Journal of Chromatography, 481 (1989) 135–146
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 823

TEMPERATURE-INDUCED CHANGES IN REVERSED-PHASE CHROMATOGRAPHIC SURFACES

C_8 AND C_9 POLYMERIC LIGANDS

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(First received July 28th, 1988; revised manuscript received July 26th, 1989)

SUMMARY

The temperature-dependent polarity of polymeric C_8 and C_9 stationary phases, preconditioned with acetonitrile and then contacted with water, is studied using the fluorescence of sorbed pyrene as a probe. The fluorescence vibronic band ratio, sensitive to the polarity of the probe surroundings, shows a temperature-dependent hysteresis which is indistinguishable from the retention behavior of these materials. The stationary phase polarity decreases when passing through the hysteresis, which is consistent with the loss of conditioning solvent from the interface but inconsistent with an extended structure for the alkyl ligands. Solvent retention in the stationary phase and its temperature dependence appear to require local ordering of the polymeric phase.

INTRODUCTION

One of the more challenging aspects of developing a comprehensive model for reversed-phase liquid chromatography has been to determine the role of the alkylated surface in the selective retention of solute molecules. While mobile phase effects dominate solute retention[1,2], the contribution of the surface to retention has been the subject of intensive investigation and interest[3–13]. These chromatographic experiments have provided insight into the structure and behavior of bonded hydrocarbon layers and have extended our ideas of the stationary phase beyond a homogeneous, hydrocarbon layer. A picture of the stationary phase as a dynamic surface, responding to conditions in the mobile phase and affecting solute retention, is beginning to emerge.

Models of the stationary phase as an alkyl "grass" or "bristles"[5] attached to the solid support, or as a hydrocarbon "blanket" covering the silica gel[3] are being replaced by models of a heterogeneous surface incompletely covered by chemically bonded alkyl ligands. Lochmüller and Wilder[4] have suggested a microdroplet configuration for the bonded layer, where alkyl ligands are aggregated by hydrophobic interactions

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0021-9673/89/$03.50

into clusters of organic material. If the solute is sufficiently small to be contained within these clusters, the stationary phase would appear as islands of non-polar material distributed on a polar silica surface. The organization of bound ligands appears to influence shape selectivity of retention[11,12] and depends on the bonding to the silica surface, the degree of surface coverage, and the underlying substrate. Distribution of solvent components into the stationary phase has also been studied, and the resulting effects on solute retention have been described[6–10].

Transfer of organic modifier from the mobile phase into the stationary phase is responsible for changes in the environment of a solute sorbed to the bound layer[13–16] and for changes in the phase volumes[6,9]. The intercalation of organic modifier into the stationary phase may, in addition to a change in the polarity of the bonded layer, be responsible for conformational changes in the surface as well. While studying the effects of temperature and conditioning solvent on polymeric C_8, C_9 and C_{10} stationary phases with a totally aqueous mobile phase, Gilpin and Squires[7] noted an interesting hysteresis in the temperature dependence of solute retention, following preconditioning with organic solvent. They showed in a subsequent study that organic modifier, initially retained by the stationary phase when the mobile phase is abruptly changed from neat organic modifier to water, is released[8] when a sufficiently high temperature is reached. The temperature at which the solvent was released correlated with the alkyl chain length and functionality of the organic solvent[7,17]. A mechanism proposed to account for the observed behavior was that solvent molecules were trapped in the stationary phase by collapse of the alkyl ligands[7] into an aggregated, metastable state[17] when exposed to water. Raising the temperature of the stationary phase above a transition temperature was postulated to cause restructuring of the surface ligands into an extended or "bristle" configuration[7,8], releasing trapped organic modifier which is replaced by water molecules between the hydrocarbon chain, now in an extended, more stable surface structure[17].

In the present work, the surface environments of C_8 and C_9 polymeric stationary phases, under conditions of the above temperature-dependent hysteresis, are examined using the fluorescence emission of sorbed pyrene as a probe. The vibronic band structure of the fluorescence from pyrene is quite sensitive to the polarity of the molecule's local environment[18]. As the solvent polarity around pyrene is increased, the intensity of the vibronic origin of the weak, highest energy L_b transition (band I at 374 nm) is increased due to symmetry lowering perturbations from the solvent environment which allow mixing with the much stronger L_a transition dominating the third vibronic peak (band III at 385 nm)[19]. As a result, the ratio of the intensities of the third and first peaks, III:I, varies inversely with increases in the polarity of microenvironment of the pyrene probe[14,18]. The vibronic band ratios of pyrene fluorescence have been used to study the interfacial microenvironments of polymeric and monomeric C_{18} stationary phases by sorption of the probe to the hydrophobic surface[14–16]. The results of these studies showed the effects on C_{18} stationary phases of organic modifier and the heterogeneity of the sorption environments as a function of overlaying solvent. In the present work, the polarity of polymeric C_8 and C_9 stationary phases, preconditioned with acetonitrile and then contacted with water, is investigated as a function of temperature using sorbed pyrene as a probe. The results indicate that the polarity of the stationary phase *decreases* upon heating, which is not consistent with an extended structure for alkyl ligands in contact with the totally aqueous mobile phase.

EXPERIMENTAL

Chemicals

Pyrene was obtained from Aldrich and used after recrystallization from ethanol–water solution. Reversed-phase high-performance liquid chromatography (HPLC) analysis of the compound using a 10-cm × 4.6 mm I.D., 10-μm particle column revealed no resolvable impurities. Acetonitrile (HPLC grade) was obtained from MCB and Burdick & Jackson. Water was purified in-house using a Corning still (MP-1) and a Barnstead, four-cartridge Nanopure system. Decane and 1,2-ethanediol were obtained from Fisher and used without further purification. Polymeric C_8 and C_9 stationary phases were prepared in-house by reacting either *n*-octyltrichlorosilane or *n*-nonyltrichlorosilane with 10-μm irregular silica (Partisil-10) having a surface area of approximately 320 m^2 g^{-1} and a mean pore diameter of 9.6 nm before derivatization. The trichlorosilane reagents were obtained from Petrarch and used without further purification. The silica was stirred in water-saturated toluene for 2 h. After filtering, dry toluene and the silane reagent were added to the silica and refluxed for 2 h. Carbon loading of the resulting polymer phase was determined to be 8.4 and 7.5% for C_8 and C_9, respectively, by M-H-W Laboratories (Phoenix, AZ, U.S.A.).

Column preparation

The stationary phase was contained in a 43 × 1.5-mm I.D. precision bore quartz tube (Wilmad Glass). The quartz microcolumn was supported in a brass cuvette assembly. Both the cell and the packing procedure have been described in detail in earlier publications[15,20].

Before an experiment, the stationary phase was conditioned by passing large volumes of acetonitrile through the column (approximately 50 ml or 2000 column volumes) at a flow-rate of 1 ml min^{-1}. Pyrene was introduced to the column with acetonitrile–water (50:50, v/v) as the mobile phase. The concentration of pyrene in the mobile phase was adjusted to a value of *ca.* 3.0 μM, which gives a surface concentration low enough to avoid excimer formation (to assure that no probe–probe interactions are present) but high enough to emit a strong fluorescence signal with a small fraction of shot noise. When the pyrene concentration on the column reached equilibrium (as determined by a steady-state fluorescence signal), the mobile phase was changed to 100% water for the remainder of the experiment. The contributions to the fluorescence signal from pyrene in the mbile phase were extremely small due to the high capacity factor conditions for 100% water mobile phase conditions. Using *in situ* fluorescence measurement of the frontal elution of pyrene through a spectroscopic microcolumn, the capacity factor (k') for pyrene on a C_{18} phase has been measured to be $6.0 \cdot 10^3$ (refs. 21 and 22). While the lower carbon loading of the C_8 and C_9 stationary phases studied here would result in a factor of two smaller k', the mass fraction of pyrene in the mobile phase under these conditions would still be less than 0.1%.

Fluorescence measurements

Emission spectra of pyrene sorbed onto the stationary phase were measured at 2.5 nm resolution on a Farrand Model 201 spectrofluorimeter (Farrand Optical, New York, NY, U.S.A.). The excitation wavelength used through the experiment was 335 nm. Fluorescence emission spectra were recorded on a strip chart. To obtain a measure of the stationary phase polarity, the intensity ratio of the third vibronic band to the highest energy, first band was calculated. The reported results were typically obtained from the average of seven spectra. At a particular temperature, the precision of the vibronic band intensity ratios was normally 0.3% relative standard deviation.

The brass cuvette, when placed in the spectrometer, was in thermal contact with a circulating fluid bath (Haake, Model L). The temperature of the cell could be set between 10 and 110°C (± 0.25°C) using a Haake temperature controller (Model D3). In a typical experiment, the column would be raised from 14 to 65°C and then cooled down to approximately 15°C. A series of spectra were recorded every 2.5–5.0°C when the temperature was being increased and every 5.0–15.0°C when the sample was cooling down. The magnitude of the temperature change between the series of spectra was determined by the behavior of the stationary phase; *i.e.* the rate of change of surface polarity. After a series of spectra at a particular temperature were recorded the system was allowed to reach the next temperature and kept there for 10 min to ensure satisfactory equilibration; during this period the solvent flow was discontinued. Aqueous moble phase at a flow-rate of 1.0 ml min^{-1} was then pumped through the column for 2 min to remove any released organic modifier. The flow-rate was reduced to 0.5 ml min^{-1} while the spectra were being recorded.

The temperature dependence of pyrene vibronic bands was also measured in free solution in four different solvents in a standard 1-cm quartz fluorescence cel (Wilmad Glass). The solvents used covered a wide range of polarity: decane, 1,2-ethanediol, acetonitrile and water. The conditions were the same as on-column experiments except

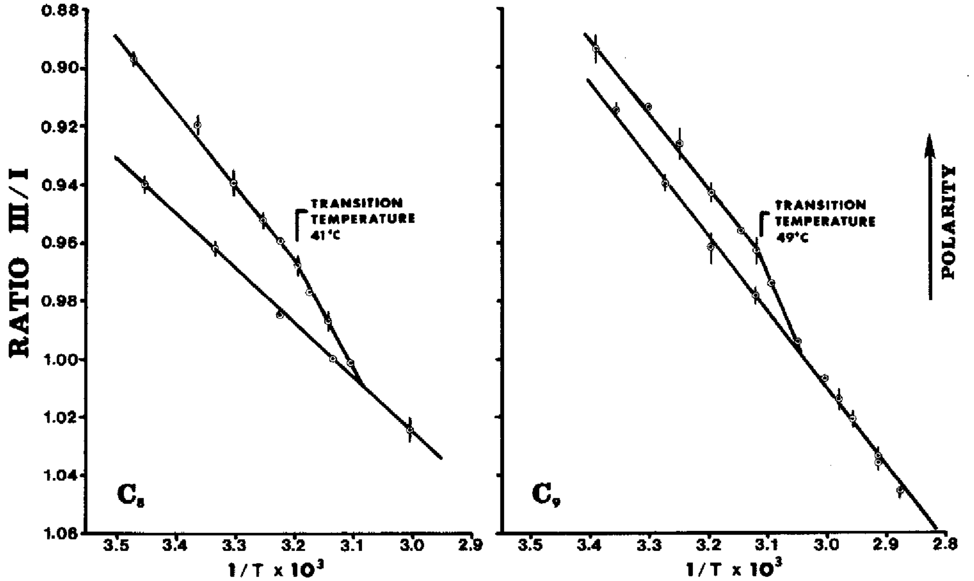

Fig. 1. Temperature-dependent stationary phase polarity as measured by the fluorescence vibronic band ratios of pyrene sorbed to C_8 and C_9 polymeric surfaces in contact with 100% aqueous mobile phase. Upper curve represents surface polarity immediately after preconditioning with acetonitrile. Lower curve represents surface polarity following temperature excursions above 41°C for C_8 and 49°C for C_9, respectively.

that the solution was not flowing. Spectra were obtained from 15 to 105°C for decane and 1,2-ethanediol, between 15 and 75°C for acetonitrile and between 15 and 95°C for water.

RESULTS AND DISCUSSION

Temperature dependence of stationary phase polarity

The effect of temperature (T) on the fluorescence vibronic band ratio of pyrene sorbed to a C_8 or C_9 polymeric surface in contact with water is plotted *versus* $1/T$ in Fig. 1 for comparision with the retention behavior. When the temperature of the stationary phase, preconditioned with acetonitrile, was being raised, the change in polarity of the surface was linear with $1/T$. This behavior was followed at a higher temperature, by a sigmoidal transition of the surface polarity towards less polar values. At still higher temperatures, the surface polarity again varied linearly with $1/T$. Upon cooling, the surface polarity increases with a linear dependence for temperatures as small as the initial conditions. Since the non-linear portion of the curve is not repeated, the stationary phase at the end of the experiment is less polar than at the beginning. The temperature where the surface polarity undergoes a non-linear change is dependent on the chain length of the surface-bound hydrocarbons. The temperatures characteristic of the on-set of this non-linear region are 41 and 49°C ($\pm$ 1°C) for the C_8 and C_9 polymeric surfaces, respectively.

Gilpin and co-workers[7,8,17] have noted a similar temperature dependence in the retention of phenol and resorcinol on C_8, C_9 and C_{10} stationary phases. Following preconditioning with pure organic modifier and elution with a totally aqueous mobile phase, increasing temperature causes the log of the capacity factor, $\ln k'$, to decrease linearly with $1/T$; at a chain length-dependent transition temperature $\ln k'$ undergoes a sigmoidal drop in magnitude. Following this transition, $\ln k'$ again varies linearly with $1/T$, along a line which is offset from the original temperature dependence. Two examples of the temperature dependent retention behavior of phenol and resorcinol from ref. 7 have been reproduced in Fig. 2, which allow the similarity between the retention data and the surface polarity to be compared (see Fig. 1). Further verification of the strong correlation between these two experiments is the agreement of the transition temperatures which were observed; the on-set temperatures for the non-linear decrease in $\ln k'$ were reported to be 40.7 and 51.8°C ($\pm$ 1.5°C) for the C_8 and C_9 stationary phases, respectively[7]. The transition temperatures for surface polarity and solute retention, therefore, agree within their uncertainty bounds.

Surface structural changes with temperature

The temperature-dependent hysteresis in surface polarity and solute retention appears to be related to the preconditioning with organic modifier, in this case acetonitrile. By collecting and analyzing fractions of the aqueous mobile phase following the transition temperature, Gilpin *et al.*[8] have showed that the conditioning solvent was initially retained in the alkyl layer and released upon heating. In interpreting this observation, however, the authors suggested that solvent retention is by physical entrapment and that its release was associated with a significant rearrangement of the alkyl chain conformation. They assert that the chains evolve from a "collapsed state" with "solvent entrapment within the bonded hydrocarbon

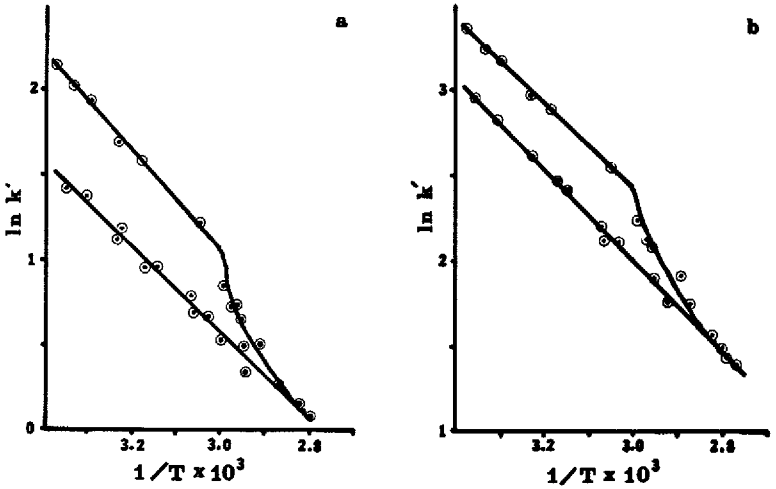

Fig. 2. Temperature-dependent retention of (a) phenol and (b) resorcinol on C_{10} polymeric reversed phases with 100% aqueous mobile phase; surfaces were preconditioned with acetonitrile. Data are replotted from Figs. 1c and 2c of ref. 7.

layer during formation" to a more stable, chain-extended or "bristle" configuration where "entrapped" conditioning solvent is released and replaced by water[8,17].

The surface polarity change indicated by sorbed pyrene fluorescence is consistent with the observed loss of conditioning solvent from the interface, but *not* with a chain conformation where alkyl ligands extend into the aqueous solution. Although less polar than water, the preconditioning solvent is considerably more polar than the bound hydrocarbonaceous ligands. Removal of solvent molecules from the bound layer would decrease the average polarity of the stationary phase as observed in the pyrene fluorescence. The irreversible decrease in polarity which takes place in the transition region can, therefore, be attributed to the departure of intercalated solvent from the stationary phase leaving a less polar environment. This interpretation is consistent with other spectroscopic studies of the effects of conditioning solvent on stationary phase environments[13,15,16]. On the other hand, the observed decrease in polarity upon heating confirms that the conditioning solvent is not replaced by water, at least in regions of the stationary phase occupied by a sorbed, hydrophobic solute. These results cast doubt about the existence of a extended chain or "bristle" configuration for the stationary phase layer under aqueous moble phase conditions. Instead, the surface environment both before and after the transition temperature appears to exist in a collapsed state[4], where environment differences are primarily determined by the presence or absence of the preconditioning solvent. This interpretation appears to be reasonable in terms of hydrophobic interactions between the hydrocarbon ligands and water, and the capability of amphiphilic molecules such as typical reversed-phase organic modifiers to reduce surface tension at hydrophobic layer–water and hydrophobic layer–silica interfaces.

In addition to the non-linear decrease in surface polarity accompanying the loss of conditioning solvent, a systematic lowering of the stationary phase polarity is observed as the system temperature is increased. This trend is observed both before

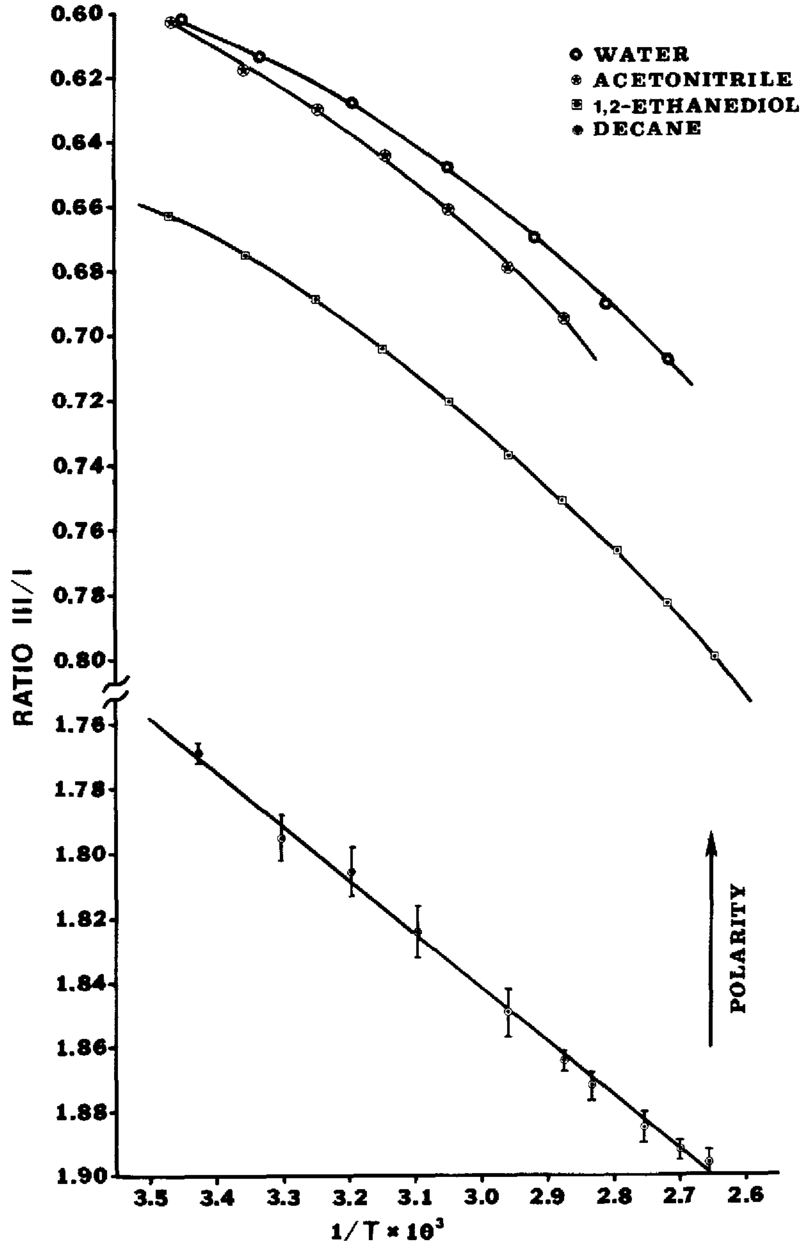

Fig. 3. Fluorescence vibronic band ratios of pyrene as a function of temperature in four solvents: water, acetonitrile, 1,2-ethanediol and decane. Precision of the data in the curves for water, acetonitrile and 1,2-ethanediol is about the size of the plotted points; reproducibility of the data in the decane is indicated by the error bars.

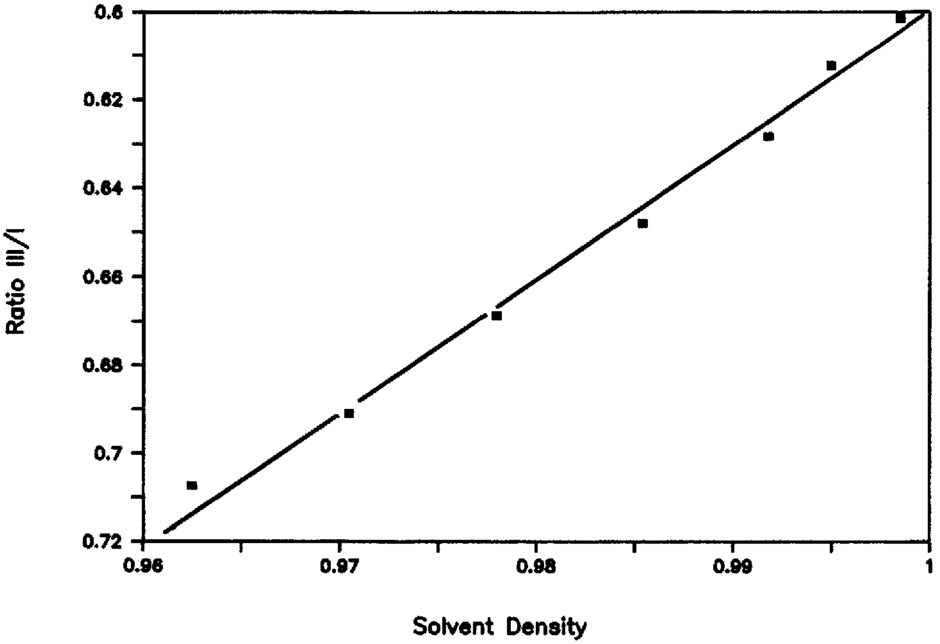

Fig. 4. Fluorescence vibronic band ratios of pyrene *versus* temperature-induced changes in the density of the solvent. The solvent is water, and the temperature range is identical to that shown in Fig. 3.

and after the loss of conditioning solvent at the interface. To better understand this trend, the effect of temperature on the vibronic band ratios of pyrene fluorescence was measured in four solvents of differing polarity, and the results are plotted in Fig. 3. For each of the solvents, a similar reduction in the environmental polarity of the probe is observed with increasing temperature. This trend would indicate a lowering of solvent-induced perturbations which are responsible for mixing the weak L_b transition, which dominates the highest energy vibronic band, with the much stronger L_a transition in pyrene[18,19]. The observed trends in polarity with temperature likely corresponds to the decrease in the density of the solvents at higher temperature temperature. The reduction in density would effectively increase the size of the solvent cage or the free volume of the solvent and thereby increase the average distance between pyrene and its perturbing neighbors, the final result being a decrease in the dielectric constant of the probe's immediate surroundings.

The density dependence of the vibronic band ratios for pyrene fluorescence has been included in an empirical response model by Stahlberg and Almgren[14] where the III to I intensity ratio of pyrene in a solvent was found to correlate well with the solvent's dipole moment divided by its molecular volume. This model also appears to be valid for temperature-induced changes in solvent density as shown in Fig. 4, where the III to I ratio of pyrene in water varies linearly with density over the same temperature range as the data in Fig. 3. A similar decrease in the apparent dielectric constant with increasing temperature is also observed in both sets of stationary phase data. The slopes of the surface environment curves in Fig. 1 are of the same magnitude as the slopes of the free solution curves in Fig. 3. While the stationary phase layer would not duplicate the behavior of free solution, the similarities in response are enough to suggest an analogy. The environment of pyrene sorbed to a polymeric stationary phase responds to a change in temperature in a manner analogous to thermal expansion of a bulk solvent. Studies of the temperature dependence of monomeric stationary phases[23], on the other hand, reveal quite different behavior for these surfaces, where little sensitivity to temperature change is observed following loss of conditioning solvent. The lack of a response to temperature for the monomeric stationary phase is consistent with a symmetry perturbation of pyrene's excited states which is interfacial in nature and thus depends less significantly on the density of its surroundings. If differences in this behavior are similarly interpreted, then the slope of the C_9 polymer stationary phase temperature dependence being steeper than C_8 in Fig. 1 could relate to how much of the probe's environment is of bulk *versus* interfacial character. Differences in the temperature dependence of surface environments, therefore, appear to carry information about surface structures and sorption mechanisms. More complete discussion of these issues follows in a separate article[23].

Organic modifier in alkylated silica environments

The lowering of the polarity of C_8 and C_9 stationary phases upon loss of conditioning solvent provides strong evidence that the surface structure does not evolve from a collapsed to an extended configuration upon heating. As a result, a mechanism other than "physical entrapment" for the retention and loss of the conditioning solvent must be considered. Regardless of changes in alkyl chain conformation, physical entrapment of organic solvent by an alkyl layer is not a likely mechanism for solvent retention because of molecular diffusion. Taking the viscosity of the stationary phase from excimer formation rates to be 19 cP (ref. 24)[a], and the thickness of the hydrocarbon layer to be less than 2.0 nm, diffusion rates through the alkyl layer are fast. Even if the solubility of organic solvent in the hydrocarbonaceous overlayer were only 0.01% of its concentration at the underlying silica surface, the diffusion rate calculated from this viscosity and solubility reveals that more than 99% of "trapped" organic solvent would diffuse from the stationary phase into the mobile phase in less than 1 ms. Even if transport of the solvent through the stationary phase were as slow as molecular diffusion through a crystalline hydrocarbon solid[26], trapped solvent would still be released in less than 1 s. Physical entrapment, therefore, cannot account for solvent retention in the stationary phase, independent of changes in ligand conformation.

Another mechanism which could account for the retention of an amphiphilic solvent under these conditions relates to the reduction in surface tension of the alkyl chain–water interface and possibly the alkyl chain–surface silanol interface. The addition of mutually miscible solvent molecules at these interfaces lowers the surface free energy, and interfacial excess concentrations are, therefore, selectively adsorbed[27]. If this process were a simple two-phase adsorption equilibrium, however, the lack of organic modifier in the totally aqueous mobile phase would cause its slow but

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[a] More recent studies using pressure jump and nuclear magnetic resonance relaxation methods place the value closer to 2 cP (ref. 25).

eventual loss from the stationary phase for any finite values of the adsorption equilibrium constant. An abrupt decrease in the concentration of retained solvent upon increasing the temperature could only arise if the enthalpy of adsorption were unrealistically large in magnitude.

These two observations, the stable retention of organic solvent and the well-defined temperature at which the solvent is lost from the surface phase, strongly suggest ordering of the solvated interface. While the irregular, porous structure of the underlying silica substrate would prevent long-range order of bound ligands, nevertheless, short-range ordering of alkyl chains and intercalated solvent could support a stable, nematic phase which minimizes the surface energy. An analogy could be drawn between the short-range order of these bonded chains and the local intramolecular ordering of polymer molecules containing relatively long, linear side-chains; in free solution, these macromolecules have no long-range orientational order as in the porous silica matrix, but there exists strong correlation in the conformations of neighboring side-chains[28]. Above a particular temperature, one would expect such a system to exhibit a nematic–isotropic phase transition where conformational order is lost together with, in this case, the intercalated solvent.

Considerable evidence exists in polymeric reversed-phase chromatographic materials for surface structures which have local conformational order. In Gilpin and Squires' pioneering observations[7] of temperature dependent retention hysteresis in C_8, C_9 and C_{10} phases, it was found that observation of the hysteresis required a significant surface coverage by alkyl chains, and that the magnitude of the hysteresis increased dramatically with increasing carbon percentage above a critical coverage. Studies of the effects of temperature on densely-grafted C_{22} (ref. 29) and C_{28} (ref. 30) polymer phases showed similar, persistent effects of conditioning solvent on elution with aqueous mobile phase and temperature-dependent hysteresis in retention.

Wise and co-workers[11,12,31] have showed that C_{18}, polymer stationary phases grafted at high-carbon loadings on large-pore silica supports produce unusually large selectivity for planar solutes compared to monomeric phases; drawing on an analogy to retention on liquid crystalline phases in gas chromatography, they conclude that such polymeric phases are considerably more "ordered". The relationship between the observed selectivity and the pore diameter of the substrate argues for conformational order over a significant range. Such ordering also requires a high density of alkyl ligands with a surface configuration produced by polymeric bonding of the phase. Similar conclusions can be drawn from a comparison of the temperature-dependent retention and sorption environment hysteresis in C_6, C_7 and C_8 monomeric stationary phases, where *neither* the persistent retention of conditioning solvent nor a distinct transition temperature for its accelerated loss are observed[23].

From differences in solute retention[7] as shown in Fig. 2, stationary phase affinity for solutes is affected by the retention of solvent. Since the mobile phase is identical for both upper and lower curves of Fig. 2, differences in retention (after correcting for changes in the phase volume ratio) reflect only changes in the partial molar free energy of a solute in the stationary phase[29,30]. From the effects of the acetonitrile on the surface polarity, one might expect that the enthalpy of sorption of molecules such as phenol and resorcinol would be increased by the presence of acetonitrile the hydrocarbon layer. The slopes of the straight-line portions of the upper and lower curves of Fig. 2, however, do not differ from each other by more than the error of estimation when fit to a linear equation[32]. The partial molar enthalpy of sorption of these molecules, therefore, does not change significantly upon loss of the conditioning solvent.

In contrast to the negligible differences in slope, in the intercepts of the straight-line portions of respective upper and lower curves of Fig. 2 are statistically distinguishable. The differences are equivalent to a lowering of the partial molar entropy of sorption by $-2.0\,(\pm 1.0)$ cal. $\mathrm{mol}^{-1}\,\mathrm{K}^{-1}$ not counting changes in the phase volume ratio. Using quantitative data for the amount of lost solvent[8], the carbon loading of the stationary phase and an estimate of its mass from the column size and packing method[7], the relative change in volume of the stationary phase due to loss of organic solvent is less than 5%, corresponding to a 2% gain in mobile phase volume. The resulting change in the phase ratio accounts for less than -0.2 cal $\mathrm{mol}^{-1}\,\mathrm{K}^{-1}$ of the change in partial molar entropy of sorption upon loss of solvent.

The lowering of the sorption entropy upon loss of the preconditioning solvent supports the existence of a more ordered stationary phase in the presence of the adsorbed organic solvent. Under these conditions, the effect of a solute molecule in the stationary phase layer is to locally disrupt this order and to increase the overall entropy of the system. Above the transition temperature, when the conditioning solvent is lost, the effect of solute sorption on the stationary phase order is less and the partial molar entropy of sorption is lowered. Thus, thermodynamic data available from temperature-dependent retention of solutes also support the model of a structured, polymeric stationary phase layer in the presence of a conditioning solvent. Monomeric stationary phases, on the other hand, show distinctly different behavior, more characteristic of disordered structures; a comparative study of monomeric surfaces will consider the differences in more detail[23].

ACKNOWLEDGEMENTS

This work was supported in part by the Office of Naval Research. Fellowship funds (to J.M.H.) from the Alfred P. Sloan Foundation are also acknowledged.

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