CHROM. 25 230

Review

Phase transitions of reversed-phase stationary phases

Cause and effects in the mechanism of retention

John F. Wheeler

Department of Chemistry, Furman University, Greenville, SC 29613 (USA)

Thomas L. Beck, S.J. Klatte, Lynn A. Cole[*] and John G. Dorsey[*]

Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221-0172 (USA)

ABSTRACT

We provide a somewhat historical perspective on the work that has been accomplished that supports the suggestion of phase transitions in reversed-phase liquid chromatographic bonded phases. In addition to this general review, we consider from both a practical and theoretical viewpoint the implications of such transitions in the rational design of improved reversed-phase separations. Analytical theory and molecular simulation approaches to chromatographic stationary phases and related systems are reviewed. Initial results are presented on the temperature, density and chain length dependences of stationary phase properties. The role of computer simulation in analyzing transition behavior and retention processes is discussed.

CONTENTS

---

* Corresponding author.
[*] Present address: Glaxo Inc. Research Institute, 5 Moore
  Drive, Research Triangle Park, NC 27709, USA.

1. INTRODUCTION

As our experience with elution using reversed-phase chromatographic supports has expanded over the last decade, so too has our understanding of the inherent structure associated with n-alkyl bonded phases. Numerous investigators have reported evidence for what have collectively been termed as temperature-dependent phase transitions in both polymeric and monomerically bonded alkyl chains; however, the specific impact of the proposed transitions in affecting the mechanism of retention has not been fully elucidated. The approaches used to investigate this behavior have varied considerably, and the interpretation of the data has at times seemed conflictory. Moreover, although many of the pioneering studies leading to suggestion of such transitions were accomplished in the early 1980s, to our knowledge, a comprehensive review of this subject has never been pursued. In keeping with the theme of this special issue, our goal is to first provide a somewhat historical perspective on the work that has been accomplished that supports the suggestion of phase transitions in reversed-phase liquid chromatographic bonded phases. In addition to this general review, we wish to consider from both a practical and theoretical viewpoint the implications of such transitions in the rational design of improved reversed-phase separations for the next decade. Analytical theory and molecular simulation approaches to chromatographic stationary phases and related systems are reviewed. Initial results are presented on the temperature, density and chain length dependences of stationary phase properties. The role of computer simulation in analyzing transition behavior and retention processes is discussed.

2. INDIRECT (CHROMATOGRAPHIC) EVIDENCE FOR A PHASE TRANSITION

Early comprehensive treatment of the temperature-dependent structural association of alkyl ligand bonded phases was provided by Morel and Serpinet [1–3] in a series of papers appearing in the early 1980s. In their original work, densely grafted (>4.0 μmol/m^2) C_{18} bonded silicas were investigated by gas chromatographic Van 't Hoff analysis (log V_R vs. $1/T$) using low-surface-area, but large-diameter silica supports (100–250 μm) as substrates for monomeric derivatization [1]. By considering changes in retention volume as a function of temperature using n-alkanes as test solutes, the authors observed a reproducible shift in linearity between 5 and 30°C in the otherwise linear Van 't Hoff profile (see Fig. 1). To explain this apparent change in retention, they proposed that a *reversible* fusion-like transition was occurring within the alkyl phase that resulted in an altera-
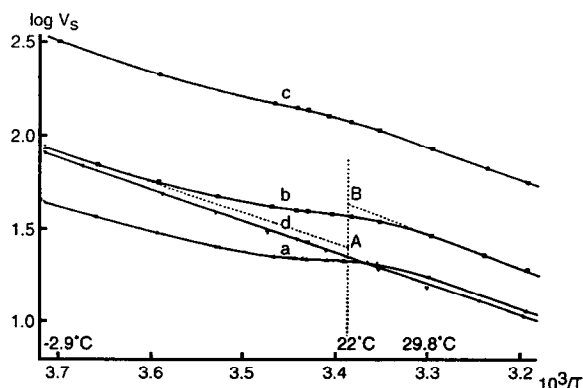

Fig. 1. Variation of the log of the absolute retention volume of hexane per unit mass of substrate (V_s) as a function of $1/T$. a, b, c and d represent various surface areas, pore sizes and bonding densities of C_{18}. A and B show the difference in retention volume across the transition. From ref. 1.

tion of the number and nature of the retention sites [1]. Given the variability that has been associated with the use of Van 't Hoff analysis for studying retention in RPLC [4–6], it is interesting to note that even in this early work in which the alkyl chains were non-solvated by mobile phase, totally linear Van 't Hoff plots were occasionally observed for what were termed as aberrant bonded silicas, and no obvious explanation could be found for the apparent inability of some bonded phases to crystallize .

In a follow-up paper published shortly after the original work, Morel and Serpinet [2] considered similarly densely grafted C_{22} as a test phase for probing non-linear Van 't Hoff behavior by this same inverse GC technique, and again observed behavior consistent with a change in the retention process as a function of temperature. In this case, however, the observed transition appeared much more abruptly and at a higher temperature between 40 and 48°C as long as the bonding *density* remained above 4.18 μmol/m^2. Extrapolation of the two distinct linear sections above and below the proposed transition temperatures for both C_{18} and C_{22} yields a relatively higher degree of retention for the alkane solutes at higher temperatures, which again may be attributed to permitting the solute increased access to inner sites of the stationary phase.

Among the other early chromatographic evidence for the occurrence of phase transitions for alkyl chains grafted to silica was a contribution provided by Kessaissia et al. [7], who used GC under temperature conditions ranging from −140 to +120°C to consider the retention volume–temperature profile of a series of laboratory-made alkyl ligands (C_3, C_6, C_{10}, C_{16} and C_{20}) bonded to 40–100 μm esterified silica of surface area 100 m^2/g. Although the bonding density was considerably lower (i.e., 3.6 μmol/ m^2) for these phases than in the work of Morel and Serpinet [1–3], reproducible non-linear Van 't Hoff data were obtained for solutes consisting of methane, nitrogen and argon for each phase investigated. A total of three transitions were observed, two that remained constant (i.e., − 20 and − 80°C) for all alkyl chain lengths, and a

third that increased as the chain length increased, ranging from − 75°C for C_3 to − 40°C for C_{16}.

Although the early GC work provided some insight into unusual temperature–retention behavior associated with solutes partitioning into reversed-phase supports, none of the aforementioned experiments were performed under normal reversed-phase separation conditions in which the stationary phase was solvated. At approximately the same time, several other well established groups published reports showing non-linear Van 't Hoff behavior using direct LC measurements [8–11]. In this case, however, deviation from linearity was not attributed to change due to crystallization or melting of the hydrocarbon stationary phase, but rather to an *irreversible* reorganization of chain structure as a function of alkyl ligand solvation. For example, Gilpin and Squires [8] demonstrated a non-linear dependence of retention on temperature using polymeric bonded phases (C_8, C_9, C_{10}) under totally aqueous elution conditions with polar solute probes [8] (see Fig. 2). Although the observed Van 't Hoff plots were indeed non-linear, the forces responsible for the non-linearity were determined to be associated with the immediate solvation environment (i.e., water *versus* acetonitrile), and once reorientation was established, no subsequent deviations from linearity were observed [8]. Thus, in apparent conflict to the GC data, this study recognized an entirely different type of phase transition phenomenon.

At this point it is useful to make an important clarification. In considering Van 't Hoff plots in RPLC, we find from even these first reports that we must keep in mind at least two important factors: (1) it is imprudent to directly compare chromatographic behavior from systems with completely different alkyl ligand bonding chemistries (e.g., monomeric C_{18} *vs.* polymeric C_8), and (2) as we have recently reported [4,5], although non-linear Van 't Hoff behavior *may* be indicative of a change in retention mechanism, simple morphological changes in the bonded layer do not necessarily change the intrinsic mechanism.

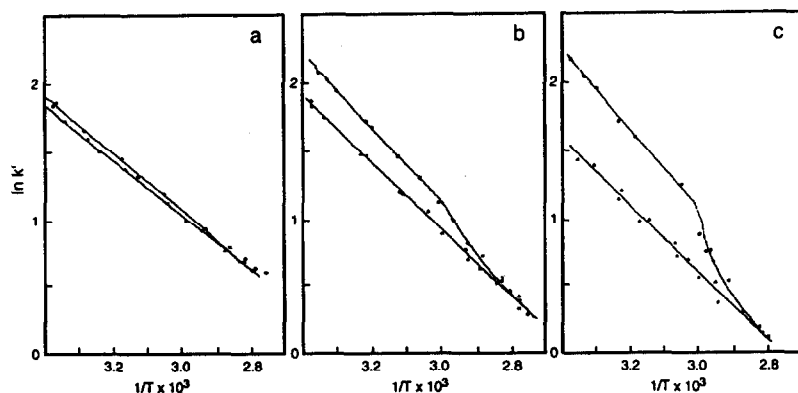In an attempt to address the first issue, Morel

Fig. 2. Plots of ln k' vs. $1/T$ for resorcinol obtained on C_{10} bonded silica for various bonding densities. Evaluation cycle: Upper curves = 1st and 2nd run data; lower curves = rerun data. Conditioning solvent: acetonitrile; mobile phase: water. From ref. 8.

and Serpinet's third contribution [3] in this area included LC data in addition to GC data for the retention of several structurally diverse solute types on high-bonding-density C_{22} derivatized silica. Although non-linear Van 't Hoff plots were obtained consistent with their earlier work, the onset of the deviation from linearity was quite dependent on mobile phase composition, increasing for C_{22} from an observed transition temperature of 46°C in pure methanol to 54°C in mobile phases containing >50% water. So called short-chain and non-linear alkanes (*e.g.*, *n*-dodecane, cyclododecane) demonstrated two linear segments in the Van 't Hoff plot with a hysteresis consistent with the earlier GC work; long-chain alkanes (*e.g.*, tetradecane), demonstrated only a single change in slope at a temperature considerably lower than the 46°C transition temperature (see Fig. 3). Careful comparison of these two systems reveals that linear extrapolation of the higher-temperature Van 't Hoff section for the shorter-chain solutes yields a log k' intercept consistent with higher retention than what is experimentally observed, whereas linear extrapolation of Van 't Hoff plots for the long-chain solutes yields an intercept lower than the experimentally observed value. Thus, once again it is clearly demonstrated that deviations from Van 't Hoff linearity are associated with change in solute type and solvent makeup, and not only a reversible melting of the stationary phase. In this case, the explanation provided by the authors is that the longer-chain solutes can

better insert into the stationary phase, particularly at lower temperatures where thermal agitation is more restricted, therefore relative retention is increased over the extrapolated segment from that of the higher temperatures. Conversely, short-chain solutes are not able to insert to the same extent, therefore as proposed
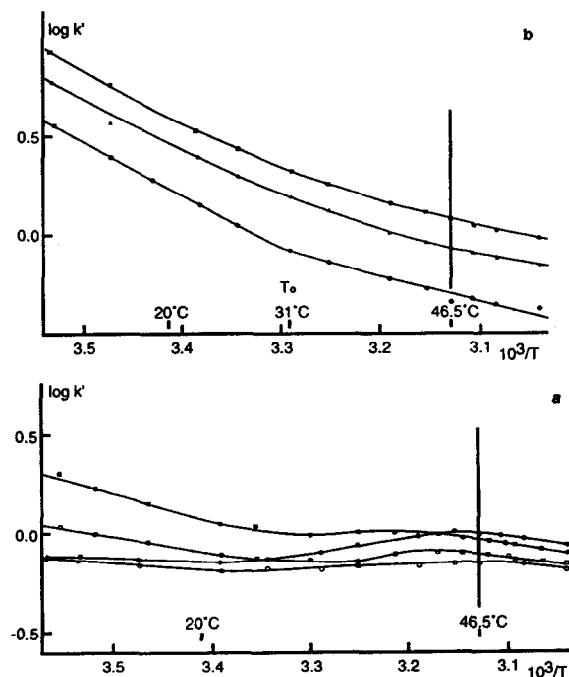

Fig. 3. Variation of log k' with $1/T$ for a pure methanol mobile phase and (a) different short-chain and non-linear solutes, and (b) different long-chain and linear solutes. From ref. 3.

in the earlier GC studies [2], the melting transition of C_{22} at 46°C provides new retention sites in the stationary phase that result in an overall higher degree of retention than what would be predicted from high-temperature extrapolation [3].

Since these early studies a number of other chromatographic systems have been investigated with respect to observed non-linearities in the Van 't Hoff behavior of solutes, presumably from reversible and non-reversible changes in the stationary phase structure as a function of temperature and mobile phase composition [4–6,12–17]. For example, since the original work by Gilpin and co-workers [8,9], Yang and Gilpin have reexamined the temperature dependency of retention for both monomeric and polymeric supports, and found both supports to exhibit non-linear Van 't Hoff behavior consistent with their earlier work which they attribute to chain reorganization as a function of solvent pre-treatment [15]. From the quality and quantity of the collective evidence, it is probably safe to assume that the mechanism of retention of solutes in reversed-phase stationary phases is indeed affected by column temperature, at least in some instances. Questions we must yet address are (1) is there direct evidence for a single-phase melting transition or chain reorganization for commonly used alkyl ligands (e.g., C_{18}), and (2) can the observations that have been made be used to our advantage to design improved separations.

3. DIRECT EVIDENCE FOR REVERSED-PHASE STATIONARY PHASE TRANSITION

As discussed above, non-linearity in Van 't Hoff analysis using both GC and LC as probes does provide evidence for the occurrence of phase transitions, particularly for long alkyl chains ($> C_{16}$) densely grafted ($> 3.5 \ \mu mol/m^2$) onto chromatographic silica. However, direct studies of the alkyl surface certainly lends credibility to this assumption. Among the earliest comprehensive attempts at directly studying the phase transition phenomenon was a contribution provided by Hansen and Callis [18], although Riedo et al. [19] may also be credited with

examining this behavior using contact angle measurements as early as 1978. In the work by Hansen and Callis, differential scanning calorimetry (DSC) was employed to examine conventionally bonded C_{18} and C_{22} alkyl ligands at intermediate bonding densities of 2.0 to 2.5 $\mu mol/m^2$, with or without additional free ligand physisorbed at the surface. From DSC thermograms of the pure C_{18}, a well defined endothermic transition was noted at 31°C; however, C_{18} bonded to silica at the intermediate bonding densities used in this study failed to show evidence of any transitions [20]. When the apparent bonding density was increased through the physisorption of free organosilane, however, a distinctive bulk phase transition did occur in the dry silica at a temperature of 18°C. If non-bonded silica were treated with free organosilane to permit physisorption alone, a transition was noted at 22°C. It is useful to once again point out that all of these studies were conducted without the influence of chromatographic mobile phase. In the presence of methanol, even the medium-bonding-density phase demonstrated phase transitions of a different type at a temperature of 50–60°C, which were attributed to an expulsion of methanol (i.e., solvent release) from the hydrocarbon lattice due to solvation, instead of a crystallization inherent in the alkyl ligand by itself [18].

Since the original work by Hansen and Callis, there have been several other alkyl ligand studies performed using DSC. Morel and co-workers [12,20] investigated the entire range of alkyl chain lengths from C_1 to C_{22} by DSC and NMR in addition to their inverse GC method, and they likewise found calorimetric evidence of enthalpic transitions for chain lengths $> C_{16}$ (e.g., 18°C for C_{18}) for dry densely grafted silicas bonded monomerically. The authors noted, however, that the transition enthalpy was considerably lower than that observed for free alkane, and surmised that only part of the bonded chain was actively participating in the observed transition. More recently, Jinno and co-workers [14,21] studied polymeric C_{18} phases by DSC and found evidence supportive of a phase transition for dry stationary phase at approximately 45°C, compared with monomeric silica, in which they

observed a weaker transition at 35°C (see Fig. 4).

In addition to the DSC data, a number of other investigators have used some rather elegant approaches in an attempt to elucidate the apparent melting of the n-alkyl silicas [16,22–24]. Included in this partial list are the Fourier transform (FT) IR studies by Sander et al. [23], who examined the fraction of gauche bonds in alkyl-derivatized silicas to demonstrate the degree of ordering associated with wetting solvents versus dry silica or non-wetting solvents, consistent with Gilpin and Squires' [8] earlier observation. In the work by Jinno et al. [21], diffuse reflectance FT-IR and cross-polarization magic-angle solid-state ^{13}C NMR were used to further elucidate their DSC findings. From these data they summarize that while solvent does

affect chain organization, evidence for a melting-like phase transition is also very strong, and the effect of temperature and chromatographic retention on changes in solute structure cannot be ignored. Bliesner et al. [25] have just recently completed additional work using ^{13}C NMR of solvated high-density monomeric C_{18} phases that provides additional supportive evidence of a melting-like phase transition.

4. EFFECTS OF PHASE TRANSITIONS ON REVERSED-PHASE RETENTION

After more than a decade of work in this area, evidence supporting important molecular changes in the structural arrangement of alkyl chain silicas as a function of temperature has become convincing. In synopsis, we may be
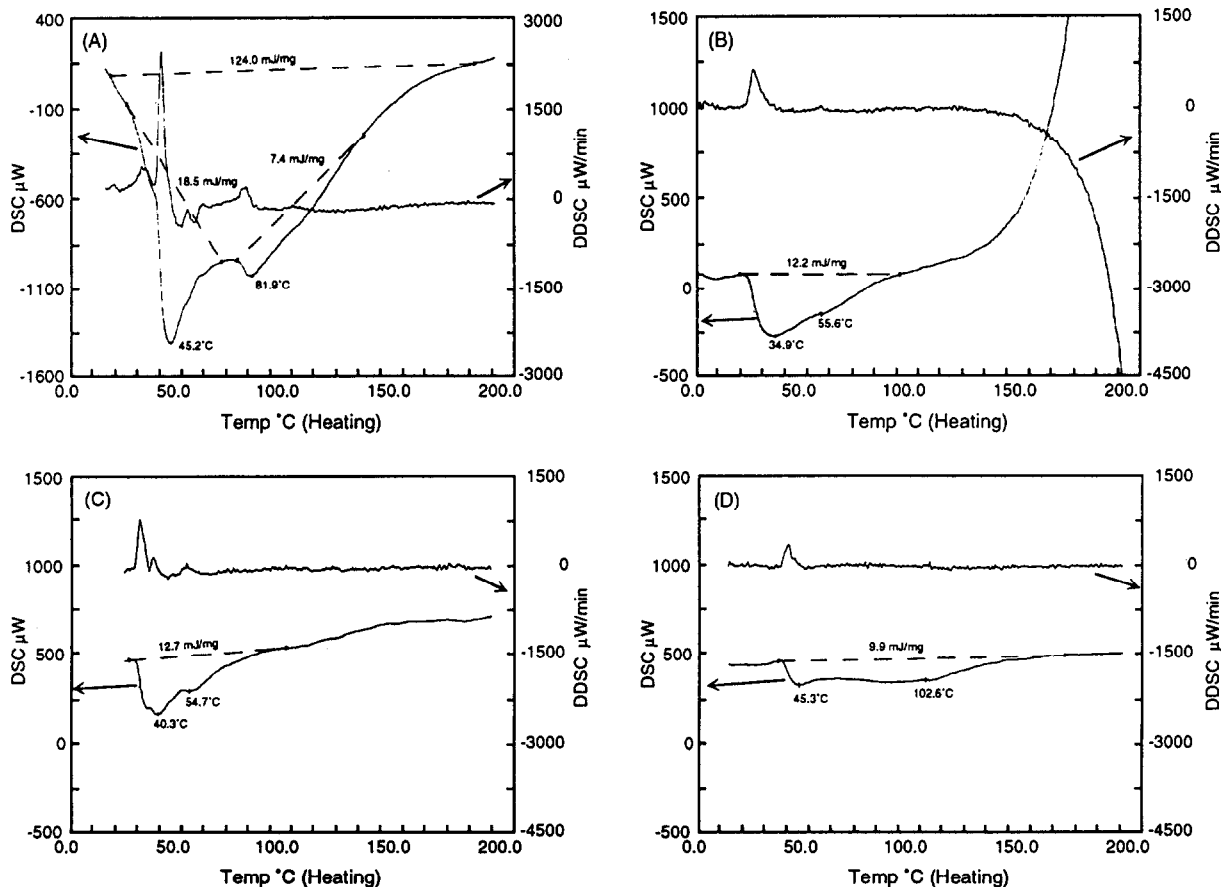

Fig. 4. DSC charts for four different stationary phases: (A) Vydac 201 TPB-5; (B) monomeric C_{18}, (C) diphenyl; (D) pyrenylethyl. For conditions see ref. 14. From ref. 14.

reasonably assured that a reversible melting-like transition of long-chain hydrocarbon ligands does occur for high bonding densities (> 3.5 μmol/m^2), and that for C$_{18}$ the change occurs somewhere in the vicinity of room temperature for monomeric silicas, slightly higher for polymeric silicas. Longer-chain ligands undergo this transition at correspondingly higher temperatures than shorter-chain silicas, and a critical length of 16 or more carbons is required to see such a melting transition. On the other hand, temperature-associated chain reorganization accompanied by solvent release also occurs as a function of solvent composition, and this irreversible process is most significantly noticed for polymerically bonded silicas. In this case, long alkyl chains are not required for the two states, although the ultimate effects on retention may be of greater magnitude for increased chain lengths. Both of these phase transition phenomena can contribute to non-linear Van 't Hoff behavior, which simply implies that the retention process is not completely constant over all temperature ranges for these materials. Finally, effects of both solute and solvent cannot be necessarily disregarded, as they also change as a function of column temperature and affect the linearity of a Van 't Hoff format.

Having compiled a rather formidable array of evidence supporting the idea of phase transitions in RPLC, the next important question involves ultimate effects on retention. It is perhaps first important to reiterate that we are considering a special class of materials. Linear Van 't Hoff relationships have been assumed for RPLC for many years. Such relationships in all likelihood do exist, at least over narrow temperature ranges, for medium- or low-bonding-density phases with traditional organosolutes separated in traditional hydro–organic mobile phases, although as we have recently shown [4,5], this too is very much mobile phase dependent. As we have seen, however, for high-bonding-density materials (whether they be monomeric or polymeric) a linear Van 't Hoff relationship may not always be obtained.

It is perhaps most useful to consider the specific chromatographic advantages of these materials as compared with the traditional com-

mercial materials. In addition to the aforementioned work by Jinno and co-workers [14,21] examining retention of polyaromatic hydrocarbons (PAHs), Sander and Wise [17] have demonstrated differences in shape selectivity for polymeric phases for the separation of planar vs. non-planar molecules as a function of temperature. High-bonding-density phases were shown to provide improved selectivity over low- or medium-density phases, and subambient temperatures provide the highest selectivity among the high-density phases, presumably because planar molecules can better insert into the ordered phase associated with the more crystalline alkyl chain. Sentell and Henderson [6] also recently published results in this area, showing non-linear Van 't Hoff data for high-density monomeric phases that correlates with improved PAH and polyphenyl selectivity (see Fig. 5). In addition to this work, our group [26] has shown improved separations on high-density phases with respect to both methylene selectivity and phenyl selectivity. We have recently rigorously
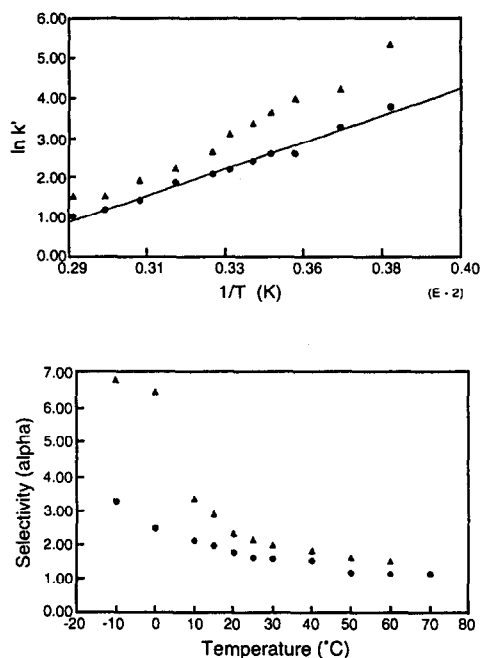

Fig. 5. Variation of ln k' with $1/T$ for a high-density ($\blacktriangle$) and low-density ($\bullet$) monomeric C$_{18}$ phase for the solute 2,3-benzanthracene, and selectivity versus temperature plots for the high-density ($\blacktriangle$) and low-density ($\bullet$) monomeric C$_{18}$ phase for 2,3-benzanthracene vs. triphenylene. From ref. 6.

examined [4,5] the Van 't Hoff relationship of traditional organosolutes on well characterized phases with respect to both bonding density and solvent composition, and find that both stationary phase and mobile phase can significantly contribute to non-linear temperature profiles. A natural extension of this work is now to consider molecular theory and computer simulation as an approach to better understand the nature of these relationships for the rational design of better separations.

5. THEORY OF PHASE TRANSITIONS IN CHROMATOGRAPHIC SYSTEMS

The traditional classification of phase transitions was developed by Ehrenfest [27]. Under this scheme, first-order transitions (solid–liquid, liquid–vapor, etc.) involve simultaneous coexistence of phases (with equal free energies) and discontinuous internal energies. Second-order transitions, on the other hand, have continuous internal energies. Thus there is no latent heat. In more recent times, the rigorous definition of phase transition for bulk systems has been softened to describe diverse phenomena such as melting of lipid bilayers in biophysical systems [28] and small atomic clusters [29]. Other transition phenomena of relevance to the current paper are wetting phenomena in interfacial systems [30] and the glass transition [31] (there is currently a great deal of debate concerning the ultimate origins of the glass transition and its relation to bulk equilibrium phase transitions). It is a looser definition of phases and phase transitions which we adopt in relation to chromatographic stationary phases. That is, we observe a range of phenomena which clearly indicate transitions in the chromatographic system as functions of temperature, density, solvent composition and solute. The RPLC system cannot be expected to display bulk first- or second-order phase transition behavior; hence, we do not try to impose the rigorous terminology of phase transitions. It is more likely, due to the inherently disordered nature of the interface, that the observed transitions are more akin to glass transition behavior or liquid ↔ disordered solid

transitions than to bulk equilibrium phase transitions. In the present theoretical section, we discuss work of relevance to chromatography which can impact on a molecular level understanding of retention. This behavior includes temperature dependent transitions observed in stationary phases.

6. BACKGROUND

Consider the typical RPLC system at the sub-silica pore length scale. Alkane chains are chemically bonded via silanization reaction to pendant OH groups on the silica surface. The surface is rough and disordered at the molecular level [32]. This contrasts with flat metal surfaces to which alkyl thiol self assembled monolayers are attached at high density [33-35]. While the OH surface density is 8 μmol/m^2, the maximum bonding density is approximately half this value. This density corresponds to inverse surface densities of 40 Å^2/chain, where 20 Å^2/chain is close packing.

One might thus expect that, as a chromatographic system is cooled to low temperatures, the chains would freeze into a disordered or glassy configuration on the silica surface. The FT-IR experiments of Sander et al. [23] support this picture. Both the inherent roughness and disorder of the silica surface and the sub-maximal bonding density likely play roles in the observed non-crystalline state of the alkanes. In addition, at elevated temperatures, when the chain tails undergo large amplitude, liquid-like motions, the constraint of low bonding density suggests substantial available free volume for insertion of solute (see below).

A multicomponent solution, consisting typically of water–cosolvent (acetonitrile or methanol) and solute mixtures passes through the column and the detailed molecular interactions at the mobile phase–stationary phase interface lead to observed retention behavior. In fact, the free energy of solute transfer between phases is directly measurable as long as a good estimate of stationary phase volume is known [36].

Thus RPLC is a complex solution phase problem involving a wide range of structural features

and molecular interactions. As discussed above, it is likely that each component of the system cannot be ignored in developing a molecular level theory of transition behavior and effects on separation. It is the purpose of our theoretical work to examine the microscopic interactions and dynamics which drive separation processes. We first briefly review analytical theories of interphase structure and RPLC. These theories do not directly relate to phase transition behavior due to inherent assumptions in the theories. Then we review computer simulation methods as related to phase transition behavior in alkane fluids and model interphases. Finally, we discuss the expected role of simulation in unraveling the complex interplay of solute, solvent and stationary phase in chromatographic retention.

6.1. Analytical theory

If analytical theory of isotropic fluids is difficult, theory of molecular solution interphases is even more challenging. This is because, while it has been shown that perturbation theories about a hard sphere reference system give qualitatively (and often quantitatively) correct results for isotropic fluids [37], the anisotropy of an interface draws into question when and to what extent mean field perturbation theories are appropriate. Interfacial systems which have received theoretical attention are wetting phenomena [30], surface phase transitions of mono- and multi-atom layers [38], polymer brushes [39], liquid crystals at surfaces [40] and molecular interphases [41-44]. The most pertinent to RPLC stationary phases are the latter three.

Polymer brushes consist of diblock copolymers; one (short) segment binds strongly to the surface while the other longer segment protrudes into the solvent. In the limit of good solvent, attractive interactions between chains can be ignored since chain-chain and chain-solvent interactions have equal weights. Such polymers can substantially modify the surface properties of the solid. Typically, the bonding density is quite low in relation to chromatographic stationary phases. However, the chains are much longer.

The most successful theory of these interphases is self consistent field theory [39]. The chains are treated as infinitesimally narrow hard sphere chains which undergo a free propagation into solution, i.e. they are free to follow any path as long as they do not violate the excluded volume restriction. Paths with more bends have a larger kinetic energy. The free energy of the system is then minimized at a given temperature under the constraints of attachment at one end, excluded volume, and finite length of the chain. Path integral methods, first developed in quantum mechanics [45], are utilized. Predictions are obtained for the segment density profile $\rho(z)$ (generally parabolic) of the entire chain and of the tail segment. Molecular dynamics simulations of an equivalent model imply that the theory is relatively accurate under the (rather strong) assumptions given above [46]. In our simulations (below) we find qualitatively similar profiles when attractive chain-chain and chain-surface interactions are turned off, but observe different profiles when the full molecular potentials are used. Since RPLC stationary phases are most often in contact with at least modestly poor solvent, it appears a purely repulsive model is not sufficient to capture subtle structural features which may be of importance in chromatography.

Liquid crystals are systems which display liquid-like translational order but solid-like orientational order. They are prototypes for molecular assembly in membranes, and are also crucial in microelectronic devices. Rigid tube molecules comprise one class of liquid crystals and may exist in bulk as isotropic, nematic, and/or smectic phases [28]. It is also known that preferential ordering may occur near an interface (solid-liquid, liquid-vapor) [40]. For example, a collective tilt occurs for some liquid crystals near an interface. Cai and Rice [43] have formulated a density functional theory which captures the essential features of tilt transitions in monolayers of Langmuir-Blodgett films. In this theory, the chain molecules are treated as rigid rods which interact with each other and the surface by Lennard-Jones induced dipole-induced dipole potentials. These are realistic potentials for non-ionic and non-dipolar fluids. The authors found critical values for chain-surface potentials and

surface densities which lead to collective tilts. In computer simulation studies (below) it has been found that above roughly 25 $Å^2$/chain for alkanes, no collective tilt is observed; the chains have enough available free volume to move into disordered configurations. Hence RPLC stationary phases would not be expected to display collective tilts. We observe this to be the case in our molecular dynamics simulations.

Theories have been developed to directly model alkane interphases [36,41,42,44]. The various approaches and relationships between methods are discussed in refs. 36 and 41. Theories of RPLC [36,41,44] have utilized lattice methods, common to liquid theories, to treat the stationary phase, solvent and solute. Each of the lattice theories contains excluded volume interactions, as no two chain segments or molecules can lie on the same lattice site. In the theory of Dill and co-workers, a mean field approach is taken for the chain–chain interactions. The chain heads are constrained to lie on a flat surface, and the interphase–solvent surface is abrupt. The solute–solvent, solvent–solvent and solute–chain interactions are treated in the regular solution approximation. The theory predicts an entropic expulsion of solute at high chain density due to a free energy cost for chain reorganization, in agreement with experiment. In the theory of Bohmer et al. [41], the solvent may penetrate into the stationary phase. The degree of solvent penetration depends on the solvent quality in reference to alkane solvation.

In each of the lattice theories, the chain–chain interactions are treated purely as repulsive excluded volume interactions; explicit inclusion of all attractive interactions would lead to hopeless complexity in the models of interphase structure. Due to the nature of the lattice model and the fact that no free volume exists in the theory, these theories are unable to address the issue of phase transition behavior (i.e. from liquid-like to solid- or glass-like) of the stationary phase chains. The chains may access all regions of configuration space accessible to them as long as no chain overlap occurs; the distinction between solid and liquid is not meaningful under these assumptions.

6.2. Computer simulation

Computer simulation provides an alternative theoretical approach for complex chemical problems. The advantage of simulation coupled with statistical mechanical theories of fluids is that the assumptions of the theory are more clearly defined than in analytical theory. In addition, one gains a more detailed picture of the underlying molecular processes occurring in the physical system than is typically possible experimentally. Thus simulation provides an intermediate approach which utilizes input both from theory (motivation for simulations to test theoretical assumptions) and experiments (computations can be directly correlated to experimental data, resulting in improved models). The most pressing limitations of simulation are the constraints of rather short time scales (1–10 ns) and length scales (100 Å). Therefore only phenomena which occur over these or shorter scales are approachable by simulation on present-day supercomputers. While chromatographic experiments are conducted at much longer time and length scales than those listed above, the local molecular processes of relevance to chromatography, namely those that occur at the mobile phase–stationary phase interface, may be modeled accurately over these scales.

As an example of the important role of simulation in the theory of fluids, one of the landmark initial discoveries was the observation of "freezing" of hard spheres, which have no attractive interatomic potentials [47]. Quotes are used since it is still the subject of controversy whether hard spheres actually freeze. The arguments center on the stability of the solid phase. Nevertheless, it is certainly true that continuous systems with only excluded volume interactions can undergo a transition from liquid to solid-like states due only to geometric effects. While the hard sphere liquid is a much simpler fluid than the stationary phases of chromatography, the finding raises challenging issues for understanding phase transitions in interphases: (1) What roles do the attractive, repulsive, and torsional components of the potential play in the structural and dynamical properties of the chains (in

particular the phase behavior of the chains)? (2) Can one observe a solidification of tethered chains (with increasing density) in the absence of attractive forces (as would be expected in good solvent)? At a practical level, can realistic molecular simulations coupled with statistical mechanical theory reveal microscopic origins of observed temperature- and density-dependent retention behavior in RPLC, and what role phase transitions?

Below, we discuss the molecular dynamics (MD) and Monte Carlo (MC) methods and how they may be applied to RPLC theory. In addition, we review related simulation work on liquid alkanes, Langmuir–Blodgett films, and self assembled monolayers, systems of direct relevance to our work. Then we discuss initial results from one of us (T.L.B.) on the temperature- and density-dependence of structure and dynamics in chromatographic stationary phases.

6.3. Molecular dynamics and Monte Carlo methods

The methods of computer simulation of atomic and molecular liquids are exhaustively reviewed in ref. 48. In MD simulation of classical systems, the Newtonian equations of motion are solved numerically by integration of the coupled differential equations. The necessary input is a set of initial conditions (positions and velocities), masses and the interatomic potentials. Generally, an initial equilibration period is followed by a data collection run. Both equilibrium and dynamical quantities are calculated along a trajectory. Various constraints and alterations in system conditions can be modeled depending on desired conditions, such as constant temperature, pressure or volume. Often it is advantageous to constrain certain bonds, such as C–C bonds in alkanes, to constant values, since these bonds do not contribute much to effects of relevance to solvation. This allows one to use a much longer time step, since other chain modes are of lower frequency. In addition, in many simulations of alkanes, a single CH_2 group is collapsed into one united atom, which also reduces computation time. Periodic boundary

conditions are employed to limit finite size effects. In the RPLC system two-dimensional xy periodic boundary conditions apply. Typical simulations are run for periods up to the ns time scale. Longer-time-scale phenomena are not approachable with present-day computers. Physical quantities which are computed include: thermodynamic variables such as temperature (mean kinetic energy) and pressure (virial theorem); radial distribution functions $g(r)$; dynamical quantities like mean square displacements (which give diffusion constants), time correlation functions, and power spectra. Direct comparison with experiment can often be made via thermodynamic measurements, scattering experiments and spectroscopy. In addition to calculations of measurable quantities, direct visualization of trajectories can often lend insight into molecular level processes.

MC methods center on the use of random numbers to model a random walk on the potential surface which characterizes the system. The general method was developed by Metropolis et al. [49]. Only equilibrium quantities are directly calculable with MC methods. No further discussion of MC methods will be given since most work relevant to this paper has been done with MD methods. The reader is referred to ref. 48 for details.

6.3.1. MD simulations of alkane phases

Long chain alkanes (C_nH_{2n+2}, $n = 10$–26) exhibit several solid phases and a liquid phase in the bulk [50–53]. The solid phase at low temperature is crystalline. As the temperature is increased, the chains pass into states, termed rotator phases, where the all trans chains rotate about the long molecular axis. Defects appear predominantly at the chain ends. Even and odd carbon numbers display different structural properties. At least four rotator phases are observed experimentally by IR spectroscopy, neutron scattering, and X-ray scattering. At elevated temperatures, the alkanes eventually pass to an isotropic liquid phase, which is a bulk first-order transition. The bulk systems are of course at nearly close packed densities.

Several groups have undertaken MD studies of

the solid and liquid phases of n-alkanes. Ryckaert and Klein [54] examined long-chain alkanes (infinite chains in periodic boundary conditions). The system potentials include segment–segment Lennard–Jones interactions, bending potentials and torsional potentials. They calculated chain radial distribution functions, lattice constants, and time evolution of chain angles. Translational diffusion occurred along the chain direction at elevated temperatures, but no transition from orthorhombic phase to rotator phase was observed at 400 K as in experiments. In succeeding studies Ryckaert et al. [55] found a transition to the rotator phase with the development of increasing longitudinal chain motion and significant numbers of conformational defects, mostly at the chain ends. Padilla and Toxvaerd [56] carried out MD simulations of fluid pentane and decane. They examined liquid structure and mechanisms for diffusion. They found that torsional potentials have a substantial influence on relaxation rates of the chains in the fluid. Rigby and Roe [57] studied onset of glassy behavior in extensive simulations of dense alkane fluids. They cooled chain systems from high temperatures through the glass transition. They focused in particular on development of orientational order (and its length scale) through the transition.

6.3.2. MD simulations of Langmuir–Blodgett (LB) films and self assembled monolayers (SAMs)

LB films and SAMs are the systems of most direct relevance to chromatography since they consist of alkane chains either adsorbed or chemically bonded to liquid or solid surfaces. The densities obtained for the high-density phases of these systems are typically near close packed, namely 20–25 Å^2/chain. Several groups have carried out computer simulations of these systems in the last five years. Harris and Rice [58] performed MD simulations of a model LB film. The surface was treated as a uniform polarizable medium with an integrated Lennard–Jones potential. The chain heads were allowed to move freely in the xy directions. The authors

observed a low-density vapor phase and a high-density phase (< 23 Å^2/chain), but no liquid expanded phase, as is found experimentally. They attributed this to inaccuracies in modeling the surface potential. In the high-density phase, oscillatory segment-by-segment density profiles $r(z)$ were observed indicative of chain ordering. In addition, collective tilt angles of 20–30° away from the surface normal occurred. In subsequent work, Collazo and Rice [59] explored the effect of surface anisotropy on chain structure and observed appreciable influence of the surface on interphase structure.

Klein and co-workers [33–35] have carried out a series of MD studies of high-density alkyl thiol SAMs on flat gold surfaces. A first paper [33] examined basic issues of structure and dynamics of the chemically bonded chains. They found that the nature of the sulfur bond to the metal surface affected both equilibrium and dynamical properties of the chains. Oscillatory z profiles and collective tilts were observed, similar to the LB films examined by Rice and co-workers. A second study [34] probed the temperature dependence of the SAM system. At low temperatures an orientational solid phase appeared which passed through several transitions to three defineable rotator phases between 200 and 500 K (octane melts at 216 K and octadecane at 301 K). Klein's group has also simulated a model of an LB film and has obtained power spectra for chain vibrational motions. [60]

Bishop and Clarke [61] studied the system size dependence of tilt angles in monolayer films. They found that 64 (high-density) chains were necessary to obtain fully converged tilt angles. However, even 16 chains give results not drastically different from larger systems. Toxvaerd and co-workers [62–64] have recently presented simulations of LB monolayers. They computed surface pressures, tilt angles and structure factors. In addition, they examined transitions from collectively tilted to disordered states as the surface density was decreased. They found that above 25 Å^2/chain, no collective tilt occurs. Thus chromatographic stationary phases cannot be expected to display such behavior.

6.3.3. MD simulations of chromatographic stationary phases

We have initiated theoretical studies of chromatographic systems by utilizing MD simulation and statistical mechanical theory [65,66]. We treat the stationary phase alkane chain system similarly to the models discussed above for LB films and SAMs. Since the silica surface is rough and the chemical attachments are thus to some extent random, we introduce disorder in two ways. First, the chains are placed randomly in the xy plane on the surface with excluded volume constraints (no two chains are allowed to overlap). In a sense, this mimics the synthetic process as the chains are added one by one until the desired bonding density is reached. Second, the head groups are located randomly in a 2 Å range above the surface. The surface potential itself is taken as an integrated Lennard–Jones potential, so in principle it is flat. Future studies will focus on the role of surface anisotropy on interphase structure. The surface potential parameters were obtained from heat of adsorption data of alkanes on silica. The chains are started in an all-*trans* configuration at high temperature (500 K) where the chains immediately begin to undergo rapid large-amplitude motions and sample frequent *trans* ↔ *gauche* transitions. The system is then gradually cooled by velocity scaling to low temperatures at rates between 3.4 K/ps and 0.023 K/ps. These rates are necessarily many orders of magnitude greater than experimental time scales, but are consistent with or slower than the rates employed by Rigby and Roe [57] in studies of glass transition behavior in alkanes.

Our first series of simulations [65] examined the temperature dependence of a 40 Å^2/chain (4 μmol/m^2) C$_8$ system in the absence of solvent. The chains were cooled gradually to a temperature of 107 K, and initial conditions were thus generated to begin trajectories at several intermediate temperatures. Our findings can be summarized as follows. The system passes slowly with decreasing temperature from a fully liquid-like state to a low-temperature disordered state. Conversely, when heated from 100 K upwards, the disordered solid-like phase passes gradually

to a liquid-like phase between 200 and 300 K, consistent with DSC experiments [24]. The high-temperature phase is liquid-like in the sense that the chain tails exhibit substantial mobility on short (ps) time scales. The z density profiles exhibit no clear layer by layer ordering as for LB films and SAMs but rather display several random peaks at low temperature. The profiles soften with increasing temperature, but the mean phase width remains roughly 10 Å, in agreement with neutron scattering experiments [67]. Order parameter profiles S_n, which provide a measure of degree of chain tilt away from surface normal, differ from those predicted in Flory–Dill theory [68], presumably due to the inclusion of attractive surface and chain–chain potentials. The simulation values are near to or below zero reflecting a substantial collapse towards the surface, while the theoretical values are small and positive. The chains have an average of 20% gauche defects even at low temperatures, in agreement with IR experiments [23]. This value rises to roughly 30% above room temperature. We observe substantial structural and dynamical gradients in passing away from the surface (Fig. 6). The chain tails display nearly gas-like radial distribution functions and large mobilities. In regions near the surface, ordering is apparent in the radial distribution functions, and reduced mobility occurs. This effect has been found in
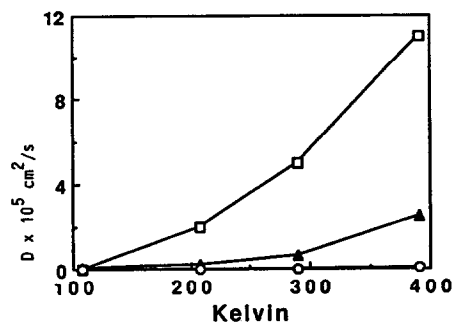

Fig. 6. Diffusion constants for C$_8$ chains at 4 μmol/m^2 as a function of temperature for three carbons ($\square$ = tail carbon; $\blacktriangle$ = middle carbon; $\bigcirc$ = first carbon) along the chains. The diffusion constants were obtained from the mean square displacement as a function of time for a time period on the order of 1.5 ps.

electron paramagnetic resonance (EPR) [69] experiments.

In a second series of studies [66], we have simulated C_{18} stationary phases at several densities and temperatures. A surprising ordering appears in simulations of these longer-chain systems. The z profiles exhibit a layering, not atom by atom but in three large-density peaks passing away from the surface (Fig. 7). A given chain may contribute density to any or all three of the density layers. At lower density (2.5 μmol/m^2), only two peaks appear. This would suggest that segment density fills in layer by layer near the surface until a given layer is largely filled. Such profiles are more familiar from studies of wetting [30] and from simulations of free alkanes on gold surfaces [70]. We have begun to examine which contributions to the potential give rise to the ordering, and find that each contribution (surface, chain–chain, repulsive, attractive) must be included to obtain the observed profiles. This suggests that mean field, hard sphere approaches for the interphase may fail to incorporate important aspects of microscopic forces (we obtain results consistent with the mean field theory when we remove the attractive portions of the potential). A rough surface likely will lead to destruction of the possibility of clear layering on the surface; future work will examine the role of surface anisotropy on the density profiles. A phase width of approximately 16 Å is observed, also consistent with neutron diffraction data [67]; this width is substantially shorter than the fully extended chain

length of 24 Å. The chains display dynamical gradients at room temperature with increasing z as for the C_8 system. The chain tails exhibit fully liquid-like behavior at room temperature. Initial analysis of diffusion constants at several temperatures imply that the C_{18} system freezes more abruptly than the corresponding C_8 system in the temperature range 300→200K. DSC experiments [24] exhibit a clear heat capacity peak for C_{18} chains in the temperature region 300→250K, while the C_8 system displays only a broad and weak peak in the 300→200K range. Therefore our results are at least qualitatively consistent with these data.

We have thus obtained extensive simulation data on the temperature- and density-dependent properties of chromatographic stationary phases. The results are consistent with available DSC, neutron scattering, IR, NMR [32] and EPR experimental data. The alkane interphases in the absence of solvent undergo gradual transitions with increasing temperature over a large temperature range from solid-like to liquid-like behavior. Upon cooling, initial results suggest that the longer-chain systems freeze more strongly (and over a narrower temperature range) into a disordered solid than shorter-chain systems. Solvent effects may shift melting temperatures and accentuate differences between different chain lengths. The next step is to explore the role of observed melting transitions in retention behavior. This will involve inclusion of solvent and simple solutes in the simulations.
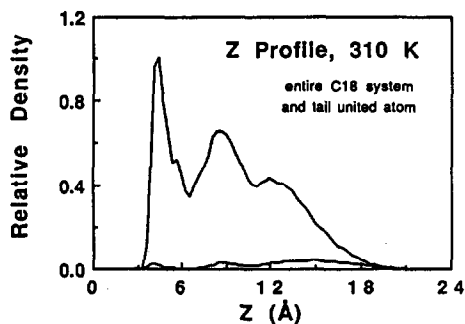
7. ROLE OF THEORY IN UNDERSTANDING PHASE TRANSITION AND SOLVENT EFFECTS ON RETENTION

Non-linear Van 't Hoff plots indicate temperature dependences to enthalpies and entropies (and hence chemical potentials) of transfer of solute from mobile to stationary phases. Certainly the phase of the stationary phase will have an appreciable impact on the retention mechanisms. If the chains are essentially frozen into a disordered state on the surface, a solute molecule will find it quite difficult to penetrate into the alkane phase, unless substantial open cavities exist for the solute to move diffusively in the

Fig. 7. Segment density profile for all CH$_2$ segments and for all carbons and for the tail carbons as a function of distance from the surface.

disordered matrix. This is unlikely since the chains collapse to a large extent onto the surface so as to create a dense liquid-like or solid-like phase. The solute would likely interact with the stationary phase by adsorption in the solid limit. On the other hand, at higher temperatures, where the chains undergo substantial mobility and create a fluctuating liquid-like environment, a solute sees a fluid environment where it can imbed itself into the alkane phase. It is implied by experiment and our simulations that the longer-chain stationary phases exhibit transitions from low-temperature disordered solids to (tethered) liquids over temperature ranges in regions near room temperature. In addition, our simulations reveal structural and dynamical gradients passing away from the surface, an issue which has not been extensively addressed in relation to chromatography. The solvent appears to have some affect on transition temperatures (experimentally) [32]. We plan to insert solvent into our simulation and examine solute chemical potentials at several temperatures and at several z locations in the interphase region by statistical mechanical theory developed by Widom [71].

In recent work on the hydrophobic effect on solvation of simple hard sphere solutes in water, Pratt and Poharille [72] have coupled MD simulation with scaled particle theories of liquids for determination of solute chemical potentials. An important feature of the theory is analysis of distributions of transient cavities in the molecular fluid. The authors compared cavity distributions for alkane fluids and water, and found that, while water has more total accessible free volume, the volume is distributed about a narrower range of sizes. Thus it takes more work of cavity formation for an atomic sized hard sphere solute to be inserted into water than into the alkane fluid. This finding provides a clear microscopic explanation of structural origins of hydrophobic solubility effects. We plan to utilize similar methods in studying cavity distributions at several z locations in the interfacial region. In addition, examination of cavity distributions for non-spherical solutes may lend insight into origins of shape selectivity in chromatography. (We note that this theoretical approach relates more directly to entropic effects than enthalpic effects,

since one implicitly inserts a hard sphere solute. Enthalpic contributions can subsequently be included by thermodynamic integration if desired.) These topics are the subject of ongoing research. In addition, dynamical processes at the interface have important implications for such issues as diffusional peak spreading, etc. We believe MD computer simulation will continue to provide important additions to existing experimental and analytical theoretical understanding of RPLC.

8. CONCLUSIONS

For the past ten years, various groups with both theoretical and practical interests have looked at phase transitions of reversed-phase chromatographic stationary phases. What have we learned in this time? We now know that the observation of phase transitions is a function of virtually every chromatographic variable; bonded phase alkyl chain length, chain density, type of bonding reaction (monomeric or polymeric), mobile phase solvent(s) and the choice of solutes. This leads to a great deal of contradictory information in the literature, and the critical comparison of this information must be done with a great deal of care. It is clear that a single experimental approach is not sufficient to understand these phenomena. Van 't Hoff plots alone are not an indication of changes in stationary phase structure, curvature can arise from other thermodynamic processes than stationary phase changes, DSC experiments are generally not performed under the influence of mobile phase conditions. A further critical issue is the effect of pressure on solvation of the stationary phases. Experiments where the stationary phase is wetted under ambient pressure may not extrapolate to actual chromatographic conditions. This is a question that is beginning to be addressed by the groups using spectroscopic methods to probe the stationary phase. There is also much that can be learned by studying the literature dealing with self-assembled monolayers. While these structures are at a much higher chain density than chromatographic stationary phases, the experimental approaches to studying these structures can be used beneficially by chromatographic scientists.

332

J.F. Wheeler et al. / J. Chromatogr. A 656 (1993) 317–333

What is becoming more clear, both from the experimental and theoretical studies which are underway, is that the term phase transition must be used very loosely. That is, the observations are not of sharp, distinct changes in structure or morphology of the bonded layer. Rather, they are a more diffuse change, going from a solid like to a liquid like state, or *vice versa*. Furthermore, because of the constraints imposed by the bonding and because of the rather low bonding densities achieved, these transitions do not occur over a narrow temperature range, rather, they are more diffuse, resulting in phases which may exhibit properties of both limiting phases.

What is left to learn? Certainly some of the spectroscopic approaches, such as NMR, which are being brought to bear on this problem can offer a great deal of new information. These experiments may allow, for the first time, the direct probing of alkyl chain configuration under actual chromatographic conditions with realistic mobile phases and pressures. Molecular dynamic simulations offer a very new picture of the stationary phase. The results of these studies will likely guide experimentalists in terms of both planning experiments and interpreting results. Finally, there is the ultimate practical question, which is, if we finally fully understand the structure of the stationary phase and the partitioning process leading to retention, how can we use this information to design better separations?

REFERENCES

1 D. Morel and J. Serpinet, *J. Chromatogr.*, 200 (1980) 95.
2 D. Morel and J. Serpinet, *J. Chromatogr.*, 214 (1981) 202.
3 D. Morel and J. Serpinet, *J. Chromatogr.*, 248 (1982) 231.
4 L.A. Cole and J.G. Dorsey, *Anal. Chem.*, 64 (1992) 1317.
5 L.A. Cole and J.G. Dorsey, *Anal. Chem.*, 64 (1992) 1323.
6 K.B. Sentell and A.N. Henderson, *Anal. Chim. Acta*, 246 (1991) 139.
7 Z. Kessaissia, E. Papirer and J.-B. Donnet, *J. Colloid Interface Sci.*, 79 (1981) 257.
8 R.K. Gilpin and J.A. Squires, *J. Chromatogr. Sci.*, 19 (1981) 195.
9 R.K. Gilpin, M.E. Gangoda and A.E. Krishen, *J. Chromatogr. Sci.*, 20 (1982) 345.
10 A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
11 W.E. Hammers and P.B.A. Verschoor, *J. Chromatogr.*, 282 (1983) 41.
12 P. Claudy, J.M. Letoffe', C. Gaget, D. Morel and J. Serpinet, *J. Chromatogr.* 329 (1985) 331.
13 D. Morel, J. Serpinet and G. Untz, *Chromatographia*, 18 (1984) 611.
14 K. Jinno, T. Nagoshi, N. Tanaka, M. Okamato, J.C. Fetzer and W.R. Biggs, *J. Chromatogr.*, 436 (1988) 1.
15 S.S. Yang and R.K. Gilpin, *J. Chromatogr.*, 449 (1988) 115.
16 J.W. Carr and J.M. Harris, *J. Chromatogr.*, 481 (1989) 135.
17 L.C. Sander and S.A. Wise, *Anal. Chem.*, 61 (1989) 1749.
18 S.J. Hansen and J. Callis, *J. Chromatogr. Sci.*, 21 (1983) 560.
19 F. Riedo, M. Czencz, O. Liardon and E. Kováts, *Helv. Chim. Acta*, 61 (1978) 1912.
20 D. Morel, K. Tabar, J. Serpinet, P. Claudy and J.M. Letoffe, *J. Chromatogr.*, 395 (1987) 73.
21 K. Jinno, T. Ibuki, N. Tanaka, M. Okamato, J.C. Fetzer, W.R. Biggs, P.R. Griffiths and J.M. Olinger, *J. Chromatogr.*, 461 (1989) 209.
22 J.P. Beaufils, M.C. Hennion and R. Rosset, *Anal. Chem.*, 57 (1985) 2593.
23 L.C. Sander, J.B. Callis and L.R. Field, *Anal. Chem.*, 55 (1983) 1068.
24 J.C. van Miltenburg and W.E. Hammers, *J. Chromatogr.*, 268 (1983) 147.
25 D.M. Bliesner, S.T. Shearer and K.B. Sentell, *Anal. Chem.*, submitted for publication.
26 K.B. Sentell and J.G. Dorsey, *J. Chromatogr.*, 461 (1989) 930.
27 H.B. Callen, *Thermodynamics*, Wiley, New York, 1960.
28 P.J. Collings, *Liquid Crystals*, Princeton University Press, Princeton, NJ, 1990.
29 T.L. Beck and T.L. Marchioro II, *J. Chem. Phys.*, 93 (1990) 1347.
30 D.E. Sullivan and M.M. Telo de Gama, in C.A. Croxton (Editor), *Fluid Interfacial Phenomena*, Wiley, New York, 1986.
31 C.A. Angell, J. Clarke and L.V. Woodcock, *Adv. Chem. Phys.*, 48 (1981) 397.

32 L.C. Sander and S.A. Wise, *CRC Crit. Rev. Anal. Chem.*, 18 (1987) 299.

33 J. Hautman and M.L. Klein, *J. Chem. Phys.*, 91 (1989) 4994.

34 J. Hautman and M.L. Klein, *J. Chem. Phys.*, 93 (1990) 7483.

35 J.P. Bareman and M.L. Klein, *J. Phys. Chem.*, 94 (1990) 721.

36 J.G. Dorsey and K.A. Dill, *Chem. Rev.*, 89 (1989) 331.

37 J.-P. Hansen and I.R. McDonald, *Theory of Simple Liquids*, Academic Press, New York, 1986.

38 A. Zangwill, *Physics at Surfaces*, Cambridge University Press, New York, 1988.

39 S.T. Milner, T.A. Witten and M.E. Cates, *Macromolecules*, 21 (1988) 2610.

40 T.J. Sluckin and A. Poniewierski, in C.A. Croxton (Editor), *Fluid Interfacial Phenomena*, Wiley, New York, 1986.

41 M.R. Bohmer, L.K. Koopal and R. Tijssen, *J. Phys. Chem.*, 95 (1991) 6285.

42 I. Szleifer, A. Ben-Shaul and W.M. Gelbart, *J. Phys. Chem.*, 94 (1990) 5081.

43 Z. Cai and S.A. Rice, *J. Chem. Phys.*, 96 (1992) 6229.

44 C. Yan and D.E. Martire, *J. Phys. Chem.*, 96 (1992) 7510.

45 R.P. Feynman and A.R. Hibbs, *Quantum Mechanics and Path Integrals*, McGraw-Hill, New York, 1965.

46 M. Murat and G.S. Grest, in R.J. Roe (Editor), *Computer Simulation of Polymer Systems*, Prentice-Hall, Englewood Cliffs, NJ, 1991.

47 B.J. Alder and T.E. Wainwright, *J. Chem. Phys.*, 27 (1957) 1208.

48 M.P. Allen and D.J. Tildesley, *Computer Simulation of Liquids*, Oxford University Press, Oxford, 1987.

49 N. Metropolis, A.W. Rosenbluth, M.N. Rosenbluth, A.H. Teller and E. Teller, *J. Chem. Phys.*, 21 (1953) 1087.

50 G. Zerbi, R. Magni, M. Gussoni, K.H. Moritz, A. Bigotto and S. Dirlikov, *J. Chem. Phys.*, 75 (1981) 3175.

51 G. Ungar, *J. Phys. Chem.*, 87 (1983) 689.

52 G. Ungar and N. Masic, *J. Phys. Chem.*, 89 (1985) 1036.

53 F. Guillaume, J. Doucet, C. Sourisseau and A.J. Dianoux, *J. Chem. Phys.*, 91 (1989) 2555.

54 J.-P. Ryckaert and M.L. Klein, *J. Chem. Phys.*, 85 (1986) 1613.

55 J.-P. Ryckaert, M.L. Klein and I.R. McDonald, *Phys. Rev. Lett.*, 58 (1987) 698.

56 P. Padilla and S. Toxvaerd, *J. Chem. Phys.*, 95 (1991) 509.

57 D. Rigby and R.J. Roe, in R.J. Roe (Editor), *Computer Simulation of Polymers*, Prentice-Hall, NJ, 1991.

58 J. Harris and S.A. Rice, *J. Chem. Phys.*, 89 (1988) 5898.

59 N. Collazo and S.A. Rice, *Langmuir*, 7 (1991) 3144.

60 J.P. Bareman, G. Cardini and M.L. Klein, *Mat. Res. Soc. Symp. Proc.*, 141 (1989) 411.

61 M. Bishop and J.H.R. Clarke, *J. Chem. Phys.*, 95 (1991) 540.

62 S. Karaboni and S. Toxvaerd, *J. Chem. Phys.*, 96 (1992) 5505.

63 S. Karaboni and S. Toxvaerd, *J. Chem. Phys.*, 97 (1992) 5876.

64 S. Karaboni, S. Toxvaerd and O.H. Olsen, *J. Phys. Chem.*, 96 (1992) 4965.

65 S.J. Klatte and T.L. Beck, *J. Phys. Chem.*, 97 (1993) 5727.

66 S.J. Klatte and T.L. Beck, in preparation.

67 L.C. Sander, C.J. Glinka, and S.A. Wise, *Anal. Chem.*, 62 (1990) 1099.

68 K.A. Dill, J. Naghizadeh and J.A. Marqusee, *Ann. Rev. Phys. Chem.*, 39 (1988) 425.

69 M.D.E. Forbes, T.L. Myers, K.E. Dukes and H.D. Maynard, *J. Am. Chem. Soc.*, 114 (1992) 353.

70 T.K. Xia, J. Ouyang, M.W. Ribarsky and U. Landman, *Phys. Rev. Lett.*, 69 (1992) 1967.

71 B. Widom, *J. Chem. Phys.*, 39 (1963) 2808.

72 L.R. Pratt and A. Poharille, *Proc. Natl. Acad. Sci. U.S.A.*, 89 (1992) 2995.