

**CHAPTER 5 “NMR HOMONUCLEAR DIPOLAR RELAXATION  
THEORY: ANISOTROPIC MOLECULAR TUMBLING”**

## 5.1 Summary

This chapter is an introduction for chapters 6-7, both of which utilize the theories discussed here. Presented is a treatment of nuclear magnetic resonance (NMR) dipolar relaxation theory for homonuclear interactions for both isotropic and anisotropic molecular tumbling. Methods are also presented for using these theories to simulate the nuclear Overhauser effect (NOE).

## 5.2 Introduction

NMR is a powerful spectroscopic technique for studying molecular systems. Data from NMR spectroscopy can give a wealth of information about structure and dynamics. In recent years, this technique has become a useful tool for molecular biochemists in determining the three dimensional structures of biomolecules, such as proteins and nucleic acids.

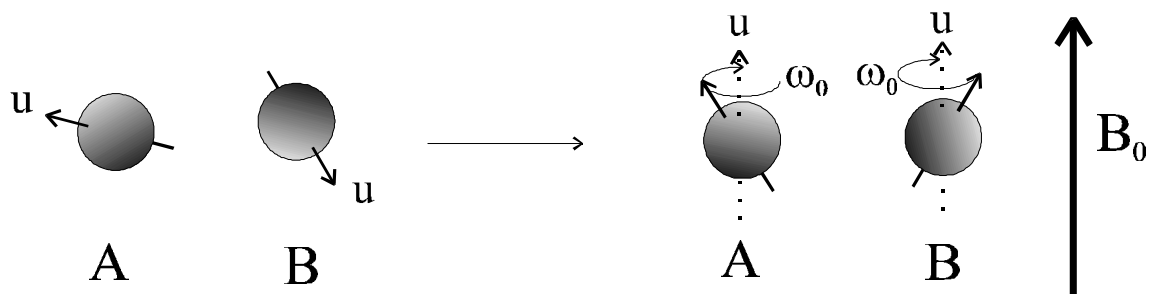
Measurement of NOE in NMR spectroscopy can be used to calculate the distances between nuclei to determine the three-dimensional structures of molecules. NOE is derived from through-space dipolar relaxation that is induced by time-dependent fluctuating magnetic fields and is dependent on the distance between two dipoles. For solution-state NMR, these fluctuations mainly result from molecular rotational diffusion spinning the nuclear dipole moments that have aligned with a strong external  $B_0$  magnetic field. The current theories and practices in biomolecular structure determination often make the assumption that an isotropic rotation model can adequately describe this molecular rotational diffusion. This assumption is not valid for extended shape

biomolecules such as long DNAs, which are better described as having two rotational diffusion rates, one for the long axis and one for the short axis.

The effect of the anisotropic rotation is examined in this chapter theoretically in terms of the effect on the NOE and its interpretation in distance calculations.

### 5.3 Homonuclear NMR relaxation theory

As mentioned earlier, the NOE is a through-space dipolar relaxation process between magnetically active nuclei. The theories behind NMR relaxation (Abragam & Pound, 1953; Solomon, 1955) will be developed in this section for the special case of two rigid, isolated spins. The concept rate matrix treatment will be described, which allows for later application of these theories to multi-spin systems, with coupled relaxation properties.

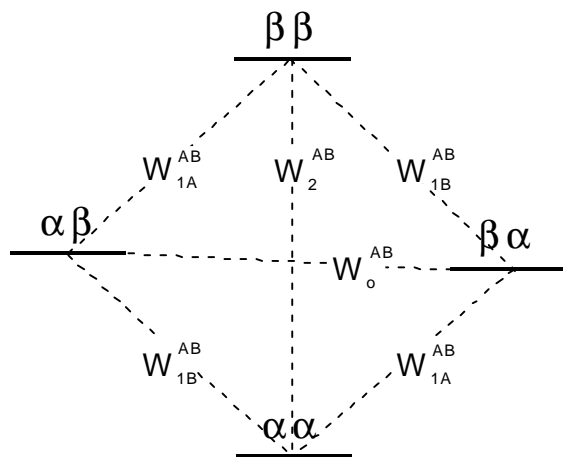


**Figure 5. 1** Two magnetic nuclei placed in an external  $B_0$  field

Consider two nuclei (as shown in figure 5.1), A and B, that have an inherent magnetic dipole moment  $\mathbf{u}$ , which have been placed in a strong external magnetic field  $B_0$ . The magnetic dipole moment will precess under the torque induced by the  $B_0$  field at the nuclei's characteristic Larmor frequency given by  $\omega_0 = -\gamma B_0$  ( $\gamma$  is the gyromagnetic ratio for that spin, with a value of  $26.7520 \times 10^7$  rad  $T^{-1} s^{-1}$  for proton). The net magnetic moment of the precession will lie parallel to the  $B_0$  field (defined as the z-axis) and is

represented by the dotted arrow in figure 5.1. The energy for the interaction between the magnetic dipole and the external magnetic field is given by  $E(m) = -g\mathcal{B}_0\hbar m$ , where the allowable values for the quantum number  $m$  are  $+I, (+I-1), \dots, (-I+1), -I$ . For spins with a quantum number  $I = \frac{1}{2}$  (such as the biologically relevant  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  nuclei),  $m = +\frac{1}{2}$  or  $-\frac{1}{2}$  which gives the allowable energies for the spin to be proportional to  $\pm \frac{1}{2}\hbar$ . These energies are abbreviated as  $\alpha$  and  $\beta$ , respectively.

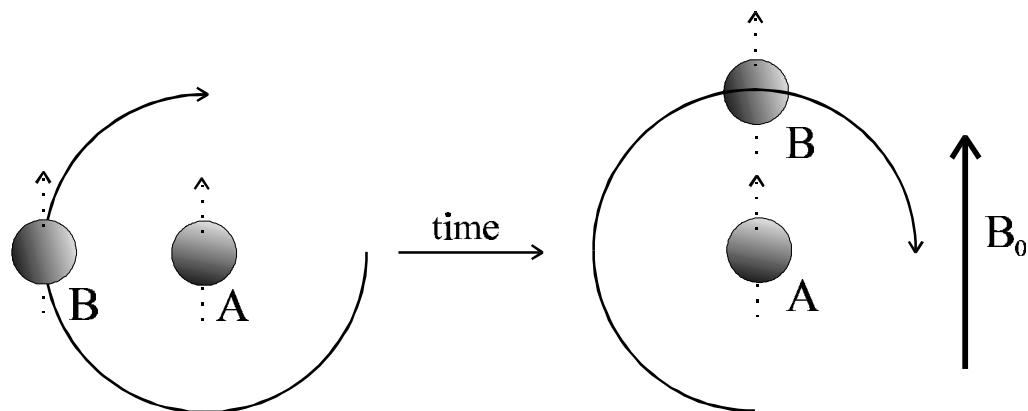
Thus, the energy of the two spin system can be described as one of four possible energy states ( $\alpha\alpha$ ,  $\alpha\beta$ ,  $\beta\alpha$  or  $\beta\beta$ ) and the changes in energy of the system are described by the energy diagram shown in figure 5.2 below.



**Figure 5.2** Energy diagram for two nuclei of spin  $\frac{1}{2}$

It is these transitions between energy states that give rise to all of NMR relaxation theory. The rate of a transition occurring between any of the above energy states is given by the function  $W$ , as shown in figure 5.2. The phenomenon of dipolar relaxation occurs because of time-dependent fluctuations in the magnetic field surrounding a nucleus. These fluctuations can arise from a number of molecular properties, such as molecular

tumbling in solution, dynamical motions between nuclei or librational atomic motions. For the simple case of a rigid two-spin system, the magnetic field fluctuations are completely described by the molecular tumbling, as shown in the figure below.



**Figure 5. 3 Time-dependent magnetic field fluctuations due to molecular rotation**

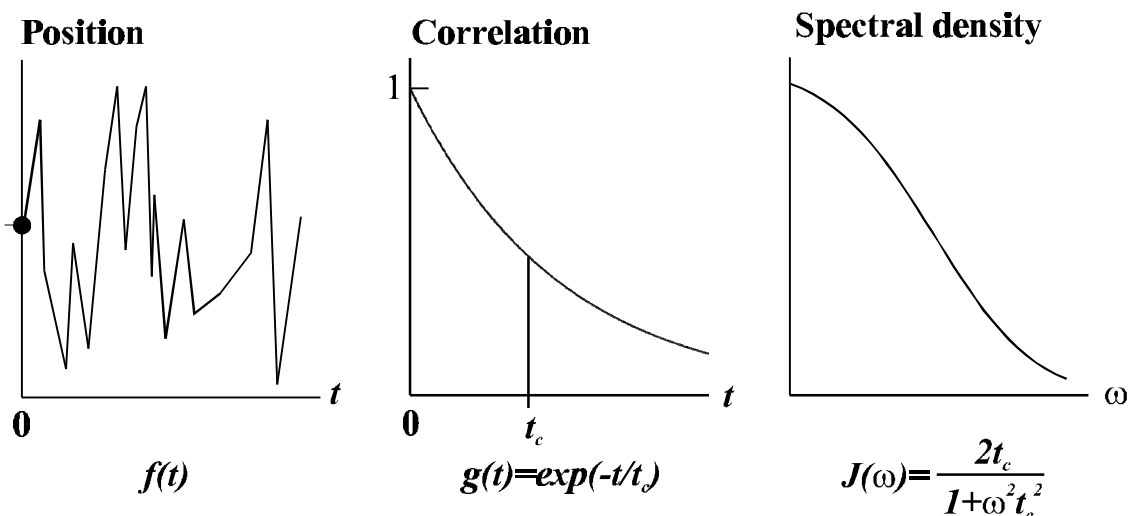
A complete description of NMR dipolar relaxation thus requires an accurate mathematical description of the frequencies of these magnetic field fluctuations. The frequency domain function used for this purpose is known as the “spectral density function” and can be derived from Brownian motion theory for particles.

### 5.3.1 The spectral density function for isotropic rotation

Bloembergen, Purcell and Pound (1948) first described the spectral density function for isotropic motion. They used a model of a randomly orienting internuclear vector that is attached to a sphere undergoing isotropic rotational diffusion in a continuous medium. This model is similar to that developed by Debye (1929) for dielectric relaxation. Full mathematical treatments on the derivation of this spectral density function have been well reviewed (Solomon, 1955; Ernst, *et al.*, 1987; Hennel & Klinowski, 1993; Schmidt-Rohr & Spiess, 1994) and will not be presented here. It is the

intention of this section to give a qualitative description of the concepts involved in molecular orientation mathematics.

The mathematical description of isotropic molecular reorientation involves three functions, position,  $f(t)$ , correlation,  $g(t)$ , and spectral density  $J(\omega)$ . A graphical representation of these three functions is shown below,



**Figure 5. 4 Functions of molecular reorientation**

The position function is simply a measure of motional movement as a function of time for a *single* particle in a molecule. If this motion is due to Brownian thermal movements, as shown above,  $f(t)$  will appear to be random. However, the ensemble average of the position of many particles will be described by an exponential decay,

$$g(t) = \langle f(0)f(t) \rangle = \exp(-t/t_c). \quad 5.1$$

This ensemble average is called the correlation function, with the time constant for the decay,  $t_c$ , defined as the “correlation time”.  $g(t)$  is a probability function that describes the chances of finding a particle near the original position,  $f(0)$ , in the ensemble, and has been described as a measure of the ‘position memory’ of a particle. With increasing time

this probability diminishes as the positions of particles become less correlated to their starting positions  $f(0)$ .

The spectral density function is the frequency domain representation of this correlation function, and as such they are mathematically interconvertible by the Fourier transform,  $FT$ ,

$$J(\omega) = FT(g(t)) = FT(\exp(-t/t_c))$$

$$= \int_0^{\infty} \exp(-t/t_c) \cos(\omega t) dt = \frac{2t_c}{1 + \omega^2 t_c^2} \quad 5.2$$

### 5.3.2 Transition rates

With a mathematical definition of the spectral density function, the energy state transition rates can be expressed as functions of  $J_{AB}$  by the Solomon equations (Solomon, 1955) (assuming  $\omega_A = \omega_B = \omega$ , a homonuclear interaction),

$$W_{1A}^{AB} = \frac{3}{4} q_{AB} J_{AB}(\omega_{0A}) = \frac{3}{4} q_{AB} J_{AB}(\omega), \quad 5.3$$

$$W_{1B}^{AB} = \frac{3}{4} q_{AB} J_{AB}(\omega_{0B}) = \frac{3}{4} q_{AB} J_{AB}(\omega), \quad 5.4$$

$$W_0^{AB} = \frac{1}{2} q_{AB} J_{AB}(\omega_{0A} - \omega_{0B}) = \frac{1}{2} q_{AB} J_{AB}(0), \quad 5.5$$

$$W_2^{AB} = 3q_{AB} J_{AB}(\omega_{0A} + \omega_{0B}) = 3q_{AB} J_{AB}(2\omega). \quad 5.6$$

The derivation of these expressions comes from perturbation theory and the Hamiltonian of the motion of the particles. Notice that the Solomon equations include a "rate" constant  $q_{AB}$ , which is derived from the coulombic interaction of two dipoles, and is defined as,

$$q_{AB} = \frac{1}{10} g_A^2 g_B^2 h^2 r_{AB}^{-6} \left[ \frac{m_0}{4p} \right]^2. \quad 5.7$$

The  $r^{-6}$  term assumes that there are no distance fluctuations between the nuclei **AB**. If fluctuations do exist, then a more complex definition of  $r$  would be contained in the spectral density function  $J_{AB}$ . The rate constant is often conveniently represented as  $56.9 \cdot r^{-6}$  (in units of  $s^{-1} \text{ ns}^{-1} \text{ \AA}^{-6}$ ) (note the  $s^{-1}$  and  $\text{ns}^{-1}$  component, see appendix 5.7.1 for the derivation and dimensional analysis).

The time dependent change of population ( $dN$ ) of any of the four energy states shown in figure 5.2 can be calculated by multiplying the appropriate population ( $N$ ) by the transition rate, which can be positive or negative depending on whether it is adding or removing magnetization. This is shown by the following equations,

$$\frac{dN_{bb}}{dt} = -(W_{1A}^{AB} + W_2^{AB} + W_{1B}^{AB})N_{bb} + W_{1A}^{AB}N_{ab} + W_2^{AB}N_{aa} + W_{1B}^{AB}N_{ba}, \quad 5.8$$

$$\frac{dN_{ab}}{dt} = -(W_{1A}^{AB} + W_0^{AB} + W_{1B}^{AB})N_{ab} + W_{1A}^{AB}N_{bb} + W_0^{AB}N_{ba} + W_{1B}^{AB}N_{aa}, \quad 5.9$$

$$\frac{dN_{ba}}{dt} = -(W_{1B}^{AB} + W_0^{AB} + W_{1A}^{AB})N_{ba} + W_{1B}^{AB}N_{bb} + W_0^{AB}N_{ab} + W_{1A}^{AB}N_{aa}, \quad 5.10$$

$$\frac{dN_{aa}}{dt} = -(W_{1B}^{AB} + W_2^{AB} + W_{1A}^{AB})N_{aa} + W_{1B}^{AB}N_{ab} + W_2^{AB}N_{bb} + W_{1A}^{AB}N_{ba}, \quad 5.11$$

The experimentally observable magnetization,  $I_z$ , will be the difference between the populations of the  $\alpha$  and  $\beta$  energies for each spin **A** or **B**,

$$I_{z,A}K = (N_{aa} + N_{ab}) - (N_{ba} + N_{bb}), \quad 5.12$$

$$I_{z,B}K = (N_{aa} + N_{ba}) - (N_{ab} + N_{bb}). \quad 5.13$$



$K$  is a normalization constant. Substitution of equations 5.8-5.11 into 5.12 and 5.13 gives (see appendix 5.7.4 for the algebra) equations 5.14 and 5.15,

$$K \frac{dI_{z,A}}{dt} = -(W_2^{AB} + 2W_{1B}^{AB} + W_0^{AB})I_{z,A} + (W_0^{AB} - W_2^{AB})I_{z,B}. \quad 5.14$$

$$K \frac{dI_{z,B}}{dt} = -(W_2^{AB} + 2W_{1A}^{AB} + W_0^{AB})I_{z,B} + (W_0^{AB} - W_2^{AB})I_{z,A}. \quad 5.15$$

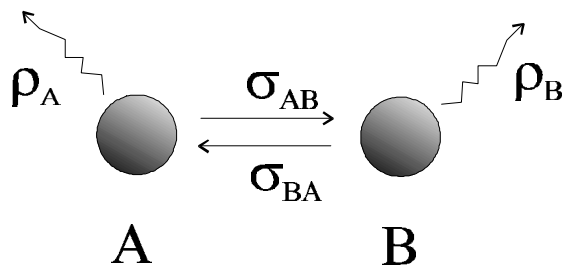
These equations show that for spin A, magnetization is taken away at a rate of  $-(W_2 + 2W_{1B} + W_0)$  and is transferred to spin B at a rate of  $(W_0 - W_2)$ . These values have been given special names and symbols,

$$r_A = -(W_0^{AB} + 2W_{1B}^{AB} + W_2^{AB}), \quad 5.16$$

$$r_B = -(W_0^{AB} + 2W_{1A}^{AB} + W_2^{AB}), \quad 5.17$$

$$S_{AB} = S_{BA} = W_0^{AB} - W_2^{AB}. \quad 5.18$$

Where  $r$  is denoted the “spin-lattice relaxation” rate and  $S$  is denoted the “cross-relaxation” rate. Mutual energy coupling between the two spins occurs in the cross-relaxation, or “spin-flip” transitions (see figure 5.2). It is this cross-relaxation term that gives rise to the nuclear Overhauser effect. Graphical representation of these relaxation parameters between spins A and B is shown below.



**Figure 5.5** The NMR relaxation parameters  $S$  and  $r$

The cross-relaxation rate,  $S_{AB}$ , can now be expressed in term of the spectral density function. Notice that  $\nu_{bA} \approx \nu_{bB}$  for nuclei of the same element (protons, for instance) to give,

$$S_{AB} = \frac{56.9}{r^6} [J(0) - 6J(2\nu)]. \quad 5.19$$

Expansion of this equation with the definition of the isotropic rotation spectral density function, eq. 5.2, gives,

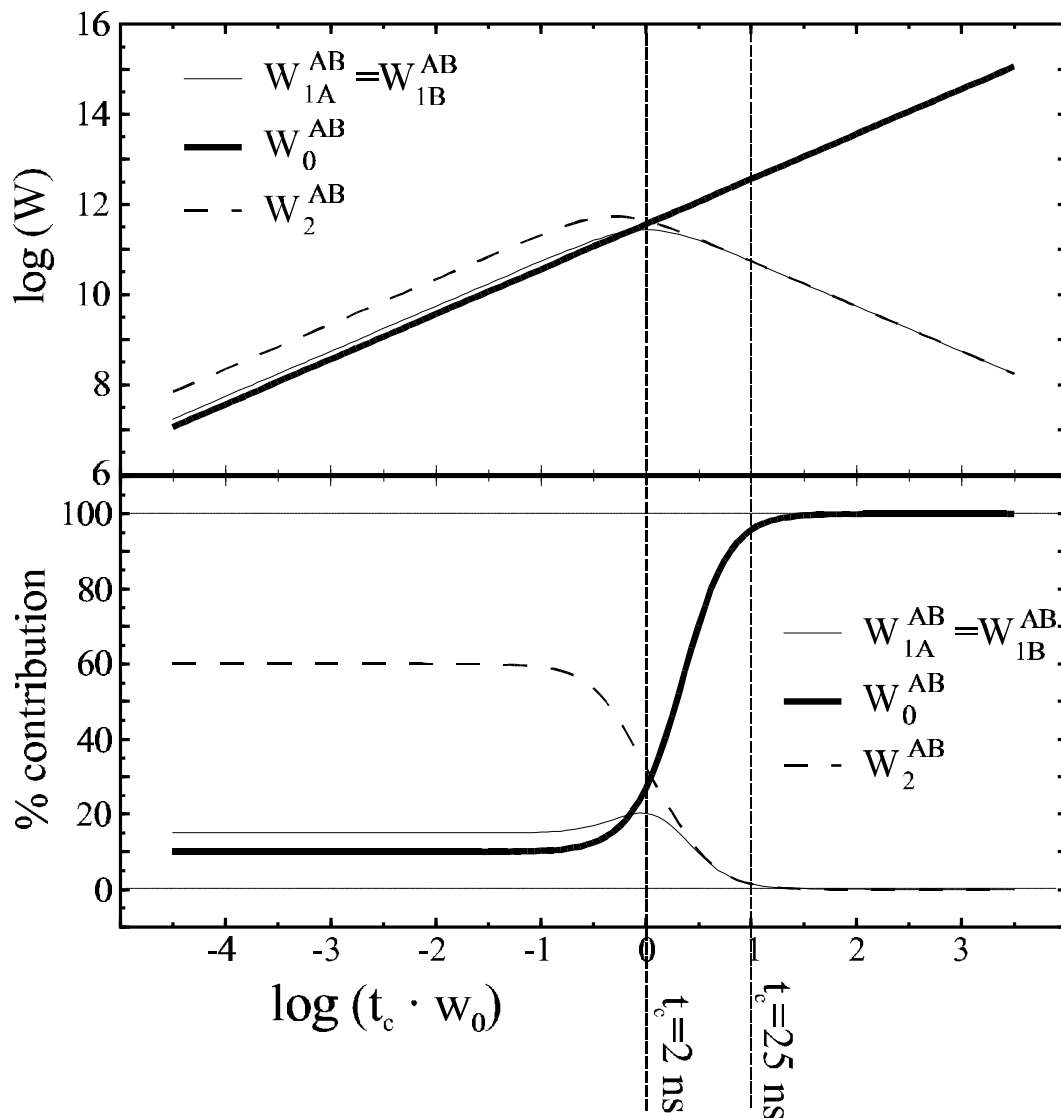
$$S_{AB} = \frac{56.9}{r^6} \left[ 2t_c^{AB} - \frac{12t_c^{AB}}{1 + (2\nu t_c^{AB})^2} \right]. \quad 5.20$$

This is then a complete description of the cross-relaxation rate of any rigid, isotropically rotating spin pair. Often this equation simplified further by making the assumption that we will only consider large, slowly rotating molecules ( $t_c \gg 1/2\nu$  the slow motion limit), which causes the  $6J(2\nu)$  term to approach zero, leaving,

$$S_{AB} = -\frac{56.9}{r^6} [2t_c^{AB}]. \quad 5.21$$

However, a plot of the transition probability functions with increasing  $t_c$  values (as can be seen in figure 5.6) demonstrates that this assumption may not be completely valid for the size biomolecules (with  $t_c$  between 1 and 10 ns) studied here. There may be a significant contribution to the cross-relaxation from the  $W_2$  transition, and thus eq. 5.20 is the preferred definition of  $S_{AB}$ .

Similarly, the value of  $r_A$  can be expanded in terms of this spectral density function by combining equation 5.16 with 5.3, 5.4, 5.5, 5.6 and 5.7,



**Figure 5.6** Two-spin energy transition rates

Transition rates for the energy diagram (fig 5.2) have been calculated using  $W_0=0.5qJ(0)$ ,  $W_1=3qJ(2\nu_b)$  and  $W_2=0.75qJ(\nu_b)$ , substituting the spectral density function for isotropic rotation. The script "W.pl" (see Chapter 7) was written for this purpose. A range of values for  $t_c$  were plotted, assuming a 500 MHz NMR spectrometer ( $\nu_b = 500 \times 10^6 \text{ s}^{-1}$ ). The top graph is the actual values for each  $W$  function, while the lower graph shows the percentage contribution of each transition rate. For the size DNA molecules used in these studies, the  $t_c$  values calculated ranged from 2 to 25 ns, and is represented by the vertical dotted lines, notice that for cross-relaxation, ( $W_0-W_2$ ) one cannot make the assumption that the  $W_2$  term is negligible.

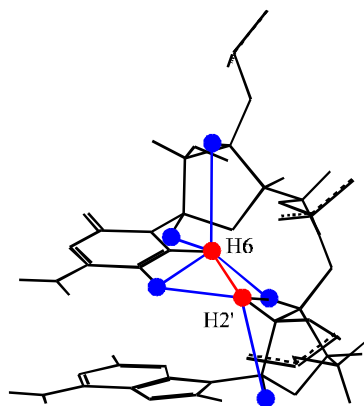
$$\begin{aligned}
 r_A &= -\left( \frac{1}{2} q_{AB} J_{AB}(0) + 2 \left( \frac{3}{4} q_{AB} J_{AB}(\omega) \right) + 3 q_{AB} J_{AB}(2\omega) \right), \\
 &= -5 q_{AB} [J_{AB}(0) + J_{AB}(\omega) + J_{AB}(2\omega)], \\
 &= -5 \cdot \frac{56.9}{r^6} \left[ 2t_c + \frac{2t_c}{1 + \omega^2 t_c^2} + \frac{2t_c}{1 + (2\omega)^2 t_c^2} \right].
 \end{aligned} \tag{5.22}$$

### 5.3.3 The relaxation rate matrix

These two relaxation processes can be followed with the use of a 2x2 "relaxation" or "rate" matrix,  $\mathbf{R}$ , of form (Keepers & James, 1984; Ernst, *et al.*, 1987) (see appendix 5.5.2 for the derivation of the rate matrix from chemical exchange theory),

$$\mathbf{R} = \begin{vmatrix} r_A & s_{AB} \\ s_{BA} & r_B \end{vmatrix}. \tag{5.23}$$

The advantage of using the rate matrix for representing the relaxation processes is that it offers a convenient method of multiple (more than two) spin coupled relaxation. Dipolar relaxation in NMR often involve many spins that are in close proximity to each other, as shown below in figure 5.7 for the H6-H2' protons in A-form RNA.



**Figure 5.7 Multiple spin coupling in nucleic acids**

The H6-H2' distance is important in nucleic acid structure determination because it is one of the few distance restraints which interconnects adjacent nucleotides in standard helical regions of the structure. Besides the two protons of interest, there are five other protons within 3.5Å of the pair. It is important that the distance calculations used to determine RNA structure take into account these multiple spin partners.

The rate matrix is a general method of describing any number of coupled relaxation rate processes, and as such it can be expanded to include more spins. The expanded rate matrix allow for all these additional rate processes to be accounted for simultaneously, and is given by,

$$\mathbf{R}(N \times N) = \begin{vmatrix} r_{1,1} & S_{2,1} & \dots & S_{N,1} \\ S_{1,2} & r_{2,2} & \dots & S_{N,2} \\ \dots & \dots & \dots & \dots \\ S_{1,N} & S_{2,N} & \dots & r_{N,N} \end{vmatrix}, \quad 5.24$$

with the more general definitions for  $r$  and  $S$ ,

$$r_{i,i} = - \sum_{j(i \neq j)} (W_0^{i,j} + 2W_1^{i,j} + W_2^{i,j}), \quad 5.25$$

$$S_{i,j} = W_0^{i,j} - W_2^{i,j}. \quad 5.26$$

The T1, or longitudinal, relaxation time is a measure of the rate at which the z component of the magnetization returns to the equilibrium state. It can be calculated from these relaxation matrix parameters and goes as the inverse of the sum of the  $r$  with all possible  $S$  rates.

$$\frac{1}{T1} = r_i + \sum_{j(i \neq j)} S_{ij} \quad 5.27$$

which gives,

$$\frac{1}{T1} = -2 \sum_{j(i \neq j)} (W_1^{i,j} + W_2^{i,j}) \quad 5.28$$

#### 5.4 Measured NOE volumes and the relaxation matrix

Measurement of the homonuclear relaxation matrix by NMR comes from the interpretation of the volume intensities from NOESY experiments. In fact, the volume matrix ( $\mathbf{V}$ ) is fundamentally related to the relaxation matrix in that they are of the same dimension (both are  $N \times N$  with  $N$  equal to the number of protons in the molecule). The diagonal elements of the relaxation matrix correspond to the autopeaks of the volume matrix, and the off-diagonal elements of the relaxation matrix correspond to the crosspeaks of the volume matrix. The two are related by the following equation,

$$\mathbf{V}(t_{mix}) = \mathbf{V}(0) \exp[\mathbf{R}t_{mix}]. \quad 5.29$$

With  $\mathbf{V}(0)$  being the intensities of the autopeaks at a mixing time of 0.

The power of the relaxation matrix  $\mathbf{R}$  comes from the fact that it offers a way of calculating the intensities of a NOESY spectrum by simultaneously solving all the relaxation rate equations for every nucleus. If one assumes that the rate matrix truly represents all the relaxation properties of the system, it is theoretically possible to back-calculate NOE intensities from a molecular structure model.

##### 5.4.1 Mathematic considerations

As discussed previously, the intensities of all resonances in the NOESY spectrum are represented by an  $N \times N$  matrix  $\mathbf{V}(t_m)$ , with the intensity of the autopeaks as the  $\mathbf{V}_{i,i}$  elements and the crosspeaks as the  $\mathbf{V}_{i,j(i \neq j)}$  elements. The zero-time intensity matrix  $\mathbf{V}(0)$

is a matrix with zero value off-diagonal terms and diagonal terms which represent the intensity of the autopeaks at a  $t_{mix} = 0$ .

This matrix equation can be solved by diagonalizing the rate matrix  $\mathbf{R}$  to determine the eigenvalue matrix  $\mathbf{\Lambda}$ , and the corresponding eigenvector matrix  $\mathbf{X}$  (see appendix 5.6.3 on solving simultaneous rate equations). This leads to the following,

$$\mathbf{V}(t_{mix}) = \mathbf{V}(0)\mathbf{X} \cdot \exp(-\mathbf{\Lambda}t_{mix}) \cdot \mathbf{X}^{-1}, \quad 5.30$$

that can be used to directly calculate the intensity matrix  $\mathbf{V}$ .

It is apparent that this “relaxation matrix” method of predicting NOE volumes will only be as successful as the model used in building the relaxation rate matrix  $\mathbf{R}$ . It is in this matrix that any and all assumptions made about the relaxation processes of the system are placed. In fact, as discussed previously, any assumptions in the relaxation theory arise from the model used to build the spectral density function.

Thus far it has been assumed that isotropic motion can adequately describe the rotational diffusion of the molecule. This assumption, however, is not true for molecules with extended hydrodynamical shapes such as DNA. The rotational dynamics of these molecules cannot be accurately described using the isotropic definition of the spectral density function.

## 5.5 Anisotropic molecular tumbling

A molecule undergoing anisotropic molecular tumbling, such as a long thin cylinder, will actually have two correlation times describing its motion; one about the short axis of rotation ( $t_s$ ) and one about the long axis ( $t_l$ ) of rotation. Unlike the isotropic dipolar interactions, the effective correlation time any particular pair of nuclei experience will be dependent on the angle they make with respect to the principal axis of rotation.

If, for instance, an isolated pair of nuclei form a vector that lies parallel to the principal axis of rotation, the dipolar interactions they experience will be independent of the rotation about the principal axis and will be described by the short axis rotation. However, an isolated pair of nuclei that form an interaction vector that lies perpendicular to the principal axis of rotation will experience some geometric mean of the long and short axis rotation.

This angular dependence of the correlation time for a pair of dipoles in an anisotropically rotating molecule must be represented in the definition of the spectral density function.

### 5.5.1 The spectral density function for anisotropic rotation

Woessner (1962) derived the spectral density function for an anisotropically rotating molecule. The derivation will not be presented here, as it is rather lengthy. This is a summation of the results,

$$J(\omega) = a_1 J(\omega \tau_1) + a_2 J(\omega \tau_2) + a_3 J(\omega \tau_3), \quad 5.31$$

where,

$$J(\omega \tau) = \tau / (1 + \omega^2 \tau^2) \quad 5.32$$

and the amplitudes,  $a_i$  are given by,

$$a_1 = 0.25 (3 \cos^2 b - 1)^2 \quad 5.33$$

$$a_2 = 3 \cos^2 b \sin^2 b \quad 5.34$$

$$a_3 = 0.75 \sin^4 b. \quad 5.35$$

The angle  $b$  is the angle the **AB** vector makes with the principal axis (see appendix 5.7.2 for a discussion of finding this axis vector by calculating the inertia tensor) of the molecule. The correlation times  $\tau_{1,2,3}$  are composite correlation times defined by,



$$t_l = t_L \quad 5.36$$

$$t_2 = 6t_L t_S / (t_L + 5t_S) \quad 5.37$$

$$t_3 = 3t_L t_S / (2t_L + t_S) \quad 5.38$$

This new spectral density function can then be used in place of the isotropic definition to give new equations for the spin-lattice ( $S_{AB}$ ) and cross-relaxation ( $r_A$ ) rates for the elements of the relaxation rate matrix  $\mathbf{R}$ ,

$$S_{AB} = 56.9 r_{AB}^{-6} [6a_1 J(2\omega, t_1) + 6a_2 J(2\omega, t_2) + 6a_3 J(2\omega, t_3)] \\ - [a_1 J(0, t_1) + a_2 J(0, t_2) + a_3 J(0, t_3)], \quad 5.39$$

$$r_A = \sum_{j(i \neq j)} 5 \cdot 56.9 r_{i,j}^{-6} (a_1 J(0, t_1) + a_2 J(0, t_2) + a_3 J(0, t_3)) + \\ (a_1 J(\omega, t_1) + a_2 J(\omega, t_2) + a_3 J(\omega, t_3)) + \\ (a_1 J(2\omega, t_1) + a_2 J(2\omega, t_2) + a_3 J(2\omega, t_3)). \quad 5.40$$

## 5.6 Discussion

The NMR theory for understanding homonuclear dipolar relaxation has been presented in this chapter. The rotational tumbling rate of a molecule is an important component of this dipolar relaxation process, as it is the principal mechanism that induces the fluctuating magnetic fields responsible for dipolar relaxation. An accurate description of the rotational motion of a molecule is thus necessary in order to interpret any experimental manifestations of the dipolar relaxation.

The NOE is an important probe of molecular structure because the intensity of the NOE is related to the spatial proximity between the two nuclei. The NOE arises as a consequence of dipolar relaxation, and as such, it is important for the interpretation of NOE data that the molecular tumbling of the molecule be understood. A measured NOE between two nuclei can only be interpreted as a distance restraint in the context of a rotational dynamics model. For nucleic acids, a description of this rotational dynamics as isotropic may not be adequate, and the spectral density function proposed by Woessner (1962) is preferred.

This chapter is presented as the theoretical basis for the next few chapters, which will discuss the use of these theories in the simulation of NOE intensities from structural and dynamical models as well as their use in methods of structural refinement.

## 5.7 References

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## 5.8 Appendix

### 5.8.1 Cross relaxation rate constant calculation:

The cross-relaxation rate constant,  $q$ , is often represented as the value 56.9, I often wondered from where that number came. Relaxation theory gives us the constant  $q_{AB}$ , which can be derived from magnetic point charges,

$$q_{AB} = \frac{1}{10} \frac{g_A^2 g_B^2 \hbar^2}{r^6} \left( \frac{\mu_0}{4\pi} \right)^2 \quad 5.A.1$$

with,

$$\begin{aligned} J &= \text{Joules (kg} \cdot \text{m}^2 \cdot \text{s}^{-2}) \\ T &= \text{tesla (kg} \cdot \text{s}^{-2} \cdot \text{A}^{-1}) \\ g_H &= \text{gyromagnetic ratio for proton} \\ &= 26.7520 \times 10^7 \text{ (rad} \cdot \text{T}^{-1} \cdot \text{s}^{-1} \text{ or rad} \cdot \text{kg}^{-1} \cdot \text{s}^2 \cdot \text{A} \cdot \text{s}^{-1}) \\ \hbar &= \text{planck's constant} \\ &= 6.626208 \times 10^{-34} \text{ (J} \cdot \text{s)} / 2\pi \\ &= 1.054593 \times 10^{-34} \text{ (kg} \cdot \text{m}^2 \cdot \text{s}^{-2} \cdot \text{s)} \\ \mu_0 &= \text{permeability constant} \\ &= 4\pi \cdot 1 \times 10^{-7} \text{ (kg} \cdot \text{m} \cdot \text{s}^{-2} \cdot \text{A}^{-2}) \\ r &= \text{distance between the spins (\AA or } 1 \times 10^{-10} \text{ m)} \end{aligned}$$

The number 56.9 is derived,

$$\begin{aligned} &\frac{(26.7519 \times 10^7 \text{ rad} \cdot \text{kg}^{-1} \cdot \text{s}^2 \cdot \text{A} \cdot \text{s}^{-1})^4 (1.0545938 \times 10^{-34} \text{ kg} \cdot \text{m}^2 \cdot \text{s}^{-2} \cdot \text{s})^2 (12.56637 \times 10^{-7} \text{ kg} \cdot \text{m} \cdot \text{s}^{-2} \cdot \text{A}^{-2})^2}{10(4\pi)^2} \\ &= 5.696 \times 10^{-50} \text{ (s}^{-2} \text{m}^6) \\ &= 5.69 \times 10^{10} \text{ (s}^{-2} \text{A}^6) \\ &= 56.9 \text{ (s}^{-1} \text{ns}^{-1} \text{A}^6) \end{aligned}$$

### 5.8.2 Determining the principal axis: the inertia tensor calculation

Determining the principal axis of rotation for a hydrodynamical particle is of fundamental importance for the calculations involving NMR relaxation of anisotropically rotating molecules. Ideally, it is the diffusion tensor that would give the best measure of

this axis. However, the diffusion tensor is quite complicated in that it requires knowledge about the frictional components of the solvent and solute. The inertia tensor, on the other hand, is a simpler calculation and is probably very accurate in predicting the principal axis of rotation in most cases. The inertia tensor requires only knowledge of the structure (or structural model) and masses of the atoms of the molecule in question.

The inertia tensor is an important relation in rotational dynamics. For example, angular momentum ( $\mathbf{L}$ ) is related to angular velocity ( $\mathbf{w}$ ) by means of the inertia tensor,

$$\mathbf{L} = \{\mathbf{I}\} \cdot \mathbf{w} , \quad 5.A.2$$

and torque ( $\mathbf{\Gamma}$ ) is related to angular acceleration ( $\mathbf{\alpha}$ ) by the inertia tensor,

$$\mathbf{\Gamma} = \{\mathbf{I}\} \cdot \mathbf{\alpha} . \quad 5.A.3$$

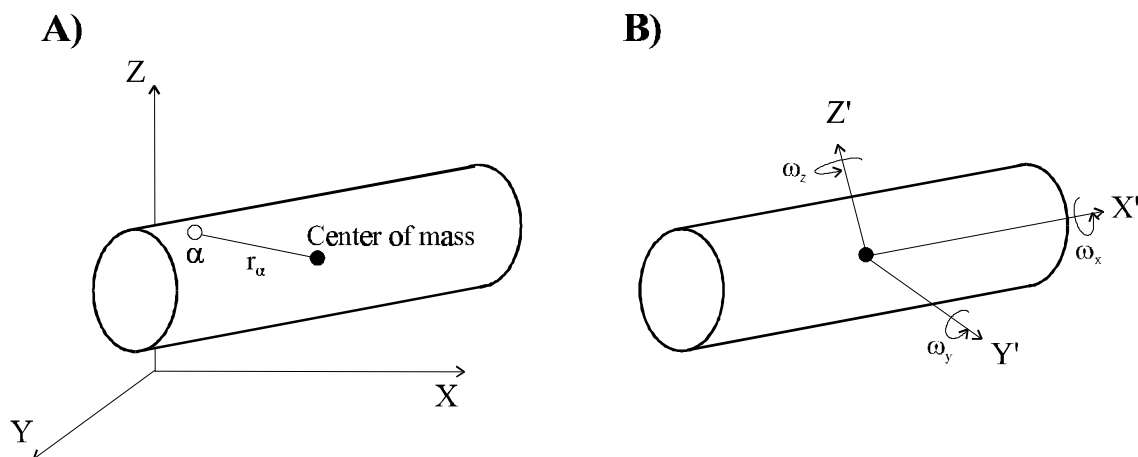
In a sense, the inertia tensor relates rotational variables much like mass relates non-rotating variables ( $\mathbf{P} = m\mathbf{v}$  and  $\mathbf{F} = m\mathbf{a}$ ). The inertia tensor is a measure of how much “apparent rotational mass” an object has.

The inertia tensor is a 3x3 matrix in which the nine elements are composed of the X,Y or Z cartesian coordinates of a particle  $\alpha$  and the distance from that particle to the center of mass of the object,  $r_a$ . (Read chapter 10 of Marion and Thornton’s “Classical Dynamics” book (1965) if you are interested in the derivation of the equations for the inertia tensor).

$$\{\mathbf{I}\} = \begin{vmatrix} \sum_a m_a (r_a^2 - x_a^2) & -\sum_a m_a x_a y_a & -\sum_a m_a x_a z_a \\ -\sum_a m_a x_a y_a & \sum_a m_a (r_a^2 - y_a^2) & -\sum_a m_a z_a y_a \\ -\sum_a m_a x_a z_a & -\sum_a m_a z_a y_a & \sum_a m_a (r_a^2 - z_a^2) \end{vmatrix} , \quad 5.A.4$$

The inertia tensor is characterized by diagonal elements  $I_{11}$ ,  $I_{22}$  and  $I_{33}$  that are known as the “moments of inertia” and the 6 independent off-diagonal elements,  $I_{12}$ ,  $I_{13}$ , etc, are

termed the “products of inertia” (notice that this matrix is Hermitian,  $I_{12} = I_{21}$ ). The initial Cartesian coordinate system (x,y,z) may be of arbitrary origin as shown in figure 5.14 A, meaning the object can be displaced to any position without changing the result of the inertia tensor calculation.



**Figure 5.8 The inertia tensor and a symmetrical top**

The “principle axes of inertia” is defined as the axis coordinate system ( $x', y', z'$ ) in which the off diagonal terms for  $\{\mathbf{I}\}$  vanish,  $I_{i \neq j} = 0$ . This diagonalized inertia tensor,  $\mathbf{I}'$ , is calculated from the inertia tensor by finding a transformation matrix,  $\lambda$ , such that,

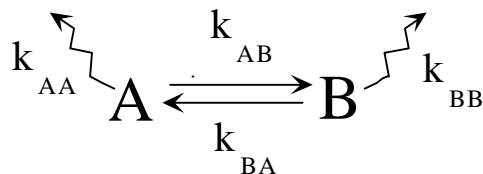
$$\mathbf{I}' = \lambda \mathbf{I} \lambda^{-1}. \quad 5.A.5$$

When the inertia tensor is transformed in this manner, the three eigenvalues of  $\mathbf{I}$ ,  $I_1$ ,  $I_2$  and  $I_3$  (solved using methods described in appendix 5.6.3) are known as the “principle moments of inertia”. Examination of the relative values of  $I_1$ ,  $I_2$  and  $I_3$  gives much information on the shape of the body. If  $I_1 = I_2 = I_3$  the body is a “spherical top”. If  $I_1 = I_2 \neq I_3$  then the body is termed a “symmetrical top” (DNA and other cylindrical molecules fall into this category). Finally, if  $I_1 = 0$  and  $I_2 = I_3$  then, for instance, the body may be two point masses connected via a weightless shaft, this is known as a “rotor”.

### 5.8.3 Solving coupled rate equations, eigenvalues and eigenvectors

Many mathematical problems deal with solving simultaneous rate equations, such as chemical exchange and NMR relaxation. The concentration (or magnetization) of a species may be dependent on the interactions of many other species which are all undergoing rate processes. This appendix is a short discussion of how to exactly solve the simple case of chemical exchange in a two species system as well as the methods of solving N species problems via approximate methods.

The simplest case of two species in chemical exchange is presented (Eigen & DeMaeyer, 1963; discussions with Pat Vaccaro & Donald Crothers). Assume two chemical species, A and B, which can interconvert at a rate of  $k_{AB}$  in which both species also undergo an external decay with rates  $k_{AA}$  and  $k_{BB}$  respectively. This is analogous to the situation of relaxation processes in NMR (think cross-relaxation and  $T_1$ ).



The kinetic differential equations for the chemical exchange process would be (assuming  $k_{AB} = k_{BA}$ ),

$$\frac{d[A]}{dt} = -k_{AB}[A] - k_{AA}[A] + k_{AB}[B] = -(k_{AB} + k_{AA})[A] + k_{AB}[B], \quad 5.A.6$$

$$\frac{d[B]}{dt} = -k_{AB}[B] - k_{BB}[B] + k_{AB}[A] = -(k_{AB} + k_{BB})[B] + k_{AB}[A], \quad 5.A.7$$

which can be rewritten in matrix form,

$$\frac{d}{dt} \begin{bmatrix} [A] \\ [B] \end{bmatrix} = \begin{bmatrix} -(k_{AB} + k_{AA}) & +k_{AB} \\ +k_{AB} & -(k_{AB} + k_{BB}) \end{bmatrix} \begin{bmatrix} [A] \\ [B] \end{bmatrix}. \quad 5.A.8$$

Symbolically, this matrix equation becomes,

$$\frac{d}{dt} \mathbf{C} = \mathbf{RC}, \quad 5.A.9$$

with a "concentration matrix"  $\mathbf{C}$  and a "rate matrix"  $\mathbf{R}$ . Solving matrix equations of this type requires diagonalization of the matrix  $\mathbf{R}$  to determine a set of eigenvalues, which can then be placed back into equation 5.19 to determine the values for  $\mathbf{C}$ , the eigenvectors.

All matrix operators, such as  $\mathbf{R}$ , can be diagonalized by a similarity transformation  $\mathbf{T}$ ,

$$\mathbf{T}^{-1}\mathbf{RT} = \boldsymbol{\lambda}, \quad 5.A.10$$

in which  $\boldsymbol{\lambda}$  is a diagonal matrix (a diagonal matrix is any matrix with off-diagonal term of zero). The transformation matrix is unique in that,

$$\mathbf{T}^{-1}\mathbf{T} = \mathbf{TT}^{-1} = \mathbf{E} \quad 5.A.11$$

where  $\mathbf{E}$  is the unity matrix (a diagonal matrix with diagonal elements  $E_{i,i}=1$  and off-diagonal elements  $E_{i,j}(i \neq j)=0$ ).

Define a new matrix,  $\mathbf{y}$ , as,

$$\mathbf{T}^{-1}\mathbf{C} = \mathbf{y}. \quad 5.A.12$$

Multiply both sides of equation 5.19 by  $\mathbf{T}^{-1}$  and place the unit matrix between  $\mathbf{R}$  and  $\mathbf{C}$  to give,

$$\mathbf{T}^{-1} \frac{d}{dt} \mathbf{C} = \mathbf{T}^{-1} \mathbf{R} \mathbf{T} \mathbf{T}^{-1} \mathbf{C} \quad 5.A.13$$

Now, using the definitions for  $\boldsymbol{\lambda}$  and  $\mathbf{y}$  found in equations 5.A.10 and 5.A.12 respectively,



$$\frac{d}{dt} \mathbf{y}_i = -\lambda_i \mathbf{y}_i, \quad 5.A.14$$

which have solutions of,

$$y_i = y_{i,0} \exp(-\lambda_i t) \quad 5.A.15$$

A new vector,  $\mathbf{C}'$ , is defined as  $\mathbf{MCM}^{-1}$  and is known as the normal coordinate. Which yields,

$$\frac{d}{dt} \mathbf{C}' = -\lambda \mathbf{C}', \quad 5.A.16$$

with specific values of,

$$\frac{d}{dt} \begin{pmatrix} C'_1 \\ C'_2 \\ C'_N \end{pmatrix} = -\lambda \begin{pmatrix} C'_1 \\ C'_2 \\ C'_N \end{pmatrix}. \quad 5.A.17$$

The matrix matrix can be expanded, term for term, as,

$$\begin{aligned} C'_1 &= C'_1 \exp(-\lambda_1 t) \\ C'_2 &= C'_2 \exp(-\lambda_2 t) \\ C'_N &= C'_N \exp(-\lambda_N t) \end{aligned} \quad 5.A.18$$

The process of diagonalizing a matrix is accomplished by setting the determinant of the matrix to zero and solving for  $\lambda$ ,

$$\det(\mathbf{R}) = \det \begin{vmatrix} -(k_{AB} + k_{AA}) - \lambda & k_{AB} \\ k_{AB} & -(k_{AB} + k_{BB}) - \lambda \end{vmatrix} = 0 \quad 5.A.19$$

$$[-(k_{AB} + k_{AA}) - \lambda][-(k_{AB} + k_{BB}) - \lambda] - (k_{AB})^2 = 0 \quad 5.A.20$$

$$\lambda^2 + (k_{AA} + 2k_{AB} + k_{BB})\lambda + k_{AA}k_{AB} + k_{AA}k_{BB} + k_{AB}k_{BB} = 0$$

Which gives (using the quadratic equation to solve for the roots of  $\lambda$ ) the eigenvalue (or only non-trivial) solutions of the problem (in this case, two,  $\lambda_1$  and  $\lambda_2$ ),

$$|_{1 \text{ and } 2} = \frac{-(k_{AA} + 2k_{AB} + k_{BB}) \pm [k_{AA}^2 + 4k_{AB}^2 - 2k_{AA}k_{BB} + k_{BB}^2]^{1/2}}{2}. \quad 5.A.21$$

The coefficients from the vector matrix, or the eigenvectors, can now be determined by substituting each eigenvalue back into the matrix equation  $\mathbf{RT}=\mathbf{0}$ ,

$$\begin{vmatrix} -(k_{AB} + k_{AA}) - |_{1} & k_{AB} \\ k_{AB} & -(k_{AB} + k_{BB}) - |_{1} \end{vmatrix} \begin{vmatrix} C_{a1} \\ C_{b1} \end{vmatrix} = \begin{vmatrix} 0 \\ 0 \end{vmatrix}, \quad 5.A.22$$

and solving the equations for  $C_{a1}$  and  $C_{b1}$  such that  $[A](t)=C_{a1}\exp(-|_{1}t)$  and

$$[B](t)=C_{b1}\exp(-|_{1}t).$$

This approach gives an exact solution to the problem of two species chemical exchange, however, as the number of coupled equations increase, it becomes exceedingly difficult to solve the matrices exactly and methods of approximation are required. These approximation approaches include the Jacobi rotation-transformation matrix method and those available in the LAPACK software for computers.

#### 5.8.4 Calculation of two-spin state populations

This mathematical transformation is the algebraic substitutions of equations 5.8-5.13. Start from the definitions for the time-dependent change in population (equations 5.8-5.11) and for the NMR observables,  $I_{z,A}$  and  $I_{z,B}$  (equations 5.12 and 5.13). The time derivative of equation 5.14 gives,

$$\frac{d}{dt}(KI_{z,A}) = \frac{d}{dt}(N_{bb} + N_{ba} - N_{ab} - N_{aa}), \quad 5.A.23$$

$$K \frac{dI_{z,A}}{dt} = \frac{dN_{bb}}{dt} + \frac{dN_{ba}}{dt} - \frac{dN_{ab}}{dt} - \frac{dN_{aa}}{dt}. \quad 5.A.24$$

For which the definitions of  $dN_{bb}/dt$ , etc., given in equations 5.8-5.11 are substituted,

$$\begin{aligned}
K \frac{dI_{z,A}}{dt} = & -(W_{1A}^{AB} + W_2^{AB} + W_{1B}^{AB})N_{bb} + W_{1A}^{AB}N_{ab} + W_{1B}^{AB}N_{ba} + W_2^{AB}N_{aa} \\
& + W_{1A}^{AB}N_{bb} - (W_{1A}^{AB} + W_0^{AB} + W_{1B}^{AB})N_{ab} + W_0^{AB}N_{ba} + W_{1B}^{AB}N_{aa} \\
& - [W_{1B}^{AB}N_{bb} + W_0^{AB}N_{ab} - (W_{1B}^{AB} + W_0^{AB} + W_{1A}^{AB})N_{ba} + W_{1A}^{AB}N_{aa}] \\
& - [W_2^{AB}N_{bb} + W_{1B}^{AB}N_{ab} + W_{1A}^{AB}N_{ba} - (W_{1B}^{AB} + W_2^{AB} + W_{1A}^{AB})N_{aa}] \quad 5.A.25
\end{aligned}$$

Grouping the  $N_{bb}$ ,  $N_{ab}$ ,  $N_{ba}$  and  $N_{aa}$  terms respectively,

$$\begin{aligned}
K \frac{dI_{z,A}}{dt} = & (-W_{1A}^{AB} - W_2^{AB} - W_{1B}^{AB} + W_{1A}^{AB} - W_{1B}^{AB} - W_2^{AB})N_{bb} \\
& + (W_{1A}^{AB} - W_{1A}^{AB} - W_0^{AB} - W_{1B}^{AB} - W_0^{AB} - W_{1B}^{AB})N_{ab} \\
& + (W_{1B}^{AB} + W_0^{AB} + W_{1B}^{AB} + W_0^{AB} + W_{1A}^{AB} - W_{1A}^{AB})N_{ba} \\
& + (W_2^{AB} + W_{1B}^{AB} - W_{1A}^{AB} + W_{1B}^{AB} + W_2^{AB} + W_{1A}^{AB})N_{aa} . \quad 5.A.26
\end{aligned}$$

$$\begin{aligned}
K \frac{dI_{z,A}}{dt} = & (-2W_2^{AB} - 2W_{1B}^{AB})N_{bb} + (-2W_0^{AB} - 2W_{1B}^{AB})N_{ab} \\
& + (2W_{1B}^{AB} + 2W_0^{AB})N_{ba} + (2W_2^{AB} + 2W_{1B}^{AB})N_{aa} . \quad 5.A.27
\end{aligned}$$

Factoring out the common terms,

$$K \frac{dI_{z,A}}{dt} = 2(W_2^{AB} + W_{1B}^{AB})(N_{aa} - N_{bb}) + 2(W_0^{AB} + W_{1B}^{AB})(N_{ba} - N_{ab}) . \quad 5.A.28$$

The sum of equations 5.10 and 5.11 gives,

$$2(N_{bb} - N_{aa}) = I_{z,A} + I_{z,B} , \quad 5.A.29$$

whilst the difference of the two gives,

$$2(N_{ba} - N_{ab}) = I_{z,A} - I_{z,B} . \quad 5.A.30$$

Substitution of equations 5.36 and 5.37 into 5.35 yields,

$$K \frac{dI_{z,A}}{dt} = -(W_2^{AB} + W_{1B}^{AB})(I_{z,A} + I_{z,B}) - (W_0^{AB} + W_{1B}^{AB})(I_{z,A} - I_{z,B}), \quad 5.A.31$$

$$K \frac{dI_{z,A}}{dt} = -(W_2^{AB} + 2W_{1B}^{AB} + W_0^{AB})I_{z,A} + (W_0^{AB} - W_2^{AB})I_{z,B}. \quad 5.A.32$$

The same treatment will yield the following for  $dI_{z,B}/dt$ ,

$$K \frac{dI_{z,B}}{dt} = -(W_2^{AB} + 2W_{1A}^{AB} + W_0^{AB})I_{z,B} + (W_0^{AB} - W_2^{AB})I_{z,A}. \quad 5.A.33$$