DIFFERENCES IN CARBON 13 NUCLEAR MAGNETIC RESONANCE SPECTRA FOR MONOMERIC AND POLYMERIC OCTADECYL DERIVATIZED SILICA COLUMN PACKINGS FOR LIQUID CHROMATOGRAPHY

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SUMMARY

   Two different types of reversed-phase octadecyl derivatized silica (C_{18}) packings have been compared using cross-polarization, magic-angle spinning carbon 13 nuclear magnetic resonance spectrometry (^{13}C NMR) on dry solids and high-resolution ^{13}C NMR on suspensions in chromatographic solvents. Differences in the NMR spectra of the dry monomeric and polymeric C_{18} packings confirmed the differences in the manufacturing procedures for endcapping the C_{18} packings. The high-resolution studies on suspensions showed differences in peak shapes and areas for the packings. In pure methanol as opposed to pure chloroform the peaks due to the alkyl chain on the polymeric C_{18} packing were almost lost in the baseline, while the peaks for the monomeric packings were decreased much less. In both cases, the decreases in the sizes of these peaks reflected decreased mobility of the groups in the alkyl chain and suggests that the chain is lying on the surface. The differences in the spectra for the packings provides a basis for rationalizing the differences in chromatographic retentions reported by others for polycyclic aromatic hydrocarbons.

INTRODUCTION

   High-performance liquid chromatography (HPLC) has become one of the preferred methods for separation and analysis of polycyclic aromatic hydrocarbons (PAHs). In 1971, Schmit *et al.*[1] first described the use of a chemically bonded octadecylsilica (C_{18}) for the separation of PAHs. Since then, a majority of the separations of PAHs reported in the literature have employed reversed-phase C_{18} columns[2-4]. A comparison of retention data and reports by Wise *et al.*[5], and Ogan and Katz[6] indicates that C_{18} columns from different manufacturers provide not only different separation efficiencies but different retention characteristics and selectivities for

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PAHs. Even the nature of the organic layer attached to the silica surface, *i.e.* monomeric or polymeric C_{18}, affects the retention characteristics and selectivities for PAHs.

Wise[7] has summarized the retention characteristics of more than 100 PAHs on both monomeric (Zorbax ODS) and polymeric (Vydac 201TP) C_{18} materials. Several interesting trends were observed by Wise. He observed that the separation of chrysene and benz[*a*]anthracene varied with the nature of the C_{18} layer. For the monomeric materials, as expected, chrysene eluted prior to benz[*a*]anthracene with the greatest separation factor. On polymeric phases, the reverse elution order of these two solutes was observed.

Polyphenyl arenes exhibit significantly different retention characteristics on monomeric *versus* polymeric C_{18} materials. Katz and Ogan[8] and Wise *et al.*[5] have illustrated the behavior of a polyphenyl arene, 9,10-diphenylanthracene, on different C_{18} columns. On the monomeric columns 9,10-diphenylanthracene eluted after benzo[*a*]pyrene, whereas on polymeric materials it eluted prior to benzo[*a*]pyrene. Wise *et al.*[6] suggested that the elution order reversal for triphenylene and benzo[*c*]phenanthrene on monomeric and polymeric C_{18} materials may be due to nonpolarity. They also observed that polyphenylarenes exhibited greater retention than corresponding fused ring PAHs having an equivalent number of rings. Sleight[10] and Peaden *et al.*[11] have also observed that on monomeric C_{18} phases large PAHs in carbon black extracts elute approximately according to their molecular weights. In both studies, the compounds were generally of similar PAHs classes having planar structures.

More recently, Fetzer and Biggs[12] have examined the effect of planarity on chromatographic retentions of PAHs. The degree of the planarity of several members of these PAHs was found to depend on the solvent in which the sample was dissolved. (The degree of non-planarity of PAHs was estimated from the resolution between the UV–VIS spectral bands. The non-planar PAHs have shallower valleys between the band peaks.) According to Fetzer and Biggs[12], the reversed-phase behavior of these compounds is profoundly effected by the changes in planarity.

Fetzer and Biggs[13] have also observed that these PAHs show distinctive differences in retention behavior on a polymeric Vydac 218TP5 packing compared to monomeric IBM ODS or Zorbax ODS columns. On a Vydac polymeric packing elution of the 9-ring tetrabenzo[*a,c,d,j,l,m*]perylene before the 8-ring compounds, and the elution of 7-ring peropyrene after the 8-ring compounds indicated a much different separation mechanism than a simple two-phase partition mechanism. Another interesting observation made by Fetzer and Biggs[14] was that a Vydac 218TP5 polymeric C_{18} packing retained planar PAHs longer than the non-planar, for a given number of cyclic carbon atoms. This was observed to be more prominent in a methanol–chloroform (60:40) system.

In the present study, an attempt has been made to examine the differences between monomeric (Ultrapak™-ODS) and polymeric (Vydac 218TP5) C_{18} packing materials using cross-polarization magic-angle spinning (CP-MAS) solid-state carbon 13 nuclear magnetic resonance (^{13}C NMR) techniques. High-resolution ^{13}C NMR spectra on suspensions of monomeric and polymeric C_{18} phases in chromatographic solvents were also obtained. The effects of the solvent on the physical nature and orientation of the C_{18} chain for both monomeric and polymeric phases have been discussed in terms of the relative areas and peak shapes.

EXPERIMENTAL

Materials and chemicals

The commerically available packings were: Ultrapak-ODS 10 μm (Altex, Berkeley, CA, U.S.A.) and Vydac protein and peptide C_{18} 218TP5 packing (Separation Group, Hesperia, CA, U.S.A.).

Deuterated solvents were purchased from Aldrich (Milwaukee, WI, U.S.A.). Other solvents were Baker HPLC-grade (J. T. Baker, Phillipsburg, NJ, U.S.A.) and were filtered through 0.5-μm pore size millipore filter type FH (Millipore, Bedford, MA, U.S.A.).

Instruments

The solid-state CP-MAS ¹³C NMR spectra were recorded using a MSL200 Fourier transform (FT)-NMR spectrometer (Bruker Instruments, Billerica, MA, U.S.A.). This system was equipped with an Aspect 2000 computer (48K, 24 bit) system and operated at 50.3 MHz for ¹³C.

The high-resolution ¹³C NMR of the suspensions of packings were recorded using a FX270 FT-NMR spectrometer (JEOL, Tokyo, Japan).

Procedures

Solid-state ¹³C NMR spectra. Spectra of the bonded C_{18} packings were obtained using a MAS solid-state probe. A detailed procedure is described in an earlier study from this laboratory[15].

High-resolution ¹³C NMR spectra of suspensions. These experiments were conducted on 0.8–1.0 g of stationary phase which was weighed directly into a 10-mm NMR tube. Of the desired mixture of solvents *ca.* 5–6 ml was added and the tube was hand-shaken until proper mixing was obtasined[16]. A ¹³C NMR spectrum of this suspension was run as explained in ref. 15.

RESULTS AND DISCUSSION

Solid-state NMR

Fig. 1 shows a CP-MAS solid-state ¹³C NMR spectrum of Ultrapak-ODS silica packing material that was run in the absence of a solvent. Due to the enhanced CP-MAS capabilities, a number of peaks were sufficiently distinct to be identified in the alkyl chain. The assignments of the peaks in this spectrum were deduced from the ¹³C NMR Sadtler reference spectra of octadecane[17], the ¹³C solution spectra of pure octadecylchlorosilane reagent, and from the assignments suggested by Bayer *et al.*[18]. The broad shoulder at about 2.0 ppm may represent some physically adsorbed –CH_2 on the silica surface.

Fig. 2 shows a similar CP-MAS solid-state ¹³C NMR spectrum of the Vydac 218TP5 C_{18} packing. It is very clear from the figure that there were significant differences between these two C_{18} packings. Table I shows a comparison of ¹³C NMR shifts of dimethyloctadecylchlorosilane reagent in C^2HCl_3 *versus* the solid-state ¹³C NMR shifts of Ultrapak-ODS and Vydac 218TP5 C_{18} packings. The assignments of the solid-state spectra were based on the completely decoupled ¹³C NMR shifts of the silane recorded in C^2HCl_3 at 67.80 MHz. In comparing the ¹³C chemical shifts
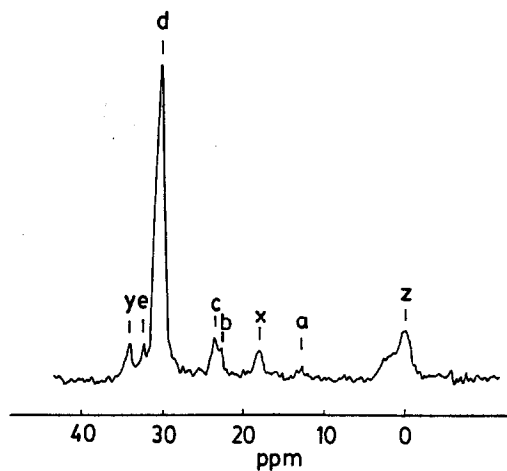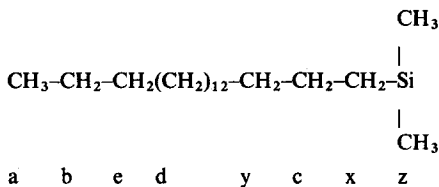
Fig. 1. CP-MAS solid-state ^{13}C NMR of UltraPak-ODS using the MSL200-FT-NMR spectrometer at 50.3 MHz anmd the solid probe. The assignments of the peaks in this spectrum of ODS silica are as follows:

$$
\begin{array}{c}
\text{CH}_3 \\
|
\end{array}
$$

CH$_3$–CH$_2$–CH$_2$(CH$_2$)$_{12}$–CH$_2$–CH$_2$–CH$_2$–Si

$$
\begin{array}{c}
| \\
\text{CH}_3
\end{array}
$$

a   b   e   d   y   c   x   z

where: a = 12.89 ppm; b = 22.85 ppm; c = 23.66 ppm; d = 30.33 ppm; e = 32.39 ppm; x = 18.20 ppm; y = 34.13 ppm; z = 0.09 ppm.
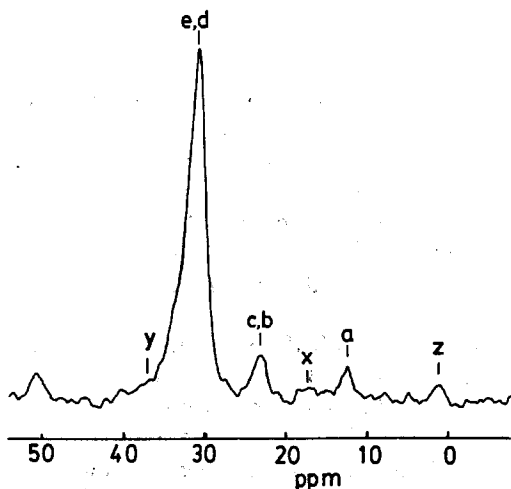

Fig. 2. CP-MAS solid-state ^{13}C NMR of Vydac 218TP5 using the MSL200-FT-NMR spectrometer. Peaks: a = 12.42 ppm; b, c'= 23.15 ppm; d, e = 30.87 ppm; z = 1.19 ppm; y is hidden; x = 18.2 ppm.

TABLE I

COMPARISON OF DIMETHYLOCTADECYLSILANE ¹³C NMR PEAK POSITIONS WITH UL-
TRAPAK-ODS AND VYDAC 218TP5 C_{18} PACKINGS

$$CH_3$$
$$|$$
$$H_3C-CH_2-CH_2(CH_2)_{12}-CH_2-CH_2-CH_2-Si-Cl$$
$$|$$
$$CH_3$$

a   b   e   d   y   c   x   z

Peak assignments	Dimethyloctadecylsilane in chloroform solution ¹³C NMR chemical shifts (ppm)*	Ultrapak-ODS CP-MAS ¹³C NMR (ppm)**	Vydac 218TP5 CP-MAS ¹³C NMR (ppm)**
a	14.14	12.89	12.42
b	22.71	22.85	23.15 (b, c)
c	23.01	23.66	
d	29.74, 29.56, 29.38	30.33	30.87 (e, d)
e	31.95	32.39	
x	19.04	18.20	18.32
y	32.99	34.13	– ***
z	1.65	0.09–2.0	1.19

    * Chemical shifts referenced to external tetramethylsilane.
   ** Chemical shifts references to poly(dimethyl silane).
  *** Peak y merged into peak d.

of Ultrapak-ODS and Vydac C_{18} packing in the solid-state with those of dimethyl-
octadecylchlorosilane, we have to consider the differences in the chemical environ-
ment of the silicon atom. The solid reagent has a chlorine attached to its silicon,
whereas the monomeric packing will have the oxygen of a surface silanol; the poly-
meric packing may have up to two oxygens (and sometimes no methyl group). The
differences in chemical shifts for carbon x, ($Si-CH_2$) is about 1 ppm. The chemical
shifts of the terminal methyl carbon for both packings is lower by about 2 ppm.

In Fig. 2, peak x was found to be almost buried in the baseline, and peak b
merged into peak c and peak y and e were either absent or merged into peak d at
30.87 ppm. Another important difference was that peak z (the two methyls on the
silicon) for the Vydac packing was much smaller than that for the Ultrapak-ODS
packings. For the remaining peaks, the chemical shifts differed from those in Fig. 1.
Furthermore, an additional resonance at about 50 ppm was observed, probably due
either to $Si-OCH_3$, which could have been generated by slow solvolysis during stor-
age in methanol, or to adsorbed methanol used in the washing procedure. Thus, the
two C_{18} packings were distinctly different.

Vydac 218TP5 packing is prepared by reacting a dichloromethyloctadecylsi-
lane reagent with silica and then endcapping with hexachlorodisilane. Hence, Vydac
218TP5 packing should have fewer $-CH_3$ groups on the silica from the silane as was
evident from the size of peak z. On the other hand, Ultrapak-ODS packing, which
is synthesized by reacting a dimethyloctadecylchlorosilane reagent with a silica sur-
face followed by trimethylchlorosilane as an endcapping reagent has more $-CH_3$
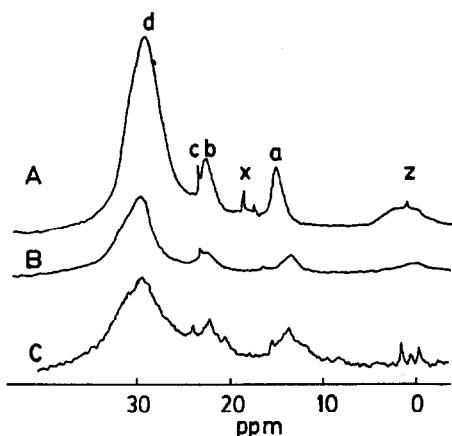groups attached to silicon from the silane reagent.

Fig. 3. ^{13}C FT-NMR spectra of Ultrapak-ODS in varying compositions of methanol–chloroform using the FX270 FT-NMR spectrometer at 20000 Hz and the locking agent, $^{2}H_{2}O$. (A) 100% chloroform, (B) chloroform–methanol (1:1) and (C) 100% methanol. All the parameters were constant in these three spectra.

High-resolution ^{13}C NMR on suspensions

Fig. 3 shows spectra obtained for Ultrapak-ODS packing in different compositions of a methanol–chloroform mobile phase. It has been proposed by Yonker *et al.*[19] and McNally and Rogers[20] that width at half-height of the bulk –CH$_2$ peaks can be used as a measure of the liquid-like nature of the bonded alkyl chain. Greater peak widths are a result of less freedom of movement due to interactions of the bonded phase chain with the surface, including other bonded-phase chains and unreacted silanols while narrow peaks indicate a more liquid-like nature. The peak widths encountered in this study are wide (as large as 10 ppm) when compared to typical solution NMR values (1 ppm and less).

As seen in Fig. 3, maximum chain mobility was obtained in 100% chloroform (A) for the ODS packing. In 100% methanol (C) the bulk –CH$_2$ peak and some of
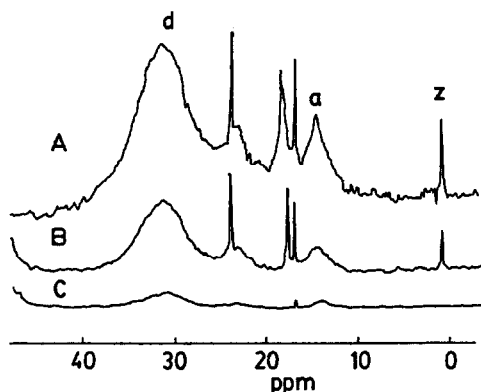

Fig. 4. ^{13}C FT-NMR spectra of Vydac 218TP5 in varying compositions of methanol–chloroform using the FX270 FT-NMR spectrometer at 20000 Hz. (A) 100% chloroform, (B) chloroform–methanol (1:1) and (C) 100% methanol. NMR operating parameters and expansions were the same as in Fig. 3.

the other peaks were wider while still other peaks were absent. In methanol–chloroform (1:1) (B) there was no significant difference in line shape for most of the peaks as compared to 100% chloroform (A).

Fig. 4, shows similar spectra of Vydac 218TP5 packing when suspended in the same solvents. It was clear that as one changed the solvent from pure chloroform to pure methanol the peak width at half-height increased for the bulk $-CH_2$ peaks as well as for the β-CH_2 and terminal methyl groups. In pure methanol, the peaks were almost lost in the baseline. These drastic changes in peak shape clearly indicated a greater solvent effect on this packing than on Ultrapak-ODS.

Table II shows the relative areas of the bulk $-CH_2$, β-CH_2 and terminal methyl groups in the same three different solvents for the Ultrapak-ODS and Vydac C_{18} packings. Note the dramatic decrease in the peak areas for all three peaks as the solvent polarity changed from pure chloroform to chloroform–methanol (1:1) to pure methanol. The changes in area were especially large for the Vydac packing. This table clearly suggests that in 100% chloroform the C_{18} alkyl chain was freely moving and was interacting with the solvent, but, as the percentage of methanol increased the molecular motion of the bonded alkyl chain became more restricted. In pure methanol, virtually no peak was seen for either the terminal methyl or β-CH_2 peaks of Vydac C_{18} packing suggesting that the alkyl-bonded chain is lying flat on the surface. On the other hand, with the monomeric Ultrapak-ODS packing there were noticable differences in peak asrea under three different solvents but the changes were not that drastic. Particularly, the area of the bulk $-CH_2$ peak, in chloroform–methanol (1:1) and pure methanol, changed only by a very small amount suggesting that the change in solvent composition had little effect upon the orientation of the monomeric C_{18} chain.

Chromatographic studies by Fetzer and Biggs[12] on Vydac 218TP5 polymeric C_{18} packing under identical conditions revealed an increase in the capacity factor, k', for the PAHs in pure methanol. Fetzer and Biggs[14] and others[5–7] have observed that on a polymeric C_{18} packing (particularly the Vydac packing) the planar PAHs had higher k' values than the non-polar. Fetzer and Biggs[14] have also observed that, irrespective of the number of cyclic ring carbons, planar PAHs were held longer on Vydac 218TP5 C_{18} packing. On the other hand, on a monomeric C_{18} packing, they observed that the elution order of PAHs paralleled their molecular weight or the number of cyclic ring carbons. They also observed that, in pure chloroform on Vydac 218TP5 polymeric C_{18} packing, the relative k' values of both planar and non-planar PAHs was much smaller, but the planar PAHs were held longer than the non-planar.

CONCLUSIONS

The results shown here demonstrate that changes in the shape, location, area and in some cases, the presence of ¹³C NMR peaks representing different portions of the bonded alkyl chain in reversed-phase LC packings occur with changes in the solvent composition. These changes depended upon the type of C_{18} packing. For a monomeric C_{18} packing, less effect of solvent composition was observed than for a polymeric C_{18} phase. In the case of Vydac 218TP5 polymeric C_{18} packing, the anomalous retention behavior for the planar *versus* non-planar PAHs reflects differences in the orientation of the stationary phase as well as solvent-solute interactions which

TABLE II

PEAK LOCATION AND AREA UNDER THE PEAK FOR BULK –CH_2, β-CH_2, AND TERMINAL CH_3 GROUPS FOR ULTRAPAK-ODS AND VYDAC 218TP5 IN CHLOROFORM–METHANOL SOLVENT

Stationary phase	Solvent	Bulk –CH_2		β-CH_2		Terminal CH_3	
		Peak position (ppm)*	Area (cm²)	Peak position (ppm)*	Area (cm²)	Peak position (ppm)*	Area (cm²)
Ultrapak-ODS	100% Chloroform	30.54	31.96	22.95	4.08	14.26	2.73
	Methanol-chloroform (1:1)	29.70	17.1	22.37	3.64	13.12	2.46
	100% Methanol	30.46	13.23	23.00	1.43	13.81	1.63
Vydac 218TP5	100% Chloroform	31.49	42.12	23.82	2.8	14.89	6.5
	Methanol-chloroform (1:1)	31.00	16.2	23.33	1.1	14.08	2.6
	100% Methanol	31.00	3.77	23.23	—**	14.14	—**

* Chemical shift measured within ±0.1 ppm with reference to tetramethylsilane and ^2H_2O as locking agent.
** For Vydac in 100% methanol, it is very difficult to compute areas for β-CH_2 and terminal CH_3 peaks, since they are very broad and flat.

affect the planarity of the solute. Because the polymeric phases exhibited greater selectivity and a greater correlation with the shape of the PAH solute than the monomeric phases, the polymeric materials must be more "ordered". Another possibility is a liquid-crystal-like effect or "ordering" of the C_{18} chains on polymeric phases.

Another important conclusion is the fact that by using CP-MAS ¹³C NMR of solid phases one can not only distinguish between various structural elements on the surface but also differentiate between their modes of preparation. The results of the original manufacturing procedures and their subsequent treatment with trimethyl silylating reagent or any other endcapping reagents should be investigated further.

REFERENCES

1 J. A. Schmit, R. A. Henry, R. C. Williams and J. F. Dieckman, *J. Chromatogr. Sci.*, 9 (1971) 645.
2 S. A. Wise, S. N. Chester, H. S. Hertz, L. R. Hilpert and W. E. May, *Anal. Chem.*, 49 (1977) 2306.
3 T. Nielsen, *J. Chromatogr.*, 170 (1979) 147.
4 J. Chmielowiec and A. E. George, *Anal. Chem.*, 52 (1980) 1154.
5 S. A. Wise, W. J. Bonnett and W. E. May, in A. Bjorseth and A. J. Dennis (Editors), *Polynuclear Aromatic Hydrocarbons: Chemistry and Biological Effects*, Battelle press, Columbus, OH, 1980.
6 K. Ogan and E. Katz, *J. Chromatogr.*, 188 (1980) 115.
7 S. A. Wise, in A. Bjorseth (Editor), *Handbook of Polynuclear Aromatic Hydrocarbons: High-Performance Liquid Chromatography for the Determination of Polycyclic Aromatic Hydrocarbon*, Marcel Dekker, New York, 1983, p. 183.
8 E. Katz and K. Ogan, *J. Liquid Chromatogr.*, 3 (1980) 1151.
9 S. A. Wise, W. J. Bonnett, F. R. Guenther and W. E. May, *J. Chromatogr. Sci.*, 19 (1981) 457.
10 R. B. Sleight, *J. Chromatogr.*, 83 (1973) 31.
11 P. A. Peaden, M. L. Lee, Y. Hirata and M. Novotny, *Anal. Chem.*, 52 (1980) 2268.
12 J. C. Fetzer and W. R. Biggs, *J. Chromatogr.*, 322 (1985) 275.
13 J. C. Fetzer and W. R. Biggs, *J. Chromatogr.*, 295 (1984) 161.
14 J. C. Fetzer and W. R. Biggs, personal communication, Chevron Research Company, Richmond, CA, 1986.
15 P. Shah, T-B. Hsu and L. B. Rogers, *J. Chromatogr.*, submitted for publication.
16 M. F. Burke, Personal communication, Department of Chemistry, University of Arizona, 1985.
17 *The Sadtler Standard Spectra*, Sadtler Research Laboratories, Philadelphia, PA, 1985.
18 E. Bayer, K. Albert, J. Reiners, M. Nieder and D. Muller, *J. Chromatogr.*, 264 (1983) 197.
19 C. R. Yonker, T. A. Zwier and M. F. Burke, *J. Chromatogr.*, 241 (1982) 257.
20 M. E. McNally and L. B. Rogers, *J. Chromatogr.*, 331 (1985) 23.