Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 298 (2005) 143–152
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Utilization of pure nuclear quadrupole resonance spectroscopy for the study of pharmaceutical crystal forms

S.C. Pérez [a], L. Cerioni [a], A.E. Wolfenson [a,*,1], S. Faudone [b], S.L. Cuffini [b,1]

[a] *Facultad de Matemática, Astronomía y Física, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina*
[b] *Agencia Córdoba Ciencia, Unidad CEPROCOR, Alvarez de Arenales 230, Córdoba, Argentina*

Received 23 August 2004; received in revised form 23 December 2004; accepted 11 April 2005
Available online 23 May 2005

Abstract

Solid-state physical characterization of a pharmaceutical substance is necessary for successful development and approval of the final product. Different physical analytical techniques are available to do so: X-ray diffraction (XRD), IR, Raman, DSC, TG and NMR. Moreover, all of them detect the presence of excipients perturbing the analysis of the pure substance in low doses. In order to study polymorphism and pseudo polymorphism of drug, this paper introduces possible applications of pure nuclear quadrupole resonance, as a non-destructive technique in qualitative and quantitative approaches. Chlorpropamide and diclofenac sodium were used as examples. Unlike the mentioned techniques, the nuclear quadrupole resonance (NQR) signal of pharmaceutical compounds is not perturbed by the presence of solid excipient or other substances unless they possess resonance frequencies in the same frequency range of the compound studied.

Keywords: Pure nuclear quadrupole resonance; Pharmaceutical drugs; Quantitative analysis; Qualitative analysis; Polymorphism and pseudopolymorphism

1. Introduction

Most pharmaceutical products are typically administered in solid dosage form (over 70%) and their physicochemical properties are of high importance in establishing therapeutic efficacy. It is well-known that different polymorphic forms have different properties. Therefore, a good characterization of polymorphism is necessary.

Classical and usually employed methods for the study of pharmaceutical polymorphs include X-ray crystallography, optical microscopy, thermal methods, infrared spectroscopy, solubility and dissolution rate measurements, solid-state NMR (Saindon et al., 1993; Tishmack et al., 2003), near-IR spectroscopy, and Raman spectroscopy (Findlay and Bugay, 1998; Anquetil et al., 2003; Szelagiewicz et al., 1999).

* Corresponding author. Tel.: +54 351 4334051; fax: +54 351 4334054.
E-mail address: wolf@famaf.unc.edu.ar (A.E. Wolfenson).
[1] Fellow of CONICET, Argentina.

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.04.015

In the present work**,** pure nuclear quadrupole resonance (NQR) is introduced as another technique for the investigation of different crystal forms with important advantages in low dose pharmaceutical products. To demonstrate the potentiality of this technique, chlorpropamide and sodium diclofenac were used as test drugs.

2. Theory

Pure NQR is based on the interaction of the nuclear quadrupole moment, used as a sensor, with the electric field gradient (EFG) generated by the surrounding charges (Slichter, 1990). This interaction gives place to discrete energy levels that can be detected, as in NMR by measuring the absorption (dispersion) of a signal in a resonant circuit that contains the sample studied. In this sense, this technique is equivalent to NMR, where discrete energy levels are generated by the interaction of the nuclear magnetic moment with a static external magnetic field.

From a theoretical point of view, it is necessary to begin with a description in terms of the classical charge density of the nucleus ρ. Classically, the quadrupole interaction energy E^2 of a charge distribution density ρ with a potential V due to external sources is:

$$E^2 = \frac{1}{6}\sum_{i,j} V_{i,j} Q_{i,j}$$

where:

$$V_{i,j} = \left.\frac{\partial^2 V}{\partial x_i \partial x_j}\right)_{r=0},$$

$$Q_{i,j} = \int (3x_i x_j - \delta_{i,j} r^2)\rho \, \mathrm{d}v$$

To obtain a quantum mechanical expression for the quadrupole coupling, only the replacement of the classical ρ by its quantum mechanical operator is necessary. The quadrupolar Hamiltonian is then given by:

$$H_\mathrm{Q} = \frac{1}{6}\sum_{ij} Q_{ij} V^{ij} = \frac{1}{6}\frac{eQ}{I(2I-1)}\sum_{ij}\left(\frac{3}{2}(I_i I_j + I_j I_i) - \delta_{ij} I^2\right) V^{ij}$$

where Q_{ij} is the nuclear quadrupole moment tensor, V^{ij} the electric field gradient tensor and $I > 1/2$ is the nuclear spin.

In terms of principal axes of the potential V, such that $V^{ij} = 0$ if $i \neq j$, it results:

$$H_\mathrm{Q} = \frac{e^2 qQ}{4I(2I-1)}\left[(3I_z^2 - I_2) + \eta(I_x^2 - I_y^2)\right]$$

where symbols η and q, called as the asymmetry parameter and the field gradient, respectively, are defined by the equations:

$$eq = V^{zz}, \qquad \eta = \frac{V^{xx} - V^{yy}}{V^{zz}}$$

For an axially symmetric field gradient, the energy levels are given by:

$$E_\mathrm{m} = \frac{e^2 qQ}{4I(2I-1)}(3m^2 - I(I+1))$$

This result holds for both half-integral and integral spins. The energy levels are, thus, doubly degenerated in m. For half-integral spins, there are $I + 1/2$ energy levels all doubly degenerated; while for integral spins, there are $I + 1$ energy levels, where I of these are doubly degenerated, and only one of them with $m = 0$ is non-degenerated. For ^{35}Cl, $I = 3/2$, the resonance frequency is:

$$V_\mathrm{Q} = \frac{e^2 qQ}{2\hbar}$$

NQR frequencies of chlorine atoms in C–Cl bonds are usually in the range 28–43 MHz, while the frequencies of ^{14}N atoms are usually in the range 0.5–3.5 MHz.

The fact that the EFG is due to the distribution of electrical charges in the crystal makes it a very sensitive technique to any structural or dynamical modification that occurs in the crystal. Therefore, different polymorphic forms of a solid compound will have different well-defined NQR frequencies.

Finally, it is important to mention that: (1) the number of lines is proportional to the number of non-equivalent nuclei in the crystal cell and (2) the area under the NQR line is proportional to the number of resonant nuclei at that frequency, and therefore, the intensity of the signal is proportional to the sample size.

Another important characteristic is the line width. Line broadening is mainly due to two factors. The

first one, named homogeneous line broadening (of the order of a few kHz), has its origin in dipolar interactions. The second one, named inhomogeneous line broadening, is due to many factors, such as impurities, vacancies, dislocations, disorder, etc. For this reason, NQR line widths could range from 1 kHz to hundreds or thousands of kHz (disordered systems, amorphous systems).

Other NQR parameters are, as in NMR, the spin–lattice relaxation time (T_1) and the spin–spin relaxation time (T_2). The study of temperature dependence of all NQR parameters makes also possible to detect phase transitions as well as dynamic modifications (molecular reorientations) (Perez and Brunetti, 1995; Meriles et al., 1997; Schneider et al., 1996).

3. Experimental

Chlorpropamide and dichlofenac used in the experiments were provided by Marsing Co. Ltd. A/S and Calao Resfar Division, respectively. The samples measured were not only pure raw materials but also a mixture with excipient (lactose) and commercial tablets.

Non-specific sample preparation for NQR measurements was needed. The sample containers were glass cylinders of 2 cm length and diameter 1 cm. These glass tubes were put inside the resonant coil (Fig. 1). Commercial tablets were introduced directly inside the coil wrapped with Teflon tape.

^{35}Cl NQR measurements were done using a Fourier transform pulse spectrometer (Fig. 1) with a Tecmag

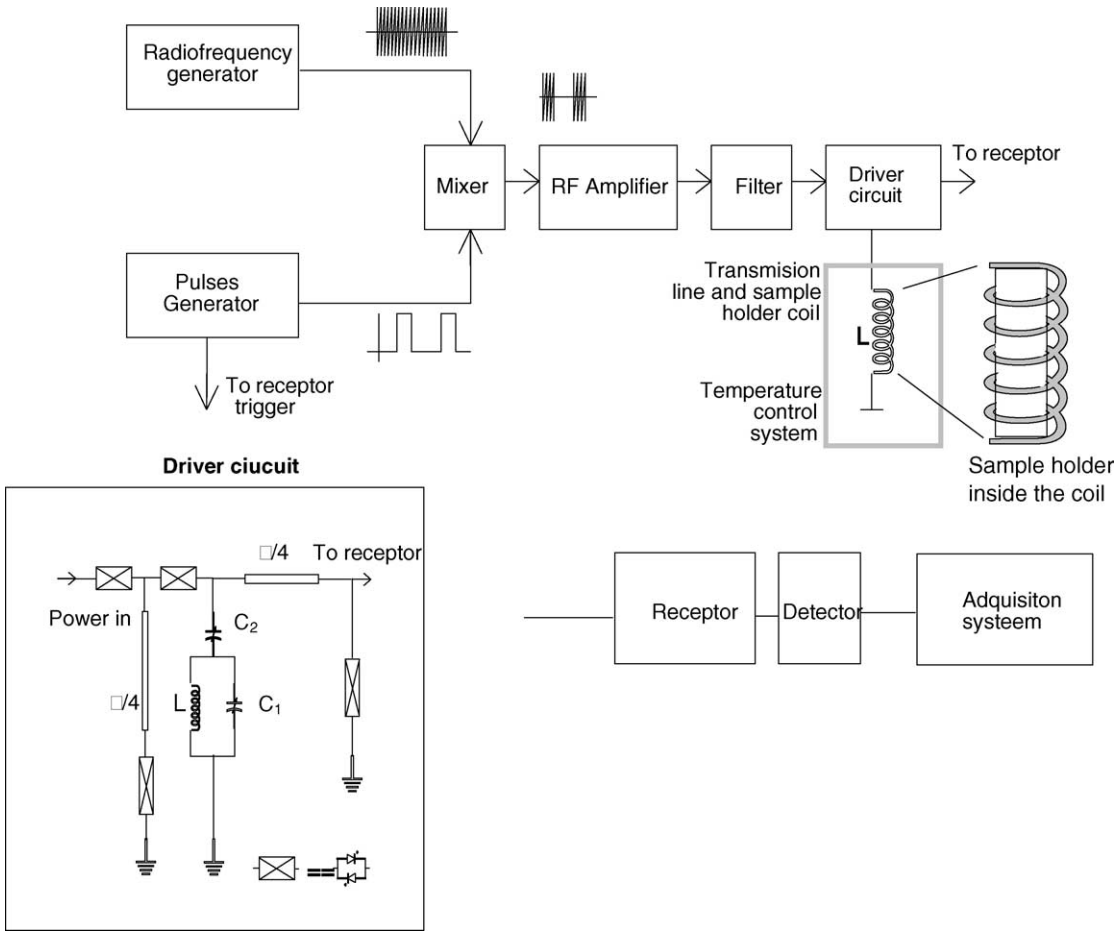

Fig. 1. Schematic representation of an NQR spectrometer.

NMRkit II multi nuclei observe unit, and a Tecmag Macintosh-based real time NMR station. The line shape was obtained by using Spin–Echo Fourier Transform mapping spectroscopy (Bussandri and Zuriaga, 1998). The measurements were made upon the echo by the standard two-pulse $\pi/2$–100 μs–π sequence. The number of average was 5000 and $\pi/2 = 15$ μs.

3.1. T_1 effects

A repetition rate $t_w \geq 10T_1$ is needed with the aim that magnetization is fully recovered after each shot. As an example, $t_w = 100$ ms was set up for chlorpropamide A and C. Their T_1 values are 3.5 and 1.5 ms, respectively.

3.2. T_2 effects

Since all the measurements were made upon the echo at $\tau = 100$ μs, the effect of T_2 on the signal is always the same. Therefore, the intensity of each line must be corrected multiplying by the corresponding factor $e^{2\tau/T_2}$.

In the case of chlorpropamide, $T_2 = 720$ μs for A form, and $T_2 = 630$ μs for C form. In dichlofenac, $T_2 = 422$ μs for the lowest frequency, and $T_2 = 643$ μs for the two upper lines.

X-ray patterns were recorded on Bruker D8 Advance Diffractometer using Cu Kα tube (λ = 1.5418 Å).

4. Results

4.1. Qualitative NQR spectroscopy

Two pharmaceutical compounds containing Cl nuclei have been chosen to present NQR technique. These are: chlorpropamide (Polymorph A) ($C_{10}H_{13}ClN_2O_3S$) and dichlofenac sodium ($C_{11}H_{12}Cl_2N_2O_5$). Fig. 2 shows the NQR spectra of these two compounds at room temperature, and in Table 1 the NQR frequency values are detailed. Chlorpropamide A has only one resonance frequency. It is known that in Polymorph A, there are four molecules in the unit cell (Koo et al., 1980). Since only one line is observed, it is possible to say that all chlorine atoms (one for each molecule) are equivalent in agreement with crystallographic data. In the case of dichlofenac, three lines are observed with area relationship 2:1:1.

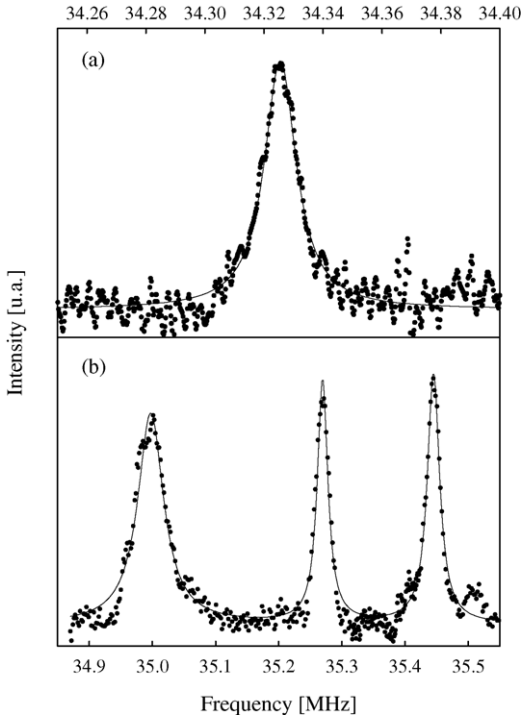

Fig. 2. ^{35}Cl NQR spectra of pure: (a) chlorpropamide A and (b) diclofenac sodium.

Even though no structural information is available, it is possible to infer the existence of two nonequivalent molecules in the reduce cell. Each molecule has two chlorine atoms and it would be expected to have two resonant frequencies; however, the spectrum shows three peaks. Since one of the peaks has double area compared to the other two, this means that two chlorine atoms are contributing to this resonance, and the other two peaks are associated to different chlorine atoms. Therefore, four chlorine atoms in the reduce cell are needed to explain this spectrum. This implies two molecules in the reduce cell.

Table 1
NQR frequency of chlorpropamide A and diclofenac sodium at room temperature

Compound	^{35}Cl NQR frequency (MHz)
Chlorpropamide	34.325
Diclofenac sodium	34.997
	35.269
	35.445

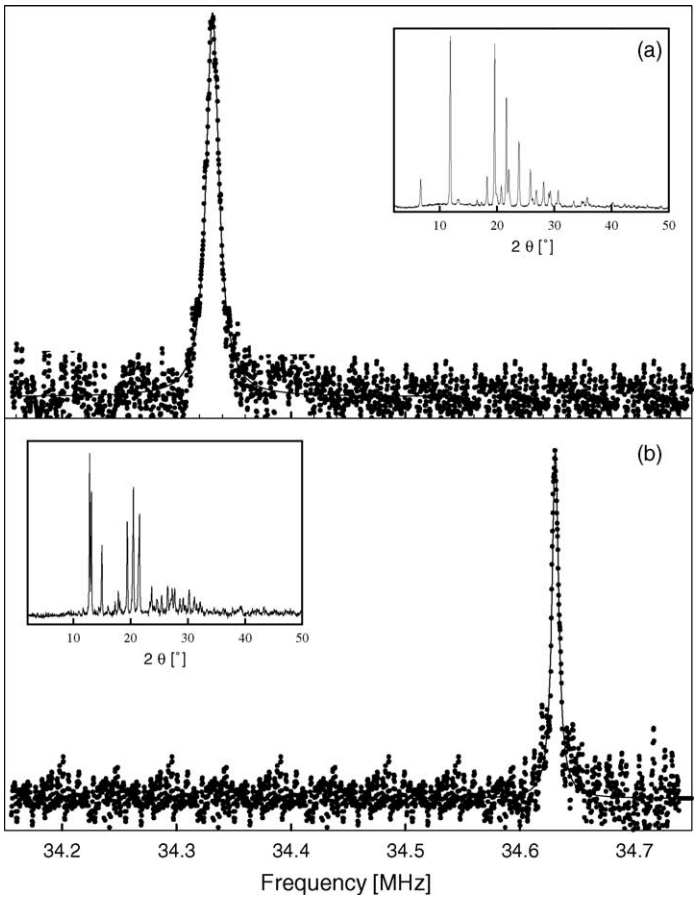

Fig. 3. ^{35}Cl NQR spectra and X-ray patterns of pure chlorpropamide: (a) Polymorph A and (b) Polymorph C.

Similarly, it is also possible to identify different polymorphic forms. In Fig. 3 the spectra of chlorpropamide Polymorphs A and C are compared to their respective X-ray patterns. It can be noticed that not only their frequencies (34.325 MHz for Polymorph A and 34.631 MHz for Polymorph C at room temperature) but also their line width are different (chlorpropamide A: 18 kHz, chlorpropamide C: 8 kHz). The frequency difference is 306 kHz, and it is easily detected by an NQR spectrometer, since it has around 1 kHz frequency resolution. It is important to remember that in NQR, energy is transferred to the nuclear two-level system through a syntonized resonant circuit. Thus, only those nuclei with that resonance frequency will absorb energy. To be sure that there is not a mixture of polymorph, it is necessary to irradiate at both frequencies. If only one polymorph is present, a signal will be gotten at one of the frequencies and not at the other.

Each crystal structure of a molecular crystal presents a spectrum with specific frequencies so that NQR can differentiate not only different compounds but also their polymorphs. As a consequence, it could be used to identify testing if NQR spectra libraries of pure substances were created. In literature, NQR table already exits for many molecular crystals (Chihara and Nakamura, 1997).

4.2. Variable temperature NQR spectroscopy

Polymorph C was obtained by heating Polymorph A from room temperature up to 110 °C. At this temperature, it was necessary to wait for about

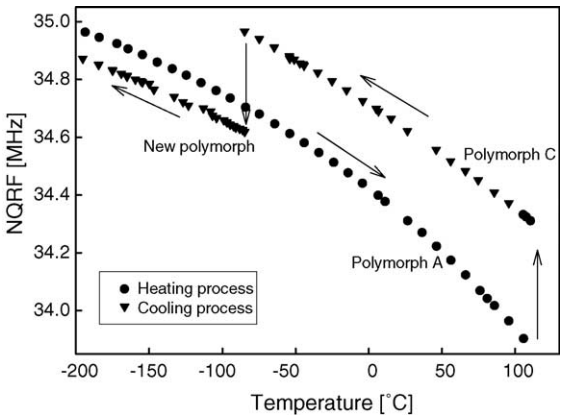

Fig. 4. Temperature dependence of ^{35}Cl NQR frequency: (●) heating process of chlorpropamide Polymorph A from −196 to 110 °C; the upper arrow indicates the transition to Polymorph C; (▼) cooling process of chlorpropamide Polymorph C from a temperature of 110 °C to −85 °C; the down arrow shows the transition to other polymorph different from A and C, which is stable until −196 °C.

half an hour to complete the transition from A to C. After that, Polymorph C is stable until the fusion temperature is reached. Fig. 4 shows the temperature dependence of the NQR frequency in this process. If the heating process is stopped above 110 °C but below the fusion temperature, and then the sample temperature is decreased, it is observed that Polymorph C is stable down to −85 °C. At this temperature, a phase transition occurs to another polymorphic form different from Polymorph A, since its frequency does not match with the corresponding frequency of Polymorph A. Therefore, temperature dependence of NQR frequency allows detecting phase transitions.

4.3. Quantitative NQR spectroscopy

As mentioned above, the area under the NQR line is proportional to the number of quadrupole nuclei present in the sample. Therefore, NQR could be used as a quantitative technique after an appropriate calibration. In order to do the calibration, the peak areas for different known amounts of sample have to be determined by numerical integration. It is important to notice here that parameters, such as τ, t_w, high power of the spectrometer, etc., . . ., must be kept fix through the experiments. Fig. 5 shows a calibration curve of our spectrometer for pure chlorpropamide A. It is observed that it is possible to quantify over than 20 mg of pure active agent.

For this experiment five different glass tubes containing the amount of pure sample, indicated in Fig. 5, were prepared. Two extra glass sample holders were prepared in order to test the calibration. They were both prepared as mixture of lactose with pure chlorpropamide A. One (sample A) had a total mass of 852.9 mg with only 98.2 mg of chlorpropamide, and the other (sample B) had a total mass of 843.7 mg; however, only 52.5 mg were chlorpropamide. Using

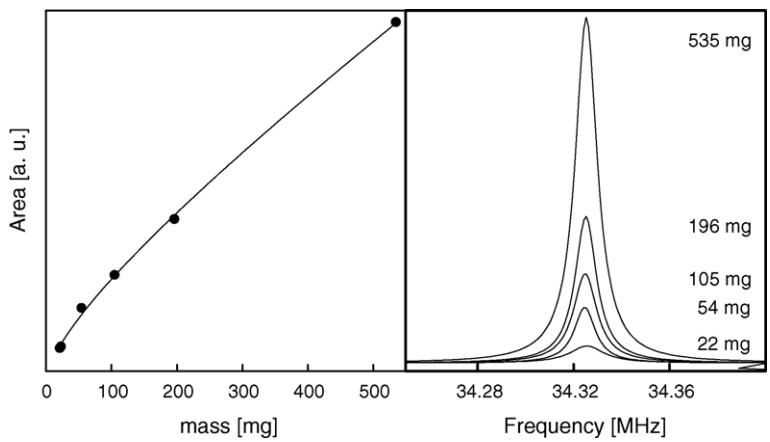

Fig. 5. NQR spectrometer calibration using chlorpropamide Polymorph A. *Right graph:* calibration curve. *Left graph:* fit of NQR spectra obtained with the different mass used to calibrate.

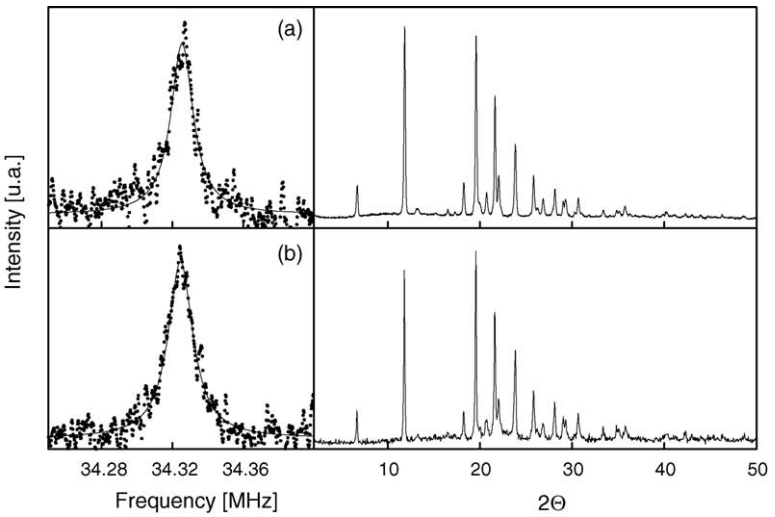

Fig. 6. ^{35}Cl NQR spectra and X-ray pattern of: (a) 250 mg of pure chlorpropamide Polymorph A and (b) commercial tablet containing 250 mg of chlorpropamide Polymorph A and excipients.

the calibration curve, the peak area values of both samples correspond to 93.4 and 49.8 mg of chlorpropamide, respectively. This means a discrepancy in mass determination of approximately 5 %.

It is also possible to detect as low as 10 mg of chlorpropamide. In this case, 15,000 averages are needed so as to improve the S/N ratio, but still the error associated with the peak area determination is high compared to that of samples over 20 mg. These detection and quantification limits will depend on the characteristics of the spectrometer and on the compound itself (i.e. number of quadrupole nuclei in the volume unit, impurities, etc.).

Due to the fact that NQR uses as a sensor a quadrupolar nucleus belonging to the compound under study, the spectrum is not modified by the presence of other pharmaceutical active agents or excipients in the sample unless chemical interactions exist between them or they have resonance frequencies in the same range. As an example, no difference was observed in the comparison of the spectra of a glass tube containing 250 mg of pure chlorpropamide and commercial tablet

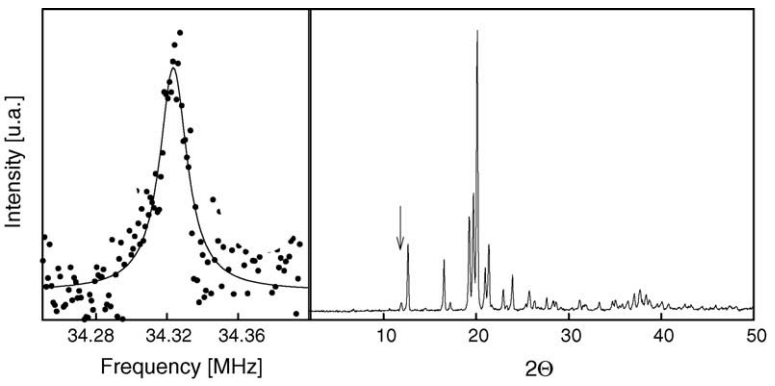

Fig. 7. ^{35}Cl NQR spectra and X-ray pattern of sample containing a mixture of pure chlorpropamide Polymorph A (21 mg) and excipient (lactose 900 mg). In the X-ray pattern, the arrow indicates the one peak corresponding to chlorpropamide that could be clearly identified.

(containing excipients) with an equal dose of the active agent (Fig. 6). Other techniques frequently used in the characterization of pharmaceutical compounds are seriously affected by the presence of excipients, especially dealing with small amounts of the active agent, such as X-ray data. Fig. 7 presents the NQR spectra and X-ray pattern of 21 mg of chlorpropamide A mixed with 900 mg of lactose. While NQR spectrum is not affected, X-ray pattern is affected by the overlapping of peaks corresponding to the lactose and clearly only one peak of chlorpropamide can be identified. At least 10 peaks are advisable in X-ray diffraction to identify the crystal structure of a compound. Unfortunately, no example of pharmaceutical compounds or excipients with resonance frequencies in the same range is available. Nevertheless, it is possible to mention an example of a molecular crystal, where three different phases of a same compound coexist. This is the case of *p*-chlrofluorobenzene (Cerioni et al., 2004). When this compound is quenched to liquid nitrogen temperature, a coexistence of phases appears with frequencies in the same range. The reference shows how this problem can be solved, and the frequencies assigned to each of the crystal phases.

It is also possible to determine the percentage of each crystalline polymorph in a mixture of them using the area under the NQR peaks. For this experiment, six glass tubes containing the mixture of Polymorphs A and C were prepared in different proportions. After acquiring the NQR lines at both resonance frequencies, the percentage of form C in the total sample was possible to calculate as the area of Polymorph C peak over the sum of the area of both peaks. Fig. 8 shows these results as a function of the percentages obtained by weight. NQR results are in good agreement with those obtained by weight. It is important to note that a previous calibration for the percentage determination is not necessary.

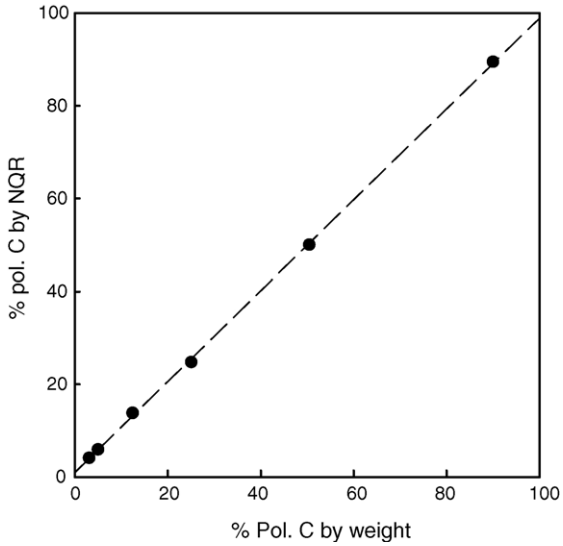

Fig. 8. NQR percentage vs. weight determination percentage of Polymorph C in samples containing mixtures of Polymorph C/Polymorph A.

Finally, the quantification process of a mixture of crystalline and amorphous forms in a sample consists in the determination of the amount of crystalline form, since the amorphous phase is quite difficult to detect (very broad NQR line of about hundreds or thousands of kHz). Therefore, a previous calibration of the spectrometer for the crystalline form, and a quantification of the total amount of active agent presents in the sample using typical pharmacopeial techniques are necessary. As an example, mixtures of crystalline (form A) and amorphous chlorpropamide with lactose were prepared (see Table 2). In Fig. 9, the spectra of

Table 2
Amorphous content determination

	Sample 1		Sample 2	
	By weight	By NQR	By weight	By NQR
Excipient (lactose) (mg)	653		640	
Crystalline sample (mg)	98	93[a]	50	46[a]
Amorphous sample (mg)	102	107[b]	150	154[b]
% amorphous	51	53	75	77

[a] Crystalline sample weight obtained using the calibration curve shown in Fig. 5.
[b] Amorphous sample weight obtained by difference between the total sample weight and crystalline sample weight.

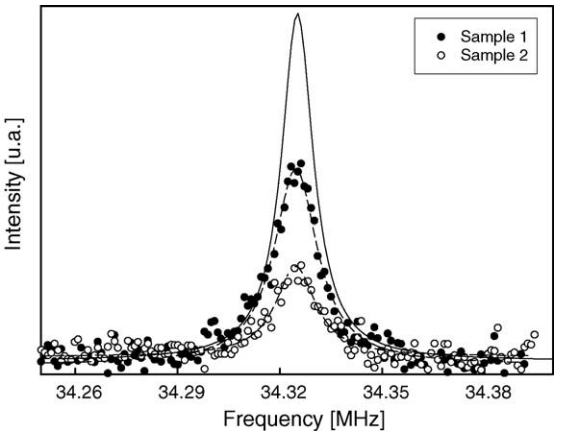

Fig. 9. Quantification of amorphous/crystalline mixtures by NQR. The spectra of two different amorphous/crystalline/excipient mixtures are shown: (●) 98 mg/102 mg/653 mg and (◯) 150 mg/50 mg/640 mg. The solid line indicates the signal corresponding to 200 mg of pure chlorpropamide Polymorph A. The signal intensity loss corresponds to the amorphous part of the sample.

two different crystalline/amorphous/lactose mixtures are shown compared to that corresponding to a total amount of chlorpropamide in crystalline state. The differences in area represent the part of sample in amorphous state. Also, in this case, the limit for crystalline form quantification is the same mentioned above.

5. Advantages (disadvantages) of technique

In order to detect NQR, the compound has to be in solid state and possess a quadrupolar nucleus, i.e. N, Cl, Br, I, etc. ($I > 1/2$). At first sight, it could seem that the use of this technique is very limited, but it is important to notice that many of the pharmaceutical compounds have nitrogen atoms, or are in chlorhydrate form.

The NQR spectrum is unique for each substance, since it is due to the EFG distribution of the sample itself, and usually, no more than three or four peaks characterize the spectrum.

No special sample preparation is needed. The only problem could be associated with finite-size effects, i.e. when the surface/bulk ratio of the grains is large, the line is quite widened. In this case, a recrystallization of the compound would be needed to observe the signal.

Another problem may arise if the excipients or other active agents have resonance frequencies in the same range. It is quite improvable that this happens, since it would be necessary that not only they have the same quadrupole nucleus than the substance studied, but also that they have similar EFG distribution. This is more likely to happen with different phases of the same compound; however, even in this case, it is possible to assign the frequencies to different crystal states.

6. Conclusions

Applications of NQR technique in solid-state characterization of pharmaceutical compounds have been presented. This technique could be used to identify and quantify different polymorphs of pharmaceutical agents. The detection limit for the studied drugs in the present work is 10 mg, and the quantification limit is 20 mg, with an error of 5%. It is important to emphasize that NQR measurements could be carried out with the presence of excipients in the sample without spectrum modifications probably in most cases. The properties of NQR technique allow to study not only pure raw materials but also different mixtures, such as several active agents, different polymorphs, and amorphous/crystalline mixture. For this reason, NQR could be a powerful technique to detect soild-state uniformity content in intermediate and final products.

Acknowledgements

The authors want to thank SeCyT-UNC, CONICET and Agencia Córdoba Ciencia of Argentina for financial support.

References

Anquetil, P.A., Brenan, C.J., Marcolli, C., Hunter, I.W., 2003. Laser Raman spectroscopic analysis of polymorphic forms in microliter fluid volumes. J. Pharm. Sci. 92, 149–160.

Bussandri, A., Zuriaga, M., 1998. Spin–echo mapping spectroscopy applied to NQR. J. Magn. Res. 131, 224–231.

Cerioni, L., Pérez, S., Wolfenson, A., 2004. DTA and NQR studies of polymorphism in *p*-chlorofluorobenzene. J. Phys. Chem. Solids 65, 1133–1140.

Chihara, H., Nakamura, N., 1997. Nuclear Quadrupole Resonance Spectroscopy Data. Springer.

Findlay, W., Bugay, D.E., 1998. Utilization of Fourier transform-Raman spectroscopy for the study of pharmaceutical crystal forms. J. Pharm. Biomed. Anal. 16, 921–930.

Koo, C.H., Cho, S.I., Yeon, Y.H., 1980. Arch. Pharmacol. Res. 3, 37–49.

Meriles, C.A., Pérez, S.C., Brunetti, A.H., 1997. Nuclear quadrupole spin–lattice relaxation due to molecular reorientations in crystals with orientational disorder. J. Chem. Phys. 107, 1753–1758.

Perez, S.C., Brunetti, A.H., 1995. An NQR study of thermally activated motions of nitro groups and of the phase transition in 2,6-dinitrochlorobenzene. Phys. Condens. Matter 7, 101–110.

Saindon, P.J., Cauchon, N.S., Sutton, P.A., Chang, C.J., Peck, G.E., Byrn, S.R., 1993. Solid-state nuclear magnetic resonance (NMR) spectra of pharmaceutical dosage forms. Pharm. Res. 10, 197–203.

Schneider, J., Wolfenson, A., Schürrer, C., Brunetti, A., 1996. Partial disorder and molecular motion of 4-chlorobiphenyl studied by 35Cl NQR and Raman spectroscopy. Phys. Rev. B 53, 9045–9051.

Slichter, C.P., 1990. Principles of Magnetic Resonance, third ed (Chapter 10).

Szelagiewicz, M., Marcolli, C., Cianferani, S., Hard, A.P., Vit, A., Burkhard, A., von Raumer, M., Hofmeier, U.Ch., Zilian, A., Francotte, E., Schenker, R., 1999. In situ characterization of polymorphic forms: the potential of Raman techniques. J. Therm. Anal. Cal. 57, 23–43.

Tishmack, P.A., Bugay, D.E., Byrn, S.R., 2003. Solid-state nuclear magnetic resonance spectroscopy: pharmaceutical applications. J. Pharm. Sci. 92, 441–474.