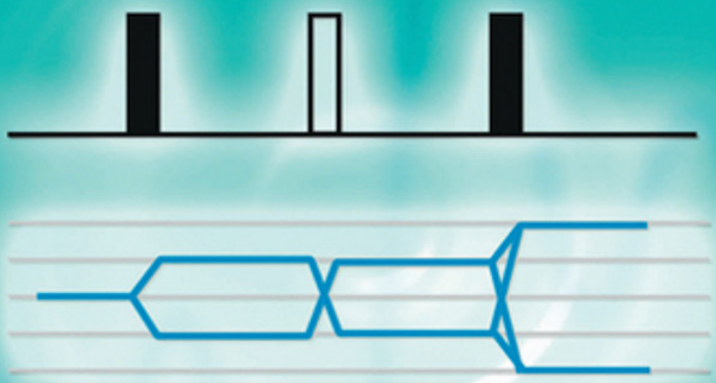
Understanding NMR Spectroscopy

SECOND EDITION



James Keeler

WILEY

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– Second Edition

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WILEY
A John Wiley & Sons, Ltd Publication

This edition first published 2010

Registered office
John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, United Kingdom

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Reprinted in January 2011
October 2012

Library of Congress Cataloging-in-Publication Data

Keeler, James.
Understanding NMR spectroscopy / James Keeler. – 2nd ed.
p. cm.
Includes bibliographical references and index.
ISBN 978-0-470-74609-7 (cloth) – ISBN 978-0-470-74608-0 (pbk.)
1. Nuclear magnetic resonance spectroscopy–Textbooks. I. Title.
QD96.N8K44 2010
543'.66–dc22
2009054393

A catalogue record for this book is available from the British Library.

ISBN 978-0-470-74609-7 (cloth)
ISBN 978-0-470-74608-0 (pbk.)

Set in 10pt Sabon by the author using LaTeX

Printed and bound in Singapore by Markono Print Media Pte Ltd

Preface

I am very pleased to have the opportunity to produce a second edition of *Understanding NMR Spectroscopy*, not least as I have been encouraged by the many kind comments that I have received by users of the first edition. For all its undoubted flaws, the book has clearly been found to be useful in helping people to get to grips with the theory of NMR.

I have resisted the temptation to add a great deal of additional material or to make the discussion more technical. However, I have included a new chapter which covers two topics which, in retrospect, seemed to be serious omissions from the first edition. The first topic is how product operators can be extended to describe experiments in AX_2 and AX_3 spin systems, thus making it possible to discuss the important APT, INEPT and DEPT experiments often used in ^{13}C spectroscopy.

The second topic is spin system analysis i.e. how shifts and couplings can be extracted from strongly coupled (second-order) spectra. In the early days of NMR this kind of analysis was all but essential since the low field strengths then available meant that spectra were often strongly coupled. The current use of much higher fields means that strong-coupling effects are less common, but they have not gone away completely. It therefore remains important to be aware of such effects and their consequences for the appearance of spectra. In a related topic, I also discuss how the presence of chemically equivalent spins leads to spectral features which are somewhat unusual and possibly misleading. In contrast to strong-coupling effects, these features are independent of the field strength and so are not mitigated by the move to higher fields.

The chapter on relaxation has been reorganised, and a discussion of chemical exchange effects has been introduced in order to help with the explanation of transverse relaxation. Finally, I have added a short section on double-quantum spectroscopy to Chapter 10.

The use of two-colour printing will, I hope, both improve the clarity of many of the diagrams and improve the appearance of the printed pages.

I am very much indebted to Dr Andrew Pell (now at the École Normale Supérieure de Lyon) who found time between completing his PhD and starting a postdoctoral position to help me in the preparation of this edition. Andy worked on adding colour to the figures, produced some additional simulations for Chapter 9, recorded all of the experimental spectra and commented on the new sections. I am also grateful to Dr Daniel Nietlispach (Department of Biochemistry, University of Cambridge) for once again providing very useful and perceptive comments on the new material.

Cambridge, January 2010

Preface to the first edition

I owe a huge debt of gratitude to Dr Daniel Nietlispach and Dr Katherine Stott (both from the Department of Biochemistry, University of Cambridge) who have read, corrected and commented on drafts of the entire book. Their careful and painstaking work has contributed a great deal to the final form of the text and has, in my view, improved it enormously. I am also grateful to them for their constant enthusiasm, which sustained and encouraged me throughout the project. I could not have wanted for two more constructive and helpful readers.

Special thanks are also due to Professor Nikolaus Loening (Lewis and Clark College, Portland, Oregon) who, at short notice and with great skill, provided all of the experimental spectra in the book. His good humoured response to my pernickety requirements is much appreciated. Andrew Pell (Selwyn College, Cambridge) also deserves special mention and thanks for his skilled assistance in producing the solutions manual for the exercises.

I would like to acknowledge the support and advice from my collaborator and colleague Dr Peter Wothers (Department of Chemistry, University of Cambridge): he remains both my sternest critic and greatest source of encouragement. I am also grateful to Professor Jeremy Sanders (Department of Chemistry, University of Cambridge) for his much valued support and advice.

My appreciation and understanding of NMR, such that it is, has been very much influenced by those I have been fortunate enough to work alongside, both as research students and collaborators; I thank them for their insights. I would also like to thank Professor Malcolm Levitt (Department of Chemistry, University of Southampton), Professor Art Palmer (Columbia University, New York) and Dr David Neuhaus (MRC LMB, Cambridge) for tirelessly answering my many questions.

This book grew out of a series of lecture notes which, over a number of years, I prepared for various summer schools and graduate courses. On the initiative of Dr Rainer Haessner (Technische Universität, Munich), the notes were made available on the web, and since then I have received a great deal of positive feedback about how useful people have found them. It was this, above all, which encouraged me to expand the notes into a book.

The book has been typeset by the author using LaTeX, in the particular implementation distributed as MiKTeX (`www.miktex.org`). I wish to express my thanks to the many people who develop and maintain the LaTeX system. All of the diagrams have been prepared using *Adobe Illustrator* (Adobe Systems Inc., San Jose, California), sometimes in combination with *Mathematica* (Wolfram Research Inc., Champaign, Illinois).

Finally, I am delighted to be able to dedicate this book to Professor Ray Freeman. I was lucky enough to have started my NMR career in Ray's group, and what I learnt there, both from Ray and my fellow students, has stood me in good stead ever since. My appreciation for Ray has continued as strong as ever since those early days, and I am pleased to have this opportunity to acknowledge the debt I owe him.

Cambridge, July 2005

Dedicated to

Professor Ray Freeman FRS

Contents

1

What this book is about and who should read it

This book is aimed at people who are familiar with the use of routine NMR for structure determination and who wish to deepen their understanding of just exactly how NMR experiments 'work'. It is one of the great virtues of NMR spectroscopy that one can use it, and indeed use it to quite a high level, without having the least idea of how the technique works. For example, we can be taught how to interpret two-dimensional spectra, such as COSY, in a few minutes, and similarly it does not take long to get to grips with the interpretation of NOE (nuclear Overhauser effect) difference spectra. In addition, modern spectrometers can now run quite sophisticated NMR experiments with the minimum of intervention, further obviating the need for any particular understanding on the part of the operator.

You should reach for this book when you feel that the time has come to understand just exactly what is going on. It may be that this is simply out of curiosity, or it may be that for your work you need to employ a less common technique, modify an existing experiment to a new situation or need to understand more fully the limitations of a particular technique. A study of this book should give you the confidence to deal with such problems and also extend your range as an NMR spectroscopist.

One of the difficulties with NMR is that the language and theoretical techniques needed to describe it are rather different from those used for just about all other kinds of spectroscopy. This creates a barrier to understanding, but it is the aim of this book to show you that the barrier is not too difficult to overcome. Indeed, in contrast to other kinds of spectroscopy, we shall see that in NMR it is possible, quite literally on the back of an envelope, to make exact predictions of the outcome of quite sophisticated experiments. Further, once you have got to grips with the theory, you should find it possible not only to analyse existing experiments but also dream up new possibilities.

There is no getting away from the fact that we need quantum mechanics in order to understand NMR spectroscopy. Developing the necessary quantum mechanical ideas from scratch would make this book rather a

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hard read. Luckily, it is not really necessary to introduce such a high level of formality provided we are prepared to accept, on trust, certain quantum mechanical ideas and are prepared to use these techniques more or less as a recipe. A good analogy for this approach is to remember that it is perfectly possible to learn to add up and multiply without appreciating the finer points of number theory.

One of the nice features we will discover is that, despite being rigorous, the quantum mechanical approach still retains many features of the simpler *vector model* often used to describe simple NMR experiments. Once you get used to using the quantum mechanical approach, you will find that it does work in quite an intuitive way and gives you a way of 'thinking' about experiments without always having to make detailed calculations.

Quantum mechanics is, of course, expressed in mathematical language, but the mathematics we will need is not very sophisticated. The only topic which we will need which is perhaps not so familiar is that of complex numbers and the complex exponential. These will be introduced as we go along, and the ideas are also summarized in an appendix.

1.1 How this book is organized

Optional sections are marked like this:

Optional section ⇒

The ideas we need to describe NMR experiments are built up chapter by chapter, and so the text will make most sense if it read from the beginning. Certain sections are not crucial to the development of the argument and so can be safely omitted at a first reading; these sections are clearly marked as such in the margin.

Chapter 6, which explains how quantum mechanics is formulated in a way useful for NMR, is also entirely optional. It provides the background to the product operator formalism, which is described in Chapter 7, but this latter chapter is written in such a way that it does not rely on anything from Chapter 6. At some point, I hope that you will want to find out about what is written in Chapter 6, but if you decide not to tackle it, rest assured that you will still be able to follow what goes on in the rest of the book.

The main sequence of the book really ends with Chapter 8, which is devoted to two-dimensional NMR. You should dip into Chapters 9–13 as and when you feel the need to further your understanding of the topics they cover. This applies particularly to Chapter 10 which discusses a selection of more advanced ideas in two-dimensional NMR, and Chapter 11 which is concerned with the rather 'technical' topic of how to write phase cycles and how field gradient pulses are used.

Quite deliberately, this book starts off at a gentle pace, working through some more-or-less familiar ideas to start with, and then elaborating these as we follow our theme. This means that you might find parts of the discussion rather pedestrian at times, but the aim is always to be clear about what is going on, and not to jump over steps in calculations or arguments. The same philosophy is followed when it comes to the more difficult and/or less familiar topics which are introduced in the later chapters. If you are already familiar with the vector model of pulsed NMR, and are happy with thinking about multiplets in terms of energy levels, then you might wish to jump in at Chapter 6 or Chapter 7.

Each chapter ends with some exercises which are designed to help your understanding of the ideas presented in that chapter. Tackling the exercises will undoubtedly help you to come to grips with the underlying ideas.

1.2 Scope and limitations

In this book we are going to discuss the high-resolution NMR of liquid samples and we will concentrate, almost exclusively, on spin-half nuclei (mainly ^{1}H and ^{13}C). The NMR of solids is an important and fast-developing field, but one which lies outside the scope of this book.

The experiments we will choose to describe are likely to be encountered in the routine NMR of small to medium-sized molecules. Many of the experiments are also applicable to the study of large biomolecules, such as proteins and nucleic acids. The special multi-dimensional experiments which have been devised for the study of proteins will not be described here, but we note that such experiments are built up using the repertoire of pulsed techniques which we are going to look at in detail.

The existence of the chemical shift and scalar coupling is, of course, crucial to the utility of NMR spectroscopy. However, we will simply treat the values of shifts and coupling constants as experimentally derived parameters; we will have nothing to say about their calculation or interpretation – topics which are very well covered elsewhere.

1.3 Context and further reading

This is not a 'how to' book: you will find no advice here on how to select and run a particular experiment, nor on how to interpret the result in terms of a chemical structure. What this book is concerned with is how the experiments work. However, it is not a book of NMR theory for its own sake: rather, the ideas presented, and the theories introduced, have been chosen carefully as those most useful for understanding the kinds of NMR experiments which are actually used.

There are many books which describe how modern NMR spectroscopy is applied in structural studies, and you may wish to consult these alongside this text in order to see how a particular experiment is used in practice. Two useful texts are: J. K. M. Sanders and B. K. Hunter, *Modern NMR Spectroscopy* (2nd edition, OUP, 1993), and T. D. W. Claridge, *High-Resolution NMR Techniques in Organic Chemistry* (Elsevier Science, 1999).

There are also a number of books which are at roughly the same level as this text and which you may wish to consult for further information or an alternative view. Amongst these, R. Freeman, *Spin Choreography* (Spektrum, 1997) and F. J. M. van de Ven, *Multidimensional NMR in Liquids* (VCH, 1995) are particularly useful. If you wish to go further and deeper into the theory of NMR, M. H. Levitt, *Spin Dynamics* (2nd edition, John Wiley & Sons, Ltd, 2008) is an excellent place to start.

The application of NMR to structural studies of biomolecules is a vast area which we will only touch on from time to time. A detailed account of this important area, covering both theoretical and practical matters, can be

found in J. Cavanagh, W. J. Fairbrother, A. G. Palmer III, M. Rance and N. J. Skelton, *Protein NMR Spectroscopy* (2nd edition, Academic Press, 2007).

At the end of each chapter you will also find suggestions for further reading. Many of these are directions to particular chapters of the books we have already mentioned.

1.4 On-line resources

A solutions manual for the exercises at the end of each chapter is available on-line via the *spectroscopyNOW* website:

`http://www.spectroscopynow.com/nmr`
follow the 'Education' link from this page

A list of corrections and amendments will also be available on this site, as well as other additional material. It will also be possible to download all of the figures (in 'jpeg' format) from this book.

1.5 Abbreviations and acronyms

ADC	analogue to digital converter
APT	attached proton test
COSY	correlation spectroscopy
CTP	coherence transfer pathway
DEPT	distortionless enhancement by polarization transfer
DQF COSY	double-quantum filtered COSY
FID	free induction decay
HETCOR	heteronuclear correlation
HMBC	heteronuclear multiple-bond correlation
HMQC	heteronuclear multiple-quantum correlation
HSQC	heteronuclear single-quantum correlation
INEPT	insensitive nuclei enhanced by polarization transfer
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy
RF	radiofrequency
rx	receiver
ROESY	rotating frame Overhauser effect spectroscopy
SHR	States–Haberkorn–Ruben
SNR	signal-to-noise ratio
TOCSY	total correlation spectroscopy
TPPI	time proportional phase incrementation
TROSY	transverse relaxation optimized spectroscopy
tx	transmitter

2

Setting the scene

You will probably find that much of this chapter covers topics you are familiar with or have at least come across before. The point of the chapter is, as the title says, to set the scene for what follows by reminding you of the basic language of NMR, how we describe NMR spectra and how some important quantities are defined. There is also a section on oscillations and rotations, explaining how these are described and represented mathematically. These are key ideas which we will use extensively in the rest of the book.

2.1 NMR frequencies and chemical shifts

Like all forms of spectroscopy, an NMR spectrum is a plot of the intensity of absorption (or emission) on the vertical axis against frequency on the horizontal axis. NMR spectra are unusual in that they appear at rather low frequencies, typically in the range 10 to 800 MHz, corresponding to wavelengths from 30 m down to 40 cm. This is the radiofrequency (RF) part of the electromagnetic spectrum which is used for radio and TV broadcasts, mobile phones etc.

It is usual in spectroscopy to quote the frequency or wavelength of the observed absorptions; in contrast, in NMR we give the positions of the lines in 'ppm' using the chemical shift scale. The reason for using a shift scale is that it is found that the frequencies of NMR lines are directly proportional to the magnetic field strength. So doubling the field strength doubles the frequency, as shown in Fig. 2.1 on the following page. This field dependence makes it difficult to compare absorption frequencies between spectrometers which operate at different field strengths, and it is to get round this problem that the chemical shift scale is introduced. On this scale, the positions of the peaks are *independent of the field strength*. In this section we will explore the way in which the scale is defined, and also how to convert back and forth between frequencies and ppm – something we will need to do quite often.

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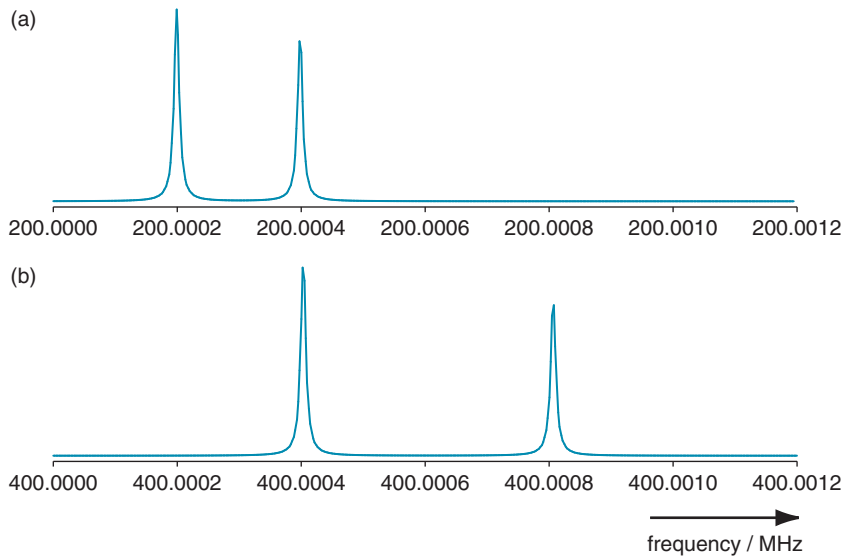

Fig. 2.1 Schematic NMR spectra consisting of two lines. In (a) the magnetic field is such that the two lines appear at 200.0002 and 200.0004 MHz, respectively; their separation is 200 Hz. The spectrum shown in (b) is that expected when the applied magnetic field is doubled. The frequency of each peak is doubled and, as a consequence, the separation between the two peaks has now also doubled to 400 Hz.

Before we look at the definition of the chemical shift it is worthwhile pointing out that the frequency at which an NMR signal appears also depends on the nuclear isotope (e.g. ^{1}H, ^{13}C, ^{15}N etc.) being studied. Also, for a given field, the NMR absorptions for a particular isotope cover rather a small range of frequencies relative to the absolute frequency of the absorption. In an experiment it is therefore usual only to measure the NMR spectrum from one particular isotope at a time.

2.1.1 Chemical shift scales

The chemical shift scale is set up first by agreeing a simple *reference compound*, a line from which is taken to define zero on the chemical shift scale. For ^{1}H and ^{13}C this reference compound is TMS. The choice of reference compound is arbitrary, but subject to careful international agreement so as to make sure everyone is using the same compound and hence the same origin on their shift scales.

The position of a peak in the spectrum is specified by measuring its frequency separation from the reference peak, and then dividing this difference by the frequency of the reference peak. As we are taking the *ratio* of two frequencies, the field dependence cancels out. The ratio thus specifies the position of a line in a way which is *independent* of the applied field, which is what we require.

Expressed mathematically, the chemical shift δ is given by

$$\delta(\text{ppm}) = 10^6 \times \frac{\upsilon - \upsilon_{\text{ref}}}{\upsilon_{\text{ref}}}, \tag{2.1}$$

where υ is the frequency of the NMR line in question and υ_{ref} is the frequency of the line from the agreed reference compound. Clearly, the line from the reference compound will appear at $\delta = 0$.

It is usual to quote chemical shifts in 'parts per million' (ppm) in order to make the numbers more convenient, and this is why in the definition of

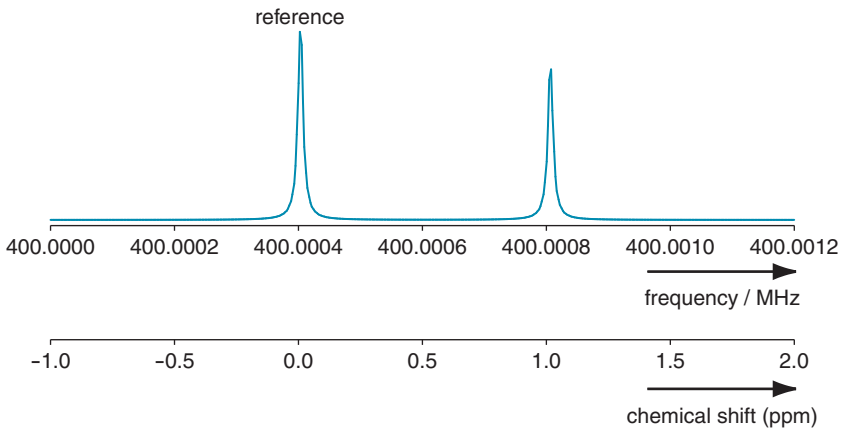

Fig. 2.2 A schematic NMR spectrum consisting of two lines is shown with both a frequency scale and a chemical shift scale, in ppm. The left-hand peak has been chosen as the reference, and so appears at 0 ppm. Note that it is usual for the ppm scale to be plotted increasing to the left, and not to the right as shown here.

δ the ratio is multiplied by 10^6. Figure 2.2 shows the schematic spectrum of Fig. 2.1 (b) on the facing page with both a frequency scale and a chemical shift scale in ppm; the left-hand peak has been chosen as the reference and so appears at 0 ppm. The right-hand peak appears at 1 ppm and it is easy to see that if a ppm scale were to be added to the spectrum of Fig. 2.1 (a), the right-hand peak would still be at 1 ppm.

The frequency scale in Fig. 2.2 increases to the right, which is the natural way to plot it, but as a consequence the ppm scale also increases to the right. This looks unusual since in NMR it is the universal practice to plot spectra with the ppm scale increasing to the left.

2.1.2 Conversion from shifts to frequencies

Sometimes we need to know the frequency separation of two peaks, in Hz. The software used to process and display NMR spectra usually has an option to toggle the scale between Hz and ppm, so measuring the peak separation is quite easy. However, sometimes we will need to make the conversion from ppm to Hz manually.

The definition of the chemical shift, Eq. 2.1 on the preceding page, can be rearranged to

$$10^{-6} \times \delta(\text{ppm}) \times \upsilon_{\text{ref}} = \upsilon - \upsilon_{\text{ref}}.$$

From this it is clear that a peak at δ ppm is separated from the reference peak by $10^{-6} \times \delta \times \upsilon_{\text{ref}}$. It follows that two peaks at shifts δ_1 and δ_2 are separated by a frequency of $10^{-6} \times (\delta_1 - \delta_2) \times \upsilon_{\text{ref}}$.

It is usual to express the frequency of the reference peak in MHz ($= 10^6$ Hz). When this is done the factor of 10^{-6} cancels and the frequency separation in Hz is simply

$$\text{frequency separation in Hz} = (\delta_1 - \delta_2) \times \upsilon_{\text{ref}}(\text{in MHz}). \qquad (2.2)$$

So, for example, if the reference frequency is 500 MHz, then two peaks at 2.3 and 1.8 ppm are separated by $(2.3 - 1.8) \times 500 = 250$ Hz.

Put even more simply, if the frequency of the reference peak is 500 MHz then 1 ppm corresponds to 500 Hz; if the reference peak is at 800 MHz, 1 ppm corresponds to 800 Hz. The conversion from ppm to Hz is therefore rather simple.

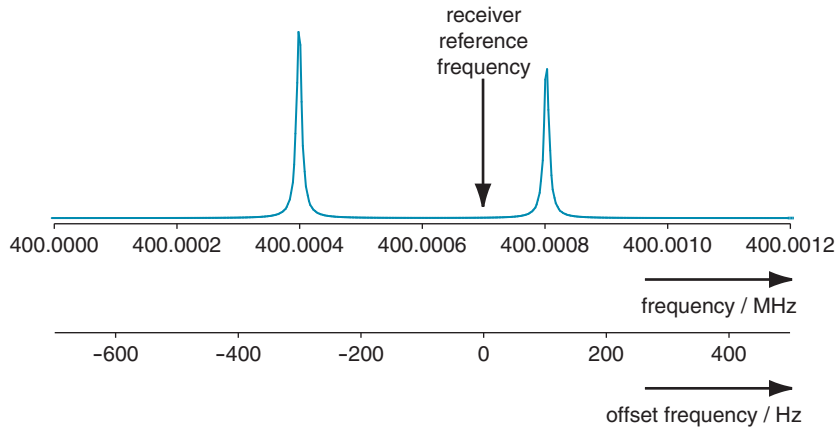

Fig. 2.3 Our two-line spectrum is shown with both a frequency scale and an offset frequency scale. The receiver reference frequency has been chosen as 400.0007 MHz, as indicated by the arrow. As a result, the right-hand peak has an offset frequency of +100 Hz and the left-hand peak has an offset of −300 Hz. It is important to realize that the choice of the receiver reference frequency is entirely arbitrary and is not related to the frequency of the resonance from the reference compound.

2.1.3 The receiver reference frequency and the offset frequency

The RF circuits in virtually all NMR spectrometers are arranged in such a way that the frequencies of the peaks in the spectrum are not measured absolutely but are determined relative to the *receiver reference frequency*. This reference frequency can be set quite arbitrarily by the operator of the spectrometer; typically, it is placed somewhere in the middle of the peaks of interest.

It is important to realize that this receiver reference frequency has got *nothing* to do with the resonance from the reference compound; the receiver reference can be placed anywhere we like. The usual arrangement is that when the full spectrum is displayed, the receiver reference frequency is in the middle of the displayed region, so the frequencies of the peaks can be positive or negative, depending on which side of the reference frequency they fall.

The frequency of a peak relative to the receiver reference frequency is called the *offset frequency* (or, for short, the offset) of the peak. This offset frequency υ_{offset} is given by

$$\upsilon_{\text{offset}} = \upsilon - \upsilon_{\text{rx}},$$

where υ is the frequency of the peak of interest and υ_{rx} is the receiver reference frequency ('rx' is the traditional abbreviation for receiver). We see from this definition that the offset frequency can be positive or negative, as exemplified in Fig. 2.3.

When calculating the chemical shift using Eq. 2.1 on page 6 it is usually sufficiently accurate to divide, not by the frequency of the line from the reference compound (υ_{ref}), but by the receiver reference frequency, υ_{rx}:

$$\delta(\text{ppm}) = 10^6 \times \frac{\upsilon - \upsilon_{\text{ref}}}{\upsilon_{\text{rx}}}. \qquad (2.3)$$

The reason for this is that NMR resonances cover such a small range relative to their absolute frequencies that, provided the receiver reference frequency is somewhere in the spectrum, the difference between υ_{rx} and υ_{ref} is completely negligible. Similarly, when converting from shifts to frequencies (Eq. 2.2 on page 7), it is generally sufficiently accurate to use the receiver reference frequency in place of υ_{ref}.

2.2 Linewidths, lineshapes and integrals

We cannot extract much useful information from a spectrum unless the peaks or multiplets are clearly separated from one another – an observation which is as true for the most complex multi-dimensional spectrum as it is for the simplest conventional ^{1}H spectrum. Whether or not two peaks are resolved will depend on the separation between them relative to their *linewidth* and, to an extent, their *lineshape*. These two properties are thus of paramount importance in NMR.

It is not uncommon for lines in NMR spectra of small to medium-sized molecules to have widths of a few Hz. Thus, compared with their absolute frequencies, NMR lines are very narrow indeed. However, what we should really be comparing with the linewidth is the *spread* of frequencies over which NMR lines are found for a given nucleus. This spread is generally rather small, so relatively speaking the lines are not as narrow as we might suppose. Indeed, NMR experiments on complex molecules are primarily limited by the linewidths of the resonances involved.

The basic lineshape seen in simple NMR experiments is the *absorption mode* lineshape, illustrated in Fig. 2.4. The lineshape is entirely positive and is symmetrical about the maximum. The breadth of the line is specified by quoting its width at half of the peak height, as is also shown in the figure.

When we first learn about proton NMR we are told that the area under a peak or multiplet, i.e. the integral, is proportional to the number of protons giving rise to that feature. It therefore follows that if two peaks are both associated with single protons, they must have the same integral and hence if one of the lines is broader it will have reduced peak height; this is illustrated in Fig. 2.5. This reduction in peak height also means that the signal-to-noise ratio of the spectrum is reduced.

As two lines get closer and closer together, they begin to overlap and eventually will merge completely so that it is no longer possible to see the two separate lines; the process is illustrated in Fig. 2.6 on the next page. The diagram shows that, by the time the separation falls somewhat below the linewidth, the merging of the two lines is complete so that they are no longer distinct. The exact point at which the lines merge depends on the lineshape.

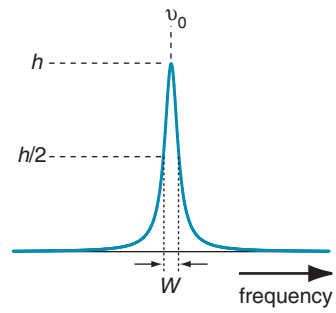

Fig. 2.4 An absorption mode lineshape. The peak is centred at υ_0 and is of height h; the width of the peak is specified by giving the width W measured at half the peak height ($h/2$).

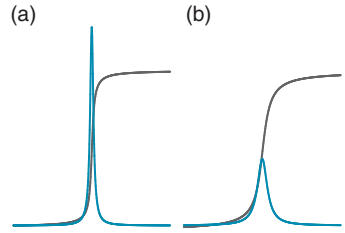

Fig. 2.5 Illustration of how the area or integral of a peak corresponding to a certain number of protons is fixed. The peak shown in (b) is three times broader than that shown in (a); however, they have the same integral (shown by the grey line). As a result, the peak height of the broader line is reduced, also by a factor of three.

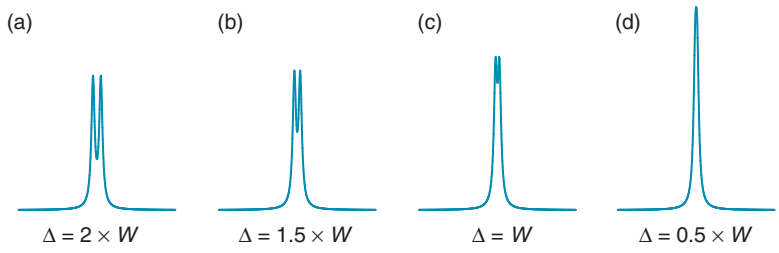

Fig. 2.6 Illustration of how the ability to resolve two lines depends on their separation relative to the linewidth. In (a) the separation Δ is twice the linewidth, W; the two peaks are clearly resolved. In (b) the separation has decreased so that it is equal to 1.5 times the linewidth and as a result the 'dip' between the two lines is less pronounced. Further reduction in the separation makes the dip even smaller, as in (c) where the separation is equal to the linewidth. Finally, in (d) where the separation is half of the linewidth, the two peaks are no longer distinct and a single line is seen.

2.3 Scalar coupling

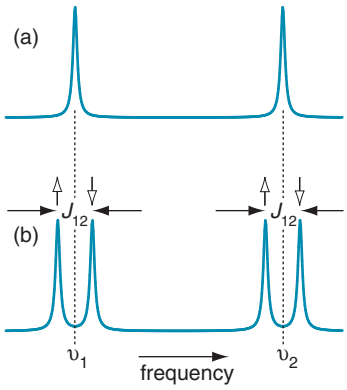

Fig. 2.7 Spectrum (a) shows two lines, at frequencies υ_1 and υ_2, from two different spins. If there is a *scalar coupling* between the spins, each line splits symmetrically into two, giving two *doublets*, as shown in (b). The splitting of the two lines in each doublet is the coupling constant, J_{12}. One way of thinking about the two lines of a doublet is to associate one line with the coupled spin being in the 'up' spin state, and the other line with the coupled spin being in the 'down' spin state; these spin states are indicated by the open-headed arrows.

Scalar or J coupling between nuclei is mediated by chemical bonds and is therefore very useful in establishing which nuclei are close to one another on the bonding framework. The presence of such coupling gives rise to *multiplets* in the spectrum; for example, as shown in Fig. 2.7, if two spin-half nuclei are coupled, the resonance from each spin splits symmetrically about the chemical shift into two lines, called a *doublet*.

Each doublet is split by the same amount, a quantity referred to as the *coupling constant*, J. It is found that the values of coupling constants are *independent* of the field strength; they are always quoted in Hz.

One way of thinking about the two lines of a doublet is to associate them with different *spin states* of the coupled spin. The idea here is that a spin-half nucleus can be in one of two spin states, described as 'up' and 'down' (in Chapter 3 we will have a lot more to say about what up and down actually mean). So, for the doublet centred at the chemical shift of the *first* spin, one line is associated with the *second* spin being in the up spin state, and the other line is associated with the second spin being in the down spin state; Fig. 2.7 (b) illustrates the idea. Similarly, for the doublet centred at the shift of the *second* spin, one line is associated with the *first* spin being up and the other line with the first spin being down.

In terms of frequencies, the line associated with the coupled spin being in the up state is shifted by $\frac{1}{2}J_{12}$ to the left, and the line associated with the coupled spin being in the down state is shifted by $\frac{1}{2}J_{12}$ to the right. So, the two lines of the doublet are separated by J_{12}, and placed symmetrically about the chemical shift.

2.3.1 Tree diagrams

If there are couplings present to further spins, the form of the multiplets can be predicted using 'tree' diagrams, of the type shown in Fig. 2.8 on the facing page. Multiplet (a) is the doublet arising from the first spin due to

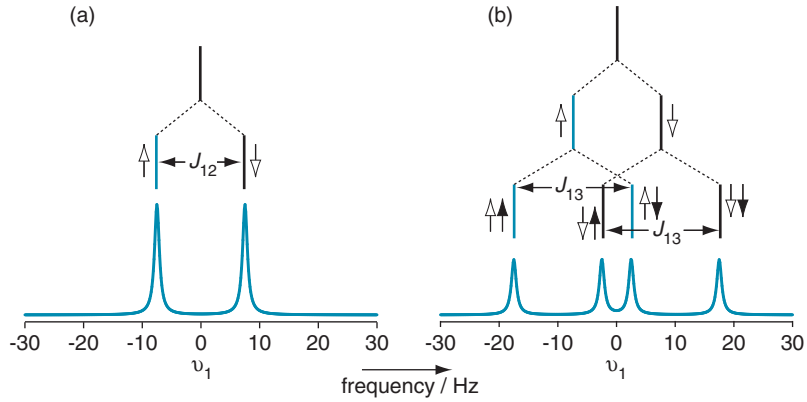

Fig. 2.8 Illustration of how multiplets are built up as a result of scalar coupling. In (a) we see a doublet which arises from the coupling of the first spin to a second spin; the coupling constant is J_{12}. The doublet can be built up using a tree diagram in which the original line at υ_1 is split symmetrically into two; the left-hand line is associated with the second spin being up (indicated by an upward pointing arrow), and the right-hand line is associated with the second spin being 'down' (a downward arrow). If the first spin is also coupled to a third spin, with coupling constant J_{13}, each line of the doublet is split once more, as is shown in (b); the resulting multiplet is called a doublet of doublets. The first branching of the tree diagram represents the coupling to the second spin and is the same as in (a). The second layer represents the coupling to the third spin: again, the line which splits to the left is associated with the third spin being up, and the one which splits to the right is associated with the third spin being down. The spin states of the second spin are shown using arrows with open heads, whereas those of the third spin have filled heads. Each line of the doublet of doublets is thus associated with particular spin states of the two coupled spins. The parameters chosen to draw the diagram were: $\upsilon_1 = 0$, $J_{12} = 15$ Hz and $J_{13} = 20$ Hz.

its coupling to the second, and over the multiplet is shown the tree diagram which can be used to construct it. At the top, we start with a line at υ_1. In the next layer down this line splits symmetrically into two: one shifted by $\frac{1}{2}J_{12}$ to the left, and one by $\frac{1}{2}J_{12}$ to the right, thus producing the doublet. These two branches can be associated with the second spin being in the up and down spin states, respectively.

If a third spin is now introduced which also has a coupling (of size J_{13}) to the first spin, we have to add another layer of branching to the tree diagram; this is shown in Fig. 2.8 (b). As before, we start with a line at υ_1. The first layer of the branching is due to the coupling to the second spin, and is exactly the same as in (a). To construct the second layer, each line from the first is split symmetrically into two but this time with the splitting being J_{13}. As before, the branch which splits to the left is associated with the third spin being up and the branch which splits to the right is associated with the third spin being down. Overall, the result is a four line multiplet, called a *doublet of doublets*.

If we assume that a branching to the left reduces the frequency of the line, and that a branching to the right increases it, we can work out the frequencies of each of the four lines of the doublet of doublets simply

by noting whether they are the result of a branching to the left or right. So, the left-most line of the doublet of doublets shown in (b) must have frequency $(\upsilon_1 - \frac{1}{2}J_{12} - \frac{1}{2}J_{13})$, whereas the next line along has frequency $(\upsilon_1 + \frac{1}{2}J_{12} - \frac{1}{2}J_{13})$ as it derives from a branching to the right due to the coupling to the second spin and a branching to the left due to the coupling to the third spin.

You should convince yourself that the doublet of doublets looks exactly the same if, in the tree diagram, you first split according to the coupling to the third spin and then according to the coupling to the second spin.

The question arises as to how we know that it is the up spin state which is associated with the line which splits to the left. In fact, whether it is the up or down state depends on the *sign* of the coupling constant; here we have chosen both couplings to be positive. In section 3.6 on page 38 we will return to the influence which the sign of the coupling has on the spectrum. However, for the moment we will simply note that the appearance of the multiplet is unaffected by the sign of the coupling.

The final thing to note from Fig. 2.8 on the previous page is that since the doublet and the doublet of doublets are both from one spin, the integral of both must be the same. So, adding the second splitting to form the doublet of doublets reduces the intensity of the lines by a factor of two.

2.3.2 Weak and strong coupling

All we have said so far about the multiplets which arise from scalar coupling is applicable only in the *weak coupling* limit. This limit is when the frequency separation of the two coupled spins is much larger in magnitude than the magnitude of the scalar coupling between the two spins.

For example, suppose that we record a proton spectrum at 500 MHz and that there are two protons whose resonances are separated by 2 ppm and which have a coupling of 5 Hz between them. As explained in section 2.1.2 on page 7, the frequency separation between the two lines is $2 \times 500 = 1000$ Hz. This is two hundred times greater than the coupling constant, so we can be sure that we are in the weak coupling limit. The coupling between different isotopes (e.g. ^{13}C and ^{1}H) is always in the weak coupling limit on account of the very large frequency separation between the resonance frequencies of different isotopes (usually of the order of several MHz).

On the other hand, if the frequency separation of the resonances from two coupled spins is comparable with the coupling constant between them, we have what is called *strong coupling*. In this limit, both the frequencies and intensities of the lines are perturbed from the simple weak coupling prediction. We will return to a more detailed discussion of the effects of strong coupling in section 12.1 on page 442 and section 12.7 on page 468.

Unless we say otherwise, everything described in this book applies only to weakly coupled spin systems. This is something of a limitation, but for strongly coupled systems the calculations for all but the simplest experiments become very much more complex and the resulting spectra are rather hard to interpret, so little is to be gained by such an analysis. In practice, therefore, we need not be too worried by this limitation to weak coupling.

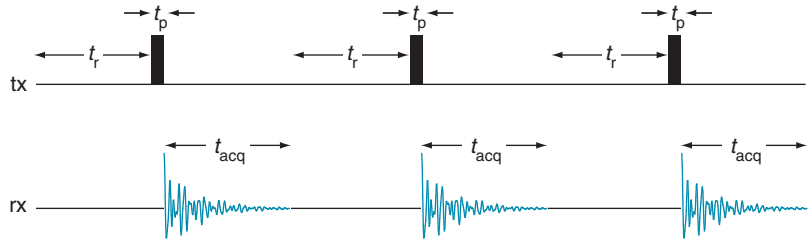

Fig. 2.9 Timing diagram showing how a basic NMR spectrum is recorded. The line marked 'tx' shows the location of high-power RF pulses; tx is the traditional abbreviation for an RF transmitter. The NMR signal is detected by a receiver during the times shown on the line marked 'rx'. During time t_r the spins come to equilibrium. A very short RF pulse is applied for time t_p and then the resulting FID is recorded for time t_{acq}. In order to improve the signal-to-noise ratio, the whole process is repeated several times over and the FIDs are added together; this process is called time averaging. Here, the experiment is repeated three times.

Confusion can arise as the term strong coupling is sometimes used to mean a coupling constant with a large *size*. Strictly, this is an erroneous use of the term.

2.4 The basic NMR experiment

The way we actually record an NMR spectrum using a pulsed experiment is shown in Fig. 2.9. First, a delay is left in order to allow the spins to come to equilibrium; this is called the *relaxation delay*, t_r. Typically this delay is of the order of a few seconds.

Next, a very short burst, typically lasting no more that 20 μs, of high power RF is applied. This excites a transient signal known as a *free induction decay* or FID, which is then recorded for a time called the *acquisition time*, t_{acq} which usually lasts between 50 ms and a few seconds. Finally, Fourier transformation of the FID gives us the familiar spectrum.

The NMR signal tends to be rather weak, so that it is almost never the case that the spectrum from a single FID has sufficient signal-to-noise to be useful. In order to improve the signal-to-noise ratio we use *time averaging*. The idea here is to repeat the experiment many times and then add together the resulting FIDs. The signal part of the FID simply adds up so that after N experiments the signal will be N times stronger. However, the noise, because it is random, adds up more slowly – usually increasing as $\sqrt{N}$. Overall, then, repeating the experiment N times gives an improvement in the signal-to-noise ratio by a factor of $\sqrt{N}$. We usually describe this by saying that N 'transients' or 'scans' were recorded. Calling each experiment a scan is something of a misnomer, but it is an historic usage which has stuck firmly.

Figure 2.10 on the following page shows the proton spectrum of quinine, whose structure is shown in Fig. 2.11 on the next page. Throughout the rest of the text, we will be using spectra of this molecule to illustrate various different experiments.

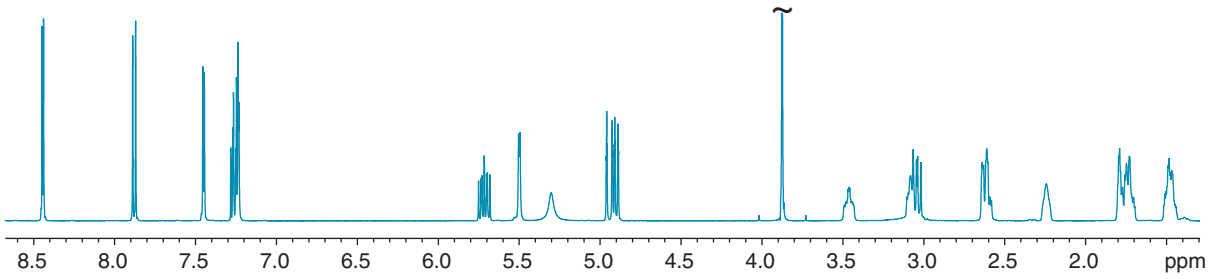

Fig. 2.10 500 MHz proton spectrum of quinine (in $CDCl_3$ solution), whose structure is shown in Fig. 2.11. The group of multiplets between 7 and 9 ppm are clearly from the aromatic ring, while those between 4.5 and 6 ppm include the protons on the double bond. The intense peak at 3.8 ppm (which has been truncated) is from the OCH_3 group.

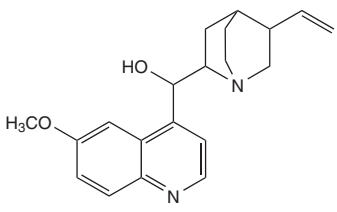

Fig. 2.11 The structure of quinine.

2.4.1 Heteronuclear NMR and broadband decoupling

In an NMR experiment we can usually only observe one kind of nucleus at a time, such as proton, ^{13}C or ^{15}N. Historically, proton NMR was the first to be exploited widely, and it is still the most recorded nucleus. As a result, all nuclei which are not protons are grouped together and called *heteronuclei*.

Scalar couplings can occur between any magnetic nuclei which are reasonably close on the bonding network. It is usual to distinguish between *homonuclear* couplings, which are couplings between nuclei of the same type, and *heteronuclear* couplings, which are couplings between nuclei of different types.

While couplings certainly provide useful information, at times they can be troublesome as the presence of many couplings will result in complex broad multiplets. This is particularly the case when observing ^{13}C spectra of organic molecules in which any one ^{13}C is likely to be coupled to several protons.

The effect of all of these ^{13}C–^{1}H couplings can be removed if, while the ^{13}C spectrum is recorded, the protons are irradiated with a *broadband decoupling* sequence. Such sequences generally involve continuous irradiation of the protons with a carefully designed repeating set of pulses of particular phases and flip angles. The most commonly employed sequence is called WALTZ–16, although there are many more which can be used. Such broadband decoupling essentially sets all of the ^{13}C–^{1}H couplings to zero, so that in the ^{13}C spectrum there is a single peak at each shift. The simplification achieved is very significant, and in addition the signal-to-noise ratio is improved as all of the intensity appears in a single line rather than being spread across a multiplet. This is well illustrated by the comparison of Fig. 2.12 and Fig. 2.13 on the facing page, which are the coupled and decoupled ^{13}C spectra of quinine.

The main issue with broadband decoupling sequences is that, as they are applied continuously during data acquisition, the sample itself may be heated to a significant degree simply by absorbing the RF power. The wider the range of chemical shifts of the nucleus being irradiated, the more power is needed and hence the more serious the heating effect. For protons,