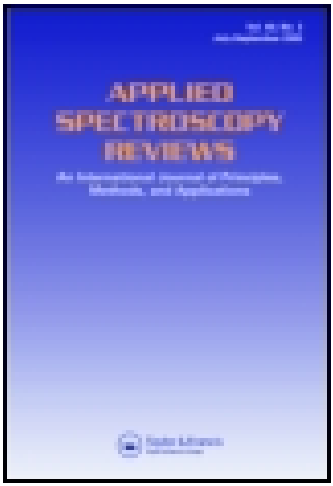
This article was downloaded by: [Duke University Libraries]
On: 05 January 2015, At: 12:34
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954
Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK




Applied Spectroscopy Reviews
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/laps20

# Applications of NMR to Pharmaceutical Technology

James S. Bernstein [a]

[a] Burroughs Wellcome Company , 3030 Cornwallis Road, Research Triangle Park, North Carolina, 27709
Published online: 15 Aug 2006.

To cite this article: James S. Bernstein (1995) Applications of NMR to Pharmaceutical Technology, Applied Spectroscopy Reviews, 30:1-2, 119-137, DOI: 10.1080/05704929508003312

To link to this article: http://dx.doi.org/10.1080/05704929508003312

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan,

sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

APPLIED SPECTROSCOPY REVIEWS, 30(1&2), 119–137 (1995)

# Applications of NMR to Pharmaceutical Technology

JAMES S. BERNSTEIN
Burroughs Wellcome Company
3030 Cornwallis Road
Research Triangle Park, North Carolina 27709

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

## Introduction

Nuclear magnetic resonance (NMR) is a spectroscopic technique well suited to determining the structure of molecules ranging from a few atoms to large proteins. The strength of NMR lies in its ability to distinguish and identify atoms on the basis of their chemical environment, and in determining the chemical bond and spatial relationship of those atoms. Thus, NMR is a powerful tool for the identification of molecules, especially those of an organic nature. In addition, NMR has quantitative applications valuable to pharmaceutical analysis.

This article surveys the uses and applications of NMR in pharmaceutical technology; it does not cover the applications to research fields such as medicinal chemistry. The pharmaceutical applications discussed here include both drug substance and dosage form applications. The purpose of the article is to describe the range of NMR applications and not the theoretical or experimental details of the technique. Many excellent texts are available for that purpose (see Bibliography for examples).

## The NMR Experiment

### NMR Transitions and Spectra

The NMR experiment generates spectra based on the transitions of the magnetic moment of atomic nuclei. A magnetic dipole moment is a characteristic of protons and neutrons, and the size and nature of the magnetic moment of an atomic nucleus depend on the numbers of protons and neutrons. Nuclei containing even numbers of both protons and neu-



Copyright © 1995 by Marcel Dekker, Inc.

trons have no magnetic moment. For hydrogen atoms with only a proton as nucleus, the magnetic moment exists in only two states (in quantum mechanics, this is referred to as a "spin ½" nucleus, with a "multiplicity" of two). This two-state situation also applies to carbon-13 ($^{13}C$) nuclei, an isotope of carbon with a natural abundance of 1.1%. Carbon-12 nuclei have zero magnetic moment and are not observable by NMR. Tables of nuclei, the size of their magnetic moment, and the multiplicity of the magnetic spin states are found in most NMR texts.

In the absence of a magnetic field, there is no difference in energy between the possible states of the magnetic moment. When a nucleus with a spin ½ magnetic moment is placed in an external magnetic field, the magnetic dipole may align either parallel or antiparallel to the applied field. The two states now differ in energy, the antiparallel state being higher. The size of the energy difference is directly proportional to the strength of the applied magnetic field.

Transitions between the two states are induced by electromagnetic radiation of frequency $\nu$ in such a way that $\Delta E = h\nu$, where $h$ is the Planck's constant and $\Delta E$ the energy difference between the two states of the magnetic dipole. At equilibrium conditions, the lower energy state has a population excess compared to that of the higher energy state, due to the Boltzmann distribution. Radiation is absorbed when this population of magnetic moments is exposed to resonant radiation. For protons in the field generated by current NMR instrumentation, the radiation frequency lies in the 60 to 600 MHz range.

The two most important nuclei studied by NMR are the proton ($^{1}H$) and the $^{13}C$ nuclei. The former has a magnetic moment which is about four times that of the latter. Thus, in a magnetic field in which 100 MHz radiation causes transitions in proton magnetic moments, only 25 MHz radiation is required to cause resonant transition in $^{13}C$ nuclei. Different resonant frequencies require different instrument conditions to observe each type of nuclei.

In the NMR experiment, a sample is placed inside a strong magnetic field and irradiated with radiofrequency radiation. The magnetic field actually experienced by a nucleus on a molecule differs slightly from the externally applied field, depending on the chemical environment of the nucleus and molecule. This microscopic magnetic environment is principally affected by the electron density at that nucleus and by the presence of other nuclei with magnetic moments. These slight changes in the effective magnetic field give rise to the effectiveness of NMR as a tool for determining molecular structure.

The magnetic field seen by a proton is different, for example, if the proton is attached to an alkane versus an alkene carbon. In practice, nearly any chemical change affects the resonant frequency of a proton up to several bonds away. Thus, a spectrum of resonant frequencies characterizes the protons in a molecule corresponding to the different chemical environments experienced by the different protons. The same effects operate for other nuclei observable by NMR. This change in resonant frequency is referred to as "chemical shift," because it is due to the molecular and chemical environment of the nuclei. The contribution of the molecular environment to the effective magnetic field is very small compared to that of the externally applied field in NMR spectroscopy. For protons in

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

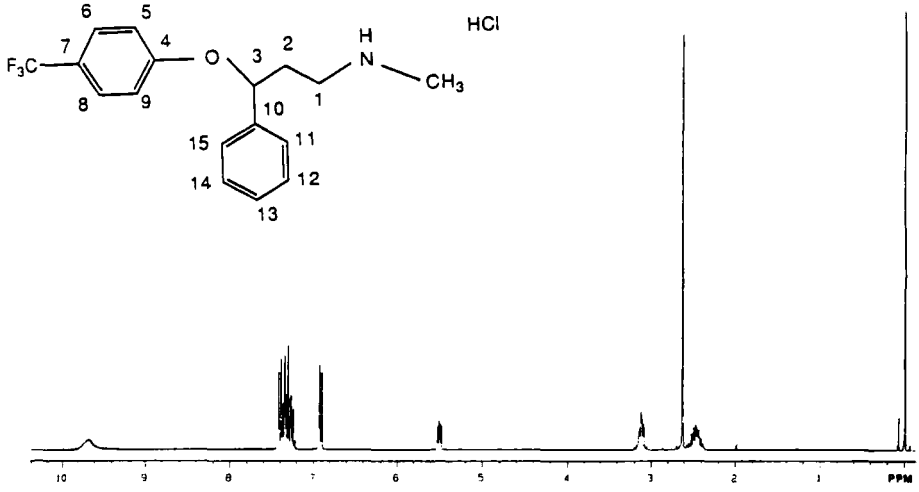



**FIG. 1.** Proton NMR spectrum of fluoxetine hydrochloride obtained at 300 MHz $CDCl_3$ as solvent. (From Ref. 1, p. 201.)

typical organic molecules, the effective field varies from the applied field by at most 0.0002%, or 20 ppm. For $^{13}C$ nuclei, the resonant frequencies of the magnetic moment transitions vary over a range of about 200 ppm of the applied field.

Figure 1 is a proton NMR spectrum of fluoxetine hydrochloride. At least ten distinct kinds of protons are observed in this spectrum; the assignments are listed in Table 1. The trends observed in this molecule hold for many molecules. For aromatic protons they are generally between 6 and 8 ppm and for aliphatic protons up to 5.5 ppm. Tables of chemical-shift correlations for protons and carbon atoms with various functional groups are found in many basic NMR and spectroscopy texts. It is noteworthy that the signals in most proton spectra are not single lines, but multiplets due to the interaction between the magnetic moments of the different types of protons with each other. When nuclei interact in this manner, the nuclei are said to be coupled.

An important aspect of most NMR experiments is the use of deuterated solvents. Because the proton signals of normal solvents would be several orders of magnitude greater than the sample resonances, almost all spectra are acquired in fully deuterated solvents. These solvents are commercially available, prepared and packaged for NMR applications. Spectrometers also make use of the deuterium NMR signal from these solvents to provide a frequency reference (known as a lock signal) for the spectrometer electronics.

The scale used in NMR experiments is expressed in units of chemical shift, sometimes referred to by the symbol $\sigma$. Chemical shift is a relative measure with units of parts per million, and allows spectra from instruments of different magnetic field strength to be directly compared.

$$\sigma = [\text{Observed frequency } (\nu) \times 10^6] / \text{Reference frequency } (\nu_0)$$

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

**TABLE 1** Proton and Carbon NMR Assignments for Fluoxetine Hydrochloride[a]

| Assignment | $^{13}C$ Chemical Shifts | $^{1}H$ Chemical Shifts |
|---|---|---|
| 1 | 46.03 | 3.12 |
| 2 | 34.49 | 2.48 |
| 3 | 77.04 | 5.47 |
| 4 | 139.72 | — |
| 5,9 | 125.76 | 6.90 |
| 6,8 | 126.77 | 7.39 |
| 7 | 123.33 | — |
| 10 | 159.74 | — |
| 11,15 | 115.88 | 7.31 |
| 12,14 | 128.99 | 7.27 |
| 13 | 128.38 | 7.24 |
| $CF_3$ | 124.21 | — |
| $N\text{-}CH_3$ | 32.89 | 2.62 |
| Exchangeables | | |
| NH, HCl | — | 9.71 |

[a]From Ref. 1.

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

Furthermore, chemists have agreed that the chemical shift of the protons of tetramethylsilane (TMS) is assigned the value zero. All spectra are acquired against this reference point, again for the purpose of being able to compare spectra from different instruments in different environments. A similar convention exists for $^{13}C$ spectra.

The NMR spectrum of a given compound depends thus primarily on first the chemical shift of the nuclei, and second on the coupling of the observed nuclei with other magnetic nuclei present. In $^{13}C$ NMR, the observed coupling patterns are always due to the interaction of the $^{13}C$ nucleus and nearby protons. The $^{1}H$–$^{13}C$ coupling patterns result in the $^{13}C$ spectrum reducing the sensitivity of the experiment and complicate the interpretation. Therefore, a technique known as decoupling is generally used in the acquisition of $^{13}C$ spectra, resulting in single-line resonances.

Figures 1 and 2 are the $^{1}H$ and $^{13}C$ spectra of fluoxetine hydrochloride, respectively, with the assignments in Table 1. Empirical knowledge about the chemical shifts of different types of protons and carbon atoms, with interpretation of the coupling patterns in the proton spectrum, can be used to arrive at a reliable determination of the structure for many simple molecules. Additionally, the relative integrated intensities of the resonances in the proton spectrum may be used to determine the number of protons of a given type (remembering that molecular symmetry can reduce the apparent number of protons present). Modern NMR techniques lessen dependence on empirical knowledge and establish bond conductivity patterns and spatial relationships to determine chemical structures.

## Instrumental Aspects

### *Continuous-Wave NMR*

Continuous-wave (CW) NMR was the first technique in which NMR was applied in the

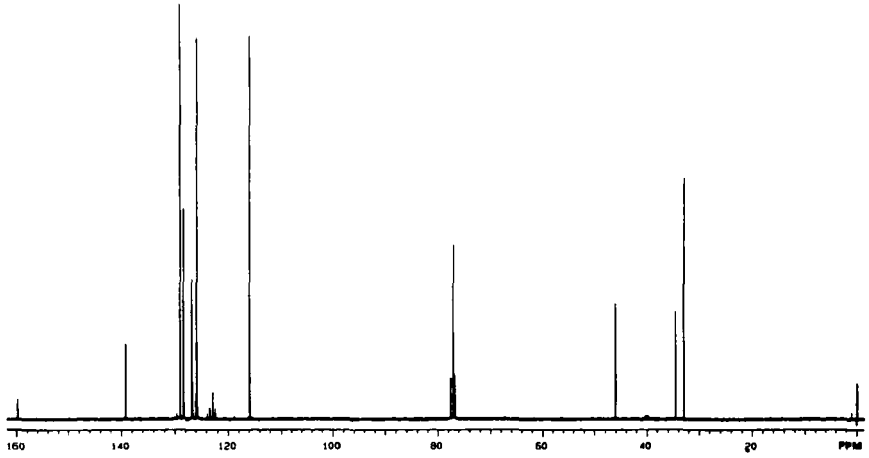



**FIG. 2.** $^{13}C$ NMR spectrum of fluoxetine hydrochloride obtained at 75 MHz in $CDCl_3$ as solvent. (From Ref. 1, p. 202.)

organic chemistry laboratory. Today, CW-NMR is available in lower resolution instrumentation designed for simple organic compound identification or for specialized applications generally in a production environment.

Conceptually, CW-NMR presents an easy means to understanding the basic NMR experiment. Previously, the NMR transition was described as the result of the interaction of the nuclear moment with electromagnetic radiation in the presence of an externally applied magnetic field. The frequency of the radiation required was determined by the chemical and physical properties of the nucleus and the strength of the surrounding magnetic field.

An experiment to determine an NMR spectrum can then be performed in one of two ways: either the magnetic field is held constant and the frequency of the radiation varied, or vice versa. In practice, it is experimentally easier in CW-NMR to vary the strength of the magnetic field and hold the frequency of the radiation constant.

The magnetic field is varied by means of secondary electromagnet coils attached to the faces of the primary magnet, whose field is fixed. The NMR spectrum is created by sweeping the magnetic field strength over the range necessary to cause all the nuclei being examined to resonate with the fixed-frequency radiation. As the different nuclei come into resonance, the spectrometer detects the absorption of the radiation and records the spectrum onto a chart. Recalling the description of the NMR experiment, the total change in magnetic field strength is only a few parts per million of the total field for observing protons, and a few hundred parts per million for observing carbon nuclei.

### *Pulse (Fourier Transform) NMR*

Pulse or Fourier transform (FT) NMR is the type of experiment performed on all modern

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

NMR instrumentation capable of the kind of resolution and sensitivity necessary for application to a broad range of samples. It acquires the spectrum in a fundamentally different way from CW-NMR, which varies the magnetic field to bring nuclei with slightly different resonant frequencies into resonance with fixed frequency radiation. FT-NMR places the nuclei in a constant magnetic field, and irradiates the sample with a short burst of radiofrequency energy, a pulse. The effect is much like striking a bell with a hammer: all of the resonant frequencies of the bell are excited by the hammer [2]. Similarly, in FT-NMR, if the pulse is short enough, all the nuclei in the sample absorb energy from the radiofrequency pulse and reemit radiation as they relax into equilibrium. In FT-NMR, it is this emission of radiation that is measured.

The term Fourier transform originates from a mathematical transformation of the same name. This mathematical treatment is used to process the data resulting from the pulsed excitation of the nuclei to give a spectrum containing the same information as that resulting from a CW-NMR instrument. Fourier transform methods depend on computers, which perform the necessary calculations.

The great advantage of FT-NMR over CW-NMR is in the speed with which data can be acquired. This derives from the fact that all resonances are observed with each pulse, rather than one at a time as in sweeping a magnetic field. Signal averaging is commonly used to improve the signal-to-noise ratio of the spectrum. An FT-NMR acquires data faster by gathering them on all resonances at once, and also because each cycle of pulse and data acquisition is shorter than a single sweep through the spectrum on a CW instrument.

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

### *Instrumentation*

The basic components of an NMR spectrometer are a magnet, a probe for placing the sample into the magnetic field, radiofrequency circuitry located in the probe to allow the irradiation of the sample and the detection of signals, radiofrequency circuitry to generate the radiation and amplify the detected signals, and a device for recording the spectrum (ranging from a chart recorder to high speed computers). Figure 3 is a block diagram of a FT-NMR spectrometer, where the probe resides inside the magnet, and the ADC converts the analog signals into digital form for processing by the computer into a spectrum. The CW-NMR instruments may use either a permanent magnet or, to reach higher fields, electromagnets. These instruments operate a proton resonant from 30 to 100 MHz. Higher field strengths and correspondingly higher resonant frequencies are obtained through the use of superconducting magnets contained in large Dewar bottles. The magnet is immersed in liquid helium at 4.2 K, and a liquid nitrogen container insulates the liquid helium container from ambient conditions.

The superconducting spectrometers are always FT-NMR systems, whereas permanent and electromagnet systems may be CW or FT systems. Modern FT-NMR instruments have sophisticated pulse generators, which are controlled and programmed by computers. Data acquisition and processing tasks are also controlled by high-speed computers; two-dimensional NMR experiments place especially large demands on the processing power of the spectrometer computers due to the large number of data points possible.

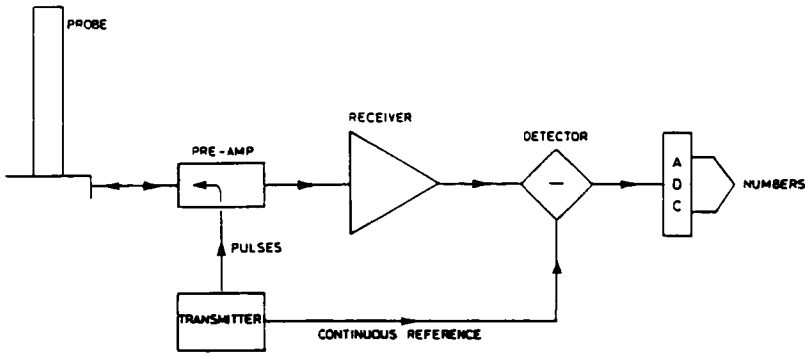



**FIG. 3.** Block diagram of an FT-NMR spectrometer pulse and detection system (ADC = ampere direct current).

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

## Experimental Techniques

Modern NMR spectroscopy has three aspects that make it possible for most of the detailed information to be learned about complex samples.

### *The Nuclear Overhauser Effect (NOE)*

When a sample is irradiated by a pulse with the nuclei present, the magnetic-moment populations are disturbed from the equilibrium described by the Boltzman distribution. The return of the population to equilibrium is known as relaxation (characterized by an exponential constant $T_1$), and occurs for magnetic moments principally through emission of radiation (which gives rise to the detected NMR signal) and dipole–dipole interactions with other magnetic moments (nuclei and unpaired electrons). The interactions with other nuclei are very short range in nature, and generally occur only for nuclei located on the same molecule. The nuclear Overhauser effect is a measure of the degree to which a nucleus contributes to the relaxation of a given nucleus. This interaction is generally a function of the distance between the interacting nuclei. Comparisons between the size of the NOE for pairs of nuclei may give relative information about the distance between the interacting nuclei. This type of information is very useful in determining conformational questions about structures of molecules and in the proper assignment of resonances.

### *Spectral Editing*

The principle application of spectral editing is the generation of distinct $^{13}C$ NMR spectra for CH, $CH_2$, and $CH_3$ carbon atoms. By inference, quaternary carbon atoms are also identified. These separate spectra greatly aid in the interpretation of complex $^{13}C$ NMR spectra.

The experiments use the coupling between abundant nuclei (usually protons) and less

abundant nuclei (usually $^{13}C$) to effect the transfer of spin polarization (energy) from the more abundant nuclei to the less sensitive or abundant nuclei. The transfer of polarization is dependent upon the number of protons attached to the carbon atom, and through the adjustment of experiment parameters the spectrometer generates spectra containing only specific types of $^{13}C$ atoms. Polarization transfer is also used to enhance the sensitivity of the NMR experiment for these less-sensitive or less-abundant nuclei, such as for $^{31}P$ and $^{15}N$.

### *Two-Dimensional Spectroscopy*

Two-dimensional spectroscopy provides the tools to assign NMR spectra and determine structures unambiguously, without recourse to empirical rules. These experiments may be divided into those that establish relationships between one type of nuclei (homonuclear experiments), and those that relate different types of nuclei (heteronuclear experiments).

Homonuclear experiments are especially useful for sorting out proton spectra; they take two forms. The most common experiment, known as COSY (COrrelation SpectroscopY), is used to establish which nuclei (usually protons) are coupled to which. By defining the strength of the coupling the experiment is sensitive to, protons coupled to each other through a given number of bonds may be identified. The correlation of protons by chemical shift (which provides functional group information) allows the chemist to determine the placement of interacting protons on the molecule. Figure 4 is a proton–proton correlation spectrum of celiprolol. Interaction between the protons is identified by the off-diagonal peaks.

Another class of homonuclear experiments correlates the coupling of nuclei (again, usually protons) with the chemical shift of the protons. This type of experiment is especially useful for sorting out complex patterns of overlapping multiplets of proton resonances.

Heteronuclear experiments are most commonly used to correlate proton and carbon NMR resonances, as seen in Fig. 5. The two-dimensional plot reveals which protons are attached to which carbon atoms. The heteronuclear experiments enhance structure determinations because the complexity of a proton spectrum is reduced when resolved into the carbon spectral window, which covers a wider range and normally has fewer overlapping resonances. The most common experiments are known as HETCOR, for HETeronuclear CORrelation. In conjunction with the proton–proton correlation experiment, the entire connectivity scheme of many molecules may be deduced using heteronuclear two-dimensional experiments. These measurements have advanced structure determinations from stating "the spectrum is consistent with the proposed structure" to "the spectral data establish the proposed structure."

Figure 4 shows the proton, carbon, proton–proton correlation, and proton–carbon correlation NMR spectra of the β-blocker celiprolol; assignments are given in Tables 2 and 3. Correlations in the proton–proton experiment are detected by off-diagonal peaks; by examining the contour plot, one can confirm the assignments given. In the proton–carbon correlation spectrum, the contour signals denote which protons are coupled (attached) to which carbon atoms.

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

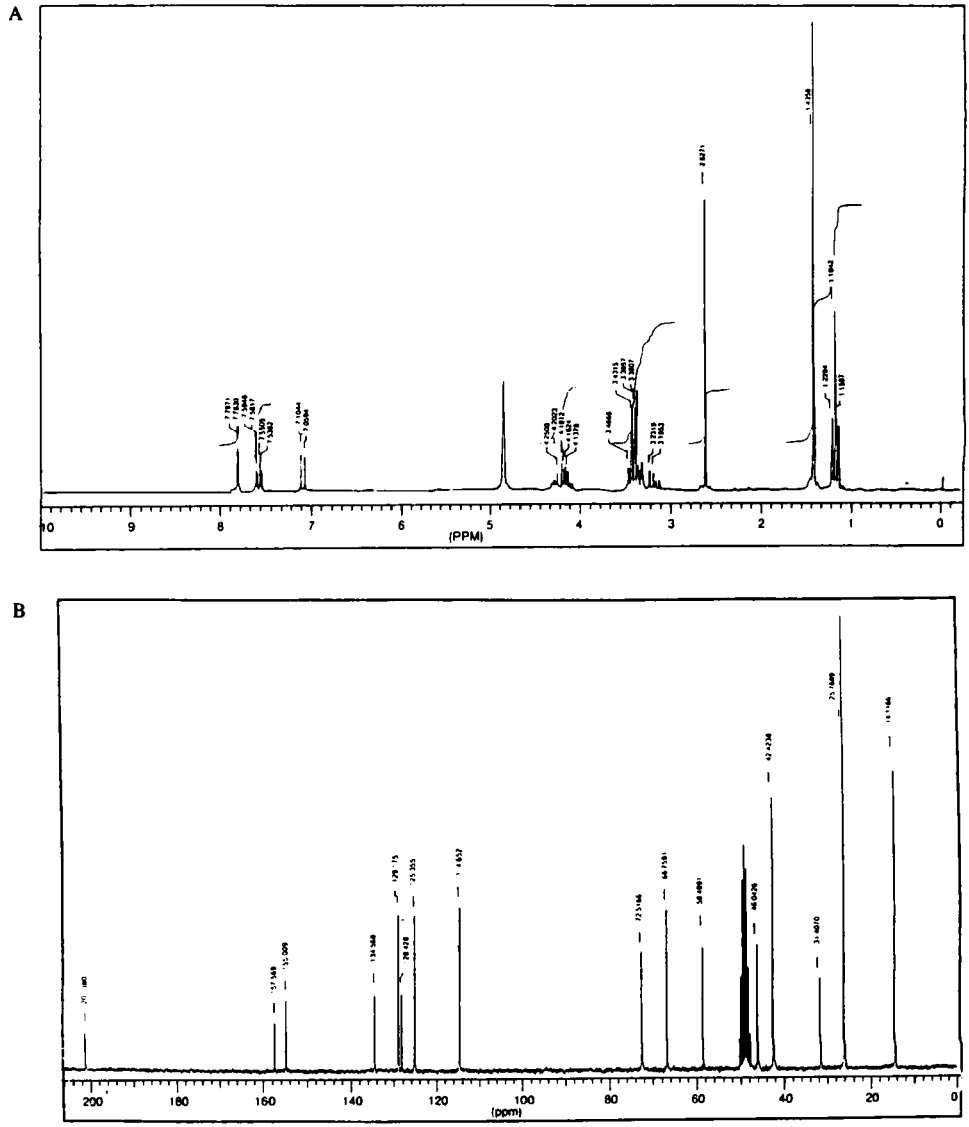



**FIG 4.** NMR spectra of celiprolol. A. Proton spectrum at 200 MHz. B. $^{13}C$ spectrum at 50 MHz. C. Proton–proton correlation spectrum. D. Proton–carbon correlation spectrum. (From Ref. 3.)

(continued)

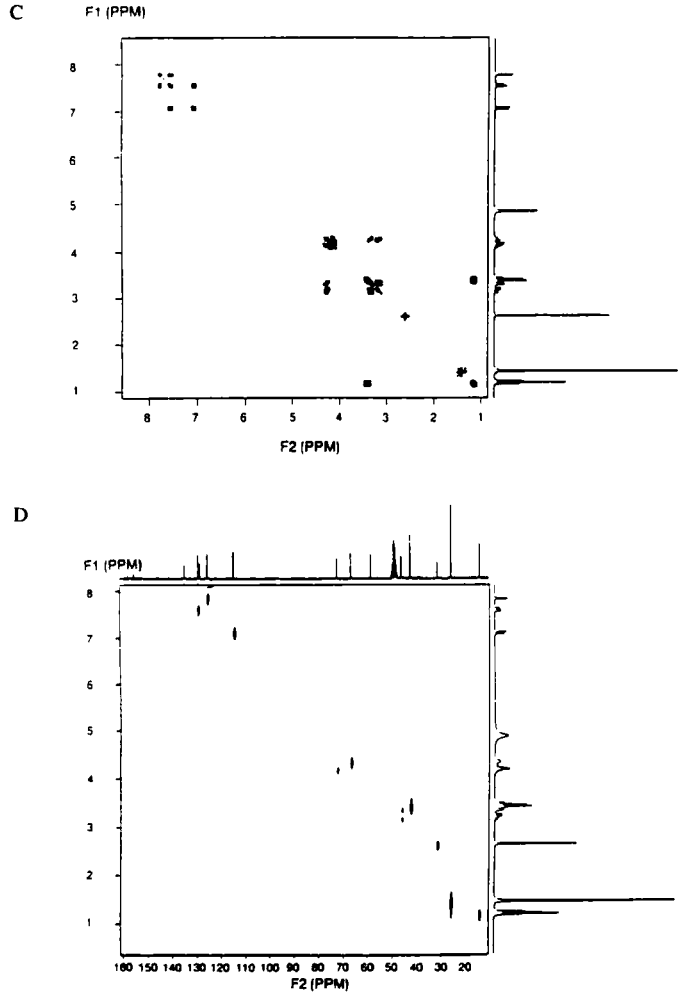



**FIG. 4.** Continued

## Solid-State NMR

Solid-state NMR offers most of the advantages and characteristics of modern NMR but at the expense of some experimental problems, and the loss of most of the utility of proton spectra. What it does provide is the carbon (and phosphorous and other less studied nuclei) NMR of solids. The earlier discussion of chemical shift noted that it was due to the microenvironment of the nucleus studied. In solution NMR, with random orientations and molecular motion, the interactions between molecules are averaged out. In the solid

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

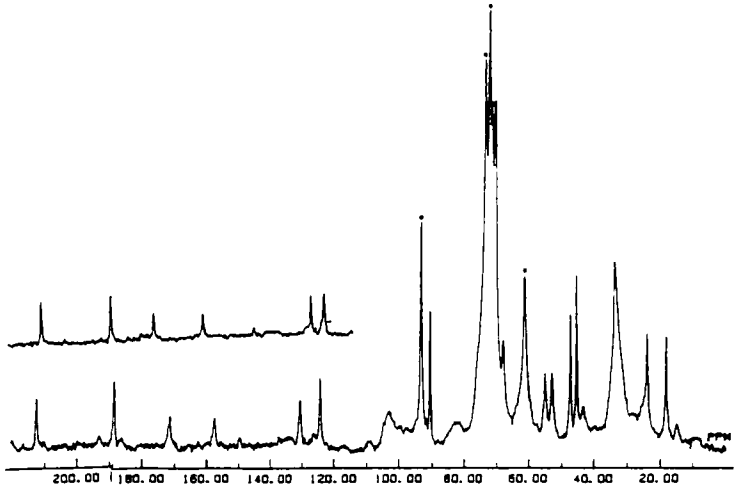
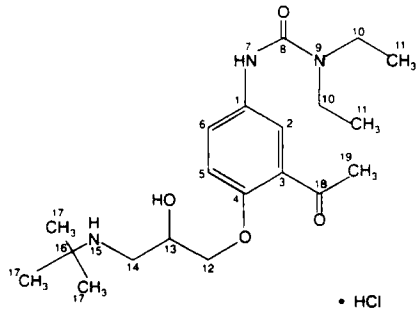



**FIG. 5.** Solid-state CP/MAS NMR spectra of prednisolone tablets from two sources; the inset spectrum shows differences in the spectra due to different morphic forms. The signals marked by dots are artifacts or excipient signals. (From Ref. 15, p. 199.)

**TABLE 2** Proton NMR Spectral Assignments of Celiprolol Hydrochloride[a]




| Proton | Chemical Shift (ppm) |
|---|---|
| H-2 | 7.79 |
| H-5 | 7.08 |
| H-6 | 7.57 |
| H-10 | 3.41 |
| H-11 | 1.19 |
| H-12,H-13 | 4.08–4.34 |
| H-14 | 3.13–3.38 |
| H-17 | 1.44 |
| H-19 | 2.63 |

[a]From Ref. 3.

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

**TABLE 3** $^{13}C$ NMR Spectral Assignments of Celiprolol Hydrochloride[a]

| Carbon No. | Chemical Shift (ppm) |
|---|---|
| 1 | 134.57 |
| 2 | 125.36 |
| 3 | 128.43 |
| 4 | 157.57 |
| 5 | 114.65 |
| 6 | 129.18 |
| 8 | 155.01 |
| 10 | 42.42 |
| 11 | 14.12 |
| 12 | 72.52 |
| 13 | 66.76 |
| 14 | 46.04 |
| 16 | 58.49 |
| 17 | 25.79 |
| 18 | 201.38 |
| 19 | 31.41 |

[a]From Ref. 3.

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

state, however, the chemical shift is anisotropic, as the molecules maintain a fixed orientation to each other. This results in different spectra for different molecular arrangements and thus NMR spectra that are characteristic of morphic forms.

If a typical solid sample, consisting of microcrystalline particles, were to be placed in a fixed position in the spectrometer probe, the spectrometer would produce a random array of anisotropically broadened lines. To average this anisotropic broadening, samples are spun at the "magic angle" of 54°44′ with respect to the applied field. This technique makes possible the acquisition of high resolution spectra.

In solid-state $^{13}C$ NMR, two other techniques are commonly applied to increase sensitivity. Since most solid-state NMR work is done with carbon nuclei, protons are continuously decoupled from the carbon nuclei to eliminate the broadening of the carbon lines due to the dipole–dipole interactions of the $^{13}C$ and $^{1}H$ nuclei, similar to normal practice in solution $^{13}C$ NMR. Furthermore, because of the long relaxation times and relatively low sensitivity of the $^{13}C$ nuclei, the technique of cross-polarization is used to transfer spin polarization from the $^{1}H$ nucleus to the $^{13}C$ nucleus. The cross-polarization technique enhances the sensitivity of the solid-state $^{13}C$ NMR experiments, and can also be used to measure relaxation phenomena in solids. The combination of cross-polarization (CP) and magic-angle spinning (MAS) yields an experiment class known as CP/MAS NMR.

## Applications

The applications of NMR are categorized here as qualitative, quantitative, or to a sample

in the solid state. Most NMR is done on solution samples, but solid-state NMR has much to offer in the study of pharmaceutical materials, including degradation chemistry, dissolution, and polymorphism.

## Qualitative Applications

Qualitative applications identify a molecule, either for the purpose of determining an unknown, or for confirming the presence of a known molecule.

### *Structure Determinations*

Structure determination is the main purpose of NMR and is covered in detail in many texts. The first step is the acquisition of proton and carbon spectra, along with edited carbon spectra to identify the CH, $CH_2$, $CH_3$, and quaternary carbons. For many simple molecules, these spectra, with empirical knowledge of chemical shifts, can be interpreted and the structure confirmed. For these and larger molecules, homonuclear and heteronuclear correlation spectra determine the connectivity between interacting protons and carbon atoms. Using the differences in magnitude between the coupling of atoms one, two, three, or more bonds apart, spectra are obtained to identify relationships between distant atoms. NOE spectra may be used to confirm spatial relationships and stereochemistry.

### *Impurities*

The identification of impurities through NMR ranges from a trivial exercise to a difficult one better suited to another technique. Identification of an unknown substance often takes place more quickly by combining information from several techniques.

A common type of impurity readily identified by NMR is a residual solvent from a manufacturing or synthetic step. Most NMR operators are familiar with the characteristic peaks of common organic solvents such as acetone, ethanol, ethyl acetate, etc. Because of the low molecular weight of the solvents compared to most bulk pharmaceuticals, the molar concentration is relatively high, and thus even minor amounts (1% weight basis) of solvents are often detectable. Spiking of the sample with the suspected solvent serves to confirm the identity of the residual solvent.

The identification of impurities similar in structure to the main component of the sample is a more difficult task. Often the most efficient means is to isolate the impurity by chromatographic or other means, and then carry out a structure determination. NMR should not be relied on in isolation; mass spectroscopy particularly is an important partner with NMR in the structural determination of compounds.

If the impurity cannot be separated from the principal component, NMR correlation experiments may be used to identify which atoms are bonded to each other, and thus belong to the same molecule. If the resonances of one component are known, the remaining resonances may be used to build up the structure of the impurity.

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

## *Identification Testing by NMR*

The FDA lists infrared spectroscopy, NMR, and mass spectrometry as techniques suitable for identification of bulk materials [4]. The key advantages of a technique for identification are the information richness of the spectrum, the uniqueness of the spectrum, and the ease with which the spectrum may be acquired.

The spectroscopic identification of a bulk material is a common need in the development and manufacture of pharmaceuticals. Although infrared spectroscopy is most commonly used for this purpose, NMR is also well suited to this task. Either the proton or carbon spectrum may be used, depending on which gives the most positive identification. The spectrum that displays the higher number of unique signals provides an identification better differentiated from related materials. The spectrum of the sample may either be compared to a reference spectrum or to the spectrum of reference material newly acquired each time a sample is run.

The most significant complication (or advantage) of IR spectroscopy is that morphic form differences may cause changes in the IR spectrum. Mass spectroscopy (MS) has the ability to generate spectra from very small quantities of samples and is widely accepted as an identification technique, especially when coupled with chromatography, such as in GC-MS.

Most frequently, NMR spectroscopy obtains spectra from liquid solutions, and thus is blind to morphic form variations. For samples that are soluble in a suitable solvent at concentrations higher than 0.1 mg/mL, spectra can be acquired in a few minutes. Sample preparation consists of dissolving the sample in about a 1 mL of solvent and filtering the solution into a clean sample tube. Solid-state NMR spectra are an excellent means of identifying morphic forms.

The direct correlation of the spectrum with the functional groups of the sample molecule is a clear advantage of NMR. The absence of or change in a functional group normally results in changes in the chemical shift in proton or carbon resonances located adjacent to the functional group. If a question as to the authenticity of a sample is present, the NMR spectra provides significant clues to the chemical difference between the references and sample structures.

## *Validation of NMR Identification*

Validation is a test of the method's ability to perform with accuracy and reproducibility. Spectroscopic identifications typically rely on the analyst to visually compare two spectra and make a judgment as to whether they match. Validation of the method focuses on sample preparation ruggedness and the sensitivity of the technique to detect a difference in the sample spectrum from the reference spectrum. A reference spectrum is obtained from a sample whose authenticity and purity have been established through extensive analysis.

For NMR, the primary factors in sample preparation are the moisture content, concentration, and contaminants such as inorganic or particulate material. Variation in moisture content affect the width and intensity of proton signals arising from exchangeable

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

protons, such as hydroxyl or amine protons. The concentration influences the signal-to-noise ratio of the spectrum, and may cause small changes in the chemical shifts of nuclei whose magnetic environment change when molecules associate. An example of this behavior would be a highly aromatic and planar molecule subject to stacking in solution. Inorganic contaminants cause broadening of NMR resonances due to spin-orbit coupling, resulting in more rapid relaxation of all nuclei in the sample. The best way to obtain reproducible NMR results for identification is to use a consistent source of high-purity deuterated solvents, a consistent and effective means of filtering particulates, and taking the time to weigh samples to achieve consistent solution concentrations.

If the NMR spectrum contains a sufficient number of lines to suggest uniqueness, it is most important to show how the spectrum differs from closely related compounds such as synthetic precursors or compounds of the same chemical class. Spectra of the related compounds are compared for differences from the target molecule, and the spectra archived for future reference.

## Quantitative Applications

### *Substances, Intermediates, Ingredients, and Products*

Quantitative measurements by NMR are broad in scope, but are usually not the technique of first choice. As described below, NMR can be used for rough quantitative proton determination without much effort, but for precisely quantitative operations only when attention is paid to several experimental factors. The advantage of NMR lies in the quickness with which an approximate method may be developed, and in applicability to any organic compound observable by NMR. Its disadvantages lie in the cost of high-field instrumentation, a lack of sensitivity to trace impurities, and in experimental factors capable of introducing bias into the determination.

### *Basis of Quantitative NMR*

Proton NMR accounts for most quantitative NMR applications, because the response of protons is nearly equal regardless of chemical shift or coupling to other nuclei, with the important exception of exchangeable protons. Functional groups such as hydroxyl and amine groups frequently show less than unity response due to exchange with deuterium atoms in solvents such as $D_2O$.

This near-unity response means that the ratio of the areas of proton resonances are usually accurate enough to determine the relative numbers of protons belonging to different functional groups. By extension then, the relative proportions of a mixture of compounds can be estimated to an accuracy of perhaps $\pm 20\%$. Better accuracy requires developing experimental conditions and (usually) the selection of an internal standard to fit the particular analysis at hand.

Most quantitative NMR assay methods are based on an internal standard, such as described in the assay of amyl nitrite bulk drug and inhalant in *USP XXII* [5]. An internal standard is accurately weighed and added to a solution of an accurately weighed sample

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

or a dosage form unit. The spectrum is obtained under specific conditions and the integrals are measured. The assay is then calculated as in Eq. (1).

$$\text{Assay } (\%) = 100\% * (A_{spl}/A_{std}) * (W_{std}/W_{spl}) * (EMW_{spl}/EMW_{std}) \quad (1)$$

where $A$ is the integral of the sample or internal standard signal, $W$ the weight of the internal standard or sample introduced into the NMR tube, and $EMW$ the equivalent molecular weight of the sample or internal standard. The equivalent molecular weight is the molecular weight divided by the number of protons producing the signal integrated.

Selection of the internal standard is dictated by convenience, availability in high purity, solubility in a solvent in which the sample is readily soluble, and stability (both on the shelf and in solution). A simple spectrum is desirable with a signal (usually a singlet) not subject to exchange phenomena and well resolved from any signals arising from the sample. NMR methods are also used to determine the relative proportions of two compounds, such as diastereomers; for these relative measurements, an internal standard is not needed.

The development of instrumental conditions affording suitable precision and accuracy, is the least appreciated and most important part of the practice of quantitative NMR. Method development begins with the selection of an internal standard and identification of possible resonances to base the method on. Ideally, these resonances are separated from other resonances by at least 40 times the full width at half height of the resonance; compromise on this separation may reduce the accuracy of the method. Next, the $T_1$ relaxation times of the protons under consideration in the sample and the internal standard must be measured, a routine experiment on modern spectrometers. To ensure a complete return to equilibrium between NMR pulses, at least five $T_1$ periods must be allowed to elapse between pulses to avoid nonequal responses from the protons used. (Common NMR conditions for the acquisition of spectra for identification and structure determination use instrument conditions in such a way that the proton resonances are in a state of partial saturation; this gives the maximum rate of increase of signal to noise. In a single molecule, the differences in relaxation rates are usually not big enough to interfere with determining the relative numbers of protons.) If sufficient sensitivity is available, a single NMR pulse may result in a spectrum of adequate signal-to-noise ratio, and thus avoid any question of unequal response due to unequal relaxation rates.

Other instrumental parameters that affect quantitative NMR methods are the phasing of the spectrum, unequal effects of the electronic filters internal to the spectrometer, and baseline artifacts. Discussing control of these phenomena is beyond the scope of this article, but because both are more difficult to control over a wider region of the spectrum, quantitative methods are often improved by selecting resonances within a ppm or two of each other for quantitative measurements.

The $^{13}C$ satellites of proton NMR peaks are an important means of quantitatively measuring sample components less than few mole percent of the major component. These peaks arise from the coupling of the proton resonances with the 1.1% of $^{13}C$ carbon atoms. The coupling constant of $^{1}H$–$^{13}C$ interactions is typically 100 to 200 Hz; the inter-

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

action of the spin-½ $^{13}C$ nuclei with the proton resonance results in a doublet, centered at the location of the unsplit proton resonance, with the satellite peaks symmetrically located 50 to 100 Hz to both sides of the main proton line. Each $^{13}C$ satellite peak has 0.55% of the intensity of the proton signal from which it arises (not true in samples intentionally enriched in $^{13}C$!). These $^{13}C$ satellite peaks are convenient for the quantitation of impurities with mole percents of a few percent or less, as it is more accurate to compare two integrals nearly equal in area than comparing the integral of a small impurity to that of a line with an intensity orders of magnitude greater.

Quantitative NMR is more complicated for carbon NMR, but is occasionally useful. Two phenomena are more severe in carbon NMR than in proton NMR, namely, unequal $T_1$ relaxation times and NOE effects (which are nearly equal for all protons). These phenomena can be reduced by relaxation reagents, but methods based on such techniques must be validated against other means of quantitation, such as chromatography.

### *Limited Examples*

Quantitative analysis may be used to determine solvent residues, and for the assay of bulk drug, impurities, and drug product. When quantitative methods are used for the approval of final pharmaceutical materials, the methods should be checked for validity against established methods.

Many quantitative methods for drugs and drug products have been developed for lower field (90 and 60 MHz) instrumentation, both FT and CW. Examples include the USP XXII methods for amyl nitrite (bulk and products) [5], for phenytoin drug products [6], dicycloamine drug products [7], and for the enantiomeric purity of naproxen [8].

Higher field instrumentation was applied in the analysis of heparin preparations for levels of dermatan sulfate [9], the analysis of Busulfan tablets [10], and the estimation of the cis/trans isomeric ratio of chlorprothixene [11].

A final example is the use of 75 MHz $^{13}C$ NMR and 300 MHz $^{1}H$ NMR to determine the purity of vitamin E raw materials and reference standards [12].

## *Validation of Quantitative Methods*

If used for regulatory purposes, NMR methods must be subjected to validation just as an HPLC or FTIR method would be. Ruggedness of the assay to experimental conditions, differences between operators, sample preparation, reproducibility, and accuracy are some of the areas to be examined. A particularly troublesome area may be the selection of integration regions and the sensitivity of the assay to base-line artifacts. A higher level of confidence can be reached when the NMR method can be verified against an accepted method such as chromatography or titration.

### *Semiquantitative Assay of Solvents and Impurities*

A common quantitative application is the rapid estimation of residual solvent concentrations in bulk substances. As mentioned before, many common solvents have distinc-

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

tive resonances, usually with sharp lines. If the time is taken to set experimental conditions to obtain quantitative results (the smaller solvent molecule protons often have longer $T_1$ times than the sample molecule protons), useful results can be obtained very quickly. For trace solvents, comparison against $^{13}C$ satellite peaks from sample resonances may be useful.

## Stereochemical Analysis

### *Lanthanide Shift Reagents*

Splitting of NMR resonances of enantiomers is caused by association with a chiral coordination complex of a lanthanide series ion, such as tris[3-(heptafluoropropylhydroxymethylene)-(-)-camphorato]europium (III). A variety of these complexes are available commercially. Most are moisture sensitive, hence application is limited to organic-soluble compounds. NMR resonances are broadened and shifted with the help of these reagents in a concentration-dependent manner, hence application requires some care.

### *Chiral Solvating Agents and Other Reagents*

These reagents, of which 2,2,2-trifluoro-1-(9-anthryl)ethanol is the most successful, have advantages over the lanthanide agents, when applicable. Resonances are not broadened and the resolved spectra are easy to interpret, as only minor changes in chemical shifts occur. Because the agents depend on association between the sample molecule and the solvating agent, measurements must be made in nonpolar solvent systems. These reagents are commercially available.

In recent years, there have been several reports of the use of cyclodextrin species to resolve chiral species by NMR. Cyclodextrins form inclusion complexes under suitable conditions; since the cyclodextrin molecule is chiral, it follows that the inclusion process is stereoselective. An example is the use of γ-cyclodextrin to determine the enantiomeric purity of a 2-aminotetralin derivative [13].

### *Diastereomers*

Unlike enantiomers that have identical NMR spectra in the absence of a chiral environment, diastereomers have distinct chemical properties, including NMR spectra. However, the splitting between the resonances of a pair (or more) of diastereomers may be rather small, depending on the distance between chiral centers and the degree of affect the chiral centers have upon the configuration of the molecule. Quantitative measurements of diastereomer ratios may be made by careful measurement of signals from corresponding nuclei.

## Solid-State NMR Applications

Applications of solid-state NMR fall into two broad categories: studies of morphic forms or structures, and studies of solid-state reactions.

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

Solid-state NMR has been used to identify and quantitate polymorphic forms in bulk substances and directly in dosage forms such as capsules and tablets. Advantage can be taken of the chemical differences between active compounds and typical pharmaceutical excipients to selectively remove peaks due to excipient carbon atoms and thus simplify the spectra of formulated drugs. Besides true polymorphism, solvates and hydrates can also be differentiated. Solid-state reactions studied include solvation, interactions with excipients, and polymerization [14]. Figure 5 is the solid-state CP/MAS $^{13}C$ NMR spectrum obtained from prednisolone tablets from different sources, showing the differences in the morphic character of the drug substance in the two tablets [15].

Downloaded by [Duke University Libraries] at 12:34 05 January 2015

## Bibliography

Derome, A. E. *Modern NMR Techniques for Chemistry Research*, Pergamon Press, New York, 1987.

Levy, G. C., and Nelson, G. L., *Carbon-13 for Organic Chemists*, Wiley-Interscience, New York, 1972.

## References

1. Risley, D. S., and Bopp, R. J. In: *Analytical Profiles of Drug Substances*, Vol. 19 (K. Flory, ed.), Academic Press, San Diego, 1990, p. 203.
2. Derome, A. E., *Modern NMR Techniques for Chemistry Research*, Pergamon Press, New York, 1987, p. 11.
3. Mazzo, D. J., Obetz, C. L., and Schuster, J. E. In: *Analytical Profiles of Drug Substances*, Vol. 20 (K. Flory, ed.), Academic Press, San Diego, 1991, pp. 252–259.
4. Food and Drug Administration, *Guideline for Submitting Documentation in Drug Applications for the Manufacture of Drug Substances*, Food and Drug Administration, Washington, 1987, p. 37.
5. *United States Pharmacopeia XXII*, The United States Pharmacopeial Convention, Rockville, MD, 1990, pp. 95–96, 1590–1594.
6. Hanna, G. M., *Drug Dev. Ind. Pharm.*, 10(3): 341–354 (1984).
7. Hanna, G. M., *J. Assoc. Off. Anal. Chem.*, 67(2): 222–224 (1984).
8. Hanna, G. M., and Lau-Cam, C. A., *J. AOAC Int.*, 75(3): 417–423 (1992).
9. Neville, G. A., Mori, F., et al., *J. Pharmaceut. Sci.*, 78(2): 101–104 (1989).
10. Turczan, J., and Lau-Cam, C. A., *Spectr. Lett.*, 17(6–7): 353–359 (1984).
11. Rosler, K-H. A., Wright, J., et al., *Pharm. Res.*, 6(8): 706–708 (1989).
12. Baker, J. K., and Myers, C. W., *Pharm. Res.*, 8(6): 763–770 (1991).
13. Redenti, E., Fronza, G., et al., *Chirality*, 4:404–405 (1992).
14. Bugay, D. E., *Pharm. Res.*, 10(3): 317–327 (1993).
15. Saindon, P. J., Cauchon, N. S., et al., *Pharm. Res.*, 10(2): 197–203 (1993).