. 1.

also important points to consider when comparing two different packing materials. If one assumes a geometric model of the silica surface in which there are eight accessible silanol groups per 100 Å^2 (refs. 13 and 14), then the maximum surface coverage would be 13.3 μmol C/m^2. For RP-18, the surface coverage is 5.5 μmol C/m^2, and the degree of derivatization is *ca.* 42%. For RP-8, the surface coverage is 4.0 μmol C/m^2 and the degree of derivatization is *ca.* 30%. These values are not absolute but are dependent on the geometric model chosen, but the trends in surface coverage and degree of derivatization are significant.

In order to understand the solvation mechanism of RP-8, one must begin with a geometric model of its surface. With the assumption of 8 silanols per 100 Å^2, it is reasonable to assume that the bonded structure for these sites will be the one of lowest energy for the number of sites occupied by the silane. That is, the monochlorosilanes will bind as far away from each other as possible. These sites will be at the corners of an isosceles triangle with the base *ca.* 10.0 Å and the sides *ca.* 11.2 Å long. The remaining silanols are either sterically hindered from any interactions or hydrogen bonded to the solvent present, *e.g.*, water, methanol, acetonitrile or THF. This geometric model accounts for 3 silanols per 100 Å^2 for silane binding. The RP-8 surface with 30% derivatization has 2.5 silanols per 100 Å^2 which are chemically bonded; this is in close agreement with the above postulated model Therefore, a comparison between the model and the RP-8 surface can be undertaken. The physical phenomena inherent in this model will manifest themselves for the case of RP-8 through the amount of structuring of the bonded moiety by the loss of degrees of freedom on bonding to the surface, and the residual silanol activity of the surface.

Under 100% aqueous conditions, the C_8 chain will tend to increase the entropy of the surrounding water molecules by decreasing its surface area through intermolecular and intramolecular interactions[15]. Intramolecular interactions are limited owing to the rigidity imparted to the molecule on binding to the surface, and the limits imposed by the C_8 chain length. This structuring of the C_8 moiety on binding to the surface is borne out through ^{13}C nuclear magnetic resonance investigations of the RP-8 surface[16]. Intermolecular interactions are restrained owing to the limits imposed by the geometric model of the surface. The distance between C_8 moieties as determined from the model are 10.0 and 11.2 Å, respectively, while the C_8 chain length is *ca.* 12.0 Å. The C_8 chain will have difficulty undergoing efficient intermolecular interactions with neighboring chains, because of the C_8 chain length and the distance between bonded chains. Also, the hydrophilic surface, from the remaining silanols, will not allow a close approach of the lipophilic C_8 chain. Both of these reasons will combine in decreasing the amount of C–C overlap for efficient dispersion interactions between C_8 chains. As the percentage of methanol increases in the mobile phase (0–20%), there is enough methanol present to overcome the weak interchain dispersion interactions and solvate the C_8 chains. This results in the enrichment of the non-aqueous modifier in the stationary phase under low methanol concentrations as seen in Table II, for 20% methanol in the stationary phase. On solvation by methanol, the RP-8 assumes a "brush"-like structure, with the mobile phase having direct access to the residual silanols on the silica surface. As the concentration of methanol in the mobile phase increases above 20%, the solvation of the RP-8 surface is dominated by its residual silanols. Any solvent molecules that can effectively hydrogen bond to the residual silanols present between the C_8 chains will be brought into the

stationary phase. Methanol and water, being hydrogen-bond donors or acceptors, will bring other water and methanol molecules with them into the stationary phase. Therefore, hydrogen bonding is the chemical driving force behind stationary phase formation. This mechanism is dependent on mass action: the higher the percentage of methanol in the mobile phase, the more methanol can be brought into the stationary phase by solvent molecules already present, and the more methanol brought into the stationary phase, the more water can be brought along with it. A synergistic effect is prevalent between the methanol and water, which aids the stationary phase formation. This explains the results in Table II and Fig. 2 of the continuing increase in the water present in the stationary phase throughout the entire mobile phase compositional range. Dispersion interactions between C_8 and methanol play a major role in solvation for low concentrations of methanol modifier (between 0 and 20%). Once methanol has initially solvated the C_8 chain, opening the C_8 structure by breaking any weak interchain dispersion interactions, the synergistic effect due to the residual silanols on the surface takes over and continues throughout the solvent compositional range.

RP-18 has the same geometric surface model of 8 silanols per 100 Å, but the bonding arrangement of the silanes are different. In order to explain the experimental data of RP-18 in which *ca.* 3.3 sites per 100 Å are bonded by silanes, a model of the RP-18 system must have at least four non-sterically hindered silanols with which to chemically bond through. An arrangement of this nature can be made within the constraints of 100 $Å^2$ by making the silanols be at the corners of a square with free silanols surrounding them. This proposed model will have four unhindered silanols for binding, with four silanol groups remaining. One consequence of this model is that the C_{18} chains are bonded closer to one another than the C_8 chains. So, unlike the RP-8 material, the carbon chains in RP-18 are long enough to undergo effective intermolecular interactions, and probably weaker intramolecular interactions even with the first 6–8 carbons being rigid due to bonding. This picture of the surface is similar to Lochmuller's[16].

The solvation of RP-18 is a meld of two mechanisms. The first is a mechanism similar to RP-8 involving the residual surface silanols, and the second involves the increased dispersion interactions for RP-18 due to the larger carbon surface area of the chain as compared to RP-8. From 0–60% methanol modifier concentrations the solvation is similar to RP-8 in that residual silanols on the surface not sterically hindered by the C_{18} chains dominate the solvation. This mimics the RP-8 solvation process with one major exception, which occurs at low methanol modifier concentrations, <20%. For RP-18 under low methanol modifier concentrations, most of the intramolecular interactions and all of the intermolecular interactions between chains are intact. Therefore, the methanol does not open the C_{18} structure as it does the C_8 structure, with the resulting increase in methanol solvation for RP-8 over RP-18. The residual silanols between the clumps of C_{18} chains dominate the solvation mechanism up to *ca.* 70% methanol modifier concentration through their ability to hydrogen bond with either water or methanol. As the percentage of methanol in the mobile phase increases, the intermolecular interactions between chains are "unzipped" from top to bottom by the mass action effect of the methanol. When the solvent-chain interactions overcome the intra- and intermolecular interactions of the chain, the C_{18} becomes more erect and "brush"-like. This "brush"-like structure increases the

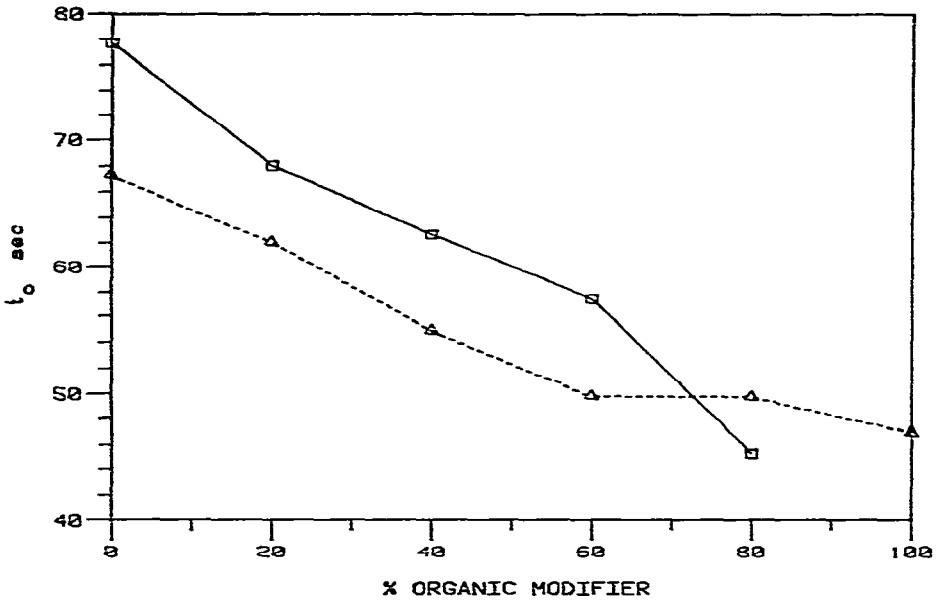

Fig. 3. Elution time for a non-retained peak (t_0) *versus* percentage of organic modifier in the mobile phase for RP-18 (△) and RP-8 (□). t_0 was determined by eqn. 4.

ability of the C_{18} chain to undergo dispersion interactions with methanol, as there is a larger carbon surface area available for Van der Waals' interactions with solvent molecules for C_{18} than for C_8. This increased solvation of the C_{18} by the second mechanism is reflected in the more "liquid"-like spectra seen in ^{13}C nuclear magnetic resonance experiments[16]. At *ca.* 70% methanol in the mobile phase the second mechanism of solvation begins to dominate. This "unzipping" of the C_{18} interactions is completed and methanol now has access to the increased carbon surface area. Methanol can effectively solvate the C_{18} with a resulting "brush"-like structure. These events will cause an enrichment of the C_{18} in organic modifier. Also, with the more open structure methanol can remove any water from between the chains. This hypothesis explains the jump in the percentage of methanol in the stationary phase which is seen in Table I for RP-18 at an 80% concentration of methanol modifier.

There is one final point to be discussed which is relevant to both RP-18 and RP-8, and that is stationary phase formation at 100% water in the mobile phase. From Tables I and II a large stationary phase volume is found at this value for the mobile phase composition. A possible explanation for this observation is two-fold: (1) the residual silanols present on the silica surface are involved in hydrogen bonding with the water present from the mobile phase and (2) water can be trapped on the substrate surface by a "tent" of C_{18} or C_8 chains. This trapping of water is caused by the "freezing" of the bonded chains. This "freezing" of the bonded chains results from the intra- and/or intermolecular interactions amongst the chains themselves. Such interactions would be expected to be energetically favorable when compared only with the interactions between the water matrix and the hydrocarbon. At 100% water in the mobile phase an adsorption mechanism is most likely responsible for chromatographic retention, in contrast to a partition mechanism in a solvation layer.

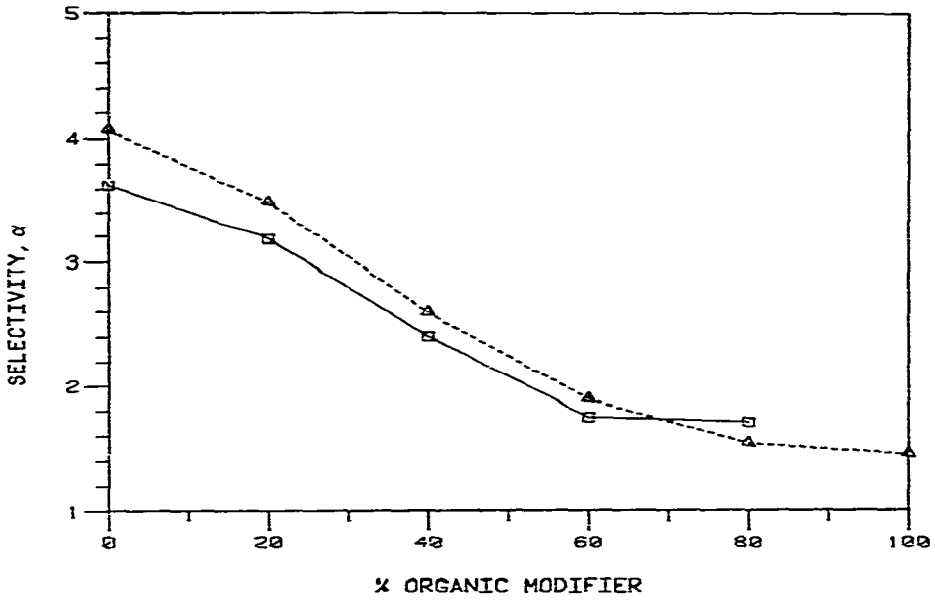

Fig. 4. Selectivity (α) of RP-18 (△) and RP-8 (□) for ΔCH_2 *versus* percentage of organic modifier in the mobile phase. α was determined by eqn. 4.

Further work is being carried out to determine the exact mechanism which is responsible for chromatographic selectivity at 100% water in the mobile phase and also the conditions under which a partitioning mechanism begins to contribute.

Another interesting phenomenon is the change in α between RP-8 and RP-18 (see Fig. 4). The relative retention (α) in this experiment is based on the selectivity of the stationary phase for a change of one methylene group, ΔCH_2, in the homologous series of alcohols. RP-18 has the highest initial selectivity (Table I), but a cross-over occurs at approximately 70% methanol, and from then on RP-8 has the largest α value. A possible explanation for this cross-over of α between packing materials is the effect of surface coverage and chain length in controlling the stationary phase composition. The enrichment of the stationary phase with non-aqueous modifier does have a definite effect on the separations process. The higher the concentration of methanol in the stationary phase, the more "non-polar" the stationary phase appears to the solute, relative to the mobile phase (see Tables I and II). The more "non-polar" the stationary phase, the smaller the difference in free energy of transfer ($\Delta\Delta G_{2-1}$) for a change of one methylene group between the N and $N + 1$ homologues. Thermodynamically, $\Delta\Delta G_{2-1} = -RT\ln\alpha$ (ref. 6); if $\Delta G_2 < \Delta G_1$, then $\alpha > 1$ or $k'_2 > k'_1$. If $\Delta G_2 = \Delta G_1$, then $\Delta\Delta G_{2-1} = 0$, and $\alpha = 1$ or $k'_2 = k'_1$, and no separation of these two solutes will occur under this set of stationary and mobile phase conditions. The more "non-polar" the stationary phase becomes owing to enrichment of the non-aqueous modifier by the bonded moiety, the less discrimination the stationary phase has for the change in one dispersion interaction (ΔCH_2). Thus α approaches unity or the retention times of the two solutes become more equal.

RP-8 has the greater concentration of methanol in the stationary phase up to

70% methanol modifier concentration, and therefore RP-8 will have a smaller selectivity for ΔCH_2 than RP-18. At 70% and higher methanol modifier concentrations the concentration of methanol in the stationary phase is kept constant for RP-8 owing to the synergistic effect of the methanol and water for the RP-8 materials. At this point RP-8 then becomes the more selective packing material for a ΔCH_2 group because it appears more "polar" to the mobile phase than does RP-18. At methanol modifier concentrations above 70%, RP-18 can effectively enrich the stationary phase in methanol, owing to the "unzipping" of the C_{18} chains. This presents a larger carbon surface area to the solvent molecules for undergoing dispersion interactions, thus presenting to the solutes, a more "non-polar" stationary phase than the mobile phase, with a concomitant decrease in selectivity for a ΔCH_2 group for RP-18.

The α values of both RP-8 and RP-18 plateau at 1.7 and 1.5, respectively. These plateaus are reached for both packing materials, at a point where the enrichment of the stationary phase in methanol no longer changes the overall selectivity characteristics of the stationary phase for ΔCH_2 group, which for methanol occurs at higher modifier concentrations, because of the weakness of the dispersion interaction of methanol compared with more "non-polar" solvents such as acetonitrile, THF and dioxane[2]. The strength of the dispersion interaction of the solvent will ultimately govern how rapidly a bonded chain will be solvated and when the α plateau will be reached.

In conclusion, solvation and stationary phase formation for RP-18 and RP-8 are dynamic processes controlled through a combination of two mechanisms. The extent of control exercised by these two mechanisms is dependent on the residual silanol activity and length of carbon chain bonded to the surface. For RP-8 the low surface coverage by the silane allows the residual silanols to dominate stationary phase formation. Dispersion interactions between the solvent and C_8 chain dominate stationary phase formation only at low methanol modifier concentrations, whereas for RP-18, with its higher surface coverage and longer carbon chain, residual silanols play a decreasing role in stationary phase formation for methanol modifier concentrations from 0 to 60%. The increased carbon surface area of the C_{18} chain plays an increasing role in stationary phase formation for methanol modifier concentrations from 0 to 100%, with the cross-over point between these two mechanisms occurring at *ca.* 70% methanol for RP-18. The data presented in this paper can be contrasted with the work of McCormick and Karger[6], where for RP-8 the reported minimum V_m was found at *ca.* 60% methanol in the mobile phase. The differences probably result from the different manner in which t_0 has been measured. The linearization of a homologous series of *n*-alcohols depends only on the retention of the solute species for the determination of t_0. Therefore, V_m is independent of the possible retention of any mobile phase component. If V_m^{max} values for the data reported in Tables I and II are chosen at 0% organic modifier, *i.e.*, no solvation, then for RP-18 $V_m^{max} = 1.12 \pm 0.07$ ml and for RP-8 $V_m^{max} = 1.29 \pm 0.07$ ml. These values are reasonable when compared with the values reported and calculated from McCormick and Karger[6] and Berendsen *et al.*[5].

These results clearly show that the selectivity of a bonded material is determined by the extent of solvation of the substrate materials as well as the bonded moiety. Solvation of the stationary phase is dependent on the specific and non-specific interactions of the mobile phase components for the substrate and bonded moiety.

Further investigations involving other organic modifiers should provide a better understanding of the question of relative solvent strengths in determining the chromatographic characteristics of bonded materials.

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