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EXAMINATION OF THE EFFECT OF SOLVENT COMPOSITION ON BONDED PHASE LIQUID CHROMATOGRAPHY PACKINGS BY ^{13}C FOURIER TRANSFORM NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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SUMMARY

Commercially available bonded phase liquid chromatographic (LC) packings have been examined using ^{13}C Fourier transform nuclear magnetic resonance spectroscopy (^{13}C FT-NMR). Conditions inside the NMR sample tube were set up to closely mimic the actual conditions inside a LC column. To eliminate any effects of deuterated solvents on the chromatographic packings, an internal reference tube containing the deuterated locking reagent was placed inside the sample tube. Three peaks were seen when ^{13}C FT-NMR analysis was conducted of C_8 and C_{18} packings. The first peak, represented the free methyl group of the carbon chain, the second peak represented the β-CH_2 group, the remaining peak, which was usually very large, was the bulk $-CH_2$ peak representing the remaining carbons in the chain. Changes in the shape and size of these peaks with changes in solvent composition of the mobile phase reflected movement of the free methyl group and the remaining CH_2 groups in the chain and may be indicative of a conformational change of the bonded phase.

INTRODUCTION

Models have been proposed for the orientation and configuration of the alkyl-bonded surface of liquid chromatographic (LC) stationary phases[1–3]. Initially, these models were supported by chromatographic retention data[4,5], fluorescence[6,7] and infrared (IR) spectroscopy[8–12]. More recently ^{13}C Fourier transform nuclear magnetic resonance spectroscopy (^{13}C FT-NMR) has been used to study the physical and chemical properties of bonded phase LC packings[13–22].

Specifically, the two most common models that have been suggested are: the rigid or "brush" configuration, and the "folded" or associated configuration[1]. The brush configuration suggests that each alkyl chain exists independently, extending outward from the surface of the silica, not interacting with other chains. The associated configuration suggests that the alkyl chains form an aggregated sheath that

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is coiled so as to minimize the surface area in contact with the polar mobile phase[2].

Lochmüller and Wilder[2] and Tananka *et al.*[23] have supported this folded model by citing the hydrophobic association of alkanes in aqueous solutions at low solute concentrations and the likelihood that bonded alkanes would interact similarly in aqueous–organic mobile phase. In addition, the brush model would require solvent structuring to isolate each bristle, an energetically unfavorable condition. Gilpen and co-workers[1,18,19] have more recently described a dynamic model in which the alkyl phase bonded to the surface can undergo changes in orientation and mobility with changes in certain parameters, *i.e.*, temperature, solvent, and composition of the bonded chain.

The current study is an attempt to examine this dynamic model. It is unique in that it uses conventional FT-NMR techniques, to mimic as closely as possible the actual conditions in a LC column. Generally, cross polarization–magic angle spinning (CP–MAS) studies have been used to generate the most information about solid stationary phases. The main disadvantage of those CP–MAS studies is that they must be carried out in the absence of solvent, and therefore do not represent actual LC conditions. Conventional solution FT-NMR studies have been conducted on surfaces modified by *n*-alkylchlorosilanes and triethoxy-(alkylamino)silanes[16,18,20,21].

Burke[13] has proposed using the size width at half height, $w_{\frac{1}{2}}$, of the bulk $-CH_2$ peaks as a measure of the liquid-like nature of the bonded alkyl chain. Narrow peaks, having small $w_{\frac{1}{2}}$ values, indicated a more liquid-like nature. Although, the peak widths encountered in the present study are wide (as large as 10 ppm) compared to those for typical solutions (1 ppm and less), a change in $w_{\frac{1}{2}}$ value can be used to estimate the effect of a change in solvent composition on the bonded alkyl chain. Generally, a change in the $w_{\frac{1}{2}}$ value of the bulk $-CH_2$ peak is easier to monitor because of its size. Our work has been conducted on commercially available reversed-phase LC packings.

EXPERIMENTAL

Materials

The C_{18} commercially available packing materials examined were: Ultrapak™-ODS, 10 μm, Lot No. 81-1, %C = 17.49 (Beckman, Norcross, GA, U.S.A.), LiChrosorb RP-18, 10 μm, Batch No. 2406, %C = 17.61 (Alltech, Norcross, GA, U.S.A.), and Polygosil 60-10, 10 μm, Batch No. 9051, %C = 7.40 (Bodman Chemicals, Doraville, GA, U.S.A.). The C_8 packings were: Polygosil 60-10, 10 μm, Batch No. 8051, %C = 5.76 (Bodman Chemicals, Doraville, GA, U.S.A.), and Adsorbosphere 5 μm, Lot No. 018403, %C = 6.39 (Alltech). A C_2 packing, LiChrosorb RP-2 (Alltech), was also examined.

Deuterated solvents purchased from Aldrich (Metuchen, NJ, U.S.A.) were ^2H_2O (99.8 atom % ^2H) and C^2HCl_3 (99.8 atom % ^2H). Other solvents were HPLC-grade (J. T. Baker, Phillipsburg, NJ, U.S.A.), and they were filtered through 0.5-μm pore-size filters type FH (Millipore, Bedford, MA, U.S.A.).

Water was purified by a two-stage deionization process followed by passage through an activated charcoal column to remove trace organics before being distilled. The water was then filtered through 0.45-μm filters, type HA (Millipore, Bedford, MA, U.S.A.).

Spectrometers

Two FT-NMR instruments from JEOL (Tokyo, Japan) were used during different phases of this study. One was a Model JNM-FX90Q spectrometer and the other a Model FX270 spectrometer. NMR tubes were 17.8 × 0.5 or 1.0 cm from Wilmad (Buena, NJ, U.S.A.).

Procedures

The NMR experiments were conducted on 0.8–1.0 g of the commercially available alkyl-bonded silicas that were weighed directly into 10-mm NMR sample tubes. Approximately 5–6 ml of the desired mixture of solvents was added as a solution and the tubes hand-shaken until a uniform suspension, topped by a 1–2 cm portion of solvent, was obtained. The internal reference, a 5-mm NMR tube containing the deuterated locking reagent was then carefully inserted inside the sample tube. The tubes were then ultrasonicated for 30 sec to 1 min to remove trapped air. The sample was usually allowed to stand for 24 h (shorter times are sometimes indicated) before the NMR analysis was run.

The packing material was rejuvenated after each use according to the procedure recommended by Burke[13]. The packing was filtered and rinsed with five 25-ml portions of filtered HPLC-grade methanol. The washed packing was dried overnight at 40–100°C under vacuum. In some cases, the complete procedure had to be repeated because the NMR spectrum revealed extraneous solvent peaks.

Each spectrum was the accumulation of 20 000 scans except where noted. The radio-frequency pulse width was 15.0 μsec with a flip angle of 45°. The pulse was followed by a 1.9 msec pre-delay and a 278.5 msec acquisition time; the post-delay was 2.0 sec. Any differences in these pulse conditions are noted. An internal lock of C^2HCl_3 was used for the FX90Q measurements; ^2H_2O was used for the FX270 measurements. All spectra were broadband decoupled. To compare spectra under equivalent conditions, both the normalized gain, *N*-gain, and the sensitivity, *Y*-gain, had to be controlled. For different values of *N*-gain, the *Y*-gain was adjusted; usually a factor of two was involved.

RESULTS AND DISCUSSION

In the spectra that follow, the peak assignments are as follows:

Octane

CH_3–CH_2–CH_2–CH_2–CH_2–CH_2–CH_2–CH_3

a b d c c d b a

a = 14.1 ppm c = 29.8 ppm

b = 23.1 ppm d = 32.3 ppm

Octadecane

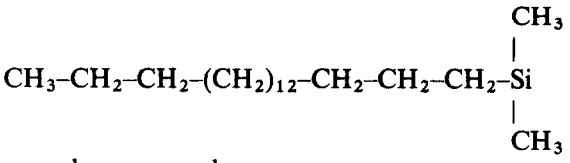
CH_3–CH_2–CH_2–CH_2–$(CH_2)_{10}$–CH_2–CH_2–CH_2–CH_3

a b e c d c e b a

a = 14.2 ppm c = 29.6 ppm e = 32.2 ppm

b = 22.9 ppm d = 30.0 ppm

ODS silica



a = 12.89 ppm	d = 30.35 ppm	y = 34.13 ppm
b = 22.88 ppm	e = 32.42 ppm	z = 0.29 ppm
c = 23.69 ppm	x = 18.20 ppm	

The assignments of the peaks for ODS silica were deduced from the ^{13}C NMR Sadtler reference spectra of octane and octadecane[24] and from the ^{13}C and ^{29}Si CP–MAS assignments of Bayer *et al.*[21] for chemically modified silica gels. Figs. 1 and 2 show a ^{13}C NMR spectrum of Ultrapak™-ODS and one of Polygosil C_8, each in acetonitrile (100%). The peaks at 1.7 and 117.4 ppm can be attributed to the acetonitrile. The three peaks at 14, 23, and 30 ppm have been attributed to the free methyl group on the end of the chain, the β-CH_2 group and the remaining detectable carbons, respectively. Fig. 1 shows a merging of peaks c, d, and e from the octadecane spectrum simply because the short relaxation times associated with solide cause band broadening. Fig. 2 shows a similar merging of peaks from c and d from octane.

Fig. 3 shows a CP–MAS spectrum of Ultrapak™-ODS in which five additional peaks became sufficiently distinct to be identified in the alkyl-bonded chain. These observations were possible because of the absence of solvent in this spectrum and the

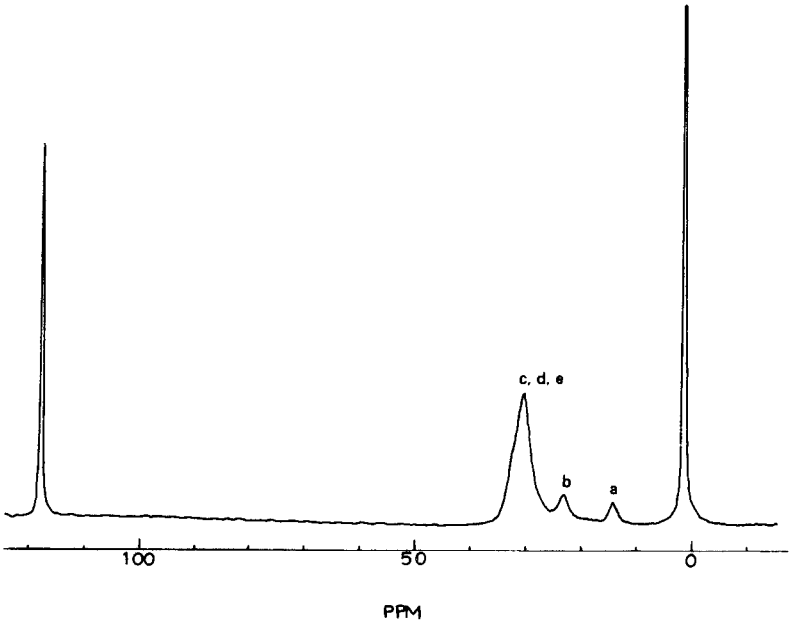


Fig. 1. ^{13}C spectrum of Ultrapak™-ODS in 100% acetonitrile using the FX270 at 20 000 Hz; locking agent, ^2H_2O; pulse width, 7500 μsec; delay, 0.5 μsec; and total acquisition time, 204.8 msec.

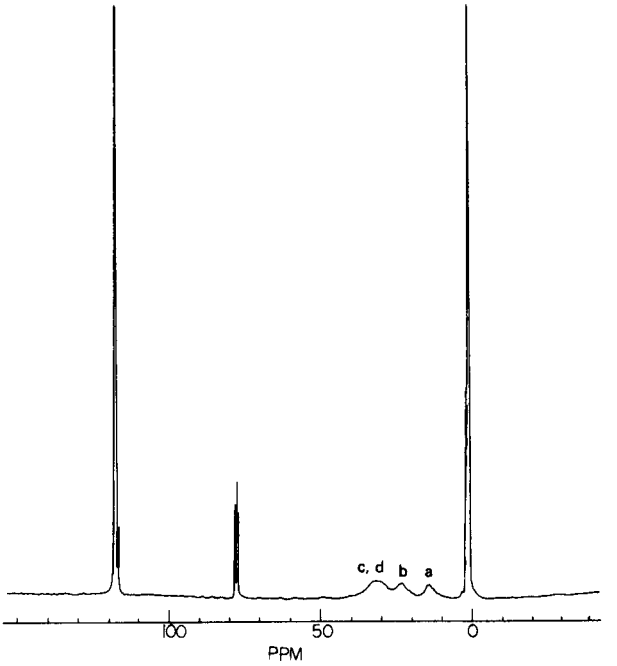


Fig. 2. ^{13}C spectrum of Polygosil C_8 in 100% acetonitrile using the FX270 at 20 000 Hz; locking agent, C^2HCl_3 (accounts for peaks near 77 ppm); pulse width, 7.500 μsec; delay, 0.5 μsec; total acquisition time, 204.8 msec.

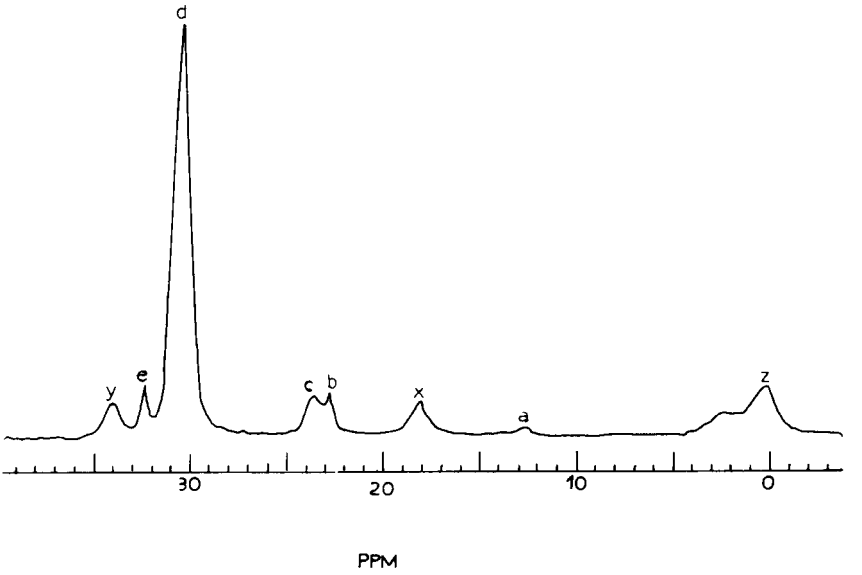


Fig. 3. CP–MAS spectrum of solid Ultrapak™-ODS using the FX270 at 25 000 Hz and the solids probe; pulse width, 6.00 μsec; delay, 19.7 μsec; total acquisition time, 204.8 msec.

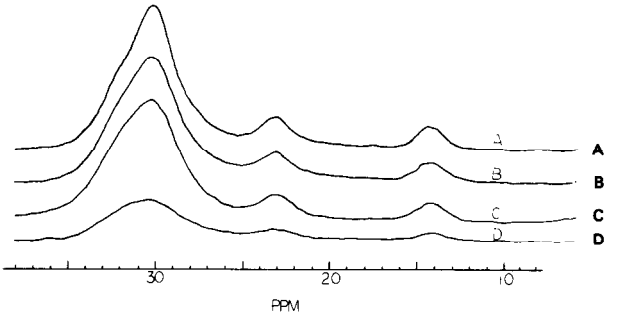


Fig. 4. ^{13}C spectra of Ultrapak™-ODS in different compositions of acetonitrile using the FX270 with the operating parameters shown in Fig. 1 for A, 100% acetonitrile; B, acetonitrile–water (75:25); C, acetonitrile–water (50:50); and D, acetonitrile–water (25:75). Note that the region of the solvent peaks has been omitted.

greater capability of CP–MAS. This spectrum supports the identification of the free end methyl, the β-CH_2 and the bulk $-CH_2$ peaks. The loss of these additional peaks in the solution spectra (Figures 1, 2 and 4–6) are the results of the short relaxation times of carbons in the vicinity of the silica surface. However, because the solvent is missing, this method clearly can not be used to monitor changes in chain movement due to a solvent change.

Fig. 4 shows representative spectra obtained for different compositions of acetonitrile–water in contact with Ultrapak™-ODS. Note that the bulk $-CH_2$ peak got wider with increasing water content in acetonitrile, thus indicating a decrease in the liquid-like nature of the bonded chain with an increase in water. The actual $w_{\frac{1}{2}}$ values for the bulk $-CH_2$ peaks can be found in Table I.

Also visible in Fig. 4 for 100% acetonitrile is the existence of a shoulder on the downfield side of the bulk $-OCH_2$ peak. This shoulder represents the γ-CH_2 peak that normally appeared near 32 ppm in the octane and octadecane spectra. With a higher water concentration in the mobile phase, this shoulder merged with the bulk $-CH_2$ peak as seen in Fig. 4 (B, C, and D). The peak locations of the bulk $-CH_2$ peak also showed a gradual downfield shift as the solvent composition changed from 100 to 25% acetonitrile. Both the shift of the peak location and the merging of the

TABLE I

PEAK LOCATIONS AND WIDTH AT HALF HEIGHT IN ppm FOR ULTRAPAK™-ODS IN ACETONITRILE–WATER MIXTURES

ppm Values known to ±0.1 ppm.

Acetonitrile (% in water)	*Bulk $-CH_2$ peak location*	*$w_{\frac{1}{2}}$ Values*		
		Bulk $-CH_2$	*β-CH_2*	*Free CH_3*
100	28.08	3.68	2.42	1.91
75	28.79	3.97	2.36	1.89
50	29.01	4.48	2.53	1.91
25	29.37	4.77	2.54	1.91

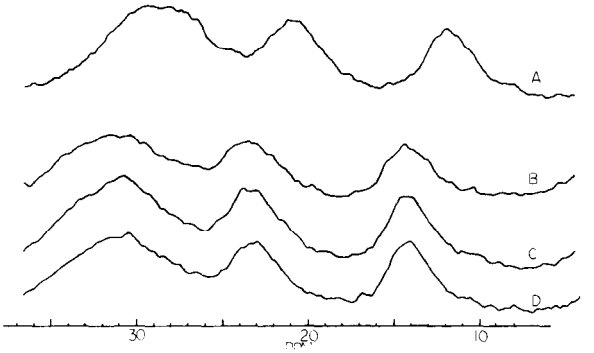


Fig. 5. ^{13}C spectra of Polygosil C_8 in different compositions of acetonitrile using the FX270 at 20 000 Hz; locking agent, ^2H_2O. A, 100% acetonitrile; B, acetonitrile–water (75:25); C, acetonitrile–water (50:50); and D, acetonitrile–water (25:75).

bulk and γ-CH_2 peaks support the loss of the liquid-like nature of the bonded chain with a decrease in organic component in the solvent.

The $w_{\frac{1}{2}}$ values of the free methyl and the β-CH_2 peaks showed little or no change with the percentage of acetonitrile as seen in Table I. This is in keeping with Burke's prediction that the free methyl peak represents only one carbon and remains, even after a change in solvent composition, restricted in motion only by being part of an anchored chain.

Fig. 5 shows corresponding spectra for Polygosil C_8 packing. Because of the lower signal-to-noise ratios in Fig. 5, trends are not as distinctly visible. The $w_{\frac{1}{2}}$ values are no longer indicative of the merging of the bulk and γ-CH_2 peaks, and no shoulder is visible, even at the 100% acetonitrile concentration. Most probably the merging has already taken place. Moreover, the loss of resolution between the bulk $-CH_2$ and β-CH_2 peaks indicates these two peaks merged as the concentration of water in-

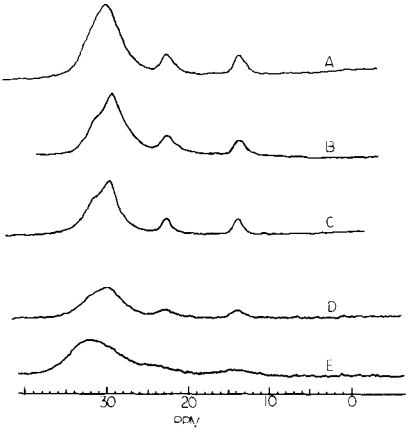


Fig. 6. ^{13}C spectra of LiChrosorb RP-18 in different compositions of *p*-dioxane–water using the FX270 at 20 000 Hz; locking agent. ^2H_2O. A, 100% *p*-dioxane; B, *p*-dioxane–water (75:25); C, *p*-dioxane–water (50:50); D, *p*-dioxane–water (25:75); and E, 100% water.

creased. Also, the free methyl peak maintained or increased somewhat its sharpness with a decrease in organic component in the mobile phase. That behavior supports Burke's idea that the free methyl peak that corresponds to the last carbon in the chain, actually gains some freedom of movement at intermediate concentrations in which part of the chain is anchored, (less restriction by the rest of the chain). The terminal methyl only loses its mobility or liquid-like nature at very low percentages of organic solvent. From our experimental data, that point was below 25% acetonitrile.

Fig. 6 shows the NMR spectra of LiChrosorb RP-18 in different compositions of *p*-dioxane and water. As seen for Ultrapak™-ODS in acetonitrile, the γ-CH_2 and bulk $-CH_2$ peaks merged with increasing water concentration (B, C, and D). The β-CH_2 peak merged on the upfield side of the bulk $-CH_2$ peak at a still greater water concentration (E). It is interesting to note that in 100% water virtually no peak was seen for either the free methyl or β-CH_2 groups. This is an indication that even the free methyl group is eventually restricted by the presence of pure water.

These same trends were examined on two other C_{18} packing materials using dioxane–water mixtures. The $w_{\frac{1}{2}}$ values and peak locations determined can be found in Table II. An additional peak was seen the high concentrations of dioxane for

TABLE II

PEAK LOCATIONS AND WIDTH AT HALF HEIGHT IN ppm FOR ULTRAPAK™-ODS, LICHROSORB RP-18, AND POLYGOSIL C_{18} IN *p*-DIOXANE–WATER MIXTURES

ppm Values known to ±0.1 ppm.

	Dioxane (% in water)	*Bulk $-CH_2$ peak location*	*$w_{\frac{1}{2}}$ Values*		
			Bulk–CH_2	*β-CH_2*	*Free CH_3*
Ultrapak™-ODS	100	30.58 (30.52)*	4.81	2.92	2.10
	75	30.79 (32.44)*	4.08	2.58	2.10
	50	30.14 (32.30)*	4.57	2.40	1.80
	25	32.37	6.91	**	4.81
LiChrosorb RP-18	100	30.79	4.67	2.54	1.88
	75	30.14 (32.45)*	4.11	2.33	1.76
	50	30.14 (31.87)*	4.11	2.05	1.60
	25	29.50 (32.52)*	5.38	3.04	2.28
	0	32.52	7.36	**	6.65
Polygosil C_{18}	100	28.43 (30.60)*	3.64	2.15	2.22
	75	30.36	3.70	2.22	1.61
	50	29.63	5.56	5.68	4.45
	25	29.28	12.41	**	**

* Visible shoulder.

** Not distinguishable from other peaks or baselines.

Ultrapak™-ODS and Polygosil C_{18}. This peak located near 0.3 ppm, indicates a C–Si bond. *p*-Dioxane, having a lower polarity index (4.8) than acetonitrile (5.8), allowed more of the bonded-alkyl carbon chain to behave as a liquid and, therefore, the C–Si bond became visible in the ^{13}C NMR.

This same feature was noted when a C_2 bonded chain was examined in two solvents having different polarity indices. Methanol, having a higher polarity index (5.1) than chloroform (4.1), showed only one peak, that correspoonds to the presence of the C–Si (0.11 ppm) bond. However, chloroform exhibited two peaks, one at the C–Si peak location (0.62 ppm) and the other at the free methyl group location (17.7 ppm).

CONCLUSION

The present study demonstrated that the shapes, locations and even the presence of ^{13}C NMR peaks, representing different portions of the bonded-alkyl chain in reversed-phase LC packings, changed with the solvent composition. The effects were consistent in that a greater concentration of an organic solvent and a longer bonded chain led to greater mobilities being observed for the groups at the free end of the chain. The observed effects indicated conformational changes in the folded or associated configuration, as suggested by Gilpen[1]. Additional studies using ^{13}C NMR of reversed-phase packings are currently being conducted.

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