

## Review

# Nuclear magnetic resonance and electron spin resonance spectroscopic investigations of reversed-phase liquid chromatographic retention mechanisms: stationary phase structure

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### ABSTRACT

Recent studies have demonstrated that stationary phase structure and conformation play important roles in reversed-phase liquid chromatographic (RPLC) behavior. Nuclear magnetic resonance (NMR) and electron spin resonance (ESR) spectroscopic techniques can provide a unique perspective for studying RPLC stationary phases. They not only provide information about the types of molecular dynamics and interactions ongoing at the stationary phase surface, but also about their temporal domains. This paper reviews NMR and ESR spectroscopic studies that have been performed to better understand the structure and mobility of RPLC stationary phases. The effects of ligand morphology, mobile phase composition and temperature on alkyl chain mobility and conformation are discussed. The advantages and limitations of spectroscopic techniques for these types of investigations are also reviewed.

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## 1. SPECTROSCOPIC TECHNIQUES AS PROBES OF MOLECULAR INTERACTIONS IN RPLC

Although reversed-phase liquid chromatography (RPLC) has remained the predominant liquid chromatographic separation technique for well over a decade, the dynamics and structure of RPLC bonded phases and their concomitant effects on retention and selectivity are still very active areas of research. Mobile phase parameters are the most obvious factors controlling chromatographic retention, and in earlier theories describing RPLC mechanisms the stationary phase was considered to play a passive and completely inert role in retention and selectivity. However, more recent studies have demonstrated that stationary phase structure can play an important role in chromatographic behavior [1–6], particularly for compounds which are closely related in morphology [7,8]. In the study of RPLC bonded phase structure, there is no true “macroscopic phase”; *i.e.* its composition and properties vary according to microscopic location on the silica surface as well as with alkyl ligand depth. Another complication in studying these systems is that they are “solids” that are not in thermodynamic equilibrium with respect to phase changes (in the same sense as glassy polymers [9]).

As might be expected, stationary phase conformation has primarily been studied via chromatographic experiments; selectivity studies utilizing solutes with very similar structures are particularly pertinent [5,7,8,10]. Chromatographic measurements provide essential information about the microscopic state of the bonded phase as a function of the type and bulk composition of mobile phase, temperature, stationary phase and support surface chemistry and modification, and solute hydrophobicity and polarity. However, the nature of such measurements dictates that stationary phase structural information be drawn by *inference*, via interpretation of the chromatographic results. Spectroscopic techniques, on the other hand, provide a much more direct means of studying the stationary phase surface. These types of studies not only provide information about different types of molecular interactions occurring at the stationary phase

surface, but also about the various temporal domains of these interactions. Just as importantly, spectroscopic measurements can be made *independently* of chromatographic measurements; this orthogonality often leads to spectroscopic information that is entirely complementary to that obtained chromatographically. The combined approach provides a much more complete and realistic picture of molecular level contributions to RPLC retention and selectivity than can be obtained from chromatographic measurements alone. Spectroscopic experiments are also quite versatile, as they can be utilized to inspect intrinsic stationary or mobile phase properties directly, stationary or mobile phase components can be labelled with a spectroscopically active moiety, or interactions of spectroscopically active solute probes with the chromatographic system can be examined.

## 2. PROBE SITES FOR SPECTROSCOPIC STUDIES

### 2.1. Stationary phase probes

There are numerous advantages to using spectroscopic measurements to study the properties of native chemical groups in the stationary phase support material or in the moieties which are covalently bonded to it. Since the groups are already present, samples can be prepared with minimal expense, time and effort. If there is an abundance of the species to be studied, signal to noise is enhanced, thereby increasing sensitivity as well as decreasing the required experimental time. Because measurements are made on the native material, the system is virtually identical to actual chromatographic systems (although in reality spectroscopic measurements are rarely carried out under chromatographic pressure or flow conditions) and therefore the presence of the probe does not perturb the system. However, since the environments of similar groups on the silica support surface often differ in very subtle ways, distinguishing between signals from very proximal yet chemically non-equivalent groups is often difficult, leading to an “average” value for similar but not identical chemical groups. This results in measurements of the average overall state of the sites rather than of

the individual states of the specific sites and can therefore lead to the very generality in the overall information obtained that spectroscopic measurements are intended to avoid. Also, many groups of interest either give very weak spectroscopic signals or are present in such low abundance in the native material that measurements on them require prohibitively long experiments.

In order to avoid the above disadvantages, many researchers have incorporated spectroscopic labels into the stationary phase. Labelling a specific stationary phase site enables that precise location to be studied. The addition of labelling for groups which are naturally low in abundance or which provide weak spectroscopic signals generally also results in much enhanced signal to noise. However, the disadvantages to using labelled probes must also be recognized. Chemical attachment of the probe to the stationary phase usually involves a multistep synthetic procedure; often the probe molecule must itself be synthesized and purified prior to attachment. Since yields for many of the synthetic procedures are often low and the reagents expensive, the advantages to the spectroscopic measurement gained by adding the probe must outweigh the monetary and temporal investment of the synthetic procedures. A potentially more serious drawback to labelling the stationary phase surface can result if the probe contains a functional group necessary for spectral enhancement that is chemically different from the functional groups making up the bonded phase. Spectroscopic measurements are often affected by the presence of chemical groups on probe molecules that can undergo more specific chemical interactions (*i.e.* hydrogen bonding, dipolar and/or  $\pi$ - $\pi$  interactions) and may exhibit artifacts of these specific interactions. In reversed-phase materials, stationary phase ligand-solute interactions are rather weak and non-selective dispersive interactions. If specific or non-dispersive interactions between probe functional groups on adjacent sites occur to a different extent than interactions in the native bonded phase system, then the probe groups do not serve as accurate models of bonded phase interactions. Alternately, the probe molecules may themselves perturb the native stationary phase system sufficiently (*e.g.*

by steric or specific interactions) to result in a stationary phase structure that is not equivalent to that existent under normal operating conditions.

## 2.2. Mobile phase probes

In RPLC there is no distinct interfacial boundary between the stationary and mobile phases because of the formation of a solvation layer, which results from the dynamic equilibrium between mobile phase components and the grafted alkyl chains as well as the silica support. Preferential sorption of mobile phase components by the stationary phase results in a layer whose thickness and composition varies with the distance from the bonded ends of the alkyl chains. The stationary phase alkyl chains are not isotropic; they exhibit ordering due to their covalent attachment to the rigid silica substrate surface and this increases with alkyl chain surface coverage [1–3]. Due to these varying degrees of chain interaction, mobile phase components will penetrate into the stationary phase in a non-uniform manner [4]. The overall structure of the RPLC solvation layer is thus a function of the bulk mobile phase composition, the length of the bonded alkyl chain, the degree of alkyl derivatization of the silica surface, the system temperature and pressure, and the numbers and types of residual silanols remaining on the silica surface following derivatization.

Because of the profound effect of the mobile phase on stationary phase structuring and conformation, spectroscopic measurements on mobile phase components can provide useful information about the solvation (or “wetting”) microenvironment of the stationary phase surface, so long as certain cautions are followed. Direct measurements for solvent molecules in contact with stationary phases will of necessity be a weighted average of the bulk and associated solvent, if the exchange between these sites is fast relative to the time frame of the spectroscopic experiment [11], as will often be the case. However, if the spectroscopic measurements for the bound solvents are quite different (temporally or spectroscopically) from those for the bulk solution solvents, the spectroscopic signals mea-

sured for the associated solvent species can often be deconvoluted from those for the bulk solvent species. Also, any spectroscopic measurement of the interactions of a species of interest with an RPLC bonded phase will be representative of the *average* degree of interaction with the bonded phase surface. This is because any chromatographic solute, including mobile phase components, will participate in varying degrees of association with such a heteroenergetic surface as a RPLC stationary phase [11]. Although the use of labelled mobile phase components can offer unique perspectives for studying solvent interactions with bonded phases, the same cautions as mentioned for labelled stationary phase probes hold true for labelled mobile phase materials.

### 2.3. Solute probes

By using a spectroscopically active solute to probe chromatographic interactions, the role of stationary phase structure in retention and selectivity can be probed *in situ*. By prudent choice of the probe molecule, the roles of specific types of interactions involved in retention (dipolar,  $\pi$ - $\pi$ , dispersive, hydrogen bond donor or acceptor, steric) can be elucidated. However, the same constraints that apply to chromatographic experiments apply to the analogous spectroscopic experiments. In choosing a solute probe for studies of analytical scale separations, the concentration of the probe must be kept sufficiently low as to ensure that linear chromatography conditions are operative, *i.e.* partitioning is taking place in the linear portion of the distribution isotherm. Interactions between the solute and competing stationary phase sites (residual silanols *vs.* bonded phase alkyl ligands) must be able to be deconvoluted if the individual contribution from each type of site is to be determined. The vast majority of the solute must also be in the stationary phase as opposed to the mobile phase, *i.e.* the distribution coefficient for the solute must be very large, or the problem of measuring the average spectral behavior for solute distributed in both the stationary and mobile phases is again present. Another important consideration is that the probe must be present in such a low

concentration compared to that of the stationary and mobile phase components at the bonded phase surface that the interfacial layer will behave in the presence of the solute probe exactly as it does when none of the probe molecule is present. This means that it cannot induce ordering of stationary phase ligands or expulsion of mobile phase components from the solvation layer. Although this assumption may be true in a macroscopic sense, it cannot strictly be true at the level of the bonded phase microenvironment [12].

## 3. SPECTROSCOPIC TECHNIQUES

### 3.1. Nuclear magnetic resonance spectroscopy

This review will discuss nuclear magnetic resonance (NMR) and electron spin resonance (ESR) spectroscopic studies that have been carried out on RPLC chromatographic systems. Other spectroscopic techniques are discussed in a complementary paper contained in this volume [13]. NMR has become one of the most prominent spectroscopic techniques in modern chemistry. The transition frequency for a nucleus in a particular environment is an extremely delicate probe of that environment [9] and therefore NMR spectroscopy provides a wide range of information. This information can be divided into four general categories [14]:

(i) Sites on a molecule or surface can be identified via NMR parameters that affect the spectrum, such as quadrupolar couplings or different chemical shifts. Under many circumstances, quantitative information about the spectral intensities for each site can be obtained; this is helpful for structural determinations [14].

(ii) Relationships between sites can also be investigated via determination of any existent correlation. Information provided by these types of NMR studies can be static in nature, such as bond distances or angles; or dynamic, such as correlation of motions at different sites [14].

(iii) Because NMR is sensitive to motion through its effects on line shape as well as via nuclear relaxation mechanisms, it is an excellent technique for studying dynamical phenomena

with times ranging from picoseconds to hours [14].

(iv) With non-invasive NMR imaging techniques, information about density, flow and spatially localized chemical compositions can also be obtained [14]. By using ingeniously designed NMR probe heads amenable to flowing systems, Bayer and co-workers [15–23] have carried out extensive *in situ* imaging of the chromatographic process.

Both solid- and solution-state NMR experiments have been used to study the structure and dynamics of RPLC bonded phases. Solution-state NMR measurements on suspensions of bonded phase materials in relevant solvent systems provide information about motions in the grafted alkyl ligands in the range of the Larmor frequency of the  $^{13}\text{C}$  nuclei (MHz); application of the spin lock in  $^{13}\text{C}$  cross-polarization (CP) experiments on dry state stationary phase materials opens the kHz motional regime to experimentation [24]. In liquids, rapid random motions bring about a natural averaging of line-broadening influences; however, broadening of spectral resonances is more of a problem for NMR measurements on solid materials. Besides the resultant loss of spectral resolution, distribution of the spectral intensity over a broader line width also causes sensitivity problems [25]. In bonded phase materials, closely neighboring atoms (generally protons) in the lattice can cause very strong dipolar–dipolar coupling interactions, which result in severe broadening of individual nuclear resonances [24,26]. Additionally, chemical shift anisotropy interactions result in a chemical shift distribution for a particular type of site. Since the chemical shift for a given nucleus is dependent on the geometric relationship between the nucleus's local environment and the direction of the static magnetic field, the entire chemical shift range spanned by all possible orientations of the molecules in a solid [25]. Each of the elements of the shielding tensor for each geometric relationship results in a slightly different chemical shift, which results in an observed overall broad resonance, as shown in the  $^{13}\text{C}$  spectrum of a dry state  $\text{C}_{18}$  bonded phase material in Fig. 1a. For a nucleus experiencing a

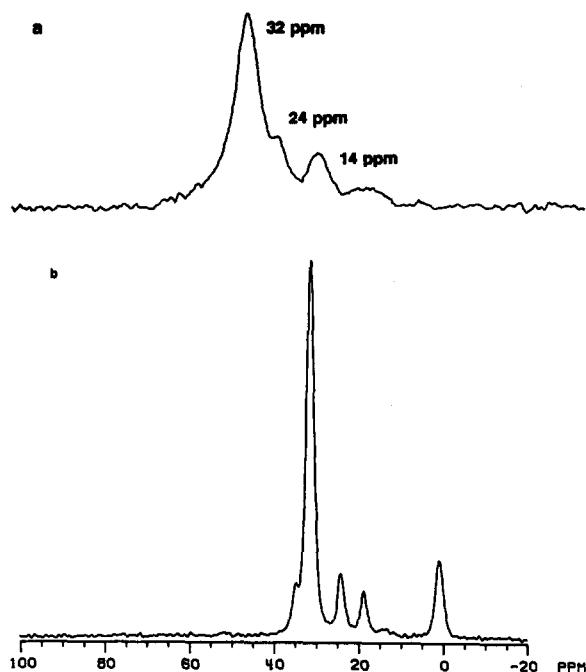


Fig. 1.  $^{13}\text{C}$  NMR spectra of a high chain density monomeric  $\text{C}_{18}$  reversed-phase material (LT1) prepared in our laboratory. (a) Solution-state spectrum of the dry material. (b) Solid-state cross-polarization magic angle spinning spectrum of the same material. Note the improved resolution and narrower resonances.

number of broadening interactions, the resultant overall spectrum will be a combination of all of the possible spectra [9]. Therefore, in order to obtain useful information, the influences on the spectrum must be deconvoluted [9] by experimental conditions, sophisticated data processing techniques and/or computer simulations. High-power decoupling of adjacent protons at their resonance frequency and spinning the sample very rapidly (2–5 kHz) at the magic angle (MAS) are the respective experimental methods for compensating for dipolar–dipolar and chemical shift anisotropy interactions in solid state spectra [26]. Rapid spinning of the sample at the magic angle mechanically accomplishes the same sort of line narrowing that rapid, random motional averaging does in liquid samples [25].

An additional problem in measuring nuclear relaxation rates for solid-state dynamic studies is the often very long (minutes to hours) spin–

lattice relaxation times ( $T_1$ ) in solids; this is particularly true for  $^{13}\text{C}$  nuclei [25]. However, double resonance experiments in which the less abundant nucleus ( $^{13}\text{C}$  or  $^{29}\text{Si}$  for RPLC bonded phases) is excited *indirectly* via the numerous surrounding protons (cross-polarization) enable the “time bottleneck” of inefficient spin-lattice relaxation to be overcome [24,25]. CP experiments are very effective for static or immobile species, but not for mobile, liquid-like species [25]. An added benefit of proton–carbon or proton–silica cross-polarization is that the weaker signals from the less abundant nucleus are enhanced [12]. The combined advantages of the CP-MAS experiment are illustrated in Fig. 1b. CP-MAS techniques also allow for very good selectivity, since only those nuclei which are in close contact with protons are efficiently polarized in this type of experiment [24]. For  $^{29}\text{Si}$ , the species detected are therefore those at or near the surface, providing a great degree of specificity in determining the effects of preparation methods on the silica gel or bonded phase structure [27,28]. If the contact time between the nucleus of interest and the protons is varied, additional measures of nuclear mobility ( $T_{\text{CH}}$ ,  $T_{\text{SiH}}$  and  $T_{1\rho\text{H}}$ ) can also be measured in the CP-MAS experiment [24].

Signal quantitation of individual resonances via CP-MAS techniques is somewhat difficult, however, because the signal amplitude of individual resonances depend on the contact time applied, and the contact times which provide maximum signal of individual nuclear sites are often different due to disparities in the relaxation times for the different sites. However, if the relaxation times of the individual types of nuclear sites are known, their distribution ratios can be determined [24]. Therefore CP-MAS experiments can be used to elucidate surface–backbone chemistry and structure, characterize ligands that are attached to the bonded phase support and determine overall molecular and segmental chain motion [29]. One important drawback to experiments on liquid chromatographic bonded phases under CP-MAS conditions is that the effects of mobile phase solvation on their structure and mobility are not considered under wetting and pressure conditions that

accurately represent those in liquid chromatographic systems. Under RPLC operating conditions, cooperative effects from the latter factors can be profound. However, solid-state NMR measurements can be thought of as a “bridge” between the data on crystalline solids that techniques like X-ray diffraction can provide and data for materials in the liquid state or in solution, wherein the chemical reactivity is usually of more interest [25].

Dynamic NMR experiments in the solution state are invaluable for determining bonded phase mobility under solvated conditions, and therefore more nearly approximate actual RPLC systems. Although not as many individual resonances of distinct sites in the stationary phase can be delineated as in the solid-state experiments, useful information about the dynamics of four to five different regions in a typical bonded phase alkyl ligand can be obtained from solution state experiments. The most useful parameter measured is the spin–lattice relaxation time,  $T_1$ , of the nuclei of interest, since it is inversely proportional to  $\tau_c$ , the nuclear correlation time. The correlation time is a measure of how long it takes the nucleus to rotate through one radian and is therefore a measure of molecular motion. In the solution state, as the correlation time increases (*e.g.* molecular motion decreases) the longitudinal relaxation time,  $T_1$ , decreases. Greater molecular mobility is therefore indicated by an increase in  $T_1$ . Determination of solution-state  $T_1$  for nuclei of interest is generally carried out via standard inversion recovery experiments [30].

$^{29}\text{Si}$  chemical shifts have been detected over a range of 400 ppm, and provide valuable information about the structural elements of silica surfaces. For example, the  $^{29}\text{Si}$  chemical shifts of free and geminal silanol groups are sufficiently different to distinguish between these groups on silica surfaces; this is not possible using infrared spectroscopy [27].  $^{13}\text{C}$  chemical shifts extend over a range of 200 ppm. Unfortunately, for long-chain silanes, the methylene groups in the central portion of the alkyl ligand (for example,  $\text{C}_4$  through  $\text{C}_{15}$  in the octadecyl ligand, where numbering begins at the point of ligand attachment to the silica support) exhibit essentially

identical chemical shifts even under CP-MAS conditions, since substitution effects from neighboring carbons with differing groups are only effective up to the carbon  $\gamma$  to them [24].

### 3.1.1. Silica and alkylsilyl bonded phase structure

In some of the earliest reported solid-state NMR studies on RPLC bonded phases and supports, Maciel and Sindorf [28,31] performed  $^{29}\text{Si}$  CP-MAS experiments which confirmed that the presence of siloxane, alkylsiloxy and single and geminal silanol groups gave rise to distinct  $^{29}\text{Si}$  chemical shift values. Since the use of  $^{29}\text{Si}$  NMR for determining the hydroxyl content of silica materials is based on the properties of Si nuclei, this method is much less sensitive to the effects of adsorbed or bulk molecular water than more conventional techniques. They also studied silanol reactivity with  $^{29}\text{Si}$  CP-MAS experiments, and found that when reacted with hexamethyldisilazane (HMDS), the fraction of single silanol groups derivatized was  $0.39 \pm 0.01$ ; that for geminal sites was  $0.65 \pm 0.02$ . Calculations predicted that the reaction rate for geminal hydroxyl sites is faster than that for single hydroxyls; this is the most probable reason that the former are more reactive, since more of them will have had a chance to react before the steric constraints become large [31]. Köhler *et al.* [32] used  $^{29}\text{Si}$  CP-MAS-NMR and diffuse reflectance Fourier transform infrared spectroscopy (FT-IR) to study the relative reactivity of silica hydroxyl groups with methyl silylating agents and their measurements also indicated that geminal silanol groups reacted first during silanization. They determined that two of the silicas studied (Zorbax and Nucleosil) initially contained 32% geminal silanols and 68% single silanols. Upon heating, peak intensities shifted from hydrogen bonded to unbonded silanols, due to dehydration of the surface and the resultant additional siloxane bonds. From the relative intensity of the isolated silanol peak as well as the observation that the pH of the material did not change appreciably upon heating, unbonded or isolated silanol groups were thought to be the last to be removed as well as the chief cause of acidic behavior, due to their

isolation in a “field” of electron withdrawing siloxane bonds. Köhler *et al.* [32] concluded that isolated acidic silanols are responsible for the adsorption of basic compounds on both silanized and non-silanized supports.  $^{29}\text{Si}$  solid-state  $T_1$  values have also been used to determine the effects of acid pretreatment on the silica surface, which results in both amorphous regimes and polycrystalline domains [33].

Quantitation of various types of bonded phase surface species resultant from different synthetic procedures can be achieved via careful CP-MAS measurements [34,35]. Alkyl derivatization of the silica support is accomplished by reaction of silica hydroxyl groups with a silane with one or more reactive sites; the reversed-phase materials resulting from the former type of synthesis are commonly described as monomeric and the latter polymeric. [These descriptors of the silylation procedure will be used in the remainder of this review, and the latter term should not be confused herein with the polymeric *support* materials which are used in place of silica in some RP-type stationary phases.] By performing Gaussian simulations of experimental  $^{29}\text{Si}$  CP-MAS spectra, Pfeiderer *et al.* [35] were able to deconvolute individual contributions to broad resonances, and hence obtain quantitative information about the relative distribution of the various types of silane ligands resulting from the reaction conditions and reagents used. If the synthesis is carried out under an inert argon atmosphere, difunctional silanes result in monodentate surface species and trifunctional silanes primarily result in bidentate species; without the argon atmosphere bidentate or cross-linked monodentate modification occurs with either reagent. In general, their  $^{29}\text{Si}$  studies indicated that the larger number of functional groups on the trichlorosilane increase the probability of the ligand reacting with two neighboring silanol groups [35]. Cross-polarization contact times also indicate that the ligands resultant from dichlorosilanes are more mobile than those from trifunctional reagents. Sindorf and Maciel [36] suggest that the presence of molecular water not only dictates the course of derivatization reactions with polymeric silanes, but may also be essential in determining the extent to which the

silylation occurs. Their data semiquantitatively supports a model wherein the polymeric silane phase initially has a silicon bond order of one; following exposure to water the bond order increases to a value between 1.0 and 1.5 [36].

$^{29}\text{Si}$  NMR characterization also indicates that these phases undergo condensation reactions with unreacted silane moieties or even with the wash solvents from the synthetic process (particularly ethanol). Cross-linking can occur via polymerization of the silane with unreacted ligand functional groups perpendicular to or along the surface; the former is a lesser product, since it is subject to a greater degree of steric hindrance [35]. The predominance of the latter type of polymerization offers a plausible explanation for the enhanced selectivity for planar and linear solutes than is often noted for polymeric phases [10]. Condensation and polymerization play a minor role during the initial silica derivatization. However upon ageing in the dry state, or particularly under HPLC operating conditions, where the ligands are more mobile and therefore more likely to interact, cross-linking is an important modification process for bonded phases prepared with polyfunctional silane reagents [35,37].

Gangoda and Gilpin [38] note that although CP-MAS experiments provide interesting results, they do not address the role of solvation on bonded phase structure and dynamics and therefore are not representative of actual chromatographic conditions. However, alkyl ligands in bonded phase systems exhibit broad solution state resonances, and for low surface coverage bonded phases this problem is compounded by the weak signal which results from the small amount of carbon contained in the material as well as the low natural abundance of  $^{13}\text{C}$  nuclei [38]. Selective labeling can therefore provide a simpler means for interpretation of individual resonance contributions in solution state spectra. Gilpin and Gangoda [39] prepared  $\text{C}_7$  and  $\text{C}_9$  trichlorosilane-modified silicas with varying degrees of surface coverage and  $^{13}\text{C}$  enrichment at the terminal position. In comparison to  $^{13}\text{C}$  NMR spectra of these materials obtained in 100%  $^2\text{H}_2\text{O}$ , when small amounts of dioxane

were added to the bonded phases in  $^2\text{H}_2\text{O}$  the effect on the line shape was a dramatic sharpening of the resonance. The materials were then washed with methanol, dried at elevated temperatures and the  $^2\text{H}_2\text{O}$ -dioxane NMR experiments repeated. When the subsequent loss of the sharpened signal is then considered in combination with the lack of silane resonances in the methanol wash, their conclusion of cross-polymerization after exposure to solvents is similar to that offered by Pfeiderer *et al.* [35]. As expected, when monomeric reagents were used no change in the bonded phase resonance line shape was observed after exposure to solvents [39].

Hetem *et al.* [40] used  $^{29}\text{Si}$  solid-state NMR, elemental analysis and chromatographic studies to investigate the long term stability of  $\text{C}_{18}$  stationary phase materials which had approximately equal carbon contents but were made with two different types of silica substrates and mono-, di- and trifunctional silanes. They found that upon exposure to aqueous buffer solutions with high (8.4) or low (3.0) pH, dehydration reactions in the di- and trifunctional modified materials caused more multidentate surface and neighbor linkages to form than were present in the initial material, accompanied by a slight increase in the number of surface silanol groups. Multidentate linkage with adjacent silanes caused considerably less hydrolysis of the anchored ligands to occur, particularly for the trifunctional silane, than for materials made from the monofunctional silanes and subjected to the same buffers. The basic buffer caused a larger degree of condensation to occur as well as considerable dissolution of the silica substrate, particularly for the trifunctional phase. Chromatographic retention and methylene selectivity were also diminished due to loss of alkyl ligand, with the latter parameter more affected by ligand loss. Polar group selectivity was much affected in the monofunctional phase and little affected in the polymeric phases [40]. Claessens *et al.* [41] also used  $^{29}\text{Si}$  CP-MAS-NMR, chromatographic behavior and elemental analysis to study the stability of bonded phases of various chain lengths, prepared with mono- and trifunctional silanes, under chromatographic operating condi-



tions. They found that for any given type of silane, the *monofunctional* silanes produced more stable linkages (contrary to other reports in the literature) and that longer-chain phases were more stable than shorter ones.

Albert *et al.* [34] used comparisons of the resonance widths at half-height ( $\Delta w_{1/2}$ ) for neat octadecyldimethylmethoxysilane, the silane in contact with silica and the silane bonded to the same silica to develop a solution-state NMR method for discriminating between chemically bonded and adsorbed silanes on silica surfaces. For the neat silane in benzene,  $\Delta w_{1/2}$  was 4.4 Hz for the bulk chain ( $C_4$ – $C_{15}$ ) resonance; if a solution containing an amount of silane equivalent to that in a bonded phase material was added to silica, the line width broadened to 16 Hz. However, if the silane was chemically attached to the silica surface,  $\Delta w_{1/2}$  was 90 Hz, due to the greatly restricted motion of the central portion of the silyl alkyl chain. Gilpin and Gangoda [42] have reported similar observations.  $^{13}\text{C}$  Chemical shifts can also be used for this purpose. Gilpin and Gangoda [29] noted that the chemical shifts of the  $^{13}\text{C}$  resonances of labelled methyl and terminal methylene groups for  $C_7$ ,  $C_9$ ,  $C_{12}$  and  $C_{13}$  monomeric and polymeric silanes and bonded phases were virtually the same. This is not surprising, since the presence of the silica surface and the heterogeneity in the types of polymeric ligand attachment to the surface will have little effect on the chemical environment of groups which are located far from the silica surface. The chemical shift of labelled carbons located closer to the point of ligand attachment to the silica surface, however, were observed to move to slightly lower fields with respect to those measured for the corresponding silane. These peaks were also observed to be asymmetric [38]. Gilpin and Gangoda invoke Palmer and Maciel's explanation [43], which considers both homogeneous and heterogeneous effects on peak shape and chemical shift. The latter effect comes about from variations in bonding chemistry and the existence of different types of reaction sites, and therefore exhibits a much larger effect at or near surfaces than at the terminus of the bonded alkyl ligands

[43]. Gangoda and Gilpin [38] have observed similar effects on peak widths for bonded phases synthesized from monomeric and polymeric silane reagents.

$^{13}\text{C}$  Chemical shifts can even provide information about the surface density of the bonded alkyl ligands. Albert and Bayer report that for long-chain alkyl ligands ( $n \geq 18$ ) at surface loadings  $\geq 2.9 \mu\text{mol}/\text{m}^2$ , two  $^{13}\text{C}$  resonances are noted for the terminal methyl groups of  $C_{18}$  phases, which result from the distribution of alkyl ligands in a more rigid environment as well as one in which there is more motional freedom [24]. This indicates that there is heterogeneity in the alkyl ligand surface coverage even at relatively high alkyl chain densities.

### 3.1.2. Alkyl ligand mobility studies

One of the most useful applications of NMR techniques to RPLC systems is the study of bonded phase mobility. Sindorf and Maciel [44] have used  $^{13}\text{C}$  CP-MAS-NMR to study the molecular motion of *n*-alkylsilanes bonded to silica surfaces under dry state conditions. Methyl carbons cross-polarize at noticeably slower rates than methylene carbons in similar chemical environments. They attributed this to the rapid rotation of the methyl group about the terminal C–C bond, which partially decouples the methyl carbons and protons, and they identified methyl rotation as being the most likely source of proton spin-lattice relaxation [44]. Cross-polarization efficiency (and rate) decreased with increasing distance from the surface along the alkyl chain up to eight carbons; beyond this point the CP rates were essentially the same. This is interpreted as meaning the molecular motion increases (and/or becomes less anisotropic) toward the free end of the chain. However, motion does not become completely liquid-like for carbons far removed from the surface. This suggests that some sort of interactions (with the silica surface or with other attached moieties) might restrict the modes of motion that are available for these groups. Overall, motional behavior for methyl and methylene environments in silica-attached alkylsilanes is very heterogeneous; however, CP rates for carbons at equal distances from surface-

attached silicon atoms were similar for monomeric  $C_8$  and  $C_{18}$  silane systems [44].

One of the most widely applied NMR methods used for studying RPLC bonded phase mobility is measurement of the spin-lattice relaxation times ( $T_1$ ) of specific alkyl ligand moieties. Using the standard inversion-recovery method, Gilpin and Gangoda [29] have made solution state  $^{13}C$  spin-lattice relaxation time ( $T_1$ ) measurements in [ $^2H_3$ ]acetonitrile for  $C_7$ – $C_{13}$  monomeric and polymeric bonded phases with various  $^{13}C$ -labelled positions. In the solution state the alkyl ligands have motion in the motional narrowing regime, wherein the larger the  $T_1$  value, the more mobile the corresponding carbon. Gilpin and Gangoda found that the bonded phases exhibited lower  $T_1$  values for the terminal carbon than the native silanes, with virtually the same spin-lattice relaxation times for the terminal carbon of monomeric or polymeric bonded phases with similar surface coverages regardless of the overall chain length. Since the  $T_1$  values were virtually the same, yet the lines were broader for the polymeric phases, the terminal methyl groups are likely equally mobile in both types of phases, but the broader resonances for the polymeric phases are again due to their less homogeneous morphology. Gilpin and Gangoda also found that as polymeric  $C_{12}$  surface coverage was increased, a significant *decrease* in the  $T_1$  of the terminal carbon occurred. They gave as a possible explanation that steric interaction of the alkyl chains at higher surface coverages led to hindered end rotation, and suggested that there might be a critical alkyl surface density beyond which this rotation becomes hindered [29].

Gilpin and co-workers usually approached their  $^{13}C$  NMR studies by labelling alkyl groups with  $^{13}C$  at certain chain positions, particularly at the terminus. However, Bayer and co-workers [45,46] argue that measurements of the dynamic nature of the central portion of the bonded alkyl chains are more relevant to the general aspects of overall chain motion, and therefore to the chromatographic process, than measurements taken on the methyl group of the chain, where motion will be dominated by rotation about the end bond. Albert *et al.* [46] applied conventional solution state  $^{13}C$  NMR to slurries of  $C_{18}$  bonded

phase materials suspended in acetonitrile and acetonitrile–water mixtures. Even without  $^{13}C$  enrichment, three types of bonded phase alkyl groups could be distinguished in the slurry spectra—a peak corresponding to the methyl carbon, one corresponding to the terminal methylene and a broad envelope of peaks corresponding to the  $C_4$  to  $C_{15}$  methylene groups. They determined that the overall mobility of the ligand in contact with various solvents decreased in the order benzene > acetonitrile > acetonitrile–water mixtures, particularly for those mixtures containing 50–90% (v/v) water [46]. Increasing the aqueous content of the mobile phase leads to more restricted chain conformations because the hydrophobic alkyl groups attached to the surface “try to intermingle with each other near the silica surface” [34]. Mobility also decreased with increasing alkyl surface coverage and markedly so when the bonded phase was endcapped. Chain mobility is affected by surface coverage because the available space for a single chain decreases with increasing surface coverage. Endcapping has an even greater effect, because the bulky trimethylsilyl (TMS) groups cause a larger degree of steric interference between atoms that are close to the point of surface attachment. In all cases in which the alkyl chains exhibited decreased mobility, increased solute retention was also observed [34,45,46]. Therefore, NMR data could be directly correlated to HPLC data, since a more rigid alkyl chain conformation exhibited increased solute interactions and led to longer retention [34].

Albert *et al.* [47] used both solid and solution state  $^{13}C$  NMR to study the dynamic behavior of monofunctionally derivatized  $C_4$ – $C_{20}$  alkyl silicas, all with surface coverages of *ca.*  $3.5 \mu\text{mol}/\text{m}^2$ . In the solid state experiments, CP-MAS techniques were used to measure  $^1H$  spin-lattice relaxation parameters in the rotating frame,  $T_{1\rho}$ . If a distinct environment of a flexible alkyl chain has motion in the kHz range, spin diffusion is reduced by this high degree of motional freedom in the solid state, and  $T_{1\rho H}$  of that environment becomes a useful probe of spin dynamics [47].  $T_{1\rho H}$  values increased from the point of chain attachment at the surface to the terminal alkyl group; this was explained as being due to in-

creased motional freedom within the chain with increasing distance from the silica surface, although there is anomalous behavior at 346 K for carbons 3 and 4 in a C<sub>6</sub> phase (see Fig. 3 in ref. 47). The highest mobility was observed for the C<sub>6</sub> and C<sub>8</sub> phases; in general, phases with either shorter or longer alkyl ligands exhibited less motion freedom, with the C<sub>18</sub> and C<sub>20</sub> phases being the least mobile. The C<sub>4</sub> phase exhibited lower mobility than the C<sub>10</sub> phase. If the stationary phases are then suspended in a suitable solvent (in this case, benzene), molecular motions of the chain carbons in the MHz range can be studied by measuring the <sup>13</sup>C spin-lattice relaxation time. In the latter case, the same general trends held true as in the CP-MAS experiments, indicating that the bonded phase alkyl groups exhibit similar motional behavior in the solid state to that exhibited when suspended in benzene [47]. This means that the alkyl chains of the bonded phases exhibit a liquid-like behavior even in the solid state [24]. The authors caution that their results are only valid for monolayers with high surface densities which result from monofunctional silane derivatization of silicas with pore sizes of 100 Å [47].

<sup>2</sup>H NMR linewidth studies have been used by Gangoda *et al.* [48] to characterize the motions of deuterated sites on monomeric C<sub>12</sub>, C<sub>10</sub> and C<sub>7</sub> alkylsilicas. Deuteration was located at the methyl, terminal methylene, C-1 or C-4 position. A trimethyldecyl silane, labelled at the fourth position on the alkyl chain, was also *physically* coated onto the same support. Just as they had observed for <sup>13</sup>C [38], the bonded phases labelled at the 1 or 4 positions exhibited the broadest resonances. Line width was almost identical at these two sites, which indicates that motions in these chain locations are strongly correlated, most likely by tight packing; this would prevent the chain segments from moving as individual units [48]. Line shapes narrowed for the labelled group located at the distal end. This indicates that in this chain position, additional motions contributed to the motional averaging process, with several bond rotations involved. The physically coated sample exhibited a very sharp liquidlike resonance, reflecting complete average of quadrupole interactions.

This means that the observed line broadening for the chemically attached groups is not just due to magnetic field anisotropy effects caused by the silica present in the sample. Because the line shapes were Lorentzian rather than uniaxial powder patterns, complex motional considerations, such as slow motions and the absence of unique axes of reorientation, must be present [48]. The latter could be due to a distribution of chain packing along the silica surface, resulting from heterogeneous reactivity of the silica support. A model that fits the line width data depicts the bonded chains near the surface (through at least the first four carbons) as being more rigid and restricted in motion, with greater translational and conformational dynamics at the chains' ends, most likely due to rotational contributions. Slopes from the Arrhenius plots ( $\ln T_1$  vs. temperature<sup>-1</sup>) were consistent with differences in motional averaging between the free end of the chains compared to those positions closer to the anchored end. The NMR data indicated that there are at least two different motional domains along the alkyl ligand chain [48]. Although these studies are interesting, they can only be used as preliminary approximations for C<sub>18</sub> stationary phase systems, since even the longest alkyl chain used in this work (C<sub>12</sub>) is considerably shorter than C<sub>18</sub>, and contact interactions between neighboring ligands are diminished for shorter alkyl chains.

### 3.1.3. Solvent effects on RPLC bonded phase structure

3.1.3.1. *Stationary phase studies.* Gilpin and Gangoda [49] measured <sup>13</sup>C  $T_1$  values and nuclear Overhauser enhancements (NOE) for a terminally labelled monomeric dodecyl silica in contact with nine different deuterated neat solvents. Changes in  $T_1$  as a function of viscosity ( $\eta$ ) can be used to elucidate the solvent's relative influence on total and segmental motion of the labelled chain segment. If  $T_1$  is plotted *versus*  $1/\eta$  and the slope approaches one, little or no segmental motion is present; the slope decreases as the importance of segmental motion to total motion increases [49]. The slopes measured by Gilpin and Gangoda [49] fell into two solvent groups. The "low slope" neat solvents (slope  $\approx$

0.36 s-cP) included acetone, acetonitrile, dioxane and methanol; the “high slope” neat solvents ( $\approx 0.87$  s-cP) were benzene, carbon tetrachloride, chloroform, dimethyl sulfoxide, and tetrachloroethylene. Gilpin and Gangoda stated that because of the magnitude of their slope values, the “high slope” solvents must interact with the bonded alkyl stationary phase ligands in such a way as to enhance overall chain motion. Similarly, they concluded that the “low slope” solvents caused a loss of overall chain mobility which took place “by a combination of poor solvating character and/or enhanced structuring ability of the contact liquids” [49]. However, since it is well known that acetonitrile, which is one of the “low slope” solvents in this study, solvates RP bonded phases very well, the latter explanation is the more plausible. They further suggest that the small value for the slope suggests a much greater degree of structuring near the silica surface due to strong solvent interactions with residual silanols [49]; however, since the  $T_1$  values that Gilpin and Gangoda measured are for the *terminal* alkyl group this interpretation seems a bit speculative. Although the NOE values that they measured for the terminal carbon position ( $\approx 2.48$ ) were less than the theoretical maximum value of 3.0, the differences in measured NOEs between solvents was statistically insignificant and there was no significant correlation between NOE and bulk solvent viscosity. Gilpin and Gangoda concluded that on an individual basis, changes in the type of solvent had no observable effect on the  $^{13}\text{C}$  NOE for the methyl group, and that the underlying cause of NOE values less than the theoretical maximum was a distribution of correlation times as a result of non-isotropic motion [49]. Although these experiments are important, extending them to binary hydro-organic mixtures commonly used as RPLC mobile phases is crucial for true relevance to reversed phase systems.

Gangoda and Gilpin [50] carried out wide-line  $^2\text{H}$  NMR  $T_1$  studies on a polymeric  $\text{C}_{12}$  phase ( $^2\text{H}$ -labelled in the terminal methylene position) which was placed in contact with a variety of solvents. The spectrum of the bonded phase in contact with methanol–water (60:40) had virtually the same line width and  $T_1$  as that of the dry

state material. When placed in contact with neat methanol or hexane, the line sharpened considerably (a bit more for hexane) and the  $T_1$  and line width values indicated a greater degree of motional averaging, *i.e.* an increased contribution from collective motions (and greater disorder) than for the other solvents. The spin–lattice relaxation rate,  $T_1^{-1}$ , was found to be linearly related to the square of the line width at half-height ( $\Delta w_{1/2}$ ), suggesting that the motional averaging at the deuterium-carbon bond comes about from a combination of fast and intermediate molecular motions, with the fast reorientations likely due to bond rotational isomerizations [50].

McNally and Rogers [51] studied the effect of solvent composition on  $\text{C}_8$  and  $\text{C}_{18}$  bonded phases with solution state  $^{13}\text{C}$  NMR by monitoring the  $^{13}\text{C}$   $\Delta w_{1/2}$ . For the  $\text{C}_8$  bonded phase, resonances were very broad in contact with all solvents used. In contact with an acetonitrile or dioxane binary mixture with water, the  $\Delta w_{1/2}$  of the bulk methylene ( $\text{C}_4$ – $\text{C}_{15}$ ) group of the  $\text{C}_{18}$  phases widened and its chemical shift moved slightly downfield with increasing mobile phase aqueous content. For 100% acetonitrile and for mixtures with  $\geq 50\%$  *p*-dioxane, a shoulder appeared at a slightly higher resonance on the bulk methylene peak; this is attributed to the methylene group  $\gamma$  to the terminal end. The shift of the bulk peak location, its increasing peak width and the merger of the shoulder with the bulk peak are all interpreted as supporting evidence of the loss of the liquid-like nature of the bonded phase chain with increasing water content. In contrast, the terminal methylene and methyl groups show little to no change with solvent composition until the mobile phase composition is almost entirely aqueous, indicating that the motion of even those groups at the terminus of the alkyl ligand is eventually restricted in the presence of pure water. In 100% dioxane, an additional resonance appeared at a chemical shift corresponding to that for the silyl methyl groups; since *p*-dioxane is less polar than acetonitrile, it allows the locations very near the attached end of the alkyl ligand to exhibit liquid-like behavior [51]. This is indicative of chain extension and the resultant decreasing steric

hindrance to motion near the silica surface; we have observed this in our laboratory (Fig. 2) for a high chain density monomeric  $C_{18}$  phase in contact with neat methanol [52].

Shah *et al.* [53] used NMR to compare monomeric and polymeric  $C_{18}$  stationary phases under both dry state and solution conditions. Solution state samples were prepared by suspending the bonded phases in chloroform, methanol or a 1:1 mixture of the two. For the monomeric phase, the peak shapes broadened and peak areas decreased in the order neat chloroform < chloroform–methanol < neat methanol. The effects were similar and much more pronounced for the polymeric phase, with all peaks almost lost in the baseline for 100% methanol. They attributed this to changes in chain orientation for the alkyl ligands. The dynamic model proposed for the polymeric phase was that the chains freely move and interact when chloroform is solvent, but molecular motion was more restricted as the methanol content increased. In 100% methanol, the loss of intensity for the terminal methylene and methyl peaks was thought to result from the alkyl chains lying flat on the surface of the silica. Because the changes were much less drastic under all conditions for resonances in the monomeric phase, they stated that the solvent composition had little effect on the monomeric bonded phase chain orientation [53].

Maciel *et al.* [54] have conducted comprehensive CP-MAS-NMR studies on the lineshape and relaxation parameters of “high-loading” ( $1.68 \mu\text{mol}/\text{m}^2$ ) and “low-loading” ( $0.441 \mu\text{mol}/\text{m}^2$ ) monomeric  $C_{18}$  modified silicas. The effects of alkyl chain surface loading in both the dry state and under “saturation” conditions with neat solvent vapors were compared.  $^{13}\text{C}$  Line widths for the dry state samples were smaller for the higher loading phase and for those phases that had been in contact with methanol; this was especially apparent for the silyl methyl groups. Their interpretation was that methanol either gives a more highly ordered structure to the octadecylsilyl (ODS) moiety, or enhances motion within the ODS moiety and thereby “averages out” some of the chemical shift dispersion of each carbon position [54]. They also noted two peaks in the  $^{13}\text{C}$  resonances for methanol.

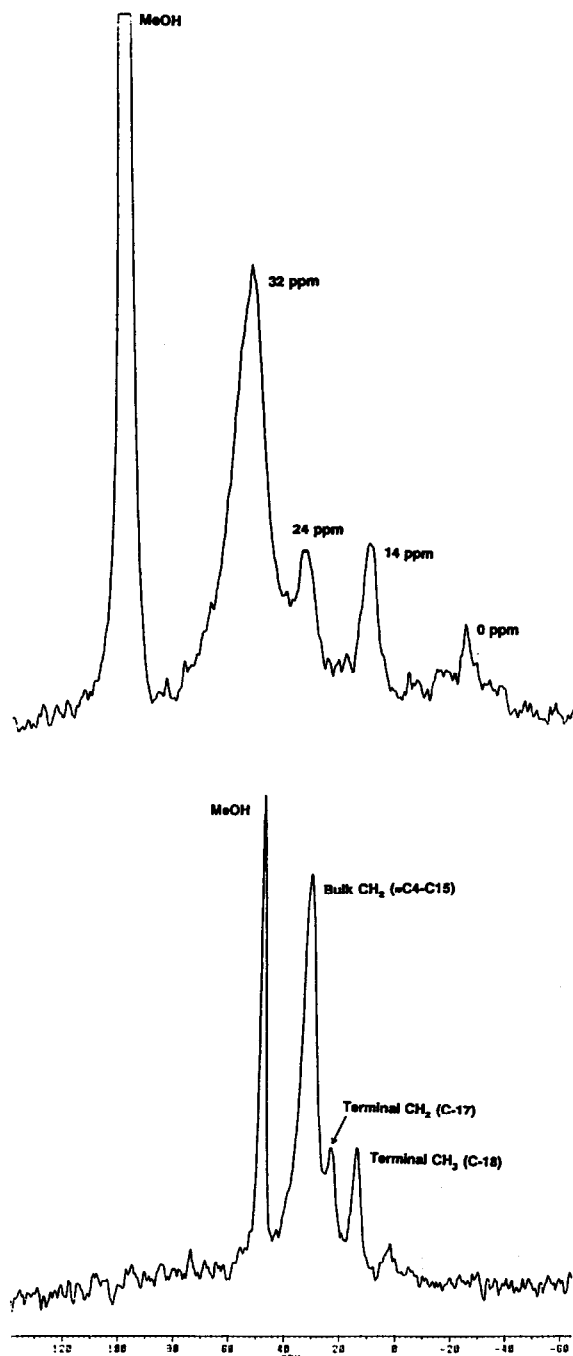


Fig. 2. Comparison of the solution state  $^{13}\text{C}$  NMR spectra of a high chain density monomeric  $C_{18}$  reversed-phase material (LT1) prepared in our laboratory in contact with 100% methanol (upper panel) and with methanol–water (80:20) (lower panel). Note the resonance for the silyl methyls (0 ppm) that is evident when in contact with 100% methanol; this resonance does not appear in the lower panel.

The sharp peak was assigned to physisorbed methanol and the broader peak to chemisorbed methanol. For  $^{13}\text{C}$  and  $^1\text{H}$ ,  $T_1$  values measured for all of the methanol-saturated samples were smaller than for the corresponding dry state samples; in the dry state the higher loading samples had smaller  $T_1$  values. Under these conditions, smaller  $T_1$  values indicate that spin-lattice relaxation is more efficient. Based on their results, Maciel *et al.* speculated about possible stationary phase models. They described the high-loading sample as resembling a “bristle brush”; the lower loading sample had less steric constraints, which could allow a more disordered “blanket” configuration. This allows two opposite scenarios to explain the effects of methanol addition: either strong interactions between the methanol hydroxyl groups and the silica could displace the Van der Waals interactions between the surface and the  $\text{C}_{18}$  groups, causing them to become more extended, or adsorption of methanol on the silica surface could reduce its hydrophobicity and thereby enhance the likelihood that the  $\text{C}_{18}$  chains would lie down on the methanol-covered surface [54].

In an attempt to ascertain the likelihood of these possibilities, Zeigler and Maciel [55] continued the above studies, using both single-pulse (SP) excitation measurements, commonly used for liquids, as well as CP techniques, commonly used for solids. SP and CP spectra exhibit different relative peak intensities for carbons in different portions of the alkylsilane chains. In the SP spectra, resonances near the free end of the  $\text{C}_{18}$  chain are more intense than those in the intermediate ( $\text{C}_4$ – $\text{C}_{15}$ ) region. In the CP spectra, the resonances near the bound end of the chain are more intense. This occurs because each of the two different excitation methods is more efficient for a different state of molecular motion. The SP pulse sequence serves to generate magnetization from spin-lattice relaxation and therefore depends on the presence of motional components at the Larmor frequency (50 MHz in this case). Therefore, this type of excitation is most efficient when vigorous molecular motions are present. CP excitation requires the presence of a static component of  $^1\text{H}$ – $^{13}\text{C}$  dipolar interaction, which can result from lack of motion

(which will *not* be the case for  $\text{C}_{18}$  alkyl ligands at room temperature) or from nonrandom motion, which is motion that is highly isotropic, yet does not result in effective motional averaging over the three orthogonal spatial dimensions. Since in every case the SP spectra give the same intensity patterns for the carbons at the free ends of the ligands and the CP spectra give the same intensity patterns near the bound end, in every case the bound ends of the silane chains have more static interaction than the distal ends [55].

In order for normal  $^1\text{H}$ -coupled  $^{13}\text{C}$  spectra to exhibit complete motional averaging of the  $^1\text{H}$ – $^{13}\text{C}$  dipolar coupling and its concomitant line narrowing effects, the frequency of random motion of the resonance of interest must be greater than  $\approx 23$  kHz [55]. Therefore, if a  $\text{CH}_x$  moiety exhibits sharp resonances in a  $^1\text{H}$ -decoupled CP-MAS spectrum *as well as* in the  $^1\text{H}$ -coupled spectrum, it must be moving rapidly (*i.e.* at a frequency  $\geq 23$  kHz) in all three spatial dimensions. For the high-loading  $\text{C}_{18}$  silica saturated with  $[\text{}^2\text{H}_3]\text{acetonitrile}$ , the resonances for  $\text{C}_4$ – $\text{C}_{15}$ ,  $\text{C}_{16}$ , and the unresolved  $\text{C}_2/\text{C}_{17}$  resonance remained narrow, indicating that the unbound ends are more mobile than the bound ends [55]. When the proton-coupled spectrum of the low-loading sample was run in the dry state, all peaks were broad; the analogous spectrum for the high loading sample showed sharp peaks for  $\text{C}_4$ – $\text{C}_{15}$ ,  $\text{C}_{16}$ , and  $\text{C}_{17}$ . The latter resonances for the low-loading sample sharpened when water,  $[\text{}^2\text{H}_3]\text{acetonitrile}$  or  $[\text{}^2\text{H}_6]\text{benzene}$  was present; the high-loading sample spectra showed no significant change and the polarity of the solvent appeared to have no effect on the degree of sharpening. Overall, this means that for the dry-state, low-loading sample, no significant portion of the  $\text{C}_{18}$  chain is randomly moving with a motional frequency of  $\geq 23$  kHz. For higher surface loading or in the presence of solvent, the unbound ends of the chains move sufficiently rapidly and randomly to average the  $^1\text{H}$ – $^{13}\text{C}$  dipolar interaction [55].

Zeigler and Maciel speculated further on a model to explain these observations. For the low-loading sample in the dry state, if a substantial portion of the chains interact with the surface, they could spend a relatively large portion

of time lying along it. Rather than being stationary, they would have constrained motion around a “mean silane binding site”. When solvents are added to the system, they may compete for surface binding, forcing the C<sub>18</sub> chains to spend a larger fraction of time moving in the space above the silica surface. This would cause more complete averaging of the <sup>1</sup>H–<sup>13</sup>C dipolar interaction, and thereby give rise to sharper peaks in the proton-coupled spectrum [55]. Increased loading has the same effect, because there would be less available silica surface per alkyl ligand for interaction. Since the addition of solvent did not change the high-loading sample spectrum, and solvent polarity did not affect the low-loading sample proton-coupled spectrum, it appeared that regardless of the type of solvent, once the chains are displaced from the surface, solvents have little effect on their configuration and dynamics [55]. However, this latter interpretation seems counterintuitive with respect to solvent type. For very highly loaded phases (alkyl chains densities  $\geq 3.0 \mu\text{mol}/\text{m}^2$ ), the case might be made that there would be little partitioning of any type of solvent into the chain interphase, due to interactions between neighboring chains that must be overcome in order for any solute to penetrate the chain structure [6]. However, at the surface loading levels of these phases (0.441 and  $1.68 \mu\text{mol}/\text{m}^2$ ), the rationale for the same types of chain configuration and dynamics in acetonitrile and benzene as in water is much less convincing, unless the manifestation of the same type of motional averaging in water as in the other two solvents is due to placement of the alkyl chains in close proximity to each other either by almost perpendicular extension (for the organic solvents) or by assuming a “collapsed” chain structure in aqueous media. The sometimes contradictory conclusions arising from these sorts of studies do not prove that they are unsuitable for studying alkyl chain motion in these systems, but they do demonstrate that the results are often difficult to interpret [55].

Zeigler and Maciel [56] have also studied the dynamics of monomeric octadecyl silicas via line shape analysis of <sup>2</sup>H-labelled stationary phases (labelled at positions 1, 9 and 10), both in the dry state and when saturated with neat solvent

vapors. Computer-generated spectral simulations used to deconvolute the motional effects on the experimental line shapes allowed motional models to be made. The C-1 position in the low-loading sample exhibited very restricted motion in the dry state. This constraint was either removed or lessened by addition of solvents or by increasing the C<sub>18</sub> loading level. They explain this as being due to interaction (presumably Van der Waals) between the C<sub>18</sub> chains and the silica surface. Increasing the loading level would displace the C<sub>18</sub> chains from the surface, thereby allowing the motion to become less constrained. Addition of solvent to the low-loading sample also appeared to displace the chains from the surface, either because of an interaction of the (non-polar) solvent molecules with the alkyl chains or replacement of the chains on the silica surface with the (polar) solvent. Simulations indicated that at room temperature the internuclear vector between the <sup>2</sup>H nucleus and its attached C is undergoing reorientation at rates near the upper limit of “intermediate motion” ( $10^6 \text{ s}^{-1}$  for these conditions). These simulations also indicated that the motional rate at the 9,10 position for the low-loading material is only about twice that for the C-1 position; the relative rates are the same upon addition of solvent, although they are twice as fast. Added solvents allow the alkyl chains to exist in a wide variety of configurations, with reorientation between configurations occurring within a very rapid motional regime (30 MHz or more) [56].

The high-loading sample was changed much less with the addition of liquids. Acetonitrile increased the motional rate of the 9,10 position slightly; water decreased the mobility, probably due to hydrophobic clustering. In the presence of neat cyclohexane, the C-9 and C-10 positions exhibited an unusually high degree of ordering and decreased mobility, which is likely due to cyclohexane association with the chains, decreasing the available free volume for motion. Under the same conditions, simulations for the C-1 position of the alkyl ligand indicated unusually rapid and random motion at the *anchored* end of the ligand. This suggests that the addition of cyclohexane to the high-loading phase allowed the chains to assume a well-ordered “brush”-

type configuration, reducing the orientational/motional constraints on the C-1 carbon that would exist when a larger fraction of the alkyl chains were in strained orientations near the silica surface; *i.e.* cyclohexane served as an “annealing agent” that alleviated some of the strain normally present in this position. The chains in the high-loading phase did not move as rapidly as those in the low-loading phase in the presence of solvents. The interpretation is that a variety of surface conformations are present, but very few of them have chain segments interacting with the silica surface to any significant degree. In the presence of water, the alkyl chains aggregate, causing their motion to be impeded by interactions with other nearby chains [56].

$T_{1\rho}$  values for various C and H positions indicated that the addition of acetonitrile increased chain motion of the high-loading sample. Zeigler and Maciel [57] hypothesized that this is because acetonitrile can interact with both the polar surface and the non-polar chains; under such conditions this solvent would increase the mobility of the  $C_{18}$  chains and make the stationary phase more accessible to solutes than either water or cyclohexane could. The measured line widths were also compared to the “natural” line widths predicted from the measured  $T_2$  (spin-spin relaxation time) values [57]. Since the actual values exceeded the expected ones by a factor  $\geq 2.8$ , an inhomogeneous line broadening effect is taking place. The most likely reason is chemical shift dispersion, which indicates that the carbon nuclei in the  $C_{18}$  chains are present in a variety of surface environments. This would indicate that the irregular surface topography of the silica probably forces the  $C_{18}$  chains into a variety of conformations and interactions with other components of the  $C_{18}$  silica system [57].

When solvents are added, the highest percentage of the more randomly moving chain components in the C-1 position were for the low-loading phase in contact with acetonitrile, followed by cyclohexane, water, and then the dry state [56]. For this position in the high-loading phase, only cyclohexane caused a significant ( $\approx 10\%$ ) contribution from the randomly moving component to be present. Motion in the C-1 position for the latter phase best fits a

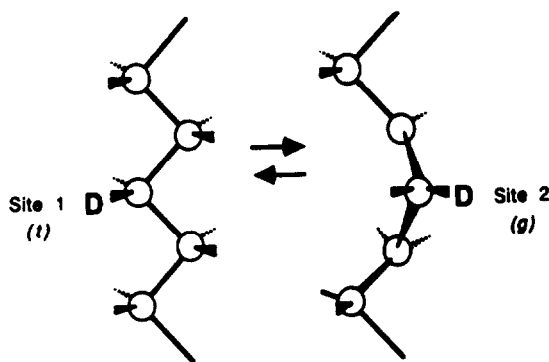


Fig. 3. Kink-3-bond motion implemented as the “two-site jump” computational model for bonded phase alkyl chain re-orientations between *trans* (*t*) and *gauche* (*g*) orientations. From ref. 56 (© 1991 American Chemical Society).

constrained two-site jump model (Fig. 3), regardless of solvent. For the C-9,10 positions, the low-loading phase exhibited essentially random motion in all solvents, with cyclohexane exhibiting the largest effect. For the high-loading phase, the addition of acetonitrile or cyclohexane did not affect significantly the dry state distribution of motion between the more constrained, two-site jump model and the random motion model;  $\approx 85\text{--}90\%$  of the motion was best modeled by the former. However, in cyclohexane the experimental line width for the random motion fraction was narrowed; this solvent is thought to decrease steric hindrance for the randomly moving portion of the central chain, but not to affect significantly chain segments moving with the two-site jump motion [56]. This indicates that even the non-polar cyclohexane solvent does not significantly penetrate the more densely packed portions of the alkyl chain. It may also be noted that when water was used as contact solvent, the fraction of chain sites moving with the two-site jump model in the high-loading phase *increased* and that for the random motion decreased [56]. This is likely a further indication of chain clustering in aqueous solvent media. However, the effects of contact solvent on chain structure and mobility interpreted from these studies must be applied to real RPLC systems with caution, since equilibration of hydrophobic bonded phase materials with saturated vapors of the neat solvents at ambient pressure



will be quite different from bonded phase solvation in condensed hydro-organic solvent mixtures under the flowing high-pressure conditions present in operating chromatographic systems.

Zeigler and Maciel [57] also made  $^{13}\text{C}$   $T_1$  measurements at a lower magnetic field strength (25 MHz), which were *ca.* two times smaller than those measured at 50 MHz. This indicated that the molecular motions are in a regime not much greater than 50 MHz, since  $T_1$  would be independent of the Larmor frequency if  $\tau_c$  is much greater than the Larmor frequency of the observed  $^{13}\text{C}$  nucleus. A comprehensive assessment indicated that the only  $^{13}\text{C}$  motional frequency regime in which all of the data were consistent was that for 50 MHz and slightly above, with a distribution of correlation times.  $^{13}\text{C}$  NOE values were also measured at both field strengths. They indicated that the alkyl ligand carbons move with an average correlation frequency value near 50 MHz but do not fit an NOE model based on isotropic motion [57].

The main obstacle to interpreting the  $^{13}\text{C}$ -based relaxation data quantitatively as a function of surface loading and solvent addition is that only small changes in the measured relaxation parameter (0–50%) occur as the sample preparation parameters are systematically varied, with changes in the former sometimes smaller than the estimated variance in the relaxation data. Therefore, conclusions drawn must be somewhat qualitative [57]. With the exception of the terminal position,  $T_1$  values for different chain locations did not vary by more than a factor of five for different portions of the chain; therefore motional rates between those types of chain segments also do not differ by more than a factor of five. For the methyl group, the very large  $T_1$  values result from chain reorientations and the rapid local rotations about the end bond at room temperature [57].

Zeigler and Maciel's experiments have made it evident that any model that can adequately reconcile experimental data and simulations will involve a distribution of motions in the bonded alkyl ligand, suggesting that these systems have very nonuniform structural and dynamical characteristics. This leads to their assessment that "Any realistic picture of the  $\text{C}_{18}$  silica

surface should most likely be a statistical model that encompasses a variety of surface structures, with the precise nature and population of each type dependent on the surface loading level and the presence or absence of added liquids" [56]. The general pattern from Zeigler and Maciel's line shape and relaxation studies is that the  $\text{C}_{18}$  surface is complex and heterogeneous and that there are no dramatic changes in the motional frequency spectrum for the various carbons in the system as the sample characteristics are varied. This indicates that the substantial differences in the proton-coupled  $^{13}\text{C}$  MAS spectrum for the dry low-loading silica and those for the other samples may be related to changes in the *mode* of motion, rather than the frequency spectrum of the motion. Changes in mode of motion could not be accounted for in all of the equations used in their work, because they are based on isotropic models of motion [57].

**3.1.3.2. Mobile phase studies.** In order to characterize the types and degrees of interactions between mobile phase components and the stationary phase, NMR techniques can also be applied to studying nuclei present in the mobile phase. To put this approach in perspective, it should be clarified that NMR measurements made on mobile phase components cannot generally be used to *quantitate* the *amount* of sorbed organic modifier in the stationary phase systems, but rather are used *qualitatively* to determine the *extent* to which mobile phase components interact with the stationary phase. For NMR  $T_1$  studies on any solute, it is expected that a distribution of relaxation times would result from the varying degrees of solute association with such a heteroenergetic surface as a silica-based RPLC stationary phase [58]. Additionally, extremely precise quantitative measurements via integration of NMR peak areas are very difficult (if not impossible) to make on these materials, due to non-Gaussian broadening of the solvent resonances.

The spin-lattice relaxation time for any quadrupolar nucleus is dominated by intermolecular effects; other relaxation mechanisms such as intramolecular dipole-dipole interactions and quenching by paramagnetic impurities compete poorly if at all [11]. Similar to  $^{13}\text{C}$ , as the

solution-state correlation time for a quadrupolar nucleus increases (*e.g.* molecular motion decreases), the longitudinal relaxation time,  $T_1$ , decreases. This can make  $T_1$  values for quadrupolar nuclei sensitive measures for monitoring the binding of the parent molecule to another species present, even if the amount bound is a relatively small fraction of the total species present [11]. In going from a three-dimensional bulk fluid to a two-dimensional surface, the  $T_1$  of the quadrupolar moiety of interest in the mobile phase is expected to decrease [11] and therefore the degree of reduction can be used to gauge the degree of association of the mobile phase component with the stationary phase. Although it may be expected that relaxation time measurements at the bonded phase–mobile phase interface might suffer from interface-induced inhomogeneities in the magnetic field, in practice  $T_1$  measurements are relatively insensitive to magnetic field inhomogeneities [59].

Because it is a quadrupolar nucleus that is readily available and therefore more tractable for NMR studies, deuterium ( $^2\text{H}$ ) is a popular nucleus for studying intermolecular interactions in chemical and biological systems. The first researchers to use  $^2\text{H}$   $T_1$  studies to examine stationary phase solvation from the perspective of the mobile phase were Marshall and McKenna [11]. They measured the solution-state longitudinal relaxation times ( $T_1$ ) of deuterium in  $^2\text{H}_2\text{O}$ –acetonitrile mixtures over a composition range of 0% to 50%  $^2\text{H}_2\text{O}$  as a function of the volume-to-volume (v/v) ratios of the binary solutions as well as for samples of the mobile phases combined with various chromatographic supports. By comparing the  $T_1$  in the solutions alone to those for the same solutions in contact with the stationary phases, Marshall and McKenna qualitatively gauged the degree of water association with the stationary phases. If the  $T_1$  values for a particular component in a binary solution are different than the  $T_1$  values for the solution component in neat form, this is indicative that in the binary solution, neat solvent interactions are disrupted by interactions with the second solvent. Reduction of  $^2\text{H}$   $T_1$  values of solution components when the solution is in contact with a stationary phase *versus* the  $T_1$  values in the mobile phase

solution alone indicate that there is reduction in the motional freedom of the  $^2\text{H}$ -containing molecules relative to the bulk solution due to association of the solution species with the stationary phase [11]. Marshall and McKenna found that the  $T_1$  values for the solvent mixtures decreased with increasing percent  $^2\text{H}_2\text{O}$  in the contact mixture and that the amount of water associated with the stationary phase seemed to decrease with *increasing* amounts of water in the mobile phase. In predominantly aqueous mobile phases, the hydrophobic alkyl stationary phase chains were thought to assume a highly collapsed or folded configuration in order to minimize their surface contact with the polar mobile phase. This self-association would cause some degree of expulsion of both organic co-solvent and water [11].

For the samples that they studied, the relative amount of water associated with both a monomeric  $\text{C}_1$  and a monomeric  $\text{C}_{18}$  bonded phase in acetonitrile–water systems was a function of the amount of water in the bulk mobile phase; *i.e.* the composition of the stationary phase solvation layer appeared to be a continuous function of mobile phase composition. They point out that numerous studies support the view that the bare silica surface, as well as the surface after derivatization, is heterogeneous. Since the heterogeneity of the derivatized surface is likely a function of native silanol diversity, it can therefore be assumed that any chemically modified silica surface is heterogeneous. “Such a surface will necessarily cause a heteroenergeticity in interaction with mobile phase and solute” [11]. The comprehensive explanation that Marshall and McKenna offer for stationary phase solvation layer formation is that variations in the mutual solubility of the alkyl-derivatized surface–organic co-solvent–water system may be an important factor in describing the relationship between capacity factor and percent water in the mobile phase [11]. Although their experiments are significant and interesting, they covered only a limited range of solvent compositions. Marshall and McKenna did not perform measurements at acetonitrile concentrations less than 50%, since uniform wetting of the stationary phase was not possible at the latter solution compositions when

the stationary and mobile phase mixtures were combined at atmospheric pressure. In addition to observing  $^2\text{H}_2\text{O}$  relaxation times, they also measured the  $T_1$  for  $[\text{}^2\text{H}_3]\text{acetonitrile}$  under a few of their experimental conditions [11].

Bliesner and Sentell [60,61] have recently expanded on Marshall and McKenna's work by measuring  $^2\text{H}$   $T_1$  for  $[\text{}^2\text{H}_4]\text{methanol}$ ,  $[\text{}^2\text{H}_3]\text{acetonitrile}$  and  $^2\text{H}_2\text{O}$  in binary aqueous mixtures and for those mixtures in contact with monomeric  $\text{C}_{18}$  bonded phases. Since the sample preparation method that they used equilibrates the stationary and mobile phases at pressures comparable to those attained under chromatographic operating conditions, their experiments much more nearly approximated wetting under real chromatographic conditions and thereby overcame the problem of wetting the stationary phase samples with highly aqueous mobile phases.

In Bliesner and Sentell's work, the  $^2\text{H}$   $T_1$  was first measured at 10% or smaller volume increments over the entire binary composition range for the methyl deuterons of  $[\text{}^2\text{H}_4]\text{methanol}$  and  $[\text{}^2\text{H}_3]\text{acetonitrile}$  and for  $^2\text{H}_2\text{O}$  in the binary solvent mixtures. If stationary phase is combined with the binary solutions at pressures comparable to those under chromatographic operating conditions, and the longitudinal relaxation times are re-measured, it becomes useful to compare the change in the  $^2\text{H}$   $T_1$  for the solvent component in contact with the stationary phase versus that observed in the solution alone. This comparison in effect normalizes the relaxation data for the former case, because bulk solution phenomena that will affect the observed  $T_1$  in both systems (e.g. intermolecular interactions between the aqueous and organic solution species) are accounted for by the bulk solution measurements. The observed change in the  $T_1$  of a solution component upon combining the bulk solution with the stationary phase is therefore the result of the effects of that component's interactions with the stationary phase. For a two-site rapid exchange model, the  $T_1$  values observed are a weighted average of those for free solution species and for those solution species associated with the stationary phase [11]. Therefore the magnitude of the decrease in the

$T_1$  of a solution component when the solution is placed in contact with stationary phase provides qualitative information about the relative fraction of that solution species associated with the stationary phase at the particular solution composition. Similarly, when all experimental variables except for the surface morphology of the stationary phase are held constant, comparison of solution component relaxation times for identical solutions in contact with different stationary phases is possible. A larger change in  $T_1$  of a solution species for one stationary phase system compared to that for another implies that there is a larger degree of association of that species with the former stationary phase.

Measurements of the change in the  $^2\text{H}$   $T_1$  (i.e.  $T_1$  for the solution minus  $T_1$  for the solution in contact with the bonded phase; hereafter referred to as  $\Delta T_1$ ) for each of the individual solution components as a function of binary solution composition confirmed that organic modifier interactions with monomeric  $\text{C}_{18}$  stationary phases are much more complex than  $^2\text{H}_2\text{O}$  interactions, and are distinctly different for methanol when compared to acetonitrile. For both a high ( $4.4 \mu\text{mol}/\text{m}^2$ ) and a low ( $1.4 \mu\text{mol}/\text{m}^2$ ) chain density  $\text{C}_{18}$  monomeric phase in contact with methanol–water mobile phases, the degree of water associated with the stationary phase was almost constant and was limited, yet the degree of methanol association was considerably greater and was a function of the amount of methanol present in the bulk mobile phase. In contact with either stationary phase,  $[\text{}^2\text{H}_4]\text{methanol}$   $\Delta T_1$  for bulk mobile phase compositions ranging from ca. 30% to 80% methanol was relatively constant, as well as less than  $\Delta T_1$  for any other range of bulk methanol–water compositions. This composition region corresponds to that in which water-methanol associated complexes predominate in Katz and co-workers' [62,63] proposed models of solution structure in methanol–water bulk mixtures. Hydrogen bonding interactions are stronger in water–methanol associated species than in self-associated methanol species, and the former solution interactions are stronger and more directed than the predominantly dispersive interactions between methanol and  $\text{C}_{18}$  stationary phase

ligands. Additionally, the extremely small  $\Delta T_1$  values for  $^2\text{H}_2\text{O}$  in this composition range indicated that accessibility to the support surface silanols is likely to be minimal [61]. Consequently, a limited and relatively constant degree of methanol association with the stationary phase was expected and observed over this mobile phase composition range.

For mobile phase compositions containing greater than 80% methanol, the methanol  $^2\text{H}$   $\Delta T_1$  increased with the volume percent of methanol in the bulk mobile phase, and the magnitude implied a significantly greater degree of methanol association with the stationary phase than was exhibited in the intermediate composition region. At nominal methanol volume fractions above *ca.* 0.8, the volume fraction of the less polar self-associated methanol species in bulk solution increases linearly, commensurately with the decrease in water–methanol associated complexes [62,63]. Since the self-associated methanol solution species present in this composition region are not hydrogen bonded with water, they are much more likely to experience dispersive interactions with the  $\text{C}_{18}$  chains of the stationary phase [64–66]. Uptake of methanol should enable the bonded alkyl chains to assume a more extended configuration [4,67] and thereby allow a greater degree of penetration of both mobile phase components with the stationary phase. However, the  $^2\text{H}_2\text{O}$   $\Delta T_1$  behavior indicated that the degree of  $^2\text{H}_2\text{O}$  association with the stationary phase was little changed over this composition region. This signifies that any stationary phase chain extension from methanol uptake is not so pronounced as to allow surface silanol groups to effectively compete for hydrogen bonding interactions with solution species. Therefore the much larger increase in [ $^2\text{H}_4$ ]methanol  $\Delta T_1$  in this range is more logically attributable to more effective dispersive interactions between the bonded phase alkyl ligands and the self-associated methanol species [61].

A marked increase in [ $^2\text{H}_4$ ]methanol  $\Delta T_1$  and a smaller increase in the  $^2\text{H}_2\text{O}$   $\Delta T_1$  are observed for mobile phase compositions with less than 30% methanol. Contact angle measurements have confirmed that pure water does not wet  $\text{C}_{18}$  bonded silica surfaces and that the addition of

20% methanol to pure water only brings about partial wetting [68]. In predominantly aqueous mobile phases, the hydrophobic alkyl stationary phase chains are thought to assume a highly collapsed or folded configuration in order to minimize their surface contact with the polar mobile phase [4,5,68]. This would cause mobile phase components to become entrapped within the collapsed stationary phase structure (most likely within narrow-necked silica pores) and thereby result in a distinct change in their  $T_1$ . Therefore it is demonstrated that when [ $^2\text{H}_4$ ]methanol and  $^2\text{H}_2\text{O}$  are placed in contact with monomeric  $\text{C}_{18}$  stationary phases, changes in  $T_1$  for both of these species are largely due to bulk solution microstructure and its resultant effects on bonded phase solvation and structure.

For acetonitrile–water contact solutions, the degree of association with both stationary phases was much less for water than for acetonitrile. However, the degree of water association with the stationary phase was a function of bulk mobile phase composition, whereas the degree of association of acetonitrile was relatively constant regardless of the mobile phase composition. This was opposite from the case with methanol–water mobile phases [61]. When acetonitrile is added to bulk aqueous solutions, it enters cavities in the well-defined water structure until these sites are occupied. As the amount of acetonitrile is further increased, it becomes increasingly self-associated in aggregates or loosely defined clusters [67,69]. Although this results in bulk solution microheterogeneity, the acetonitrile species experience a relatively homogeneous solution environment over a large binary composition range, due to their extensive self-association [62,69,70]. It is therefore quite reasonable that [ $^2\text{H}_3$ ]acetonitrile exhibits a relatively constant degree of association behavior ( $\Delta T_1$ ) with monomeric  $\text{C}_{18}$  stationary phases throughout the composition range.

The  $\Delta T_1$  behavior for  $^2\text{H}_2\text{O}$  in contact with both stationary phases changed rapidly in the solution composition region from 80–100% acetonitrile, and this cannot be attributed solely to solution properties in the absence of the stationary phase surface, since the physical properties of acetonitrile–water mixtures change slowly and

monotonically over the entire composition range [11]. Because the  $^2\text{H}$  chemical shifts observed in Marshall and McKenna's [11] as well as Bliesner and Sentell's [61] studies for  $^2\text{H}_2\text{O}$  in contact with the bonded phase materials were in all cases virtually identical to the corresponding chemical shifts in the mobile phase binary solutions, extreme changes in the chemical environment of the  $^2\text{H}_2\text{O}$  molecules, such as would be expected if the majority of them were directly hydrogen bonded to surface silanols, cannot be predominant [11]. The increased association of water with the  $\text{C}_{18}$  bonded phases in this composition region must therefore be due to a combination of hydrogen bonding of  $^2\text{H}_2\text{O}$  with residual silanols as well as the formation of water-acetonitrile mixed species complexes in the acetonitrile-rich alkyl chain environment [11]. At low acetonitrile concentrations, acetonitrile is likely entrapped within silica pores by a relatively collapsed chain structure [5], just as is the case for comparable methanol compositions. The  $^2\text{H}_2\text{O}$  results reported by Bliesner and Sentell [61] for mobile phases containing in excess of 50% (v/v) of acetonitrile were in excellent agreement with the findings of Marshall and McKenna [11].

Finally, the extent of association of mobile phase components with the two stationary phases in Bliesner and Sentell's studies can be compared for the organic modifiers *versus* the aqueous component [60,61]. There was more difference between the low and high chain density stationary phases in the degree of association for the organic modifier mobile phase components than for the aqueous component. This implies that the effects of chain density on the formation of the solvation layer in RPLC are more important for less polar mobile phase components such as methanol or acetonitrile than for water and further supports earlier chromatographic observations on the partitioning behavior of organic modifier components of the mobile phase within the RPLC stationary phase interphase [71]. Bliesner and Sentell's work reinforces the view that the association of mobile phase components with the stationary phase that causes the formation of a solvation layer in RPLC is a complex phenomenon that is largely determined

by the competition between the relative strengths of solvent-solvent and solvent-stationary phase interactions. This is illustrated by the distinct correlations between bulk solution structure and stationary phase solvation by individual solution components [60,61].

Ellison and Marshall [58] have also made  $^2\text{H}$  and  $^{14}\text{N}$   $T_1$  measurements on  $[^2\text{H}_3]\text{acetonitrile}$  in contact with monomeric  $\text{C}_{18}$  phases as well as with bare silica to determine the surface fluidity in RPLC systems. The overall intent of their studies was to obtain a better understanding of the relative roles of surface *versus* bulk diffusion in the overall transport of species in HPLC separations. Although spin-lattice relaxation for both  $^2\text{H}$  and  $^{14}\text{N}$  are dominated by the quadrupolar relaxation mechanism, that for  $^2\text{H}$  in  $[^2\text{H}_3]\text{acetonitrile}$  is modulated by rotations with axes both parallel and symmetric to the principal symmetry axis, meaning that the  $^2\text{H}$  correlation time does not represent any particular mode of rotation. In contrast,  $^{14}\text{N}$  relaxation in  $[^2\text{H}_3]\text{acetonitrile}$  is modulated by a single mode of rotation (reorientation of the principal symmetry axis) and therefore its  $T_1$  is indicative of only this mode of motion. Because of this, only the  $^{14}\text{N}$  reorientational correlation time is required for determining the perpendicular diffusion constant describing the frequency of reorientation of the principal symmetry axis. The correlation times for both  $^2\text{H}$  and  $^{14}\text{N}$  are required for determining the analogous parallel diffusion constant [58].

Ellison and Marshall examined the surface fluidity of ODS and silica phases by comparing the correlation times of the probe nuclei in the bulk and surface phase. The  $T_1$  values of the surface-bound nuclei were determined using a two-phase rapid-exchange model (weighted average of surface-bound and bulk relaxation rates) and the measured  $T_1$  values of the nuclei in suspensions of  $[^2\text{H}_3]\text{acetonitrile}$  and the bonded phase [58]. In order to determine the weighting factor for the surface-bound species, the silica-to-solvent mass ratio was determined via gravimetric and chromatographic displacement methods and used to calculate the mole fraction of the acetonitrile in the ODS phase. However, the mass of  $\text{C}^2\text{H}_3\text{CN}$  associated per unit weight

of bonded phase is very prone to error in measurement, since it is calculated from the volume of  $C^2H_3CN$  sorbed onto the stationary phase, which is obtained experimentally by subtracting the column void volume,  $V_0$ , and  $V_{extracolumn}$  from  $V_{total}$ . The uncertainties associated with the determination of the former volumes, particularly  $V_0$ , will propagate a large degree of uncertainty in the volume of sorbed  $C^2H_3CN$  thus obtained. Another assumption made in this work was that theories of bulk relaxation could be applied to bound species (*i.e.* that the model for quadrupolar relaxation is unaffected by dimensionality or geometric consideration). Ellison and Marshall feel that this is reasonable, given the high degree of fluidity of the interphase (which they describe as “pseudo-3-D”) and the non-directed dispersive interactions in the ODS chains; the relaxation rates should in this case be affected by intermolecular interactions only [58]. If these assumptions hold, the ratio of the bulk and surface relaxation rates is proportional to the viscosity ratio, and the surface viscosity of the acetonitrile in the stationary phase can be calculated from the above and the viscosity of the bulk acetonitrile.

From their  $^{14}N$   $T_1$  experiments, Ellison and Marshall predicted that the surface viscosity of the  $C^2H_3CN$  in contact with an ODS phase was 13 times that of bulk  $C^2H_3CN$ ; for contact with bare silica the surface viscosity was 30 times that of the bulk solvent. The increased viscosity of the surface bound solvent was shown to be attributable to the molecular interactions in the *hydrocarbon* portion of the ODS surface. The stronger and more directed dipole–dipole or hydrogen-bonding interactions that would take place on the silica surface would be expected to result in a greater degree of restriction of rotational motion than dispersive interactions with the ODS phase would cause. When the  $^2H$   $T_1$  data was examined, the surface viscosities were 2.5 times smaller than those from the  $^{14}N$  data. This was explained as being due to a lower bulk phase activation energy for  $^2H$  relaxation; it also indicated that parallel reorientations of the  $C^2H_3CN$  were less affected by the surface than perpendicular diffusional reorientations [58]. The predicted surface viscosity of the ODS indicated that the surface environment is rela-

tively fluidlike, with significantly higher fluidity than reported from fluorescence studies using a surface-sorbed pyrene probe [72,73]. The drawback to this type of study is that the data rely on not only the choice of convention for *defining* the void volume and the volume of sorbed mobile phase components but also on their accurate measurement. However, such studies are extremely useful in following trends in delineating the diffusional aspects of solvation layer formation.

#### 3.1.4. Temperature effects on RPLC bonded phase structure

Kelusky and Fyfe [74] have used solid-state wide-line  $^2H$  NMR to probe molecular motion in perdeuterated dimethylalkoxysilanes ranging from one to sixteen carbons in length which were chemically attached to silica. All of the spectra that they observed were narrowed from the rigid limit due to partial motional averaging of the quadrupole interaction, but the amount of motional averaging varied with the sample. For the methoxy-, ethoxy- and isopropoxysilicas, cooling to temperatures below 150 K gave the eventual appearance of a rigid line shape. At higher temperatures, these materials generated quadrupole splitting patterns that would be expected for methylene and for a rapidly rotating methyl group. The  $C_8$  and  $C_{16}$  alkoxy groups exhibited motionally narrowed line shapes down to 120 K. The resonances for these groups manifested the greatest degree of narrowing, and therefore the most motion, of all of the groups studied, although the motions are not well defined. Their line shapes also showed contributions from a broad and a narrow component. From the relative intensities of the two components, the narrow line with the longer  $T_1$  was assigned to the methyl group, whereas the broader line with the shorter  $T_1$  was thought to arise from the methylene groups [74].

Adding solvents made substantial changes in the line shapes. The addition of methanol added a narrow line superimposed on a line shape similar to that seen for the solid; the narrow line made a substantial contribution to the amount of total spectral intensity. This suggests that the solvent “solubilized” only a portion of the sur-

face silane species present. The remainder of the species was left unchanged, with the same line shape as exhibited in the absence of solvent. The narrow line superimposed on the solid-state resonance that was noted when methanol was added, and the fact that the effect of methanol was much more pronounced than for hexane or benzene, even for the long-chain silanes, suggested that the surface of the modified silicas was still fairly polar [74]. This may be due to the use of perdeuterated *alkoxysilanes* in synthesizing the modified silicas, resulting in a more polar ligand than found in typical RPLC bonded phases. For hexane, the presence of substantial motional narrowing at temperatures (178 K) well below the freezing point of the solvent suggested that there is only limited penetration of the solvent molecules into the surface-immobilized species [74].

The temperature dependence of  $^{13}\text{C}$   $T_1$  for labelled monomeric and polymeric bonded phases over the range of *ca.* 298–373 K (*ca.* 25–100°C) was examined by Gangoda *et al.* [75]. Arrhenius plots were made in order to find the activation energy ( $E_a$ ) under both dry and solvated conditions. The dry state activation energies were equivalent (within experimental error) for all labelled chain positions and were similar to those that had been measured by others for *trans-gauche* conformational changes in *n*-alkane systems [75]. Activation energies in the solvated state were significantly larger, with ethylene glycol (the more structuring solvent) exhibiting a greater effect than carbon tetrachloride. This was explained as being due to enhanced chain-solvent structuring (ordering) near the stationary phase surface, resulting in a loss of freedom of the immobilized groups. For the methyl group,  $E_a$  increases were relatively less than for adjacent methylene groups, due to gradients in motional freedom [75].

Zeigler and Maciel [56] have examined the temperature dependence of  $^2\text{H}$  line shapes in labelled  $\text{C}_{18}$  monomeric bonded phases in order to investigate the nature of their alkyl chain motions. At  $-125^\circ\text{C}$ , both the high- ( $1.68 \mu\text{mol}/\text{m}^2$ ) and low-loading ( $0.441 \mu\text{mol}/\text{m}^2$ ) silicas exhibited nearly static  $^2\text{H}$  line shapes. When temperature was increased (to  $-100^\circ\text{C}$  for low-loading;  $-75^\circ\text{C}$  for high-loading), the peak shape

corresponded to motional behavior of a two-site jump through an angle of  $109.5^\circ$  (Fig. 3), with unequal populations of the two jump sites. This indicated a *trans-gauche* reorientation of the  $^2\text{H}$ -C pair, with one orientation (*trans*, which is also more highly populated) more favored because of steric interactions. As temperature was further increased ( $-50$  to  $25^\circ\text{C}$ ), the resonances became a combination of the latter shape and a rounded triangular shape; with further temperature increase the triangular shape became dominant. The triangular line shape will only come about if the alkyl chains move with an inhomogeneous *distribution of geometries*. Zeigler and Maciel believe that this is due to silica surface structural environments that have varying degrees of *trans* and *gauche* contributions to the mean chain orientations, due to there being different degrees of chain crowding in these environments; *i.e.* inhomogeneity in the alkyl ligand distribution, and this must be factored into any accurate alkyl chain reorientational model [56]. As can be seen from Fig. 4, alkyl chain rotation jumps in an environment with more *gauche* bonds (more “bent”) will “sweep out” a substantially larger motional volume than in an environment with a higher degree of *trans* bonds.  $\text{C}_{18}$  chains in the more sterically crowded environments are likely to move in the more restricted two-site motion; those in environments with greater accessible volume undergo motion characterized by the alkyl chain rotational jump model that is more favored when a larger proportion of *gauche* configurations is present. The simulations for the low- and high-loading phases indicate that both types of materials have identical *types* of motion, but that the low-loading phase has a higher percentage of the more randomly moving chains at all temperatures [56].

Sander *et al.* [76] have made FT-IR measurements of dry-state monomeric bonded alkyl ligand conformations as a function of temperature. They found that  $\text{C}_4$ ,  $\text{C}_8$ ,  $\text{C}_{12}$ ,  $\text{C}_{18}$  and  $\text{C}_{22}$  alkyl ligands all had a large population of conformers with kink defects, with disorder comparable to that in a liquid. For the  $\text{C}_{18}$  bonded phase at  $44^\circ\text{C}$ , there was a considerable reduction in the number of kink defects per chain compared to the pure liquid alkane, although the former still contained several *gauche* bonds per

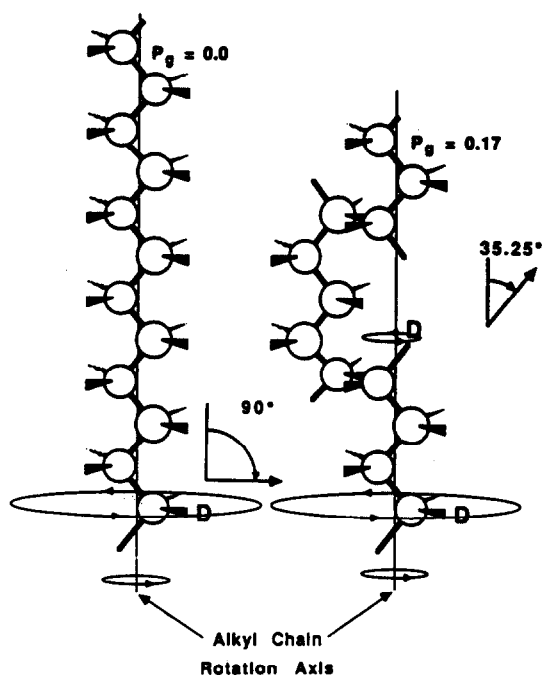


Fig. 4. Depiction of differences in chain motion for the alkyl chain rotational jump model for alkyl ligands with all-*trans* conformations ( $P_g = 0$ ) and with *gauche* conformers ( $P_g > 0$ ). From ref. 56 (© 1991 American Chemical Society).

chain [76]. Measurements on the native silane and the corresponding alkane were taken at  $10^\circ\text{C}$ , which is below their freezing point, and provided spectral evidence of a phase transition. However, the bonded phase spectra were virtually unchanged. This indicates that the population of the various rotational isomers of the anchored alkyl chains is not strongly affected at this temperature. At  $-30^\circ\text{C}$ , chain ordering was exhibited by the  $\text{C}_{18}$  bonded phase, yet there were still symptoms of chain kink disorders. For both  $\text{C}_{18}$  and  $\text{C}_{22}$  dry-state bonded-phase materials, no FT-IR evidence of a phase transition was found [76]. If methanol-water solutions ranging from 100 to 70% methanol were added to the  $\text{C}_{18}$  silicas at room temperature, a marked degree of chain straightening resulted, with the changes in chain conformation comparable for all of the solvent compositions. Sander *et al.* attributed the latter effect to intercalation of methanol between the alkyl ligands, with the

packing of mobile phase molecules between the alkyl chains favoring increased chain ordering [76]. It would have been very interesting to extend their temperature-dependent FT-IR measurements to these wetted samples; however, no such work was reported. As will be discussed below, the combination of reduced temperature, high alkyl chain densities and structuring from contact with an alcohol-water mobile phase can bring about a much more profound effect on chain ordering than any of these effects alone.

Albert *et al.* [77] have made dry state  $^{13}\text{C}$  CP-MAS measurements which indicated that both temperature and chain density have a marked effect on the distribution of *trans* and *gauche* bonds in monomeric  $\text{C}_{18}$  ligands. The mobility of the methyl chain segment is especially sensitive to changes in the alkyl chain conformation. For phases with alkyl chain surface densities  $\geq 2.9 \mu\text{mol}/\text{m}^2$ , there were two resonances for the methyl group, which is evidence of two populations with different mobilities. From molecular modeling studies, the more rigid population was thought to exhibit an all-*trans* conformation in the  $\text{C}_1$ - $\text{C}_4$  segment of the  $\text{C}_{18}$  ligand; the more flexible chains had a higher population of *gauche* bonds. The population of the all-*trans* conformation increased with decreasing temperature, and when the temperature was lowered to  $-10^\circ\text{C}$ , the resonance for the more rigid domain became apparent for even a lower chain density ( $2.4 \mu\text{mol}/\text{m}^2$ ) phase. This was interpreted as direct proof for the interconversion of more mobile alkyl ligands with a higher population of *gauche* conformers to ordered alkyl ligands with all-*trans* conformations [77].

Because of the important effects of mobile phase solvation on bonded phase ordering, solution-state  $^{13}\text{C}$   $T_1$  measurements are essential for studying the effect of temperature on the structure and conformation of solvated RPLC bonded phase ligands. Overall ligand mobility can be examined, since in the solution state, decreasing  $T_1$  implies decreasing molecular motion. More importantly, the temperature dependence of  $T_1$  for spin-1/2 nuclei such as  $^{13}\text{C}$  is indicative of the primary mechanism for nuclear



relaxation, and thereby gives more specific information about molecular motion. Dipole–dipole and spin-rotation interactions are the primary relaxation mechanisms for  $^{13}\text{C}$ , with the former greatly predominating in most cases [30]. Typical liquid-state organic molecules relax via the dipolar mechanism and in this case if  $T_1$  is plotted *versus* inverse temperature ( $1/T$ ), a linear relationship with a negative slope is obtained [78]. Although it is generally a very secondary relaxation mechanism for spin-1/2 nuclei, in certain small top symmetrical molecules or rapidly rotating functional groups, spin-rotation can make a significant contribution to relaxation [78–80]. For example, a number of previous NMR studies have indicated that motion of the methyl group of the alkyl ligands in RPLC bonded phases is dominated by rotation about the end bond [29,44–46,48,57,74]. When spin-rotation is the dominant relaxation mechanism, a plot of  $T_1$  *versus*  $1/T$  yields a positive slope. Gillen *et al.* [78] have demonstrated that for methyl iodide at reduced temperatures, dipole–dipole interactions dominate the relaxation of the methyl group, resulting in the typical negative slope observed in a  $T_1$  *vs.* inverse temperature plot. However, the contribution of spin-rotation to relaxation of the rapidly rotating methyl carbon is larger at higher temperatures, producing a slightly positive slope in this portion of the observed  $T_1$  *vs.*  $1/T$  plot. Therefore, although it is normally regarded as an insignificant contribution to nuclear relaxation for typical liquid-state organic molecules, in some systems spin-rotation becomes an influential contribution to the overall relaxation mechanism as temperature increases; at high enough temperatures it can dominate the relaxation process [78]. In a chemical system such as an anchored alkyl ligand wherein there is a possibility of contribution to relaxation from both of these mechanisms for the chain segments at the free end of the ligand, monitoring the temperature dependence of the slope of the  $^{13}\text{C}$   $T_1$  *vs.*  $1/T$  plot for various carbons along the bonded alkyl ligand in the stationary phase should make it possible to observe abrupt changes in the rotational freedom of the end methyl groups as a function of temperature. Such changes would indicate that a

drastic change in stationary phase structure (*e.g.* a phase transition) may be occurring.

Sentell and Henderson [8] had observed distinct curvature between 25 and 30°C in Van 't Hoff plots of polycyclic aromatic hydrocarbon and polyarene isomers for a high alkyl chain density ( $4.4 \mu\text{mol}/\text{m}^2$ ) monomeric  $\text{C}_{18}$  stationary phase with a methanol–water (80:20) mobile phase, as shown for pyrene in Fig. 5a. Fig. 5b shows that chromatographic selectivity for linear and/or planar *vs.* bent and/or non-planar solute isomers also increased dramatically on this stationary–mobile phase system at temperatures below 30°C [8]. One explanation for these observations was a “phase transition” of the

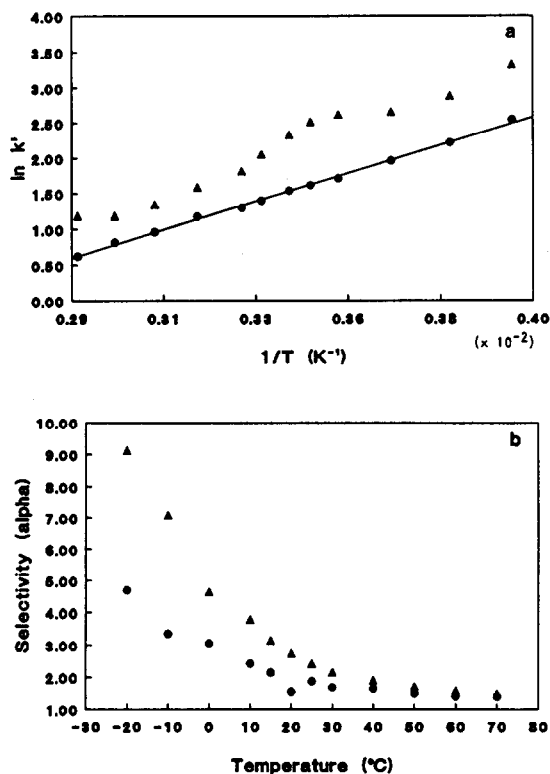


Fig. 5. (a) Van 't Hoff plots for pyrene with a high chain density [ $4.4 \mu\text{mol}/\text{m}^2$ ] ( $\blacktriangle$ ) and a low chain density [ $1.5 \mu\text{mol}/\text{m}^2$ ] ( $\bullet$ ) monomeric  $\text{C}_{18}$  stationary phase and methanol–water (80:20) mobile phase. Note the curvature in the plot for the high density phase. (b) Selectivity plots for *p*-terphenyl *vs.* *o*-terphenyl for a high chain density [ $4.4 \mu\text{mol}/\text{m}^2$ ] ( $\blacktriangle$ ) and a low chain density [ $1.5 \mu\text{mol}/\text{m}^2$ ] ( $\bullet$ ) monomeric  $\text{C}_{18}$  stationary phase and methanol–water (80:20) mobile phase. From ref. 8.

bonded octadecyl chains from an isotropic state with a large population of *gauche* conformers at higher temperatures to a more ordered and extended liquid-crystalline-like state with a larger population of *trans* conformers at lower temperatures.

Bliesner *et al.* [52,81] therefore made  $^{13}\text{C}$   $T_1$  solution-state measurements on this stationary phase (slurried with the same mobile phase) over the same temperature range as the chromatographic experiments in order to study the motional effects of temperature for different segmental regions of the alkyl ligand. To serve as a basis for comparison for the bonded phase results,  $T_1$  experiments first were carried out from 5–70°C on neat dimethyloctadecylchlorosilane. For the neat silane, a plot of  $T_1$  versus inverse temperature exhibited a negative slope for all four of the carbon resonances present in the spectrum ( $\text{C}_{4-15}$ ,  $\text{C}_{16}$ ,  $\text{C}_{17}$  and  $\text{C}_{18}$ ; Fig. 6a), even though the material exists as a solid at temperatures below 27°C and as a liquid at temperatures above [81]. In contrast, as shown in Fig. 6b, the  $^{13}\text{C}$   $T_1$  measurements over the same temperature range for the high chain density stationary phase slurried in methanol–water (80:20) provided evidence of a distinct change in ordering in this material. When  $T_1$  for the bulk chain ( $\text{C}_4\text{--}\text{C}_{15}$ ) carbons was plotted versus inverse temperature, the small negative slope was in agreement with that expected for a dipolar relaxation mechanism [52,81]. However,  $T_1$  vs.  $1000/T$  plots for both the terminal methylene ( $\text{C}_{17}$ ) and methyl ( $\text{C}_{18}$ ) carbons exhibited distinct curvature and an accompanying change in slope at *ca.* 35°C, which indicated that although spin-rotation is the predominant relaxation mechanism for  $\text{C}_{17}$  and  $\text{C}_{18}$  at temperatures greater than 35°C, dipolar relaxation is dominant at lower temperatures. This suggests that the spin-rotational component of relaxation for the terminal methylene and methyl carbons is becoming hindered at temperatures less than 35°C due to a distinct increase in ordering of the bonded phase chains [*i.e.* a phase transition] which restricts rotational mobility [52,81]. As Albert *et al.* [77] have described, this can be attributed to a large increase in the population of *trans* conformations with decreasing temperature. These data are a further dem-

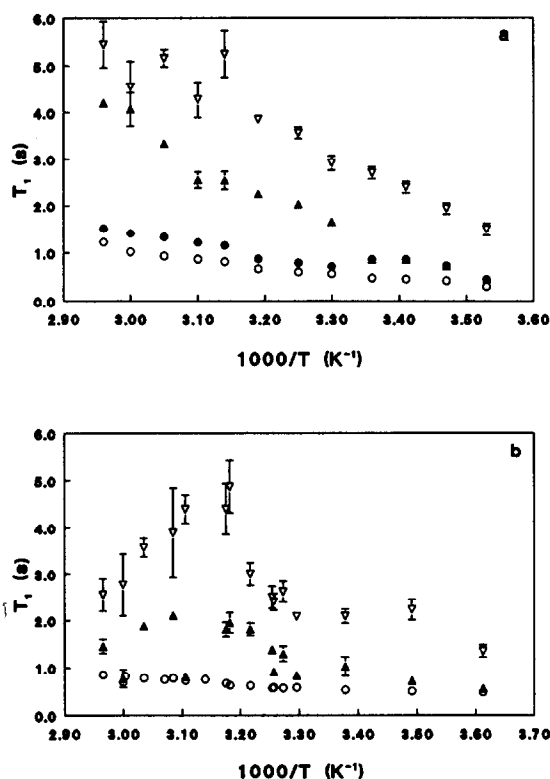


Fig. 6. Plot of  $^{13}\text{C}$  spin-lattice relaxation time ( $T_1$ ) versus inverse temperature for  $^{13}\text{C}$  resonances corresponding to alkyl ligand chain positions in (a) neat dimethyloctadecylchlorosilane and (b) a high chain density [ $4.4 \mu\text{mol}/\text{m}^2$ ] monomeric  $\text{C}_{18}$  stationary phase (LT1) in contact with methanol–water (80:20) mobile phase. The chain segment positions plotted are  $\text{C}_4\text{--}\text{C}_{15}$  (○),  $\text{C}_{16}$  (●),  $\text{C}_{17}$  (▲) and  $\text{C}_{18}$  (▽).

onstration of the excellent utility of NMR measurements for providing corroborating information about stationary phase structure that is both independent from and complimentary to chromatographic measurements.

### 3.2. Electron spin resonance spectroscopy

Although it is a powerful spectroscopic technique for studying chemical environments, ESR spectroscopy (also known as electron paramagnetic resonance spectroscopy or EPR) has been applied only sparingly to studying chromatographic systems [82]. The  $^{14}\text{N}$  splitting constants of nitroxide free radicals are affected by both solvent polarity and the extent of hydrogen

bonding. The coupling constant measures the interaction between the nitrogen nuclear spin and the unpaired electron in the NO bond and is sensitive to the polarity of the nitroxide's environment [83]. Monitoring changes in the coupling constant as a function of bulk mobile phase composition allows exploration of the microscopic solvation composition of the bonded phase system [82]. ESR has two important advantages for the study of RPLC systems. Because of its sensitivity, very small amounts of the label or probe can be used in the experimental system, bringing about minimal perturbation. Also, the experiments can be carried out in flowing, pressurized systems which much more accurately reflect actual chromatographic operating conditions than most spectroscopic measurements [82].

There are two main types of ESR experiments that can be carried out on RPLC systems. In the spin-probe experiment, a paramagnetic species of relatively small size is introduced into the chromatographic system, often as a dilute solute in the mobile phase [82]. When the ESR spectrum is acquired, information about motional domains of the probe's environment(s) is obtained from line widths and spectral shape. For example, rotational effects on the probe by the host environment give information about the ordering in that environment [83]. For spin-label studies, a small percentage of the stationary phase ligand moieties have a paramagnetic center chemically bonded to the units. In this situation, the spectroscopic information acquired about the label is taken to be representative of the motional characteristics of the unlabelled ligand species as well [82]; these techniques are particularly useful for monitoring rotational mobility [84]. A serious drawback to ESR experiments, especially those of the spin-label variety, is that the labelled units (typically nitroxides) are quite polar with respect to typical reversed-phase alkyl ligands [84]. Therefore the labels will interact to a greater extent with polar species in the stationary and mobile phases. Also, the *types* of specific interactions that the labels undergo are much different than those which alkyl ligands undergo, which are generally non-specific in nature. Another disadvantage is

that both the spin labels and probes are generally large molecular species which can cause local perturbations in the stationary phase environment. Therefore, extension of the interpretations obtained from spin-label ESR experiments to typical RPLC bonded phases must be done with great care.

Gilpin *et al.* [85] used static ESR experiments with a spin label directly attached to silica to demonstrate that the effect of solvation on the label's surface conformation could be monitored via the spectral line shapes and coupling constants. Malcom *et al.* [84] carried out the first static spin-label studies on RPLC-like stationary phase materials that were prepared via a monomeric-type synthesis with C<sub>6</sub> and C<sub>11</sub> alkyl spacers between the silica and the label. In the dry state, the motion of the label in the bonded phase materials was severely hindered. Upon addition of neat benzene or methanol, the motional mobility of the label increased by two orders of magnitude. The other change in the spectra was the presence of two motional domains for labelled sites [84]. The cause of these two domains is the presence of mobile ligands which are able to bend toward the silica surface, enabling the label to hydrogen bond with residual silanols. This was attributed to heterogeneity in the surface derivatization of the silica (*i.e.* alkyl ligand "patches" on the silica surface). As would be expected, the longer chain length C<sub>11</sub> label was better able to interact with silanols than the C<sub>6</sub> label. In methanol, the C<sub>6</sub> probe exhibited very little interaction with the silanols, since methanol can very effectively compete for surface hydrogen bonding sites; the C<sub>11</sub> label exhibited less interaction with the silanols in methanol than in benzene [84].

Miller *et al.* [82] next performed ESR studies under dynamic (flowing) conditions. For the C<sub>6</sub>-labelled silica, the changes in the spectral anisotropy with the introduction of water to the system (*e.g.* a change from 100% methanol to methanol–water, 50:50) indicated that the rigidity of the system had increased. They also studied the effect of mobile phase flow by monitoring the amplitude of the central line of the nitroxide label as a function of time. It increased under constant flow until a steady state amplitude was

reached, and then slowly declined after flow was terminated. This indicated that the labelled environment was undergoing a reorientation which was dependent upon pumping methanol through the system [82]. It was shown experimentally that pressure alone was not the cause of this. Possible explanations advanced were differences in wetting under pressure (improbable for pure methanol, which is expected to be a fairly effective wetting solvent) or a buildup of charge on the surface causing a reorientation of the alkyl chains, such as seen in liquid crystals. A “streaming potential” can be caused by flowing streams of non-aqueous solvents in contact with non-metallic surfaces. In a system with plastic and glass fittings, such as was used by Miller *et al.*, dissipation of such a charge would be slow [82]. This is supported by fact that for the methanol–water (50:50) mobile phase, the increase in amplitude was smaller than for the pure methanol contact solvent.

Spin-probe studies were also carried out using mobile phases containing  $10^{-4}$  M concentrations of probes in the 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) family which were pumped through a highly loaded polymeric  $C_{18}$  phase [82]. The amine-containing TEMPO compound showed the least mobility, particularly with increasing water content in the mobile phase, and showed evidence of being sorbed at two different types of sites. This was attributed to distribution in two regions where a large degree of hydrogen bonding can occur, such as the mobile–stationary phase interface and the surface silanols [82]. The immobility of the probe indicated at least some degree of interaction with the latter type of site. TEMPO was the most non-polar probe, and for hydro–organic mobile phases containing 25, 50, 75 or 100% methanol its coupling constant was unchanged, yet different from that obtained for the probe in a pure hydrocarbon (octane) environment. Miller *et al.* concluded that this was because the stationary phase became well solvated and maintained a constant water content in mobile phases with  $\geq 25\%$  methanol. They also stated that from the magnitude of the coupling constant, the solvation environment of the probe is the same as that for methanol, and therefore the probe resides in

“pools” of methanol imbedded in the stationary phase [82]. By correlating the coupling constants for the other probes in contact with the stationary phase at particular mobile phase compositions with the coupling constants for the probes in the mobile phase solutions, they determined the “effective concentration of methanol in the zone where the probe is sorbed” [82]. These were 90% methanol for TEMPO and 60% methanol for the amine-modified and alcohol-modified TEMPO probes [82]. Unfortunately, they made measurements for only four mobile phase compositions, none of them containing less than 25% methanol, so a great degree of extrapolation must be carried out to arrive at these “effective concentration” values.

Miller *et al.* [86] performed more extensive ESR spin-probe experiments in an attempt to determine the types of solvation regions present in monomeric and polymeric  $C_{18}$  stationary phases in contact with methanol–water mobile phases. Probes in the TEMPO family (TEMPO as well as derivatives with an amine, alcohol or keto group) were pumped through the bonded phases at concentrations ranging from  $10^{-4}$ – $10^{-5}$  M and the ESR spectra were acquired. Since these probes differ significantly in polarity, they were assumed to intercalate into the stationary phase in different regions of the interphase, and therefore serve as probes of the polarity in these regions. Miller *et al.* concluded that for any given mobile phase composition, there were at least four diverse environments which differed in their local aqueous content. Region I, which is where polar solutes resided when the methanol content of the bulk mobile phase was  $\geq 50\%$ , was located at the interface between the bonded alkyl ligands and bulk mobile phase. The region *near* the silica surface (II) was where moderately polar solutes intercalated when the bulk mobile phase aqueous content was 50% or higher; it had aqueous character and a high microviscosity. Region III was located *at* the silica surface, where there is surface-bound water and silanols; hydrogen-bonding solutes interacted with this region when the water content of the bulk mobile phase was  $\geq 50\%$ . The interior of the hydrated chains (region IV) was where non-polar solutes were intercalated, with a solvation environment

characteristic of the organic modifier regardless of the bulk mobile phase composition. Line width measurements also indicated that upon going from 100% methanol to methanol–water (75:25), the order of both types of C<sub>18</sub> bonded phases increased, probably due to an increase in chain alignment [86].

In dynamic experiments, the probes were injected into the mobile phase and the evolution of their ESR signal was followed as they passed through the stationary phase [86]. The signal indicated that the probes sensed two different environments as they passed through the stationary phase. The rotational fast axis of the solute changed without a change in the mean rotational time or the solvation character of the probe interaction sites. This was taken to mean that the change in rotational character was due to the solute probe interacting with bonded phase alkyl chains existing in different configurational states, *i.e.* with different degrees of alignment [86]. This is in good agreement with Zeigler and Maciel's [56] two-site models for bonded phase chain motion (discussed above) which are attributed to inhomogeneities in the ligand surface density.

Wright *et al.* [83] have also used spin probes to study the relationship between the microscopic order in the probe's intercalation environment and the surface coverage of monomeric C<sub>18</sub> bonded phases. The spin probe used in this work was cholestane with a paramagnetic center (dimethyloxazolidine-N-oxyl; DOXYL). The cholestane portion of the probe makes it a large, rather "flat" moiety which will intercalate into the chain interphase; the rotation of the DOXYL group is anisotropic with the fast axis parallel to the long axis of the molecule. It is therefore expected to be a relatively sensitive probe to ordering of the alkyl ligands. When either acetonitrile or methanol containing the probe was pumped through the bonded phases used, the line widths and shapes of the probe spectra indicated that the molecular order of the bonded phases increased as a function of surface chain density [83]. The rotational correlation times were slower in acetonitrile than in methanol for all surface coverages, which indicated a greater degree of bonded phase solvation and an accompanying increase in microviscosity in the

acetonitrile solvent. Rotational anisotropy factors indicated that methanol solvation brings about a more ordered environment, yet the results are anomalous for acetonitrile, which is likely due to its microheterogeneity in bulk solution [83].

#### 4. CONCLUSIONS

A number of overall conclusions about RPLC bonded phase structure and mobility can be drawn from the NMR and ESR studies discussed in this review.

(1) Cross-linking of adjacent alkyl ligands on bonded phases prepared using polyfunctional silane reagents continues to occur *subsequent* to the initial synthetic procedure, and are accelerated under typical RPLC operating conditions [35,37,39]. These cross-linking reactions may be larger contributors to the irreproducible retention behavior that has often been noted for polymeric bonded phases than the variability in surface morphology from the initial synthetic procedure.

(2) For bonded phase alkyl ligands with eight or more carbons, the frequency of alkyl ligand motion is never in the mobility regime for solids, even at very low temperatures (*e.g.* 120 K) [74]. Mobility of individual chain segments increases with distance from the point of surface attachment [29,44,47,74,75]. Even though bonded alkyl ligands exhibit mobility that is considered to be liquid-like over a wide temperature range, the motion is not completely isotropic, even for those chain segments most distal from the point of surface attachment. This non-isotropic motion results in a distribution of correlation times [44]. Temperature affects the degree of *trans* and *gauche* contributions to mean chain orientations, and the proportion of *trans* conformers increases with decreasing temperature [56,76]. There is still evidence of chain kink disorders at temperatures as low as  $-50^{\circ}\text{C}$  [56,76,77].

(3) Interactions with the surface or neighboring ligands restrict the available modes of chain motion [29,83]. Temperature changes the degree of contribution of the rotational component of motion for the terminal methylene and methyl groups, with rotation about the end

bonds hindered at subambient temperatures. This is especially evident at high ligand surface densities [52,81].

(4) Motional behavior is heterogeneous over the length of the alkyl chain ligand, but the distribution of motional behavior as a function of segment location is similar for different chain lengths so long as there are at least eight chain segments [44]. There is less mobility for very short or very long ligands [47]. In all cases, the four carbons closest to the silica surface are very rigid and motionally restricted. Motions in these chain locations are strongly correlated by tight packing, and this prevents chain segments from moving as individual units [48]. Translational and conformational dynamics are much greater at the chain ends [29,44] for both monomeric and polymeric bonded phases, and this is due to the additional freedom of motion in this chain region as well as the rotational contributions to this motion [48]. The combination of fast and slow chain motions at the distal end of the ligand results in motional averaging, with fast chain motions resulting from rotational contributions to motion [50]. Except for the end methyl, motional rates of individual chain segments do not differ by more than a factor of five over all positions in the chain; overall, the molecular motional frequency is in the regime of *ca.* 50 MHz [57].

(5) At ambient temperature, averaging of  $^1\text{H}$ - $^{13}\text{C}$  dipolar interactions due to random motions at the chain ends occurs in  $\text{C}_{18}$  bonded phases when in contact with a neat organic solvent as well as for high ligand density materials in the dry state [47,55]. Under such conditions Zeigler and Maciel [55,56] have found that most of the alkyl ligand (from the fourth chain segment to the chain end) is displaced from lying along the silica surface, and is randomly moving with a motional frequency  $\geq 23$  kHz. For low chain density materials, the random motional component is not present in the dry state and the ligand chains are modeled as lying along the surface of the silica support [55]. Differences in motion between these two cases are thought to be due to changes in the *mode* of motion, rather than changes in the *frequency* spectrum of the motion [56,57]. Because the majority of current

models are based in isotropic motion, these differences have thus far not been able to be accounted for in spectral simulations [57]. A more realistic model would be a statistical one that includes distributions of different chain structures, with the nature and population of each distribution determined by the surface chain density of the bonded phase as well as the type of contact solvent [56].

(6) RPLC chain ligands exhibit no unique axes of reorientation and some spatial regimes undergo relatively slow motions [24,48]. Chemical shift dispersion and inhomogeneous line broadening effects indicate that the carbon nuclei in the chain ligands exist in a variety of surface environments [57]. These effects are thought to be due to a distribution of chain packing along the silica surface, brought about by heterogeneity in the ligand distribution and the irregular surface topography of the silica support [48,56,57].

(7) Addition of neat organic solvents to RPLC bonded phases generally increases configurational reorientations of chain segments to a very rapid motional regime ( $\geq 30$  MHz), particularly for low chain density materials; the effect of solvents on high chain density bonded phases is considerably less [56]. As would be expected, the greater the wetting ability of the solvent, the greater the increase in ligand mobility [49,51,53]. Solvent effects on mobility are much more profound for methylene units near the silica surface than for segments near the chain terminus [51,56]. Contact solvents with a greater degree of bulk solvent structure, such as methanol or methanol-water mixtures, cause more restricted chain motion (*i.e.* more chain ordering) [82,83] than less ordered bulk solvents [53,54]. However, random motions in bonded phase alkyl ligands are greatly decreased when in contact with water. This is attributed to hydrophobic clustering of the alkyl ligands, resulting in a "collapsed" chain structure [51,56].

(8) As has been indicated by other spectroscopic and chromatographic studies, ligand extension is more pronounced for bonded phases with high ligand chain densities [54]. In the presence of organic solvents, particularly non-polar ones, the alkyl ligands assume a more extended chain

configuration. Even at high ligand surface densities, a variety of chain surface conformations are present, but very few of these surface conformations have chain segments interacting with the surface to any significant degree [56].

(9) NMR and ESR measurements have not been able to precisely quantitate the composition of the bonded phase solvation layer. However, the solvation environment at the bonded phase surface for any particular mobile phase composition has been shown to be extremely dependent on the microstructure of the bulk mobile phase solution [11,60,61]. The microviscosity in the bonded phase solvation layer is considerably greater than that in the bulk solvent [58]. The polarity of the bonded phase solvation layer varies with the composition of the bulk mobile phase as well as the spatial position (“depth”) along the alkyl chain [82,86].

A number of these conclusions have previously been proposed from interpretations of chromatographic studies and from theoretical models of the bonded phase surface; however, the above conclusions which have been drawn from NMR and ESR spectroscopic studies provide concrete and specific supporting evidence for many of these prior chromatographic and theoretical models. The conclusions discussed in this review are sometimes contradictory, but this does not necessarily indicate that NMR and ESR studies are unsuitable for studying alkyl chain motion in RPLC bonded phase systems. Rather, the possible contradictions serve to demonstrate that the results from such studies are often difficult to interpret [55] and therefore should be compared to complementary information obtained from orthogonal techniques whenever possible.

##### 5. FUTURE PROSPECTS FOR THE USE OF ESR AND NMR TO STUDY RPLC BONDED PHASE STRUCTURE

From the above discussion on the broad-based utility of ESR spectroscopy to study microscopic structure and polarity in RPLC systems, it is likely that more investigations utilizing this technique will be carried out in the future. The ability to set up “flowing state” experiments is especially attractive, and enables a reasonable

approximation of RPLC operating conditions to be made. In the light of Miller *et al.*'s [82] intriguing flow-dependent experiments, measurements made under flowing conditions may be more relevant for accurately probing the microenvironment of the stationary phase than has previously been thought. However, when ESR spin labels are used in these experiments, the presence of a spin label on the stationary phase surface introduces a significant perturbation to the local surface environment. Since spin-probe experiments are therefore likely to be more accurate for studying molecular interactions in RPLC than spin-label experiments, synthesis of a greater variety of spin-probe molecules with varying chemical and morphological parameters is essential to future ESR studies.

With the high degree of interest that has already been displayed in using NMR to study RPLC systems, use of this technique for probing the molecular interactions involved in RPLC separations will undoubtedly continue. As has been noted throughout this paper, one experimental aspect which has received insufficient attention is that studies should be made in which the contact solvents for the stationary phase are true binary hydro-organic mixtures rather than neat solvents. The effects of pressure on mobile phase solvation of the stationary phase and on alkyl ligand mobility have also been ignored thus far in NMR studies; this is an area that will soon be pursued in our laboratory. Further applications of flowing and imaging NMR systems [15–23] to stationary phase studies will certainly continue to grow.

Some of the most exciting experiments on the NMR horizon for RPLC systems are the application of more sophisticated and computer-intensive NMR techniques, such as the multidimensional and multiple quantum experiments which have found recent applications in biochemical and materials science conformational studies. Maciel's group has begun using  $^1\text{H}$  CRAMPS techniques and intermolecular cross-polarization to study specific solvent-stationary phase interactions [55].  $^1\text{H}$  experiments combining MAS with multiple-pulse line narrowing (CRAMPS) result in solid-state spectra with considerably narrower lines than normally attained with more

traditional CP-MAS methods. CRAMPS spectra of silica resulted in resolvable peaks for physisorbed water and for two distinguishable types of silanol protons, which lie in close physical proximity ( $<10 \text{ \AA}$  apart) on the surface [87,88]. CRAMPS-based relaxation experiments can also be designed to explore the dipole interaction between nuclei that can be distinguished via CRAMPS [87,88]. These techniques will no doubt be utilized more routinely in the near future, and applications to  $^{13}\text{C}$  nuclei will be particularly useful for bonded phase studies.

Fyfe *et al.* [89] have recently used two-dimensional high-resolution solid-state heteronuclear NMR correlation spectroscopy not only to study the functionality of methyl-substituted silica gels, but also to try to describe the distribution of the functionality throughout the matrix. In this type of NMR spectroscopy, spatial correlations between different types of  $^1\text{H}$  and  $^{29}\text{Si}$  nuclei (*i.e.* nuclei attached to different types of functional groups) are made through their dipolar interactions. By identification of the sources of the polarization transfers, the connectivity between  $^1\text{H}$  and  $^{29}\text{Si}$  nuclei of different types can be established [89]. These types of experiments will no doubt be extended to other nuclei in RPLC systems, because they should allow more detailed study of surface distributions of alkyl groups, local solute–stationary phase interactions and local solvation environments of bonded phase materials.

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