Collagen is the major protein component of connective tissues. Its primary function is structural (1) with collagen fibrils imparting mechanical stability to tissues. Of the different types of collagen, type I is found in tendon, skin, and bone (1, 2). The type I collagen molecule consists of two α1 (1) chains and one α2 chain. These three chains wind around each other to form a triple helix (2), having a length of 3000 Å and a diameter of 15 Å. No other longer than 30 Å and a diameter of 15 Å. The sequence Gly-X-Y is repeated throughout each α-chain except for short nonhelical segments at the ends of the molecule. Because of steric interactions, glycine residues occupy positions within the interior of the helix whereas the side chains of X and Y residues are on the surface (3). As a result of interactions among the side chains, collagen molecules associate to form fibrils (3). In the fibril, collagen molecules are staggered relative to one another by ~670 Å, and they are covalently cross-linked at their nonhelical ends (1, 2). The resulting network of staggered cross-linked helical molecules produces high tensile strength fibers (1, 2). The staggering of collagen molecules results in gap and overlap regions in the fibril (Fig. 1) which may have special significance in bone (1, 2). The major constituents of bone are mineral (hydroxyapatite) and the organic matrix, more than 90% of which is collagen (5). It has been hypothesized that mineral initially deposits in the gap region and then spreads to fill all of the available space in the fibril (4).

Although much information about the three-dimensional structure and arrangement of collagen molecules in the fibril has come from x-ray diffraction and electron microscopic studies (4, 6, 7), these methods provide only a static view of the collagen molecule in the fibril. Recently, NMR studies have provided the first information about molecular dynamics in reconstituted (uncross-linked) type I collagen fibrils (8-11). These studies show that the backbone of the collagen molecule undergoes restricted but rapid reorientation probably about its long axis (8, 9). In addition, there is rapid side chain motion, indicating that the fibrillar structure is stabilized by multiple sets of interactions (10).

In the present communication, we report the first study of collagen peptide backbone dynamics in intact soft and hard tissues. The collagen peptide backbone is labeled in various tissues by injecting [1-13C]glycine into rats, and the carbonyl line shapes observed in proton-decoupled 13C NMR spectra are analyzed to address the following question. How is the collagen peptide backbone mobility, observed in reconstituted fibrils, affected by (a) the presence of cross-linking in soft tissue and (b) the mineralization of collagen in bone?

**MATERIALS AND METHODS**

[1-13C]Glycine (90 atom % 13C) was purchased from Merck Isotopes (Canada) and characterized by elemental and amino acid analyses and by mass spectrometry. Reconstituted collagen fibrils were labeled by means of chick calvaria tissue culture: the protein was characterized by using the method described in Ref. 9. The sample was equilibrated with 0.02 M Na2HPO4 and packed into an NMR tube. Rat calvaria and tail tendon were labeled by injecting a 0.9% NaCl solution of [1-13C]glycine (2.5 M) subcutaneously above the calvaria in the following manner. Initially, we injected 30 μl of glycine solution into the 3-day-old rats for 5 days. Injection volume was increased to 50 μl for the next 7 days and finally 100 μl were injected from day 15 through day 20. The rats were killed on the 21st day. Calvaria were taken out and the periostum was scraped off. The calvaria were then defatted, washed, cut in small pieces, equilibrated with 0.15 M NaCl, and finally packed into an NMR tube. Tendons were pulled out of the tail and washed...
with 1% Triton solution in 0.45 M NaCl. They were defatted, washed, equilibrated with 0.15 M NaCl, and then packed into an NMR tube. Control samples of tendon and calvaria were obtained in a similar manner except that unlabeled glycine was used for injection. The amino acid composition of collagen in calvaria and tail tendon was determined by analyses of protein hydrolysates using a Durum automatic amino acid analyzer (3). Mineral content in 21-day-old rat calvaria was determined to be 55% from the difference in weight of the calvaria sample before and after burning it to ash by increasing the temperature in stages to 800°C. This result indicates that mineralization is essentially complete in 21-day-old rat calvaria (12). The mineral was characterized as hydroxyapatite by elemental and x-ray diffraction analyses (12). Atomic absorption spectrophotometry (Perkin-Elmer 603) was used for determining Ca	extsuperscript{++} content in mineral and the phosphate analysis was performed by using the method described in Ref. 13.

The per cent incorporation of 	extsuperscript{13}C was determined to be 12% in the tissues and 50% in the reconstituted collagen by gas chromatography/mass spectrometry analyses of the N-acetylmethyl ester derivatives of the amino acids obtained after hydrolyzing the proteins with 6 N HCl at 110°C for 24 hr (9).

	extsuperscript{13}C magnetic resonance spectra were obtained on a home-built solid state spectrometer employing a wide bore Oxford-250 superconducting magnet (5.9 Tesla) operating at 62.98 MHz for 	extsuperscript{13}C nuclei. The Hartmann-Hahn condition ($\gamma_1 B_1 = \gamma_2 B_2$) is established by observing the proton-enhanced spectrum of an adamantane sample with 1.0 M NaCl. The power levels in the $B_1$ and $B_2$ channels were adjusted using precision attenuators at the input of the amplifiers, and the largest amplitude adamantane signal was taken as the NOE value (18). Within experimental error, we were unable to detect anisotropy in either TI or NOE values.

Free induction decays were collected using quadrature detection with a 100-kHz spectral window. An exponential filtering of 200 Hz was used to improve sensitivity. Constant sample temperature (within ±1°C), was attained by passing nitrogen gas through the probe Dewar. The gas temperature was regulated by a Variar temperature controller. The sample temperature was measured before and after each experiment using a copper/Constantan thermocouple placed in the gas stream 1–2 mm above the NMR sample tube.

Spin-lattice relaxation times ($T_1$) were measured using an inversion-recovery pulse sequence ($180^\circ - t - 90^\circ - T_1$). $T_1$ values were calculated from the integrated intensities of the carbonyl resonance using a least squares fit of the data. NOE values were determined as follows. A spectrum was obtained with the protons saturated continuously but decoupled only during acquisition of free induction decay. The second spectrum was obtained by decoupling the protons during acquisition of free induction decay with the proton rf gated off otherwise. After normalizing the gated decoupled and continuously irradiated spectra to the same number of acquisitions, the former was multiplied by a scale factor and then subtracted from the continuously irradiated spectrum in the computer. The scale factor that yielded a null difference signal in the carbonyl region of the spectrum was taken as the NOE value (19). Within experimental error, we were unable to detect anisotropy in either $T_1$ or NOE values.

FIG. 1. Illustration of the D stagger arrangement of molecules in collagen fibril and the gap and overlap regions.

FIG. 2. Proton-enhanced and decoupled 62.98-MHz 	extsuperscript{13}C NMR spectra of mineralized rat calvaria a, natural abundance; b, labeled with [1-13C]glycine. In each case, the spectrum is taken at 22°C with 3-ms contact time and 2-s repetition rate.

FIG. 3. Comparison of the 62.98-MHz 	extsuperscript{13}C NMR spectra of [1-13C]glycine-labeled collagen from different tissues at 22°C. Proton-enhanced and decoupled spectra: a, rat tail tendon; b, demineralized rat calvaria; c, mineralized rat calvaria; 1-ms contact time and 2-s repetition rate. 90° - $t$ proton-decoupled spectra of: d, rat tail tendon; e, demineralized rat calvaria; f, mineralized rat calvaria. $t = 20$ s.
RESULTS

A 62.98-MHz natural abundance $^{13}$C spectrum of rat calvaria collagen is shown in Fig. 2a. Although this spectrum was obtained with proton dipolar decoupling, a well defined chemical shift powder pattern is not seen because of the poor resolution and the small signal to noise ratio obtained in natural abundance. In contrast, an axially symmetric chemical shift powder pattern is clearly seen in the carbonyl region of the spectrum of rat calvaria collagen labeled with [1-$^{13}$C]glycine (Fig. 2b). Well resolved chemical shift powder patterns are also seen in the spectra of demineralized calvaria and rat tail tendon collagen (Fig. 3). As is seen in the figure, the line shape observed for each sample using the 90° -t sequence is almost identical with the line shape obtained using Hartmann-Hahn matched cross-polarization. Principal values ($\sigma_{xx}$, $\sigma_{yy}$, $\sigma_{zz}$) of the chemical shift tensors of the glycine carbonyl carbon are measured from these spectra and are listed in Table I. In each case, the $\sigma_{xx}$ values are established by a computer simulation of the line shape. Also listed in the table are the glycine carbonyl carbon powder line widths, $\Delta$, defined as $\Delta = \sigma_{xx} - \sigma_{zz}$.

At 22 °C, the residual powder line width of the glycine carbonyl carbon in rat calvaria collagen (140 ppm) is significantly larger than the line width of demineralized rat calvaria (120 ppm), rat tail tendon (124 ppm), and reconstituted chick calvaria collagen (108 ppm). In contrast, at -35 °C the same value of $\Delta$ (145 ppm) is measured from the spectra of all collagen samples (Fig. 4). This value of $\Delta$ is close to the static value of $\Delta$ measured for crystalline glycyl-glycine and polyglycine, 150 and 142 ppm, respectively. The small differences in $\Delta$ for collagen at -35 °C, glycyl-glycine, and polyglycine are probably a consequence of differences in sequence and conformation (19).

There is no apparent distortion of the glycyl carbonyl line shape in collagen due to $^{14}$N-$^{13}$C dipolar coupling. These spectra were taken at 5.9 $^T$ (62.98 MHz) and at this field the average contribution of the static heteronuclear ($^{14}$N-$^{13}$C) coupling to $^{13}$C line width was calculated to be 21.6 ppm using Equation 3.56 in Ref. 20. The glycine carbonyl line widths measured (in parts/million) for the collagen samples at 22 °C and 1.4 $^T$ (15.09 MHz) are the same as those obtained at 22 °C and 5.9 $^T$, in spite of the fact that the calculated static $^{14}$N-$^{13}$C coupling has an average value of 90 ppm at 1.4 $^T$. This result is strong evidence that $^{14}$N spin-lattice relaxation time ($T_1$) is short enough at 22 °C to effectively decouple the peptide nitrogen from the glycine carbonyl carbon. Therefore, the contribution of $^{14}$N-$^{13}$C dipolar coupling to the $^{13}$C line shapes, observed at 22 °C and 5.9 $^T$, is negligible. This statement also applies to the 62.98-MHz spectra obtained at -35 °C since at this temperature the low field (1.4 $^T$) spectra show only minor broadening (10-15 ppm) due to $^{14}$N-$^{13}$C dipolar coupling.

Table II summarizes the $T_1$ and NOE values measured for the glycine carbonyl carbon in the collagen samples. These quantities are measured using the procedures described under "Materials and Methods."

**DISCUSSION**

Previous $^{13}$C and $^1$H NMR studies (8, 9, 11) have shown that the peptide backbone is flexible in fibrils of reconstituted chick calvaria collagen. Since it is known from x-ray and electron microscopic investigations that collagen fibrils are highly organized in the direction parallel to the molecular

### Table I

**Principal values of the chemical shift tensor elements for glycine peptide carbonyl carbon in glycyl-glycine and in collagen from different tissues**

| Sample                     | $\sigma_{xx}$ | $\sigma_{yy}$ | $\sigma_{zz}$ | \(\Delta = \sigma_{xx} - \sigma_{zz}\) |
|---------------------------|---------------|---------------|---------------|----------------------------------------|
| Glycyl-glycine            | -72.7         | -4.6          | 77.3          | 150                                    |
| Mineralized calvaria      | -69.7         | -5.6          | 75.3          | 145                                    |
| Tail tendon*              | -60.7         | -3.0          | 63.7          | 124                                    |
| Reconstituted collagen fibril* | -59.3      | -1.4          | 60.7          | 120                                    |
| Mineralized calvaria      | -67.7         | -4.6          | 72.3          | 140                                    |
| Tail tendon               | -69.7         | -5.6          | 75.3          | 145                                    |
| Reconstituted collagen fibril* | -59.3      | -1.4          | 60.7          | 120                                    |

* Temperature = -35 °C.

### Table II

**Comparative measurements of $T_1$ and NOE values for glycine peptide carbonyl carbon of collagen from different tissues**

| Sample                      | $T_1^*$ | $T_1^+$ | NOE |
|-----------------------------|--------|--------|-----|
| Mineralized rat calvaria    | 4.3    | 9.6    | 1.8 |
| Demineralized rat calvaria  | 7.3    | 1.6    | 1.6 |
| Rat tail tendon             | 3.0    | 6.9    | 1.4 |
| Reconstituted collagen fibril* | 2.0   | 1.6    |     |

* Measured at 15.09 MHz.

**DISCUSSION**

Previous $^{13}$C and $^1$H NMR studies (8, 9, 11) have shown that the peptide backbone is flexible in fibrils of reconstituted chick calvaria collagen. Since it is known from x-ray and electron microscopic investigations that collagen fibrils are highly organized in the direction parallel to the molecular
axis, it has been assumed that molecular motion is primarily a consequence of reorientation about the long axis of the molecule. Therefore, the molecule does not have a fixed azimuthal orientation but rather it rapidly samples a distribution, $p(\gamma)$, of azimuthal angles, $\gamma$. We define the root mean square fluctuation in azimuthal angle, $\gamma_{rms}$ as

$$
(\gamma_{rms})^2 = \langle \gamma^2 \rangle = \int_{0}^{\pi} p(\gamma) (\gamma - \gamma_m)^2 d\gamma
$$

(1)

where $\gamma_m$ is the mean value of $\gamma$. If $\gamma_{rms} \leq 0.7$ radian, we show in the Appendix that it is not necessary to specify $p(\gamma)$ explicitly since the NMR line shape depends only upon $\gamma_{rms}$. In the case of a two-site model in which azimuthal orientations $\gamma_1$ and $\gamma_2$ are equally populated, $\gamma_{rms} = (\gamma_1 - \gamma_2)/2$. The previous NMR studies (9, 11) of lathrytic collagen showed that $\gamma_1 - \gamma_2 = 30^\circ$ which implies that $\gamma_{rms} = 15^\circ$ for the uncross-linked and unmineralized collagen fibrils.

The present study investigates the effect of cross-linking and mineralization on the peptide backbone motion in the collagen fibril. Of the four samples studied, the smallest value of $A$ (108 ppm) is observed for the reconstituted lathrytic chick calvaria collagen fibrils which are neither cross-linked nor mineralized. In contrast, the largest value of $A$ (140 ppm) is observed for the cross-linked and mineralized collagen fibrils in rat calvaria. Intermediate size values of $A$ (120–124 ppm) are observed for cross-linked collagen fibrils in rat tail tendon (not mineralized) and demineralized rat calvaria. Since all samples have the same line shapes and equal $A$ values at $-35^\circ$C we ascribe the difference in $A$ values observed at room temperature to differences in molecular mobility in the various samples at 22°C. Our problem is to determine $\gamma_{rms}$ for the various samples from an analysis of the observed NMR line shapes.

Values of $\gamma_{rms}$ can be obtained by calculating the effect of motion on the line shape of the glycine carbonyl carbon. The detailed procedure for determining $\gamma_{rms}$ is given in the Appendix. In brief this procedure is as follows. First, we assume that the orientation of the glycine peptide carbonyl chemical shift tensor is the same as that found for the model peptide glycyl-glycine. Second, we assume that the orientation of the glycyl molecular axis in collagen is the same as that in the collagen model peptide (Pro-Pro-Gly)$(\alpha)_{22}$ (21). Third, the principal components of the static carbonyl chemical shift tensor are taken to be those we have measured for all collagen samples at $-35^\circ$C. Fourth, we use the above information to write the shift tensor in the coordinate system fixed in the triple helix for any value of the azimuthal angle, $\gamma$. Fifth, if $\gamma$ assumes more than one value, as a consequence of rapid reorientation of the molecule, the averaged shift tensor is calculated and diagonalized as described in the Appendix. The resulting principal elements of the tensor yield the motions averaged line shape which is compared with the experimental line shape. Small adjustments ($\leq 10^\circ$) are made in the orientation of carbonyl shift tensor to achieve the best agreement between calculated and observed line shapes.

The results of the calculation are shown in Table III, which lists the values of $\gamma_{rms}$ obtained for collagen in different samples. Comparison of Tables I and III shows that our model not only gives the correct value of the experimental line widths ($\Delta$) in every case, but also predicts the observed principal elements of the shift tensor as well. The calculated spectra using these principal elements are in excellent agreement with the experimental spectra as shown in Fig. 5.

| $\gamma_{rms}$ in defined in Equation 1 | $\gamma_{rms}$ ppm | $\Delta$ psi- | $\gamma_{rms}$ ppm |
|---|---|---|---|
| $-69.7$ | $-5.6$ | $75.3$ | $145$ | $0^\circ$ |
| $-67.7$ | $-4.6$ | $72.3$ | $140$ | $14^\circ$ |
| $-60.7$ | $-2.6$ | $63.3$ | $124$ | $31^\circ$ |
| $-59.4$ | $-1.2$ | $60.6$ | $120$ | $33^\circ$ |
| $-53.7$ | $0.8$ | $54.3$ | $108$ | $41^\circ$ |

**TABLE III**

Calculated principal values for chemical shift tensor elements and the corresponding value of $\gamma_{rms}$

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The values of $\gamma_{rms}$ listed in Table III show that reorientation is slightly more restricted in cross-linked fibrils ($\gamma_{rms} = 33^\circ$) than in reconstituted (uncross-linked) fibrils ($\gamma_{rms} = 41^\circ$). However, in mineralized collagen fibrils the reorientation is significantly more restricted, $\gamma_{rms} = 14^\circ$. Before discussing the implications of these results, we will comment upon the accuracy of the $\gamma_{rms}$ values listed in Table III. We will also compare the $\gamma_{rms}$ value calculated in the present study for the reconstituted collagen fibrils with that obtained previously from $^{13}$C relaxation studies (8, 9).

As noted earlier, we have assumed that the orientation of the glycyl peptide carbonyl shift tensor and the orientation of the glycyl molecular axis can be obtained from data on model compounds. In fact, small differences in orientation...
between the model compounds and the collagen molecule are expected because of differences in crystal packing, molecular conformation, and amino acid sequence. We find that if the values of the Euler angles (see Appendix) are varied by ±30° in our calculation it is still possible to reproduce the observed line widths (although not usually the line shapes) but the \( \gamma_{\text{rms}} \) values obtained differ from those in Table III by as much as 20%. Significantly, for a given variation in Euler angles, \( \gamma_{\text{rms}} \) values calculated for all collagen samples experience nearly the same fractional change. In a related calculation, we obtain \( \gamma_{\text{rms}} \) values, each greater by \( \approx 5^\circ \) than those listed in Table III, if a static line width of 150 ppm (as found for glycyglycine) rather than 145 ppm is assumed.

The uncertainty in the calculated values of \( \gamma_{\text{rms}} \) may partly explain the discrepancy between \( \gamma_{\text{rms}} \) observed for the reconstituted collagen fibrils from \(^{13}C\) relaxation data (\( \gamma_{\text{rms}} = 15^\circ \)) (8, 9) and the value obtained from line shape analysis (\( \gamma_{\text{rms}} = 41^\circ \)). However, a plausible physical argument also explains the fact that the value of \( \gamma_{\text{rms}} \) obtained from the line shape analysis is substantially larger than the \( \gamma_{\text{rms}} \) value obtained from spin-lattice relaxation data. The spin-lattice relaxation time is determined by motions on the time scale of \( 10^{-8} \) s. In contrast, any motion on the time scale of less than \( 10^{-1} \) s induces motional narrowing of line shape. Since large amplitude motions would be expected to encounter more steric hindrance than small amplitude motions, only the latter take place on the fast (\( T_1 \)) time scale. Therefore a \( T_1 \) measurement will be sensitive only to the fast small amplitude motions whereas the line shape will be sensitive to larger amplitude slower motions as well.

We have attempted to determine the amplitude of the rapid motions in the intact labeled collagen samples by analyzing the measured spin-lattice relaxation times listed in Table II. As expected, the \( T_1 \) values increase as one goes from the reconstituted collagen to the mineralized collagen samples and are about 1 order of magnitude larger than the natural abundance aliphatic \( T_1 \) values (not listed). Unfortunately, the carbonyl carbon \( T_1 \) values are so large that the longitudinal relaxation may be affected by \(^{13}C\)-\(^{13}C\) spin-diffusion (22). Therefore, we have not attempted a quantitative analysis of the data in Table II. We are instead measuring spin-lattice relaxation times of collagen labeled with [2-\(^{13}C\)]glycyl. Since in this case the labeled carbon is directly bonded to two protons, the \( T_1 \) values are much shorter than those reported in Table II. Hence, spin-diffusion should not complicate analysis of the data and quantitative estimates of \( \gamma_{\text{rms}} \) obtained from relaxation data, should be available shortly.

**Conclusion**

The results presented in Table III show that fluctuations in azimuthal orientation (as measured by \( \gamma_{\text{rms}} \)) are smaller in cross-linked tendon and demineralized calvaria collagen fibers than in reconstituted collagen fibers. In addition, \( \gamma_{\text{rms}} \) is slightly larger for the demineralized calvaria collagen fibers than for the tail tendon fibers. X-ray fiber diffraction studies (23) show that average equatorial diffraction maxima are 16, 15.3, and 15 Å, respectively, for reconstituted, demineralized bone and tendon collagen fibers. It therefore appears that \( \gamma_{\text{rms}} \) is correlated with the intermolecular separation of collagen molecules in the fibers that are not mineralized. Finally, we note that \( \gamma_{\text{rms}} \) for mineralized calvaria collagen is much less than that for the demineralized calvaria. Evidently, the presence of mineral is the source of the small value of \( \gamma_{\text{rms}} \) observed for collagen fibers in the intact rat calvaria.

The preliminary relaxation data in Table II suggest that rapid small amplitude fluctuations in azimuthal orientation persist in calvaria collagen. Currently, relaxation times of [2-

**Appendix**

The chemical shift powder line shape is completely determined by the principal elements of the chemical shift tensor, \( \sigma_{zzzz} \), \( \sigma_{zzxx} \), and \( \sigma_{zzyy} \) (24–26). In the presence of rapid reorientation, the chemical shift tensor is averaged and the principal elements of the averaged shift tensor determine the motionally averaged line shape (26). We describe here in detail our calculation of the peptide carbonyl line shape averaged by reorientation about the helix axis in collagen. We further show that the motionally averaged line shape is completely determined by the root mean square fluctuation in azimuthal angle when this quantity is small.

The principal components of the peptide carbonyl shift tensor are obtained from the low temperature spectra of the collagen samples. The shift tensor, \( \sigma_{zzzz} \), in the triple helix axis system (as defined for (Pro-Pro-Gly)\(_{10}\) in Ref. 21) is given by

\[
\sigma_{zzzz} = \mathbf{R}_{pp} \sigma \mathbf{R}_{pp}^{-1}
\]

where \( \sigma \) is the shift tensor in the PAS and \( R \) is the three-dimensional rotation matrix (26) that transforms cartesian tensor components from the PAS to the helix axis system and is given by

\[
R = \begin{pmatrix}
cos \alpha \cos \beta \cos \gamma & \sin \alpha \cos \beta \cos \gamma & -\sin \beta \\
-sin \alpha \sin \gamma & \cos \alpha \sin \gamma & 0 \\
\cos \alpha \sin \beta \sin \gamma & -\sin \alpha \cos \beta \sin \gamma & \sin \beta \sin \gamma
\end{pmatrix}
\]

where \( \alpha, \beta, \) and \( \gamma \) are the Euler angles, and rotations through these angles bring the PAS into coincidence with the helix axis system (26) (Fig. 6).

The elements of the \( R \) matrix are calculated in a straightforward manner since the orientation of the shift tensor PAS has been determined in the molecular frame of the glycyl residue and orientation of the glycyl molecular frame in the helix axis system is provided by the crystal structure of (Pro-Pro-Gly)\(_{10}\) (21). Once evaluated, elements of the \( R \) matrix yield the values of the Euler angles, \( \Omega = (\alpha, \beta, \gamma) \), that transform the PAS to the helix system and we find that \( \Omega = \)

**Fig. 6. Representation of Euler angles (\( \alpha, \beta, \gamma \)) rotation through which the principal axis system (\( P \)) of the carbonyl carbon of glycine into coincidence with the collagen helix axis system (\( H \)).**
(5.8°, 123.5°, 160.4°). The Euler angles \((\beta, 180° - \gamma)\) are the polar angles \((\theta, \phi)\) that define the orientation of \(Z_P\) in the helix axis system.

Examination of Eq. 6 shows that reorientation about the helix \((Z_H)\) axis causes the angle \(\gamma\) to vary. If the fluctuations in \(\gamma\) are rapid, then the averaged chemical shift tensor, \(\bar{\sigma}_H\), is calculated as (26)

\[
\bar{\sigma}_H = \int p(\gamma) \sigma_H d\gamma
\]

where \(p(\gamma) d\gamma\) is the probability that the PAS has azimuthal orientation in the range between \(\gamma\) and \(\gamma + d\gamma\). We diagonalize \(\bar{\sigma}_H\) to obtain the principal values of the motionally averaged shift tensor and these in turn yield the motionally averaged powder pattern.

It appears from Equation A1 that the averaged powder line shape depends upon the detailed form of \(p(\gamma)\). However, for small amplitude fluctuations, \(\bar{\sigma}_H\) depends only upon the root mean square fluctuation in \(\gamma\). This is readily shown by expanding \(\sigma_H\) in Taylor series about \(\gamma_0\), the mean value of \(\gamma\).

\[
\begin{align*}
\sigma_H &= \sigma_H(\alpha, \beta, \gamma_0) + \sum_{n=1}^{\infty} \left( \frac{\partial^2 \sigma_H}{\partial \gamma^n} \right) \frac{(\gamma - \gamma_0)^n}{n!} \\
\end{align*}
\]

Substituting this expression into Equation A1 yields

\[
\begin{align*}
\bar{\sigma}_H &= \sigma_H(\alpha, \beta, \gamma_0) + \sum_{n=1}^{\infty} \left( \frac{\partial^2 \sigma_H}{\partial \gamma^n} \right) <\gamma^n>/n! \\
\end{align*}
\]

where

\[
<\gamma^n> = \int p(\gamma) (\gamma - \gamma_0)^n d\gamma
\]

Since we are expanding \(\sigma_H\) about \(\gamma_0\), \(<\gamma> = 0\), therefore,

\[
\begin{align*}
\bar{\sigma}_H &= \sigma_H(\alpha, \beta, \gamma_0) + \sum_{n=1}^{\infty} \left( \frac{\partial^2 \sigma_H}{\partial \gamma^n} \right) <\gamma^n>/2 + \ldots \\
\end{align*}
\]

(A5)

So to a first approximation, \(\bar{\sigma}_H\), depends upon \(<\gamma^n>\). It should be noted that if \(p(\gamma)\) is an even function, \(e.g.\) if both the sites are equally populated in a two-site jump model, \(<\gamma^n> = 0\), where \(n = 1, 3, 5, \ldots\)

We have tested Equation A5 by calculating the motionally averaged line shapes using 2-, 4-, and 8-site jump models with \(<\gamma^n>\) fixed. We find that if \(\gamma_m \leq 40°\), the calculated averaged shift tensor elements change by less than 1 ppm as the number of sites changes from two to eight. We also find that the best agreement between calculated and observed line shapes occurs when \(\beta = 133.5°\) rather than the 123.5° calculated from the model compound data.

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