

Q-BAND ELECTRON SPIN RESONANCE STUDIES OF SLOW MOTIONS IN BILAYERS WITH HIGH ORDER

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Q-band electron spin resonance (ESR) experiments on macroscopically oriented multilayers of dimyristoyl lecithin/cholesterol (2:1 molar ratio)/cholestane spin label, order parameter $S \approx 1$, have been carried out in the temperature range -32°C to $+20^{\circ}\text{C}$. The spectra were simulated by a numerical solution of the stochastic Liouville equation using Padé approximants. The simulations show that the rates of reorientation of the spin label molecules about their long axes fall within the slow-motion regime. At low temperatures the molecular motion is best described in terms of a small step diffusion model, while at the higher temperatures a random jump model or an intermediate jump model provides a better description.

Keywords: electron spin resonance; cholestane spin label; lipid bilayers; slow motions; reorientational motion.

Introduction

Current knowledge of the physical properties of biological membranes stems to a large extent from studies of model membranes prepared from isolated lipids. Probe techniques, in which extraneous reporter molecules are embedded in the lipid systems have proved invaluable in these studies [1,2]. In particular, electron spin resonance (ESR) spectroscopy has been widely used in studies of the restricted anisotropic motions of nitroxide spin labels in the membrane systems [2]. The spin labelled derivative of 3α -cholestane, 3-doxylcholestane (CSL) has proved particularly useful in this respect as its ESR spectra reflect the overall motion of this rigid molecule [2-7]. As the axis of the largest principal hyperfine splitting ($A_{zz} \sim 33 \text{ G}$ [7]) is oriented perpendicular to the long axis of the molecule, this spin probe is optimally sensitive to rotation about the long molecular axis [3].

The disadvantage of the spin-label technique is that the information about the orientational order and dynamics of the molecules cannot often be extracted in a simple way from the observed ESR spectra [3,7-10]. This is particularly difficult

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in the slow-motion region, when the rotational correlation time, τ , characterizing the molecular dynamics is long on the ESR time scale, $\tau \gtrsim 2 \times 10^{-9}$ s. The problem has, however, largely been overcome in recent years with the development of fast numerical algorithms for the solution of the stochastic Liouville equation which provides the basic description of the ESR spectra [10–12]. It has thus become feasible to simulate the observed ESR spectra directly, without recourse to calibration curves or comparison with standard spectra.

In this paper we report a Q-band ESR (35 GHz) study of the axial reorientational motion of CSL molecules incorporated into cholesterol-rich bilayers of dimyristoylphosphatidylcholine (DMPC). These systems have been shown to possess an almost perfect orientational order, with the order parameter $S \approx 1$ [4]. Consequently the long molecular axis of each CSL molecule lies parallel to the normal to the plane of the bilayer and the molecules are restricted to undergo an axial rotational motion.

The ESR spectra are found to be sensitive to the axial motions of the molecules down to temperatures well below the phase transition of pure DMPC bilayers. Spectral simulations show that in the temperature range -30°C to $+20^\circ\text{C}$, the axial rates of motion fall within the slow-motion regime. Furthermore, the computed spectral lineshapes appear to be markedly sensitive to the details of the motional model used in the calculations. Thus at low temperatures the experimental spectra are found to be best described in terms of a small step stochastic brownian diffusion model [8,10,13], while at higher temperatures a random jump model or an intermediate jump model [13] is found to provide a better description.

Our results show that the Q-band ESR spectra are sensitive to changes of the reorientational motions of the spin labels even far below the phase transition of the bilayers. Thus Saturation-Transfer measurements are not necessary for studying the reorientational dynamics of our system down to -10°C .

Materials and Methods

DMPC and cholesterol were obtained from Calbiochem and Sigma, respectively. The cholestane spin label was obtained from Syva.

All the experiments were carried out on multibilayers of DMPC/cholesterol (2:1 molar ratio) which contained 1 mol% cholestane. The three components were mixed in chloroform and the solvent was removed overnight under vacuum. The lipid mixture was allowed to equilibrate at a relative humidity of 98% by standing for ≈ 24 h at 40°C over a saturated solution of K_2SO_4 . Macroscopically oriented multibilayers were prepared by gently rubbing a small aliquot of the material between two microscope glass coverslips (thickness ≈ 0.2 mm) measuring about 1×8 mm, at a temperature slightly above that of the phase transition of DMPC. The macroscopic alignment was monitored by optical microscopy. One sandwich in a standard Q-band tube was used in the experiments, in order to minimise the effects on the Q factor of the cavity. Dehydration of the sample was kept to a minimum by placing a saturated K_2SO_4 solution in a capillary above the sample outside the cavity.

The ESR spectra were recorded with a Varian E-line Q-band spectrometer equipped with a cylindrical cavity. A nitrogen gas flow system was used for temperature regulation of the cavity as a whole and a fine wire copper/constantan thermocouple, placed just above the cavity, was used for temperature measurement. The temperature is subject to an error of up to 5°C due to thermal gradients.

The spectra were recorded with a top-top modulation width of 0.6 Gauss at a frequency of 100 kHz. A microwave power of 0.5 mW was used. The reference arm was used to tune the microwave bridge. It was found necessary to adjust the tuning on changing the temperature. The sample was oriented with the applied static magnetic field lying either parallel to the normal to the plane of the sample \vec{n} (Fig. 1), 0° spectra, or perpendicular to \vec{n} , 90° spectra. This was achieved by rotating the magnet. The orientations were selected by seeking two magnet positions on either side of the required orientation which yield identical spectra. The magnet was then rotated to the half-way orientation.

The spectral simulations were carried out as described by us previously [10], by solving the stochastic Liouville equation numerically using Padé approximants. However, in the case of the low-temperature spectra, where we assume that no axial motion takes place (powder spectra), the simulations were carried out following Hemminga [4].

Theoretical aspects

Within the formalism of the stochastic Liouville equation, the reorientational motion of the spin label is described in terms of the operator Γ_{Ω} defined by [8]

$$\frac{dP(\Omega_0/\Omega t)}{dt} = -\Gamma_{\Omega}P(\Omega_0/\Omega t) \quad (1)$$

where $P(\Omega_0/\Omega t)$ is the orientational conditional probability function subject to the boundary condition $P(\Omega_0/\Omega 0) = \delta(\Omega_0 - \Omega)$. Here Ω denotes the set of three

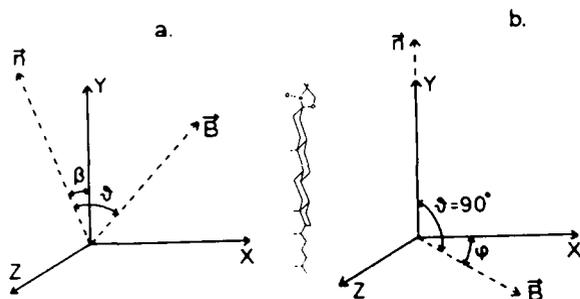


Fig. 1. (a) The molecular axis system for the cholestane spin label molecule. The cholestane molecule is also shown. The x -axis is along the N-O-bond, the z -axis along the π -orbital of the radical electron and the y -axis along the long axis of the molecule. \vec{n} is the normal to the multilayer system. (b) When the order is perfect ($\beta = 0^\circ$) and $\theta = 90^\circ$, then \vec{B} lies in the xz -plane. In this case ϕ is defined as the angle between \vec{B} and the x -axis.

Euler angles $\{\alpha\beta\gamma\}$ that transform the laboratory frame into the molecular frame at time t . $\Omega_0 \equiv \{\alpha_0\beta_0\gamma_0\}$ is defined analogously for $t=0$. The details of the molecular motion are contained in the explicit form of the operator Γ_Ω .

We shall here only consider the case of axial rotational motion (planar rotor) for which only the angle γ is time dependent. In this case $P(\Omega_0/\Omega t)$ can be expanded in terms of the functions $e^{in\gamma}$ [10,13] so that

$$P(\gamma_0/\gamma t) = \sum_n c_n(t) e^{in\gamma} \quad (2)$$

These functions are eigenfunctions of the operator Γ_γ ,

$$\Gamma_\gamma e^{in\gamma} = \lambda_n e^{in\gamma} \quad (3)$$

Because of the symmetry of the spin Hamiltonian [8,10] the summation in Eqn. (2) need be carried out only over even values of n , $n = 0, \pm 2, \pm 4 \dots$

Stillman and Freed [13] derived expressions for the eigenvalues λ_n , Eqn. (3) for three models for axial motion. These will be considered here in detail.

(i) Small step stochastic Brownian diffusion model

$$\lambda_n = n^2 D_{\parallel} \quad (4)$$

where D_{\parallel} is the diffusion coefficient for rotation of the spin label about its long axis.

(ii) Random jump model

$$\lambda_n = \tau_v^{-1} (1 - \delta_{n0}) \quad (5)$$

Here the label undergoes random, uncorrelated, orientational jumps with a mean residence time τ_v in any given orientation.

(iii) Intermediate jump model

$$\begin{aligned} \lambda_n &= \tau_\phi^{-1} n^2 [1 + (\tau_j/\tau_\phi)n^2]^{-1} \\ &= \bar{\tau}_j^{-1} n^2 \phi_j [1 + \phi_j n^2]^{-1} \end{aligned} \quad (6)$$

In this case τ_j is the mean time between reorientational jumps, as τ_v in model (ii), but $\phi_j = \tau_j/\tau_\phi$ is a measure of the main jump angle. In the limit $\phi_j \rightarrow 0$, jumps of infinitesimal angle, the result for the diffusion model is recovered, while for $n^2 \phi_j \gg 1$, the result is equivalent to that of the random jump model.

Simulated spectra for a 90° orientation using the Brownian diffusion and random jump models are shown in Figs. 2a and 2b, respectively. The spectra show the

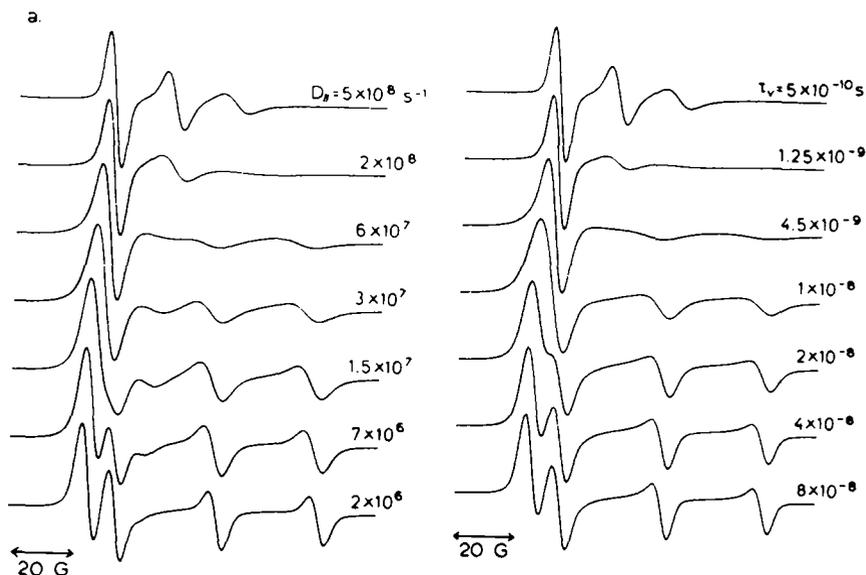


Fig. 2. Simulations with the Brownian diffusion model (a) and with the random jump model (b). The parameters from Table I are used. $\Delta G = 3.6$ G.

changes in the ESR line shape as a function of the rates of reorientation. The values of the g - and A -tensors as well as that for T_2 used in the simulations were chosen so as to reproduce the observed low temperature spectra (Table I). A small Gaussian broadening was also applied in order to improve the resemblance to the experimental spectra.

The simulations show that both models yield the same ESR spectra in the limit of very slow axial rotation. However, in the slow-motion regime the spectral lineshapes are markedly sensitive to the motional model. The differences are most pronounced for $2 \times 10^6 < D_{\parallel} < 8 \times 10^7$ and $4 \times 10^{-8} > \tau_v > 8 \times 10^{-9}$. As the rates of axial rotation increase further the lineshapes become insensitive to the detailed mechanisms of the motion. Thus in the motional-narrowing limit the lineshapes

TABLE I

THE COMPONENTS OF THE A AND g TENSORS AND VALUES FOR T_2 , OBTAINED FROM THE BEST FIT OF THE LOW TEMPERATURE SPECTRUM (FIG. 5)

These were used in the other simulations.

$A_{xx} = 5.5$ G
 $A_{yy} = 6.1$ G
 $A_{zz} = 33.5$ G

$g_{xx} - g_{zz} = 0.0060$
 $T_{2,x} = 4.7 \times 10^{-8}$ s
 $T_{2,x} = 1.1 \times 10^{-7}$ s

cannot be used to discriminate between the two models. This lack of sensitivity arises because in the latter regime the contribution to the spectra of eigenfunctions with $n > 2$ becomes negligible. Consequently the random jump model with $\tau_j^{-1} = 4D_{\parallel}$ will yield the same spectral lineshape as the Brownian diffusion model.

The sensitivity of the spectral lineshapes in the slow-motion regime on the details of the axial motion can best be illustrated with the intermediate jump model. Figure 3 shows simulated spectra as a function of the mean jump angle ϕ_j . The jump time τ_j for a given ϕ_j was chosen so as to keep the total width of the spectrum constant.

Results and Discussion

(i) 0° spectra

The underlying assumption of this study is that the cholestane molecules are perfectly aligned with their long axes lying perpendicular to the membrane surface. The molecules can therefore only undergo axial reorientational motions. This is in agreement with the findings of previous studies [4,7]. Under these conditions the 0° spectra consist of three overlapping lines having the same shape and with a centre-to-centre separation A_{yy} . The experimental spectra are shown in Fig. 4a for a number of temperatures, together with spectra simulated under the assumption of perfect ordering. In simulating the spectra we found it necessary to use a composite lineshape – a convolution of a Lorentzian and a Gaussian shape. The width of the Lorentzian component, Δ_L , is related to the electron relaxation time T_2 by $\Delta_L = 2(\gamma_e \sqrt{3} T_2)^{-1}$. The Gaussian component, of peak-to-peak width of the first derivative Δ_G , reflects the broadening due to unresolved hyperfine coupling of the electron and intramolecular protons.

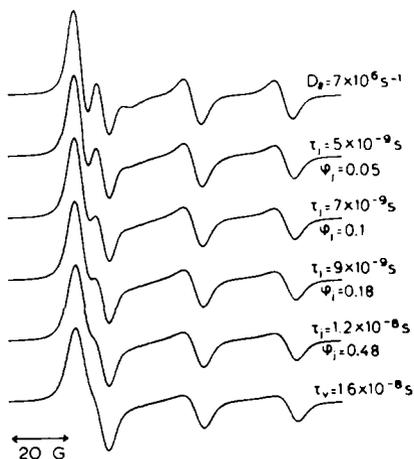


Fig. 3. Simulations with the intermediate jump model for different values of the mean jump angle ϕ . The parameters of Table I are used, with $\Delta_G = 3.6$ G.

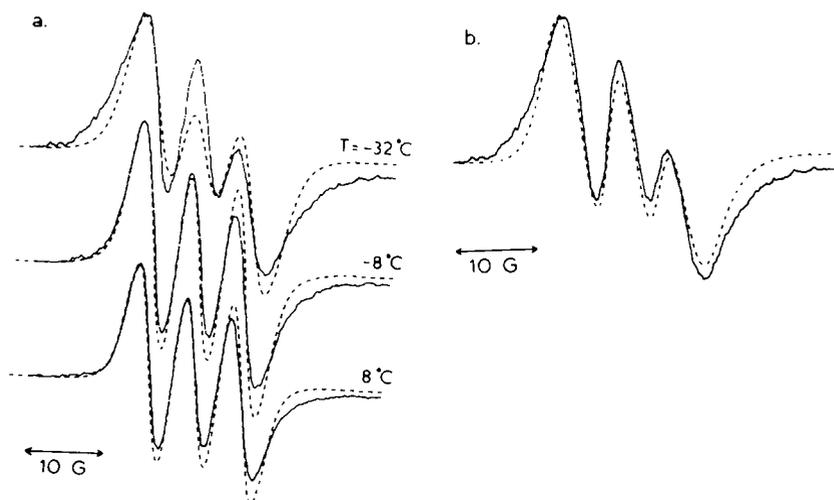


Fig. 4. —, experimental 0° spectra; ----, simulated 0° spectra. (a) Simulations under the assumption of perfect order, $A_{yy} = 6.1$ G, for $T = -32^\circ\text{C}$: $T_2 = 4.7 \times 10^{-8}$ s, $\Delta_G = 4.4$ G, for $T = -8^\circ\text{C}$: $T_2 = 4.7 \times 10^{-8}$ s, $\Delta_G = 3.2$ G, for $T = 8^\circ\text{C}$: $T_2 = 6.6 \times 10^{-8}$ s, $\Delta_G = 3.2$ G. (b) Simulation with $S = 0.95$, $A_{yy} = 5.1$ G, $T_2 = 1.1 \times 10^{-7}$ s, $\Delta_G = 4.4$ G.

The agreement between the experimental and simulated spectra is good at high temperatures, but worsens progressively as the temperature is lowered to -32°C . We note that the line splitting $A_{yy} = 6.1$ G is essentially temperature independent, and that the spectral changes are due primarily to a broadening of the lines. This could be explained in terms of two effects. Firstly, the onset of electron spin-spin broadening as a result of clustering of spin label molecules on lowering the temperature and secondly, changes in the texture of the sample and misalignment of the membrane planes. The influence of the latter effect on the spectra can be calculated at -32°C , where the axial motion of the molecules has been slowed down to the powder limit [4], see below. We note that in this limit we cannot distinguish between microscopic and macroscopic misalignment in the sample. We consider a misalignment in the orientation of the long axes of the molecules (taken to be the y -axis) relative to the director (Fig. 1). On taking β as the angle between the molecular y -axis and the director, we can introduce an orientational distribution function $f(\beta)$ of the form

$$f(\beta) = e^{+\lambda P_2(\cos\beta)} / \int_0^\pi e^{+\lambda P_2(\cos\beta)} \sin\beta d\beta \quad (7)$$

$$P_2(\cos\beta) = \frac{1}{2}(3 \cos^2\beta - 1) \quad (8)$$

$$S = \int_0^\pi f(\beta) P_2(\cos\beta) \sin\beta d\beta \quad (9)$$

We have chosen this function in preference to the more usual Gaussian distribution [2,4,5,7] as it satisfies the symmetry properties of the sample. A good agreement between experiment and simulation can be obtained, Fig. 4b, in the powder limit on taking $\lambda \sim 20$, corresponding to an order parameter $S \sim 0.95$. For this case we find a lower value of $A_{yy} \sim 5.1$ G as a result of the contributions from the $A_{zz} \cong 33$ G component of the hyperfine tensor. Thus even at -32°C the degree of alignment of the long axes of the cholestane molecules relative to the director is extremely high. The result indicates that our samples can be described using the assumptions stated above to a good approximation over the entire temperature range studied here. Furthermore, we shall show below that the 90° spectrum at -32°C is only marginally influenced by a misalignment corresponding to $S \sim 0.95$.

(ii) 90° spectra

The experimental 90° spectra in the temperature range -32°C to $+20^\circ\text{C}$ are shown in Figs. 5 and 6a. This temperature range lies below the phase transition temperature 23°C of pure DMPC bilayers. The spectral lineshapes and line positions change down to the lowest temperature studied. We shall assume that the spectrum at -32°C corresponds to the powder limit (no effective motion) and use it to extract the values of the g and A tensor components. However, as the magnetic field and microwave frequency have not been measured, only the difference $g_{xx} - g_{zz}$ can be obtained from the spectrum.

The best simulated spectrum calculated on assuming perfect orientational ordering, $S = 1$, is shown in Fig. 5. It was found that the experimental lineshapes could be satisfactorily reproduced only on taking either Δ_L or Δ_G to be explicitly dependent on ϕ , the angle of rotation about the long axes of the molecules, Fig. 1b. We have chosen to use an angle dependent $T_2(\equiv\Delta_L)$ process

$$T_2^{-1} = T_{2,x}^{-1} \cos^2 \phi + T_{2,z}^{-1} \sin^2 \phi \quad (10)$$

and an isotropic Gaussian broadening.

The effects of misalignment, seen in the 0° spectra, cause but small changes in the simulated 90° spectrum. On introducing an orientational distribution function

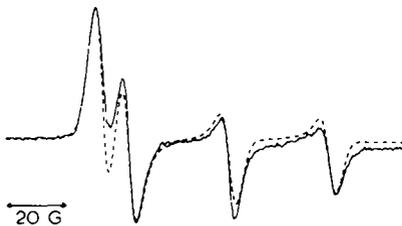


Fig. 5. —, experimental 90° spectrum for $T = -32^\circ\text{C}$; ----, simulated 90° powder spectrum under the assumption of perfect order. The parameters of Table I were used with $\Delta_G = 4.8$ G.

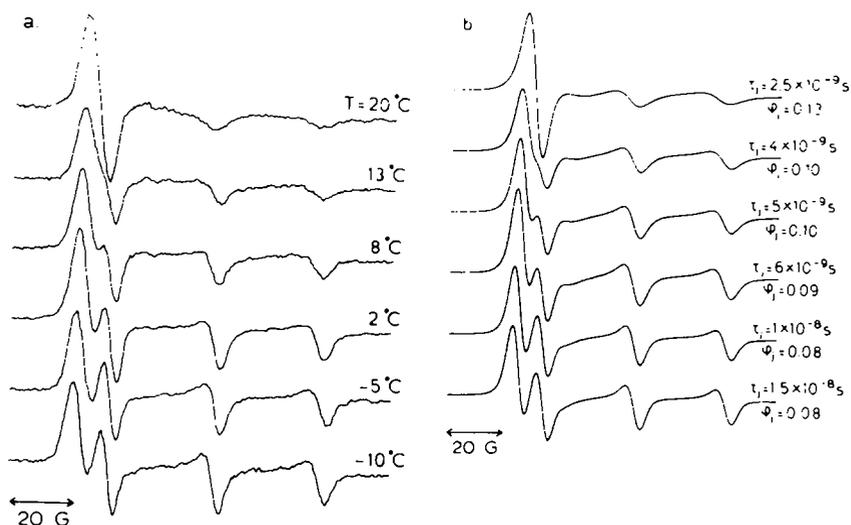


Fig. 6. (a) Experimental 90° spectra at several temperatures. (b) Simulations with the intermediate jump model which agree best with the experimental spectra of Fig. 6a. The parameters of Table I are used. For the first four spectra: $T_{1,x} = 6.6 \times 10^{-8}$ s, $T_{1,z} = 3.3 \times 10^{-7}$ s, for the last two spectra: $T_{1,x} = 4.7 \times 10^{-8}$ s, $T_{1,z} = 1.1 \times 10^{-7}$ s. ΔG is respectively: 2.4 G, 2.4 G, 2.4 G, 3.0 G, 3.0 G, 3.6 G.

for β , Eqn. (7), with $S \sim 0.95$ into the calculations and using the same values for the g and A tensor components, only small changes in the simulated spectra are apparent. The total width of the spectrum decreases by 0.8 G with minimal changes in the lineshapes. These changes are significantly smaller than those observed on raising the temperature. We can thus conclude, that our working model based on perfect order yields a good description of the experimental spectra.

On raising the temperature from -32°C to -6°C , the total width of the spectra decreases by 3.0 G mainly as a result of a shift in the line positions. This trend is well reproduced by simulations using the Brownian diffusion model, (cf. Fig. 2a), but not if the random jump model is used, Fig. 2b. The Brownian diffusion model, however, fails to describe the changes in the lineshapes, particularly the flattening of the high field lines, on raising the temperature further. The random jump model provides a better description of the lineshapes, although it does not account satisfactorily for the temperature dependence of the shifts and the depths of the high field lines.

The simulations indicate strongly that the mean angle of rotation of the molecules about their long axes is neither random nor infinitesimally small. We have, therefore, carried out simulations using the intermediate jump model in which the mean jump angle can be varied. These spectra are shown in Fig. 6b. Although the agreement with experiment is not perfect, the principal features of the experimental

spectra can be reproduced over the entire temperature range. The discrepancies partly arise from the imperfect simulation of the powder spectrum.

Two important conclusions can be drawn from the results presented above. Firstly, the experimental spectra clearly fall within the slow-motion regime. Secondly, the mean angle of rotation of the cholestane molecules about their long axes is temperature dependent. The molecules execute larger angular excursions at the higher end of the temperature range studied here, but the amplitudes are damped on cooling down to the powder limit, while the mean jump time τ_j increases. These conclusions are in agreement with X-band studies using cholestane molecules labelled with ^{14}N and ^{15}N nuclei (A.J. Dammers, unpublished results).

The results presented here cannot be compared to those of previous studies [7] where the spectral simulations were performed on the assumption that the motional rates satisfied the motional-narrowing limit.

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