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Nuclear Magnetic Resonance Spectrometry of Alkyl Ligands Immobilized on Reversed-Phase Liquid Chromatographic Surfaces

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Reversed-phase chromatographic surfaces were prepared with ^{13}C enrichment at the terminal and adjacent carbon positions as well as the fourth carbon position by using labeled *n*-alkylchlorosilanes. In all cases single resonances were observed arising from the site of the enrichment. Similar values for chemical shift were noted before and after bonding for the terminal methyl and adjacent methylene carbons. For a given surface coverage measured ^{13}C spin–lattice relaxation times were relatively constant for the terminal methyl carbon for the various midrange chain lengths studied. However, a decrease in spin–lattice relaxation time was noted with increasing alkyl surface density. These as well as other observed results support the idea of significant chain interaction at higher surface coverage. Finally, rotation about the terminal C–C bond seems to be a major factor to spin–lattice relaxation for the terminal carbon.

The nonpolar stationary phases used in reversed-phase liquid chromatography generally are prepared by reacting *n*-alkylchlorosilane with porous silica materials. Both mono- and trireactive silanes have been utilized. On a commercial basis monochlorosilane reactions have evolved as the routes of choice due to ease and reproducibility of the synthetic process compared to multireactive chemistry. Regardless of the mode of preparation, a more detailed description of both the chemical and physical properties of the bonded surface is paramount to understanding the nature of solute–surface and solvent–surface interactions.

To date a host of models have been proposed to explain the modified chromatographic surface. Initially, these models were more or less static in nature. The static models generally have fallen into either the "bristle" or "blanket" configurations popularized by Halasz and co-workers (*1*) and Hemestberger et al. (*2*), respectively. More recently dynamic descriptions have emerged. Gilpin and co-workers have suggested that the bonded groups can undergo changes in orientation and mobility depending on various parameters such as temperature and solvent type and composition (*3–5*). Likewise, Lochmüller and Wilder have proposed that bonded alkyl chains cluster or aggregate under certain conditions (*6*).

Historically, most of the earlier surface models were based only on chromatographic evidence. More recently, however, the idea of probing the chromatographic surface with various spectroscopic methods has become extremely popular. Besides infrared (*7–13*), fluorescence (*14*, 15), and photoacoustic (*13*, *16–18*) spectroscopy, NMR spectrometry is a technique where considerable research interest has emerged (*19–29*). This has been true especially with the advent of cross polarization and magic angle spinning (CP-MAS).

Generally CP-MAS studies have fallen into three classes: (1) elucidation of surface-backbone chemistry and structure, (2) characterization of attached ligands, (3) determination of overall molecular and segmental chain motion. Bayer (*25*), Hays (*26*), and Leyden (*13*) and co-workers have used solids NMR to characterize several types of chemically modified surfaces in terms of chemical shift information. In each study the observed spectra for the attached ligands were similar to the corresponding ligand in the liquid state. Maciel et al. (*22*, *24*, *27*, *28*) have examined various alkylchlorosilane modified silica in an effort to obtain backbone-bonding information as well as motional information about the surface bound hydrocarbons. Not only did this work support previously proposed reaction models (*30*), but it presented evidence for segemental motion (*24*). Additionally, Sindorf and Maciel have discussed the importance of methyl rotation as a mechanism for relaxation of the protons based experimentally on observed changes in carbon–hydrogen cross-polarization rates (*24*). Likewise, Slotfeldt-Ellingsen and Resing (*21*) using only cross polarization have shown that phenyl-attached surface groups undergo changes in orientation with temperature. Additionally, they found that the degree of motion increased when longer methyl hydrocarbon spacer arms were used to place the phenyl groups further from the surface. To date, although considerable information has been generated by using solids NMR techniques, the main disadvantage of CP-MAS is that the experiments are carried out in the absence of solvent, an experimental condition which is more analogous to those which exist in gas chromatography rather than liquid chromatography.

One of the first studies employing conventional solution FT-NMR techniques was carried out by Tanaka and co-workers (*19*). In these studies chemical shift, NOE, and T_1 values were reported for surfaces modified with triethoxy(alkylamino)silanes. Although interesting, a major problem with these earlier studies was that the materials were prepared in such a manner that they little resembled the thin surface films used for chromatographic supports. Thus the NMR results reported were for a cross-linked siloxane polymeric system into which silica particles had been incorporated. As a consequence of the synthetic procedures employed by Tanake and co-workers, the relatively narrower resonances reported since have been shown by others (*23*) not to be indicative of truly surface immobilized thin layers. In such systems board resonances have been noted.

The effective application of conventional ^{13}C FT-NMR to chromatographic type surfaces is limited due to problems of signal intensity and multiple line collesence arising from the broad resonances of similarly shelfed signals. The advantage of selective ^{13}C enrichment to eliminate resonance overlap and to reduce signal acquisition times has been demonstrated previously (*23*, *29*). Changes in resonance line shape and width as a function of position of labeling along the chain and surface-backbone chemistry also have been reported (*23*, *29*). Likewise, these same investigations have demonstrated the existence of at least two different types of attachment (i.e., surface and bulk polymerization) when trireactive silanes are used to prepare modified silica surfaces. Further, the preferential enrichment of the surface with the organic component

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Table I. Synthesized Surfaces

labeled silane[a]	% carbon[b]	notation	% enrichment
$Cl(CH_3)_2Si(CH_2)_7{}^*CH_3$	7.4	8M8(7.4)	25
$Cl(CH_3)_2Si(CH_2)_9{}^*CH_3$	7.9	10M10(7.9)	25
$Cl(CH_3)_2Si(CH_2)_{11}{}^*CH_3$	9.5	12M12(9.5)	50
$Cl(CH_3)_2Si(CH_2)_{11}{}^*CH_2CH_3$	9.8	13M12(9.8)	25
$Cl(CH_3)_2Si(CH_2)_3{}^*CH_2(CH_2)_7CH_3$	8.3	12M4(8.3)	25
$Cl_3Si(CH_2)_7{}^*CH_3$	9.5	8T8(9.5)	25
$Cl_3Si(CH_2)_9{}^*CH_3$	10.4	10T10(10.4)	25
$Cl_3Si(CH_2)_{11}{}^*CH_3$	10.8	12T12(10.8)	50
$Cl_3Si(CH_2)_{11}{}^*CH_3$	13.7	12T12(13.7)	50

[a] Asterisk denotes the position of enrichment. [b] Bound carbon.

of mixed aqueous–organic LC mobile phase systems have been examined.

In the current study by applying these same labeling techniques spin–lattice relaxation times have been measured for several surface attached hydrocarbons. Likewise, these measurements have been made in terms of position of labeling, surface attachment chemistry, and degree of coverage.

EXPERIMENTAL SECTION

Materials. Labeled chlorosilanes were synthesized via barium carbonate routes as reported previously (*31*, *32*). Before use the reagents were purified by vacuum distillation and their identity was verified by several techniques which included infrared spectroscopy and nuclear magnetic resonance spectrometry. Subsequently, all surface modifications were carried out on 10-μm Lichrosorb SI-60 silica (E. Merck Labs) also as described in earlier work (*29*). Following preparation a portion of each of these materials was analyzed for bound carbon (Huffman Laboratories, Wheatridge, CO).

NMR Experiments. NMR experiments were carried out on 0.5–0.8 g of modified silica from each reaction batch. The materials were placed in standard 10-mm tubes, appropriate solvents added, and the contents thoroughly mixed with a mechanical vortex shaker. Following this, each tube was left to stand undisturbed for at least 6 h before spectra were acquired. All experiments were carried out under proton decoupled conditions at ambient temperature on a Varian FT-80 spectrometer. The normal spectra were acquired using square wave modulation decoupling while the T_1 measurements were made under pseudorandom conditions. Both were carried out with a bandwidth of 2000 H_z (i.e., frequency 66.67). Depending on the experiment either $CDCl_3$ or CD_3CN was employed as solvent.

RESULTS AND DISCUSSIONS

A summary of the surfaces which were synthesized and studied in the current work appears in Table I. Shown in the first and second columns are the labeled silanes used to prepare each of these materials and the degree of surface coverage reported as percentage of bound carbon, respectively. The corresponding short notation used to identify each surface throughout the remainder of this paper is summarized in column three and is based on the following. The first number denotes the length of the attached carbon chain. The subsequent letter refers to the bonding chemistry utilized to prepare each surface (i.e., M and T correspond to monochloro- and trichloro- chemistry, respectively). The number which follows is used to indicate the site of the ^{13}C enrichment (i.e., position along the chain from the Si atom). The final number which appears in parentheses corresponds to the surface coverage. Also listed in Table I is the degree of enrichment for the various silanes.

Representative spectra obtained from $CDCl_3$ for the monochloro-modified surfaces, 12M12(9.5), 10M10(7.9), and 8M8(7.4) appear in Figure 1. Likewise, shown in Figure 2 are the spectra for the 12T12(10.8), 10T10(10.4), and 8T8(9.5) surfaces. This latter series of material was similar to those shown in Figure 1 except that they were prepared by using trireactive instead of monoreactive chemistry. Also included

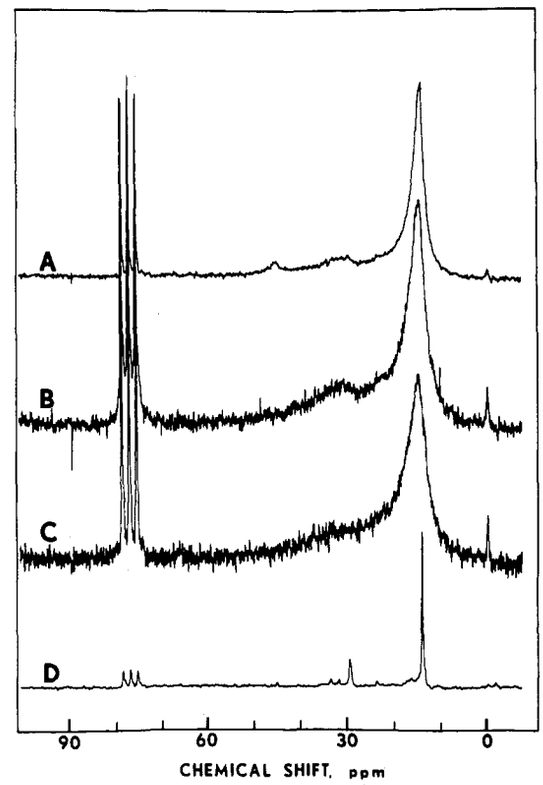

Figure 1. Spectra of monochloro-modified surfaces with terminal labeling: solvent, $CDCl_3$; surfaces (A) 12M12(9.5), (B) 10M10(7.9), (C) 8M8 (7.4); stabilized liquid (D) $(CH_3)_3Si(CH_2)_{11}{}^*CH_3$.

in both Figures 1 and 2 is the corresponding spectrum for the control compound $(CH_3)_3Si(CH_2)_{11}{}^*CH_3$ which was obtained by reacting terminally labeled *n*-dodecylchlorosilane with excess methylmagnesium iodide. The narrow resonance line which was observed for this latter compound in the presence of the base silica demonstrates that line broadening for the attached groups is not due to the presence of silica but rather arises from chemical attachment. Others also have observed line broadening as a result of bonding rather than the physical presence of the matrix material (*33*).

Chemical shift values for the compounds shown in Figures 1 and 2 are summarized in Table II. As expected for all terminally labeled carbons there was no observed change in chemical shift due to bonding. Reported shift values (*26*) for the terminal methyl of similarly modified surfaces obtained by using CP-MAS agree well with the data listed in Table II.

Shown in Figure 3 are spectra obtained from surfaces with labeling incorporated at various positions along the bonded chain. As in the case of the terminally labeled surfaces chemical shift values for the adjacent methylene carbon (i.e., for the 13M12(9.8) material) were relatively unaffected by bonding. Similar values for chemical shift before and after bonding for far removed carbons also have been reported by others (*22*, *23*). The observed shifts to low field for the surface

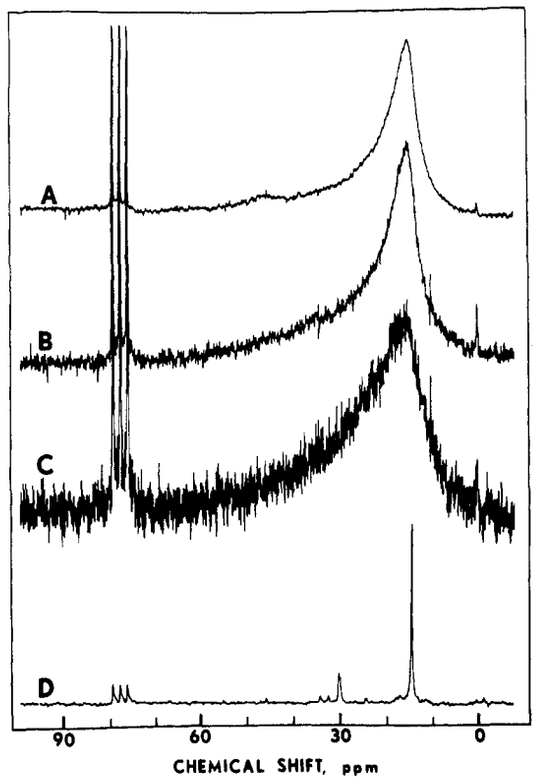

Figure 2. Spectra of trichloro-modified surfaces with terminal labeling: solvent, $CDCl_3$; surfaces (A) 12T12(10.8), (B) 10T10(10.4), (C) 8T8(9.5); stabilized liquid (D) $(CH_3)_3Si(CH_2)_{11}{}^*CH_3$.

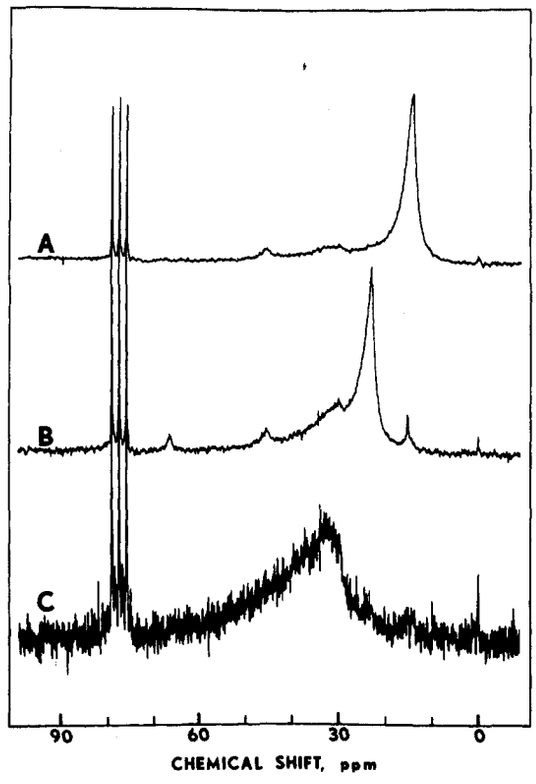

Figure 3. Spectra of monochloro-modified surfaces with labeling at various positions: solvent, $CDCl_3$; surfaces (A) 12M12(9.5); (B) 13M12(9.8); (C) 12M4(8.3).

with the fourth position labeling also agree with CP-MAS results (*22*, *23*).

As shown in Figures 1–3 the shapes of resonance lines were dependent on the position of labeling. The observed resonances were symmetrical for the terminal and adjacent carbons. However, at the fourth position definite asymmetry was observed. These data can be explained by considering both homogeneous and heterogeneous effects as discussed by Palmer and Maciel (*34*). For terminal carbons and adjacent methylene units which are removed a sufficient distance from surface, heterogeneous effect arrising from variations in the backbone bonding chemistry and the existence of different types of reaction sites contribute very little. On the other hand such effects play a dominate role at or near the surfaces and are illustrated by the resonance profile shown in Figure 3 for the material with the fourth position carbon label. Likewise, comparisons of trichlorosilane-modified surfaces prepared with labeling near the surface also have shown similar asymmetry (*23*).

Typical inversion recovery spectra are shown in Figures 4 and 5. Spin–lattice relaxation times (T_1) were measured for the 8M8(7.4), 10M10(7.9), 12M12(9.5), 13M12(9.8), 12T12-

Table II. Chemical Shift Values

sample	chemical shift: silane[a]	chemical shift: surface
8M8(7.4)	14.1	14.1
10M10(7.9)	14.1	14.1
12M12(9.5)	14.1	14.1
13M12(9.8)	22.7	22.7
12M4(8.3)	29.3	33.0
8T8(9.5)	14.1	14.4
10T10(10.4)	14.2	14.4
12T12(10.8)	14.1	14.6

[a] Unreacted modifying reagent.

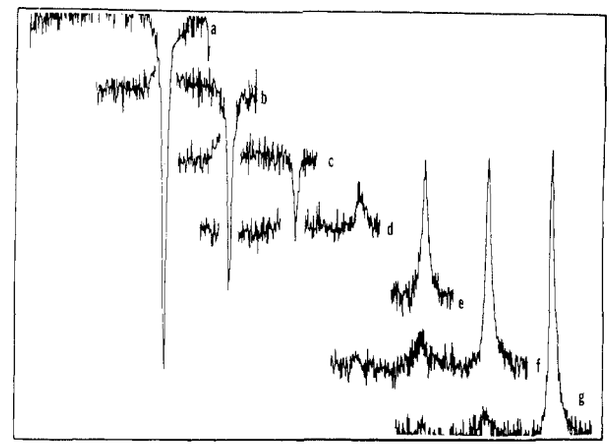

Figure 4. Inversion-recovery spectra for 12M12(9.5) surface: solvent, CD_3CN; pulse delay (in seconds) (A) 0.5, (B) 1.0, (C), 1.5, (D) 2.0, (E) 2.5, (F) 3.0, (G) 3.5.

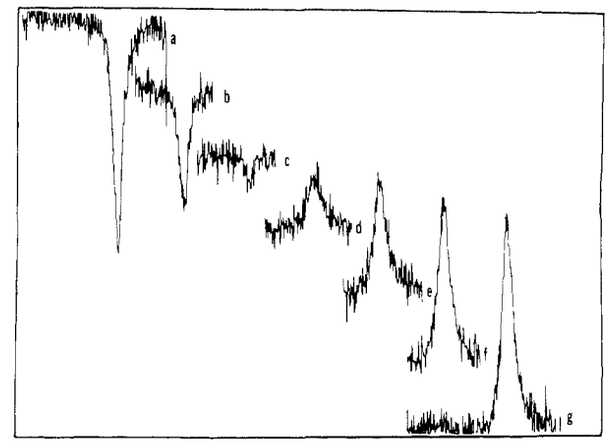

Figure 5. Inversion-recovery spectra for 12T12(10.8) surface: solvent, CD_3CN; pulse delay (in seconds) (A) 0.5, (B) 1.0, (C) (1.5, (D) 2.0 (E) 2.5, (F) 3.0, (G) 3.5.

Table III. Spin-Lattice Relaxation Times

sample	normalized carbon	T_1 values[a]
8M8(7.4)	0.74	3.37 ± 0.02
10M10(7.9)	0.66	3.22 ± 0.15
12M12(9.5)	0.68	3.50 ± 0.12
13M12(9.8)	0.65	2.02 ± 0.07
12T12(10.8)	0.90	3.25 ± 0.10
12T12(13.7)	1.14	2.66 ± 0.19
$(CH_3)_3Si(CH_2)_{11}{}^*CH_3$	*b*	5.94 ± 0.54
$(CH_3)_3Si(CH_2)_{11}{}^*CH_3$ + silica	*b*	4.92 ± 0.07

[a] Spin-lattice relaxation time in seconds. [b] Unreacted stabilized monomer.

(10.8), and the 12T12(13.7) surfaces in CD_3CN. Likewise, T_1 was determined for the unreacted terminally labeled control compound, *n*-dodecyltrimethylsilane. These latter measurements were carried out in $CDCl_3$ in the presence and absence of silica. All T_1 results have been summarized in Table III along with values for normalized carbon (i.e., percentage bound carbon/hydrocarbon chain length) for each surface.

In the case of the surfaces prepared with monochloro chemistry, similar values for normalized carbon were obtained. Comparable coverage per unit surface area is not unreasonable since the bound hydrocarbon chains do not differ radically in size. Likewise, the spin–lattice relaxation times arising from the terminal methyl carbons of these materials as well as from the terminal methyl carbons of materials prepared with trichlorosilane attached chains of similar coverage (i.e., the 12T12(10.8) surface) were not significantly different. These results suggest that for terminal methyl carbons which are removed sufficiently from the surface that spin–lattice relaxation time is nearly independent of chain length and backbone chemistry for surfaces with similar coverages. In such cases the principal source of motion arises from rotation about the end bond. Additionally, these observations are consistent with the data of Sindorf and Maciel (*24*) who, using CP-MAS NMR techniques, found for similar unsolvated systems little difference in cross-polarization rates for end carbons removed roughly eight bonds or more from the surface.

A reduced T_1 value was observed for the terminally adjacent carbon labeled surface 13M12(9.8). This result is consistent with expected reductions in carbon mobility along the alkyl chain in the direction of attachment.

In the current system, since the bonded chains were attached to a rigid matrix, the overall molecular motion was slow. In the case of the terminal methyl carbons, the predominant motion was rotation about the end bond and was independent of chain length for the bonded groups studied. However, as the surface coverage was increased, a point was reached where a significant decrease in the spin–lattice relaxation time was observed as seen by comparing differences in T_1 (i.e., 3.25 vs. 2.66) between the 12T12(10.8) and 12T12(13.7) surfaces, respectively. A possible explanation for this might be steric interaction of the alkyl chains at higher surfaces coverage, which might lead to hindered end rotation. Gilpin et al. (*3–5*) have suggested the interaction of hydrocarbon chains in models which explain thermally induced orientational changes for similar surfaces. Such changes have been observed chromatographically at normalized surfaces coverages similar to that on the 12T12(13.7) surface. These results thus suggest the idea of a critical alkyl surface density were terminal end rotation is significantly hindered.

CONCLUSIONS

The above results demonstrate the advantages of selective labeling of the bonded phase coupled with conventional solution FT-NMR. These techniques thus provide a means of investigating solvent effects on surface bound layers. As in the case of T_1 measurements, NOE and T_2 can be determined for the bonded carbons. These latter measurements can be compared to the liquid-state counterpart to provide additional understanding of bonded alkyl mobility. These studies are currently in progress.

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