Model-Free Approach to the Interpretation of Nuclear Magnetic Resonance Relaxation in Macromolecules. 1. Theory and Range of Validity

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Abstract: A new approach to the interpretation of nuclear magnetic resonance relaxation experiments on macromolecules in solution is presented. This paper deals with the theoretical foundations and establishes the range of validity of this approach, and the accompanying paper demonstrates how a wide variety of experimental relaxation data can be successfully analyzed by using this approach. For both isotropic and anisotropic overall motion, it is shown that the unique information on fast internal motions contained in relaxation experiments can be completely specified by two model-independent quantities: (1) a generalized order parameter, δ, which is a measure of the spatial restriction of the motion, and (2) an effective correlation time, τ, which is a measure of the rate of motion. A simple expression for the spectral density involving these two parameters is derived and is shown to be exact when the internal (but not overall) motions are in the extreme narrowing limit. The model-free approach (so called because the δ's and the τ's have model-independent significance) consists of using the above spectral density to least-squares fit relaxation data by treating δ's and τ's as adjustable parameters. The range of validity of this approach is illustrated by analyzing error-free relaxation data generated by using sophisticated dynamical models. Empirical rules are presented that allow one to estimate the accuracy of the δ's and the τ's extracted by using the model-free approach by considering their numerical values, the resonance frequencies, and the parameters for the overall motion. For fast internal motions, it is unnecessary to use approaches based on complicated spectral densities derived within the framework of a model because all models that can give the correct value of the δ's work equally well. The unique dynamic information (δ and τ) can be easily extracted by using the model-free approach. Moreover, if one desires a physical picture of the motion, the numerical values of δ and τ can be readily interpreted within a physically reasonable model.

I. Introduction

Nuclear magnetic relaxation data on macromolecules in solution contain information concerning the nature of internal motions that occur in these systems. The usual approach1 to extracting such information involves the use of dynamical models that are based on physical intuition and/or the ease of formulation. While such analyses can be useful, there is the danger of overinterpretation of limited data and the possibility that the resulting physical picture is not unique. Models cannot be proven; they can only be eliminated.

In this paper we seek to answer the questions: (1) what is the unique information content of a given set of relaxation data and (2) how can one extract that information? In order to clarify the nature of the problem, let us consider a hypothetical example of a 13C NMR relaxation study of a lysine side chain in an isotropically reorienting protein. The relaxation of each carbon nuclei is determined by the fluctuations of the 13C-H vectors with respect to the external magnetic field. The observed quantities are determined by the Fourier transform (the spectral density) of an appropriate time-correlation function evaluated at certain frequencies whose values depend on the external field strength. To obtain the time dependence of the correlation function, which contains all the potentially available dynamic information, one would, in principle, need to perform experiments at an infinite number of magnetic field strengths. Even then the dynamic information would be limited because of the nature of the correlation function. With current NMR technology, a typical data set consists of a few numbers (say, T1's and NOE's at two magnetic fields). As a result of steric constraints and concerted motions, the dynamics of a side chain are extremely complicated, and one cannot expect to construct a detailed picture of the dynamics from a few experimentally accessible numbers that, as we shall see, may contain redundant information.

The simplest possible description of the internal dynamics of the side chain involves specifying (1) the rate (time scale) and (2) the spatial restriction of the motion of each carbon in the chain. Suppose we sit in a frame rigidly attached to the macromolecule and CTAB micelles. We also have shown that the ion-exchange formalism is adequate to describe quantitatively the effect of detergents on reactions of divalent ions.

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and observe the reorientation of the $^{13}$C–H vector associated with, say, the $C_4$ carbon. Because of intrachain and interchain steric interactions, this vector will not assume all possible orientations. That is, its motion is not isotropic but is restricted. The motion of other carbons in the chain may be more or less restricted. The time course of the trajectory of a $^{13}$C–H vector as it explores the positions accessible to it is complicated. The rate of motion of this $^{13}$C–H vector cannot be described by a single parameter (i.e., the correlation function is not a single exponential). However, different vectors in general do move at different "speeds" (i.e., the time dependence of their correlation functions differ), and it is desirable to define a correlation time that describes the rate of motion in an effective way.

In this paper we shall show that the information on fast internal motions (i.e., faster than about 0.3 ns) contained in an NMR relaxation experiment at currently available fields can be completely described by (1) a generalized order parameter, $\delta$, which is the measure of the degree of spatial restriction of the motion, and (2) an effective correlation time, $\tau_e$, which is a measure of the rate of the motion. The generalized order parameter and the effective correlation time will explicitly be defined in a model-independent way. For the special case that the overall motion can be described by a single correlation time $\tau_M$, our approach to extracting the unique information (i.e., $\delta$ and $\tau_e$) is based on the following simple expression for the spectral density:

$$J(\omega) = \frac{2}{\tau_M} \left( \frac{3\delta^2}{1 + (\tau_M/\omega)^2} + \frac{1 - \delta^2}{1 + (\tau_M/\omega)^2} \right)$$

(1)

with

$$\tau_e^{-1} = \tau_M^{-1} + \tau_e^{-1}$$

(2)

which we shall derive. Specifically, we propose to least-squares fit the experimental relaxation parameters to those calculated by using the above expression, treating $\delta^2$ and $\tau_e$ as the only adjustable parameters. We refer to this approach as "model-free" because eq 1 is derived without invoking a specific model for internal motions and because $\delta$ and $\tau_e$ are defined in a model-independent way. Once numerical values of $\delta$ and $\tau_e$ are extracted from the data, then one can consider their interpretation within the framework of a particular model. This is to be contrasted with the usual approach in which the spectral density is evaluated within the framework of a model.

The above procedure is clearly very simple to apply. The question is whether it works. The answer involves two separate issues. First, can the above two-parameter expressions reproduce the data, and second, are the resulting parameters meaningful (i.e., do $\delta$ and $\tau_e$ obtained by least-squares fitting the relaxation data agree with their exact values)? The strategy we have adopted in order to answer these questions is based on the analysis of "experimental" data that were generated by using a variety of sophisticated models of the internal dynamics (i.e., by using complicated spectral densities). The data generated in this way are error free, and $\delta$ and $\tau_e$ are known exactly. Thus, both of the questions raised above can be answered unambiguously. By analyzing a large number of sets of simulated data, we have established the range of validity and the accuracy of our approach. Basically, we have found that the data can always be reproduced. $\delta$ and $\tau_e$ are virtually exact when the overall motion is isotropic; they are close to the extreme narrowing limit; they are less than 0.01.

In the accompanying paper, we shall consider the application of our approach to actual experimental results.

The spectral density in eq 1 has the same functional form as an approximate spectral density derived by several authors.

within the framework of the diffusion in a cone model. In this model, the interaction vector is assumed to diffuse freely, with a wobbling diffusion constant $D_w$ in a cone of semiangle $\theta_0$. A major point of this paper is that eq 1 is more general than the diffusion in a cone model and is applicable to physical situations where this model is not reasonable. Stated another way, our model-free approach to analyzing experimental data (in the special case that the overall motion is isotropic) is operationally the same as using the diffusion in the cone model. However, the parameters $\delta$ and $\tau_e$ that are extracted from the data have a more general significance and need not be interpreted within the framework of this model. Our approach is also formally similar to the work of Jardetzky and co-workers (i.e., the spectral density is represented by a Lorentzian with adjustable parameters). However, as will become apparent, the theoretical justification for using such an approach and, more importantly, the interpretation of the resulting parameters are different.

The outline of this paper is as follows. In section II we describe the theoretical basis of our model-free approach to analyzing relaxation data. We first consider the case that the overall motion is isotropic; the anisotropic case is treated subsequently. We consider the general expression for the correlation function for internal motions ($C_{ij}(t)$) that describes dipolar relaxation and also quadrupolar and chemical shift anisotropy relaxation in the special case that the relevant tensors are axially symmetric. We analyze the behavior of this correlation function at short and long times. We express the infinite-time limit in terms of the generalized order parameter, $\delta$, which is a model-independent measure of the degree of spatial restriction of the motion. We introduce a single-exponential approximation to $C_{ij}(t)$ that is exact at $t = 0$ and $t = \infty$ and has the exact area. We then show that the spectral density obtained from the total correlation function constructed by using this approximation for $C_{ij}(t)$ is in fact exact when the internal motions are sufficiently fast (i.e., they are in the extreme narrowing limit). We show how the development can be generalized to incorporate fluctuations in the internuclear distance. Finally, we consider anisotropic overall motion and conclude this section by making contact with approaches that are operationally similar to ours. In section III, we establish the range of validity of our model-free approach by analyzing a large number of simulated "experimental" data sets that were generated by using a variety of sophisticated dynamical models. Empirical rules are presented that can be used to determine whether the generalized order parameters and effective correlation times extracted from the data are meaningful.

II. Theory

The relaxation due to dipole–dipole interaction between two nuclei can be described by the correlation function

$$C(t) = (D_{20}^{(2)}(\Omega_0(0))D_{20}^{(2)}(\Omega_0(t)))$$

(3)

where $D_{m}^{(2)}(\Omega)$ is a Wigner rotation matrix element and the Euler angles, $\Omega_{LF}$, specify the orientation of the unit vector, $\mu_{LF}$, connecting the two nuclei in the laboratory coordinate system. This correlation function also describes quadrupolar and chemical shift anisotropy relaxation in the special case where the relevant tensors are axially symmetric. For a system in solution, the correlation function does not depend on the index $q$ and can be rewritten by using the addition theorem for spherical harmonics as

$$C(t) = \frac{1}{3}P_2(\cos \theta)\mu_{LF}(t)$$

(4)

where $P_2(x)$ is the second Legendre polynomial,

$$P_2(x) = \frac{1}{2}(3x^2 - 1) \quad (5)$$

The spectral density, which determines the relaxation parameters, is given by

$$J(\omega) = 2 \int_0^\infty (\cos \omega t) C(t) \, dt \quad (6)$$

For $^{13}$C NMR of protonated carbons, where at currently available fields the relaxation is primarily dipolar, the relaxation times and the nuclear Overhauser enhancement (NOE) are given by

$$T_1^{-1} = \frac{h^2 \gamma^2 C^2 \gamma H}{4 \epsilon_{CH}^2} (J(\omega_H - \omega_C) + 3 J(\omega_C) + 6 J(\omega_H + \omega_C)) \quad (7a)$$

$$T_2^{-1} = \frac{h^2 \gamma^2 C^2 \gamma H}{8 \epsilon_{CH}^2} (4 J(0) + J(\omega_H - \omega_C) + 3 J(\omega_C) + 6 J(\omega_H) + 6 J(\omega_H + \omega_C)) \quad (7b)$$

NOE = $1 + \frac{\gamma H (6 J(\omega_C + \omega_H) - J(\omega_H - \omega_C))}{\gamma C (J(\omega_H - \omega_C) + 3 J(\omega_C) + 6 J(\omega_H + \omega_C))} \quad (7c)$

where $\omega_H$, $\omega_C$ are the Larmor frequencies. For $^2$H NMR of deuterated carbons, the quadrupolar tensor is axially symmetric about the C-$^2$H bond, and the relaxation times are given by

$$T_1^{-1} = \frac{3}{16} \left( \frac{e^2 q Q}{h} \right)^2 (J(\omega_D) + 4 J(2 \omega_D)) \quad (8a)$$

$$T_2^{-1} = \frac{1}{32} \left( \frac{e^2 q Q}{h} \right)^2 (9 J(0) + 15 J(\omega_D) + 6 J(2 \omega_D)) \quad (8b)$$

Note that the correlation function has the same form in both cases: $\mu_p$ points along the $^{13}$C-$^2$H bond in $^{13}$C NMR, while it points along the C-$^2$H bond in $^2$H NMR. For chemical shift anisotropy relaxation with an axially symmetric shift tensor, one has

$$C(t) = C_0(t)C(t) \quad (10)$$

where the correlation function for overall motion is

$$C_0(t) = \frac{1}{2} e^{-t/\tau_M} \quad (11)$$

where $D_M$ and $\tau_M$ are the rotational diffusion constant and correlation time of the macromolecule, respectively. The correlation function for internal motions is

$$C_I(t) = \langle P_2(\mu(0) \cdot \mu(t)) \rangle \quad (12)$$

The Internal Correlation Function. We now consider the properties of the correlation function that describes internal motions. Let us consider the relaxation of a carbon in some side chain in a protein (see Figure 1). The motion of the $^{13}$C-$^2$H vector of this carbon in the macromolecular frame is, in general, complicated. If the motion is Markovian (e.g., diffusive or jump-like), then $C_I(t)$ can be expressed as a series of exponentials

$$C_I(t) = \sum_{n=0} a_n e^{-t/\tau_n} \quad (13)$$

(13) See, for example: Table I of ref. 11.
relaxation in solution has been recently recognized in certain contexts. In this paper we show that the generalized order parameter plays a central role in the interpretation of NMR relaxation data for side chains in macromolecules.

\( \delta \) is a model-independent measure of the degree of spatial restriction of the motion. It satisfies the inequalities \( 0 \leq \delta^2 \leq 1 \). If the internal motion is isotropic (i.e., all orientations of \( \hat{\mu} \) are equally probable, \( \rho_{eq}(\Omega) = (4\pi)^{-1} \)), then

\[
\delta = 0
\]  
(20)

However, the converse is not true (see below). On the other hand, if the motion is completely restricted (i.e., \( \rho_{eq}(\Omega) = \delta(\Omega - \Omega_0) \)), then

\[
\delta = 1
\]  
(21)

In the special case that the motion is azimuthally symmetric about an axis (i.e., \( \rho_{eq}(\Omega) = \rho_{eq}(\theta) \), independent of \( \phi \), where \( \theta \) is the angle between \( \hat{\mu} \) and the symmetry axis), then

\[
\delta = \langle \rho(\cos \theta) \rangle = S
\]  
(22)

i.e., the generalized order parameter simply becomes the usual order parameter.

The order parameter \( S \) (and hence \( \delta \) ) can vanish even if the motion is not isotropic. For example, consider Woessner's model, which describes the free diffusion of \( \hat{\mu} \) about a fixed axis. If \( \delta \) is the angle between \( \hat{\mu} \) and this symmetry axis, then

\[
S_{Woessner} = \rho(\cos \theta)
\]  
(23)

since \( \rho_{eq}(\Omega) = (2\pi \sin \theta)^{-1}\delta(\theta - \theta_0) \). The order parameter for a model in which \( \hat{\mu} \) jumps among three discrete equivalent sites about the symmetry axis is also given by eq 23. We note that the order parameter vanishes when \( \delta \) is at the "magic" angle (54.7°). A similar situation arises for the diffusion in the cone model where the order parameter (see eq A3) vanishes not only when \( \theta_0 = \pi \) but also when \( \theta_0 = \pi/2 \) (i.e., when the interaction vector is restricted to move in a hemisphere). The generalized order parameter can easily be evaluated for a variety of models (see the Appendix for some examples).

We are now in a position to propose an approximation to the internal correlation function that plays a central role in our development. Figure 2 shows schematically the behavior of \( C_1(t) \) as a function of time. The simplest approximation to \( C_1(t) \) which is exact at \( t = 0 \) and at \( t = \infty \) has the form

\[
C_1^N(t) = \delta^2 + (1 - \delta^2)e^{-t/\tau_e}
\]  
(24)

where \( \tau_e \) is an effective correlation time. To determine \( \tau_e \), one must place an additional requirement on the approximate correlation function. There exist several possibilities. For the diffusion in the cone model, in which \( C_1(t) \) is exactly given by an infinite sum of exponentials, it is known that, as long as \( \delta^2 \) (\( S_{cone}^2 \)) is not too small, the single exponential approximation is very good if \( \tau_e \) is determined by requiring the area of \( C_1^N(t) \) to be exact. Because of this and for reasons that will become apparent later, we choose \( \tau_e \) so that

\[
\int_0^\infty (C_1^N(t) - \delta^2) dt = \int_0^\infty (C_1(t) - \delta^2) dt
\]  
(25)

or

\[
\tau_e(1 - \delta^2) = \int_0^\infty (C_1(t) - \delta^2) dt
\]  
(26)

In the special case that \( C_1(t) = e^{-t/\tau} (\delta = 0) \), it follows from eq 26 that \( \tau = \tau_e \). Thus, eq 26 can be regarded as the definition of an effective correlation time for an arbitrary correlation function. For future reference, we note that if expansion 13 is used for \( C_1(t) \) (a = \( \delta^2 \) since \( \tau_0 = \infty \)), the effective correlation time becomes

\[
\tau_e(1 - \delta^2) = \sum_{i=1}^{\infty} a_i \tau_i
\]  
(27)

We have seen that the generalized order parameter can be expressed as an equilibrium average and contains no information about the time scale of dynamics; it is solely a measure of the spatial restriction of the motion. The effective correlation time, on the other hand, depends both on the microscopic diffusion or jump constants and the spatial nature of the motion. For example, for the Woessner model in which the interaction vector diffuses freely about a symmetry axis with diffusion coefficient \( D \),

\[
\tau_e(1 - \delta^2) = D^{-1} \sum_{m=2}^{\infty} \sum_{l=1}^{\infty} \delta_0 (m^2 - l^2) = 3D^{-1} \sin^2 \beta \cos^2 \beta + 1/16 \sin^2 \beta
\]  
(28)

where \( \beta \) is the angle between \( \hat{\mu} \) and the symmetry axis. This property of \( \tau_e \) considerably complicates the interpretation of \( \tau_e \). In particular, while \( \delta \) has a model-independent significance, \( \tau_e \) can be related to microscopic rate (diffusion) constants only within the framework of a particular model.

It is important to note that the above approximation for \( C_1(t) \) is not equivalent to truncating the exact series expansion (eq 13) after two terms, i.e.,

\[
C_1^N(t) = a_0 + a_1 e^{-t/\tau_1}
\]  
(29)

Rather, the above approximation is the simplest form of a time-dependent Padé approximant. The area of the correlation function can be regarded as a moment of this function, and using the theory of moments, one can construct successively better approximations. For example, if in addition to \( C_0(t) \) and \( C_1(\infty) \), the area one also knows the behavior of \( C_1(t) \) at short times (i.e., \( dC_1(t)/dt \) at \( t = 0 \)) and \( \int_0^\infty dt \langle C_1(t) - \delta^2 \rangle \), one can construct the two-exponential approximation of the form

\[
C_1^N(t) = A + B e^{-Ct} + D e^{-E t}
\]  
(30)

where the coefficients are solutions to the equations

\[
A = \delta^2
\]  
(31a)

\[A + B + D = C_1(0) = 1\]

(31b)

\[BC + DE = -\langle dC_1(t)/dt \rangle_0 = \sum_{i=1}^{\infty} a_i \tau_i^{-1}\]

(31c)

\[BC^{-1} + DE^{-1} = \int_0^\infty dt \langle C_1(t) - \delta^2 \rangle = \sum_{i=1}^{\infty} a_i \tau_i\]

(31d)

\[BC^{-2} + DE^{-2} = \int_0^\infty dt \langle C_1(t) - \delta^2 \rangle = \sum_{i=1}^{\infty} a_i \tau_i^2\]

(31e)

We shall not pursue such an extension here since, as we shall see, the simplest version of this scheme (i.e., eq 24) is adequate for our purposes.

The Spectral Density. A combination of eq 11 and 24 gives the total correlation function as

\[
C(t) = \gamma_0 \delta^2 e^{-t/\tau} + \gamma_1 (1 - \delta^2) e^{-t/\tau}
\]  
(32)

with

and the corresponding spectral density as
\[ J(\omega) = \frac{2}{5} \left( \frac{\frac{\delta^2}{\tau} + \frac{(1 - \delta^2)}{\tau_e}}{1 + \frac{\omega^2}{\tau^2}} \right) \]

(34)

As discussed in the Introduction, eq 34 plays a central role in our approach to analyzing experimental data.

We now examine some limiting cases of the above spectral density. If the overall motion is considerably slower than the internal motions (\( \tau_e < \tau_M \)), eq 34 simplifies to
\[ J(\omega) = \frac{2}{5} \left( \frac{\frac{\delta^2}{\tau} + \frac{(1 - \delta^2)}{\tau_e}}{1 + \frac{\omega^2}{\tau^2}} \right) \]

(35)

Furthermore, if \( \tau_e \) is sufficiently fast and the internal motion is in the extreme narrowing limit ((\( \rho \omega^2 \)) << 1, where \( \omega \) is the largest frequency at which the spectral density must be evaluated, i.e., \( \omega_c + \omega_H \) for \( ^1^3 \)C NMR and \( 2\omega_D \) for \( ^1^H \) NMR), we have
\[ J(\omega) = \frac{2}{5} \left( \frac{\frac{\delta^2}{\tau} + \frac{(1 - \delta^2)}{\tau_e}}{1 + \frac{\omega^2}{\tau^2}} \right) \]

(36)

Note that we have not assumed that the overall motion is in the extreme narrowing limit. By using the spectral density 36 in eq 7a and 7b, we find that \( T_1 \) and \( T_2 \) for dipolar relaxation are
\[ T_{1i}^{-1} = \delta^2(T_i^{-1}) + \frac{1}{2} \gamma^2 \gamma_H^2 \gamma^2 \tau_{CH}^2 \frac{1}{1 - \delta^2} \tau_e \]

(37)

where \( (T_i)^{-1} = 1 \), \( i = 1, 2 \), are the relaxation times for an isotropically reorienting (with correlation time \( \tau_M \)) macromolecule. The analogous expression for the NOE is
\[ 2\delta^2(T_1^{-1})(\text{NOE} = 1) = 2\delta^2(T_1^{-1}) + \gamma^2 \gamma_H^2 \frac{1}{1 - \delta^2} \tau_e \]

(38)

The second term in eq 37 and 38 arises exclusively from internal motions. However, the contribution due to the overall motion is reduced by the square of the generalized order parameter resulting from internal motions.

Although we have derived the spectral density in eq 36 by using our single exponential approximation to \( C_1(t) \) (eq 24), we shall now show that this expression for the spectral density, and hence eq 37 and 38 for the relaxation times and the NOE, are actually exact when (1) the overall motion is isotropic, (2) internal motions are much faster than the overall motion and lie in the extreme narrowing limit, and (3) \( \tau_e \) is defined as the area of the correlation function (eq 26). This is an important result. It explains why our model-free approach to analyzing relaxation data works so well in many cases of interest.

To show this, we begin by noting that eq 13 for the internal correlation function can be rewritten as (since \( \delta^2 = \alpha_0 \))
\[ C_1(t) = \delta^2 + \sum_{i=1}^{M} a_i e^{-t/\tau_i} \]

(39)

The spectral density (including the overall motion) then becomes
\[ J(\omega) = \frac{2}{5} \sum_{i=1}^{M} a_i \frac{\delta^2}{\tau_i} \]

(40)

Assuming that \( \tau_M \gg \tau_i \) and evaluating the integral, we have
\[ J(\omega) = \frac{2}{5} \left( \frac{\frac{\delta^2}{\tau} + \frac{2}{5} \sum_{i=1}^{M} a_i \tau_i}{1 + \frac{\omega^2}{\tau^2}} \right) \]

(41)

Finally, assuming that the internal motions are in the extreme narrowing limit (\( (\rho \omega^2) \ll 1 \)), we have
\[ J(\omega) = \frac{2}{5} \left( \frac{\frac{\delta^2}{\tau} + \frac{2}{5} \sum_{i=1}^{M} a_i \tau_i}{1 + \frac{\omega^2}{\tau^2}} \right) \]

(42)

which is indeed identical with eq 36 when \( \tau_e \) is defined via the area criterion (see eq 27). The above result is actually more general than this derivation might suggest. A proof can be easily constructed for an arbitrary internal correlation function (not necessarily of the form given in eq 39) as long as the three conditions stated above hold.

In the above development, we have assumed that all the internal motions are faster than the overall motion (i.e., \( \tau_e < \tau_M \) for all \( i \)). What happens if some component of the internal correlation function decays more slowly than the overall motion? Suppose, for example, that in eq 39 \( \tau_1 \gg \tau_M \) but \( \tau_e < \tau_M \) for \( i = 2, 3, \ldots \). Then, proceeding as in eq 40-42, it can be seen that eq 42 should be replaced by
\[ J(\omega) = \frac{2}{5} \left( \frac{\frac{\delta^2}{\tau} + \frac{2}{5} \sum_{i=1}^{M} a_i \tau_i}{1 + \frac{\omega^2}{\tau^2}} \right) \]

(43)
where \( (T)_0 \) is the result for an isotropically reorienting sphere. We note that this equation can formally be rewritten as
\[
T_i = (T)_0
\]
where \( (T)_0 \) is the sphere result calculated by using an effective internuclear distance, \( r_{CH'} \), given by eq 46. Since \( 0 \leq |\delta| \leq 1 \),
\[
r_{CH'} = r_{CH} |\delta|^{1/3}
\]
the effective internuclear distance, \( r_{CH'} \), is always larger than \( r_{CH} \). For example, if \( r_{CH} \) is 1.09 \( \AA \) and \( \delta \) is estimated by using the diffusion in the cone model (eq A3) with a cone angle \( \theta_c \) equal to 16\( ^\circ \), then \( r_{CH'} = 1.11 \) \( \AA \). It is important to note that bending vibrations (bending motions) lead to values of \( \delta \) smaller than unity and, hence, increase the effective internuclear distance. Stretching vibrations in the harmonic approximation result in a decrease in the effective internuclear distance. Thus, the observation of the effective internuclear distance, \( r_{CH'} \), is likely to be an indication of the presence of librational motions (bending vibrations or some other fast motions that reorient the \( 1^3 \)-C-H vectors).

For the sake of completeness, we show how the theory of this paper can be formulated when fluctuations not only in the orientation but also in the internuclear separation are considered. The generalization of eq 12 is
\[
\tilde{C}_i(t) = \left( \frac{P_i(0)\tilde{C}_i(t)}{r_i(0)\tilde{r}_i(t)} \right)
\]
The general order parameter becomes
\[
\tilde{\delta}^2 = \sum_{n=-2}^{2} |C_{2n}(\Omega) / \tilde{r}_0|^2
\]
The analogue of eq 24 is
\[
\tilde{\tilde{C}}_i(t) = \tilde{\delta}^2 + (\tilde{\delta}^2 - 3)\delta^{1/3}
\]
and finally, the generalization of eq 37 is
\[
T_i^{-1} = \tilde{\delta}^2(r_0^4(\tilde{T}_i^0) + h^4 \gamma C_{2n}(\Omega - \tilde{\delta}^2)r_0^8)
\]
where \( (T)_0 \) is the relaxation time calculated for an isotropically reorienting sphere when \( r_{CH} \) is set equal to \( r_0 \).

B. Anisotropic Overall Motion. We now consider the generalization of the development to the situation where the overall motion cannot be described by a single correlation time (e.g., a cylindrical macromolecule or a random-coil polymer). When the overall motion is anisotropic, the first complication that arises is that the total correlation function cannot be rigorously factored into a product of contributions due to overall and internal motions even when it is assumed that these motions are independent (i.e., eq 10 is no longer rigorous). Nevertheless, we approximate the total correlation function as a product. Numerical evidence for the validity of this decoupling approximation will be presented later in this paper.

We shall use the same functional form for the internal correlation function as in the isotropic case (i.e., eq 24), so we are left with the problem of describing the correlation function for overall motion. For random-coil polymers, the usual approach to describing the highly anisotropic nature of the motion of the backbone involves the use of a distribution of correlation times (or equivalently diffusion coefficients). Specifically, if \( p(\tau) \) is the normalized probability that \( \tau \) is between \( \tau \) and \( \tau + d\tau \), then the overall correlation function is represented as
\[
C_0(t) = \frac{1}{2} \int p(\tau) e^{\tau t} d\tau
\]
A number of different ad hoc expressions have been used in the literature. One does not expect the resulting expression for
\[
C_0(t)
\]
to be very sensitive to the particular form of the distribution. The situation is analogous to the theory of heat capacities of solids where the Einstein, Debye, and exact normal-mode frequency distributions are very different, yet the temperature dependence of the heat capacities are rather similar (except of course at very low temperatures). In this paper we set
\[
p(\tau) = A\delta(\tau - \tau_1) + (1 - A)\delta(\tau - \tau_2)
\]
and hence
\[
C_0(t) = \frac{1}{2}A e^{\tau_1 t} + \frac{1}{2}(1 - A) e^{\tau_2 t}
\]
where \( A, \tau_1, \) and \( \tau_2 \) are adjustable parameters that can be determined by fitting the relaxation data of a nucleus that is attached to the macromolecular backbone. When \( A = 1 \), we recover the isotropic result (eq 11). It is interesting to note that eq 53 for the overall correlation function describing anisotropic motions has formally the same functional form as the total correlation function (overall and internal) for the case when the overall motion is isotropic (i.e., eq 32, \( A = \delta^2, \gamma_{TM} \rightarrow \tau_1, \) and \( \tau \rightarrow \tau_2 \)). In summary, our expression for the total correlation function when the overall motion is anisotropic is
\[
C(t) = \frac{1}{2}A(\delta e^{\tau_1 t} + (1 - A) e^{\tau_2 t}) - (1 - \delta^2) e^{\tau_1 t}
\]
The generalized order parameter \( \delta \), which describes the spatial restriction of the internal motions, is now defined with respect to a frame attached to a backbone nucleus whose anisotropic motion is described by \( A, \tau_1, \) and \( \tau_2 \).

As in the case of isotropic overall motion, it can be shown that eq 37 (where now \( (T)_0 \) are the relaxation times for anisotropic motion) is exact when \( (1) \) the internal motions are in the extreme narrowing limit and the internal correlation function decays much faster than the overall correlation function (i.e., \( \int_0^\infty C_0(t) e^{-\gamma t} dt = \int_0^\infty C(t) e^{-\gamma t} dt \) to the decoupling approximation is valid, and \( 3) \) \( \tau_0 \) is defined as the area of the internal correlation function. It is important to note that in proving the above, one does not require the overall correlation function to have the form given in eq 53 and, thus, the result holds more generally.

C. Relation to Previous Work. We shall now discuss the relation of our model-free approach to analyzing NMR relaxation data to previous work. As mentioned in the introduction, the functional form for the internal correlation function (eq 24), on which our approach is based, is identical with an approximation to the correlation function within the diffusion in a cone model (in this model \( \mu \) is restricted to diffuse in a cone of semiangle \( \theta_c \)). Specifically, if the generalized order parameter is evaluated within this model (i.e., \( \delta = \delta_{TM} \)), then the expressions are identical. It should be emphasized that the analysis of experimental data based on eq 24 is meaningful independent of whether the cone model is physically reasonable. In this regard, we mention the interesting work of Howarth, who analyzed a variety of experimental relaxation data by using the cone model. He showed that the data could be well reproduced even in cases where the cone model is not reasonable (e.g., for the carbons of an aliphatic side chain of a random-coil polymer). Our work explains why this is so: the cone angles he extracted may have no physical significance; however, the corresponding order parameters are physically meaningful.

Jardetzky and co-workers have previously considered "how much definite information on internal mobility in proteins can be deduced from NMR relaxation measurements" and proposed an approach to "mapping internal motions in proteins". Although their approach is operationally similar to ours in that the spectral density is represented as a sum of Lorentzians containing adjustable parameters, the motivation for using such expressions and interpretation of the parameters is quite different. For example, their work contains no reference to the concept of an order parameter. Moreover, they suggest that more and more experimental information (\( T_1, T_2, \) and NOE's at a large number of fields) will allow one to learn more and more about internal motions. We, on the other hand, emphasize that the information content of such data over a large range of frequencies on fast internal motions

References:
is redundant and, in fact, can be specified by just two quantities (i.e., $\beta^2$ and $\tau_g$).

Since the papers of Jardetzky and co-workers use the technical jargon of the mathematical theory of Markov processes, it is perhaps useful to summarize our understanding of their work. In their first paper, they show formally how the spectral density can be expressed in terms of the eigenfunctions and eigenvalues of a transition operator. They obtain a correlation function of the form

$$C(t) = \sum_n \alpha_n e^{-\lambda_n t}$$

and a corresponding spectral density

$$J(\omega) = 2 \sum_n \frac{\alpha_n \lambda_n}{\lambda_n^2 + \omega^2}$$

where $-\lambda_n$ are the eigenvalues of the transition operator. They then proceed to generalize the above expression to "$N$ independent motions" and obtain a spectral density of the same general form as above. The entire formal nature of this paper can be contrasted with the work of Wittebort and Szabo, who explicitly show how to construct the spectral density for a general jump model (i.e., a general discrete Markov process) and illustrate the formalism by considering the concerted motions of a lysine side chain. Jardetzky and co-workers refer to $\lambda_n^{-1}$ in eq. 55 and 56 as the correlation time for the "$n$th individual motion". However, it is well-known that simple models containing only a few types of events lead to correlation functions containing a large number of $\lambda_n$'s. For example, even in the Woesnner model (a single free internal rotation superimposed on isotropic overall motion) one has three $\lambda$'s (i.e., $\lambda_0 = 6 D_M$, $\lambda_1 = 6 D_M + D$, and $\lambda_2 = 6 D_M + 4 D$, where $D_M$ and $D$ are the overall and internal diffusion coefficients). Moreover, the exact correlation function for the slow component is in fact equal to the expressions for the relaxation parameters) is identical with our eq. 34 where $\lambda_1 = \tau_1^{-1}$, $\lambda_2 = \tau_2^{-1}$, and $\alpha_i$ is identified with the generalized order parameter ($\alpha_i = \delta^2$). However, operationally, eq. 38 and 34 are identical only if the constraint $\lambda_1 \leq \lambda_2$ is imposed, since $\tau_1 < \tau_2$ (see eq. 33).

III. Range of Validity

In this section we investigate the range of validity of the model-free approach by analyzing NMR relaxation data that were generated by using sophisticated dynamical models for which the values of $\delta^2$ and $\tau_g$ are known exactly. Using the model-free expressions for the spectral density (e.g., eq. 34 for isotropic overall motion) and treating $\delta^2$ and $\tau_g$ as the only adjustable parameters, we least-squares fit the relaxation data and then compare the resulting numerical values of $\delta^2$ and $\tau_g$ with their exact values. The rather complicated spectral densities that were used in generating the data are described in the Appendix. We have seen in the Theory section that the model-free approach is exact when the internal motions are in the extreme narrowing limit. Here we wish to quantify this result and determine how well the approach works for slower internal motions. We will give a set of empirical rules that allow one to determine the accuracy of the extracted values of $\tau_g$ and $\delta^2$ by considering only their numerical values, the parameters for overall motion, and the correlation times. This is important in the analysis of experimental data (see accompanying paper), where, of course, the exact values of the order parameter and the effective correlation times are not known. We have analyzed a large number of "simulated" data sets, and we present below the results of only a representative sample. We consider both isotropic (subsection A) and anisotropic (subsection B) overall motions.

A. Isotropic Overall Motion

We consider systems whose overall motion can be described by a single correlation time, $\tau_\text{m}$. Before presenting the results of a systematic study based on relaxation data that we have generated using the models described in the appendix, we show how well the model-free approach works for the relaxation data calculated by Levy et al. for a pseudo side chain in a macromolecule using molecular dynamics. Briefly, Levy et al. obtained trajectories describing the motion of a heptane molecule by using the diffusive Langevin equation. They mimicked a pseudo side chain in a macromolecule by isotropically reorienting the C1-C2 bond (i.e., C2 is an "r carbon" whose relaxation is described by $\tau_{\text{m}}$). Finally, they calculated $T_1$, $T_2$, and NOE for carbons C1, C2, and C3 at two fields with $\tau_{\text{m}} = 1$, 10, and 100 ns. The generalized order parameters defined in this paper can be related to "configuration averages of Wigner functions", which they tabulate. Specifically, $\delta^2$ for the carbons C3-6 is simply 4$\pi$ times the entries in their Table X. It is interesting to note that $\delta^2$ is identical with the "motional averaging scale factor" Levy and co-workers define in another paper. In Table I we present the results of the analysis of the simulated data of Levy et al. using the model-free approach. We fixed...
\(\tau_m\) at 10 ns and least-squares fit their \(T_1\) and NOE values (but not \(T_2\)'s) at the two fields simultaneously to extract numerical values of \(\delta^2\) and \(\tau_e\). We then predicted the \(T_2\) values at both fields. It can be seen that the model-free approach can reproduce the data extremely well. Moreover, the predicted \(T_2\)'s are in excellent agreement with the exact results. Finally, the generalized order parameters extracted from the relaxation data agree remarkably well with their exact values. Note, in particular, that the non-monotonic behavior of \(\delta^2\) as one goes out the chain, which appears to be a hallmark of concerted motions, is reproduced.

We have performed similar analyses of their data for \(\tau_m = 10\) and 100 ns. The results are almost as impressive \(\delta^2\) for \(C_4\) is somewhat overestimated when \(\tau_m = 1\) ns, and \(T_2\) information is required when \(\tau_m = 100\) ns to get highly accurate values of \(\delta^2\). In fact, we were able to find a misprint in a preprint of their paper (we correctly predicted that the NOE at 15 MHz for \(C_4\) with \(\tau_m = 100\) ns should be 2.93 instead of 2.83).

Why does the model-free approach work so well? Basically, because the internal motions are sufficiently fast so as to be close to the extreme narrowing limit. In this limit, the information on internal motions contained in the NMR relaxation experiment is rigorously completely specified by \(\delta^2\) and \(\tau_e\). Therefore, one should be able to extract \(\delta^2\) and \(\tau_e\) from just two experimental relaxation parameters when these parameters are known for the overall motion. Suppose we know \(T_1\)'s at two fields in the presence and absence of internal motion (i.e., \(T_1\), \(T_2\), and \(T_1\)_\(0\), \(\tilde{T}_1\)_\(0\)). In the extreme narrowing limit, these quantities are related by eq 37. Since we have two equations (one for each field) in two unknowns \(\delta^2\) and \(\tau_e\), we immediately have

\[
\delta^2 = \frac{T_1^{-1} - \tilde{T}_1^{-1}}{(T_1)_0^{-1} - (\tilde{T}_1)_0^{-1}}
\]

and

\[
\frac{\hbar^2c^2\gamma_H^2}{r_{CH}^6} \tau_e = \frac{T_1^{-1} - (\tilde{T}_1)_0^{-1} - (T_1)_0^{-1}}{T_1^{-1} - (\tilde{T}_1)_0^{-1} + (T_1)_0^{-1}}
\]

Identical equations hold for \(T_2\). If one knows \(T_1\) and NOE at one field, then, using eq 38, we have

\[
\delta^2 = \frac{(T_1)_0(2.988 - \text{NOE})}{T_1(2.988 - \text{NOE})}
\]

and

\[
\frac{\hbar^2c^2\gamma_H^2}{r_{CH}^6} \tau_e = \frac{\text{NOE} - (T_1)_0}{T_1(2.988 - \text{NOE}) - (T_1)_0(2.988 - \text{NOE})}
\]

where \((T_1)_0\) and NOE\(o\) are the relaxation parameters describing the overall motion. In favorable cases (e.g., sufficiently fast internal motions and accurate data), these equations allow one to obtain \(\delta^2\) and \(\tau_e\) directly from experimental data. We will make extensive use of these equations below and in part 2 of this series, where we will show that they work remarkably well for real systems.

In Table II we compare \(\delta^2\) calculated via eq 59a and 60a from the data of Levy et al.\(^{23}\) using (1) only the \(T_1\)'s at both fields, (2)
Table III. Model Free Analysis of $^{13}$C Dipolar Relaxation Data Generated by Using the Woessner Model$^{14}$ with $\tau_M = 10$ ns, $1/6D = 10.6$ ps, and $\beta = 70.5^\circ$.

<table>
<thead>
<tr>
<th>$\omega$, MHz</th>
<th>NOE ($1 + \eta$)</th>
<th>$T_1$, ms</th>
<th>$T_2$, ms</th>
<th>$S^2$</th>
<th>$\tau_c$, ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1.58 (1.57)</td>
<td>332 (335)</td>
<td>146 (149)</td>
<td>0.113 (0.111)</td>
<td>31.4 (31.9)</td>
</tr>
<tr>
<td>90</td>
<td>2.45 (2.45)</td>
<td>1194 (1194)</td>
<td>179 (182)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Only $T_1$'s and NOE's at high field were fitted. The $T_1$'s at high field and all the relaxation parameters at low field are predicted. The exact results are in parentheses.

ps, $(\omega_T)^2 = 5 \times 10^3$, $\tau_c/\tau_M = 2.5 \times 10^{-2}$, and eq 61 errors by $\sim 0.7\%$. If the contribution to the spectral density of the $\tau_c$ term is really frequency independent, one should be able to extract the exact values of $S^2$ and $\tau_c$ and predict very accurately the relaxation data at any other field (such that eq 61 still holds) only from, say, the measurement of two relaxation data at one field. On the basis of extensive numerical experimentation, we conjecture that if eq 61 holds to within $\pm 2\%$, this is indeed the case. Motions of this nature will be called extremely fast internal motions. In the case of extremely fast motions, eq 61 is virtually exact and predict very accurately the other relaxation data. For example, in the diffusion in a cone model with $\beta = 30^\circ$, $\tau_M = 10$ ns, $\tau_c = 100$ ns, and $\eta = 10$ ps by using eq 15 of ref 6. The order parameter for this model is 0.072. For no motion of the $d$ axis, $S^2 = 0.11$. Fitting $T_1$ and NOE values at 25 and 90 MHz by using the model-free approach, we obtained $\tau_c = 30$ ps and $S^2 = 0.11$, which correspond to no motion of $d$. Addition of $T_2$ information gives virtually identical results. In this case, for one internal motion, the slow internal motion is "invisible". We then considered the Brainard-Szabo model with all the parameters as in the above example except that $\tau_c$ was set at a value of 1 ns. Fitting $T_1$ and NOE values at both fields, we obtained $S^2 = 0.089$ and $\tau_c = 40$ ps. The addition of $T_2$ information gives $S^2 = 0.079$ and $\tau_c = 41$ ps. The effective correlation time appears to be fast, but the order parameter has a value that is intermediate between 0.11 (corresponding to rotation about the $d$ axis only) and 0.072 (corresponding to rotation and wobbling).

We now turn to the case where all the internal motions are not close to the extreme narrowing limit. In this case, both terms in the model-free expression for the spectral density depend on frequency. It is important to realize that, in general, the correlation function for internal motions is not accurately described by a single exponential. The consequences of the one-exponential approximation are particularly severe for motions that are not very restricted (small values of $S^2$) or, irrespective of the spatial extent of the motions, if the time scale of the motions is slow. For relatively slow motions, we observed that, with very few exceptions, the trend is to predict values of $S^2$ that are too large and values of $\tau_c$ that are too small.

Table IV shows the results obtained by using the model-free approach to analyze $^{13}$C relaxation data generated by using Woessner's model$^{14}$ for $\tau_M = 10$ ns, $1/6D = 99.3$ ps, and $\beta = 70.5^\circ$. For these relatively slow internal motions, it is still possible to extract fairly accurate values of $S^2$ and $\tau_c$ (in error by 25% and 15%, respectively) from a set of $T_1$ and NOE measurements at two fields. The predicted values of $T_2$ are in good agreement with the exact values at both fields (with an error of 11% at high field and of 7% at low field).

Table V shows the results obtained by fitting NMR data for $^{13}$C-H dipolar relaxation at 25.1 and 90.5 MHz in the diffusion in a cone model with $\tau_M = 10^5$, $1/6D = 7.03$ ns and 0.703 ns, and two cone angles, $\theta_2 = 36.6^\circ$ and 66.4$^\circ$. In both cases, by fitting $T_1$ and NOE at both fields, one can reproduce very accurately the exact data. The values of $S^2$ and $\tau_c$ extracted improve as the time scale of the motion gets faster. $S^2$ is about 9% larger than its true value, $1/6D = 7.03$ ns. One predicts a value in error by only 1.7% by including $T_2$ information at both fields. Notice that $\tau_c$ extracted by fitting $T_1$ and NOE values at both fields ($1/6D = 7.03$ ns) is 3.80 ns. Inclusion of $T_2$ information at both fields in the fitting procedure for $1/6D = 7.03$ ns gives $\tau_c = 4.20$ ns, which is closer to the true value of $\tau_c$. For faster internal motions, however, $(1/6D = 0.703$ ns) $\tau_c$ predicted by using $T_1$ and NOE information at both fields is 0.437 ns, i.e., only 1.6% shorter than its exact value. Inclusion of $T_2$ information has little or no effect on the extracted value of $\tau_c$. For a cone angle of $\theta_2 = 66.4^\circ$, the exact data can be reproduced, but the predicted values of $S^2$ and $\tau_c$ are not accurate for $\tau_c = 9.9$ ns. $S^2$ errs by a factor of 2, and inclusion of $T_2$ information does not improve this value. Using $T_1$ and NOE at both fields, one obtains $\tau_c = 6.80$ ns. The situation is better for the faster motion ($\tau_c = 0.99$ ns). In this case, the addition of $T_2$ information improves substantially the predicted value of $S^2$ (the error is 16% vs. 73% for the prediction based on the $T_1$ and NOE information at two fields only). For...
Tables IV and V show certain trends that extensive numerical investigations have confirmed for all the dynamical models studied. In general, one can fit NMR data from any model at all fields and arbitrary time scale and overall motion. The accuracy of the predicted values of $\gamma^2$ and $\tau_2$, however, depends crucially on the spatial restriction and time scale of the motion. As the order parameter gets smaller, the accuracy of the prediction of the value of $\gamma^2$ gets worse. For a given order parameter, the accuracy of the prediction improves as the time scale of the motion gets faster. For motions that are not fast, we conjecture that up to values of $\tau_2$ such that $(\omega\tau_2)^2 \approx 1$, where $\omega$ is the largest relevant frequency determining the relaxation data, the order parameter and $\tau_2$ can be extracted from a set of measurements of $T_i$ and NOE at two fields with an error no larger than $\pm 25\%$. For a $^{13}$ C Larmor frequency of $\approx 90.5$ MHz, one has $(\omega\tau_2)^2 \approx 1$ for $\tau_2 \approx 350$ ps. For example, for the diffusion in a cone model, for $\tau_M = 10^{-8}$ s and $1/6D_2 = 167$ ps, one predicts, using NOE and $T_i$ at 25.1 and 90.5 MHz, $\gamma^2 = 0.0898$ (exact value 0.0784) and $\tau_2 = 218$ ps (exact value 236 ps).

In applications, one is sometimes interested in internal motions that are slow on the NMR time scale, i.e., $(\omega\tau_2)^2 > 1$. There seems to be no simple criterion to determine accurately the magnitude of the error involved. We estimate, however, that order parameters larger than $\approx 0.3$ can be predicted with an accuracy of $\approx 30\%$. For order parameters larger than 0.5, the accuracy is likely to be better, probably $\approx 15\%$.

It is also interesting to consider the question whether measurements at several different fields can provide more information on the dynamics of the relaxation process. Table VI shows the results obtained for the jump model of Wittebort and Szabo for the lysine side chain attached to a spherical macromolecule. For $\tau_M = 10^{-8}$ s, data were generated at eight different magnetic fields, corresponding to Larmor frequencies ranging from 15 to 100 MHz. The table shows the results obtained by fitting $T_i$ and NOE at two fields and the results obtained by fitting $T_i$ and NOE at eight fields. The exact values of $\tau_2$ range from 278 to 31.5 ps, and $\gamma^2$ from 0.33 to 0.0046. Remarkably, the values of $\tau_2$ and $\gamma^2$ extracted by fitting $T_i$ and NOE at 25.1 and 67.9 MHz and by fitting $T_i$ and NOE at eight fields are identical. The fitted values of $T_i$ and NOE and the predicted $T_i$'s are also very close. For the $\beta$ carbon, $\tau_2 = 266$ ns and $\gamma^2 = 0.33$. The extracted values of $\gamma^2$ and $\tau_2$ are good, since $\gamma^2$ is large. For the $\gamma$ carbon, however, the exact value of $\gamma^2$ is 0.00926 and the extracted value of $\gamma^2$ is about twice as big. The $\delta$ and $\epsilon$ carbons are in the fast-motion regime, and one can extract the values of $\tau_2$ and $\gamma^2$ accurately. This example indicates that the addition of information of data measured at a number of different frequencies does not change the values of $\gamma^2$ and $\tau_2$ predicted. Some of the motions in the above example are rather fast. However, considering motions 1 order of magnitude slower, i.e., the jump model of Wittebort and Szabo with $k_1 = k_2 = k_3$, $k_1 = 10^{-8}$ s, $\tau_M = 10^{-8}$ s, we obtained similar results.

B. Anisotropic Overall Motion. In subsection II B we considered the application of the model-free approach to cases where the overall motion cannot be described by a single correlation time. The correlation function is factored in two terms, one describing the overall motion (eq 53) and the other for internal motions relative to the macromolecule, which has the usual model-free expression given by eq 32. In order to justify this approximation, we show first with an example that for anisotropic motions such "decoupling" of internal and overall motion is accurate. The expression for the correlation function for the diffusion in a cone model superimposed on anisotropic overall motion is given in the Appendix, eq A11. We now consider a correlation function of the form

$$C_{\text{decoupled}}(t) = \frac{1}{2} \exp[-(6D_0 + b^2(D_2 - D_0))t] \times (d_{90}^2(\beta_{\text{MD}})) [S_{\text{cone}}^2 + (1 - S_{\text{cone}}^2)e^{-t/\tau_2}]$$

which factors out the internal motion contribution. Equation 62 has a form similar to the isotropic case, except for the fact that the correlation function for the macromolecular motion has a more complicated structure. Table VII shows the results obtained by fitting $^{13}$ C NMR relaxation data generated at 25.1 and 90.5 MHz from eq A11 with a correlation function given by eq 62. $D_0$, $D_2$, and $\beta_{\text{MD}}$ were held fixed while $\gamma$ and $\tau_2$ were allowed to vary. Exact data correspond to $1/6D_2 = 10$ ps, $1/6D_0 = 0.5$ ps, $1/6D_0 = 2.16$ ns, $\theta_0 = 66.4^\circ$ ($\beta_{\text{MD}} = 55^\circ$) and $1/6D_0 = 2.60$ ns, $\theta_0 = 36.9^\circ$ ($\beta_{\text{MD}} = 55^\circ$). Values of $1/6D_0$ in the range 1-20 ps and of $1/6D_2$ in the range 0.1-0.5 ps are typical of DNA fragments $\sim 10^4$ base pairs long. The relaxation data can be reproduced well, and $\gamma$ and $\tau_2$ are predicted accurately in both cases. Notice that the two cases considered here are rather unfavorable, since the motions are relatively slow. For $\beta_{\text{MD}} = 15^\circ$, the amplitude of the motion is rather large, and for $\beta_{\text{MD}} = 55^\circ$, the order parameter for the total correlation function (i.e., the limit of the total correlation function as $r \rightarrow \infty$) given by eq A13 is extremely small ($3.33 \times 10^{-5}$), since $\beta_{\text{MD}}$ is close to the "magic angle". This numerical example shows that it is reasonable to separate the correlation function into a term describing the macromolecular motion and a term describing the internal motion. In applications, one has to determine the parameters for the overall motion from relaxation data for a nucleus attached to the macromolecular backbone. Such measurements may be actually available (e.g., data for $\alpha$ carbons in proteins in a helical conformation) or may
be simulated if the parameters for the motion of the macromolecule are known from other experiments (e.g., hydrodynamic or light-scattering measurements). One has to determine \( A, \tau_i \), and \( \tau_f \) in this fashion; subsequently, when data are interpreted for nuclei that have internal motions, the parameters for internal motions will be allowed to vary while \( A, \tau_i \), and \( \tau_f \) are held fixed.

Extensive numerical investigations showed that even in the case of the anisotropic motions, the internal motions can be adequately described by using the model-free approach. The accuracy of the predicted values of \( \delta^2 \) and \( \tau_2 \) depends on the time scale of the motion. However, the procedure used in determining the parameters for the overall motion introduces an additional error in the approximate correlation functions, and the inclusion of \( T_2 \) information may improve slightly the accuracy of the predicted values of \( \delta^2 \) and \( \tau_e \). We will briefly summarize the results of our numerical investigations and then present some illustrative examples.

The first case of interest is the one of extremely fast motions, i.e., motions such that eq 61 is satisfied to within \( \approx 2\% \), where now \( \tau \) is defined as \( \tau = \tau_{i}^{1} + \tau_{i}^{2} \) (recall that we assume that \( \tau_i \) and \( \tau_f \) are defined in such a way that \( \tau_i > \tau_f \)). In this case, a set of measurements (e.g., NOE and \( T_1 \)) at one field can give accurate values of \( \delta^2 \) and \( \tau_2 \) and relax data at all other fields such that eq 4 is satisfied to within \( \approx 2\% \) can be predicted accurately. For anisotropic overall motion, however, the inclusion of information at another field (e.g., \( T_1 \) and NOE) does slightly improve the extracted values of \( \delta^2 \) and \( \tau_e \), probably due to the method employed in determining the parameters for the macromolecular motion. A simple general criterion to determine the accuracy of the predicted values of \( \delta^2 \) for arbitrary values of \( \tau_i \) and \( \tau_f \) does not seem to exist; however, we estimate that as long as the overall motion is not too slow (\( \tau_f < 0.1 \mu s \)), data at one field should give \( \delta^2 \) values accurate to within \( \approx 5\% \), with the accuracy improving for larger order parameters. For fast internal motions, i.e., motions such that eq 61 is satisfied to within \( \approx 10\% \), one can extract accurate values of \( \delta^2 \) and \( \tau_f \) from a set of measurements (e.g., \( T_1 \) and NOE) at two fields. Again, the situation is slightly worse than in the isotropic case. We estimate that as long as \( \tau_f < 0.1 \mu s \), one can predict \( \delta^2 \) with an error no larger than \( \approx 20\% \), with the error being substantially smaller for larger order parameters. By including \( T_2 \) information, one obtains virtually exact answers.

Slower motions follow a pattern similar to the one observed in the isotropic case. For motions such that \( (\omega_r)^2 \approx 1 \), one can obtain fairly accurate values of \( \delta^2 \) from a set of measurements at both fields including \( T_2 \) information. For large order parameters \( (\delta^2 \geq 0.4) \), one can obtain accurate values of \( \delta^2 \) and \( \tau_e \) for motions on all time scales.

Table VIII describes results obtained for the diffusion in a cone model superimposed to an overall anisotropic motion with \( 1/6D_1 = 1 \mu s, 1/6D_2 = 0.05 \mu s, \) and \( \delta_{MD} = 15^\circ \). Exact results were generated for \( ^{13}C-^1H \) dipolar relaxation at 25.1 and 90.5 MHz: for \( \theta_0 = 66.4^\circ, 1/6D_1 = 10.9, 56.4 \) and 97.6 ps. For the fastest motion \( (1/6D_2 = 10.9 \mu s) \), we obtain an accurate prediction of both \( \delta^2 \) and \( \tau_f \) just by fitting \( T_1 \) and NOE at high field. The extracted \( \delta^2 \) value is slightly improved by fitting NOE and \( T_1 \) at both fields. For \( 1/6D_2 = 56.4 \mu s \), using \( T_1 \) and NOE at both fields, one obtains \( \delta^2 = 0.116 \) (the exact value is 0.0784), \( \tau_f = 81.9 \) ps (the exact value is 79.8 ps). By including \( T_2 \) information, one obtains \( \delta^2 = 0.0783 \) and \( \tau_f = 78.9 \) ps. For the slowest motion \( (1/6D_2 = 97.6 \mu s, \tau_f = 137 \) ps), one obtains \( \delta^2 = 0.2041 \) and \( \tau_f = 155 \) ps by fitting just NOE and \( T_1 \) at both fields; i.e., without including \( T_2 \) information, the predicted value of the order parameter is about three times bigger than the exact value. The inclusion of \( T_2 \) information, however, allows a very accurate prediction of both \( \delta^2 \) and \( \tau_f \) that differs from their exact values by 0.1% and 1.5%, respectively.

We now show that the model-free approach for overall anisotropic motions is accurate even in cases where a distribution of correlation times has been invoked (e.g., for random-coil polymers). Table IX describes \( ^{13}C-^1H \) NMR relaxation data generated for the jump model of Wittebort and Szabo for a lysine side chain.
attached to a macromolecule undergoing an overall motion associated with a distribution of diffusion coefficients given by eq A14 with $D_L = 2.5 \times 10^8 s^{-1}$ and $D_H = 5.0 \times 10^8 s^{-1}$. The motion of the $\alpha$ carbon in the laboratory frame is identical with the overall motion of the macromolecule, $T_1$ and NOE for the $\alpha$ carbon at two fields were fitted to a correlation function of the form of eq 32. The parameters for the overall motion were held fixed in the fitting procedure for carbons $\phi$ through $\epsilon$. Figure 3 shows the exact and approximate spectral densities for the overall motion plotted vs. the frequency $\omega$. The two curves are very similar in shape but tend to coincide only at the frequencies that determine the relaxation data. The inset shows the exact and approximate distributions of diffusion coefficients, $p(D)$ is plotted vs. $\log D$, where $D$ is defined by $\gamma = 1/6D$. The "double-$\beta$-function" distribution is represented by two spikes whose heights are proportional to the normalized probability of each value of $D$. The contrast between the similarity of the shape of the spectral density curves and the drastically different distributions is striking. It is also very interesting that the parameters for internal motion (predicted by using a simple double-$\beta$-function distribution of diffusion coefficients for the overall motion) are excellent in the sense that the accuracy is very similar to that achieved for internal motions on the same time scale when the overall motion is isotropic.

Table X shows a comparison of the values of $\delta^2$ and $\tau_e$ obtained in various ways from the relaxation parameters in Table IX. The exact $\delta^2$ and $\tau_e$ values were obtained from a fitting procedure, Table VII. $^{13}C$ Relaxation Data Generated by Using the Diffusion in a Cone Model Superimposed on Anisotropic Overall Reorientation

<table>
<thead>
<tr>
<th>$\rho_{MD}$</th>
<th>$\omega$, MHz</th>
<th>NOE (1 + $\gamma$)</th>
<th>$T_1$, ms</th>
<th>$T_2$, ms</th>
<th>$\delta^2$</th>
<th>$\tau_e$, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>$15^\circ$</td>
<td>25</td>
<td>1.67 (1.71)</td>
<td>46.1 (48.6)</td>
<td>0.34 (0.34)</td>
<td>0.0788 (0.0784)</td>
<td>2.83 (3.06)</td>
</tr>
<tr>
<td>90</td>
<td>1.21 (1.27)</td>
<td>184 (195)</td>
<td></td>
<td>0.35 (0.35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$55^\circ$</td>
<td>25</td>
<td>2.15 (2.10)</td>
<td>106 (107)</td>
<td>0.24 (0.24)</td>
<td>0.529 (0.518)</td>
<td>1.60 (1.64)</td>
</tr>
<tr>
<td>90</td>
<td>1.31 (1.36)</td>
<td>307 (302)</td>
<td></td>
<td>0.24 (0.24)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Data determined by using $1/6D_0 = 10 \mu$s, $1/6D_0 = 0.5 \mu$s, $1/6D_0 = 2.16 \mu$s, $\theta_0 = 66.4^\circ$ (for $\rho_{MD} = 15^\circ$), and $\theta_0 = 36.9^\circ$ (for $\rho_{MD} = 55^\circ$), fitted by using the "decoupled" correlation function of eq 62 where the only adjustable parameters were $\gamma$ and $\tau_e$. $T_1$ values are predicted. The exact results are in parentheses.

Table VIII. Model-Free Analysis of $^{13}C$ Relaxation Data Generated by Using the Diffusion in a Cone Model Superimposed on Anisotropic Overall Reorientation

<table>
<thead>
<tr>
<th>$1/6D_0$, ps</th>
<th>$\omega$, MHz</th>
<th>NOE (1 + $\gamma$)</th>
<th>$T_1$, $\delta$</th>
<th>$T_2$, ms</th>
<th>$\tau_e$, ps</th>
<th>$\delta^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.9</td>
<td>25</td>
<td>2.78d (2.79)</td>
<td>3.25 (3.50)</td>
<td>15.6d (15.5)</td>
<td>0.0835d (0.0787) (0.0784)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.97d (2.98)</td>
<td>3.24d (3.24)</td>
<td>3.25 (3.50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56.4</td>
<td>25</td>
<td>2.92b (2.94)</td>
<td>0.625b (0.621)</td>
<td>2.33b (3.46)</td>
<td>81.9b (78.9c (79.8)</td>
<td>0.116b (0.0783c (0.0784)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.93b (2.91)</td>
<td>0.665b (0.663)</td>
<td>2.33b (3.46)</td>
<td>155.5b (135c (137)</td>
<td>0.204b (0.0783c (0.0784)</td>
</tr>
<tr>
<td>97.6</td>
<td>25</td>
<td>2.95e (2.95)</td>
<td>0.372e (0.366)</td>
<td>3.44e (3.44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>2.86 (2.79)</td>
<td>0.409 (0.419)</td>
<td>3.44 (3.44)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Data determined by using $1/6D_0 = 1 \mu$s, $1/6D_0 = 0.5 \mu$s, $1/6D_0 = 2.16 \mu$s, $\theta_0 = 66.4^\circ$ (for $\rho_{MD} = 15^\circ$), and $\theta_0 = 36.9^\circ$ (for $\rho_{MD} = 55^\circ$). Exact results are in parentheses. The parameters for overall motion were $\tau_1 = 0.960 \mu$s, $\tau_2 = 0.183 \mu$s, and $A = 0.865$. $^b$ Results obtained by fitting NOE and $T_1$ at both fields. $^c$ Results obtained by fitting all relaxation data at both fields. $^d$ Results obtained by fitting NOE and $T_1$ at high field.

Table IX. Model-Free Analysis of $^{13}C$ Relaxation Data Generated by Using the Jump Model of Wittebort and Szabo for a Lysine Side Chain Attached to a Macromolecule Whose Overall Motion Is Described by a Distribution of Correlation Times, with $k_1 = k_2 = k_3 = 100$ ps, $D_L = 2.5 \times 10^8 s^{-1}$, and $D_H = 5.0 \times 10^8 s^{-1}$

<table>
<thead>
<tr>
<th>carbon</th>
<th>$\omega$, MHz</th>
<th>NOE (1 + $\gamma$)</th>
<th>$T_1$, ms</th>
<th>$T_2$, ms</th>
<th>$\delta^2$</th>
<th>$\tau_e$, ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>15.0</td>
<td>1.77 (1.75)</td>
<td>36.8 (37.0)</td>
<td>16.3 (14.1)</td>
<td>A = 0.509</td>
<td>$\tau_e = 18.8$ ns</td>
</tr>
<tr>
<td>67.9</td>
<td>1.53 (1.52)</td>
<td>185 (184)</td>
<td></td>
<td>21.3 (17.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$</td>
<td>15.0</td>
<td>2.09 (2.09)</td>
<td>73.8 (80.3)</td>
<td>41.5 (33.9)</td>
<td>0.341 (0.333)</td>
<td>256 (278)</td>
</tr>
<tr>
<td>67.9</td>
<td>2.29 (2.25)</td>
<td>213 (213)</td>
<td></td>
<td>52.9 (47.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\gamma$</td>
<td>15.0</td>
<td>2.86 (2.91)</td>
<td>226 (225)</td>
<td>202 (203)</td>
<td>0.0163 (0.00926)</td>
<td>206 (223)</td>
</tr>
<tr>
<td>67.9</td>
<td>2.81 (2.75)</td>
<td>269 (271)</td>
<td></td>
<td>224 (227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta$</td>
<td>15.0</td>
<td>2.55 (2.61)</td>
<td>407 (406)</td>
<td>281 (257)</td>
<td>0.0325 (0.0278)</td>
<td>78.4 (89.8)</td>
</tr>
<tr>
<td>67.9</td>
<td>2.82 (2.73)</td>
<td>580 (582)</td>
<td></td>
<td>325 (320)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\epsilon$</td>
<td>15.0</td>
<td>2.77 (2.79)</td>
<td>1296 (1294)</td>
<td>1055 (990)</td>
<td>0.00520 (0.0496)</td>
<td>29.9 (31.5)</td>
</tr>
<tr>
<td>67.9</td>
<td>2.92 (2.89)</td>
<td>1523 (1526)</td>
<td>1146 (1127)</td>
<td>111 (111)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ NOE's and $T_1$'s were fitted at both fields, $T_2$'s are predicted. Exact values are enclosed in parentheses.
Table X. Comparison of the Generalized Order Parameters and Effective Correlation Times Extracted from 13C NMR for the Jump Model of Wittesort and Szabo for a Lysine Side Chain Superimposed on Overall Reorientation Described by a Distribution of Correlation Times

<table>
<thead>
<tr>
<th>Carbon</th>
<th>Exact</th>
<th>Fitting Procedure</th>
<th>( T_1 ) and NOE at 15 MHz</th>
<th>( T_1 ) and NOE at 68 MHz</th>
<th>( T_1' ) s at 15 and 68 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta )</td>
<td>0.33</td>
<td>0.34</td>
<td>0.334</td>
<td>0.434</td>
<td>0.359</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>0.0093</td>
<td>0.0165</td>
<td>0.0104</td>
<td>0.110</td>
<td>0.0349</td>
</tr>
<tr>
<td>( \delta )</td>
<td>0.0275</td>
<td>0.0278</td>
<td>0.0356</td>
<td>0.0306</td>
<td>0.0345</td>
</tr>
<tr>
<td>( \epsilon )</td>
<td>0.00496</td>
<td>0.00520</td>
<td>0.00457</td>
<td>0.00805</td>
<td>0.00544</td>
</tr>
</tbody>
</table>

The exact and fitted values of \( \beta \) and \( \tau_0 \) are compared to the values obtained from the data in Table XII by using the analytical formulas given in eq 59a, 59b, 60a, and 60b. * Evaluated by using eq 59a for \( \beta \) and 59b for \( \tau_0 \). * Evaluated by using eq 60a for \( \beta \) and 60b for \( \tau_0 \). * Obtained by fitting \( T_1 \) and NOE at 15 and 68 MHz as in Table IX.

and the results obtained by using the analytical formulas given in eq 59a, 59b, 60a, and 60b are presented. The values of \( \beta \) and \( \tau_0 \) extracted from \( T_1 \) and NOE at 15 MHz by using eq 60a and 60b, respectively, are excellent since at this frequency the motions of all the carbons are close to the extreme narrowing limit. The situation is different for the values of \( \beta \) and \( \tau_0 \) extracted from \( T_1 \) and NOE at 68 MHz; at this resonance frequency, the motions of some carbons are not far from the extreme narrowing limit. The \( \beta \)’s and \( \tau_0 \)’s obtained from \( T_1 \)’s at both fields by using eq 59a and 59b, respectively, are better than the values obtained analytically from \( T_1 \) and NOE at high field, but not as accurate as the results from \( T_1 \) and NOE at low field. It is remarkable how good the general agreement between the values is, considering the simplicity of the analysis of this system where the motion is highly anisotropic. Of course the agreement between exact and calculated values would be even better for internal motions on the correlation function for overall motion. The accuracy of the \( \beta \) and \( \tau_0 \) depends essentially only on the magnitude of the order parameter and the time scale of internal motions. Even in the anisotropic case, for fast internal motions accurate values of \( \beta \) and \( \tau_0 \) can be extracted by using a straightforward fitting procedure or, for motions close to the extreme narrowing limit, from simple analytical formulas.

Acknowledgment. We have benefited from discussions with R. M. Levy and D. A. Torchia and thank R. M. Levy for sending us a preprint of his paper. GLL is partially supported by U.S. Public Health Service Grant HL-21483 awarded to Professor F. R. N. Gurd and thanks the Foundation Stiftelsen Blanceflor, Boncompagni–Ludovisi, fodd Bildt, for a fellowship.

Appendix

In this Appendix we describe the various dynamic models that we have used to generate relaxation data. We do this not only for the sake of completeness but also to contrast the complexity of the correlation functions or spectral densities of these models with the simplicity of eq 32 and 54, which are used in the model-free approach.

In Woevers’s model, a single free internal rotation is superimposed on isotropic reorientation. Specifically, the interaction vector \( \mu \) diffuses freely, with diffusion coefficient \( D_\mu \), about a symmetry axis rigidly attached to the macromolecule. The angle \( \beta \) between \( \mu \) and the symmetry axis does not change with time.

The total correlation function is

\[
C(t) = \frac{1}{2} \sum_{\alpha \epsilon \{1, 2, 3\}} \sum_{\beta \epsilon \{1, 2, 3\}} C_{\alpha \beta} \exp(-b^2 D_\mu t) \text{exp}(ib\alpha \mu_0) (A9)
\]

The order parameter in this model is

\[
\delta^2 = \sum_{\alpha \epsilon \{1, 2, 3\}} \sum_{\beta \epsilon \{1, 2, 3\}} C_{\alpha \beta} \exp(-b^2 D_\mu t) \text{exp}(ib\alpha \mu_0) \exp(ib\beta \mu_0) (A9)
\]

where \( \rho_{eq}(t) \) is the probability that at equilibrium \( \mu \) assumes...
position $i$. The effective correlation time in this model is

$$\tau_{\text{e}}(1 - \phi^2) = \sum_{\lambda = 1}^{2 \pi} \sum_{n = -2}^{2} C_{\text{rel}}^2$$  \hspace{1cm} (A10)

We now consider the situation where diffusion in a cone is superimposed on anisotropic overall motion. The macromolecule is assumed to be of cylindrical symmetry. $D_x$ and $D_y$ are the diffusion coefficients for reorientation of and about the $C_i$ axis of the molecule, respectively. The unit vector $\hat{a}$ diffuses in a cone of semiangle $\theta_0$ about a director, $\hat{d}$, which forms a fixed angle $\beta_{\text{MD}}$ with the $C_i$ axis of the cylinder. The correlation function for this model is\(^{18}\)

$$C(t) = \gamma \sum_{\lambda = 1}^{2 \pi} \sum_{n = -2}^{2} \exp[-(6D_x + b^2(D_y - D_x))t(\lambda_n^{(2)}(\beta_{\text{MD}}))^2 G_c(t)$$  \hspace{1cm} (A11)

with

$$G_c(t) = \langle D_{\text{co}}^{(2)}(\Omega(0))D_{\text{co}}^{(2)}(\Omega(t)) \rangle$$  \hspace{1cm} (A12)

where $\Omega$ is the orientation of $\hat{a}$ relative to $\hat{d}$. In generating relaxation data by using this model, we have found exact expressions for $G_c(t)$ given by Lipari and Szabo.\(^{25}\) The exact order parameter for this model is

$$\phi = \frac{1}{2} \sum_{\lambda = 1}^{2 \pi} \sum_{n = -2}^{2} \left[ C_{\text{rel}}^2 \log (D_U/D_L) \right]$$  \hspace{1cm} (A13)

where $S_{\text{cone}}$ is given by A3.

Finally, we consider the jump model of Wittebort and Szabo\(^{11}\) for the motion of a lysine side chain for the situation where the overall motion is described by a distribution of correlation times (or equivalently, a distribution of diffusion coefficients since $\tau_{\text{MD}} = (6D_{\text{MD}})^{-1}$). We use the normalized distribution function for the motion of a lysine side chain for the situation where the overall motion is described by a distribution of correlation times (or equivalently, a distribution of diffusion coefficients since $\tau_{\text{MD}} = (6D_{\text{MD}})^{-1}$). We use the normalized distribution function

$$p(D_M) = \left[ \frac{D_M}{D_U} \log \left( \frac{D_U}{D_L} \right) \right]^{-1} D_L \leq D_M \leq D_U$$

The spectral density is

$$J(\omega) = 2 \int_0^{D_L} p(D_M) \int_0^{\infty} C(t) (\cos \omega t) dt dD_M$$  \hspace{1cm} (A15)

Performing the integrations in eq A15 we obtain

$$J(\omega) = 2 \left[ \frac{2 \pi^2}{2 \pi} \sum_{\lambda = 1}^{2 \pi} \sum_{n = -2}^{2} \left[ C_{\text{rel}}^2 \log (D_U/D_L) \times \left( \frac{6(D_U - D_L)\omega}{\omega^2 + (6D_U + \lambda)(6D_L + \lambda)} \right) + \right. \left( \frac{\lambda_u}{2} \log \left( \frac{D_U^2(\omega^2 + (6D_L + \lambda_u)^2)}{D_L^2(\omega^2 + (6D_U + \lambda_u)^2)} \right) \right] \right]$$  \hspace{1cm} (A16)

The generalized order parameter is the same as in the case of isotropic overall motion (i.e., eq A9).

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**Model-Free Approach to the Interpretation of Nuclear Magnetic Resonance Relaxation in Macromolecules. 2. Analysis of Experimental Results**

**Giovanni Lipari** and **Attila Szabo**

*Contribution from the Laboratory of Chemical Physics, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205. Received November 9, 1981*

**Abstract:** In the preceding paper it has been shown that the unique dynamic information on fast internal motions in an NMR relaxation experiment on macromolecules in solution is specified by a generalized order parameter, $\phi$, and an effective correlation time, $\tau_{\text{e}}$. This paper deals with the extraction and interpretation of this information. The procedure used to obtain $\phi^2$ and $\tau_{\text{e}}$ from experimental data by using a least-squares method and, in certain favorable circumstances, by using an analytical formula is described. A variety of experiments are then analyzed to yield information on the time scale and spatial restriction of internal motions of isoleucines in myoglobin, methionines in dihydrofolate reductase and myoglobin, a number of aliphatic residues in basic pancreatic trypsin inhibitor, and ethyl isocyanide bound to myoglobin, hemoglobin, and aliphatic side chains in three random-coil polymers. The numerical values of $\phi$ and $\tau_{\text{e}}$ can be readily interpreted within the framework of a variety of models. In this way, one can obtain the same physical picture of internal motions as that obtained by using complicated spectral densities to fit the data. The numerical value of the order parameter, unlike the effective correlation time $\tau_{\text{e}}$, plays a crucial role in determining what models can be used to describe the experiment; models in which the order parameter cannot be reproduced are eliminated. Conversely, any model that can yield the correct value of $\phi$ works.

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I. Introduction

In the preceding paper\(^1\) (hereafter referred to as paper I), we addressed the question of the information content of NMR relaxation data and of the extraction of this information. We presented a model-free approach to this problem, showing that the dynamic information on fast internal motions contained in an NMR experiment is essentially specified by two parameters:

1. A generalized order parameter, $\phi$, which is a measure of the degree of spatial restriction of the motion, and
2. An effective correlation time, $\tau_{\text{e}}$, which is a measure of the rate (time scale) of the motion. These two parameters were defined in a model-independent way. For both isotropic and anisotropic overall motion we derived expressions for the appropriate spectral density (which determines the observable quantities in the NMR relaxation ex-

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