Macromolecules **1984**, *17*, 1397–1405

Magic-Angle Carbon-13 Nuclear Magnetic Resonance Study of Local Motions in Solid Poly(alkyl methacrylates)

Françoise Laupretre* and Lucien Monnerie

Laboratoire de Physico-Chimie Structurale et Macromoléculaire associé au CNRS, 75231 Paris Cedex 05, France

Joseph Virlet

Département de Physico-Chimie, CEN Saclay, 91191 Gif-sur-Yvette Cedex, France.
Received August 4, 1983

ABSTRACT: Magic-angle ^{13}C NMR line widths and relaxation times in the rotating frame have been measured for a series of solid poly(alkyl methacrylates) in order to investigate the side-chain or side-ring motions in these polymers. These experiments lead to the NMR identification of the molecular processes that are involved in the γ-transition of poly(cyclohexyl methacrylate). Results thus obtained are in good agreement with conclusions derived from mechanical measurements and use of conformational energy calculations.

The identification of the molecular processes that are involved in the mechanical transitions of bulk polymers can be deeply simplified by using high-resolution solid-state ^{13}C NMR. Under appropriate experimental conditions, this technique, which allows one to observe each carbon of the chain separately, provides information on the motion of the corresponding parts of the molecule. A particularly interesting example is that of poly(cycloalkyl methacrylates). The mechanical properties of these compounds have been extensively studied by Heijboer.[1] When the mechanical behavior of these polymers is compared with data obtained on poly(methyl methacrylate), the most striking feature is the presence of a strong mechanical loss peak. Establishment of a relation between, on the one hand, this particular transition of poly(cycloalkyl methacrylates) and, on the other hand, the molecular motions of the rings present in these polymers[1] was a painful task and required determinations of mechanical properties for a large series of poly(cyclohexyl methacrylates) modified by various substitutions of the cyclohexyl ring. The ability of high-resolution solid-state ^{13}C NMR to solve such a problem is exemplified through the present study of poly(cyclopentyl methacrylate), poly(cyclohexyl methacrylate), and poly(cycloheptyl methacrylate). Results concerning two polymethacrylates with linear side chains are also presented.

Experimental Section

Carbon-13 NMR experiments were performed at 12.07 MHz with a home-built spectrometer constructed around a 12-in. Varian electromagnet and employing external ^2H field frequency stabilization, solid-state class-A transmitters, and double-tuned single-coil probes.[2] Spectra were obtained at room temperature by using magic-angle spinning in a Henriot–Huguenard design[3] hollow rotor made of Kel-F and a spinning speed ranging from 1.5 to 2 kHz. The inner spinner volume was approximately 0.7 cm^3. All the spectra were obtained by cross polarization from the spin-locked protons followed by high-power proton decoupling. Matched spin-lock cross-polarization transfers employed H_1's of 32 kHz. Spin-lock cross-polarization times T_{CH}(SL) were determined from the rise in polarization of the carbon-13 NMR lines as a function of carbon–proton contact time. Optimum single-contact time was found to be 1–2 ms. The carbon-13 spin–lattice relaxation times in the rotating frame $T_{1\rho}{}^{13C}$ were obtained from a plot of the relative magnitude of the carbon magnetization remaining spin-locked for varying times from from 0 to 15 ms after the proton radio-frequency field was removed. The delayed decoupling sequence[4,5] was used as an assignment aid. In all the spectra, spin temperature–inversion techniques were employed to minimize base line noise and roll.[6] Flip-back[7] was also systematically used to shorten the delay time between two successive pulse sequences. About 2000 scans were needed to obtain a good signal-to-noise ratio.

Poly(cyclohexyl methacrylate) (Aldrich, 194-9) and poly(phenyl methacrylate) (Aldrich, 18, 326-2) are commercially available. Other polymethacrylates were the kind gift of Dr. J. Heijboer, Centraal Laboratorium TNO, Delft, The Netherlands. Detailed description of these compounds has been given by Heijboer[1].

Theoretical Background

Line-broadening mechanisms in glassy materials have been recently reviewed.[8] Some of them, the static ones (bulk susceptibility of the sample, chemical shift dispersions due to packing effects, bond distortion, and conformational inequivalence), induce only a small effect. For instance, the chemical shift dispersions resulting from conformational inequivalence and variation in local susceptibility are limited to 2–6 and 0.5–2 ppm, respectively.[8] More important are the line broadenings arising from relaxation processes such as motional modulation of the chemical shift anisotropy and motional modulation of the dipolar carbon–proton coupling. For an aliphatic carbon in a static magnetic field of 1.4 T, the broadening due to the motional modulation of the chemical shift anisotropy can be estimated to remain less than 7 Hz.[8] Therefore, in the case of an aliphatic carbon in a low static field and under suitable conditions of magic-angle setting and proton decoupling irradiation, the only important cause of motional line broadening comes from modulation of the dipolar C–H coupling. This mechanism is maximum when the rate of molecular motion is equal to the proton decoupling field strength in frequency units: $\omega_{1H} = \gamma_H H_{1H}$. For slow motions, this line broadening is much larger than that due to the previous "static" effects. Under the conditions that proton irradiation is applied exactly on resonance and that the sample spinning rate is much smaller than ω_{1H}, the transverse relaxation time T_{2m}, resulting from this mechanism and contributing an amount $(\pi T_{2m})^{-1}$ to the line width, is[8]

$$(T_{2m})^{-1} = \tfrac{1}{2}\langle \Delta M_{CH}{}^{(2)}\rangle_m J_m(\omega_{1H}) \qquad (1)$$

where $\langle \Delta M_{CH}{}^{(2)}\rangle_m$ is the part of the truncated carbon–proton second moment that is modulated by the motion. The spectral density $J_m(\omega_{1H})$ is assumed Lorentzian with a single correlation time τ_c:

$$J_m(\omega) = 2\tau_c/(1 + \omega^2\tau_c{}^2) \qquad (2)$$

On Figure 1 is plotted the dependence of T_{2m} as a function of correlation time τ_c for a methylene carbon in a radio-frequency field $\omega_{1H} = 2\pi \times 32$ kHz under the assumption that a tenth of the rigid-lattice second moment is modulated by the motion. This corresponds to a 7° libration. In this practical case,[9] the motional broadening is larger than 6 ppm (for a 12-MHz ^{13}C observation) for

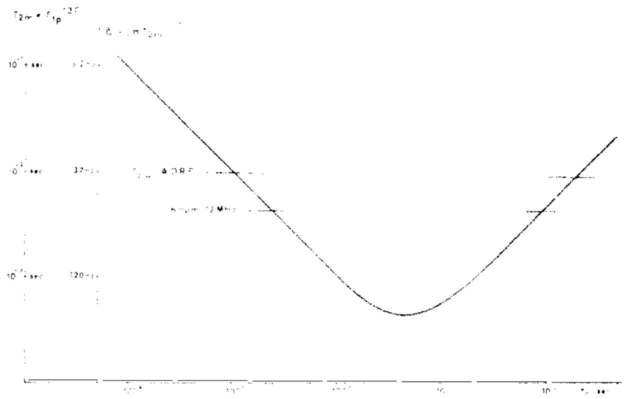

Figure 1. Dependence of the transverse relaxation time T_{2m} as a function of correlation time τ_c, as given by formulas 1 and 2, for a methylene carbon under the assumption that a tenth of the rigid-lattice second moment is modulated by the motion. $\omega_{1H} = \omega_{1C} = 2\pi \times 32 \times 10^3$ rad/s.

motions whose correlation times are in the range 10^{-4}–2×10^{-7} s. These processes will be referred later as "slow molecular motions". In the event of very high or very slow frequency motions, motional contributions to the line width are much smaller and therefore are indistinguishable from other sources of line broadening. Thus the use of the line width as a motional indicator is only practical in the case of the slow molecular motions described above. The range of accessible correlation times may be somewhat enlarged if the static line broadening is canceled out by some echo method and the motional T_{2m} measured alone.

Such slow molecular motions are also effective on the relaxation time in the rotating frame: $T_{1\rho}{}^{^{13}\mathrm{C}}$ is determined by two different processes, one resulting from "spin–lattice" effects and the other from "spin–spin" mechanisms:[10-15]

$$\left(T_{1\rho}{}^{^{13}\mathrm{C}}\right)^{-1} = \left(T_{1\rho}{}^{^{13}\mathrm{C}}{}_{\text{spin-lattice}}\right)^{-1} + \left(T_{1\rho}{}^{^{13}\mathrm{C}}{}_{\text{spin-spin}}\right)^{-1} \quad (3)$$

Spin–lattice relaxation results from the modulation of internuclear dipolar interactions by molecular motion at the rotating-frame Larmor frequency ω_{1C}. $T_{1\rho}{}^{^{13}\mathrm{C}}{}_{\text{spin-lattice}}$ is due to the same motions as T_{2m} is; it is maximum when the rate of molecular motion is equal to the carbon irradiation field strength in frequency units: $\omega_{1C} = \gamma_C H_{1C}$. Under the Hartmann–Hahn condition, $\omega_{1H} = \omega_{1C}$, so that $T_{1\rho}{}^{^{13}\mathrm{C}}{}_{\text{spin-lattice}}$ is equal to T_{2m}:[8,15,16]

$$T_{1\rho}{}^{^{13}\mathrm{C}}{}_{\text{spin-lattice}} = T_{2m} \quad (4)$$

The second mechanism, spin–spin relaxation, comes from modulation of the dipolar interactions by the spontaneous rigid-lattice proton spin fluctuations.[8,10-15] It is a depolarization process, which is exactly the reverse of the cross-polarization in an "ADRF" experiment:

$$T_{1\rho}{}^{^{13}\mathrm{C}}{}_{\text{spin-spin}} = T_{\mathrm{CH}}(\mathrm{ADRF}) \quad (5)$$

From an experimental point of view, T_{CH}(ADRF) measurements require a long and delicate nine-step procedure that has been recently carefully described.[17] In amorphous polymers, T_{CH}(ADRF)'s of methylene carbons are in the range 1–200 ms, depending on the carbon radio-frequency field strength and the dipolar local fields, which themselves are functions of internuclear distances and averaging of the dipolar interactions by fast motions.

Theoretical expressions for T_{CH}(ADRF) have been derived.[9,18] They can be written as

$$[T_{\mathrm{CH}}(\mathrm{ADRF})]^{-1} = \tfrac{1}{2}\langle M_{\mathrm{CH}}{}^{(2)}\rangle J_{\mathrm{D}}(\omega_{1C}) \quad (6)$$

where $\langle M_{\mathrm{CH}}{}^{(2)}\rangle$ is either the rigid-lattice truncated carbon–proton second moment or the second moment averaged over the fast motions, if any. (Fast motions are motions whose rate is much greater than the dipolar frequency $\gamma_C(M_{\mathrm{CH}}{}^{(2)})^{1/2}$.) Lattice sums involved in the expression of the spectral density $J_{\mathrm{D}}(\omega)$ are impossible to calculate for samples whose exact crystallographic structure is not known, and a fortiori for glasses. Therefore whatever its precise mathematical definition, the dipolar fluctuation spectral density $J_{\mathrm{D}}(\omega)$ must be approximated; the simpler approximation is an exponential function:[9,18]

$$J_{\mathrm{D}}(\omega) = \pi\tau_{\mathrm{D}} \exp(-\omega\tau_{\mathrm{D}}) \quad (7)$$

Expressions for τ_{D} have been explicitly calculated as a function of lattice sums[18] or with strong approximations as a function of proton local fields H_{L}:[9,19]

$$\tau_{\mathrm{D}} = 3^{1/2}/H_{\mathrm{L}} \quad (8)$$

Until now, in the field of polymers, a priori calculations of T_{CH}(ADRF) using eq 7 and 8 have been at best qualitative.[9] This is evidently due to approximating $J_{\mathrm{D}}(\omega_{1C})$ by an exponential in formula 7. Moreover, the strong quasi-exponential dependence of the spectral density $J_{\mathrm{D}}(\omega)$ on $\omega_{1C}\tau_{\mathrm{D}}$ makes the T_{CH}(ADRF) highly sensitive to the exact value of τ_{D}. Besides, as pointed out by Schaefer,[17] the observed T_{CH}(ADRF) of a powder sample is an average value over all T_{CH}(ADRF)'s resulting from the various orientations of the internuclear vectors in the magnetic field, and not a T_{CH}(ADRF) arising from a mean local field. The motional effects on T_{CH}(ADRF) are intricate since motions may affect both $\langle M_{\mathrm{CH}}{}^{(2)}\rangle$ and τ_{D}. However, owing to its exponential dependence on τ_{D}, for the radio-frequency field strength commonly used, T_{CH}(ADRF) is mostly sensitive to τ_{D} variations. Therefore, whenever some motional averaging of τ_{D} occurs, this averaging is the dominant effect and will override the averaging of $M_{\mathrm{CH}}{}^{(2)}$.

When calculating the variation of T_{CH}(ADRF) from one sample to another, one encounters the same dominant dependence on τ_{D}. At the present time, the best agreement between experimental and calculated T_{CH}(ADRF) has been obtained phenomenologically by Schaefer.[17] In the case of a methylene carbon, assuming a single local field $\mathrm{H_L}$ and using approximations of formulas 7 and 8, one can write eq 6 as[9,17]

$$T_{\mathrm{CH}}(\mathrm{ADRF}) = 0.000265 H_{\mathrm{L}} \exp(3^{1/2} H_{1C}/H_{\mathrm{L}}) \quad \text{ms} \quad (9)$$

where H_{L} and the applied carbon radio-frequency field H_{1C} are expressed in kHz. Schaefer et al.[17] have observed that H_{L} orientation effects can be taken into account phenomenologically by using eq 9, but by computing values relative to a known rigid-lattice polycrystalline value. Thus, with the experimental 10-ms T_{CH}(ADRF) of the methylene carbons of glycine as a reference value, for any other methylene carbon and measurements carried out under an applied carbon field of 37 kHz, one finds

$$T_{\mathrm{CH}}(\mathrm{ADRF}) = 1.09 H_{\mathrm{L}} \exp[7.0(9.2 - H_{\mathrm{L}})/H_{\mathrm{L}}] \quad \text{ms} \quad (10)$$

This equation assumes that variations of T_{CH}(ADRF) are only due to proton local field H_{L} changes, whereas the carbon–proton coupling $\langle M_{\mathrm{CH}}{}^{(2)}\rangle$, which appears in eq 6 and is included in the factor 0.000265, remains constant. From a strict point of view, this equation can describe two situations: (i) there is no motional averaging but H_{L} varies from one methylene group to another, depending on the distances of the neighboring protons; (ii) the methylene group is rigid, as may be for instance main-chain methylene groups, whereas the local field depends on side-chain protons. Side-chain motion can then reduce H_{L}, without averaging the carbon–proton coupling to a sizeable extent. However, it must be noted that eq 10 reflects the main trend of variation of T_{CH}(ADRF) since $T_{\mathrm{CH}}(\mathrm{ADRF})^{-1}$ has a linear dependence of $\langle M_{\mathrm{CH}}{}^{(2)}\rangle$ and an exponential one on H_L. Therefore, in that sense, it may be considered as a suitable approximation when both C–H and H–H couplings are modified by the motion.

Values calculated from eq 10 using proton local fields H_L determined by "proton-separated local field spectroscopy"[20] are in reasonable agreement with experimental T_{CH}(ADRF)'s derived from initial-slope determination.[17] Such measurements are lengthy and were not performed in this study.

Formula 10 is based upon the assumption of an exponential spectral density of dipolar fluctuations.[9,17] As noted above, this particular shape has no theoretical justification, but it has been shown to be able to represent experimental data obtained at ν_{1C} = 37 kHz. Therefore it seems reasonable to use the same assumption to estimate the T_{CH}(ADRF) variation when the applied carbon radio-frequency field strength is slightly different (of the order of 15%) from 37 kHz and to correct eq 10 by using eq 9.

It is very important to notice that whenever slow molecular motions are effectively dominant for $T_{1\rho}{}^{13C}$ relaxation, they also induce line-broadening effects. The range over which the effects of molecular motions are observable on $T_{1\rho}{}^{13C}$ is restricted by the spin–spin contribution whereas for T_{2m}, it is limited by the static line broadening, which is of the same order of magnitude as the spin–spin contribution to $T_{1\rho}{}^{13C}$ for H_1 values in the range 30–40 kHz (Figure 1). Owing to the experimental and theoretical difficulties in determining T_{CH}(ADRF) with enough accuracy, it appears that at low H_1's it might be easier in the case of slow motions to get information from T_{2m} measurements. Experiments to be published later are now in progress in our laboratory to remove the static chemical shift dispersion and bulk susceptibility effects and thus improve the measurement of the motional contribution to the line broadening. Other smaller "static" contributions such as off-resonance or too low power decoupling can be estimated from variations of some experimental parameters[8,15] more easily than the "spin–spin" contribution to $T_{1\rho}{}^{13C}$. Another alternative is to use sufficiently high H_1's:[13-16] The relative importance of the spin–spin contribution to $T_{1\rho}{}^{13C}$ decreases, so that $T_{1\rho}{}^{13C}$ can be dominated by spin–lattice contributions if H_1 is large enough and appears, in this latter case, as a suitable tool for the study of slow motions.

In the domain of fast motions, some complementary information can be derived from the cross-polarization relaxation time T_{CH}(SL) determined in spin-lock experiments. The rise of carbon polarization depends on the residual static dipolar interactions. When the dipolar interactions are partially averaged by fast motions, the rise of carbon polarization as a function of contact duration gets slower. Therefore, the study of the cross-polarization rate in a spin-lock experiment appears as a means, complementary to the relaxation studies, for investigating the motional behavior of solid compounds. Expressions for T_{CH}(SL) have been explicitly calculated under two different assumptions[18,21] and recently discussed in relation to their application to polymer systems.[9]

As long as the proton–proton homonuclear dipolar interaction is much stronger than the carbon–proton heteronuclear one, the amplitude of the initial nonexponential transient phenomenon is very weak and the rise of carbon polarization is mostly exponential. Such a situation is encountered in rigid organic solids for non-proton-bonded carbons such as carboxyl or quaternary carbons, or when large-amplitude motion exists, as for instance in adamantane, for any carbon, including methine and methylene ones.

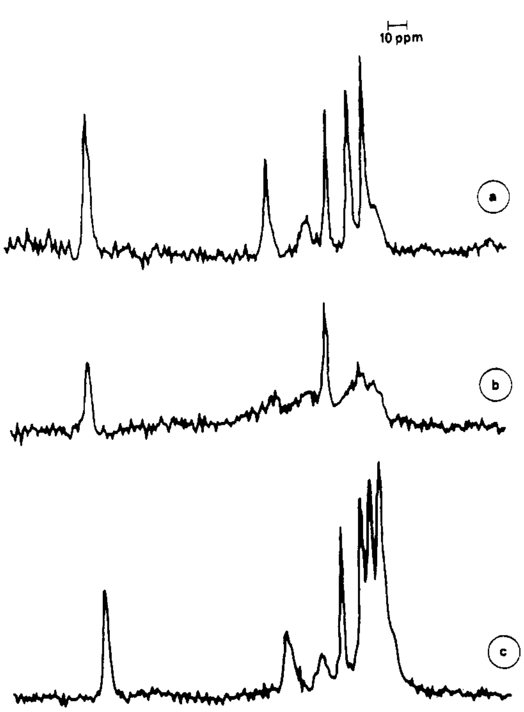

Figure 2. 12-MHz MAS CP ^{13}C NMR spectra of solid poly(cyclopentyl methacrylate) (a), poly(cyclohexyl methacrylate) (b), and poly(cycloheptyl methacrylate) (c). Matched spin-lock contact time t_c = 1 ms.

Adversely, it may happen that some protons are strongly coupled to carbons, whereas all the other protons are loosely coupled. Strongly and loosely mean that the carbon–proton coupling is higher or smaller than the proton–proton couplings. In a rigid organic compound, although the distinction between tightly or loosely coupled groups is not clear-cut, one must consider a carbon and its bound protons in a methine or a methylene group as a tightly coupled group. In such a case, the cross-polarization dynamics can no longer be described by a single cross-relaxation time. The initial step of the contact is a coherent energy transfer between the strongly coupled carbons and protons. This oscillatory transfer is damped by the coupling of the carbon and its directly attached protons to the remote protons. At the same time, this coupling induces (i) the establishment of a quasi-equilibrium state within the tightly coupled group and (ii) a cross-relaxation energy transfer from the tightly coupled carbon and protons to the remote protons viewed as a thermal bath.

At long times, the rise of polarization is well described by an exponential dependence on contact duration. Motional information is provided by the short-time behavior (first tenths of μs), which is ruled by the coherent energy transfer. In the case of a powder sample, the short-time polarization rise can be approximated by a quadratic function of contact duration.[21,22] The rise time $t_{1/2}$ to half of the total polarization depends on the strength of the dipolar interaction $\langle M_{CH'}{}^{(2)}\rangle$ of the carbon under interest and its bound protons. Values of $t_{1/2}$ as short as 20 μs for a CH_2 group or 28 μs for a CH group are indicative of rigid-lattice behavior. Longer $t_{1/2}$ values are evidence for motional reduction of $\langle M_{CH'}{}^{(2)}\rangle$.

Results and Discussion

Spectral Assignments. The magic-angle spinning (MAS) cross-polarization (CP) ^{13}C NMR spectrum of solid poly(cyclopentyl methacrylate) (Figure 2a) consists of seven lines which have been identified from left to right, in order of increasing magnetic field, as a line due to the

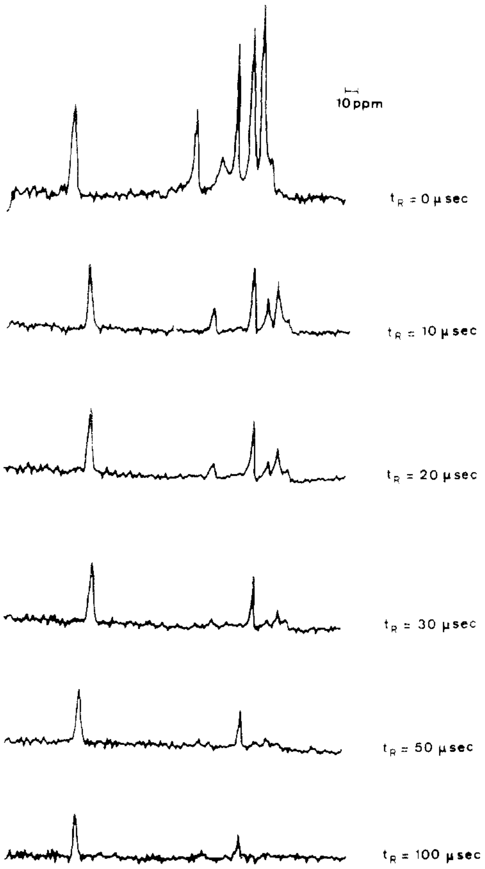

Figure 3. Delayed decoupled MAS CP ^{13}C NMR spectra of solid poly(cyclopentyl methacrylate) as a function of delay t_R. Matched spin-lock contact time t_c = 1 ms.

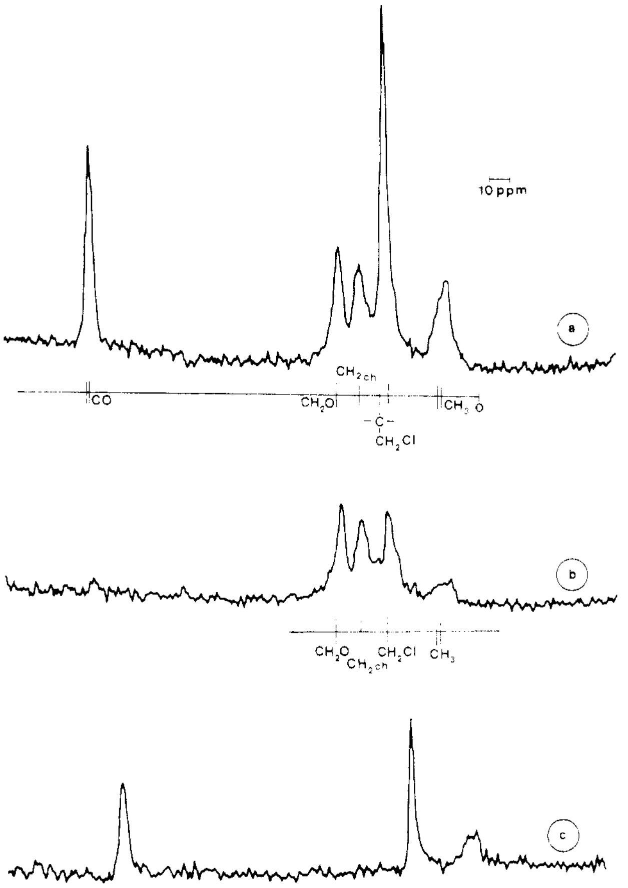

Figure 4. 12-MHz MAS CP ^{13}C NMR spectra of solid poly(chloroethyl methacrylate): (a) matched spin-lock contact time $t_c = 10^{-3}$ s (stick spectrum obtained from $CDCl_3$ solution NMR spectrum shown below the solid one); (b) $t_c = 2 \times 10^{-5}$ s; (c) delayed decoupled spectrum: $t_c = 10^{-3}$ s, $t_R = 30 \times 10^{-6}$ s.

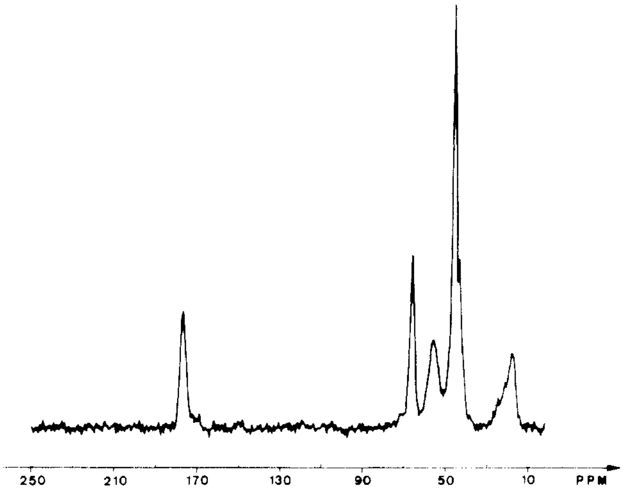

Figure 5. 75-MHz MAS CP ^{13}C NMR spectrum of poly(chloroethyl methacrylate). Rotational side bands have been suppressed by the Dixon method.[39] The use of the Dixon sequence does not introduce any distortion in the CH_2Cl line.

carboxyl carbon, a line due to the methine group of the side ring, a broad line arising from the main-chain methylene group, a line due to the main-chain quaternary carbon, two lines arising from the cyclopentyl methylene carbons, and a shoulder due to the methyl group. Assignment of the methine and methylene carbons is readily obtained by using empirical substituent effects.[23] Assignment of the quaternary carbon is supported by the delayed decoupled spectra[4,5] reported in Figure 3.

The spectra of poly(cyclohexyl methacrylate) and poly(cycloheptyl methacrylate) (Figure 2b,c) are identical with that of poly(cyclopentyl methacrylate) except for the side-ring methine and methylene lines. In the case of the cycloheptyl derivative, four sharp peaks are observed. On the contrary, for poly(cyclohexyl methacrylate), the resolution has disappeared in the side-ring methine and methylene regions. This point will be discussed in more detail later.

In the 12-MHz MAS CP ^{13}C NMR spectrum of poly(chloroethyl methacrylate) (Figure 4), the quaternary and CH_2Cl lines are not resolved. However, the fast polarization rise of a methylene carbon compared with that of a quaternary one allows the observation of the methylene line alone (Figure 4b) using a very short (20 μs) cross-polarization time. On the contrary, with a delayed decoupling sequence (Figure 4c), it is possible to observe only the quaternary and methyl carbon peaks. The assignments obtained in this way are supported by the 75-MHz spectrum shown in Figure 5.

Owing to the very low solubility of poly(cyanoethyl methacrylate) in the usual solvents, no solution spectrum of this polymer could be recorded even at 62 MHz. Here the CP MAS ^{13}C NMR spectrum of this compound was quickly and easily obtained (Figure 6). The α-CN methylene line is not apparently visible in the spectrum with 1-ms cross-polarization time. Results reported for acetonitrile[24] show that the nitrile group induces a remarkably small α-effect of 4–5 ppm. The α-CN methylene line is thus expected to be located beneath the methyl one. This conclusion is supported by the integrals of the peaks of the 1-ms cross-polarization spectrum and by the relative peak heights of the 20-μs spectrum which are mainly due to methylene carbons. (See for comparison the very small height of the methyl carbon line in the 20-μs cross-polar-

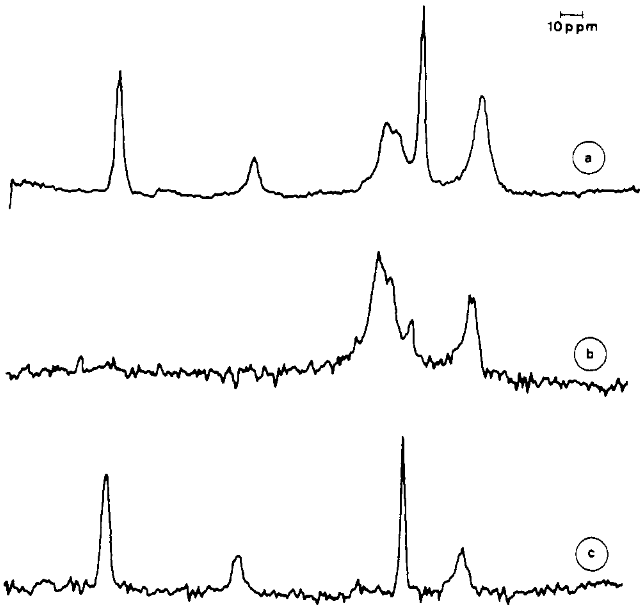

Figure 6. 12-MHz MAS CP ^{13}C NMR spectra of solid poly(cyanoethyl methacrylate): (a) matched spin-lock contact time $t_c = 10^{-3}$ s; (b) $t_c = 2 \times 10^{-5}$ s; (c) delayed decoupled spectrum: $t_c = 10^{-3}$ s, $t_R = 30 \times 10^{-6}$ s.

Table I
Rises of Carbon Polarization in a Spin-Lock Experiment

carbon	PCPMA[a] $t_{1/2}$, μs	PCHpMA[b] $t_{1/2}$, μs
C═O	250	225
quaternary	140	140
$CH_{lateral}$	37	40
$CH_{2\,lateral}$	30	30
$CH_{2\,chain}$	18	24

[a] Poly(cyclopentyl methacrylate). [b] Poly(cycloheptyl methacrylate).

ization spectrum of poly(chloroethyl methacrylate) shown in Figure 4b.)

Motional Analysis of Poly(cyclopentyl methacrylate) and Poly(cycloheptyl methacrylate). 1. Rises of Carbon Polarization in a Spin-Lock Experiment. The increase of magnetization in a spin-lock cross-polarization experiment as a function of the contact duration t_c is reported in parts a and b of Figure 7 for the various carbons of poly(cyclopentyl methacrylate) and poly(cycloheptyl methacrylate), respectively. The rise of polarization of the quaternary and carboxyl carbons is slow and obeys an exponential law over the range of contact times under study. This behavior is typical of carbons having a $\langle M_{HH}^{(2)} \rangle$ second moment higher than the $\langle M_{CH}^{(2)} \rangle$ one.

On the contrary, the methine and methylene carbons present a rapid nonexponential stepwise increase of the magnetization during the first 20–40 μs of the contact. The contact times $t_{1/2}$ at which half of the total polarization is reached are summarized in Table I. As pointed out above, in the case of carbons directly bonded to protons, these $t_{1/2}$ contact times are related to the strength of the dipolar interaction $\langle M_{CH'}^{(2)} \rangle$ of the carbon and its bound protons, and a $t_{1/2}$ value of about 20 μs is expected for a methylene carbon in a rigid lattice. Results reported in Table I show that the $t_{1/2}$ of the main-chain methylene carbons of poly(cyclopentyl methacrylate) is that of the rigid lattice. The main-chain of poly(cycloheptyl methacrylate) seems to be slightly more mobile. The ring carbon $t_{1/2}$ contact times are longer, indicating a substantial but not complete motional averaging of the dipolar interaction between carbons and directly bonded protons, achieved to approximately the same extent for the ring methine and methylene carbons. Therefore the cross-polarization data point out the existence of fast anisotropic ring motions, the frequency of which is much higher than 10^5 Hz, whereas the main-chain motions appear as frozen at the time scale of the experiment, that is much slower than 10^5 Hz.

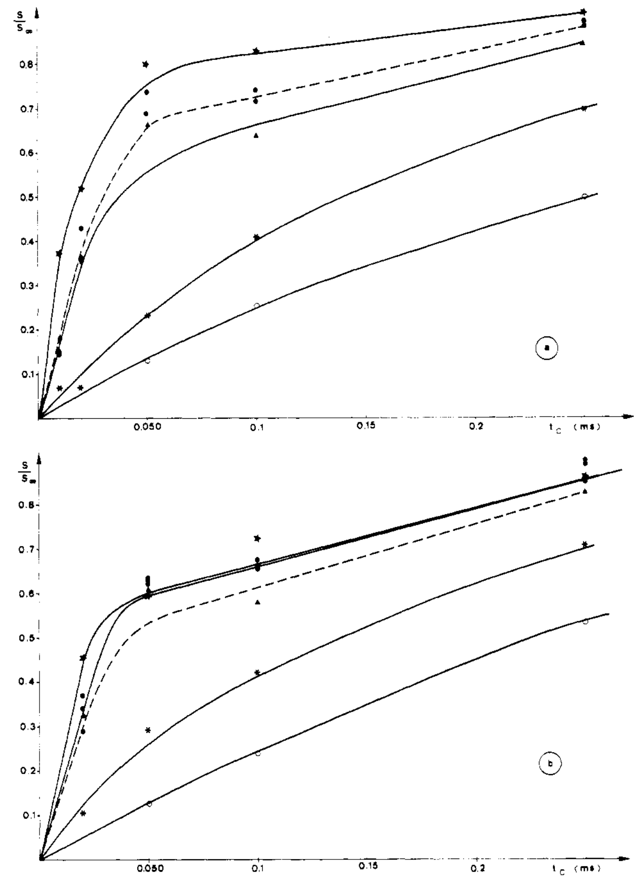

Figure 7. Variation of carbon magnetization in a spin-lock experiment as a function of contact duration t_c: (a) poly(cyclopentyl methacrylate); (b) poly(cycloheptyl methacrylate) [(★) chain CH_2; (○) C═O; (*) quaternary; (▲) side-chain CH; (●) side-chain CH_2].

Table II
$T_{1\rho}^{13C}$ (H_{1C} = 32 kHz) Spin–Lattice Relaxation Times (ms) in the Rotating Frame

polymer[a]	$CH_{2\,lateral}$[b]	$CH_{lateral}$[b]	$CH_{2\,chain}$[c]	quaternary[b]	CO[b]
PCPMA	11.1 8.6	12.0	2.0	14.3	46
PCHxMA[d]	1.1	1.5 ± 0.3	1.1 ± 0.2	7.1	27
PCHpMA	12.4 14.2 8.0	12.2	0.7	14.0	27

[a] PCPMA = poly(cyclopentyl methacrylate), PCHxMA = poly(cyclohexyl methacrylate), PCHpMA = poly(cycloheptyl methacrylate). [b] Least-squares straight line fit to relaxation data collected from t = 0.1 ms to t = 8 ms after turn off of the proton H_{1H}. [c] Least-squares straight line fit to relaxation data collected from t = 0.1 ms to t = 1 ms after turn off of H_{1H}. [d] Mean value determined over the unresolved lateral methylene lines.

2. Spin–Lattice Relaxation Times in the Rotating Frame. Experimental $T_{1\rho}^{13C}$ values are listed in Table II. As explained above, the difficulty in interpreting these results in terms of molecular motions consists in evaluating the spin–spin contribution T_{CH}(ADRF) to $T_{1\rho}^{13C}$. In the range of proton local fields H_L of interest for bulk polymers, T_{CH}(ADRF) as described by formula 9 is a decreasing function of H_L. Therefore the lowest T_{CH}(ADRF)'s, for methylene carbons, are obtained for the highest values of H_L, i.e., the most rigid polymers. For instance, using the experimental proton local fields and phenomenological expression (formula 10) determined by Schaefer[17] for an initial-slope measurement of T_{CH}(ADRF) and formula 9 to account for the carbon radio-frequency field strength dependence of T_{CH}(ADRF), one finds a 2-ms T_{CH}(ADRF) for high-density polyethylene and a 4-ms one for poly(oxymethylene) with 75% crystallinity, under a carbon radio-frequency field of 32 kHz at room temperature. In the case of glassy compounds, proton local fields are reduced, and T_{CH}(ADRF) increases. A typical example is given by the main-chain carbons of polystyrene,[17] for which the same calculation leads to a 12.6-ms T_{CH}(ADRF) at 32 kHz.

From data reported in Table II, the shortest $T_{1\rho}{}^{13C}$ values of poly(cyclopentyl methacrylate) and poly(cycloheptyl methacrylate) are obtained for the main-chain methylene carbon. Comparison of this experimental result with calculated $T_{1\rho}{}^{13C}{}_{\text{spin-lattice}}$ (Figure 1) or expected T_{CH}(ADRF) in polymer systems indicates either large-amplitude (i.e., $\geq 10°$) motions of the C–H internuclear vector with rates of the order of ω_{1C} ($\tau_c \sim 5 \times 10^{-5}$ to 5×10^{-7} s), if $T_{1\rho}{}^{13C}$ is dominated by motional effects, or, on the contrary, a high rigidity of the chain skeleton if $T_{1\rho}{}^{13C}$ is determined by the spin–spin T_{CH}(ADRF) term. The latter conclusion seems to be preferred since it coincides with the nonambiguous result deduced from the above study of the rate of growth of magnetization of the carbons in a spin-lock experiment.

The $T_{1\rho}{}^{13C}$ of the ring carbons of poly(cyclopentyl methacrylate) and poly(cycloheptyl methacrylate) are of the order of magnitude of expected T_{CH}(ADRF) for glassy polymers reported above. Indeed the ring process observed in the cross-polarization experiment will reduce the proton local fields at the sites of the side-chain carbons and lengthen the spin–spin contribution. However, the ring methine and methylene carbon $T_{1\rho}{}^{13C}$ are nearly equal (Table II). In order to interpret these data in terms of only a spin–spin contribution to $T_{1\rho}{}^{13C}$ one would need to assume either very different motional averaging of heteronuclear C–H dipolar interactions for the two kinds of protons with the same dipolar fluctuation correlation time τ_D and proton local field H_L or, on the contrary, different averaging of τ_D, the dipolar fluctuations on the methine protons becoming somewhat faster than those on the methylene protons. The former assumption is opposite to the conclusion of the cross-polarization experiment, which indicates that the C–H dipolar interactions are averaged to the same extent for both categories of carbons. The second assumption cannot be ruled out so easily: As the methine proton is on the less mobile part of the ring and closer to the chain, the strength of the proton–proton dipolar interaction is expected to be higher than that on the methylene protons. Therefore it is not unlikely that the $T_{1\rho}{}^{13C}$ values of the ring carbons of poly(cyclopentyl methacrylate) and poly(cycloheptyl methacrylate) are due to the spin–spin contribution. However, spin–lattice effects cannot be excluded without a careful determination of T_{CH}(ADRF).

Motional Analysis of Poly(cyclohexyl methacrylate). 1. Line Width Analysis. On Figure 2 are shown the MAS CP spectra of polymers of cyclopentyl, cyclohexyl, and cycloheptyl methacrylate recorded under identical conditions. The lines of the cyclopentyl and cycloheptyl ring carbons are narrow and well resolved. They are in sharp contrast with those of the cyclohexyl side group, which are so broadened that the resolution in the right part of the spectrum has disappeared. This broadening of aliphatic protonated carbon lines, which exceeds a few ppm, is a clear indication of modulation of the carbon–proton dipolar coupling by slow motions of the side ring.

2. Spin–Lattice Relaxation Times in the Rotating Frame. The $T_{1\rho}{}^{13C}$ values obtained for the various carbons of poly(cyclohexyl methacrylate) are given in Table II. In the case of the ring methylene lines, the lack of resolution of the spectrum allows measurement of only a mean relaxation time $T_{1\rho}{}^{13C}$ from the intensity variation of the whole ring methylene pattern. This mean $T_{1\rho}{}^{13C}$ of the ring methylene carbons of poly(cyclohexyl methacrylate) is 10 times shorter than those measured for the same carbons in poly(cyclopentyl methacrylate) and poly(cycloheptyl methacrylate). This shortening of $T_{1\rho}{}^{13C}$ is in agreement with the motional line broadening observed for the same carbons of poly(cyclohexyl methacrylate), and the equality $T_{1\rho}{}^{13C} \simeq T_{2m}$ is experimentally observed. Therefore both shortening of $T_{1\rho}{}^{13C}$ and line broadening point out the presence of slow molecular motions of the side ring with correlation times in the neighborhood of 10^{-6} s.

3. Nature of the Cyclohexyl Ring Motion. The slow motion of the cyclohexyl ring evidenced above may result from at least two different processes: (i) small jumps of the whole ring as a rigid body about the C–O bond and (ii) internal ring inversion. From geometrical considerations, the small-angle rotation of the ring about the C–O bond is expected to depend less strongly upon the size of the ring (n = 5, 6, or 7) than the internal ring inversion, the nature of which differs strongly according to the size of the ring.[1] Besides the latter mode is well-known to occur in cyclohexane and cyclohexane derivatives in both the solution[25] and solid[1] states. For these two reasons internal cyclohexyl inversion appears as the likely process observed in the present NMR study. It would be pleasant to confirm this identification from the relative $T_{1\rho}{}^{13C}$ values reported in Table II: Indeed conformational energy calculations[26] have shown that the chair geometry of the cyclohexyl ring with the chain in the equatorial position is the most stable conformation. In this chair conformation, 9 of the 10 C–H vectors of the methylene groups make the same angle with the C_1–O vector as the methine C–H vector does. Therefore, for this geometry, assuming jumps of the whole ring as a rigid body about the C_1–O bond, the carbon–proton dipolar interactions are modulated to the same extent as a first approximation, and the calculated $T_{1\rho}{}^{13C}(CH)/\langle T_{1\rho}{}^{13C}(CH_2)\rangle$, where $\langle T_{1\rho}{}^{13C}(CH_2)\rangle$ represents the mean value over the five methylene carbons, is approximately 2. Experimentally this ratio is 1.4 ± 0.5, somewhat smaller than the factor of 2 calculated above, and therefore rather in favor of a cyclohexyl inversion. However, the experimental accuracy in the determination of the $T_{1\rho}{}^{13C}$ values of poly(cyclohexyl methacrylate) does not allow us to consider this result by itself as a definite proof.

Comparison with Mechanical Measurements. Mechanical measurements carried out by Heijboer[1] on poly(cyclopentyl methacrylate), poly(cyclohexyl methacrylate), and poly(cycloheptyl methacrylate) have shown that these polymers present a loss maximum which has been identified with ring motions at ca. −185 °C (180 kHz), −80 °C (1 Hz), and −180 °C (1 Hz), respectively. The dependence of the loss maximum on absolute temperature and frequency ν is described by an Arrhenius equation:

$$\nu = \nu_0 \exp(-E_a/RT)$$

where R is the gas constant and ν_0 is 10^{13} Hz. The associated activation energies are 3.1, 11.3, and 6.2 kcal/mol

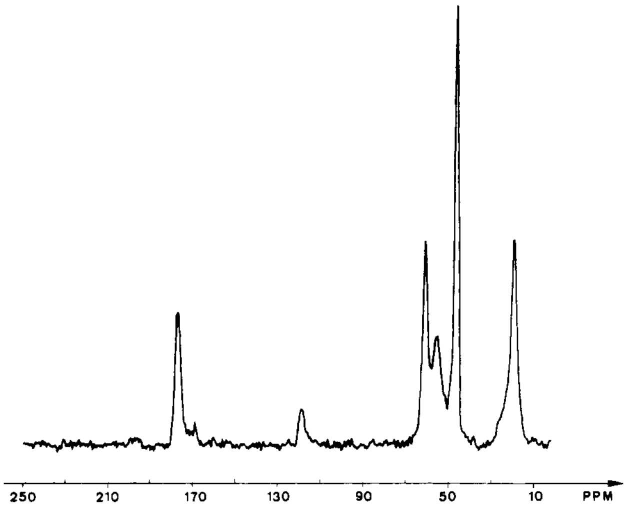

Figure 8. 75-MHz MAS CP ^{13}C NMR spectrum of poly(cyanoethyl methacrylate). Rotational side bands have been suppressed by using the Dixon method,[39] which did not induce any distortion in the C–N line.

for the cyclopentyl, cyclohexyl, and cycloheptyl derivatives, respectively. These data lead to the following values of the correlation times at 25 °C: $\tau = 3 \times 10^{-12}$ s for poly(cyclopentyl methacrylate), $\tau = 3 \times 10^{-6}$ s for poly(cyclohexyl methacrylate), and $\tau = 5 \times 10^{-10}$ s for poly(cycloheptyl methacrylate).

Thus, at room temperature, the molecular motions responsible for the mechanical losses of poly(cyclopentyl methacrylate) and poly(cycloheptyl methacrylate) are very rapid processes, having too high a frequency to broaden the line widths of MAS CP ^{13}C NMR spectra, in agreement with the absence of observable motional broadening in the spectra of these two polymers (Figure 2), but fast enough to reduce the static carbon–proton dipolar interactions, as observed from the rate of growth of polarization during the first tenths of microsecond of contact. Moreover the identification of these motions as ring-inversion modes[1] is in harmony with the fact that these static interactions are partly reduced but not completely averaged out.

On the contrary, in the case of poly(cyclohexyl methacrylate), the calculated correlation time is much longer. Its value agrees nicely with a strong line broadening of the ring carbon NMR peaks, as well as with a shortening of $T_{1\rho}{}^{13}$C. More information on the cyclohexyl ring motion will be brought by variable-temperature measurements to be published later.[27] Besides, solid-state high-resolution NMR results are in agreement with the conclusions reached by Heijboer[1] from conformational energy calculations, identifying the molecular process responsible for the γ-transition of poly(cyclohexyl methacrylate) as an internal inversion of the cyclohexyl side ring.

Side-Chain Motion of Poly(chloroethyl methacrylate) and Poly(cyanoethyl methacrylate). Spectra of poly(chloroethyl methacrylate) and poly(cyanoethyl methacrylate) at 12.07 and 75.46 MHz are shown in Figures 4, 5, 6, and 8. The full widths at midheight of the cyano carbon peak are 60 Hz at 12.07 MHz and 219 Hz at 75.46 MHz. In the case of the CH_2Cl carbon they are equal to 69 and 218 Hz, respectively. These line widths may be considered as essentially originating from only two contributions: a chemical shift dispersion term (δ in ppm) which is proportionnal to the applied static field H_0, and a dipolar broadening due to the coupling with the directly attached quadrupolar nucleus, which is not eliminated by magic-angle spinning. Quantitative calculations of the latter contribution have been reported recently[28-32] together with numerical simulations of spectra. If D_{C-N} is the dipolar coupling constant between ^{13}C and ^{14}N atoms, curves calculated in ref 32 show that the expected line width of a ^{13}C coupled to a ^{14}N is about $1.3D_{C-N}$ at 12.07 MHz and $0.244D_{C-N}$ at 75.46 MHz. Thus the δ and D_{C-N} parameters of the cyano carbon of poly(cyanoethyl methacrylate) are obtained from the coupled equations

$$12.07\delta + 1.3D_{C-N} = 60 \text{ Hz}$$

$$75.46\delta + 0.244D_{C-N} = 219 \text{ Hz}$$

which lead to $\delta \simeq 2.8$ ppm and $D_{C-N} \simeq 20$ Hz. In the case of the CH_2Cl carbon of poly(choroethyl methacrylate) the expected line width of a ^{13}C coupled to a ^{35}Cl is ca. $3.47D_{C-Cl}$ at 12.07 MHz and $2.2D_{C-Cl}$ at 75.46 MHz.[32] The same calculation as above gives $\delta \simeq 2.6$ ppm and $D_{C-Cl} \simeq$ 11 Hz.

Table III
Chemical Shift Parameters for Polymethacrylates and Related Compounds

polymer	$\Delta\sigma = \lvert\sigma_{33} - \sigma_{11}\rvert$, ppm
poly(phenyl methacrylate)	160 ± 5
poly(cyclohexyl methacrylate)	150 ± 5
poly(cyanoethyl methacrylate)	130 ± 5
methyl acetate[a]	147
acetic acid[b]	160

[a] Reference 37. [b] Reference 38.

These observed D_{C-N} and D_{C-Cl} values are very weak compared with those expected in a nitrile ($D_{C-N} = 1.48$ kHz[33]) or in a chloroethyl derivative ($D_{C-Cl} = \gamma_C\gamma_{Cl}\hbar/2\pi r^3 \simeq 540$ Hz, assuming that the C–Cl bond length is 1.767 Å). Therefore they are indicative of quasi-complete averaging of the dipolar coupling either by a motion or by a fast relaxation of the quadrupolar nucleus magnetization. Motional averaging is excluded since it would require wide-amplitude motions which would also cancel the side-chain carbon–proton coupling, and that is not observed, at least for poly(chloroethyl methacrylate) as seen in Figure 4b. On the other hand, mechanical measurements carried out on these compounds[34] have pointed out the existence of side-chain motions with correlation times of 8×10^{-7} and 2×10^{-9} s in poly(cyanoethyl methacrylate) and poly(chloroethyl methacrylate), respectively, at room temperature. Calculation of quadrupolar relaxation times T_{1Q},[35] using these correlation times and $[e^2qQ/h] = 3.87$ MHz[33] and 65.95 MHz[36] for the ^{14}N and ^{35}Cl nuclei, respectively, leads to T_{1Q} values much shorter than 1 ms, which result in a complete averaging of the nitrogen–carbon dipolar coupling. Thus the NMR line shapes under study agree with the correlation times deduced from mechanical measurements.

Motion of the Carboxyl Part of the Side Chain. A further insight into the extent of the side-chain motion may be obtained from the observation of the chemical shift tensor anisotropy of the carboxyl carbon. This anisotropy, which is suppressed by spinning at the magic angle, is partially recovered when spinning out of the magic angle (Figure 9). If β is the angle between the rotation axis and the static field, the resulting line shape is the nonspinning line shape scaled by $(3\cos^2\beta - 1)/2$ or about $2^{1/2}\epsilon$ for small values of the missetting angle ϵ between the spinning direction and the magic axis.

Data obtained for various polymethacrylates are summarized in Table III. Methyl acetate[37] and acetic acid[38] at low temperature are typical rigid examples. Although the motion of the side chain is very different in poly(cyclohexyl methacrylate) (inversion of the cyclohexyl ring), poly(phenyl methacrylate) (no such ring motion), and poly(cyanoethyl methacrylate) (rapid side-chain reorien-

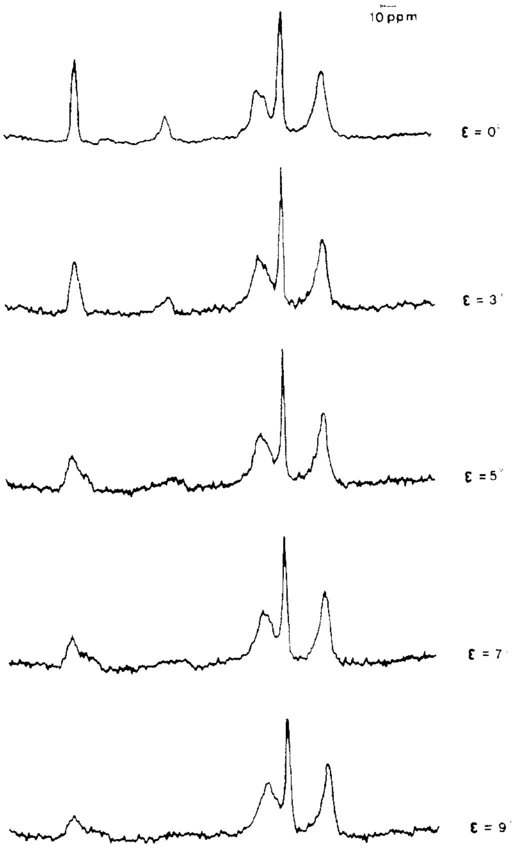

Figure 9. 12.07-MHz CP ^{13}C NMR spectra of solid poly(cyanoethyl methacrylate) as a function of the missetting angle ϵ between the spinning and magic angle axes.

tation), the observed carboxyl anisotropy is almost not affected by the nature of the side chain and is of the order of magnitude of the rigid-lattice value. Such a result had also been observed for poly(methyl methacrylate).[10] Therefore the ring or linear side-chain processes induce only very limited reorientation of the COO group in the cyclohexyl and cyanoethyl derivatives. However, within the accuracy of measurement of both the whole chemical shift tensor anisotropy and relative values of the principal components of this tensor, in the polymers under study and the model compounds,[37,38] the ability of the whole COO group to undergo a 10–20° oscillation about the bond linking the main-chain quaternary carbon to the carboxyl one cannot be ruled out.

Conclusion

The present study of polymethacrylates illustrates the ability of solid-state CP MAS ^{13}C NMR to identify the various molecular motions that are involved in the mechanical transitions of bulk polymers: Only the spectral lines of carbons belonging to the moving part of the molecule are affected and allow a detailed analysis and clear interpretation of the motion. The results thus obtained on poly(cycloalkyl methacrylates), poly(cyanoethyl methacrylate), and poly(chloroethyl methacrylate) are in good agreement with the conclusions derived from mechanical measurements and use of conformational energy calculations.[1,23] They also emphasize the interest and simplicity of line width measurements for detecting slow molecular motions in solids when small-amplitude radiofrequency fields are used. For larger H_1's, $T_{1\rho}{}^{^{13}C}$ may lead to a more precise diagnosis of these processes. Room-temperature experiments are of course very informative, but it is to be noticed that the amount of knowledge about motional processes in bulk polymers will be greatly enlarged by variable-temperature determinations. Such measurements, which lead to the thermal motional parameters and allow a higher number of compounds to be studied, are now in progress in our laboratory.

Acknowledgment. The authors are grateful to Dr. J. Schaefer for helpful discussion and communication of his manuscripts prior to publication. They thank Dr. J. P. Bayle for realizing the magic-angle spinning probe, Dr. J. Heijboer for kind gifts of polymer samples, and Drs. Muller and Forster, Bruker Analytische Messtechnik GMBH, Rheinstetten, for obtaining ^{13}C NMR spectra at 75 MHz.

Registry No. Poly(cyclohexyl methacrylate) (homopolymer), 25768-50-7; poly(cyclopentyl methacrylate) (homopolymer), 28298-90-0; poly(cycloheptyl methacrylate) (homopolymer), 28298-91-1; poly(phenyl methacrylate) (homopolymer), 25189-01-9; poly(chloroethyl methacrylate) (homopolymer), 26937-47-3; poly(cyanoethyl methacrylate) (homopolymer), 26139-78-6.

References and Notes

(1) J. Heijboer, Ph.D. Thesis, University of Leyden, 1972.
(2) J. Virlet, to be published.
(3) E. Henriot and E. Huguenard, *C. R. Hebd. Seances Acad. Sci.*, **180**, 1389 (1925).
(4) M. Alla and E. Lippmaa, *Chem. Phys. Lett.*, **37**, 260 (1976).
(5) S. J. Opella and M. H. Frey, *J. Am. Chem. Soc.*, **101**, 5854 (1979).
(6) E. O. Stejskal and J. Schaefer, *J. Magn. Reson.*, **18**, 560 (1975).
(7) J. Tegenfeldt and U. Haeberlen, *J. Magn. Reson.*, **36**, 453 (1979).
(8) D. L. VanderHart, W. L. Earl, and A. N. Garroway, *J. Magn. Reson.*, **44**, 361 (1981).
(9) T. T. P. Cheung and R. Yaris, *J. Chem. Phys.*, **72**, 3804 (1980).
(10) J. Schaefer, E. O. Stejskal, and R. Buchdahl, *Macromolecules*, **10**, 384 (1977).
(11) E. O. Stejskal, J. Schaefer, T. R. Steger, *Faraday Soc. Symp.*, **13**, 56 (1979).
(12) J. Schaefer, E. O. Stejskal, T. R. Steger, M. D. Sefcik, and R. A. McKay, *Macromolecules*, **13**, 1121 (1980).
(13) A. N. Garroway, W. B. Moniz, and H. A. Resing, *Faraday Soc. Symp.*, **13**, Chapter 6 (1979).
(14) D. L. VanderHart and A. N. Garroway, *J. Chem. Phys.*, **71**, 2773 (1979).
(15) A. N. Garroway, D. L. VanderHart, and W. L. Earl, *Philos. Trans. R. Soc. London, Ser. A*, **299**, 609 (1981).
(16) A. N. Garroway, W. B. Moniz, and H. A. Resing, Am. Chem. Soc. *ACS Symp. Ser.*, **No. 103**, 67 (1979).
(17) J. Schaefer, M. D. Sefcik, E. O. Stejskal, and R. A. McKay, submitted to *Macromolecules*.
(18) D. Demco, J. Tegenfeldt, and J. S. Waugh, *Phys. Rev. B*, **11**, 4133 (1975).
(19) L. Quiroga, These de 3eme cycle, Orsay, 1982.
(20) J. Schaefer, M. D. Sefcik, E. O. Stejskal, and R. A. McKay, *Macromolecules*, **14**, 280 (1981).
(21) L. Muller, A. Kumar, T. Baumann, and R. R. Ernst, *Phys. Rev. Lett.*, **32**, 1402 (1974).
(22) G. C. Chingas, A. N. Garroway, R. O. Bertrand, and W. B. Moniz, *J. Chem. Phys.*, **74**, 127 (1981).
(23) F. W. Wehrli and T. Wirthlin, "Interpretation of Carbon-13 NMR Spectra", Heyden and Son Ltd., London, 1976, p 37.
(24) G. A. Gray, G. E. Maciel, and P. D. Ellis, *J. Magn. Reson.*, **1**, 407 (1969).
(25) F. A. L. Anet, and A. J. R. Bourn, *J. Am. Chem. Soc.*, **89**, 760 (1967), and references therein.
(26) Reference 1, p 74.
(27) F. Laupretre, J. P. Bayle, and J. Virlet, to be published.
(28) N. Zumbulyadis, P. M. Henrichs, and R. H. Young, *J. Chem. Phys.*, **75**, 1603 (1981).
(29) A. Naito, S. Ganapathy, and G. A. McDowell, *J. Magn. Reson.*, **48**, 367 (1982).
(30) A. Samoson, E. Kundla, and E. Lippmaa, *J. Magn. Reson.*, **49**, 350 (1982).
(31) J. G. Hexem, M. H. Frey, and S. J. Opella, *J. Chem. Phys.*, **77**, 3847 (1982).
(32) E. M. Menger and W. S. Veeman, *J. Magn. Reson.*, **46**, 257 (1982).
(33) A. Naito, S. Ganapathy, and C. A. McDowell, *J. Chem. Phys.*, **74**,5393 (1981).
(34) H. A. Waterman, L. C. E. Struik, J. Heijboer, and M. P. van Duijkeren, "Amorphous Materials", R. W. Douglas and B. Ellis, Eds., 3rd International Conference on Physics of Non-

Macromolecules **1984,** *17,* 1405–1412

crystalline Solids, Sheffield, 1970, Wiley, New York, 1972, p 29.
(35) A. Abragam, "The Principles of Nuclear Magnetism", Oxford University Press, London, 1961, Chapter 8.
(36) B. P. Dailey, *J. Chem. Phys.*, **33**, 1641 (1960).
(37) A. Pines, M. G. Gibby, and J. S. Waugh, *Chem. Phys. Lett.*, **15**, 373 (1972).
(38) J. van Dongen Torman, W. S. Veeman, and E. De Boer, *Chem. Phys.*, **24**, 45 (1977).
(39) W. T. Dixon, *J. Magn. Reson.*, **44**, 220 (1981).

High-Resolution ^{13}C NMR Study of Silk Fibroin in the Solid State by the Cross-Polarization–Magic Angle Spinning Method. Conformational Characterization of Silk I and Silk II Type Forms of *Bombyx mori* Fibroin by the Conformation-Dependent ^{13}C Chemical Shifts

Hazime Saitô,*[†] Ryoko Tabeta,[†] Tetsuo Asakura,[‡] Yoshihiro Iwanaga,[‡] Akira Shoji,[§] Takuo Ozaki,[§] and Isao Ando[⊥]

Biophysics Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan, Department of Biopolymer Engineering, Faculty of Technology, Tokyo University of Agriculture and Technology, Nakamachi 2-chome, Koganei, Tokyo 184, Japan, Department of Industrial Chemistry, College of Technology, Gunma University, Kiryu, Gunma 376, Japan, and Department of Polymer Chemistry, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan. *Received August 24, 1983*

ABSTRACT: High-resolution ^{13}C NMR (75.46 MHz) spectra of silk fibroins in the solid state were recorded by the cross-polarization–magic angle spinning method with emphasis on revealing conformational features of the dimorphic structures, silk I and II, of *Bombyx mori* fibroin prepared under different conditions. It was found that the ^{13}C chemical shifts of Gly, Ala, and Ser residues of silk II samples from *B. mori* fibroin by different preparations and of cocoon samples from several silkworms gave identical values with those of corresponding model polypeptides having the β-sheet conformation. Thus, identification of the silk II type form is easily performed by examining their ^{13}C chemical shift values. The ^{13}C chemical shifts of samples having the silk I form are significantly displaced from those of the silk II form, and can be used for diagnostic purposes. As expected, the ^{13}C chemical shifts of Ala and Gly residues of the silk I samples were identical with those of (Ala-Gly)$_n$II. However, we found that none of the ^{13}C chemical shifts predicted from the crankshaft model [Lotz, B.; Keith, H. D. *J. Mol. Biol.* **1971,** *61,* 201–215] in which Ala and Gly residues are close to the β-sheet and α-helix conformations, respectively, was in agreement with the ^{13}C chemical shifts of (Ala-Gly)$_n$II and silk I type form. Instead, we found that predicted ^{13}C chemical shifts from the loose helix proposed by Konishi and Kurokawa [Konishi, T.; Kurokawa, M. *Sen'i Gakkaishi* **1968,** *24,* 550–554] on the basis of the calculated ^{13}C contour map of chemical shifts for Ala residue are in good agreement with the displacement of the ^{13}C chemical shifts.

Introduction

In our previous papers, we have demonstrated that the ^{13}C chemical shifts of a number of polypeptides and proteins in the solid state as determined by the cross-polarization–magic angle spinning (CP–MAS) method are significantly displaced depending on their particular conformations such as the right-handed α-helix (α_R-helix), left-handed α-helix, (α_L-helix), β-sheet, ω-helix, 3_1-helix, and collagenlike triple helix.[1-6] Similar observation has been made independently by Kricheldorf and co-workers.[7-11] It is clear that the ^{13}C chemical shifts of amino acid residues are not mainly determined by the sequence but by the local conformation as defined by the torsion angles (ϕ and ψ) as well as the manner of hydrogen bonding.[1-6] This view was supported by our theoretical calculation of the contour map of ^{13}C chemical shifts utilizing FPT-INDO theory.[12] This finding permits one to use the conformation-dependent ^{13}C chemical shifts as an intrinsic probe to examine conformational behavior of polypeptides and proteins in the solid[1-6] and solution[13] states as viewed from the individual amino acid residues under consideration. In particular, we previously showed that the antiparallel β-sheet (silk II) of *Bombyx mori* fibroin and the α-helix form of *Philosamia cynthia ricini* fibroin in the solid state are well characterized by the conformation-dependent ^{13}C shifts of Gly, Ala, and Ser residues with reference to those of (Gly)$_n$I, (Ala)$_n$, and sequential model peptides.[3] In a similar manner, we demonstrated that the conformation of collagen fibrils can be analyzed by the conformation-dependent ^{13}C chemical shifts, referred to those of the appropriate model polypeptides, form II of (Gly)$_n$, (Pro)$_n$, and (Ala-Gly-Gly)$_n$ and polytripeptides of (Pro-Ala-Gly)$_n$ and (Pro-Gly-Pro)$_n$.[6]

Hence, it is now feasible to carry out conformational characterization of a number of other proteins by means of the CP–MAS NMR method, if the ^{13}C chemical shifts of suitable reference polypeptides are available. The major advantage in recording the ^{13}C NMR spectra in the solid state is that the polymorphic structure of polypeptides and proteins can be studied without any problems from the disruption of their conformations by dissolving in solvents. Furthermore, the ^{13}C chemical shifts in the solid state are in many instances free from conformational fluctuation as

---

[†] National Cancer Center Research Institute.
[‡] Tokyo University of Agriculture and Technology.
[§] Gunma University.
[⊥] Tokyo Institute of Technology.

0024-9297/84/2217-1405$01.50/0