

*Annual reports on*  
**NMR Spectroscopy**

**Volume 59**



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# **NMR SPECTROSCOPY**

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**NMR SPECTROSCOPY**

Edited by

**G. A. WEBB**

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# Preface

Volume 59 of Annual Reports on NMR contains current accounts of some of the many active and exciting areas of scientific research, which have a crucial dependence on NMR measurements. I am very happy to take the opportunity of introducing the following contributions.

The volume opens with a report by L.D. Field on Multiple Quantum NMR of Partially Aligned Molecules; following this is an account on Solid-State NMR Studies of Molecular Motion by M.J. Duler; C. Odin reviews NMR Studies of Phase Transitions; Application of Multi-way Analysis to 2D NMR Data is covered by H.T. Pedersen, M. Dyrby, S.B. Engelsen and R. Bro; the final contribution is on High Resolution Protein Structure Determination by NMR and it is provided by H. Takashima. My sincere thanks go to all of these reporters and to the production staff at Elsevier for their assistance in the creation of this volume.

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February 2006

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# Multiple Quantum NMR of Partially Aligned Molecules

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*The NMR spectra of solutes partially aligned in liquid crystalline solution are significantly more complex than the spectra obtained in isotropic solution because dipolar couplings and other spectral parameters are not averaged to zero by rapid isotropic tumbling. Whereas conventional single quantum NMR spectroscopy involves the excitation and detection of*

*individual spins embedded in a spin system, multiple quantum NMR (MQNMR) involves the excitation and detection of coherence in groups of coupled spins. MQNMR spectra contain fewer transitions than single quantum spectra and, in principle, MQNMR spectra are significantly easier to analyse than single quantum spectra. This chapter describes the methods available to excite multiple quantum coherence, both selectively and non-selectively and methods available to detect, extract and analyse multiple quantum spectra. The chapter also describes the use of selective and random deuteration, combined with multiple quantum spectroscopy, to analyse the complex spectra of larger spin systems as well as applications of multiple quantum spectra in: (i) obtaining dipolar coupling constants (and hence accessing information about the shape of the spin system); (ii) studying molecular diffusion; (iii) accessing relaxation parameters which then provide information about molecular motion and mobility; and (iv) counting spins in domains and clusters. The chapter concludes with a comprehensive review of molecules, aligned in liquid crystalline solution, where MQNMR spectra have been obtained and analysed to provide information about their spin systems.*

## 1. INTRODUCTION

Since the first reports of the use of liquid crystalline materials as solvents for NMR spectroscopy, high-resolution NMR of partially aligned molecules has become an important area of NMR spectroscopy.<sup>1</sup> Liquid crystals are mesophases – their properties are intermediate between the liquid and solid phase. In the liquid crystalline phase, there is still molecular mobility (as in a liquid), but there is a degree of molecular alignment so the molecules are not oriented randomly with respect to each other and tend to align preferentially in some direction making the “liquid” anisotropic. Most compounds that form a liquid crystalline phase can act as solvents and can take up solutes without destroying the structure of the mesophase. The freedom of solute molecules to move and rotate in partially aligned solvents is restricted by the orientation of the mesophase, so the solute molecules themselves are partially oriented by the medium.

NMR spectroscopy in isotropic solution is characterised by the fact that free and random molecular reorientation averages many parameters that have a directional dependence, e.g. dipolar-coupling constants, chemical shifts, etc. Some parameters, such as dipolar-coupling constants, average to zero in isotropic solution; so once the ability of a molecule to rotate and move freely and isotropically is removed, its NMR spectra immediately become more complex.

## 2. NMR OF PARTIALLY ALIGNED MOLECULES

The NMR spectra of solutes oriented in mesophases are significantly more complex than the spectra obtained in isotropic solution because: (i) the non-zero averaging of dipolar couplings results in many more coupling constants (splittings) being expressed in the spectrum – there is a non-zero dipolar coupling constant for every pair of interacting nuclei and (ii) the magnitude of the dipole coupling constants ( $D_{ij}$ ) between a pair of interacting nuclei is generally large (up to a few kilohertz) compared to the magnitude of indirect coupling constant (typically a few hertz).

One of the main uses of NMR of partially aligned molecules has been to gain access to the dipolar coupling constants ( $D_{ij}$ ), because these can be related directly to the internuclear distances ( $r_{ij}$ ) between each pair of interacting nuclei  $i$  and  $j$ . The  $D_{ij}$  values are obtained by spectral analysis, most usually by computer simulation of experimental spectra. The  $D_{ij}$  are inversely proportional to the cube of the internuclear distances ( $r_{ij}$ ) (Eq. (1)). Providing sufficient independent  $D_{ij}$  values can be measured and the relative positions of the nuclei in the spin system can be determined then the shape of the molecule as well as its average orientation in the magnetic field can be deduced.

$$D_{ij} = -\left(\frac{h\gamma_H^2}{4\pi^2}\right) \frac{S_{ij}}{r_{ij}^3} \quad (1)$$

where  $\gamma_H$  is the proton magnetogyric ratio,  $r_{ij}$  the internuclear distance between protons  $i$  and  $j$  and  $S_{ij}$  the order of the internuclear vector between  $i$  and  $j$ .<sup>2</sup>

The order parameter  $S_{ij}$  derives from the Saupe order matrix  $S$ , which is a symmetric traceless  $3 \times 3$  tensor where the diagonal order parameters ( $S_{xx}$ ,  $S_{yy}$  and  $S_{zz}$ ) can have values between  $-0.5$  and  $1.0$ , and the off-diagonal order parameters ( $S_{xy}$ ,  $S_{yz}$  and  $S_{xz}$ ) vary between  $-0.75$  and  $+0.75$ .<sup>3</sup> The higher the symmetry of the molecule, the lower the number of independent non-zero-order parameters that are required to completely describe the orientation of the spin system.  $S_{ij}$  is  $0$  when the the  $i,j$  axis is completely non-aligned (as in an isotropic solution).  $S_{ij}$  is  $-0.5$ , where the  $i,j$  axis is orthogonal to the magnetic field and  $S_{ij}$  is  $1.0$  where the  $i,j$  axis is aligned perfectly with the magnetic field. Room temperature  $S_{ij}$  values between  $0.6$  and  $0.8$  are typical for many liquid crystalline materials.

The induced alignment of solutes oriented in liquid crystalline solution is very sensitive to small variations in temperature.  $S_{ij}$  values are temperature sensitive and since  $D_{ij}$  is proportional to  $S_{ij}$ , care must be exercised if spectra

are to be compared directly since these must be recorded sequentially under identical conditions.

### 3. MULTIPLE QUANTUM NMR AND SPECTRAL SIMPLIFICATION

For a system of coupled  $I = 1/2$  nuclei, the transition frequencies in the multiple quantum spectra are determined by the dipolar coupling constants, the scalar coupling constants and the chemical shifts of the nuclei. In theory, the spectra of order  $N-1$  and  $N-2$  contain sufficient transitions to measure all of the dipolar coupling constants and chemical shifts in an  $N$ -spin system. For additional accuracy and confidence, the  $N-3$  quantum spectrum can also be analysed to provide redundancy and more reliable estimates of the  $D_{ij}$ .

The number of transitions in an NMR spectrum increases dramatically as the number of interacting nuclei in the spin system increases. The number of transitions appearing in a 1-quantum spectrum of a spin system (without any simplifying symmetry) partially oriented in a nematic phase is expressed by Eq. (2), with some examples shown in Table 1.

$$\text{Number of transitions} = \frac{(2N)!}{[(N-1)!(N+1)!]} \quad (2)$$

where  $N$  is the number of interacting spins in the spin system.

If there is some element of symmetry in the spin system, the number of transitions is reduced. For a 6-spin system with no simplifying symmetry, aligned in an anisotropic solution, there would be 792 transitions in an  $^1\text{H}$  1-quantum spectrum. However in the spectrum of benzene,  $\text{C}_6\text{H}_6$  (a planar hexagonal 6-spin proton spin system) aligned in anisotropic solution there are only 72 transitions in the 1-quantum proton spectrum.

Severe overlap between transitions in spin systems containing more than about 7 or 8 spins (without simplifying symmetry) makes analysis of the 1-quantum spectra a virtually intractable problem. In larger spin systems, it becomes impossible to resolve or assign individual transitions for an iterative computer analysis.

**Table 1.** The number of transitions in a 1-quantum spectrum of a partially oriented spin system as a function of the number of spin  $1/2$  nuclei

Number of spins	1	2	3	4	5	6	7	8	9	10
Transitions	1	4	15	56	210	792	3003	$1.1 \times 10^4$	$4.4 \times 10^4$	$1.7 \times 10^5$