

NMR study of heterogeneity in pyridine-N-oxide...HCl crystal^a

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Abstract: An origin of narrow ¹H NMR signals in pyridine-N-oxide (PyO)...HCl crystal has been investigated by means of MAS, SPEDAS, NOESY and COSY techniques. Spectra of crystalline samples are compared with those of solid phase obtained from liquid PyO...HCl solutions (in acetonitrile/H₂O) after the heterogeneous phase separation. It has been concluded that partially resolved peaks in ¹H NMR spectra of solids are related with heterogeneity of spin system and presence of different H-bond clusters of water molecules. NOESY spectra show no cross-peaks even at very long mixing time (500 ms). This indicates there is no exchange process between spins causing different peaks, and thus the corresponding molecular aggregates are captured in “islands of mobility” without any channels sufficient for exchange. Appearance of MAS side bands as “pseudo” cross-peaks in 2D NMR spectra using MAS/COSY technique is reported. In the case of accidental coincidence of spinning frequency (ω_{MAS}) with spectral distances between some diagonal signals, intensive non-diagonal peaks are observed at the corresponding cross-positions. A misleading conclusion concerning spin coupling is easy to avoid using various ω_{MAS} .
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^a This work is dedicated to Professor Robert Blinc on the occasion of his 70th birthday.

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1 Introduction

Narrow NMR signals in solid samples have been observed in many disordered organic crystals and in some ordered crystalline phases close to the transition (melting) point [1–3]. Recently a similar behavior has been found in molecular systems trapped in nanostructural cavities [4], [5]. It is expected that modern NMR methods, such as 2D NMR together with magic-angle spinning (MAS) technique [6–11], could possibly shed new light on explaining peculiarities of molecular mobility in disordered crystals, microheterogeneous and porous media.

A pyridine-N-oxide (PyO)...HCl crystal has been chosen for this study. It is well-known molecular system that exhibits a very strong hydrogen bond (H-bond) with well-expressed ionic features [12]. Its crystals are rather easy to obtain from water as well from alcohol solutions.

In this work we report some new results concerning an origin of narrow ^1H NMR signals in PyO...HCl crystal investigated by means of MAS, SPEDAS, NOESY and COSY techniques. Spectra of crystalline samples are compared with those of a solid phase obtained from liquid PyO...HCl solutions (in acetonitrile/ H_2O) after the heterogeneous phase separation.

2 Experimental

NMR experiments were carried out on a Varian Unity plus-300 NMR spectrometer operating at 300 MHz for proton using a Doty dynamic angle-spinning (DAS) probe. Monocrystals of PyO...HCl were ground and put into ceramic rotor of DAS in dry-box. For MAS spectra the sample-spinning rate was usually 2 - 4 kHz, and the error in the spinning rate was less than ± 10 Hz. All measurements were performed at 293 K. The temperature was controlled with an accuracy of $\pm 0,5$ K.

The delayed acquisition spectra (SPEDAS) were acquired using a refocusing $(\pi)_y$ pulse to minimize phase distortion, i.e. the spin-echo sequence $\{(\pi/2)_x - \tau - (\pi)_y - \tau\}$ [9]. NOESY and COSY experiments were performed using pulse sequences shown in Figure 1.

The $\pi/2$ pulse width of $10 \mu\text{s}$ was chosen at the pulse power of 60 dB. Other parameters (delay (τ) , mixing time (t_{mix}) , number of scans (NS) , etc.) are given in figure captions. Some features of NMR spectra were processed using the *Microcal Origin* program package.

3 Results and discussion

The use of ^1H NMR spectroscopy in solids is hindered due to a very strong homonuclear coupling. Protons and other abundant nuclei with high gyromagnetic ratios form an infinite spin-coupling network. Dipolar interactions cannot be completely averaged out by MAS, and in consequence very broad signals are observed. One of ^1H MAS NMR spectra of PyO...HCl crystal is shown in Figure 2. Indeed some narrow features have been found on the broad “pedestal”. They can be caused by rapid molecular motion,

whose origin is unknown and hence has to be studied in more details.

Routine high-resolution ^1H NMR spectra of PyO...HCl solutions in acetonitrile (further - ACN)/ H_2O mixtures can be very helpful at this task. It is known that in ACN/ H_2O solution is rather inhomogeneous, and at $T = 274$ K the liquid-liquid phase transition occurs [13], [14]. It is also known, that an ionic solute in systems exhibiting this phenomenon acts as increasing pressure [15], [16] which shifts the phase separation point towards higher temperatures. We have found that solubility of PyO...HCl crystal in ACN is low – *ca.* 0.1 M at room temperature. It can increase after addition some H_2O , and e.g. PyO...HCl + 4 H_2O can be dissolved in ACN already up to 1 M. But the molecular system obtained in this way is very inhomogeneous and unstable in sense of phase transition. Even a small decrease in temperature initiates the precipitation of solid phase. We have realized this process in a NMR tube. The most important point here is that it allows to observe the NMR spectra from both – the liquid and the solid phases simultaneously. They are presented in Figure 3. Three narrow lines at 8-10 ppm belong to the PyO protons in the coexisting liquid phase (the upper phase), and three much broader signals are from protons in the solid (or gel-like) phase (the lower one). It is easy to come to this conclusion moving *rf*-coil vertically along sample and observing an interchange of intensities of narrow and broader signals.

Comparing the spectra in Figure 2 and Figure 3, we see that despite the sample used in MAS study is more “rigid” (a ground monocrystal, mentioned above) than the solid obtained from ACN/ H_2O solution, both spectra look rather similar. Namely, they consist of three lines grouped between 6 and 10 ppm. In ^1H MAS spectrum one more signal is seen at 2.9 ppm.

Another rather simple method for obtaining high-resolution ^1H NMR spectra in solids has been developed in [7–9]. The essence of their proposed single pulse excitation and delayed acquisition (SPEDAS) technique is that the magnetization of a homonuclear spin-coupled system persists much longer than transverse relaxation time (T_2), and high resolution spectra can be obtained from the residual magnetization after very long delay $\tau \gg T_2$ (usually within microseconds). On the other hand, it is known [8–10], that a SPEDAS spectrum usually exhibits phase distortion, which can be reduced by a refocusing π -pulse midway between the excitation and acquisition pulses, i.e. spin-echo pulse sequence has to be applied. SPEDAS spectrum of system under investigation is shown in Figure 4 Comparing it with ^1H MAS NMR spectrum we see that even a very long delay ($\tau = 4$ ms) did not improve the resolution of narrow signals (Figure 2a), but relative intensities of peaks changed. The peak at 2.9 ppm is changing particularly drastically. Similar behavior has been observed already in SPEDAS spectrum of fumaric acid monoethyl ester [10], where a COOH peak after a longer delay becomes weaker. It has been attributed to an increase of inhomogeneity of the Hamiltonian. There is no uniform spin temperature, so the peaks relax at different rates. This can be the case for PyO...HCl system as well.

The large scale of changes of chemical shifts at solidification indicates that those moderately narrow signals can originate from the hydrogen involved in hydrogen bond

(H-bond) only. Most probably, they arise from nuclei in the moisture together with some microscopic portion of the crystal, which may have dissolved in the moisture. This moisture was artificially introduced into solution (in order to increase the solubility of $\text{PyO} \dots \text{HCl}$ in ACN), or in the case of monocrystalline samples, it may be not completely removed during a crystal growing. The presence of water leads to appearance of various cluster states having different amounts of either $\text{NO} \dots \text{HOH}$ or $\text{H}_2\text{O} \dots \text{HCl}$ H-bonds, or bulk H_2O and even non-bonded (monomeric) water molecules. A ^1H NMR study of water in hydrophobic solvents [17] has shown that the chemical shifts of protons in manifold of H_2O states indeed can cover a large range, e.g. from 0.4-1.2 ppm (monomeric), 4.8 ppm (bulk) and to 5.4 ppm (cluster in benzene). Hence comparing these values with those measured for our system we expect that the peak at 2.9 ppm arises from monomeric water. A polar surrounding of $\text{PyOH}^+ \dots \text{Cl}^-$ pairs in our system creates a much stronger reaction field, and thus causes a larger downfield chemical shift changes (comparing with [17] data). The relaxation mechanism also differs considerably from that of peaks in 6 - 10 ppm range (see SPEDAS spectrum, Figure 4). This signal is present in a crystalline sample, but it is absent in “gel-like” structure obtained from ACN/ H_2O solution (Figs. 2 and 3). Furthermore it looks that two signals at 6.7 and 2.9 ppm in crystal (Figure 2) coalesce to one peak at 5.8 ppm in less rigid system (Figure 3). This averaging is easy to understand because the presence of a set of channels sufficient from proton exchange. In more ordered and thus more rigid crystalline sample these channels are “closed” due to a denser packing of molecules.

We expect that partially resolved peaks in ^1H NMR spectra of solids may be related to a set of dynamically different spin domains (i. e. with heterogeneity of spin system). 2D NMR NOESY method (pulse sequence presented in Figure 1) that provides valuable information concerning spin exchange ought to be applied. MAS/NOESY spectra (Figure 5) show no cross-peaks even at very long mixing time ($t_{mix} = 500$ ms). This indicates that there is no exchange process between spins causing different peaks, and thus it can be concluded that the corresponding molecular aggregates are captured in “islands of mobility” without any channels needed for exchange.

The two peaks at 7.8 and 9.8 ppm are seen in both samples (Figure 2 and Figure 3). A downfield chemical shift indicates that structures with very strong H-bonds prevail in these clusters. A more rigorous conclusion about the origin of these signals seems not possible at the moment, because it requires quantitative data concerning nuclear shielding or deshielding effects at $\text{NO} \dots \text{H-O}$, $\text{NO} \dots \text{H-Cl}$ or $\text{H-O} \dots \text{H-Cl}$ coordination, which are rather poor. The difficulties that arise at identification of certain NMR signals can be overcome by means of advanced quantum chemistry methods of calculation of magnetic screening tensor of suspected structures. These methods already give rather realistic values of chemical shifts in H-bonded systems that could be compared with experimental ones [12]. This work is in progress.

And finally, additional information concerning spin coupling in the system can be obtained by means of 2D NMR COSY technique. Its pulse sequence is also shown in Figure 1. A very interesting observation in MAS/COSY spectra can be reported here.

Namely, the appearance of MAS side bands as “pseudo” cross-peaks in 2D NMR spectra using MAS/COSY technique has been observed (Figure 6). In the case of accidental coincidence of spinning frequency (ω_{MAS}) with spectral distances between some diagonal signals, intensive non-diagonal peaks are moving away from cross-positions. A misleading conclusion concerning spin coupling is easy to avoid using various ω_{MAS} . Comparing the changes in spectra at spinning rates of 2.5 and 4 kHz (Figure 6a and Figure 6b), we see that these “pseudo” cross-peaks are moving from cross-positions away. Hence it can be concluded that there is no coupling between spins causing NMR signals in consideration.

4 Concluding remarks

MAS and 2D NMR methods have proved themselves as a very powerful tool studying molecular behavior in heterogeneous systems. They can be applied in investigations of various aspects of molecular mobility and exchange processes in nano-cavities, micro-heterogeneous and porous media. A very shocking observation of pseudo cross-peaks in MAS/COSY spectra, as far as we know, has been never reported before. Of course this finding is more important from the experimental - methodological reason. It can be a dangerous trap interpreting MAS/COSY data of systems with more complicated picture of spin coupling, when several “true” cross-peaks can overlap and mix with “pseudo” ones. Some difficulties that arise at the interpretation of the origin of certain NMR signals hopefully can be overcome by means of quantum chemistry calculations of magnetic screening of suspected structures, which are in progress.

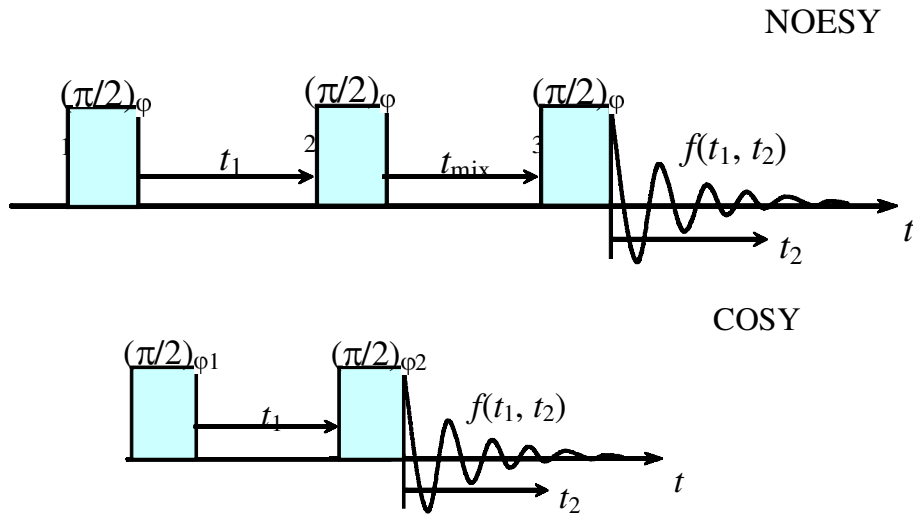
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2D NMR spectra are obtained by Fourier transform of free induction decay $f(t_1, t_2)$, where t_1 - evolution time, t_{mix} - mixing time for magnetization exchange, t_2 - acquisition time; four-phase cycling was used to improve the signal-to-noise ratio:

$\phi 1 = x, x, x, x, x, x, x, x, x, y, y, y, y, y, y, y, y, -x, -x, -x, -x, -x, -x, -x, -x, -y, -y, -y, -y, -y, -y, -y$;
 $\phi 2 = x, -x, x, -x, x, -x, x, -x, y, -y, y, -y, y, -y, y, -y$;
 $\phi 3 = x, x, y, y, -x, -x, -y, -y, y, y, -x, -x, -y, -y, x, x$.

Fig. 1 NOESY and COSY pulse sequences.

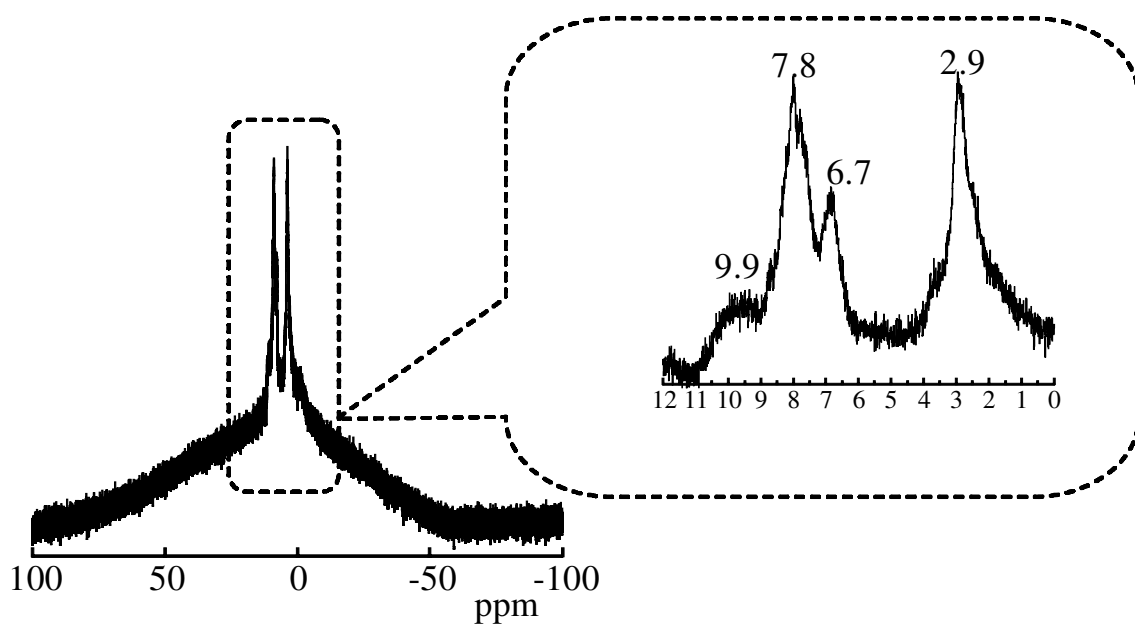
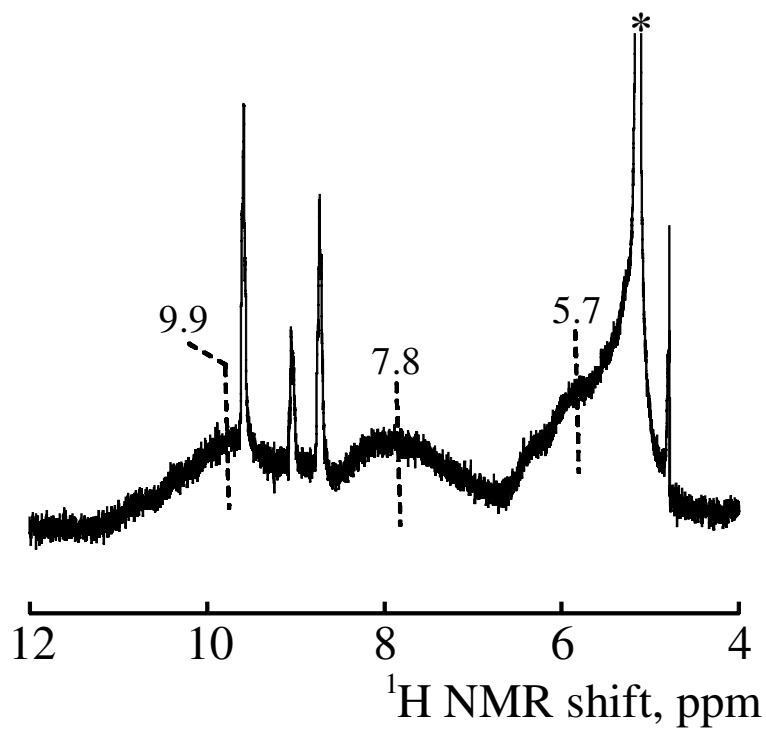


Fig. 2 ^1H MAS NMR spectrum of $\text{PyO} \dots \text{HCl}$ crystal ($T = 293\text{K}$, spinning rate $\omega_{MAS} = 4.1$ kHz).



* signal of water in insert capillary

Fig. 3 ^1H NMR spectrum of $\text{PyO} \dots \text{HCl} + 4 \text{H}_2\text{O}$ solution in acetonitrile (1M, 293 K) after heterogeneous phase separation occurred.

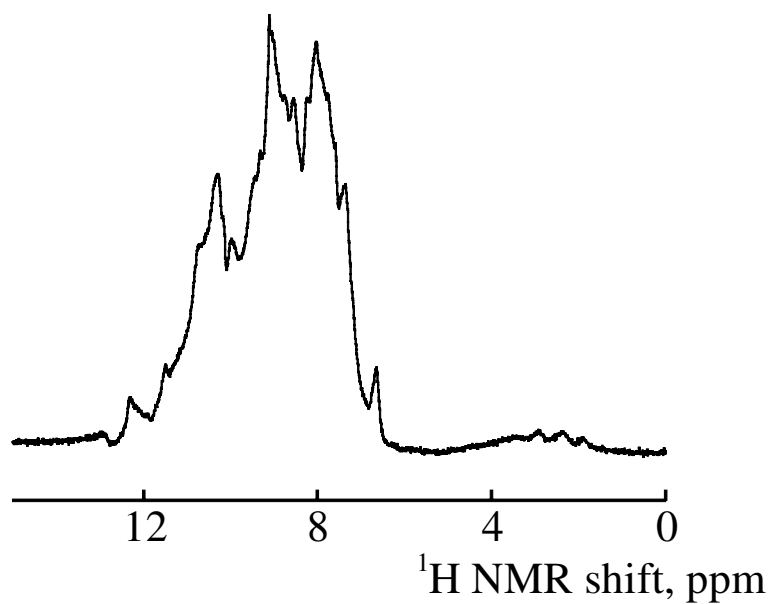


Fig. 4 ^1H spin-echo SPEDAS NMR spectrum of $\text{PyO} \dots \text{HCl}$ crystal (π -pulse was applied 2 ms after $\pi/2$ -pulse, and MAS spectrum acquired with a delay of 4 ms, $\text{NS} = 2000$, $\omega_{\text{MAS}} = 4.1$ kHz).

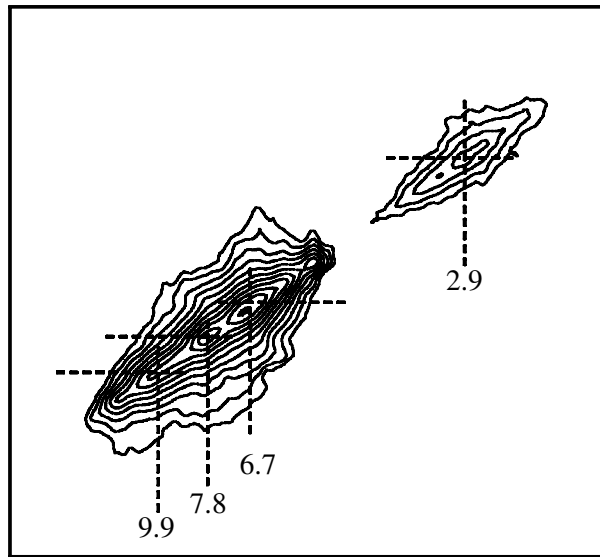


Fig. 5 ^1H MAS/NOESY spectrum of PyO...HCl crystal ($t_{mix} = 500$ ms, $\omega_{MAS} = 4$ kHz, NS = 80, peak positions (in ppm) are the same as shown on Figure 2a).

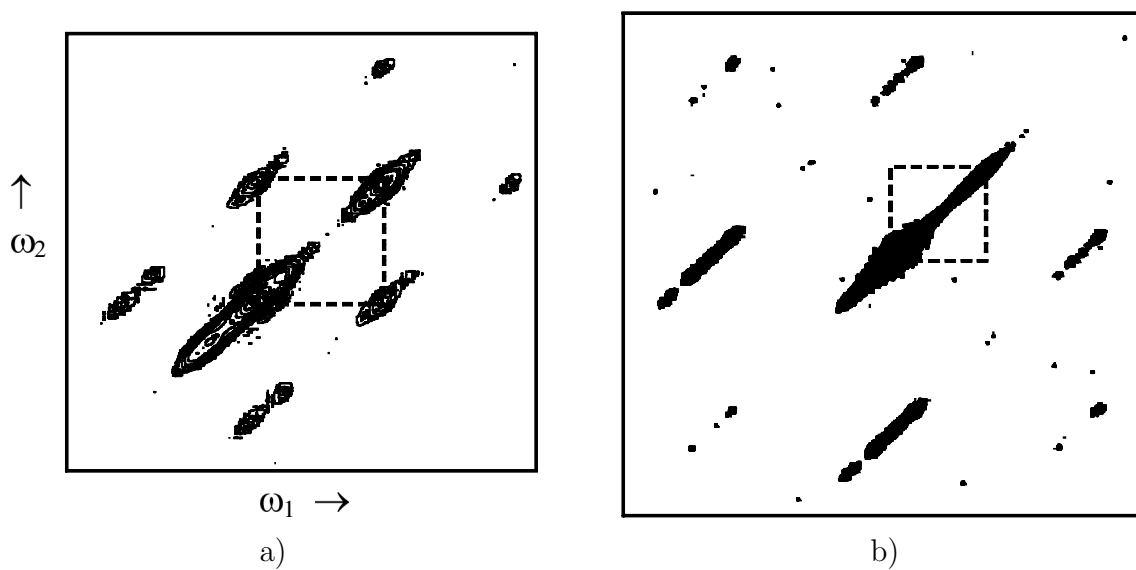


Fig. 6 ^1H MAS/COSY spectra of PyO...HCl crystal at $\omega_{MAS} = 2.5$ kHz (a) and $\omega_{MAS} = 4$ kHz (b) (peak positions are the same as shown on Figure 2a).