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RETENTION BEHAVIOUR OF LARGE POLYCYCLIC AROMATIC HYDROCARBONS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY ON A POLYMERIC OCTADECYLSILICA STATIONARY PHASE

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SUMMARY

Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy and differential scanning calorimetry experiments were performed in order to study the retention mechanism in reversed-phase liquid chromatography for large polycyclic aromatic hydrocarbons. The effects of changing the mobile phase composition and column temperature were evaluated. The results suggest that a change in mobile phase composition from pure methanol to pure dichloromethane induces further non-planarity in non-planar solutes and also slightly changes the conformation of a polymeric octadecyl stationary phase. Conversely, a change to higher column temperatures drastically changes the structure of the stationary phase from solid-like to liquid-like, with only a small change of non-planar solutes to more non-planar conformations.

INTRODUCTION

The influence of the size and shape of polycyclic aromatic hydrocarbons (PAHs) on their retention in reversed-phase liquid chromatography (RPLC) has been widely investigated[1–10] because the compounds have mutagenic and carcinogenic activity. This makes it important to determine their levels in the environment. For PAHs containing less than six rings, it is clear that retention in RPLC is controlled by their size and two-dimensional shape. With certain stationary phases in non-aqueous RPLC[9,10], in contrast, some large PAHs, such as the peropyrene-type PAHs, show anomalous elution behavior apparently not controlled simply by the size and shape of

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the molecule. Non-planar solutes that are larger in size than smaller, planar solutes are eluted earlier with higher concentrations of dichloromethane in the mobile phase on particular chemically bonded stationary phases. In systematic studies to investigate this point we found that the elution behavior of large PAHs appears to be controlled by two main factors: (1) the orderliness of the chemically bonded stationary phases and (2) the degree of solute planarity. With a polymeric, derivatized octadecylsilica (P-ODS) as the stationary phase rather than a monomerically derivatized octadecylsilica, the tendency to recognize the molecular planarity of PAHs has been found to increase[11,12]. Wise and Sander[13] have concluded that the structure of P-ODS is more rigid and slit-like (the "slot model") than that of monomeric ODS. Thus, P-ODS cannot retain or interact well with non-planar PAHs because of its slit-like structure, while planar solutes can be retained more strongly, because they more easily enter the slits. The results of our experiments also indicate that P-ODS is preferable to various bonded phases[11,14,15] for retaining planar PAHs. If the column temperature is lower than 45°C or if there is an high dichloromethane concentration (in methanol), the recognition ability for molecular planarity increases. As an explanation of these results, we have proposed a mechanism in which the temperature and mobile-phase composition induce a structural change in P-ODS. However, a more detailed description of the mechanism was impossible because direct confirmatory evidence was lacking.

A number of attempts to understand the function of the stationary phase in RPLC have been reported in which spectroscopic techniques were used, such as nuclear magnetic resonance (NMR) spectrometry[16–24], Fourier-transform infrared spectrometry (FT-IR)[25,26] and thermal analysis[27]. However, those reports concerned mainly characteristics including conformational studies of the starting silane materials and stationary phases other than octadecyl chains and sometimes no distinction was made between monomeric and polymeric ODS phases. A deficiency of those publications is also that they do not relate these data to chromatographic retention data obtained with the stationary phases. Greater understanding of RPLC retention mechanisms will come from the interpretation of chromatographic data related to the characteristics of stationary phases examined by supplemental spectroscopic techniques.

In this paper, as an extension of our previous work, the retention mechanism for large PAHs in non-aqueous RPLC with P-ODS has been investigated by spectroscopic techniques in addition to chromatographic techniques to explain the anomalous chromatographic retention behaviour observed for some PAHs in previous studies.

EXPERIMENTAL

A micro-LC system comprised of an MF-2 microfeeder (Azuma Electric, Tokyo, Japan) and an Uvidec-100-III detector (Jasco, Tokyo, Japan) was used. A microloop injector 7619 (Rheodyne, Cotati, CA, U.S.A.) was used for sample introduction. The column was a fused-silica capillary (30 cm × 0.53 mm I.D.) packed with Vydac 201 TPB5 P-ODS (Separations Group, Hesperia, CA, U.S.A.) which from our earlier work appears to be made from a trifunctional silane[15].

The peropyrene-type PAHs used as sample probes (Fig. 1), were synthesized by procedures described elsewhere[9,10]. Their purity was checked by HPLC with

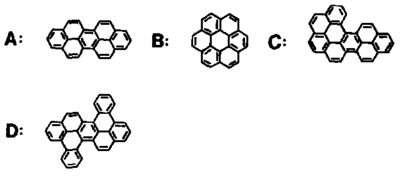


Fig. 1. Structures of peropyrene-type PAHs used in this work. A = Dibenz[*cd,lm*]perylene; B = coronene; C = benzo[*lm*]phenanthro[4,5,6-*abcd*]perylene; D = tetrabenzo[*a,cd,j,m*]perylene.

monitoring of their UV spectra using a Jasco MULTI-320 photodiode array detector, and by comparison with literature spectra[28,29]. The mobile phases were mixtures of methanol and dichloromethane or methanol and chloroform. The flow-rate was typically 4 μl/min.

Diffuse-reflectance infrared spectra were obtained on a FTS-20 instrument (Digilab, MA, U.S.A.) equipped with a temperature controller. The Vydac material was analyzed as 10% dilutions in potassium chloride which had been ground for 5 min. The depth of the sample was approximately 2.3–2.4 mm. The sample was packed by careful mounting inside the sample cup and then pressed with a flat object to pack it down. A Nichrome wire, wrapped around the detection cell, was used to increase the temperature by applying a direct current of varying voltage. Spectra were obtained at room temperature (*ca.* 20°C), as well as at 30, 40, 45, 50 and 60°C. The sample was held at the selected temperature for approximately 3 min before starting spectral collection. Spectra were the ensemble average of 256 scans taken at a resolution of 4 cm^{-1}. An MCT (mercury–cadmium–telluride) detector was used. Boxcar apodization was applied to the interferograms. Once obtained, the single-beam spectrum (between 4000 and 1000 cm^{-1}) of the sample was ratioed to the single-beam spectrum of potassium chloride at the same temperature. These reflectance data were transferred for treatment by SpectraCalc software (Galactic, Nashua, NH, U.S.A.). Once in SpectraCalc, the spectra were baseline-corrected, using either a two-point or a four-point baseline correction in the C–H stretching region. The Kubelka-Munk spectrum was then calculated from the baseline-corrected portion (between 3400 and 2600 cm^{-1}). SpectraCalc was performed on a IBM-AT-compatible computer.

The high-resolution ^{13}C NMR spectra of the suspensions of P-ODS and mobile-phase solvents, and the ^{1}H NMR spectra of coronene and tetrabenzo-[*a,cd,j,lm*]perylene, dissolved in the mobile phase solvents, were recorded with a JNM-GX270 FT-NMR spectrometer (JEOL, Tokyo, Japan). The spectra were obtained at two different temperatures, 27 and 50°C. For ^{13}C NMR, the experiments were conducted on 0.2 g of P-ODS, which was weighed directly into a 10-mm NMR tube and about 5–6 ml of the desired mixture of solvents ([^{2}H]methanol–[^{2}H]-dichloromethane or [^{2}H]methanol–[^{2}H]chloroform) were added[30]. The tube was hand-shaken until proper mixing was obtained. A ^{13}C NMR spectrum of this suspension was recorded as described in ref. 30. For the ^{1}H NMR spectra, the samples were dissolved in mixtures of [^{2}H]methanol, [^{2}H]dichloromethane and [^{2}H]-chloroform. The cross-polarization magic-angle solid-state (CP-MAS) ^{13}C NMR spectra of Vydac material were obtained with the same NMR spectrometer. The experimental conditions are as follows: observation frequency, 5600 Hz; observation point, 8192; frequency width, 27 027 Hz; scans, 5000; acquisition time, 0.038 s; pulse delay, 6 s; pulse width, 4.5 μs; irradiation frequency, 5400 Hz; standard shift, 29.5 ppm for adamantane; broadening factor, 3.00 Hz; receiver gain, 23–26; measurements at room temperature, 30, 40, 45 and 50°C.

Thermal analysis (differential scanning calorimetry, DSC) was performed with a SSC-5000 and DSC-200 thermal analyzer (Seiko Denshi, Tokyo, Japan). The measurement range was between 20 and 200°C, and the temperature programming rate was 10°C/min.

Although the stationary phase is in the presence of mobile phase during chromatography, for convenience and because of difficult technical problems, DSC and FT-IR measurements were carried out in the dry state.

Molecular structure graphics were generated by using a MV6000 System II computer (Data General, Tokyo, Japan), loaded with TUTORS[31] software which has been developed in the Chemometrics Center of Toyohashi University of Technology.

RESULTS AND DISCUSSION

Elution order change with changing mobile phase composition

The chromatographic elution orders of peropyrene-type PAHs with different mobile phase compositions were reported in our previous paper[14] but were measured again with P-ODS. The results are illustrated in Fig. 2. The mobile phase compositions ranged between 20 and 50% dichloromethane in methanol. The typical behavior shown in Fig. 2 can be interpreted to mean that the elution order of large PAHs mainly depends upon their sizes and planarity. In contrast, it has been confirmed for small PAHs that their retention is generally controlled by their sizes and shapes[8,11]. In the current work, tetrabenzo[*a,cd,j,lm*]perylene (nine rings) is always eluted earlier than dibenzo[*cd,lm*]perylene (seven rings). At higher dichloromethane concentrations the solute was eluted even earlier than coronene, the smallest molecule used in this work. This is due to the non-planarity of tetrabenzo[*a,cd,j,lm*]perylene. The non-polarity of tetrabenzo[*a,cd,j,lm*]perylene in the solid state has been confirmed by a computerized molecular display technique based on the crystal structure data obtained by X-ray

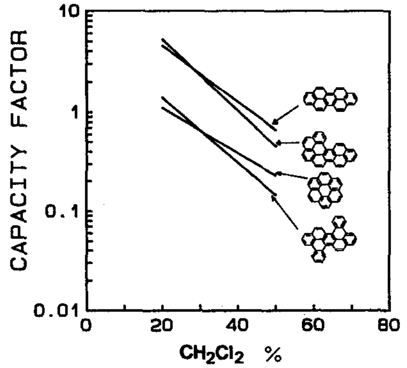


Fig. 2. Plots of log *k'* of four peropyrene-type PAHs *vs.* dichloromethane concentration in methanol as the mobile phase.

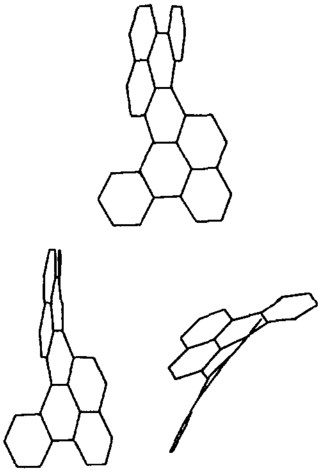


Fig. 3. Three-dimensional structure of tetrabenzo[*a*,*cd*,*j*,*lm*]perylene drawn by computer graphics.

diffraction[32]. The structure is shown in Fig. 3, where the distortion of this molecule appears to be very large. The contribution of dichloromethane to this exclusion-like retention behavior of the non-planar PAHs may be caused by the facts that, with changing mobile-phase composition, the structure of P-ODS is changing or the solute planarity is changing or both.

In order to determine which factor is dominant in non-aqueous RPLC, NMR measurements were performed. In Fig. 4, ^{13}C NMR spectra of P-ODS, suspended in

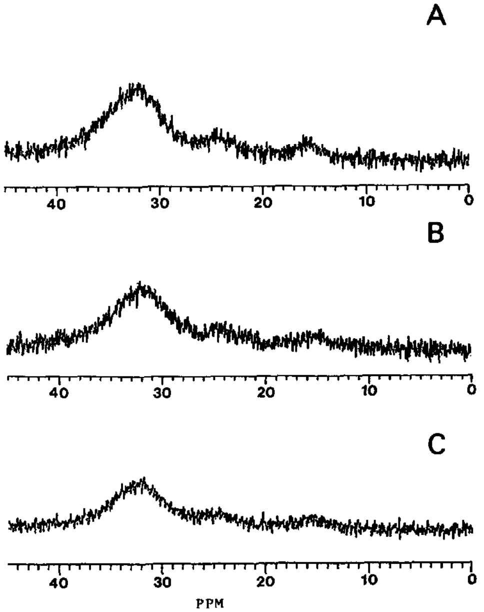


Fig. 4. ^{13}C NMR spectra of Vydac 201 TPB5 in various solvents: (A) 100% [$^{2}H_{2}$]dichloromethane; (B) [$^{2}H_{2}$]dichloromethane–[$^{2}H_{4}$]methanol (50:50); (C) 100% [$^{2}H_{4}$]methanol.

different compositions of [^{2}H]methanol–[^{2}H]dichloromethane, are shown. It has been proposed by Yonker *et al.*[33] and McNally and Rogers[34] that the width at half-height of the bulk $-CH_2$ peaks at 33 ppm[30] can be used as a measure of the liquid-like nature of the bonded alkyl chains. Wider peaks 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. As seen in Fig. 4, only small changes occur with increasing dichloromethane concentration. It appears that, as the solvent is changed from pure methanol to pure dichloromethane, the peak width and intensity increase for the peak at 33 ppm as well as for the peaks at 24 ppm (assigned to β-CH_2) and 15 ppm (assigned to terminal methyl groups[30]). These small changes might mean there is a solvent effect on this P-ODS. The work of Shah *et al.*[30] indicates that in 100% dichloromethane the difunctional polymeric octadecyl alkyl chain is moving relatively freely and interacts with the solvent. Although Fig. 4 does not show this clearly, it is reasonable to consider that, as the percentage of methanol increases, the molecular motion of the bonded-alkyl chain becomes more restricted, but not so much as was observed for a difunctional polymeric phase by Shah *et al.*[30].

As solute structure changes seem to be more reasonable in the sense of solution chemistry, ^{1}H NMR spectra were collected for representative solutes, coronene (as a planar PAH) and tetrabenzo[*a,cd,j,lm*]perylene (as a non-planar PAH), at three different [^{2}H]dichloromethane concentrations (20, 50 and 100% in [^{2}H]methanol). The results are shown in Fig. 5. The signals of tetrabenzo[*a,cd,j,lm*]perylene at 9.2 ppm were drastically shifted to higher magnetic field when the dichloromethane concentration in the mobile phase was increased from 20 to 50%. No difference in spectra was observed between 50 and 100% dichloromethane. The signals at 9.2 ppm with 20% dichloromethane showed four lines, but with 50 and 100% only two lines remained and two lines were shifted to higher field. The signals at 9.2 ppm can be assigned to two protons in the A-region of the molecular structure shown in Fig. 5. The shift of the signals to 9.05 ppm might mean that the environment of the protons has changed to be similar to those in the B-region in the structure. In contrast, the signal of coronene at 8.95 ppm did not show any shift with changing solvent composition. It is possible that tetrabenzo[*a,cd,j,lm*]perylene becomes more non-planar with increasing dichloromethane concentration. Evidence in support of this is provided by the UV spectrometric studies of Fetzer and Biggs[10]. This planarity change is the main cause of the anomalous chromatographic retention behavior. The structure change of the stationary phase seems to be a second factor contributing to the phenomena. Because the signal change in the NMR measurements in the molecular planarity study is more drastic than that observed for the stationary phase when the mobile phase compositions are changed, solute-planarity changes dominate the elution behaviour.

Elution behaviour with changing column temperature

Our previous paper[15] showed that the column temperature is an important factor in controlling the retention of large PAHs. Lower temperatures are preferable for planarity recognition. We also indicated that thermal analysis data (DSC) can be used to find out whether the structure change of P-ODS with changing temperature causes the improved recognition ability between planar and non-planar PAHs. In this work, we give more information in support of this conclusion.

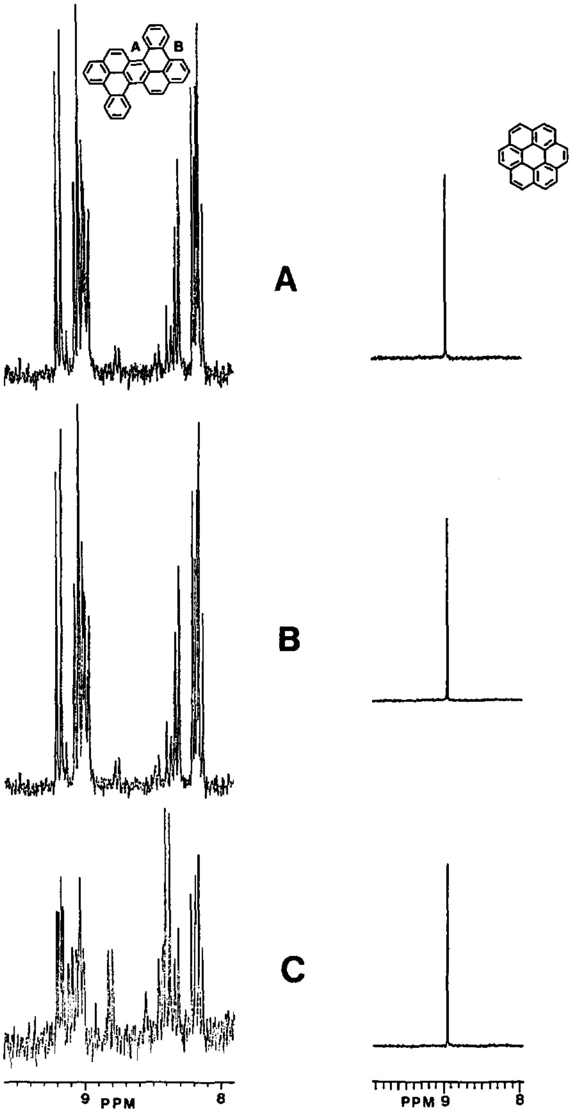


Fig. 5. ^{1}H NMR spectra of tetrabenzo[*a*,*cd*,*j*,*lm*]perylene and coronene in various solvents: (A) and (B) as in Fig. 4; (C) [$^2H_2$]dichloromethane–[$^2H_4$]methanol (20:80).

In Fig. 6, the DSC chart of P-ODS[15] is shown. The data clearly indicate that P-ODS undergoes a drastic change around 45°C, above the melting point of octadecane (29–30°C). Monomeric ODS (laboratory-made) has a weak transition at 35°C, as determined by similar DSC measurements. The DSC results indicate that a transition of P-ODS occurred at about 45°C and this may be caused by a phase transition from a solid-like to a liquid-like structure. The retention data for PAHs at various column temperatures also show large changes in behavior at about 45°C. Fig. 7 shows the Van 't Hoff plots for two planar and non-planar PAHs in the solvent

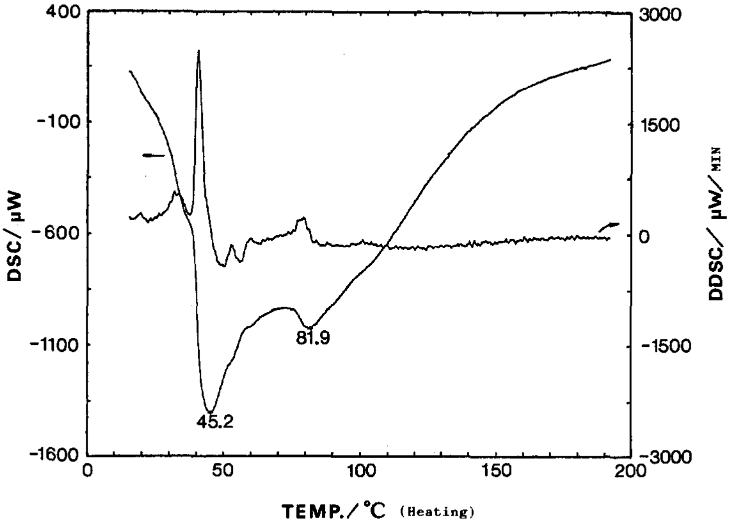


Fig. 6. DSC chart of Vydac 201 TPB5[15]. DDSC = Differential of DSC curve.

methanol–chloroform (50:50). The plots should be linear for a normal chromatographic process if the retention mechanism is constant. However, in this case, two linear relationships were found between log k' and $1/T$ (where k' = capacity factor and T = temperature), and the critical points in the Figure are at about 45°C. This indicates that the transition found in the DSC measurements causes this anomalous retention behavior of the large PAHs. The retention ratio, *i.e.*, separation factor of coronene and tetrabenzo[*a,cd,j,lm*]perylene was calculated for each column temperature and plotted as shown in Fig. 8. Planarity recognition between coronene and tetrabenzo[*a,cd,j,lm*]perylene changed with column temperature because the ratio is smaller, and increasing temperature decreased recognition ability. The critical point again seems to be between 40 and 50°C. Similar observations were reported in our previous paper[15], where different PAHs were used as the sample probes. The data shown above suggest that a critical point exists between 40 and 50°C, and this may be

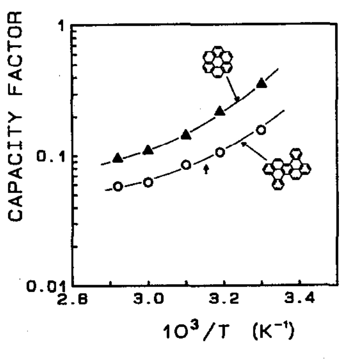


Fig. 7. Van 't Hoff plots for coronene and tetrabenzo[*a,cd,j,lm*]perylene with methanol–chloroform (50:50) as the mobile phase. The arrow indicates the critical point.

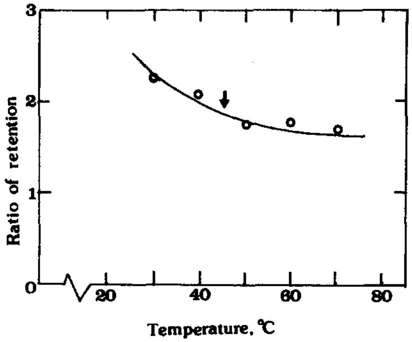


Fig. 8. Retention ratio of coronene and tetrabenzo[*a,cd,j,lm*]perylene *vs.* temperature. The arrow indicates the critical point.

explained by the existence of phase transitions of the P-ODS between these temperatures.

In order to estimate the characteristics of the transition of P-ODS at 45°C, several spectroscopic investigations were performed. First, FT-IR spectra of the stationary phase itself were collected, using the diffuse-reflectance spectroscopic technique. The spectra obtained (Fig. 9) showed an obvious change with temperature, a shift of 2 cm^{-1} in the band at 2850 cm^{-1}. Fig. 10C shows the center of gravity at the band at 2850 cm^{-1} plotted *versus* the temperature. This plot can be considered to be

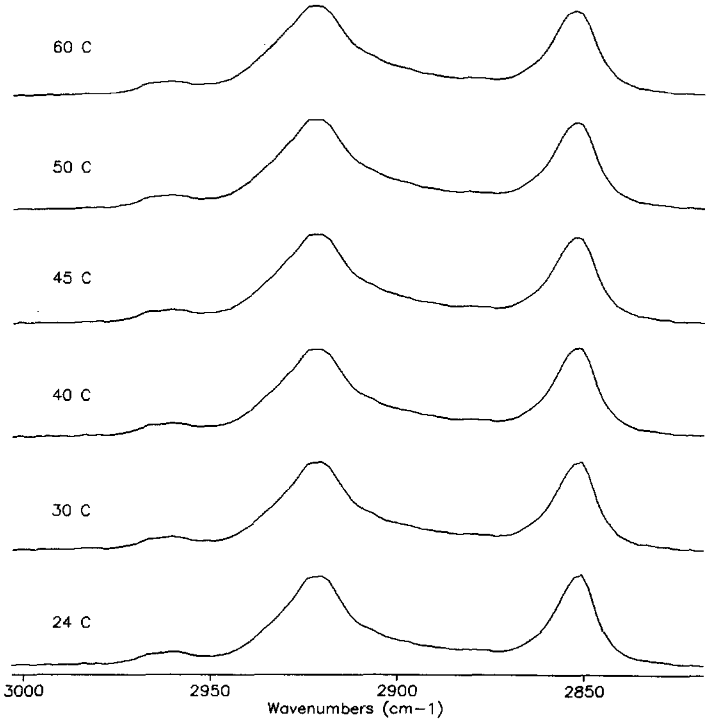


Fig. 9. Diffuse-reflectance infrared spectra of Vydac 201 TPB5 at various temperatures.

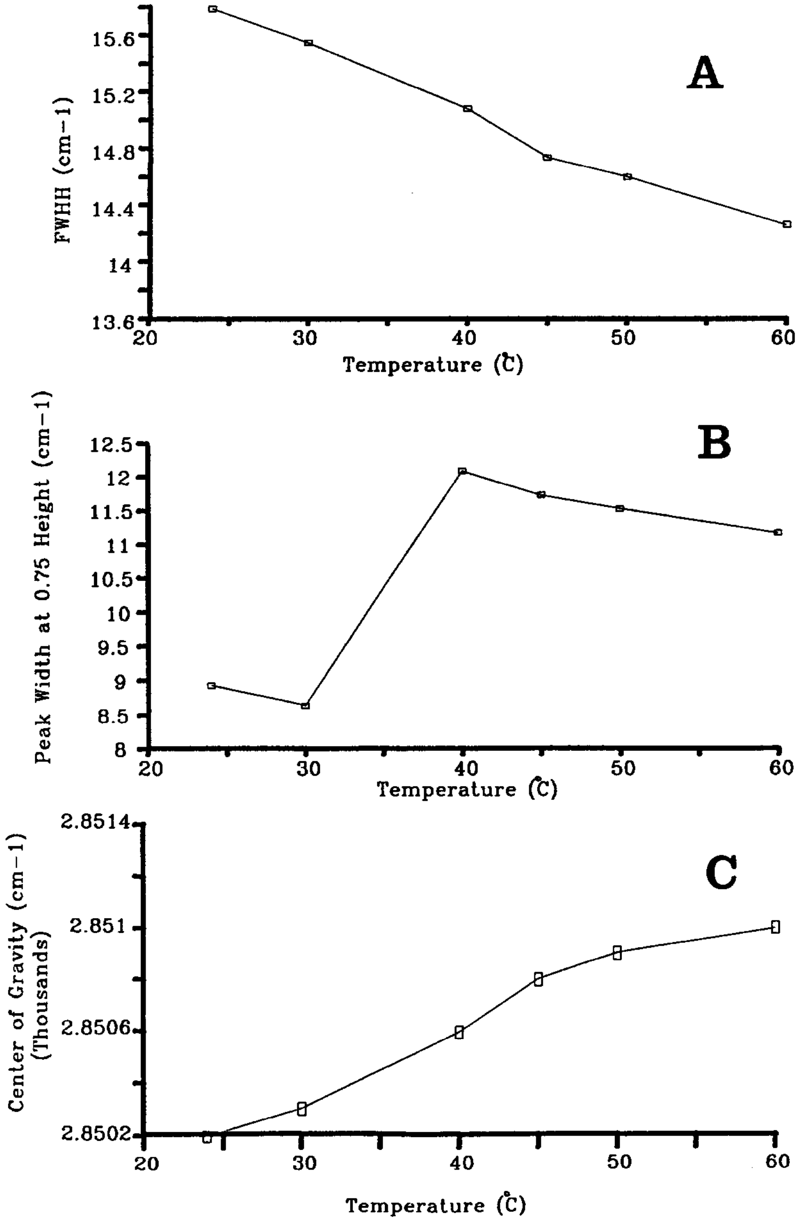
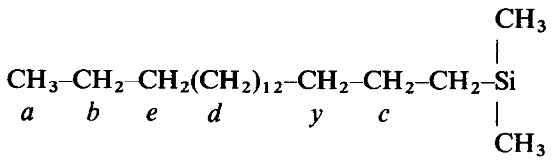


Fig. 10. Interpretation of FT-IR spectra of Vydac 201TPB5. (A) FWHH *vs.* temperature; (B) peak width at 0.75 height *vs.* temperature; (C) center of gravity *vs.* temperature.

sigmoidal, centered near 45°C. Then we tried to determine the full width of the peak. Fig. 10A shows the full width at half-height *versus* temperature. This is unusual, because the band width usually increases with temperature if the absorption is due to a single structural functionality. It is reasonable to rationalize this result in terms of two highly overlapped bands, separated by less than the full width at half-height (FWHH). The band at higher wavenumber would correspond to the more liquid-like region and that at lower wavenumber to the more solid-like region as reported for alkanes by Casal *et al.*[35]. If this type of behaviour actually describes what is happening in practice, one might expect the width at, for example, 75% height to be more likely to show temperature effects. As shown in Fig. 10B, a distinct break was found between 30 and 45°C. Therefore, it is concluded that IR spectrometric information appears to indicate some transitional structure change with increasing temperature up to 50°C. This corresponds well with the thermal transition (around 45°C) observed by DSC. Therefore, one may conclude that at 45°C some transition occurs in the CH_2 chains of the octadecyl groups.

NMR measurements gave a clearer interpretation of the transition of P-ODS. They were performed in the solid state, because CP-MAS-NMR should be more useful in understanding the conformational change of CH_2 chains. The NMR spectra are shown in Fig. 11A and B for room temperature and 50°C, respectively. Three distinct peaks are seen at 12, 22 ppm and 30 ppm (the strongest). The assignments of these signals are roughly as follows



$$\underset{a}{\mathrm{CH_3}}-\underset{b}{\mathrm{CH_2}}-\underset{e}{\mathrm{CH_2}}\underset{d}{\mathrm{(CH_2)_{12}}}-\underset{y}{\mathrm{CH_2}}-\underset{c}{\mathrm{CH_2}}-\mathrm{CH_2}-\overset{\overset{\displaystyle \mathrm{CH_3}}{|}}{\underset{\underset{\displaystyle \mathrm{CH_3}}{|}}{\mathrm{Si}}}$$

where a = 12 ppm, b = 21.5 ppm, c = 22 ppm, d, e and y = 30–33 ppm[30]. The absence of signals at 0 ppm indicates that this bonded phase is of the polymeric type, and the absence of signals at 18 and 34 ppm indicates the rigid location of this carbon chain. The details of the NMR spectral changes with temperature are summarized in Fig. 12. The most drastic change is seen at 30 ppm for the bulk $-CH_2$ signal. At room temperature, the peak has two maxima, but with increasing temperature they merge, and above 45°C the maxima at 32 ppm disappear and become a shoulder. This tendency is much clearer in Fig. 13, where the peak deconvolution procedure has been applied to three different spectra at room temperature (A), 40°C (B) and 50°C (C), respectively. The numerical data are summarized in Table I. The peak at 32 ppm shifted toward 30.8 ppm with increasing temperature. This situation is reminiscent of the case of monomeric and low-loaded polymeric ODS phases. The NMR spectrum at 50°C is very similar to that of Vydac 218 TP5 reported by Shah *et al.*[30]. Vydac 218 is a low-loaded polymeric phase and the distance between alkyl chains on the surface may be larger than that of Vydac 201. From the NMR spectra, which indicate the existence of different types of $-CH_2$ chains in the bulk, one can assume that the polymeric alkyl chain at room temperature exhibits the ordered phase suggested by Sander *et al.*[25] or "fold" like structure[16] and with increasing column temperature it becomes disordered, adopting various conformations and becoming freely mobile. Therefore, the NMR signal at 30 ppm becomes unified and similar to those of monomeric and low-loaded polymeric phases. Further studies are required in comparison with other types of ODS phases such as monomeric ones or monomeric phases with endcapping. However, the CP-MAS data suggest that the transition

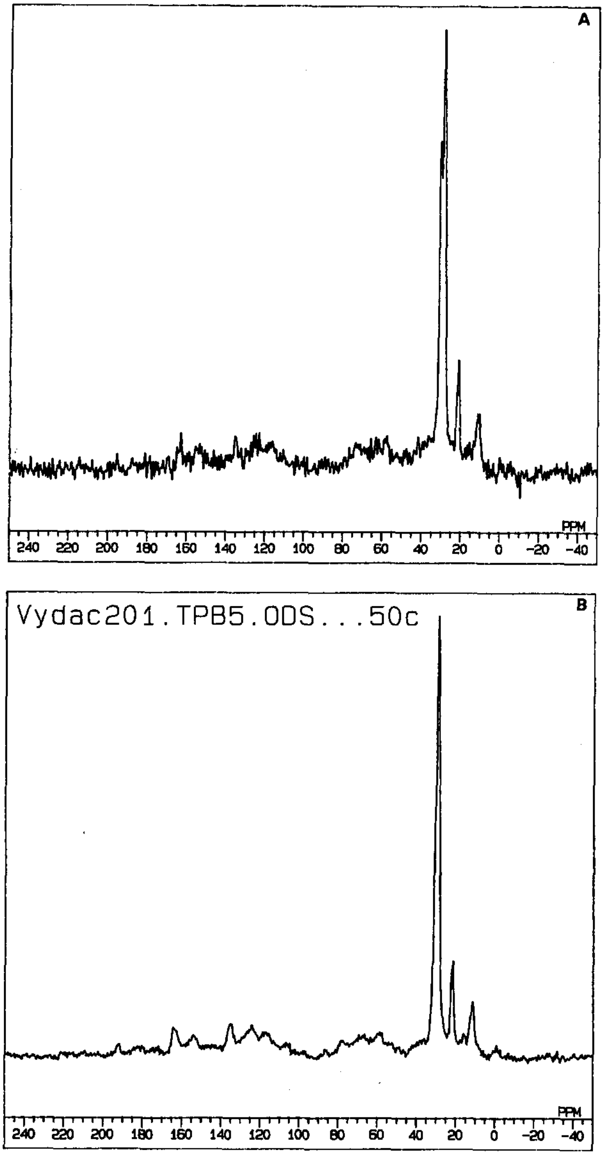


Fig. 11. CP-MAS-^{13}C NMR spectra of Vydac 201 TPB5 at room temperature (A) and 50°C (B).

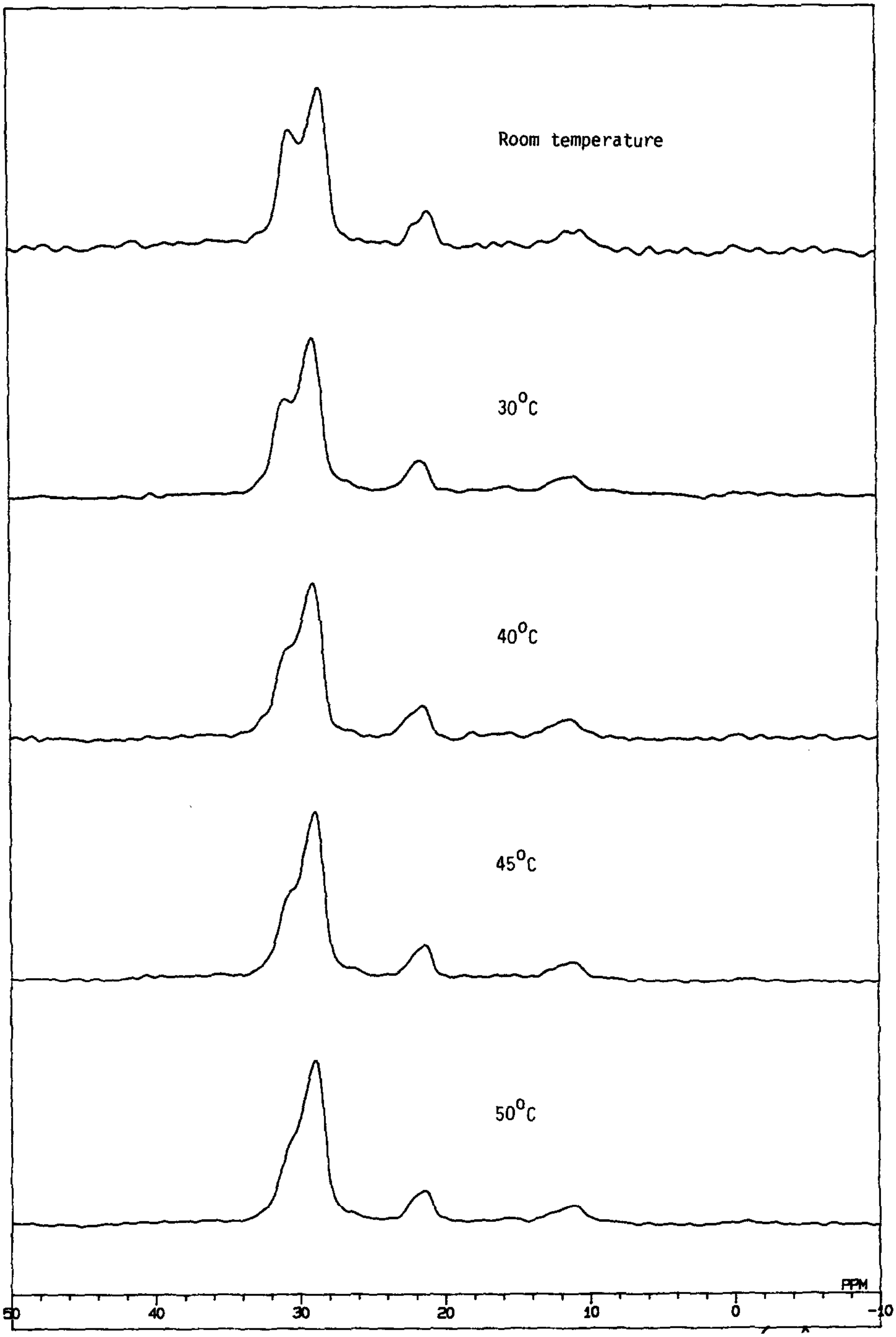
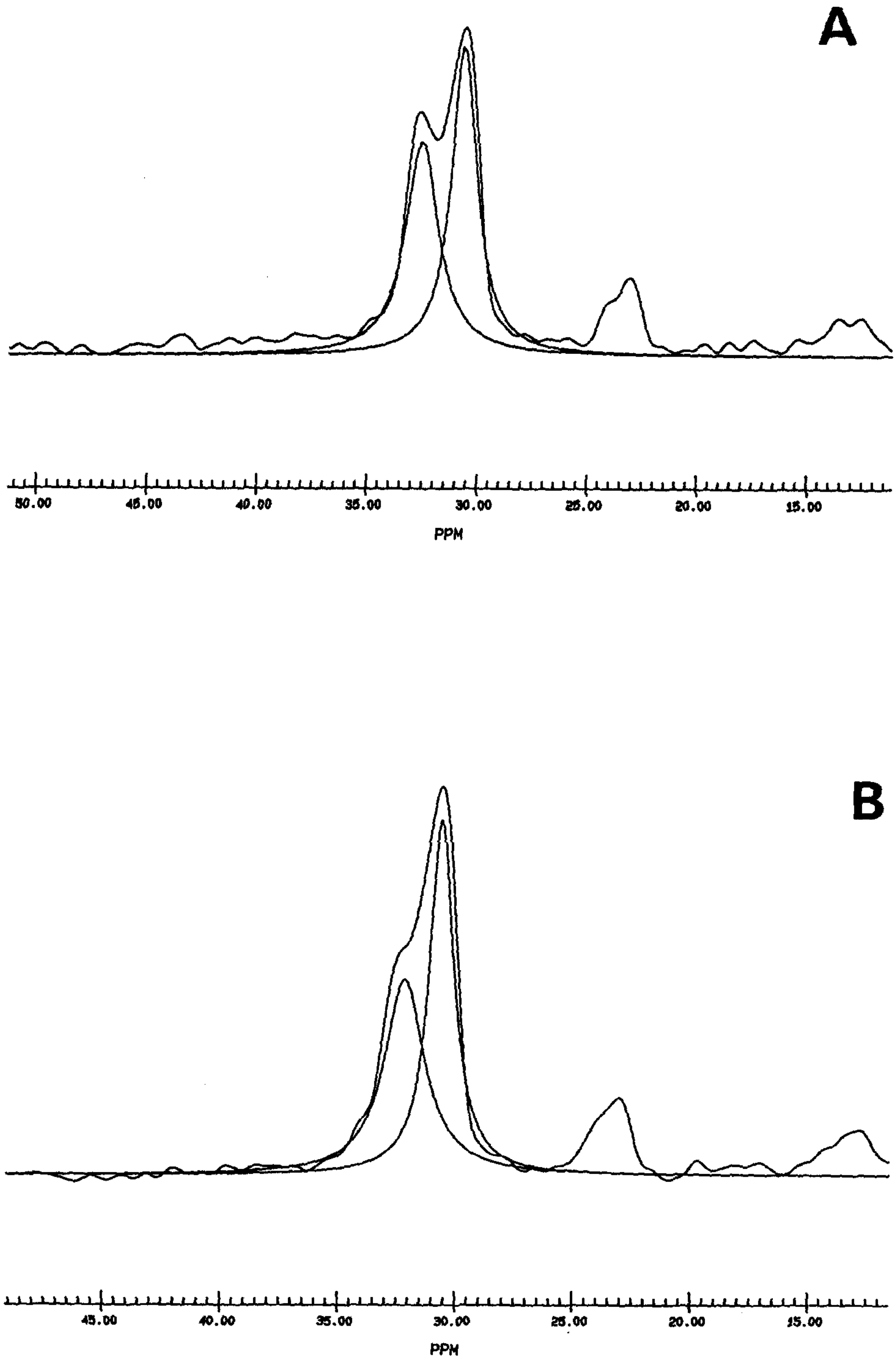


Fig. 12. CP-MAS-^{13}C NMR spectra of Vydac 201 TPB5 at various temperatures.


A
50.00
45.00
40.00
35.00
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25.00
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B
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Fig. 13.

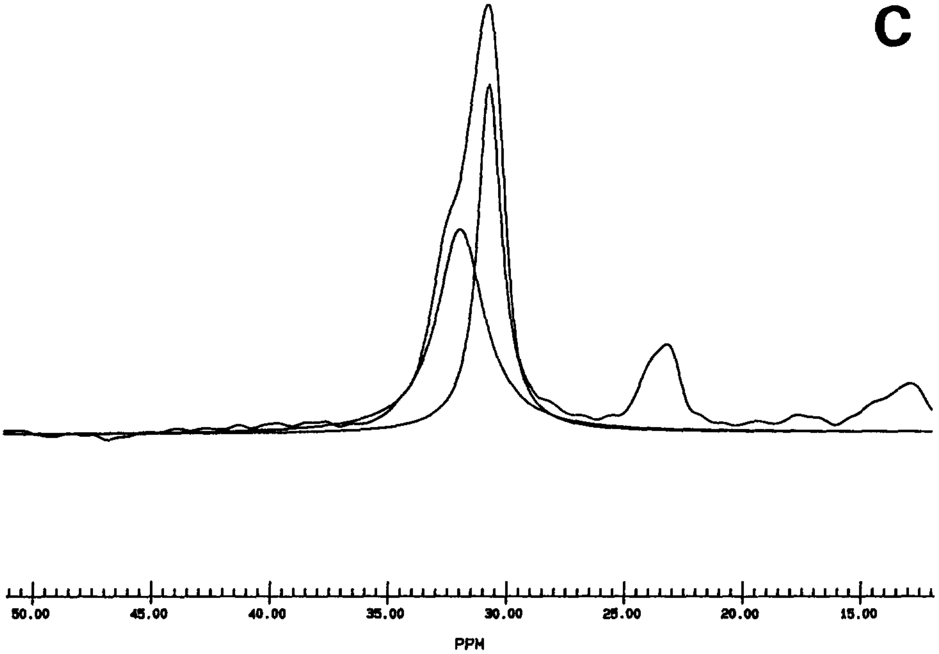

Fig. 13. Peak deconvolution for the signal at the 30 ppm at room temperature (A), 40°C (B) and 50°C (C).

around 45°C is a kind of melting from a solid-like to a liquid-like structure in the dry state stationary phase.

Fig. 14 shows the ^{13}C NMR spectra of suspended P-ODS, measured at 27 and 50°C. In this case, [^2H]chloroform was substituted for [^2H]dichloromethane, which was used in the study of the mobile phase effects on the conformation of P-ODS, because NMR measurements at temperatures higher than the boiling point of dichloromethane were not possible over long periods. The figures provide evidence to support the assumption that the structure of P-ODS changes from solid-like to liquid-like between 27 and 50°C, because the signal intensities for $-CH_2$ and bulk $-CH_2$ increase relatively, while the peak widths decreased with increasing temperature.

TABLE I

RESULTS OF PEAK DECONVOLUTION FOR CP-MAS-NMR SPECTRA OF VYDAC 201

	Ambient		*40°C*		*50°C*	
	1	*2*	*1*	*2*	*1*	*2*
Chemical shift (ppm)	32.8	30.8	32.4	30.8	32.0	30.8
Peak height	69.3	100	55.5	100	58.7	100
Peak width (Hz)	123.2	94.7	141.1	85.5	161.5	81.9
Area ratio	90.1	100	91.7	100	100	86.4

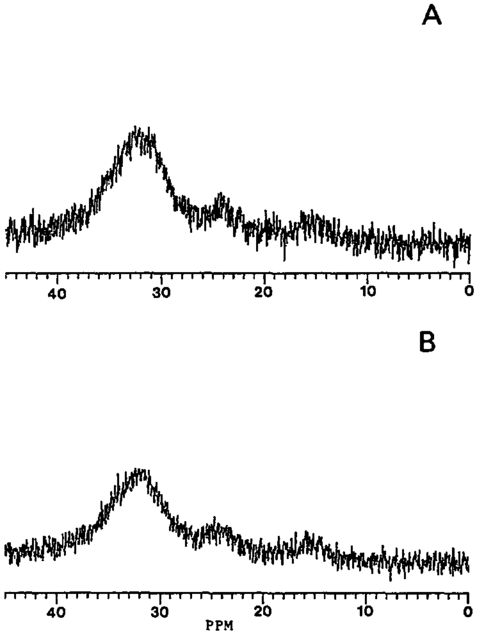

Fig. 14. ^{13}C NMR spectra of Vydac 201 TPB5 at 50°C (A) and 27°C (B).

Fig. 15 shows the NMR spectra of tetrabenzo[*a*,*cd*,*j*,*lm*]perylene dissolved in [^{2}H]chloroform–[^{2}H]methanol (50:50) at 50°C (A) and at 27°C (B) in 20% [^{2}H]chloroform in [^{2}H]methanol at 27°C (C), respectively. The change in mobile phase composition produced a drastic shift of the signals around 9.2 ppm. As previously discussed, the solvents of higher [^{2}H]dichloromethane concentration shifted two lines in the region of 9.2 ppm, which showed four lines at lower [^{2}H]dichloromethane concentrations. A similar tendency, but less than in the case of [^{2}H]dichloromethane, was seen with [^{2}H]chloroform. This is seen in spectrum C in Fig. 15, where two split signals appeared. By increasing the [^{2}H]chloroform concentration, a pair of signals at 9.2 ppm were shifted to higher magnetic field. Fig. 15B shows the four sharp lines observed when the [^{2}H]chloroform concentration was changed from 20 to 50% in [^{2}H]methanol. A change in temperature also gave a directionally similar, but smaller, signal shift at 9.2 ppm. This behaviour is identical to that observed when the [^{2}H]dichloromethane or [^{2}H]chloroform concentration in the mobile phase was increased. As discussed previously, increasing the [^{2}H]dichloromethane concentration in the solvent induced distortion of the molecular structure of tetrabenzo[*a*,*cd*,*j*,*lm*]perylene. An increase in column temperature also induced such distortion. At higher temperatures the non-planarity of the solute is increased but to a smaller extent than when the solvent is changed.

The data shown and discussed above are evidence that changing the mobile phase composition causes a drastic change in solute conformation and a small change in P-ODS conformation to a more freely mobile state. From the solid-state NMR data, it is seen that the change to higher column temperature causes a drastic change in the

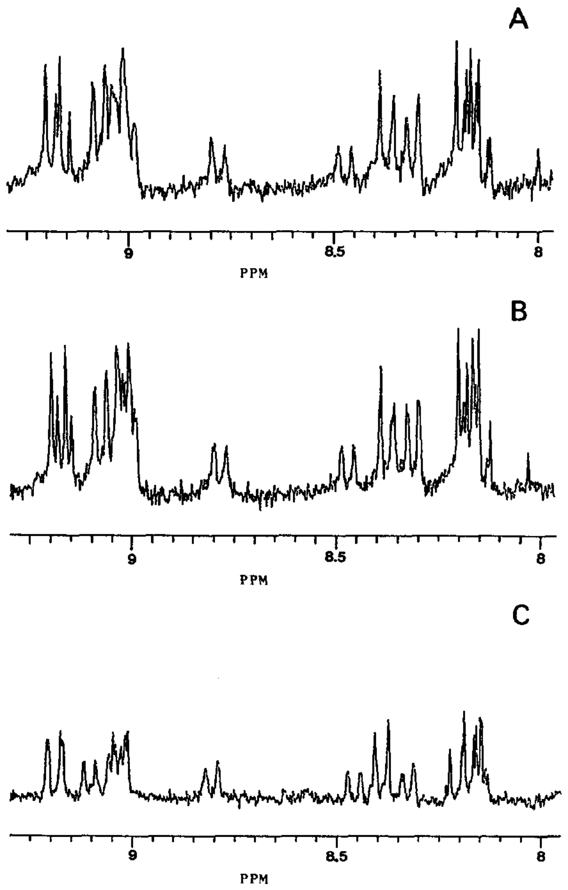

Fig. 15. ^1H NMR spectra of tetrabenzo[*a,cd,j,lm*]perylene at different mobile phase compositions and temperatures: (A) [$^2H_2$]dichloromethane–[$^2H_4$]methanol (50:50) at 0°C; (B) as (A) but at 27°C; (C) as (B) but solvent ratio 20:80.

dry stationary phase from solid-like to liquid-like (the critical temperature is about 45°C) and the solution NMR data indicate only a small change in the solute conformation. Although the suspension NMR data did not give clear information on the environment close to the actual chromatographic process, it is possible to say that the solid-like P-ODS phase is preferable for retaining planar PAHs more strongly than non-planar PAHs, and the liquid-like structure has less planarity recognition ability compared to the former state. These characters of the polymeric phase are very similar to those of bonded liquid crystalline phases[36–40]. The data shown here indicate that the ordering of the polymeric phase in the solid-like state is large enough to recognize the planarity of molecules like those of the liquid crystalline phases. This suggests that the very great influence of steric factors make the slot model[13] appropriate for the polymeric phase. The Dill[41] interphase model predicts that as alkyl surface densities increase, the corresponding configurational constraints are also increased, creating a more rigid and ordered chain packing structure. This anisotropy of the bonded chains gives rise to additional shape selectivity among solute molecules, since the molecules which can most effectively align with the chains are those which are most effectively retained. In this model, the driving force for retention is the creation of a solute-sized cavity in the stationary phase. As the bonding density and consequently chain ordering are increased the free energy required for cavity formation also increases. Greater free energy is required to insert solute substructures which are parallel to the silica–bonded chain interface than for substructures which align with the chains and are normal to the interface, therefore the selectivity for linear and planar molecules will increase with alkyl bonding density, as predicted by this theory and as shown by our experiments. However, more precise and informative suspension NMR data are required and works is in progress in our laboratory using a 400-MHz NMR instrument.

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