A ^{31}P CP/MAS NMR Study on Dehydration of Disodium Clodronate Tetrahydrate

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Purpose. ^{31}P CP/MAS NMR is used to characterize stability and changes in solid state properties of disodium clodronate tetrahydrate upon variable temperature and slow dehydration.

Methods. Variable temperature ^{31}P CP/MAS NMR spectroscopy.

Results. A fast rise in temperature leads to loss of lattice waters and produces an averaged structure characterized by a single ^{31}P NMR resonance at 398 K. Slow room temperature dehydration converts the crystalline form to an anhydrous structure with two non-equivalent phosphorus atoms. The molecular skeleton of clodronate is stable within the temperature range 296 K-398 K of experiments. Rehydration of the anhydrous samples at room temperature restores the crystalline tetrahydrate form verified by a ^{31}P CP/MAS NMR spectrum similar to that of a virginal sample.

Conclusions. Solid state NMR is a method which can offer both molecular and crystal scale information, when either bulk or dosage forms of a drug can be altered by temperature or by loss of lattice waters or solvents. The experiments are easy to perform, though time consuming, especially when low abundant nuclei are examined.

KEY WORDS: bisphosphonates; ^{31}P CP/MAS NMR; solid state nuclear magnetic spectroscopy; thermal stability; dehydration.

INTRODUCTION

Geminal bisphosphonates are drugs used in various bone, teeth and calcium metabolism diseases (1–2). Bisphosphonates are characterized by a P-C-P group and a great number of different derivatives have been developed for possible drugs. Of these compounds four bisphosphonates, namely (dichloromethylene)bisphosphonic acid (clodronate) (Figure 1), (1-hydroxyethylidene)bisphosphonic acid (etidronate), (3-amino-1-hydroxypropylidene)bisphosphonic acid (pamidronate) and (4-amino-1-hydroxybutylidene)bisphosphonic acid (alendronate) are currently in clinical use. In general the pharmaceutical solids can have several forms, in which crystalline, polymorphic, amorphous or hydrated states may occur (3). In cases where the stability or the bioavailability of the drug can be altered due to a conversion to the solid form, a suitable test for the purity of drug is needed. Also, as the possible polymorphism in solid drugs is expected more and more to be in control, an analysis method that gives structural information on molecular and crystal scale is required. On the other hand the macroscopic properties, and hence the industrial processability, can be affected by the solid form of product. Correlation between solid state NMR (Nuclear Magnetic Resonance) spectroscopy and crystalline properties has been well established and several nuclei e.g. ^{23}Na (4), ^{31}P (5), and ^{113}Cd (6) have been utilized. In pharmaceutical *NMR* analysis ^{13}C is naturally the most important nucleus, e.g. for detection of polymorphs (7). The present paper exhibits crystalline properties of disodium clodronate investigated by solid state ^{31}P NMR.

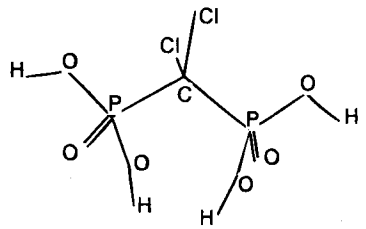

Fig. 1. Clodronic acid.

Disodium clodronate crystallizes in a triclinic (*P1*) lattice containing four lattice water molecules for each clodronate molecule (8) (Figure 2) and the water content in the total weight of the crystalline material is 20%. This study uses ^{31}P CP/MAS (Cross Polarization Magic Angle Spinning) NMR to follow the characteristics of phosphorus nuclei in several hydration states. The heteronuclear cross-relaxation rate constants T_{PH} and differences in chemical shift anisotropy (CSA) compared to X-ray diffraction data are used to characterize the two crystallographically independent P-nuclei. The changes in the chemical shifts of isotropic signals and in the chemical shift anisotropy parameters due to dehydration and variable temperature are further examined to follow alteration in the environments of the phosphorus nuclei.

EXPERIMENTAL SECTION

Disodium clodronate was received from Leiras Ltd as a microcrystalline $Na_2CCl_2(PO_3H)_2{*}4H_2O$ powder. ^{31}P CP/MAS NMR experiments were performed at 161.98 MHz on a Bruker AMX 400 spectrometer equipped with a doubletuned Bruker MAS 400 SB-BL 7 probe. Typically 200 mg of sample was packed in 7 mm zirconia rotors with KelF caps. The Hartmann-Hahn condition for ^{31}P CP/MAS was optimized by maximizing the ^{31}P NMR signal of hydroxyapatite with standard method of varying the pulse lenghts and power levels of the exciting ^{1}H pulse and the matching ^{31}P pulse. Bearing gas temperature was controlled by Bruker VT 2000 unit with internal calibration and the observed temperature stability was 0.2 K. Chemical shifts are calibrated indirectly to synthetic hydroxyapatite reso-

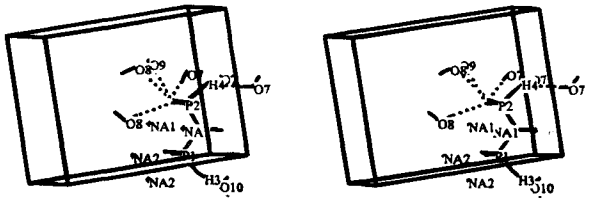

Fig. 2. Stereo view of the hydrogen bonding of lattice waters to clodronate anion.

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nating at 2.8 ppm relative from phosphoric acid. A typical spinning frequency of 4500 Hz was used. Solid state spectra were obtained using a single contact cross polarization from protons with the CP time of 1 ms. Typically 16 to 32 scans were needed to gain a good signal to noise ratio. A complete formal relaxation analysis was not done and the relaxation delay of 180 s was chosen after elementary saturation recovery tests to avoid saturation. The spectral line deconvolution was performed using Bruker Xspec v. 2.0.4 software. The estimation of chemical shift anisotropy parameters was executed with the same software using the Herzfeld-Berger method (9).

Dehydration of the disodium clodronate samples was carried out either by heating the sample in the NMR probehead in a MAS spinner closed with a cap containing a small hole, or by slow dehydration over silica gel in a desiccator at ambient temperature. The loss of lattice water was determined by weighing.

For the moisture uptake studies additional water was weighed to disodium clodronate tetrahydrate samples in airtight bottles at a normal atmospheric pressure and the system was allowed to equilibrate for two weeks at room temperature. Water was uniformly distributed, by eye, in the samples except in a sample containing nominally 14 water molecules to each clodronate skeleton, in which vaporized water formed some droplets on the walls of the flask.

RESULTS AND DISCUSSION

The NMR resonance of a nucleus in a static powder sample is a wide resonance line, the width and shape of which depend on the dipolar interactions to other nuclei and on the anisotropy in the shielding properties around the nucleus (10). To discover a high resolution spectrum of individual spins certain spectral line narrowing procedures e.g. MAS or special pulse sequences have to be applied.

The hydrogen nuclei are the major source of dipolar linebroadening in organic or other hydrogen abundant solids. The proton decoupling associated with a typical CP/MAS pulse sequence acts as a decoupling source towards hydrogen, and the residual dipolar broadening is caused by 'heavy' nuclei. When the heavy nuclei are of a low natural abundance (like ^{13}C), the homonuclear dipolar interaction is usually negligible. As the ^{31}P is a 1/2 spin nucleus of 100% abundance, an estimation of the homonuclear line broadening should be performed.

After proton decoupling the major source of dipolar broadening is caused by the P-P interaction and the value of direct dipolar ^{31}P-^{31}P coupling constant R can be calculated from equation (1)

$$R = \left(\frac{\mu_0}{4\pi}\right)\left(\frac{\hbar}{2\pi}\right)\gamma_P^2\langle r_{PP}^{-3}\rangle \qquad (1)$$

In disodium clodronate tetrahydrate with intramolecular P1-P2 distance of 3.125 Å the value of R is 640 Hz. Dipolar broadening is fully reduced with sample rotation frequency of 4500 Hz, which justifies the usage of Herzfeld-Berger method in the estimation of CSA. When the homonuclear linebroadening cannot be estimated from a known or expected structure, the centerband/sideband linewidths can be measured at various spinning frequences. When the linewidths no longer narrow at a high spinning frequency, the homonuclear dipolar contribution to linebroadening has been averaged. Disodium clodronate tetrahydrate also passed this test at spinning frequency of 4500 Hz.

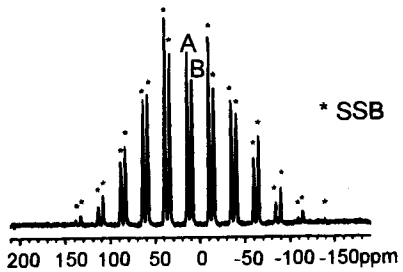

Fig. 3. ^{31}P CP/MAS NMR spectrum of disodium clodronate tetrahydrate at 296 K.

The spin-spin- or J-coupling of magnetically non-equivalent phosphorus nuclei is expected to be of same magnitude as in solution. In a ^{31}P NMR study of isopropyl esters of clodronic acid (11) four different isopropyl esters were studied by Rönkkömäki et al. and the ^{31}P-^{31}P J-coupling constants varied from 15.6 to 17.9 Hz. At this range the effect of J-coupling on the linewidth of ^{31}P in solid state is negligible.

After the dipolar broadening has been decoupled, or averaged, the remaining linebroadening is caused by chemical shift anisotropy. In a MAS experiment at moderate spinning frequencies the powder pattern breaks into an isotropic resonance surrounded with spinning sidebands (SSB) separated by the frequency of spinning. The intensity of these SSBs can be utilized to obtain the anisotropy parameters: CSA and asymmetry η, to get insight of the characteristics of the lattice site.

The ^{31}P CP/MAS NMR spectrum of disodium clodronate tetrahydrate at 296 K is presented in Figure 3. The spectrum contains two isotropic lines A and B at 14.3 and 9.1 ppm, respectively. At a spinning frequency of 4500 Hz the both lines are surrounded by five intense SSB pairs and an estimation of anisotropy was performed for both lines using all SSBs. The spectral parameters for signals A and B are presented in Table 1.

The geometrical properties of the phosphorus centered tetrahedrons of clodronate anion were estimated from the X-ray crystal structure data by Nardelli et al. (8) to identify the resonance lines of the NMR spectrum (Figure 3). The six bond angles ($C\text{-}P_h\text{-}O_{i,j,k}$, $O_{i,j,k}\text{-}P_h\text{-}O_{i,j,k}$) defining the tetrahedrons $P_hCO_{i,j,k}$ (h = 1,2) were processed statistically to gain information about the regularity of the tetrahedrons. Also the bond

Table 1. ^{31}P CP/MAS NMR Spectral Parameters for Disodium Clodronate Tetrahydrate at 296 K

	Signal	
	A	B
δ(iso)/ppm	14.3	9.0
CSA/ppm	86.3	−106.6
η	0	0.85
σ_{11}/ppm	100.6	103.7
σ_{22}/ppm	−28.9	20.7
σ_{33}/ppm	−28.9	−97.7
$\nu_{1/2}$Hz	210	215
T_{PH}/μ	254 ± 2	380 ± 20
$T_{1\rho H}$/ms	3.41 ± 0.02	4.17 ± 0.13

distances of P_h-$O_{i,j,k}$ were analyzed with a similar procedure. The selected bond lengths and descriptive statistics are presented in Table 2 a) and b).

The chemical shift of a given nucleus is a function of the molecular structure, and in phosphates the variation of the paramagnetic contribution to shielding is the dominant factor to ^{31}P chemical shift (5). In practice the chemical shift correlates with the mean P-O bond strengths or lengths. In our case the bisphosphonic group is expected to behave similarly since the difference in P1-C and P2-C bond length is small (Table 2a). The mean value of P-O bond length is slightly shorter in P1 head of clodronate and P1 is assigned to the high field signal B at 9.0 ppm. This leaves signal A at 14.3 ppm to P2.

The statistical measures for the regularity of the tetrahedrons in Table 2 are the standard deviations and ranges in bond angles and P-O bond distances. For their part, they should correlate with the chemical shielding anisotropy. Comparison between tetrahedron regularity and CSA values supports the assigment of the resonance A (CSA 86.3 ppm) to the crystal site P2 as previously and the resonance B (CSA −106.6 ppm) to P1 since the deviations from tetrahedral symmetry are bigger for the lattice site P1.

The assignment of NMR-resonances to lattice sites can also be done using the heteronuclear cross-relaxation rate constant $(T_{PH})^{-1}$ as a measure of heteronuclear dipolar interaction between protons and ^{31}P. A cross polarization sequence uses transfer of magnetization from an abundant spin system, typically protons, to a less sensitive nucleus. After the fullfilment of Hartmann-Hahn condition $\gamma_A H_{1A} = \gamma_S H_{1S}$ the flow of magnetization to a sparse spin is dependent on the number and type of interacting nuclei and on the internuclear distance. The Hamiltonian for heteronuclear dipolar interaction is written as equation (2) (12).

$$H^0_{AS} = 2\gamma_A\gamma_S\hbar^2 \sum_{i=1}^{N}\sum_{m=1}^{N} r_{im}^{-3} P_2(\cos\Theta_{im}) A_{iz} S_{mz} \qquad (2)$$

In a case like ours the simplest approach to estimate the magnitude of the thermal contact between a distinct lattice site and the abundant spin system for elementally similar sites is to sum the inverse of cubic internuclear A-S distances as in equation (3).

$$b = \sum_{i=1}^{N}\sum_{m=1}^{N} r_{im}^{-3}, \qquad \text{where } N_S = 1 \qquad (3)$$

In practice the number of abundant spins can be limited to a convenient value. We used the reconstruction of X-ray structure to measure distances from lattice phosphorus atoms P1 and P2 to the 15 closest hydrogen nuclei and the values of b are implemented in Table 2c. Several other measures for dipolar interaction like the second moment of interaction (12) or direct dipolar coupling constant (13) can also be used.

When the intensity of a ^{31}P signal is measured as a function of cross polarization time and the sample contains no direct P-H bonds the obtained curve should rise exponentially, reach a maximum and decline with common rate of $(T_1)^{-1}$ (^{1}H) (14) following the model given by Mehring (15) in equation (4).

$$I(\tau) = \frac{M^0\left(\frac{\gamma_H}{\gamma_P}\right)\left[\exp\left(-\frac{\tau}{T_{1\rho H}}\right) - \exp\left(-\frac{\tau}{T_{PH}}\right)\right]}{1 - \frac{T_{PH}}{T_{1\rho H}}} \qquad (4)$$

The observed P-signals vs. contact time obey the aforementioned behavior and are presented in Figure 4. The experimental data from Fig. 4 was fitted with a three parameter non-linear least square procedure to equation 4 and the obtained values

Table 2. Selected Bond Lengths (Å) and Angles (°) in Disodium Clodronate Tetrahydrate (8) with Crystal Structure Derived Statistics

a)P-X bond lengths

	Bond length / Å		Bond length / Å
P1-C	1.866 (3)	P2-C	1.855 (4)
P1-O1	1.484 (3)	P2-O4(H)	1.576 (3)
P1-O2	1.500 (2)	P2-O5	1.497 (3)
P1-O3(H)	1.577 (3)	P2-O6	1.496 (2)
Mean (P-O)	1.520		1.523
Stand. Dev. (P-O)	0.0497		0.0459
Range (P-O)	0.093		0.080

b)X-P-X bond angles

	Angle / °		Angle / °
C-P1-O1	106.3 (2)	C-P2-O4(H)	104.1 (3)
C-P1-O2	106.8 (2)	C-P2-O5	107.8 (2)
C-P1-O3(H)	102.6 (3)	C-P2-O6	106.9 (2)
O1-P1-O2	120.0 (3)	O5-P2-O4(H)	107.8 (3)
O1-P1-O3(H)	108.4 (2)	O6-P2-O4(H)	111.2 (2)
O2-P1-O3(H)	111.2 (2)	O5-P2-O6	118.1 (3)
Mean	109.2		109.3
Stand. Dev.	5.98		4.87
Range	17.4		14.0

c)The summed inverse of cubic P_n-H_m distance for lattice sites P1 and P2

	P1	P2
b(10^{29} m^{-3})	3.19	4.50

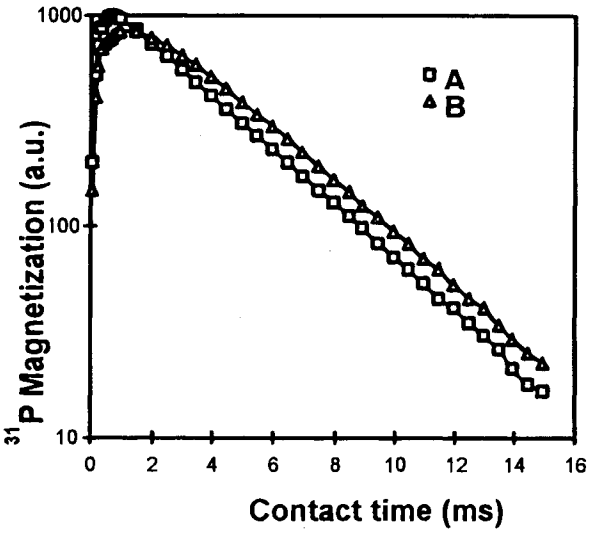

Fig. 4. Relative intensities of ^{31}P NMR signals of disodium clodronate tetrahydrate vs. CP contact time.

of T_{PH} for signals A and B were 254 ± 2 μs and 380 ± 20 μs, respectively (Table 1).

The assignment of signal A with the higher cross-polarization rate to the lattice site P2 corresponding to stronger ^{31}P-^{1}H dipolar interactions (b = $4.50*10^{29}$ m^{-3}) and signal B to P1 (b = $3.19*10^{29}$ m^{-3}), is the same as those using the chemical shift or CSA assignment.

Fast Dehydration of Disodium Clodronate Tetrahydrate

Dehydration experiments were performed at six temperatures ranging from 296 K to 398 K. The heating rate was about 20 K/min and the sample was allowed to equilibriate for 30 minutes prior to NMR-experiments. After each measurement the spinner was ejected and weighed to obtain the loss of water. The same sample was inserted and used for the next heating step. The possible hydration of sample at room temperature during weighing is possible and this was tested with a corresponding variable temperature study without intermediate ejection and weighing. The NMR spectra from both studies are identical and both the hydration from air at room temperature and the possible effects of cooling/reheating during weighing are considered negligible. Selected spectra of the effect of temperature are presented in Figure 5. Changes in the chemical shifts (δ) are minor and the relative intensity of signal A is gradually diminished as the temperature decreases. At 373 K a new resonance C at 10.6 ppm is observed. At 385 K signal C is practically the only component of the spectrum and total averaging is achieved at 398 K. Characteristics of resonances A, B and C are collected in the Table 3 with the temperatures of experiments and weight losses.

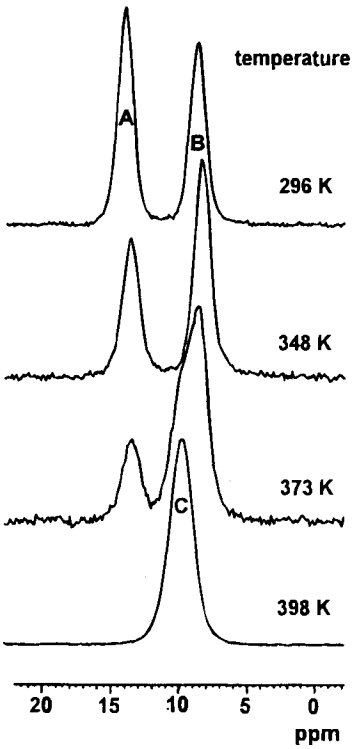

Fig. 5. ^{31}P CP/MAS NMR spectra of disodium clodronate undergoing rapid dehydration with increasing temperature.

Table 3a. Characteristics of Signal A in Variable Temperature

T/K	weight loss/%	δ(iso)/ppm	CSA/pmm	η	$\nu_{1/2}$(iso)/Hz
296	0	14.3	86.3	0	210
323	0.09	14.3	−86.3	0.95	210
348	0.52	14.2	−88.8	0.95	220
373	5.33	14.1	−94.2	0.95	248

Table 3b. Characteristics of Signal B in Variable Temperature

T/K	weight loss/%	δ(iso)/ppm	CSA/pmm	η	$\nu_{1/2}$(iso)/Hz
296	0	9.0	−106.6	0.85	215
323	0.09	9.1	−106.1	0.90	215
348	0.52	9.2	−105.6	0.85	225
373	5.33	9.3	−108.8	0.80	225

Table 3c. Characteristics of Signal C in Variable Temperature

T/K	weight loss/%	δ(iso)/ppm	CSA/pmm	η	$\nu_{1/2}$(iso)/Hz
373	5.33	10.6	−91.4	0.95	285
385	12.85	10.5	−98.7	0.85	325
398	14.35	10.6	−94.2	0.85	315

Disodium clodronate crystallizes in a layered lattice, where clodronate anions are separated by layers of lattice water. During rapid heating the water molecules are expected to loose weaker hydrogen bonds at temperatures of 323–348 K. This effect is seen in the variation of asymmetry parameter η. At these temperatures the water does not yet leave the lattice verified by low weight loss. At 373 K one lattice water on average has been lost and the lattice starts to form a new, less-hydrated phase. When the maximum temperature 398 K is reached, three water molecules have left the lattice and all material is in a new form defined by signal C. The chemical shift of signal C at 10.6 ppm is between the two initial signals A and B and the relatively small CSA of −94.2 ppm indicate an averaged structure with no major H-bonding constraints in the tetrahedral geometry of the $CP(O)_3$ group. To explain the case where one water molecule is bonded and the two phosphorus nuclei are characterized by a single resonance; there are mainly three possibilities. Either the water molecules are coordinated as bridging ligands between clodronate oxygens thus forming a chainlike structure or the water is coordinated to both ends of a single clodronate to form a cyclic structure. However, at the temperature of 398 K these models are not expected to be very stable. On the other hand, a fast exchange reaction of hydrogen between otherwise identical heavy atoms is expected to produce an averaged spectrum as in the case of ^{13}C CPMAS spectrum of solid naphthazarin, where intramolecular exchange of phenolic protons takes place (16). On this basis the averaged form is expected to have a fast exchange of water coordination between bisphosphonic groups.

Slow Dehydration of Disodium Clodronate Tetrahydrate

Slow dehydration was carried out in a desiccator at room temperature over silica gel. The spectra of the slow dehydration

studies are represented in Figure 6. As in the case of variable temperature study, the peak shifts are small. Instead, a new signal D at 6.6 ppm rises with dehydration. After total dehydration the disodium clodronate produces a spectrum of two overlapping signals at 8.4 ppm (slightly shifted signal B) and at 6.4 ppm (D). The observed upfield shift compared to original signals A and B is expected to arise from the loss of hydrogen bonding and thus stronger P-O bonding.

Characteristics of signals A, B and D from slow dehydration and excess moisture studies are presented in Table 4. The amount of water is presented as a nominal number of water molecules for a one clodronate species, the original tetrahydrate having four lattice waters.

When the virginal disodium clodronate starts to dehydrate slowly, the layered structure goes through a transitional phase. The final product is a new structure, which also carries two kinds of lattice sites for phosphorus nuclei. In principle the anhydrous form is expected to be crystalline at least on short range, but the assumption would need X-ray diffraction studies for verification.

The totally nonhydrated crystallite has two lattice sites P1′, which is formed from P1, and a new site P2′ characterized by NMR signal D. Lattice sites P1′ and P2′ differ also in the CSA and η, P2′ having a greater anisotropy. As in tetrahydrate, the sodium counterions are expected to occupy inequivalent lattice sites and thus cause the observed difference in lattice sites P1′ and P2′.

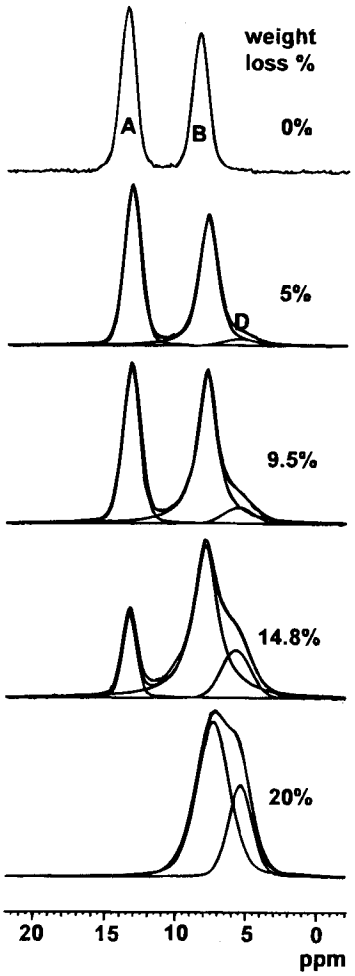

Fig. 6. ^{31}P CP/MAS NMR spectra of disodium clodronate in several dehydration states, achieved slowly in a desiccator at room temperature.

Table 4a. Characteristics of Signal A with Hydration State at 296 K

H_2O/clodronate	δ(iso)/ppm	CSA/ppm	η	$\nu_{1/2}$(iso)/Hz
1.04	14.3	83.4	0	187
2.1	14.3	83.6	0	240
3	14.3	83.6	0	230
4	14.3	86.3	0	210
5	14.2	−88.8	0.95	280
6	14.2	−88.9	0.95	280
9	14.3	−88.9	0.95	350
14	14.4	−91.9	0.95	450

Table 4b. Characteristics of Signal B with Hydration State at 296 K

H_2O/clodronate	δ(iso)/ppm	CSA/ppm	η	$\nu_{1/2}$(iso)/Hz
0	8.4	93.8	0	440
0.12	8.6	94.6	0	470
1.04	9.0	−97.3	0.95	335
2.1	8.9	−100.0	0.90	268
3	8.9	−100.2	0.90	260
4	9.0	−106.6	0.85	215
5	8.8	−111.0	0.80	280
6	8.8	−111.1	0.85	280
9	8.8	−108.4	0.85	330
14	9.0	−108.6	0.80	380

Table 4c. Characteristics of Signal D with Hydration State at 296 K

H_2O/clodronate	δ(iso)/ppm	CSA/ppm	η	$\nu_{1/2}$(iso)/Hz
0	6.4	−101.2	0.85	320
0.12	6.6	−102.9	0.80	375
1.04	6.7	−91.8	0.90	390
2.1	6.6	−86.1	0.75	400
3	6.6	−105.8	0.95	400

In both cases the start of dehydration phenomena affects mainly the spectral region characterized to lattice site P2. This implies that the first leaving lattice water molecule is located near this end of clodronate skeleton. In Nardelli's structure (8) three lattice waters, $H_2O(7)$, $H_2O(8)$ and $H_2O(9)$ have a possibility to affect the properties of P2. The hydrogen bonding to clodronate anion is presented in stereo view in Figure 2. However, $H_2O(7)$ forms a strong hydrogen bond towards clodronate oxygen with bond length of 1.81 (3) Å, and its loss is not very probable as the first dehydration step. Hydrogen bond lengths from $H_2O(8)$ and $H_2O(9)$ to clodronate are similar within the limit of errors (2.05–2.09 (±0.04) Å), and either water is equally likely to be the first lattice water to break loose.

Fast dehydration formed an averaged structure at temperature 398 K, when one lattice water molecule still remained in the solid. For comparison a tetrahydrate sample was dehydrated slowly in a desiccator to one water state and heated stepwise

(20 K/min) in a MAS spinner to 398 K. The earlier measured spectrum with three frequencies (Figure 6, weight loss 14.8%) is obtained at 295 K and it starts the conversion to averaged state at 373 K. At 398 K the fully averaged system is not yet produced, but three intense resonance lines exist at 10.6 ppm (the upcoming signal C), 8.6 ppm and 6.8 ppm (the decreasing B and D, respectively). Slow dehydration is thus assumed to form more thermally stable intermediate dehydration states than fast temperature driven dehydration. The cause of this behaviour is the slow conversion of lattice at low temperature, which enables the residual components to form a better solid packing and thus stronger hydrogen bonds.

In dehydration studies using temperature, only one heating rate (20 K/min) was examined but, theoretically, if disodium clodronate tetrahydrate is heated with a sufficiently slow heating rate, the crystalline phase would most probably undergo a change into a form characterized by signals B and D (8.4 ppm and 6.4 ppm) as obtained the in slow dehydration. With rising temperature the thermal motion would transfer the dehydrated phase into the averaged form characterized by signal C at 10.6 ppm.

Excess Water on Disodium Clodronate Tetrahydrate

When excess water is added to disodium clodronate tetrahydrate the only significant change in the characteristics of spectral parameters is the gradual broadening of isotropic peaks A and B. In single scan experiments the samples with additional water more than 5 weight % also produced a new 8 Hz wide signal without SSBs at 8.5 ppm identified as saturated disodium clodronate solution.

Additional water does not generate a new structure but gradually surrounds crystals with a saturated solution. This liquid phase was visible due to rotational forces of NMR experiments in the samples containing additional water more than 5 weight %.

The anisotropy parameters of crystalline phase are relatively constant regardless of the water content of the sample. The only change in spectral characteristics is the continuous broadening of isotropic signals. This effect can be understood to result from the dissolution of crystals. The continuous exchange between dissolved and crystalline phase tends to produce slightly different chemical environment on surface phase, which leads to broadening of isotropic signals.

In both of the dehydration procedures the changes in crystal structure are fully reversible. When the dehydrated sample is allowed to equilibrate with moisture at room temperature and studied with NMR, the obtained spectrum is similar to that of the original sample of disodium clodronate tetrahydrate.

CONCLUSIONS

Solid state NMR provides information of both the molecular structure and packing structure in a crystalline solid. The experiments themselves are easy to perform. With low abundant nuclei the experimental time can be rather long, but if only qualitative information, e.g. safe temperature limits for transport or storage, is required, less instrumental time is needed. The value of NMR lies in its capability to show chemical transformations. Our results suggest that a pharmaceutical solid, disodium clodronate tetrahydrate converts to a new form upon fast dehydration of lattice waters. Upon slow room temperature dehydration the final product is expected to contain at least short scale crystallinity with two distinct lattice sites for the phosphorus atoms of clodronate skeleton. Dehydration is fully reversible and total rehydration leads to the original crystalline disodium clodronate tetrahydrate.

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