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## ELECTRON SPIN RESONANCE STUDIES UNDER DYNAMIC MOBILE PHASE CONDITIONS ON CHEMICALLY MODIFIED SILICA

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### SUMMARY

The electron-spin resonance (ESR) techniques of spin labeling and probing are shown to be powerful methods for the study of bonded silicas used in chromatographic separations under dynamic solvent flow conditions. Microscopic information gained from these studies includes solvation composition of the bonded phase at various locales in the bonded phase, configuration of the bonded phase and solvent-flow effects. In the spin-label study, 2-(2,2-dimethyloxazolidine-N-oxy)hexylsilane (DOXYL hexyl siloxane) was used as the label. ESR spectra show changes in the motional freedom of the bonded surface as a function of mobile phase composition and elution time and demonstrate chain stiffening with the introduction of water, as inferred by other studies. In the spin-probe work, various nitroxide compounds of the 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) family were introduced under flow conditions as a 0.1 mM solution in various water-methanol mobile phases. C<sub>18</sub> sample columns were equilibrated with this mixture. Interpretation of the spectra yielded information about the solvated environment on the surface that was sampled by the probes as a function of both the mobile phase and the polarity of the probe.

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### INTRODUCTION

The development of spectroscopic techniques to study the solid-liquid interface is considered to have a high priority in the understanding of separation mechanisms in bonded phase liquid chromatography<sup>1</sup>. The microscopic views that the application of spectroscopy to bonded phase systems can give will aid the design of more efficient separation techniques.

The most useful stationary phase for liquid chromatography has been the reversed-phase material which is made by bonding long chain hydrocarbons to the porous silica surface by silanization<sup>2</sup>. These stationary phases have been studied extensively by chromatographic means<sup>3,4</sup> and more recently by various spectroscopic methods<sup>5-9</sup>. While providing valuable insights concerning the nature of the bonded silica interface, these techniques often suffer from the need to be performed under

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conditions that are not representative of those that are useful to chromatographers. As an example, in order to perform most nuclear magnetic resonance (NMR) experiments, the sample must be spinning. This makes it difficult to experiment with solvents flowing through the system. Thus, many of the initial NMR investigations were performed with the bonded phase either dry or in a solvent that wets the hydrophobic modified silica under static (ambient pressure) conditions. Most often these solvents are too "strong", *i.e.*, non-polar, to permit useful separations as chromatographic mobile phases. Mixed solvents that contain 50% or more water are seldom, if ever, useful for these studies<sup>10,11</sup>. It should be mentioned in this connection that Lochmuller *et al.*<sup>12</sup> have been able to construct a fluorescence sample cell that allowed them to maintain sample geometry and also to operate under high pressure and flowing conditions.

Other problems with spectroscopic techniques, as applied to modified silicas, include lack of sensitivity, opacity or light scattering properties of silica and a limited range of probes and labels. Often, the presence of a label, such as a large fluorescent molecule, can significantly alter the nature of the solid-liquid interface that is being characterized. The use of such molecules as probes limits their usefulness in fluorescence studies to mobile phases containing high concentrations of organic modifiers because of quenching at very high stationary phase concentrations when mobile phases with high water content are used<sup>7</sup>.

One spectroscopic technique that has only been sparingly applied to chromatographic systems is electron spin resonance (ESR) spectroscopy. The technique has been used to study a wide variety of chemical systems, such as membranes, liquid crystals, polymers, zeolites and biological macromolecules, and has been extensively reviewed<sup>14-16</sup>. The ESR studies of the dynamics, configuration and phase interface properties of systems have been carried out chiefly by two techniques: spin probe and spin label experiments. In the spin-probe experiment, a relatively small paramagnetic molecule, often a nitroxide, is introduced into the system where it is partitioned, adsorbed or intercalated into various regions. The resulting ESR spectrum contains information about the environment(s) of the probe. In a spin-label experiment, a small number of molecules in the system under study have a paramagnetic center chemically bonded to the units. The label density is kept at a very low level to avoid significant perturbation of the system so that the spectroscopic observations on the label reflect the properties of the unlabeled majority of molecules.

Early label experiments were performed on modified silicas with the use of polymers<sup>17-20</sup> and hydrocarbon modifiers under static conditions<sup>21</sup>. These studies demonstrated the existence of two dynamic domains of nitroxide labels: inside bonded clusters and on the perimeter of the cluster, capable of interacting with the unmodified silica surface. Spin probes have been used to investigate unmodified hydrated silicas<sup>22-25</sup> and have demonstrated that the nature of the water on the surface differs greatly from "bulk" water. Ion-exchange resins have also been spin probed<sup>26</sup> and have revealed very anisotropic behavior. Recently, Gilpin *et al.*<sup>27</sup> have sparsely labeled silica surfaces to probe the surface and interface dynamics of normal bonded phases. They found that the nitroxide label strongly interacted with the silanol surface in non-polar solvents, but in polar, hydrogen-bonding solvents the solvent was competitive for the surface sites. Also, Malcom *et al.*<sup>28</sup> reported a true reversed-phase that was sparsely labeled with a nitroxide. They confirmed the two site model and

were able to demonstrate dramatic changes in bonded chain mobility with the introduction of wetting solvents.

In this paper, we show the ESR technique to be very versatile and robust for the investigation of chromatographic systems. We make two important points: first, the technique is very sensitive, requiring only minimal disturbance of the system under study by the label or probe and secondly, the technique can be applied under conditions that accurately reflect those in actual chromatographic separations. The requirement that an unpaired electron must be present is satisfied by the use of nitroxide spin-labeled surfaces or nitroxide spin-probes as solutes, dissolved in typical chromatographic mobile phases. The spin-labels are synthesized *in situ* and are present in <0.5 mole percent of the untagged hydrocarbon chains, perturbing the surface very little. The spin-probes exist in a wide range of polarities and sizes, mimicking a wide variety of chromatographic behaviors. We demonstrate that considerable information about the chromatographic surface is available from ESR studies.

The dynamic label/probe studies presented here show various results of interest to chromatographers. Label studies give insight into conditioning effects and well as changes in surface configuration with mobile phase composition. Spin-probe studies give pictures of the microenvironments sampled by chromatographic solutes and offer opportunities to make traditional liquid-solid interface determinations, such as isotherm studies, by spectroscopic means.

## EXPERIMENTAL

The ESR spectra were obtained on a Varian V-4500 ESR spectrometer with 100 kHz modulation (Varian Associates, Palo Alto, CA, U.S.A.). The usual precautions were taken to insure that saturation and overmodulation effects were absent.

The dynamic ESR cell inserted into the spectrometer consisted of a 1 mm I.D. quartz tube fitted with a 1/16-in. frit at the outlet end and high pressure plastic tubing and fittings at each end. The column end fittings were made by carefully boring out the plastic coupling fittings (Model P-110; Upchurch Scientific, Oak Harbor, WA, U.S.A.) out to accommodate the frit and the nominal 2 mm O.D. quartz tube in order to provide a seal against the pressures used in the experiments. The quartz tube was filled with spin labeled silica or a typical bonded phase by aspirating the silica into the tube and then conditioning it under higher flow-rates than were actually used for running spectra in order to pack it tightly in the tube. A Beckman 110B Solvent Delivery Module (Beckman Instruments, Altex Div., San Ramon, CA, U.S.A.) was used to pump mobile phase through the column at various flow-rates ranging from 0.1 to 0.6 ml/min.

The labeled, bonded monomeric C<sub>6</sub> silica was synthesized *in situ* by a previously described method<sup>28</sup>. The label was DOXYL hexyl silane<sup>28</sup>. The silica used was Davisil (Aldrich, Milwaukee, WI, U.S.A.) which had a particle size of 36–74 μm, a pore diameter of 150 Å and a pre-derivatization surface area of 300 m<sup>2</sup>/g. The % C by weight was determined to be 5.45% which means *ca.* 1.25 bonded chains per 100 Å<sup>2</sup> or *ca.* 31% derivatized. This material is surprisingly durable. A batch made 1.5 years ago is still ESR-active. ESR spectra were recorded after equilibrating the column with either 100% methanol or 50% (v/v) aq. methanol at a flow-rate of 0.3 ml/min for 0.5 h. The mobile phases were sparged with helium to remove oxygen. After initial condi-

tioning, the column was left standing overnight, and the ESR spectra were recorded at regular intervals after pumping a mobile phase through the column. In the time-dependence studies, when a steady state had been reached (as seen by the peak heights of the spectra), the pump was shut off and the spectra monitored until the initial time zero state had been reached.

All spin-probe studies were performed in the "isotherm" mode by pumping a degassed solution (see above) of the mobile phase and chosen probe through the dynamic ESR cell. Observations were made when a time-invariant signal was observed. The stationary phase was Bond Elut C<sub>18</sub> (Analytichem International, Harbor City, CA, U.S.A.), a highly loaded polymerically modified silica marketed for solid-phase extraction. The stationary phase was packed into a 1 mm I.D. ESR quartz column, as described above, and attached to the pump for dynamic probe investigations.

The probes used were TEMPO, 4-hydroxy-TEMPO (TEMPOL) and 4-amino-TEMPO (TEMPAMINE) (Aldrich) and used as received. Solutions in various compositions of methanol and water were made at  $< 1 \cdot 10^{-4}$  M.

Chromatographic capacity factors,  $k'$ , were determined for the spin-probes and other solutes on Bond Elut C<sub>18</sub>. This stationary phase was dry-packed and conditioned with at least 100 column volumes of methanol. The column consisted of a 50 mm  $\times$  4.6 mm I.D. guard column. Methanol used in the mobile phase was filtered and water was doubly deionized and filtered. The mobile phase consisted of a 50:50 (v/v) mixture, which was sonicated and allowed to stand overnight. A 1% sodium nitrate solution was used to determine  $V_0$ .

## RESULTS AND DISCUSSION

Considerable information is available from the ESR spectrum of a free radical. The ESR spectrum of a nitroxide free radical undergoing rapid rotational reorientations ( $< 10^{-9}$  s mean rotational correlational time) contains three lines separated by the <sup>14</sup>N coupling constant (symbolized by  $a_N$ ) from the interaction of the unpaired electron with the <sup>14</sup>N nucleus in the -NO group (Fig. 1). Readily available information about the microenvironment of the system is as follows.

### *Solvent polarity*

It has been well established that the value of the <sup>14</sup>N splitting constant of nitroxides,  $a_N$ , is affected by (a) solvent polarity and (b) the extent of hydrogen bonding<sup>29</sup> and that the relationship is linear between the coupling constant and solvent polarity<sup>30</sup>. The more polar the environment of the nitroxide, the greater is the coupling constant; therefore, the coupling constant is a sensitive measure of the character of the solvation in the neighborhood of the nitroxide. Hence, measurements of the changes in the coupling constant provide a means to explore the solvation composition of the bonded phase by various spin-probes and label experiments.

### *Lineshape anisotropy and rotational correlation times*

The three lines of a nitroxide spectrum are of equal integrated intensity, but often of unequal width, hence unequal amplitude. The extent of this spectral anisotropy is related, for a given nitroxide, to the rate of rotational tumbling and to the

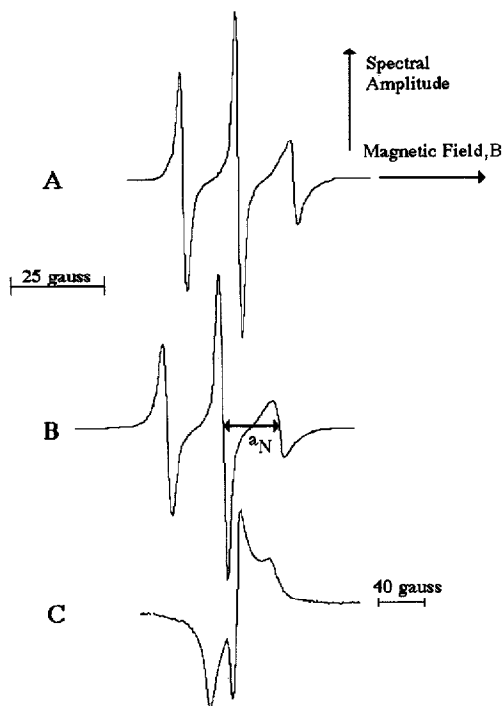


Fig. 1. ESR spectra of the  $C_6$  spin-labeled silica. Magnetic field axis increases to the right, and the three lines arise from the interaction of the electron spin with the three possible N nuclear spin states. The magnitude of the electron nuclear interaction is measured by the hyperfine coupling constant,  $a_N$ , and is sensitive to the polarity of the label environment. (A) The bonded silica under flow conditions with pure methanol; (B) the  $C_6$  bonded silica under flow conditions with 50% (v/v) aq. methanol; (C) the  $C_6$  bonded silica in the dry state (note the magnetic field axis scale is different in A and B than in C).

anisotropy of that tumbling about the three mutually perpendicular rotational axes of the molecule. In the case of small, nearly spherical molecules such as TEMPO, rotating rapidly (rates in the range of  $10^9$ – $10^{11}$   $s^{-1}$ ) in isotropic media, the three lines are of nearly equal amplitude, reflecting a rotation that is the same about the three molecular axis directions. In the case of more rapid rotation about one axis, the character of the spectrum is determined by the relationship between the rotation axis system of the molecule and the magnetic axes, fixed on the nitroxide group (Fig. 2). Rapid rotation about either the magnetic  $y$  or  $z$  axis is distinguishable from rotation about the magnetic  $x$  axis, parallel to the NO bond<sup>31–33</sup>.

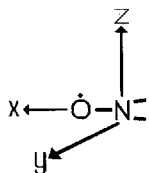


Fig. 2. Nitroxide fragment, showing the magnetic axes associated with the nitroxide group. The molecular (rotation) axes may or may not coincide with the magnetic axes on the NO group.

In the case of dilute solutions, the variation of the widths of the three spectral lines in the nitroxide radical undergoing rapid rotational motion can be used to determine the exact rotational anisotropy as well as a rotational correlation time. Such a correlation time gives information about the effective microviscosity of the environment about the radical. When the radical exhibits slower tumbling (less than  $10^9 \text{ s}^{-1}$ ), similar information is available but must be extracted by spectral simulation, based on the theory of Freed and co-workers<sup>33</sup>. In both cases, changes in rotational anisotropy can be correlated to changes in the configuration of the bonded surface.

#### *Multiple radical sites*

If there is more than one environment accessible to the radical and if the rate of exchange among the sites is not rapid, then the ESR signal consists of a superposition of signals from the different sites. Such information provides a picture of the heterogeneity of the surface, as well as information about the relative fraction in each site<sup>31</sup>.

#### *Exchange rates*

In the case of multiple sites in equilibrium with each other, the rate of jumping among the sites can be gotten from the widths of the spectral lines. If the radical concentration is high enough, radical-radical encounters become frequent enough that spin exchange begins to appear as an equal broadening of all three lines. The rate of exchange is related to, among other things, the rate of diffusion of the radicals together, *i.e.*, translational mobility.

#### *Label studies*

Label studies on C<sub>6</sub> and C<sub>11</sub> DOXYL-labeled silica have been reported recently by us<sup>28</sup>. These studies were performed statically, *i.e.*, run either on dry silica or silica wetted with a solvent. This system is typical of most previous spectroscopic studies on modified silica in that the sample is neither under pressure nor influenced by the motion of a flowing mobile phase. The results of these studies confirmed the two-site models proposed by Gilpin and co-workers<sup>6,34</sup> and the effect of the solvent on reducing the rigid nature of the bonded phase. However, our labeled silicas were the first true reversed-phase silicas reported which are ESR active and have a spacer chain of five or more methylene groups. Gilpin *et al.*<sup>27</sup> have also reported a static ESR study on silica, but this involved the use of TEMPOL which was modified through a reaction with the hydroxy group to form a silane with essentially no hydrocarbon spacer.

Typical spectra of the labeled C<sub>6</sub> silica are shown in Fig. 1 after conditioning with 100% methanol and 50% (v/v) aq. methanol as mobile phases. The first thing to note is that there is not a large difference in these spectra indicating that the labels are in relatively the same solvated environment. There is a small but measurable change in spectral anisotropy with the introduction of water indicating an increase in the rigidity of the system as expected<sup>9</sup>. Further studies are being conducted with various labeled phases, variable coverage and differing chain lengths in order to investigate bonded phase dynamics as a function of mobile-phase composition.

After initial conditioning and relaxation to a non-flow condition, upon sudden application of solvent flow and examination of the amplitude of the central line of the nitroxide label as a function of time shows that the amplitude of the center peak increases in time until a plateau is reached in about 30 min (Fig. 3). A calculation of

the area under the spectral peak shows no change with time so that the increase in amplitude is not from an increase in the number of radicals but rather must come from a narrowing of the central line. This indicates that the labeled environment is undergoing a reorientation that is somehow dependent upon pumping methanol through the system. If the pump is then shut off and the system is continually monitored as a function of time, the peak amplitude will then slowly decay over a period of several hours until the initial "relaxed" state is achieved. We have found this effect to be very reproducible in different columns. The same experiment with 50% methanol shows a similar, but greatly diminished, effect that is barely measurable in our system. The coupling constants for these series of spectra remain the same throughout the experiment indicating no significant change in the solvation of the environment of the label due to pumping the methanol through the silica.

One possible cause of the line narrowing effect with solvent flow is that the pores of the silica might not be filled at ambient pressure. This type of effect is seen in mercury porosimetry. With poorly wetting solvents, such as pure or nearly pure water, this effect has manifested itself as an increase in void volume with increasing head pressure on a column and increasing retention volume for solutes in mobile phases with high water content. (This is the opposite of what one would expect in a typical reversed-phase system<sup>35,36</sup>.) One flaw in the pore filling argument is that methanol is a very effective wetting solvent, and normally no pressure is required to wet reversed-phase silica with methanol. Pressure is another possible cause. To find out whether pressure had an effect, the column was capped off at the outlet, and the inlet was connected to a reservoir containing methanol. The reservoir was, in turn, pressurized with compressed gas up to 700 p.s.i. No change in the ESR line amplitude

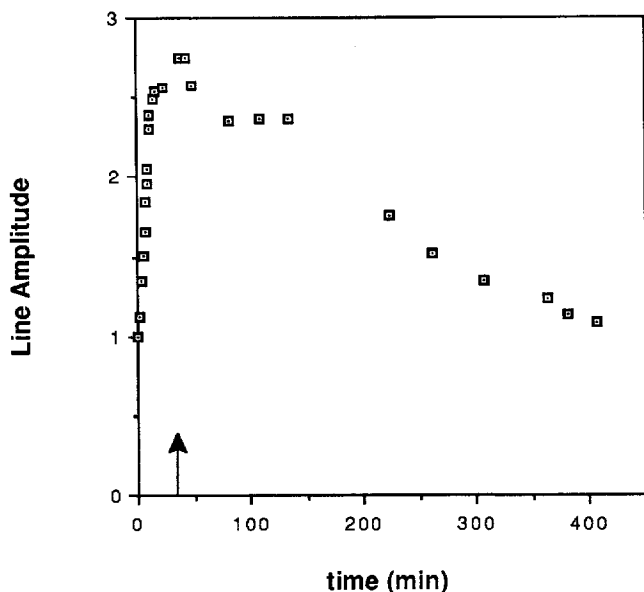


Fig. 3. Amplitude of the center line of the  $C_6$  label as a function of time, beginning from a no-flow state and going to a flow-rate of 0.6 ml/min of methanol. After the maximum was reached, the pump was turned off (shown by the arrow), and the figure shows the decay of the amplitude to the original no-flow state.

was seen after 4 h under pressure. Since the maximum head pressure in the column was estimated to be about 200 p.s.i. under flow conditions, the restructuring of the bonded silica surface is not a pressure effect.

It is possible that the flowing methanol is causing a buildup of charge on the surface which, in turn, causes a reorientation of the hydrocarbon chains, similar to the effects of a charge on liquid crystals. Since our system is in a plastic and glass column with plastic inlets and outlets, the dissipation of this charge would be slow. Streaming potential is the term applied to the potential caused by a flowing stream of liquid and charge buildup has been a problem with non-aqueous solvents in contact with non-metallic surfaces<sup>37</sup>. Discharges caused by the high-speed fueling of jet aircraft is one example of the effects of streaming potential. These effects have been noted in the packing of reversed-phase columns (*cf.*, ref. 2, p. 214). This effect is reduced when aqueous solvents are used and/or when ions are present in the solvent. This is consistent with the fact that the ESR line amplitude increase with the flow-rate is greater in the 100% methanol than with 50% aq. methanol as the mobile phase. In previous studies, Gilpin and Squires<sup>38</sup> found that changes in bonded chain orientation can have chromatographic significance. At present, we are exploring the retention and band broadening effects of the orientational changes which we observe in the ESR spectra. The outcome of this study may add to our understanding of the need to "equilibrate" a column by passing several column volumes of mobile phase through before injecting samples in order to achieve reproducibility. An interesting possibility might be the eventual ability to control column selectivity characteristics through the use of an applied electric or magnetic field to orient the bonded moieties.

### *Probe studies*

This work was performed by dissolving a small amount of a nitroxide compound, such as TEMPO, TEMPAMINE or TEMPOL in a typical chromatographic stationary phase and eluting this mixture through a test column until the ESR signal reached a maximum. It should be mentioned that at the concentrations used (*ca.*  $10^{-4}$  *M*) no ESR signal could be seen from the nitroxide alone in solution. Therefore the spectra are assumed to be representative of the nitroxide sorbed on the solvated bonded silica surface only. Examples of the spectra in 50% aq. (v/v) methanol are seen in Fig. 4. Close examination of the spectrum of TEMPAMINE indicates that it is due to the superposition of two signals from different environments. TEMPO shows no effects from a second site and, in the 50:50 solvent, TEMPOL shows a barely visible hint of a second site. In the case of TEMPAMINE, the two sites differ markedly in the rotational mobility of the probe, as seen by the differences in lineshapes. The second probe site that appears with the introduction of water is very immobile (see the "wings" in Fig. 4). These immobile probes are sorbed at sites in which they are held more energetically. Fig. 5 shows the probes in a pure water solvent. An increase in the number of probes in the more energetic environment is noted. For instance, TEMPOL now shows an appreciable immobile component, although the separation of the wings is not as great as in the case of TEMPAMINE, indicating great rotational mobility for TEMPOL<sup>39</sup>. Hence, we note that the rotational mobility of the immobile site is in order of increasing rotational rate: TEMPAMINE < TEMPOL < TEMPO. This leads to an assignment of this second site to an environment where hydrogen bonding can occur. Most likely it is a region near the



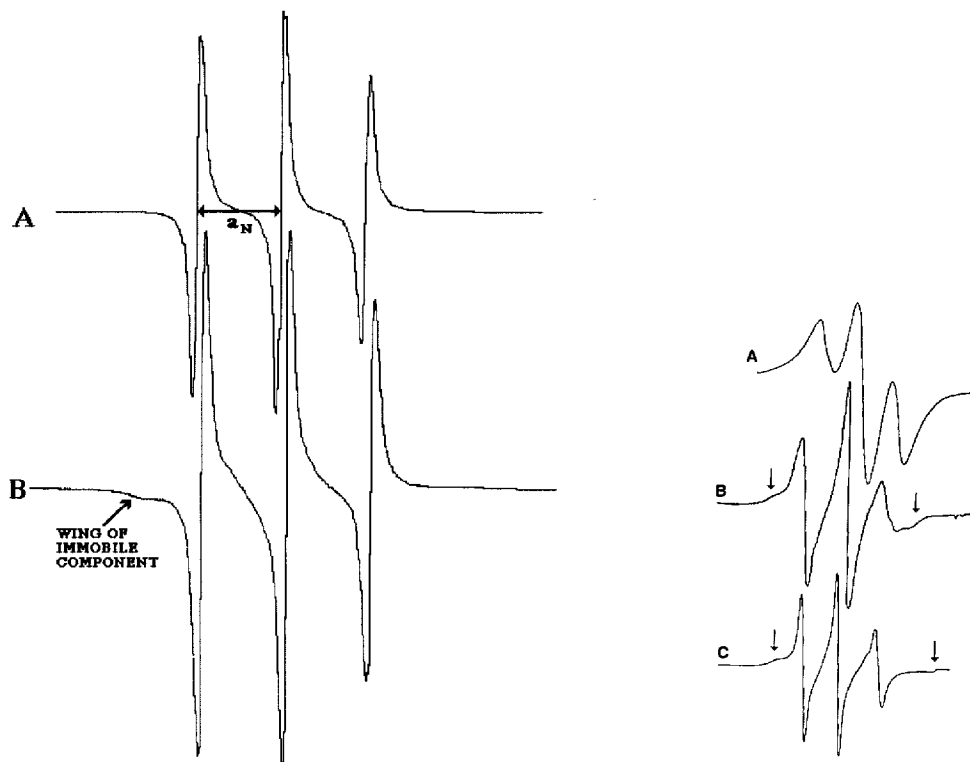


Fig. 4. ESR spectra of the TEMPO (A) and TEMPAMINE (B) spin probes, sorbed on the  $C_{18}$  bonded phase from 50% (v/v) aq. methanol mobile phase, flowing through the ESR sample-cell column. Magnetic field increases to the right. B shows a bump on the low-field side of the low-field sharp line from the second immobile site. The spectrum of TEMPOL probe is not shown, but differed little from TEMPO.

Fig. 5. ESR spectra of the TEMPO (A), TEMPOL (B) and TEMPAMINE (C) spin probes, sorbed on the  $C_{18}$  bonded phase from 100% water as the mobile phase, flowing through the ESR sample-cell column. The magnetic field increases to the right. The arrows indicate the locations of the low- and high-field extremes from probes in a second immobile site. The TEMPO spectrum is spin-exchange broadened because of the large increase in the sorption equilibrium of TEMPO in pure water as the solvent.

surface that is richer in water or a water-rich solvated region of the stationary phase, probably very near the mobile-stationary phase interface. The immobility of the two probes with polar tails probably reflects hydrogen bonding with surface silanols or imbibed mobile phase. The greater rigidity of TEMPAMINE could be due to actual proton transfer from the acidic silanol hydrogens to the amine group. We note that in bonded-phase liquid chromatography, amines will often "tail", due to interactions with the underivatized silanols. These results directly confirm that interaction.

In Fig. 6 the nitroxide coupling constant,  $a_N$ , of the free probe, TEMPO, as a function of solvent composition is plotted for the methanol-water system. Windle's<sup>29</sup> value for water is included in the figure to establish the extreme for highly polar solvents. To establish what the coupling constant would be in a pure hydrocarbon environment, we measured TEMPO  $a_N = 15.3 \pm 0.2$  G in octane at room temperature. For the free probe in solution, Fig. 6 shows, as expected<sup>29,30</sup>, that the coupling

constant increases monotonically with the water content. Using these results, we can then use the measured  $a_N$  value of the sorbed probe to determine the nature of the stationary phase solvation, *i.e.*, % methanol. Examination of the TEMPO probe behavior in the  $C_{18}$  bonded phase shows that the coupling constant is not a function of the water content of the mobile phase and that it is, within experimental error, the same as the coupling constant in pure methanol—well above the measured value in pure hydrocarbon environment of 15.3 G. We conclude that the  $C_{18}$  phase becomes well solvated with methanol above 25% methanol and that the water content of the polymeric stationary phase in which TEMPO is sorbed does not change significantly. This is consistent with the results of others, who have determined the extent of solvation of the stationary phase in reversed-phase systems<sup>35,36,40,41</sup>. It should be noted that the probe method is a vastly easier method of establishing stationary phase composition. It is interesting to note that the solvated environment for the probe is pure methanol, unmodified by the hydrocarbon bonded phase. This suggests that a relatively non-polar solute like TEMPO appears to dissolve in “pools” of organic solvent embedded in the non-polar stationary phase.

Beyond the consideration of the preferential sorption of the organic component of the mobile phase in the hydrocarbon environment, attention must be given to the nature of the solute. Table I shows a list of the capacity factors for a number of typical test solutes and the probes used in this study. It is seen that  $k'$  decreases for

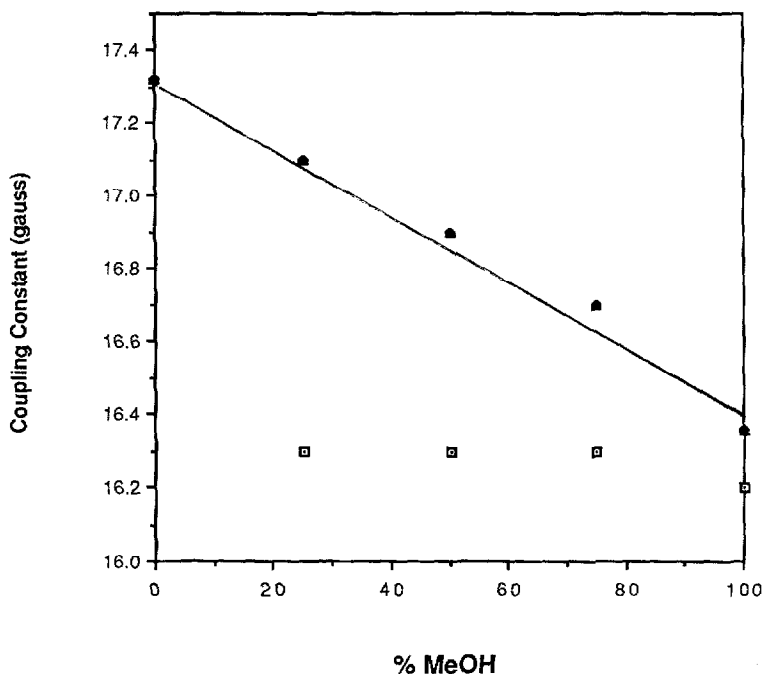


Fig. 6. Coupling constants, in gauss, for the sorbed TEMPO spin probe as a function of mobile phase composition for the methanol (MeOH)–water system in  $C_{18}$  bonded phase (squares) under flow conditions. Also shown are the values for free TEMPO, dissolved in the mixed solvent (triangles). Coupling constant for the free probe in pure water is from ref. 29.

TABLE I

CAPACITY FACTORS FOR TYPICAL SOLUTES AND ESR PROBES ON BOND ELUT C<sub>18</sub> WITH 50% (v/v) AQ. METHANOL

Solute	Capacity factor ( $k'$ )
Aniline	2.61
Acetophone	5.20
Nitrobenzene	7.13
Benzene	11.1
Toluene	25.2
TEMPOL	1.83
TEMPAMINE	2.16
TEMPO	19.9

TEMPO > TEMPOL > TEMPAMINE in the order given. It should be noted that TEMPO is quite hydrophobic, since it has a  $k'$  value of 19.9 that is comparable to toluene at 25.2 and greater than benzene at 11.1 (all determined on a C<sub>18</sub> Bond Elut phase). Previous studies have indicated that different solutes appear to contact different zones on the solvated stationary phase<sup>13,41</sup>. Using the linear correlation between  $a_N$  and % methanol (see Fig. 6) and interpolating the  $a_N$  vs. % methanol found individually for TEMPO, TEMPAMINE and TEMPOL, a value for the effective concentration of methanol in the zone where the probe is sorbed can be determined. The most non-polar probe, TEMPO, is sorbed in a surface region where the environment is *ca.* 90% methanol, even though the mobile phase contained only 50% methanol in this case. The polar probes TEMPOL and TEMPAMINE, on the other hand, are sorbed into a region that contains *ca.* 60% methanol. This type of behavior has been observed before by Schunk<sup>41</sup>, who noted significant differences in the retention of various solute probes as a function of temperature. He suggested that solute probes that penetrated the solvated surface more deeply would respond to temperature-induced phase changes at a higher temperature and *vice versa*. Our method can give similar information and we believe that we can eventually probe the surface in depth as well as determine the "topography" of the surface.

## CONCLUSIONS

ESR label studies have shown the existence of flow-induced structuring of the stationary phase. These studies also show a change in the configuration of the stationary phase upon the introduction of water into the mobile phase, demonstrating spectroscopically the chain-stiffening inferred from other techniques. The ESR probe studies have allowed us to develop a convenient polarity scale for the solute environment, based on coupling constants. This polarity scale can be used along with a variety of nitroxide probes to study the solvation of any permeable material that can be packed into a small tube. This work demonstrates the utility of the spin-label/probe technique to characterize surfaces of interest to chromatography.

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