Detection of polymorphism using ^{14}N NQR spectroscopy

V. Jazbinšek[1], J. Pirnat[1], Z. Trontelj[1], Z. Lavrič[2], S. Srčič[2]

[1]Institute of Mathematics, Physics and Mechanics, Ljubljana (IMFM)
[2]University Ljubljana, Faculty of Pharmacy (UL-FFA)

vojko.jazbinsek@imfm.si

SCIRES Zagreb, 28 June 2017

Overview

- The aim of this work was to demonstrate the value of Nuclear Quadrupole Spectroscopy (NQR) in nondestructive and reliable detecting of polymorphism in active pharmaceutical ingredients (API).
- First, I will start with a brief overview of methods that can be applied in determination and/or confirmation of polymorphism in API
- Second, I will described the NQR method applied to ^{14}N nuclei, which appears in a large number of organic and inorganic compounds, and can be therefore very effective in studying their structure, polymorphism and structural dynamics.
- Then, I will show in details results obtained on antibacterial drug sulfanilamide.
- Discussions and Conclusions.

Introduction

- Appearance of polymorphism in an active pharmaceutical ingredient (API) is an important property.
- Several methods, predominantly the spectroscopic ones
  - X-rays diffraction - XRD
  - infrared - IR
  - Raman
  - radio-frequency (RF) spectroscopic methods (NMR, NQR)

  together with differential thermal analysis are applied in determination and/or confirmation of polymorphism during the process of studies of crystallization of APIs, as well as during production, development and manufacturing of drug delivery systems and during their shelf life.
- XRD became the golden standard in determination and confirmation of polymorphism of drugs. It is the most suitable one from the point of view of polymorphism definition. However, it usually requires a special sample preparation and is less suitable when there is a need for checking the possible appearance of polymorphism during the drug production process.

Introduction

- We have noticed during our studies of different pharmaceutical products and during the detection of some counterfeited drugs and illicit materials in the last 10 years that nitrogen NQR (^{14}N NQR) reveals nondestructively, quickly and reliably the presence of a given active pharmaceutical ingredient and its polymorphism [1-4].
- In this work, we will demonstrate the value of ^{14}N NQR RF spectroscopy in nondestructive and reliable detection of polymorphism in the antibacterial drug sulfanilamide.

[1] Z. Lavrič, J. Pirnat, J. Lužnik, U. Puc, Z. Trontelj, S. Sri, ^{14}N Nuclear Quadrupole Resonance Study of Piroxicam: Confirmation of New Polymorphic Form V, *J.Pharm. Sci.* **104**: 1909-1919 (2015)

[2] J. Lužnik, J. Pirnat, J. Pirnat, V. Jazbinšek, Z. Lavrič, V. Žagar, S. Srčič, J. Seliger, Z. Trontelj, ^{14}N Nuclear Quadrupole Resonance Study of Polymorphism in Famotidine, *J. Pharm. Sci.* **103**: 2704-2709 (2014)

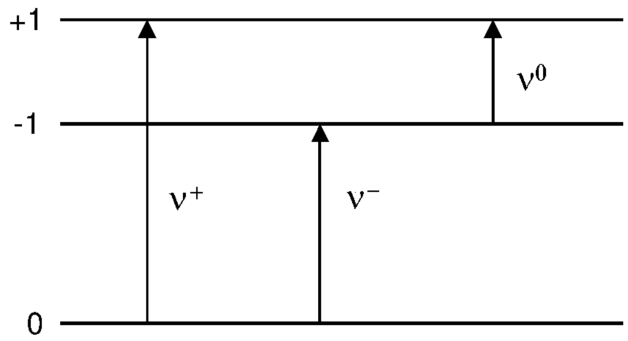
[3] Z. Lavrič, J. Pirnat, J. Lužnik, J. Seliger, V. agar, Z. Trontelj, S. Srčič, Application of ^{14}N NQR to the study of piroxicam polymorphism, *J.Pharm. Sci.* **99**: 4857-4865 (2010)

[4] J. Pirnat, J. Lunik, V. Jazbinek, V. Žagar, J. Seliger, T. M. Klapoetke, Z. Trontelj, ^{14}N NQR in the tetrazole family, *Chem. Phys.* **364**: 98-104 (2009)

Nuclear quadrupole resonance (NQR)

- Nuclear quadrupole resonance (NQR) is a nondestructive, contactless radiofrequency (RF) spectroscopic method related to nuclear magnetic resonance (NMR).
- Unlike NMR, NQR transitions of nuclei can be detected in the absence of a magnetic field (so called "Zero Field NMR").
- NQR is based on the electric interaction between nuclei with non zero electric quadrupole moment (spin ≥ 1) and the internal electric field gradient (EFG).
- Since the EFG at the location of a nucleus in a given substance is determined primarily by the valence electrons involved in the particular bond with other nearby nuclei, the NQR frequency at which transition occurs is unique for this substance.
- Solid samples in their final form (powders, granulates, tablets, pellets, etc.) can be examined without any modification, even in their original packaging.
- ^{14}N nuclei (spin 1) appears in a large number of organic and inorganic compounds, so ^{14}N NQR can be very effective in studying their structure, polymorphism, and structural dynamics. As such, the method has potential application in analysis in the areas of pharmaceutical research, quality control of manufacturing processes, and detection of illicit materials (explosives, counterfeit drugs, etc., see `http://www.conphirmer.com/` - EU FP7 Security project, Grant No. 261670)

^{14}N NQR energy levels and allowed transitions



- ^{14}N nuclei have spin I=1
- Three NQR transition frequencies:
  $\nu^{\pm} = \frac{1}{4} Q_{cc}(3 \pm \eta)$, $\nu_0 = \nu^+ - \nu^- = \frac{1}{2} Q_{cc} \eta$,

  at which transitions occur are unique for a given substance and depends only on quadrupole coupling constant Q_{cc} and asymmetry parameter η.
- The quadrupole coupling constant $Q_{cc} = \frac{e^2 qQ}{h}$ is proportional to
  - nuclear electric quadrupole moment eQ, and
  - maximal component $eq = q_{zz}$ of electric field gradient (EFG) tensor.

  where e is electron charge and h is Planck constant.
- The asymmetry parameter is defined as $\eta = (q_{xx} - q_{yy})/q$.
- Q_{cc} and η can be calculated if we know two of the above transition frequencies.

Polymorphism in antibacterial drug sulfanilamide

- Three known polymorphic forms α, β and γ
- and two chemically nonequivalent ^{14}N atoms:

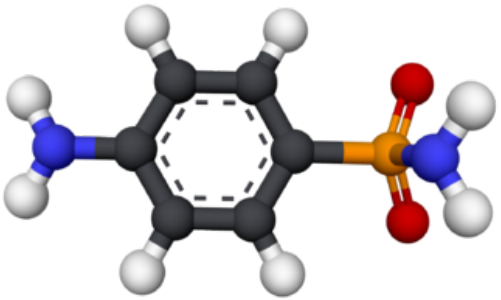
  N(1) – para amino nitrogen
  N(2) – sulfonamide nitrogen

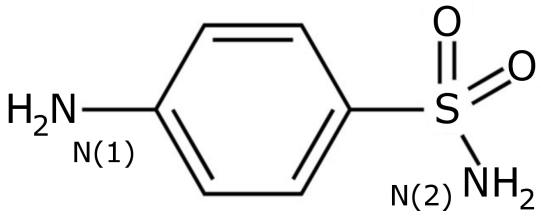
- give two sets of three transition frequencies (ν^+, ν^-, ν^0), different for each polymorph.
- Polymorphic forms α and β were obtained by crystallization of commercially available sulfanilamide (Sigma-Aldrich):

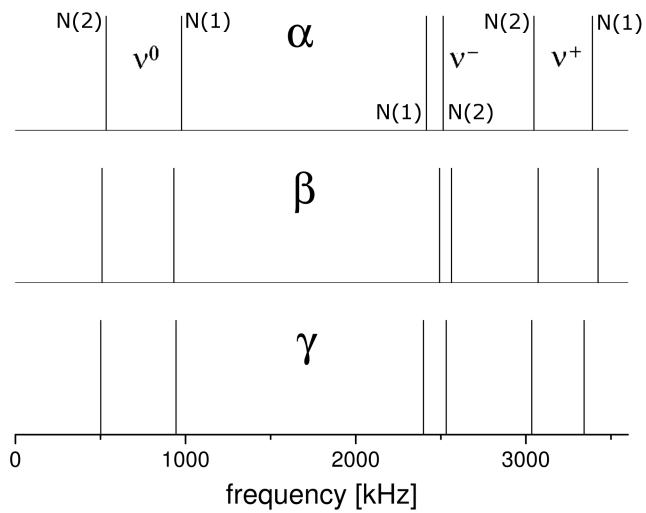
  i) in isoamyl or n-butyl alcohol for α, and
  ii) ethyl alcohol for β polymorph

  At T $>$ 390 K these two polymorphic forms exhibit a transition to γ polymorph.

- All three polymorphs are stable at room T.







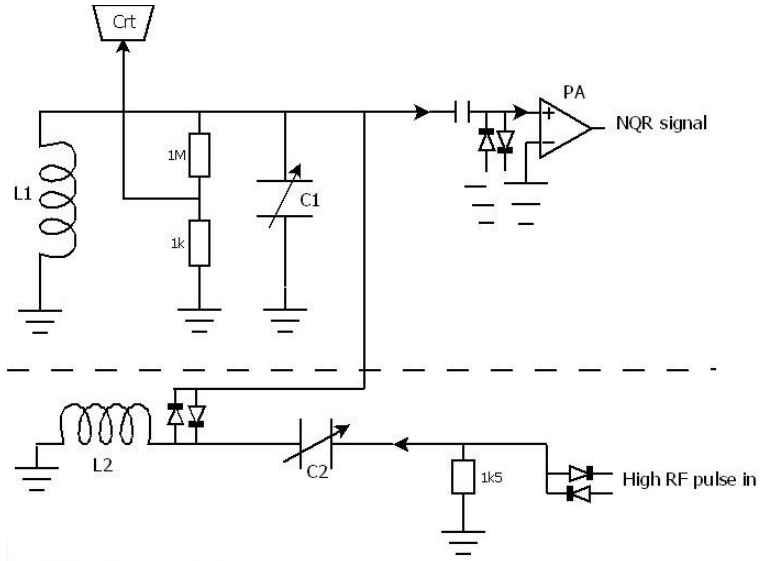
^{14}N NQR frequencies in sulfanilamide.

NQR spectrometer

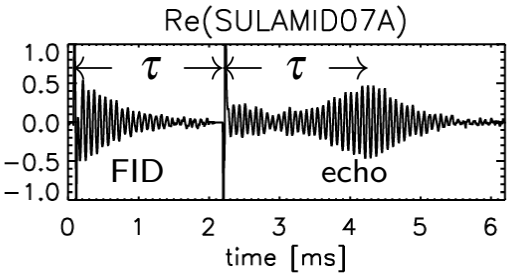
We used a standard pulsed NQR spectrometer consisting of

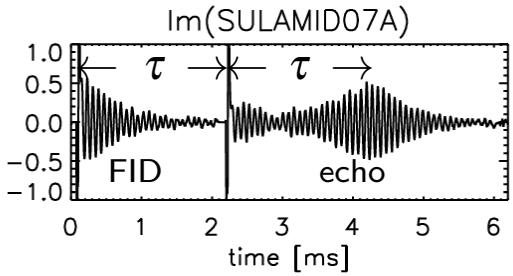
- two tunable coupled LC circuits:
  1. with a sample in the solenoid coil L1, preamplifier and home made receiver
  2. with "step-up" coil L2 attached to
     - the RF pulse programmable unit (Spin Core)
     - the power RF amplifier (Tomco)

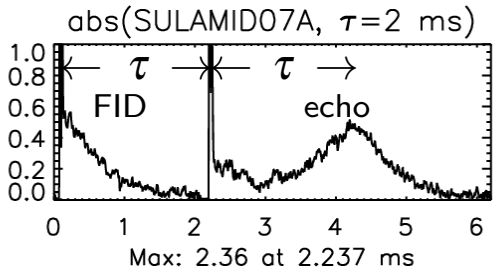
The whole spectrometer was operated from a PC.



Typical measurements: intial RF pulse generates free induction decay (FID), followed by so called refocusing RF pulse at time τ, which creates an echo at $\Delta t = \tau$.





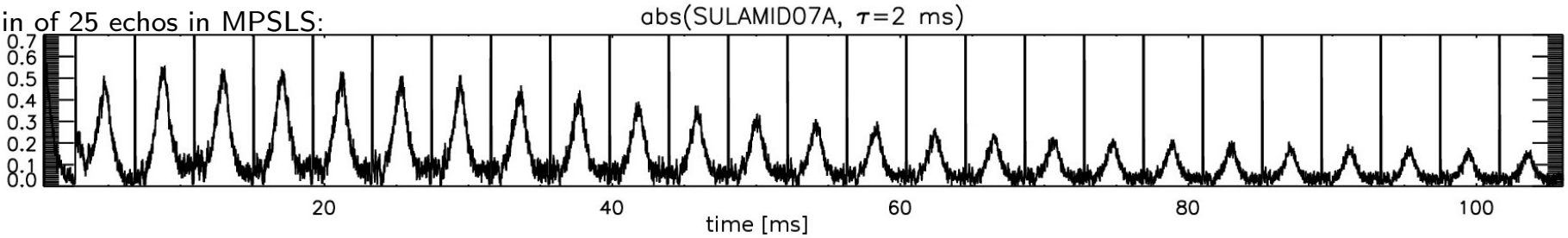


Multi-pulse spin-locking sequence (MPSLS) [5]

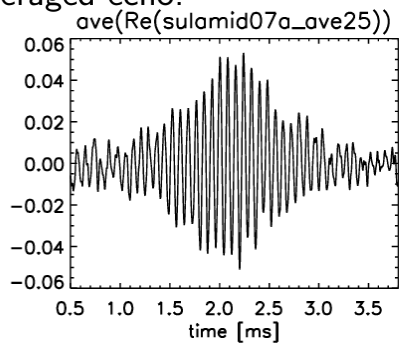
To improve S/N and to speed up these measurements, we applied the MPSLS $t_\varphi - (\tau - t_{\varphi+90} - \tau)_n$, where

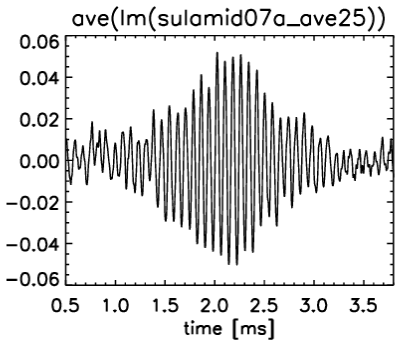
- t_φ is a duration of initial RF pulse ($\pi/2$-pulse in NMR)
- $t_{\varphi+90}$ is a duration of echo forming refocusing pulse (π-pulse in NMR), where (90) indicates a 90° phase shift relative to the previous t_φ pulse
- τ is time delay beetwen t_φ and the first $t_{\varphi+90}$ pulse. It is also a time between a given $t_{\varphi+90}$ pulse and the next echo peak.
- n is a total number of $t_{\varphi+90}$ pulses in the MPSLS sequence.

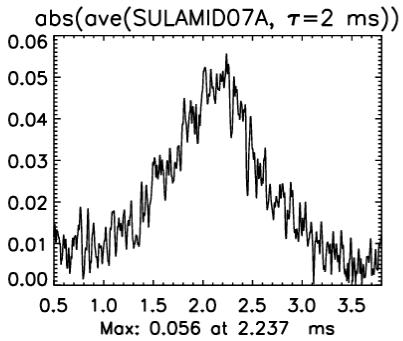
Train of 25 echos in MPSLS:

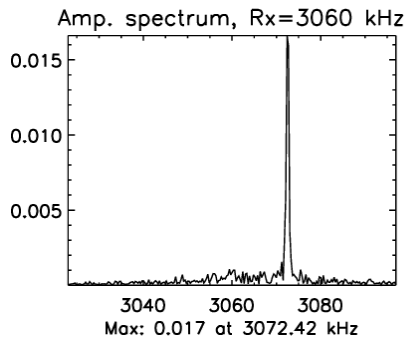


Averaged echo:





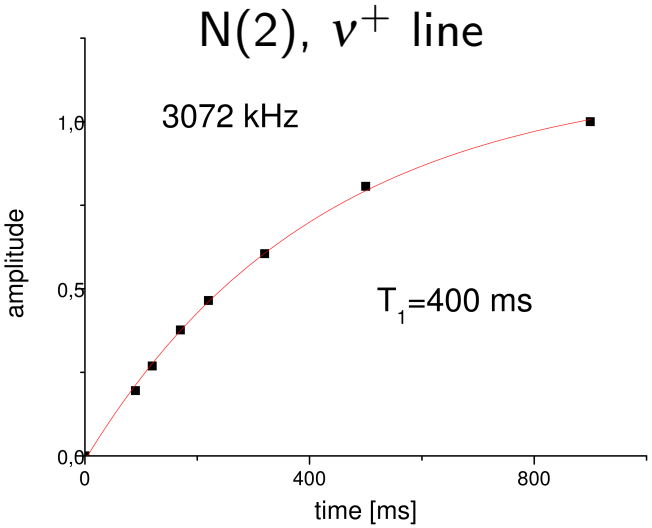


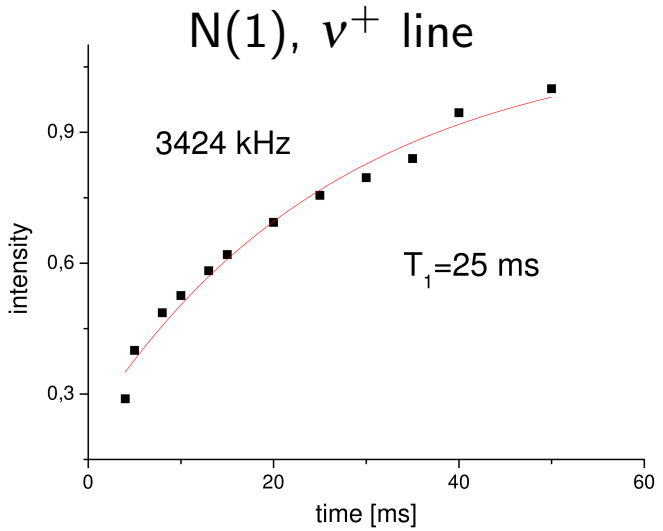


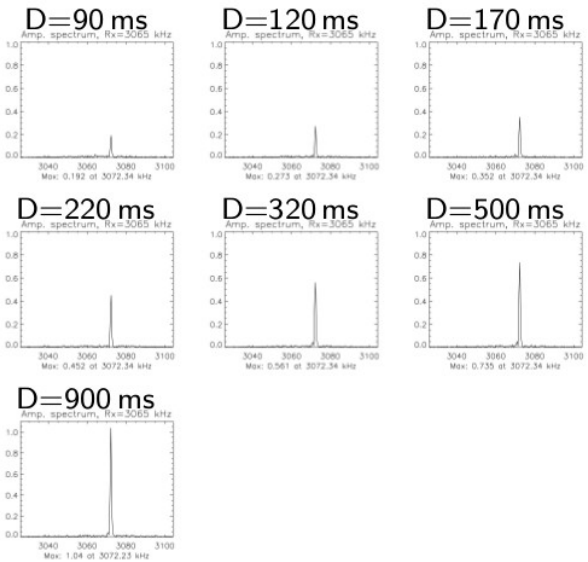
[5] R. A. Marino, S. M. Klainer, Multiple spin echoes in pure quadrupole resonance, *J. Chem. Phys.* **67**: 3388–3389 (1977)

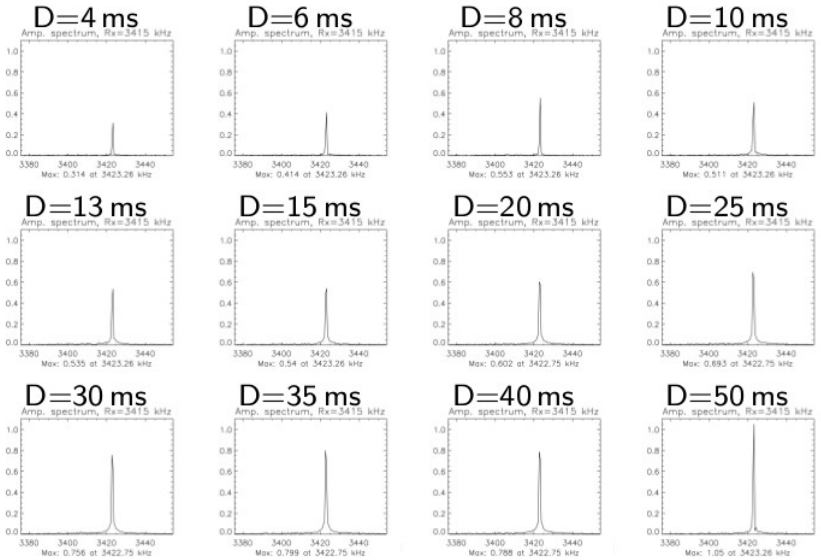
Spin-lattice ralexation time T1 for β polymorph

Measurements with increasing delays (D) between subsequent MPSLS.









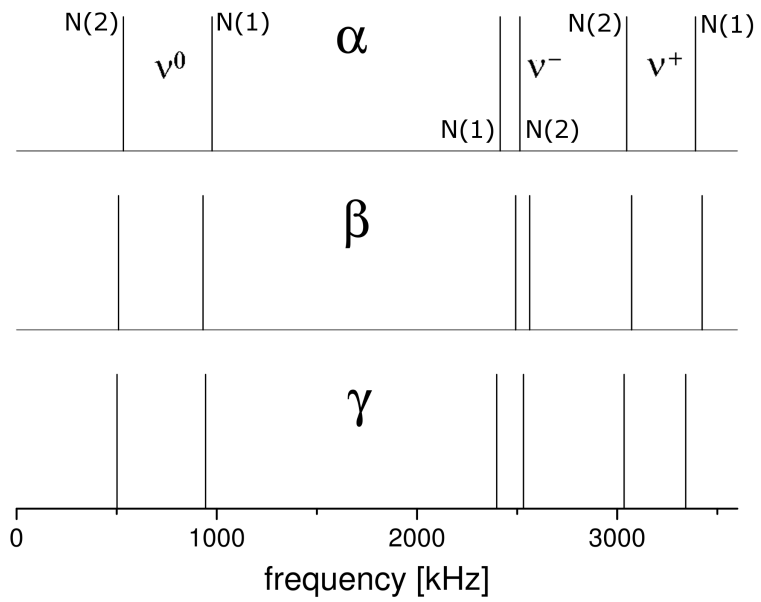
Summary of results

polymorph	atom	ν^+ [kHz]	ν^- [kHz]	ν^0 [kHz]	Q_{cc} [kHz]	η	T_1 [ms]
α	N(1)	3391	2415	976	3871	0.50	25
	N(2)	3047	2514	533	3707	0.29	400
β	N(1)	3424	2493	931	3945	0.47	25
	N(2)	3072	2563	509	3757	0.27	400
γ	N(1)	3342	2398	944	3827	0.49	25
	N(2)	3034	2532	502	3711	0.27	25

^{14}N NQR parameters of α, β and γ polymorphs of sulfanilamide at room temperature:

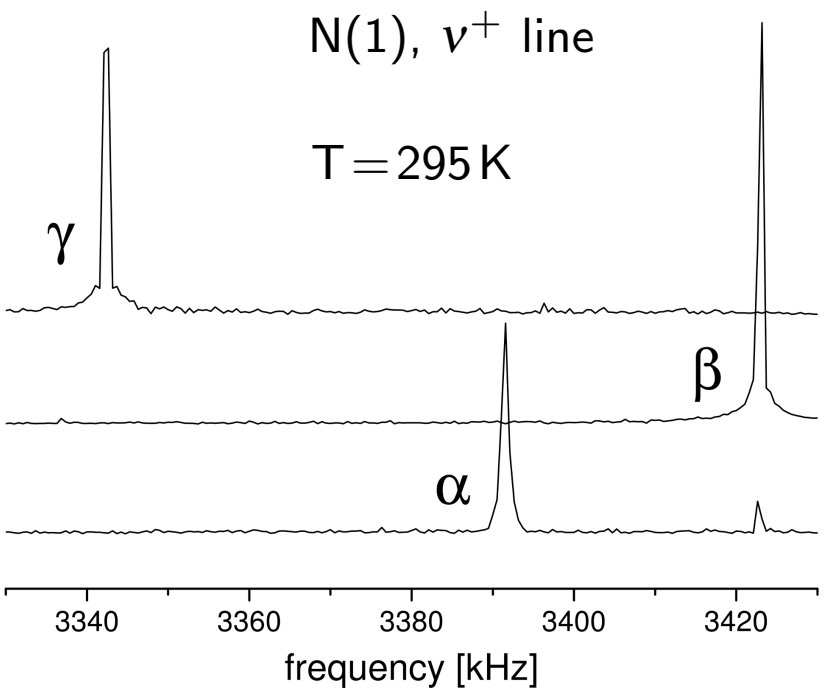
- transition frequencies (ν^+, ν^-, ν^0,)
- nuclear quadrupole coupling constants Q_{cc}
- asymmetry parameters η and
- spin-lattice relaxation time T_1

The upper rows belong to N(1) and the lower rows to N(2) atoms in sulfanilamide molecule.



^{14}N NQR transition frequencies in sulfanilamide.

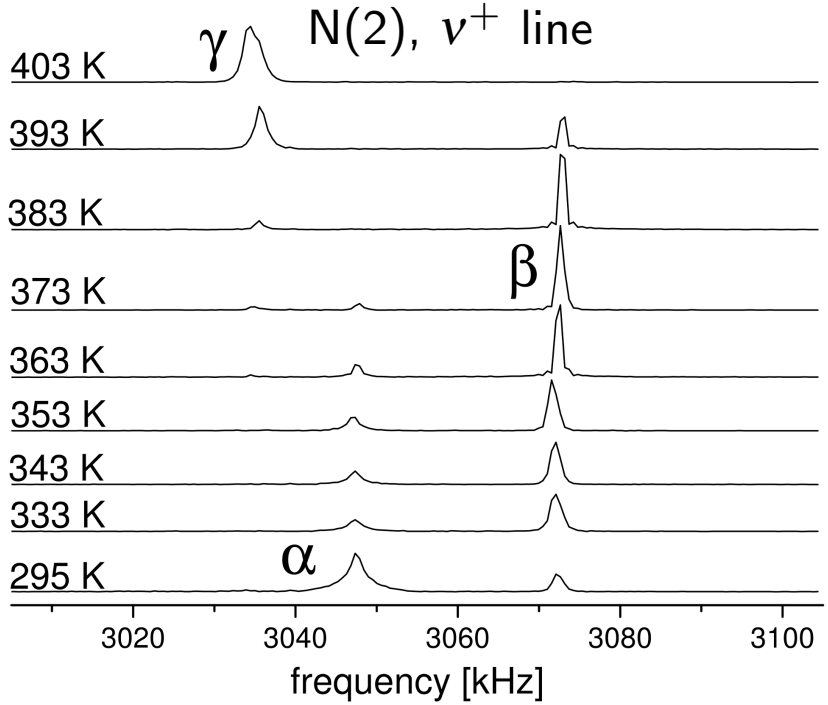
Results



Part of ^{14}N NQR spectra of nitrogen (N1) with the frequency ν^+:

- 3391 kHz for α,
- 3424 kHz for β and
- 3342 kHz for γ polymorph.

Note the traces of presence of β polymorph in the ^{14}N NQR scan of α polymorph.



^{14}N NQR spectra of nitrogen N(2) (ν^+ line) displaying a transition of the initial α polymorph at 295 K (with traces of β form) to the final γ polymorph. The sulfanilamide sample was thermally treated at different temperatures, denoted at the left side of each ^{14}N NQR scan, prior to the ^{14}N NQR measurements.

Discussion and Conclusions

- Results show that ^{14}N NQR resonance frequencies are clearly different for each polymorph in sulfanilamide. They also differ for the N(1) and N(2) nitrogen.
- ^{14}N NQR is therefore a very powerful non-destructive analytical tool for detecting polymorphism with a possibility to clearly distinguish among different polymorphs.
- The advantage of this method in comparison to other methods such as XRD, NMR, Raman and IR spectroscopy, is that in the NQR there is no special sample preparation. Solid samples in their initial forms (powders, granulates, tablets etc.) can be used even in their original package if it is not completely metallic.
- After complete ^{14}N NQR sets of transition frequencies for all possible sulfanilamide polymorphic forms are known, only one spectral line with the highest S/N ratio for each polymorph needs to be checked to identify or confirm the polymorph.
- The method is also fast enough so that the time needed for such tests is not critical. It takes only a few minutes to get a sufficiently well resolved ^{14}N NQR signal in sulfanilamide.
- In conclusion, we demonstrated a convincing use of ^{14}N NQR for quick, non-destructive and reliable determination of polymorphism appearance in solid pharmaceutical samples containing nitrogen. This method could play a role of the secondary standard besides the primary one XRD.