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Perspectives

Potential of Nuclear Quadrupole Resonance in Pharmaceutical Analysis

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Nuclear quadrupole resonance is a radio frequency (rf) spectroscopic technique, closely related to NMR, which can be used to detect signals from solids containing nuclei with spin quantum number >1/2. It is nondestructive, highly specific and noninvasive, requires no static magnetic field, and as such is currently used in the detection of explosives and narcotics. Recent technological advances in pulsed NQR methods have shortened detection times, eliminated spurious signals, and enhanced the sensitivity of detection of ^{14}N frequencies, which lie in the low rf range of 0.4−6 MHz, encouraging a wider range of "real world" applications. This Perspective highlights some of the advantages of NQR, the applications in which it could be used, such as the quantification of pharmaceuticals and the identification of polymorphs. Other roles could include detection, analysis, and quality control of pharmaceuticals at all stages of manufacture. Finally, recent advances which enhance even further the sensitivity of detection will be discussed.

PHARMACEUTICAL ANALYSIS

Organic chemical analysis is a key part of the identification, development, and quality assurance of new pharmaceuticals. For example, analytical methods play a critical role in supporting the scale-up of the synthetic route, development of the manufacture of the final dosage form, assessment of stability, and control of quality and consistency of the commercial product.[1−3]

Because of the important role of chemical analysis, there is continued interest in the refinement of existing analytical techniques and the development of new ones. The objectives of these refinement and development exercises typically include improving the speed and quality of decision-making. This can be achieved by finding ways of generating the same information more quickly or by generating additional useful information within the same time span. Products and processes need to be characterized more quickly and more fully, with the ultimate aim of decreasing development times, reducing manufacturing costs, and increasing the quality and safety of the final product.

Development in analytical chemistry proceeds not only by experimentation and application, but also by theoretical investigation. This theoretical investigation should include not only a better understanding of the physicochemical processes that underpin existing analytical methodologies, but also the identification of other physical phenomena which could form the basis of new analytical methodologies.

In this paper, we propose that recent advances in the technique of pulsed nuclear quadrupole resonance (NQR) have established it as a valuable technique in pharmaceutical analysis.

Most pharmaceuticals are marketed as solid dosage forms, for example, oral tablets, but the majority of the organic chemical analysis techniques used are solution-based. As a result, for example, HPLC, electrospray MS, and solution NMR are widely used in assessing the quantity of active agent, chemical purity, and the identification of both active agent and impurities. While such approaches enable tight control of the quality and consistency of the dosage form, they inevitably require time and effort in sample preparation and are inherently destructive in nature. Other important information, such as the polymorphic form of the active agent, is lost by solution-based methods, and so solid-state techniques such as IR, powder XRD, and solid-state NMR are used, as well.[4]

An additional area of interest in pharmaceutical analysis is that of moving the analysis closer to the process it is being used to control. Most control strategies rely on end-point testing in which the manufactured material is sampled and the samples are brought to the laboratory for testing. End-point testing imposes limits on the time scale in which process changes can be made. There is increasing interest in taking analysis from the laboratory and into the manufacturing and raw material receipt environments. Tech-

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niques such as near-IR (NIR) have made significant impacts here, but because NIR is a secondary technique, a significant calibration exercise is required before data can be interpreted in a meaningful way. Such at-line testing can also be improved by the addition of noninvasive techniques to the analytical armory so that pharmaceutical formulations can be analyzed without removal from their packaging. The development of noninvasive techniques may also prove useful in other environments, for example, hospitals, and in the detection of counterfeit products.

Such considerations lead to the conclusion that there is still benefit in developing new techniques for pharmaceutical analysis, and NQR is an example of a technique that may well hold promise in answering some of the challenges detailed above.

THE BASIC PRINCIPLES OF NQR

Nuclear quadrupole resonance (NQR) is a technique in radio frequency (rf) spectroscopy in which the signals arise from the interaction of the electric quadrupole moment of the quadrupolar nuclei in the sample with the electric field gradient (EFG) of their surroundings;[5] rf radiation excites transitions between the energy levels generated by this interaction at frequencies that are characteristic of a given material and can be used, therefore, not only to identify it but also to estimate quantity. The method is noninvasive, and signals are seen only in solids, so suspensions of materials and mixtures with other substances are eligible. Unlike the closely related technique of nuclear magnetic resonance (NMR), no static magnetic field is necessary, so remote materials and large volumes—at the moment, the record is 8000 L[6]—can be examined. The most commonly distributed quadrupolar nucleus in pharmaceuticals is ^{14}N, a spin-1 nucleus, which will be the main subject of this review. In addition, there are many other quadrupolar nuclei that are commonly found in medicines, such as ^{23}Na, ^{35}Cl, and ^{79}Br, that are equally amenable to the same methods and to which some reference will be made.

There are three allowed transitions in the general case for a spin-1 nucleus such as ^{14}N, one frequency (ν_x or ν_+) being the sum of the other two (ν_y or ν_-, ν_z or ν_0), the vast majority of which lie at the rather low radio frequencies between 0.2 and 6 MHz. These frequencies are related to quantities known as the quadrupole coupling constant and asymmetry parameter by eq 1, where (e^2qQ/h) is the nuclear quadrupole coupling constant (NQCC); e is the charge on the electron, h is Planck's constant, $q = q_{zz}$ is the maximum principal component of the electric field gradient tensor, and Q is the nuclear electric quadrupole moment. η is the asymmetry parameter defined as the difference between the other two components (q_{xx} and q_{yy}) of the electric field gradient tensor divided by q; it is a positive number lying between 0 and 1.

$$\nu_x = 3/4\frac{(e^2qQ)}{h}(1 + \eta/3)$$

$$\nu_y = 3/4\frac{(e^2qQ)}{h}(1 - \eta/3)$$

$$\nu_z = 1/2\frac{(e^2qQ)}{h}\eta \qquad (1)$$

Spin-3/2 nuclei, such as ^{23}Na, ^{35}Cl, and ^{79}Br, have two doubly degenerate levels, transitions between which give rise to just one frequency equal to

$$\nu_Q = 1/2\frac{(e^2qQ)}{h}\left(1 + \frac{\eta^2}{3}\right)^{1/2} \qquad (2)$$

Despite the low radio frequencies characteristic of ^{14}N signals, recent advances in pulsed NQR spectroscopy have significantly improved their sensitivity of detection.[6] In pulsed NQR, the ^{14}N nuclei are subject to bursts of rf radiation at or near their NQR frequency, and the resulting transient signals are monitored in the quiescent periods between pulses. The signals are generated by the interaction of the nuclear magnetic moment with the magnetic component B_1 of the rf field. They can be of two types: free induction decays (FID) and echoes. A FID is the decaying signal observed immediately following a pulse, whereas an echo is a regenerated signal with maximum intensity between rf pulses in a multiple-pulse train. The rf radiation can be generated in a number of ways: a conventional solenoid or bird-cage coil can be used to detect signals from samples dispensed in bottles, whereas tablets in blister packs could be studied by a planar single turn or spiral of copper wire or ribbon, which can also function as a receiver of the signals from the sample. Alternatively, separate transmit and receive antennae may be used. Note, however, that the samples need not be removed from their container. ^{14}N signals are usually very weak, and many responses must be accumulated to achieve an acceptable signal-to-noise ratio (SNR). For this purpose, extended trains of pulses have been developed in which the observed responses between pulses are averaged to enhance the SNR. The use of short, high-power rf pulses with active damping during the probe ring-down means that fast multiple-pulse sequences can be used to average many responses in times as short as a minute or less.[6] One example is known as pulsed spin locking (PSL) and can be represented by

$$\alpha_{0^\circ} - (\tau - \alpha_{90^\circ} - \tau -)_n$$

where α represents the pulse width, selected to optimize the signal; the subscripts denote the rf phase; τ determines the pulse spacing, which is 2τ after the first two pulses; and n determines the number of pulses in the train whose optimum value depends on the relaxation times of the material. The entire pulse sequence is repeated, often several hundred times, for further signal-averaging, depending on the quantity of material in the sample and, in the case of remote detection, its distance from the antenna. The problems posed by spurious signals, such as those observed from piezoelectric materials, have been largely eliminated by cycling the phases of the rf pulses and signals[7,8] before processing, which can be in the time domain or frequency domain after Fourier transformation. Since the pulse sequence repetition time is limited by the need to wait for the nuclear spins to recover their equilibrium magnetization and this time is determined by

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the spin−lattice relaxation time T_1, this is an important parameter that needs to be measured before any analytical measurements should be undertaken.

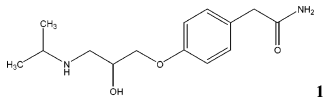
In pharmaceutical analysis, the important quantities determined by experiment are the nuclear quadrupole coupling constants (NQCC) and asymmetry parameters, which function in much the same way as the chemical shift or, rather, the chemical shift anisotropy in NMR, since they also contain information on the asymmetry through the two other components of the EFG tensor, q_{xx} and q_{yy}. Because Laplace's equation holds,

$$q_{xx} + q_{yy} + q_{zz} = 0 \tag{3}$$

there are only two independent parameters that define this tensor; unfortunately the direction cosines of these tensor components are not usually derived in measurements on powders; a single crystal would be needed.

SELECTIVITY AND IDENTIFICATION

A knowledge of the two parameters, NQCC and η, can often be used to identify a material through published lists of NQR frequencies[9] or those in related compounds. As an example, consider the antihypertensive drug Atenolol, which has the structure **1** with two different kinds of nitrogen atoms, one amine, and the other amide.



1

At room temperature, the NQR frequencies, NQCC and η, are determined as[10], I 0.47, 2.98, 3.50 MHz: NQCC = 4.32 MHz, η = 0.241, II 0.65, 1.60, 2.14 MHz: NQCC = 2.493 MHz, η = 0.433 in which the frequencies have been combined according to eq 1 so that the largest is close to the sum of the other two. A comparison of these with the listed parameters[9] for Me_2NH at 77 K (4.65 MHz, 0.169) and acetamide (2.526 MHz, 0.375) leads us to assign I to the amine group and II to the amide. These comparisons are rarely exact, or even nearly so, first, because NQR frequencies in solids are averages over all molecular and torsional modes within the molecules and so are temperature-dependent, and second, there are solid-state effects, just as in solid-state NMR, which can be rather large in the presence of hydrogen bonding to the atom containing the quadrupolar nucleus. An important difference from NMR, however, is the much greater spectral range that is obtained in NQR. There are large differences in the frequencies of nuclei in different chemical functional groups and even within the same functional group. Although this may be a disadvantage in the design of the instrument and location of the signal, it has one important consequence in pharmaceutical analysis in that NQR is likely to be a highly selective technique. It is a relatively easy matter to distinguish between different chemical species and different polymorphs; even if by coincidence the frequencies are the same, this is only likely to be true at a single temperature and in any case, the relaxation times are almost certain to be different.

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Table 1. Comparison of Theoretical and Experimental Values of the ^{14}N Quadrupole Parameters in Heroin Hydrochloride Monohydrate

	theory		experiment[a,13]	
atom	QCC (MHz)	η	QCC (MHz)	η
N(1)	−1.575	0.098	1.328	0.108
N(2)	−1.399	0.142	1.329	0.128

[a] At 4.2 K.

FREQUENCY PREDICTION

Theoretical calculations, for example, by Gaussian at the HF/6-31+G* level, can now be used to check experimental values of quadrupole coupling constants in cases for which an accurate structure for the molecule is available. Strictly speaking, such programs predict NQCC for a rigid molecule in the gas phase, but some allowance can be made for solid-state effects and hydrogen bonding by including a cluster of molecules at the configuration they adopt in the solid state. Recent examples include Gaussian calculations of the narcotics heroin and cocaine[11,12] and heroin hydrochloride monohydrate,[13] for which X-ray crystal structures and experimental frequencies[13,14] are available. In the case of the latter, two hydrogen-bonded molecules were used in the calculation, a total of 110 atoms, and NQCC and asymmetry parameters were predicted to within 15% of the experimental values, as shown in Table 1 for the two protonated nitrogen atoms, N(1) and N(2) in the two different protonated heroin cations in the unit cell**.** Note that the calculation gives the sign of the NQCC as well as their direction cosines with respect to the axial system used, information not usually available from NQR experiments.

APPLICATIONS OF NQR TO PHARMACEUTICAL ANALYSIS

Although several early papers have emphasized the importance of NQR in both qualitative[15] and quantitative[16,17] analysis and in the detection of explosives,[18] it has only recently been realized that new developments in pulsed rf spectroscopy,[19] some of which we have already referred to, and new methods of improving the signal-to-noise ratio (SNR)[20] suggest the possibility of a much wider application of NQR techniques, particularly at the low radio

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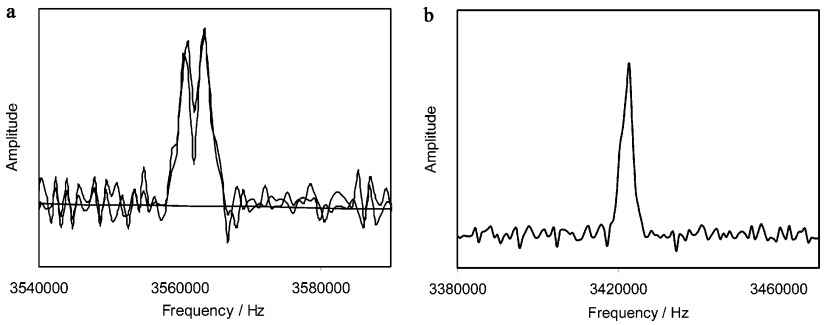
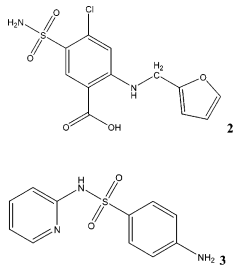


Figure 1. a. Doublet peak from phase I furosemide (Sigma powder) with frequencies 3.564 and 3.561 MHz, splitting ~2.35 kHz and line width ~1.6 kHz for both lines. b. ^{14}N singlet signal at 3.422 MHz from room temperature phase II furosemide with line width ~3 kHz.

frequencies typical of ^{14}N. We illustrate these comments by recent NQR studies of two well-known medicines, furosemide (**2**) and sulfapyridine (**3**).



POLYMORPHISM

An important aspect of pharmaceutical development is the identification and selection of the appropriate polymorphic form, because this can have significant effects on the stability, processability, and bioavailability of a pharmaceutical formulation.

As an important example, furosemide (**2**) has at least two polymorphs, for both of which X-ray crystal structures have been published,[21,22] enabling comparisons to be made with the expected point symmetry of the molecules in the solid state and providing an additional check on the polymorphic form. The crystal contains two quadrupolar nuclei, ^{14}N and ^{35}Cl, and signals from both nuclei have been detected. In this example, both polycrystalline furosemide, as supplied by Sigma, and tablets of differing size marketed as Lasix (Hoechst-Marion-Roussel) were studied and compared. Both gave rise to very similar ^{14}N NQR spectra: near 3.65 MHz, a clear doublet is seen, assigned to the sulfonamide nitrogen, with peaks at 3.564 and 3.561 MHz at room temperature (Figure 1a) and with line widths close to 1.6 kHz.

The doublet structure is predicted from the crystal structure analysis of form I.[21] In comparison with this, recrystallization of this sample from *n*-butanol gives the metastable form II when the line frequency shifts to 3.422 MHz. The change of 0.141 MHz is easily observed when line widths are only a few kilo-Hertz or less. In addition, the line is now a singlet (Figure 1b), in agreement with the prediction of the point symmetry of the molecule in this form.[22]

For furosemide, ^{35}Cl signals have been detected at 77 K;[23] only one frequency is reported, at 36.759 MHz, which appears to correspond to the line at 36.266 MHz at room temperature, an indication of the temperature effects for this nucleus to which we have already referred. Notably, this line has a very short spin−lattice relaxation of 2 ms at room temperature, a value not untypical of the higher frequencies observed for ^{35}Cl nuclei in organic compounds. As a consequence, strong signals can be obtained in a few seconds.

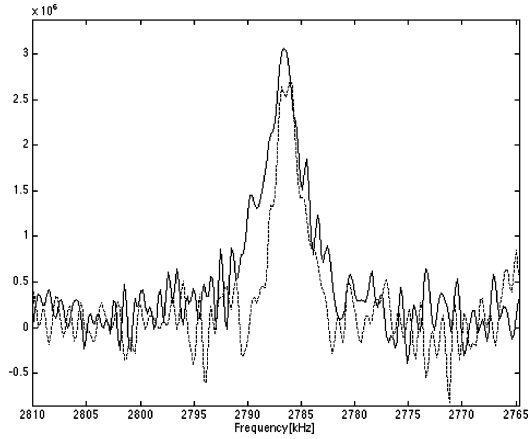


Figure 2. Comparison of the line widths for the 2.787 MHz line of sulfapyridine at room temperature for the powder sample as supplied by Sigma, and recrystallized from ethanol − - − -.

Another rather different example is the bacteriocide sulfapyridine (**3**), which also exists in different morphological forms. In this case, the NQR spectra of material as supplied by Sigma can be compared with material prepared by recrystallization from solvents such as ethanol and acetone (Table 2). Consider the 2.807 MHz line at 77 K, which has been tentatively assigned as ν_+ of the $-NH_2$ group.[24] At room-temperature, its frequency falls to 2.787 MHz, and signals at this frequency can be seen in both the sample from Sigma and that recrystallized from ethanol, indicating that they both consist of the same morphological form. However, their line widths are different, as shown in Figure 2, that of the recrystallized sample (2.5 kHz) being significantly less than that of the raw sample (4.0 kHz). The reason seems to be that, in general, NQR lines are inhomogeneously broadened, due in part to defects and crystalline imperfections but also to the presence

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Table 2. ^{14}N NQR Parameters of Sulfapyridine (Sigma) at ~25 °C

Sigma powder			from ethanol		from acetone		
ν, MHz	$\Delta\nu_{1/2}$, kHz	T_1, s	ν, MHz	$\Delta\nu_{1/2}$, kHz	ν, MHz	$\Delta\nu_{1/2}$, kHz	T_1, s
			1.480 ± 0.001	1.8 ± 0.2			
2.284 ± 0.001	5.0 ± 0.5	1.0 ± 0.1	2.283 ± 0.001	3.0 ± 0.2			
2.393 ± 0.001	3.5 ± 0.5	1.4 ± 0.1	2.392 ± 0.001	1.8 ± 0.2			
2.787 ± 0.001	4.0 ± 0.4	0.65 ± 0.5	2.786 ± 0.001	2.5 ± 0.3	2.895 ± 0.001	2.8 ± 0.3	
2.924 ± 0.001	3.8 ± 0.4	0.70 ± 0.5	2.923 ± 0.001	2.5 ± 0.3	3.060 ± 0.001	1.0 ± 0.1	0.01 ± 0.001

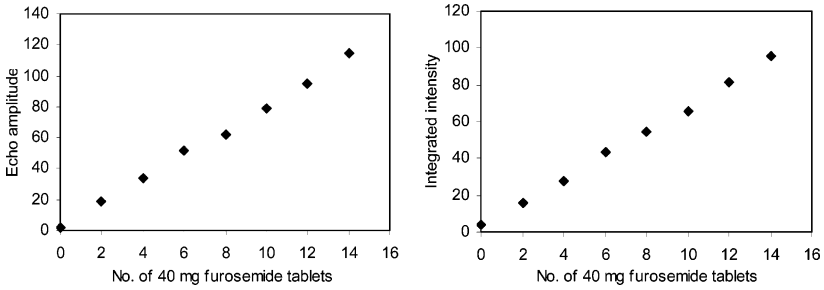

Figure 3. Variation of the ^{35}Cl echo amplitude and integrated echo intensity for furosemide from different numbers of 40-mg tablets.

of impurities or even strain in the material, factors alleviated by recrystallization. This line-broadening suggests a possible role for the technique in quality control.

Table 2 lists some of the NQR parameters at 25 °C of the three different samples of this drug examined; it is clear that the sample as supplied has the same frequencies and spin−lattice relaxation times as that recrystallized from ethanol, as expected, but different line widths, as we have indicated. It is also clear that recrystallization from acetone has produced a different form, which subsequent analysis has shown to be an acetone solvate. Both frequencies and relaxation times are now different, a convincing illustration of the solid-state effects to which we have already referred.

QUANTIFICATION USING ^{35}CL

Previous NQR experiments on inorganic compounds containing quadrupolar nuclei, such as ^{63}Cu in Cu_2O and ^{209}Bi in $BiCl_3$, showed that there was a reasonably linear dependence of peak signal intensity on sample weight.[16] These experiments were performed on a variable-frequency oscillator spectrometer, but there is reason to suppose that an equal or even better performance could be obtained from modern pulsed rf spectrometers. The ^{35}Cl NQR signals in furosemide at 36.266 MHz provides an example. For these experiments, actual tablets were used, and fast echo sequences were averaged over several scans. Standardization against a known sample was straightforward but rarely necessary; however, tablets in a dispensing bottle are best examined in an rf probe with as homogeneous an rf field as possible across the sample, for example, by the use of a coil of variable pitch.[25] In preliminary experiments, a series of eight scans was performed in which the number of tablets was increased by two between scans; the results are shown as plots of the echo amplitude and mean integrated echo intensities against number of tablets in Figure 3. Following these experiments, one sample consisting of four tablets was chosen as an unknown; from the integrated echo intensity, the mean number of tablets was estimated to be 3.82 ± 0.30, with confidence limits of 95%. These are preliminary experiments and could almost certainly be improved by better coil design and thermostating the sample and rf probe; they are now being extended to a study of the ^{14}N signal near 3.56 MHz.

IMPROVING NQR SENSITIVITY

A serious disadvantage of NQR methods involving ^{14}N has always been the relative weakness of signals detected at such low frequencies, in comparison with many hundreds of MHz common in modern NMR spectrometers. Again, recent advances in experimental techniques suggest that this problem could be considerably reduced in several ways. First, the use of cryogenic rf coils, as has already been introduced in high-field NMR, would certainly be an advantage in improving SNR, particularly at the low NQR frequencies characteristic of ^{14}N at which sample losses are minimal. Another development would exploit some of the recent signal enhancement methods developed for NQR spectroscopy,[26,27] of which the most promising is polarization-enhanced NQR (PE-NQR). In this method, the sample, containing both ^{1}H and quadrupolar nuclei, is polarized for a sufficient time in high field, say, at a frequency ν_H of 40 MHz. The field is then switched off or the sample is ejected. As its value falls, level crossing occurs between the ^{1}H levels and the quadrupolar levels (provided the latter lie at less than 40 MHz), whereupon the latter rapidly reach the same spin temperature as the former. A conventional pulsed NQR experiment in zero field would then be predicted to give a signal whose intensity was enhanced by a factor close to the ratio of the two frequencies, ν_H/ν_Q.

Unfortunately, little experimental work has so far been undertaken to check these predictions; one recent example is a PE-NQR study of *p*-nitrobenzoic acid[27] in which the protons were polarized in a magnetic field of 0.2 T (ν_H = 8.5 MHz), and an observed enhancement factor of 7 could be compared with a prediction of 7.4. The success of the method depends critically

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on the spin−lattice relaxation times of both the quadrupolar nuclei and the protons: if these are too short, it may be difficult to cycle the applied magnetic field quickly enough to capture the enhanced NQR signal. As an example of the potential advantages, the NQR signals in Figure 1 were obtained by Fourier transformation of the averaged signal from 16 PSL sequences with $\tau = 1$ ms and a repeat time of 1.5 s, in a total acquisition time of 25 s. In a successful PE-NQR experiment with a polarizing field of 0.5 T, only one PSL sequence would have been needed to achieve the same SNR in a time <0.1 s. This speed of data capture implies that the method could be used to examine signals from single tablets in a blister pack or larger samples on a production line or even within a chemical reactor. The polarizing field need not be homogeneous, provided that the entire sample experiences roughly the same polarizing field.

SUMMARY

Recent advances in instrumentation have shown that NQR shows excellent potential in the field of pharmaceutical analysis due to its simplicity, high selectivity, linear response (in certain situations), and also because it is a noninvasive technique. The high selectivity means that NQR is capable of distinguishing between very similar chemical environments, such as those occurring in different polymorphic forms. The relationship between line width and crystalline perfection indicates roles for NQR for quality control purposes and in the monitoring of, for example, recrystallization processes. The noninvasive and nondestructive nature of the technology allows analysis of the active pharmaceutical compound in situ within a formulation and within its packaging, lending itself to the "black-box" deployment of the technology in the manufacturing process, and possibly in clinical environments. Its quantitative and noninvasive nature will allow the accurate identification and quantification of tablets contained within a closed bottle. Although there are problems with NQR sensitivity (in particular, for ^{14}N), further improvements in techniques and instrumentation may help to address them.

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