

Structural Insight into the Dehydration and Hydration Behavior of Naltrexone and Naloxone Hydrochloride. Dehydration-Induced Expansion versus Contraction

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ABSTRACT: For the chemically and structurally related antagonists naltrexone and naloxone, hydrate and anhydrate forms exist for the hydrochloride salts, the generic forms that are on the market. The hydration/dehydration behavior of these salts was studied by applying hot-humidity stage X-ray powder diffraction, microscopy, differential scanning calorimetry, thermogravimetric analysis, and solid-state NMR. The combination of these techniques shows consistent results and yields a detailed conversion scheme. A new crystal structure, determined from X-ray powder diffraction data, for naltrexone hydrochloride anhydrate is presented. The structure was solved by the DASH program and was refined with TOPAS. This new structure, together with the already known structures of naltrexone and naloxone hydrochloride, enabled us to investigate the influence of the subtle molecular differences between the two antagonists and the role of water on structural properties in the solid state. All known hydrate and anhydrate forms of naltrexone and naloxone hydrochloride crystallize in the orthorhombic space group $P2_12_12_1$, although the crystal packings show clear differences. Dehydration causes in both cases no breaking of the symmetry, but the change in unit cell for naltrexone is profoundly different from that of naloxone. Dehydration of naltrexone hydrochloride tetrahydrate takes place with shrinkage of the volume of the unit cell, whereas dehydration of naloxone hydrochloride dihydrate results in an expansion of the unit cell. The H-bonding patterns corresponding to the two opioids seem to be footprints for the crystal structures and for the hydration/dehydration behavior of the two antagonists. Despite naltrexone and naloxone hydrochloride are chemically and structurally related and show similarities in their biological behavior, the overall hydration and dehydration process is fundamentally different.

1. Introduction

An agonist is a compound that binds to a specific receptor and triggers a response in the cell. An antagonist can compete with an agonist for a receptor site and can prevent an agonist from acting.¹ Narcotic antagonists are 10–1000 times more potent in enhancing receptor binding than their corresponding agonists.² The molecular structures of two antagonist opioids, naloxone and naltrexone, that are the subject of the present study are presented in Figure 1.

Naltrexone is an opioid receptor antagonist used primarily in the management of alcohol and/or opioid dependence. Synthesized in 1965 and tested during the 1970s and early 80s, naltrexone represents the pharmacotherapy for helping to eliminate drug cravings and opiate-seeking behaviors, thus preventing opiate relapse.³ Naltrexone is marketed in the generic form as its hydrochloride salt. The naltrexone hydrochloride tetrahydrate form⁴ (CSD refcode PABCEA) crystallizes in the orthorhombic system with space group $P2_12_12_1$ and cell parameters: $a = 7.768(11) \text{ \AA}$, $b = 15.926(6) \text{ \AA}$, $c = 18.099(5) \text{ \AA}$, $V = 2239.085 \text{ \AA}^3$.

Naloxone is a drug used to counter the effects of opioid overdose. Although differing only slightly from narcotics, the antagonist naloxone can completely block the analgesic and euphoric effects of agonists. The naloxone hydrochloride dihydrate^{1,5} (CSD refcode NALOXCO1) is the generic marketed form and crystallizes in the orthorhombic system with space group $P2_12_12_1$ and cell parameters $a = 7.833(3) \text{ \AA}$, $b = 13.185(5) \text{ \AA}$, $c = 18.569(5) \text{ \AA}$, $V = 1917.77 \text{ \AA}^3$. The naloxone hydrochloride anhydrate¹ crystallizes also in the orthorhombic

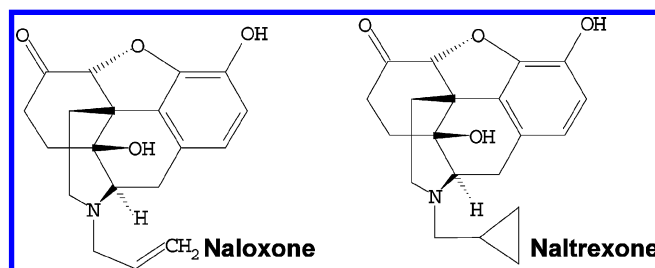


Figure 1. Molecular structure of the antagonist opioids: naloxone and naltrexone.

system with space group $P2_12_12_1$ and cell parameters $a = 17.452(4) \text{ \AA}$, $b = 14.681(4) \text{ \AA}$, $c = 7.978(2) \text{ \AA}$, $V = 2044.10 \text{ \AA}^3$. The monohydrate form⁶ of naloxone free base crystallizes in the orthorhombic system with space group $P2_12_12_1$ and cell parameters $a = 13.903(5) \text{ \AA}$, $b = 7.257(7) \text{ \AA}$, $c = 16.641(1) \text{ \AA}$, $V = 1678.99 \text{ \AA}^3$. However, the anhydrate form of naloxone free base⁶ crystallizes in the monoclinic system with space group $P2_1$ and cell parameters $a = 8.539(2) \text{ \AA}$, $b = 12.678(4) \text{ \AA}$, $c = 7.652(2) \text{ \AA}$, $\beta = 97.078^\circ$, $V = 822.16 \text{ \AA}^3$.

The well-known T-shape of opioid compounds is also observed for naltrexone and naloxone (Figure 2). The five-ring system forms two planes. The stock of the T-shape is formed by the B, C, and D rings and the arms of the T by the A and E rings. There are no significant differences in bond lengths and angles. The B and D rings take an envelope conformation, whereas the E ring has a chair conformation and the C ring is flat. The A ring has a twisted chair conformation in the known forms of naloxone and naltrexone. The different substituent on the nitrogen atom or the existence of a counter ion hardly affects the molecular structure of the opioids.

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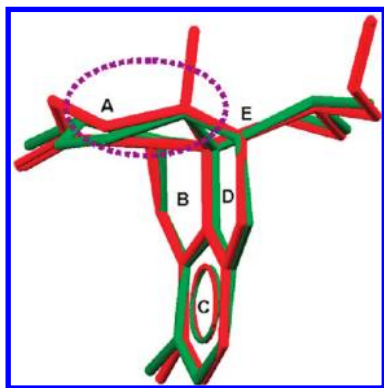


Figure 2. T-shaped five-ring system, depicting with green for the molecule of naltrexone and with red for the molecule of naloxone (the dashed circle points to the different A ring conformation).

In a previous paper, a comparison of the crystal structures of the hydrate and anhydrate forms of the agonist morphine and antagonist naloxone revealed similarities. Although they act completely different in the human body, the two agonist and antagonist opioids are shown to be crystallographically related.⁶

It is interesting to determine how the different substituents in naltrexone and naloxone influence the hydration and dehydration behavior of these antagonists, as well as on the molecular scale. The hydration/dehydration behavior of these salts was therefore studied by applying hot-humidity stage X-ray powder diffraction, microscopy, differential scanning calorimetry, thermogravimetric analysis, and solid-state NMR. The crystal structure of naltrexone hydrochloride anhydrate was determined from X-ray powder diffraction data in order to be able to study the changes in the crystal structures during dehydration.

2. Experimental Section

Materials. Naltrexone and naloxone hydrochloride were obtained from Diosynth commercial supply. The preparation procedures for the hydrate/anhydrate forms are described below.

Preparation of Naltrexone Hydrochloride Tetrahydrate. The tetrahydrate form was prepared at room temperature from a diluted aqueous HCl solution of starting material (1.5 g of NTX to 30 mL of H₂O). After complete evaporation of the solvent, needle-shaped crystals were obtained.

Preparation of Naltrexone Hydrochloride Anhydrate. Naltrexone hydrochloride anhydrate was obtained by dehydrating the tetrahydrate form at 110°C, under a vacuum (1×10^{-2} mbar) and in the presence of P₂O₅ drying agent. Before and after the experiment, argon gas was flushed into the reaction vessel. The sample was stored in a glove box under nitrogen atmosphere. Several attempts to grow crystals suitable for single-crystal diffraction failed.

Preparation of Naloxone Hydrochloride Dihydrate. The dihydrate form of naloxone hydrochloride was prepared at room temperature from a diluted aqueous HCl solution of starting material (1g of NLX to 100 mL of H₂O). After complete evaporation of the solvent, needle-shaped crystals were obtained.

Preparation of Naloxone Hydrochloride Anhydrate. Naloxone hydrochloride anhydrate was obtained by dehydrating the dihydrate form at 140°C, under a vacuum (1×10^{-2} mbar) and in the presence of P₂O₅ drying agent. Before and after the experiment, argon gas was flushed into the reaction vessel. The sample was stored in a glove box under nitrogen atmosphere.

Analytical Methodology

X-ray Powder Diffraction (XRPD). X-ray powder diffraction measurements for crystal structure determination of naltrexone hydrochloride anhydrate were performed using a Bruker D8 AXS Advance X-ray Diffractometer. The D8 was equipped with a Johansson type monochromator with a focusing curved Ge 111 crystal. A VÅNTEC-1

Table 1. Instrumental and Data Collection Parameters

| Typical Instrument Settings | |
|--|---|
| system | Bruker AXS D8 Advance $\theta/2\theta$ |
| generator | 40 kV, 40 mA |
| measuring circle (mm) | 435 |
| radiation (Å) | Cu K α_1 , $\lambda = 1.54056$ Å |
| monochromator | primary, focusing curved Ge 111 |
| geometry | transmission capillary configuration |
| sample holder | 0.5 mm glass capillary tube |
| detector | VÅNTEC-1 |
| Measuring Conditions for Structure Determination | |
| range ($^{\circ} 2\theta$) | 5–60 |
| spatial resolution (Å) | 1.5407 Å |
| step size ($^{\circ} 2\theta$) | 0.0084696 |
| step time (s) | 50 |
| total data collection time (h) | ca. 92 |
| spinning (rpm) | 15 |
| Typical Measuring Conditions for Hot-Humidity Measurements | |
| range ($^{\circ} 2\theta$) | 2–40 |
| step size ($^{\circ} 2\theta$) | 0.05 |
| step time (s) | 1 |
| humidity range (%RH) | 0–80 |
| temperature range ($^{\circ}$ C) | 25–125 |
| heating rate ($^{\circ}$ C/min) | 1 |
| carrier gas | nitrogen |

detector was used with an effective angular region of 2° . The data were collected in transmission capillary geometry using monochromatic Cu K α_1 radiation. The anhydrate sample was lightly ground, using an agate mortar and pestle and mounted in order to reduce preferred orientation effects.⁷ A 0.5 mm glass capillary tube was filled with the material and sealed in a glove box under nitrogen atmosphere. The capillary tube was spun at 15 rpm during data collection to minimize instrumental and sample packing aberrations.⁸

X-ray powder diffraction employed for investigating the hydration and dehydration behavior of the naltrexone and naloxone hydrochloride forms was done using a hot-humidity stage attached to the above-mentioned diffractometer and controlled by an Ansyco humidity and temperature control unit. The most important instrumental and data collection parameters are presented in Table 1.

Data Analysis for Crystal Structure Determination. The diffraction pattern was indexed using DICVOL91⁹ to obtain lattice parameters that were subsequently refined in a Pawley fit.¹⁰ Z matrices describing the molecular topology of the fragments in the compound were generated automatically by DASH¹¹ from the structural data of the tetrahydrate form. Twenty runs with 1×10^7 SA (simulated annealing) moves per run were performed for structure solution. To the best solution obtained after the SA process, a final full Rietveld refinement was performed using the Topas software.¹²

Microscopy. Scanning electron microscopy photographs were taken with a JEOL JSM 6330F, operating at 3 kV, 12 μ A and equipped with a secondary electron detector. To induce good conductivity of the samples, prior to SEM analysis, we coated the crystals with gold at 1×10^{-5} Torr using a Cressington 208HR Sputter Coater.

The polarized light microphotographs were taken with a Leica DM-RX microscope.

Differential Scanning Calorimetry (DSC). Differential scanning calorimetry curves were obtained using a Mettler Toledo 822 DSC with a TSO 801RO Sample Robot. The instrument was calibrated using indium standards. The samples were scanned at 1 or 5 $^{\circ}$ C/min from 25 to 280 $^{\circ}$ C under nitrogen purge at 40 mL/min in punched aluminum pans.

Thermogravimetry Analysis (TGA). Thermogravimetry analysis curves were generated using a TA Instruments Q500 TGA. The instrument was temperature calibrated using indium, tin and zinc standards. A weight calibration was performed using standard weights under nitrogen purge. The samples were scanned at 1 $^{\circ}$ C/min from 25 to 300 $^{\circ}$ C under nitrogen purge at 40 mL/min in punched aluminum pans.

Solid-State NMR Spectroscopy (SSNMR). Solid-state ¹³C cross-polarization/magic angle spinning (100.6 MHz CP/MAS) spectra for the naltrexone hydrochloride samples were obtained using a Bruker AXS 400 MHz spectrometer. The samples were packed into a 4 mm

Table 2. Lattice Parameters and Pawley Fit Results

| Lattice Parameters | | |
|-----------------------|--------------|--|
| system | orthorhombic | |
| space group | $P2_12_12_1$ | |
| a (Å) | 15.529 | |
| b (Å) | 14.991 | |
| c (Å) | 7.840 | |
| V (Å ³) | 1825.44 | |
| zero point correction | 0.0201 | |
| Pawley Fit Results | | |
| no. of reflns | 328 | |
| data points | 6309 | |
| Pawley χ^2 | 3.36 | |

rotor and spun with a rate of 12 kHz. The contact time for CP was 2.5 ms. A signal was acquired with proton decoupling.

The ¹³C NMR spectra for the naltrexone hydrochloride were obtained on a CMX-300 spectrometer (300.15 MHz for ¹H, 75.46 MHz for ¹³C) using a Bruker 4 mm double resonance MAS probe spinning at 4 kHz. The CP/MAS spectra were recorded employing a variable amplitude CP (1 ms contact time, an rf field of 64 kHz on ¹H with a ± 1 kHz ramp and a 60 kHz rf field on ¹³C). Protons were decoupled using the CM sequence with an rf field strength of 84 kHz.¹³ A 5-pulse TOSS sequence was used to suppress spinning sidebands for the amorphous and anhydrous sample.¹⁴

3. Results and Discussion

Crystal Structure Determination of Naltrexone Hydrochloride Anhydrate. Indexing, Pawley Refinement, and Simulated Annealing Process. Twenty-three reflections with low and high intensity were introduced into DICVOL91, and an orthorhombic cell with a volume of 1820.73 Å³ was found. Space group determination in DASH resulted in $P2_12_12_1$. The volume and space group $P2_12_12_1$ correspond to $Z' = 1$ (molecules per asymmetric unit). The lattice parameters obtained after indexing were subsequently refined along with background, zero point, peak shape parameters, and reflection intensities in a Pawley fit. The characteristic parameters of the orthorhombic cell obtained after the Pawley fit are presented in Table 2.

The peak shape is implemented in DASH as a convolution of Gaussian, Lorentzian, and axial divergence terms (asymmetry). Actually, the X-ray line shape is a full Voigt function, which uses two parameters σ_1 and σ_2 to describe the angle-dependent Gaussian component

$$\sigma^2 = \sigma_1^2 \sec^2 \theta + \sigma_2^2 \tan^2 \theta$$

and two parameters γ_1 and γ_2 to describe the angle-dependent Lorentzian component

$$\gamma = \gamma_1 \sec \theta + \gamma_2 \tan \theta$$

The two other asymmetry parameters are fully defined by the peak fitting procedure and cannot be refined by Pawley fitting. The sample gave sharp diffraction lines with a mean FWHM = 0.092° and a minimum value FWHM = 0.078° for the first 12 fitted peaks.

The profile Pawley χ^2 is described in DASH as

$$\chi^2 = \{ \sum_i^N w_i [y_i(\text{obs}) - y_i(\text{calcd})]^2 \} / (N - P + C)$$

where $y_i(\text{obs})$ is the observed intensity at the i th step in the powder diffraction pattern; $y_i(\text{calcd})$ is the associated calculated intensity; $w_i = 1/\sigma_i^2$, where σ_i is the standard deviation of the observed intensity at that point. The summation is performed over all N data points, P the number of parameters, and C the

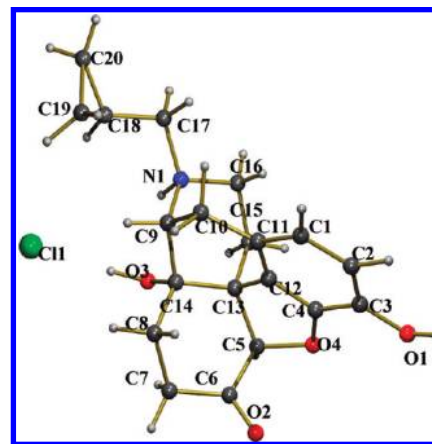


Figure 3. Molecular conformation used for the Z matrix.

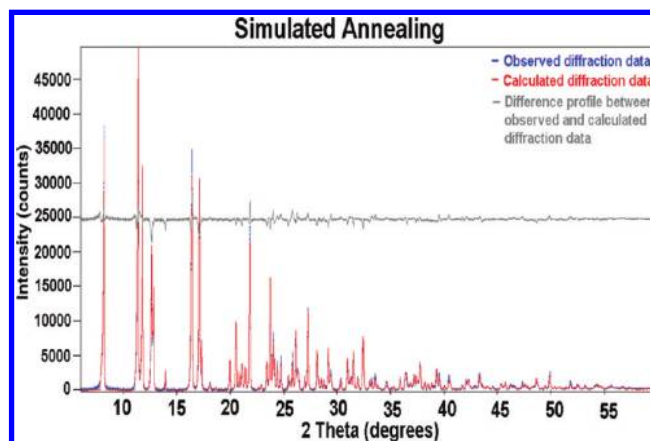


Figure 4. Fit to the diffraction data returned by the SA process.

number of parameter constraints. The profile χ^2 for the Pawley fit had a final value of 3.36.

The molecular conformation of the Z-matrix describing the molecular geometry used in the simulated annealing process is shown in Figure 3.

Naltrexone hydrochloride has two torsion angles: C18:C17:N1:C9 and C19:C18:C17:N1, optimized during the simulated annealing (SA) process. The simulated annealing process ended up with 13 solutions having low χ^2 profile values out of 20. The structure of the best solution had a profile χ^2 of 15.32. The fit of the observed and calculated diffraction data for this solution is shown in Figure 4.

Rietveld Refinement. A full Rietveld refinement¹⁵ of the best solution returned by the SA process gave a final profile $R_{wp} = 3.65$, $R_p = 2.64$ and a GOF of 2.54. The fit of the final calculated diffraction data to the observed data is shown in Figure 5.

All coordinates of the non-hydrogen atoms were freely refined, but soft restraints on bond lengths and angles were introduced in order to reduce the number of free parameters. The final crystal structure of naltrexone hydrochloride anhydrate is presented in Figure 6.

Hot-Humidity Stage X-ray Powder Diffraction (XRPD).

The conversions recorded with hot-humidity stage X-ray powder diffraction for naltrexone hydrochloride tetrahydrate are presented in Figure 7.

To study the dehydration of naltrexone hydrochloride tetrahydrate, the compound was heated in a temperature range

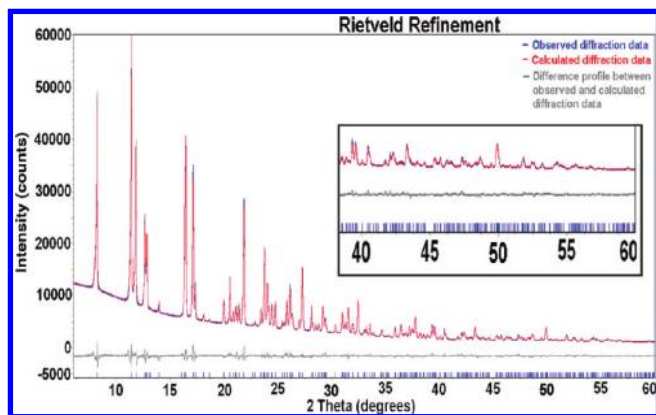


Figure 5. Fit after the final Rietveld refinement (high 2θ angle is enlarged).

between 25 and 75 °C at 0% RH and kept at 75 °C for 1 h. A complete conversion to the anhydrate form was already observed at 30 °C.

To study the reverse process, i.e., the conversion of the anhydrate to the tetrahydrate, naltrexone hydrochloride anhydrate was subjected to 40 °C and 80% RH. After 40 min this resulted in the formation of an unknown hydrate form Y. Continued exposure of form Y to 80% RH and 40 °C lead to further conversion to the tetrahydrate form. Leaving the tetrahydrate form at 40 °C and 80% RH for 12 h did not lead to other forms with a higher amount of hydration water.

The conversions recorded with hot-humidity stage X-ray powder diffraction for naloxone hydrochloride dihydrate are presented in Figure 8. To study the dehydration of the dihydrate form, the samples were heated in a temperature range between 25 and 145 °C at 0% RH and kept at 145 °C for 1 h. A complete conversion to the anhydrate form was observed at 75 °C. No intermediate forms were observed. To study the reverse process, i.e., the conversion of the anhydrate to the dihydrate form, the anhydrate was subjected to 40 °C and 80% RH. After 1 h and 20 min, this resulted in the formation of an amorphous form. Continued exposure of the amorphous form to 80% RH and 40 °C led to the formation of the dihydrate form of naloxone hydrochloride. Leaving the dihydrate form at 40 °C and 80% RH for 12 h did not lead to other forms with a higher amount of hydration water.

Microscopy. The scanning electron microphotographs of naltrexone hydrochloride samples are presented in Figure 9. Three different magnifications were used in order to emphasize the small differences between the morphology of the two forms.

The naltrexone hydrochloride tetrahydrate consists of acicular crystals found in stacked ordered aggregates with chord thickness of 2 to 18 μm (Figure 9a). The anhydrate form of naltrexone hydrochloride exhibits in general the same characteristics as the tetrahydrate form, although the anhydrate seems to consist of more defected and thinner needle crystals. The chord thickness for the anhydrate form varies from 1 to 12 μm (Figure 9b).

Naloxone hydrochloride dihydrate crystallizes in rather large pyramidal crystals (Figure 10a). Therefore, an examination using an optical microscope was possible. During a close investigation, droplets of water coming out on the surface of the crystals could be detected. It seems that the crystals of the naloxone hydrochloride dihydrate are not stable and lose at least part of the hydration water already at room temperature. When a vacuum is applied (1×10^{-2} mbar), the dihydrate pyramidal crystals break apart in order to form the anhydrate form within a few minutes. The morphology of the naloxone hydrochloride an-

hydrate crystals was investigated using scanning electron microscopy. The naloxone hydrochloride anhydrate shows needle-shaped crystals stacked in ordered aggregates with chord thickness of 2–15 μm (Figure 10b).

Differential Scanning Calorimetry (DSC). Differential scanning calorimetry curves of naltrexone hydrochloride tetrahydrate at different heating rates are shown in Figure 11. The DSC measurements of the tetrahydrate form shows an endothermic effect due to solvent loss and anhydrate formation at temperatures from 25 to 110 °C, confirming the results obtained with hot-humidity stage X-ray powder diffraction.

The anhydrate form yielded no thermal events measurable by DSC until 220 °C, where the onset of decomposition appears. The shifts recorded with 1 °C/min in comparison with the ones observed at 5 °C/min are attributed to the higher heating rates.

Differential scanning calorimetry curves of naloxone hydrochloride dihydrate at different heating rates are shown in Figure 12. The DSC measurements of the naloxone hydrochloride dihydrate show a two-step endothermic effect due to solvent loss and the final anhydrate formation at temperatures between 75 and 150 °C. Taking into account that the hot-humidity stage, XRPD data did not show the formation of another form besides the anhydrate during dehydration, which suggests that part of the water molecules can be removed from the hydrate without a structural change.

The obtained anhydrate form yielded an endothermic event measurable by DSC at 175 °C. Because of the total solvent loss and anhydrate formation at 150 °C, the recorded endothermic event at 175 °C must be due to partial decomposition of the sample. The onset of further decomposition of naloxone hydrochloride is observed at 250 °C. The shifts recorded for all the investigated forms with 1 °C/min in comparison with the ones observed at 5 °C/min are attributed to the higher heating rates.

Thermal Gravimetric Analysis (TGA). The thermal profile of the naltrexone hydrochloride tetrahydrate is shown in Figure 13. The TGA curve is denoted by a normal font line on a percentage of initial weight scale.

Dehydration of the naltrexone hydrochloride tetrahydrate was observed gradually as a mass loss of 15.57% in a temperature range from 25 to 75 °C. The dehydration of the tetrahydrate form results in the release of water with a theoretical weight loss of 16.01% close to the observed weight loss of 15.57% (3.89 molecules H_2O /molecule NTX). The investigated sample of naltrexone hydrochloride tetrahydrate decomposed in a temperature range of 220–280 °C. The TGA data confirm the results obtained with hot-humidity stage X-ray powder diffraction and DSC.

The thermal profile of the naloxone hydrochloride dihydrate is shown in Figure 14. Dehydration of the naloxone hydrochloride dihydrate was observed gradually as a mass loss of 8.81% in a temperature range from 25 to 75 °C. The dehydration of the dihydrate form results in the release of water with a theoretical weight loss of 9% close to the observed weight loss of 8.81% (1.95 molecules H_2O /molecule NLX). The investigated sample of naloxone hydrochloride dihydrate decomposed partially in a temperature range from 175 to 250 °C continued by the final decomposition process in a temperature range of 250–300 °C. The TGA data confirm the results obtained with hot-humidity stage X-ray powder diffraction and DSC; however, the separate steps in dehydration as seen in DSC are not visible here.

Solid-State NMR Spectroscopy (SSNMR). The ^{13}C CPMAS spectra of the naltrexone hydrochloride samples are displayed

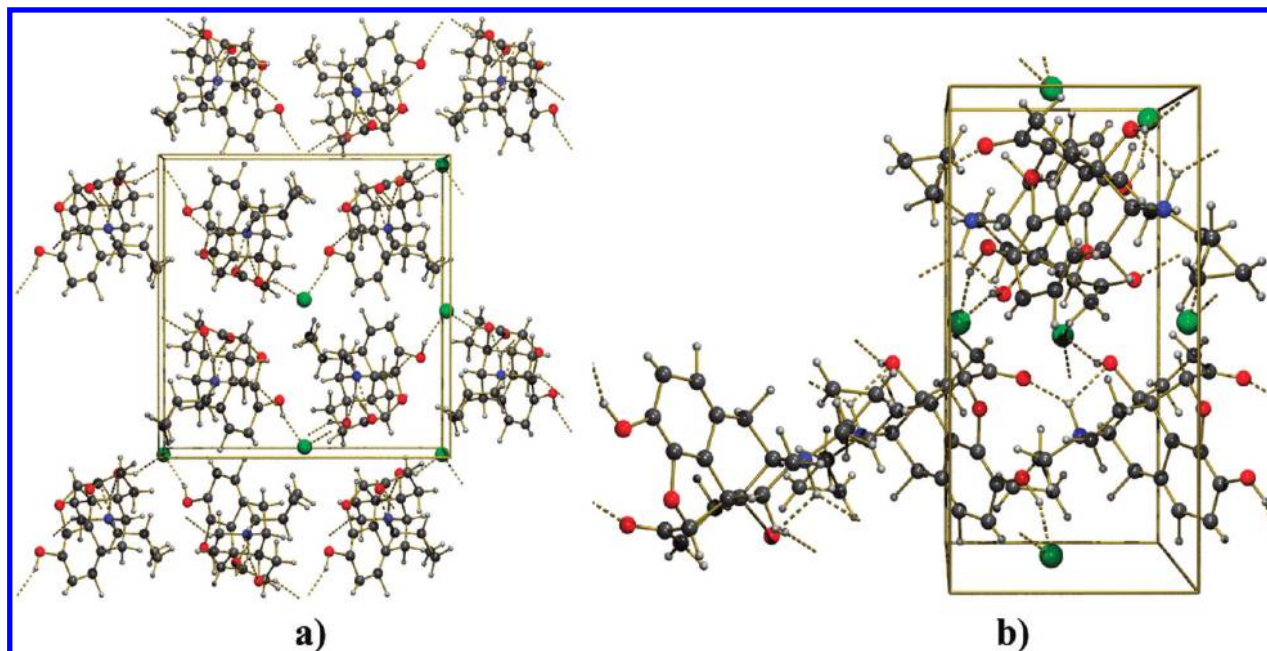


Figure 6. Crystal structure of naltrexone hydrochloride anhydrate: (a) *c*-projection, (b) *a*-projection.

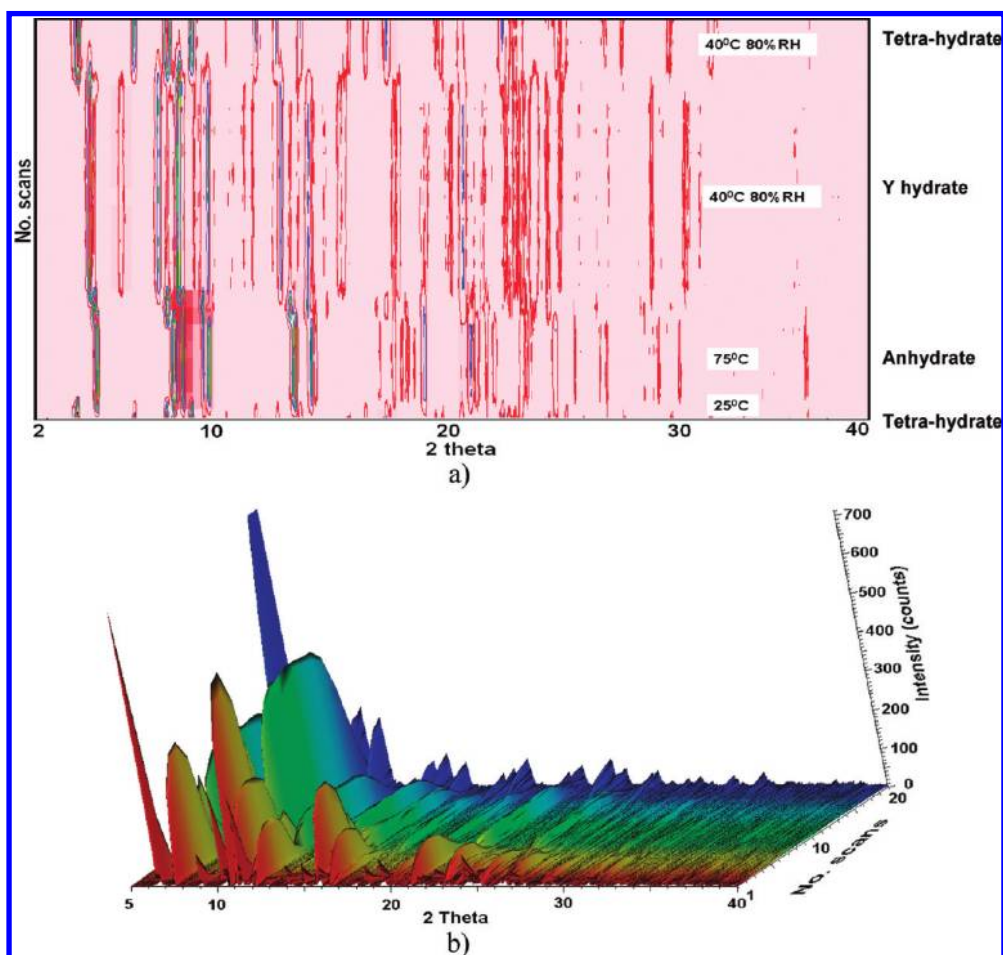


Figure 7. (a) Top view and (b) 3D pictures of the hot-humidity stage X-ray powder diffraction experiments recorded for the conversion of naltrexone hydrochloride tetrahydrate (bottom) to the anhydrate, further on to an unknown hydrate form Y and back to the tetrahydrate (top).

in Figure 15. Compared to the tetrahydrate form of naltrexone hydrochloride, the undetermined hydrate Y and the anhydrate forms show significant differences. The tetrahydrate and the anhydrate forms both have single resonances per carbon atom,

a clear indication of one molecule per asymmetric unit. However, for the undetermined hydrate Y, there seem to be three resonances per carbon atom of more or less equal intensity within each carbon species. Some groups of three carbon

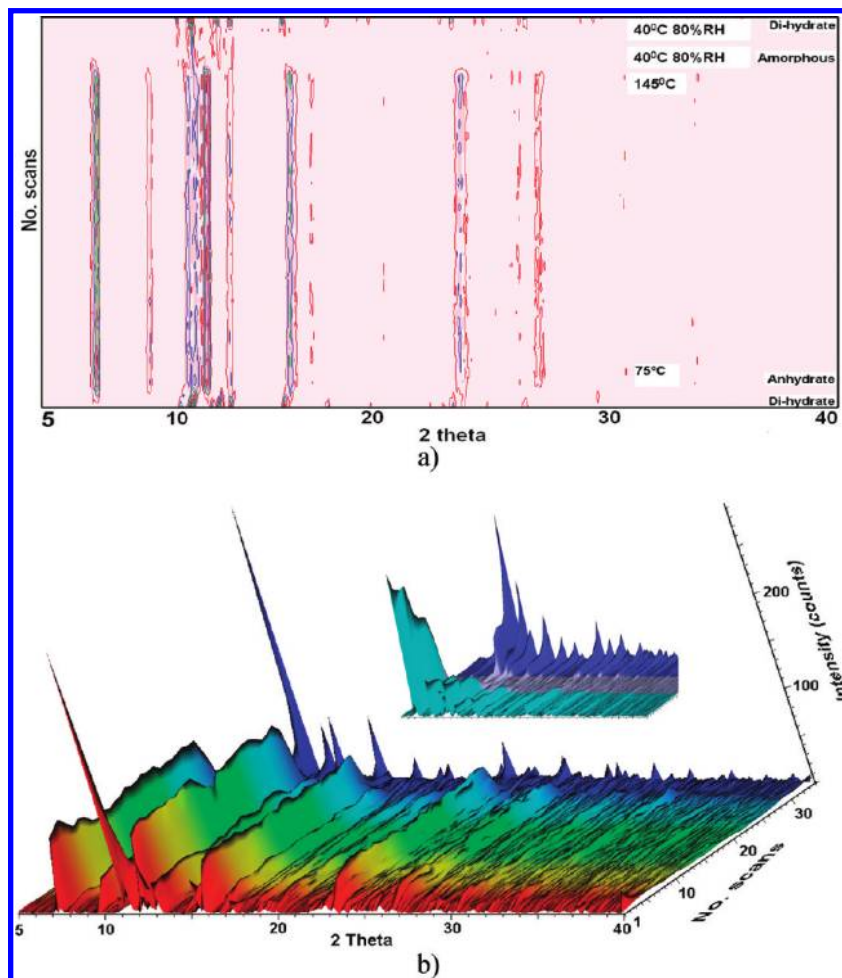


Figure 8. (a) Top view and (b) 3D pictures of the hot-humidity stage X-ray powder diffraction experiments recorded for the conversion of naloxone hydrochloride dihydrate (bottom) to the anhydrate form, further on to the amorphous form and back to the dihydrate form (top) (enlarged picture of the conversion anhydrate–amorphous–dihydrate).

resonances have shifts that neither occur in the anhydrate, nor in the tetrahydrate. This excludes the existence of a mixture of the anhydrate and the tetrahydrate and together with another unknown form. Although a mixture of three unknown compounds is still a possibility, $Z' = 3$ is much more likely for this unknown form.

The ^{13}C -NMR spectra for naloxone hydrochloride dihydrate, anhydrate and amorphous forms are shown in Figure 16. The spectra of the dihydrate and the anhydrate forms indicate the existence of one molecule per asymmetric unit. Clear differences can be observed between the dihydrate and anhydrate. The spectrum of the amorphous compound displays broad resonances, at the position of the originating anhydrate, at the position of the dihydrate, or in between. This clearly indicates the structural heterogeneity. Amorphous and materials of poor crystallinity have broader resonances because of the heterogeneity of structural forms found in the sample. This heterogeneity results in a distribution of isotropic shifts for a given carbon.

Structural Aspects of Hydration and Dehydration. The characteristic structural features of the hydrated and anhydrate forms of naltrexone and naloxone hydrochloride are presented in Table 3.

The naltrexone molecules in naltrexone hydrochloride tetrahydrate are packed in a three-dimensional network with the aid of water molecules. H-bonds are formed between the $-\text{OH}$ group attached to C(3), respectively, C(14) and water molecules (for atom numbering see Figure 3). The Cl^- is involved in

H-bonding with the water molecules and also with the $-\text{OH}$ group attached to C(14). The carboxyl group forms H-bonds only with the N atom. H-bonds between different water molecules are also observed. Obviously, the water molecules play a stabilizing role in the network formation of naltrexone hydrochloride tetrahydrate. The naltrexone molecules in naltrexone hydrochloride anhydrate are assembled in a two-dimensional network, this time with the aid of the Cl^- anion and N atom. The Cl^- forms H-bonds with the $(-\text{OH})$ groups attached to C(3) and C(14). As for the case of the tetrahydrate form, the carboxyl group forms only H-bonds with the N atom. The packing of the naltrexone molecules in naltrexone hydrochloride tetrahydrate and hydrochloride anhydrate is presented in panels a and b in Figure 17.

Comparing the SSNMR spectra of the anhydrate with the tetrahydrate, significant differences can be found in the cyclopropane substituents (C18, C19, C20 between 0 and 11 ppm) that can be easily traced back to their significantly different surroundings in the crystal lattice (presence of chlorine in the anhydrate vs hydroxyl in the tetrahydrate, see panels a and b in Figure 17). It is interesting to note that the shift of the carboxyl hardly changes between the two forms (215.9 ppm in the tetrahydrate vs 215.8 ppm in the anhydrate), in line with the fact that in both the tetrahydrate and the anhydrate this carboxyl is only involved in the same type of hydrogen bond to N. By contrast, the unknown form shows 3 peaks with different shifts

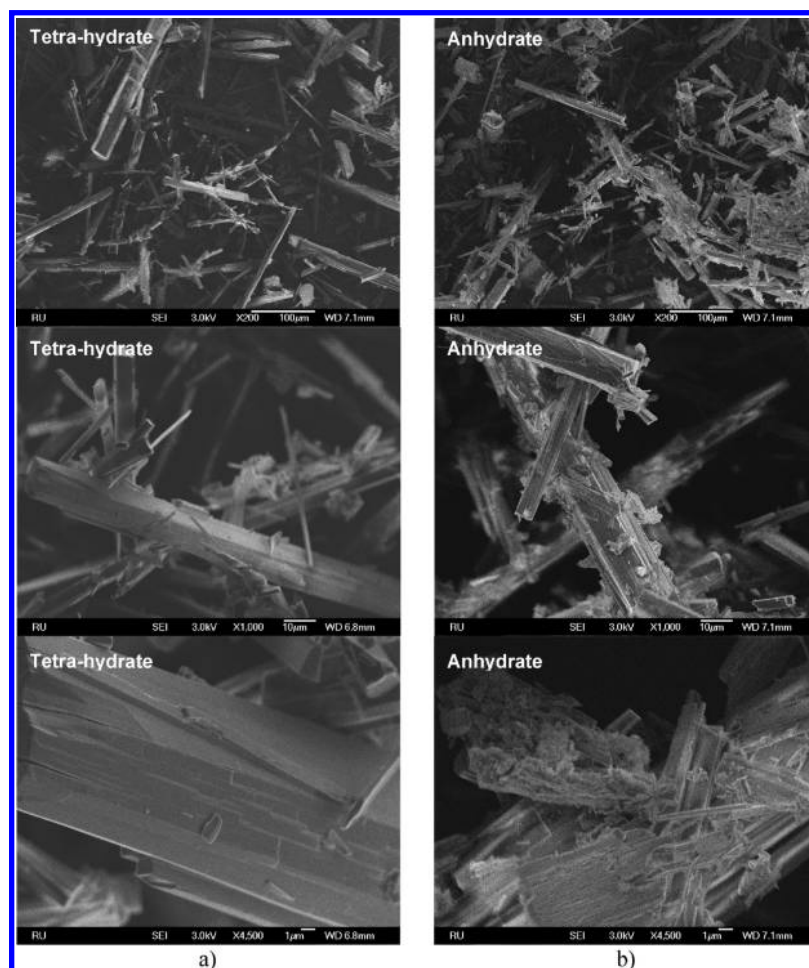


Figure 9. Scanning electron microphotographs of naltrexone hydrochloride forms at different magnifications ($\times 200$, $\times 1000$, and $\times 4500$) of (a) tetrahydrate and (b) anhydrate.

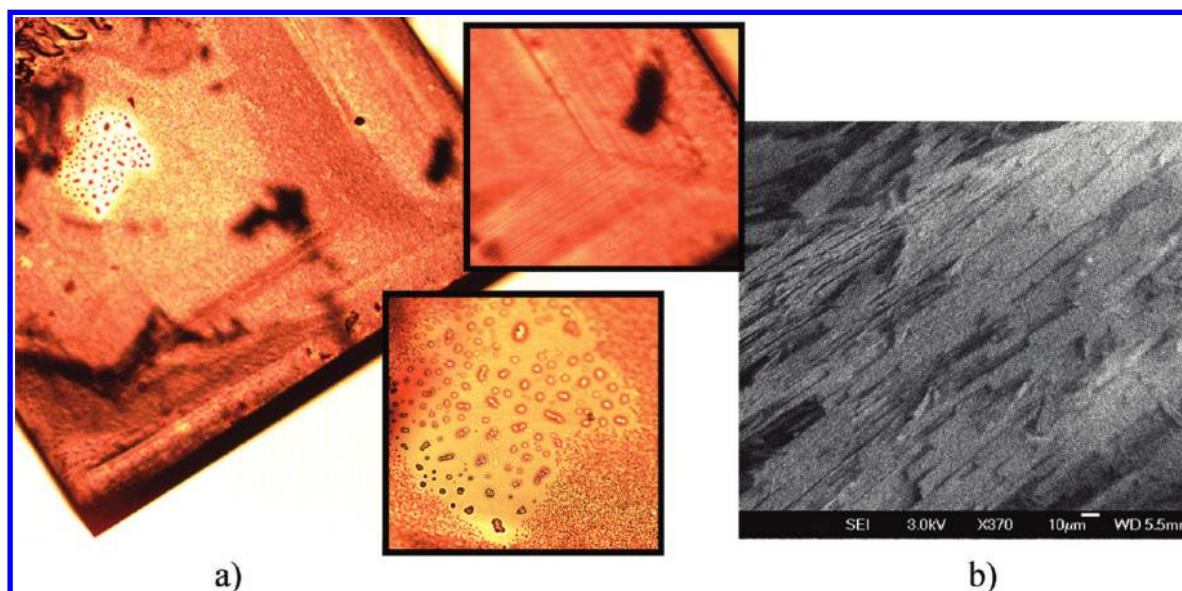


Figure 10. Microphotographs of naloxone hydrochloride: (a) dihydrate form with enlarged zoom on the water droplets (down) and the pyramidal shaped crystal (up); (b) anhydrate form at $\times 370$ magnification.

(215.0, 216.0, and 216.4), a strong indication that the hydrogen bonding in this form is significantly changed.

The naloxone molecules in naloxone hydrochloride dihydrate are also packed in a three-dimensional network with the aid of

water molecules. H-bonds are formed between the $-\text{OH}$ group attached to C(3), respectively C(14) and water molecules. The carboxyl group and the Cl^- are also involved in H-bonding with the water molecules. The N atom forms H-bonds only with the

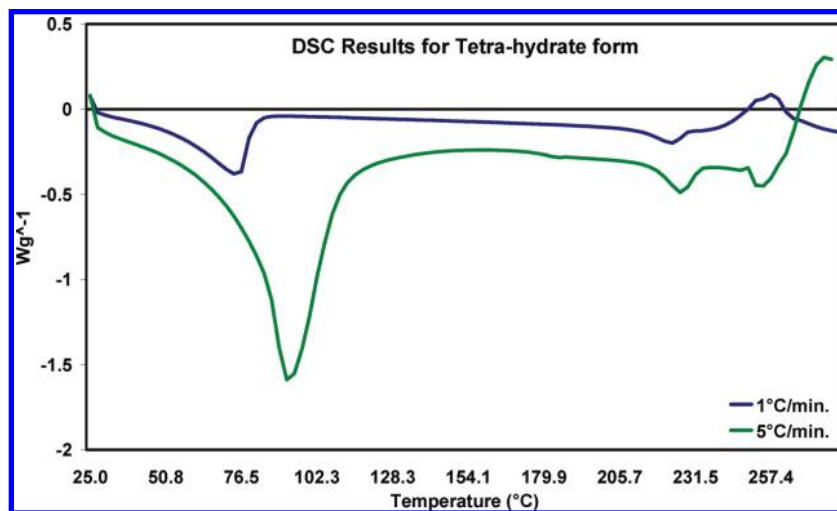


Figure 11. DSC curves with 1 °C/min (blue) and 5 °C/min (green) for naltrexone hydrochloride tetrahydrate.

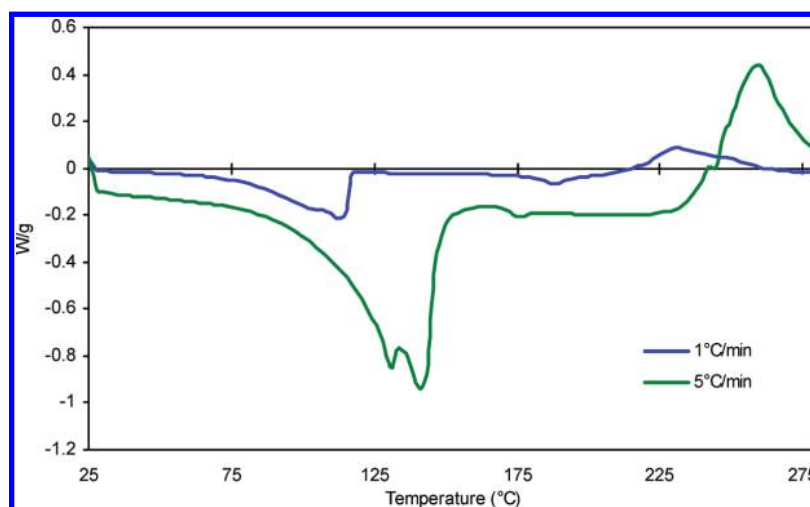


Figure 12. DSC curves with 1 and 5 °C/min for naloxone hydrochloride dihydrate.

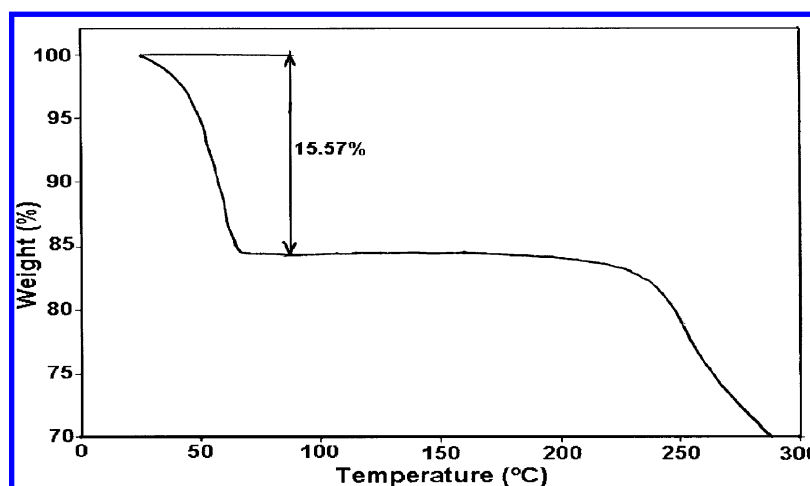


Figure 13. TGA curves with 1 °C/min heating rate for naltrexone hydrochloride tetrahydrate.

Cl^- . The naloxone molecules in naloxone hydrochloride anhydrate are also assembled in a three-dimensional network, this time with the aid of the Cl^- anion. Cl^- forms H-bonds with the N atom and with the ($-\text{OH}$) groups attached to C(3) and C(14).¹ The packing of the naloxone molecules in naloxone hydrochloride

dihydrate and hydrochloride anhydrate is presented in panels c and d in Figure 17.

The SSNMR data showed that for those carbon atoms involved in the hydrogen bonding, going from the anhydrate to the dihydrate, the chemical shift changes in the C3 hydroxyl at

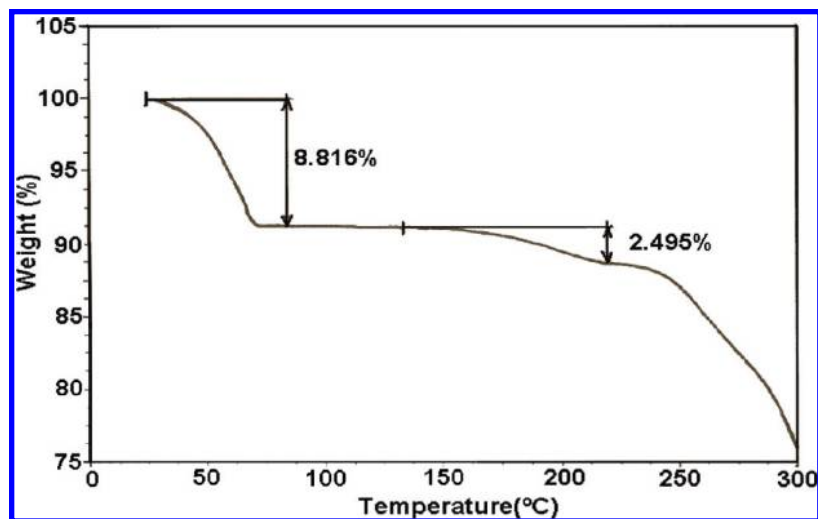


Figure 14. TGA curves with 1 °C/min heating rate for naloxone hydrochloride dihydrate.

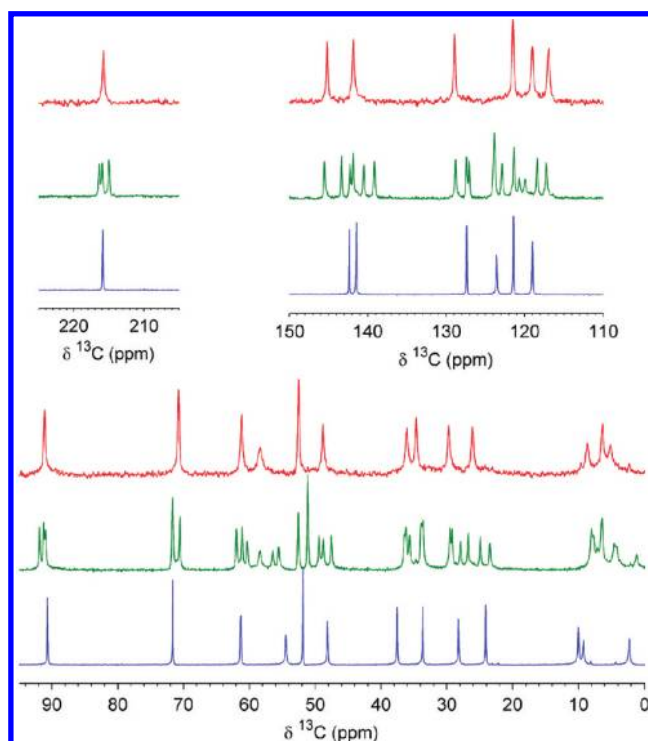


Figure 15. Solid-state ^{13}C CP/MAS NMR spectra of naltrexone hydrochloride forms: tetrahydrate (bottom spectra), anhydrate (top), undetermined hydrate (middle).

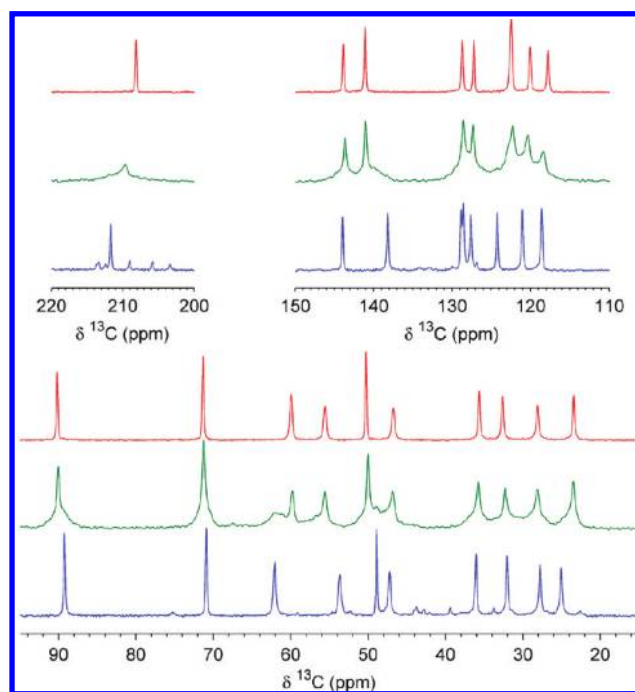


Figure 16. Solid-state ^{13}C CP/MAS NMR spectra of naloxone hydrochloride forms: dihydrate (bottom spectra), anhydrate (top), amorphous (middle). The low intensity peaks in the dihydrate spectrum are spinning side bands and not impurity signals.

Table 3. Characteristic Structural Data of the Investigated forms

| antagonist opioids | H-bond network | H-bonds formed by N atom | H-bonds formed by Cl^- anion | other H-bond |
|---------------------------------------|----------------|--------------------------|---------------------------------------|--|
| naltrexone hydrochloride tetrahydrate | 3D | carboxyl group | water C(14)–OH | C(3)–OH \cdots O _(water) C(14)–OH \cdots O _(water) OH _(water) \cdots Cl ⁻ OH _(water) \cdots O _(water) |
| naltrexone hydrochloride anhydrate | 2D | carboxyl group | C(3)–OH C(14)–OH | |
| naloxone hydrochloride dihydrate | 3D | Cl ⁻ anion | C(3)–OH water N–H | C(3)–OH \cdots O _(water) C(14)–OH \cdots O _(water) OH _(water) \cdots Cl ⁻ OH _(water) \cdots O–C(6) |
| naloxone hydrochloride anhydrate | 3D | Cl ⁻ anion | C(3)–OH C(14)–OH N–H | |

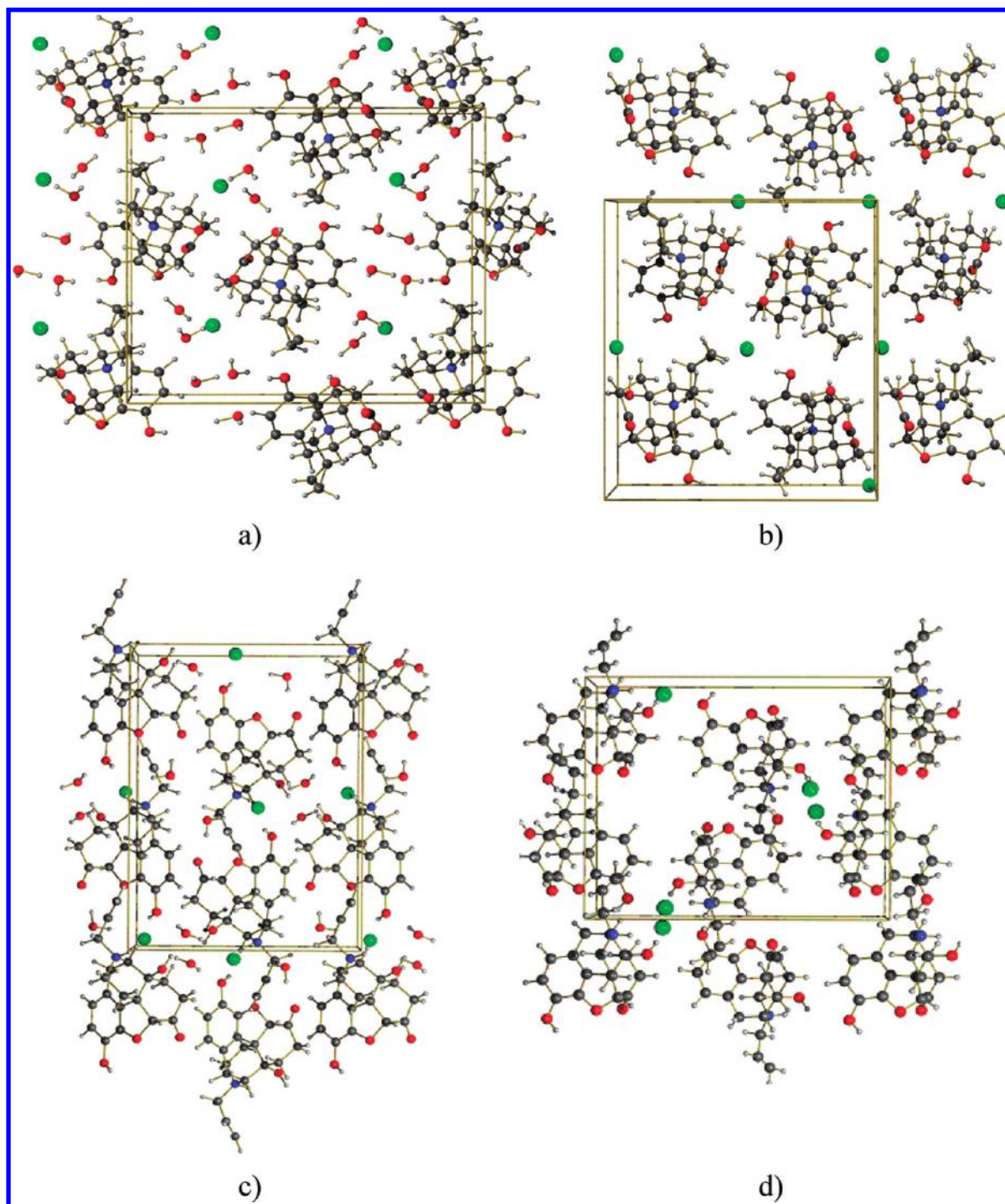


Figure 17. Crystal structures of the (a) naltrexone hydrochloride tetrahydrate, (b) naltrexone hydrochloride anhydrate, (c) naloxone hydrochloride dihydrate, and (d) naloxone hydrochloride anhydrate.

141.1 ppm shifts -2.9 ppm (becomes more shielded) and the C6 carboxyl at 208.1 ppm shifts $+3.6$ ppm in the dihydrate (more deshielding). Interestingly, also other carbon atoms are affected: C17 and C10 are not involved in hydrogen bonding but still their chemical shifts change going from anhydrate to dihydrate from 50.3 ppm to 48.9 ppm for C17 and from 23.4 ppm to 25.1 ppm for C10. Because the conformation of the naloxone molecules is virtually the same for the anhydrate and the dihydrate, these changes in chemical shift therefore reflect the effect of the differences in stacking of the molecules. One should note that all peaks that are present in the anhydrate still exist in the amorphous form. On the whole this indicates that

locally, for at least a significant number of molecules, the environment still resembles the anhydrate structure. The positions of the carboxyl on the other hand seem to be the first to be entirely affected by hydration. From the crystal structure, one can see that the carboxyls are located in the voids in the structure and are therefore most readily hydrated.

A comparison between the crystal structures of the hydrated and anhydrate forms of naltrexone and naloxone hydrochloride can now be made. By dehydrating the naltrexone hydrochloride tetrahydrate, the water disappears from the net without breaking of the symmetry, but with the lowering of the dimensionality of the H-bond network. The dehydration process of the

Table 4. Unit-Cell Parameters of the Investigated Forms Showing the Dehydration Pathway

| | naltrexone hydrochloride tetrahydrate | naltrexone hydrochloride anhydrate | naloxone hydrochloride dihydrate | naloxone hydrochloride anhydrate |
|-----------------------|---------------------------------------|------------------------------------|----------------------------------|----------------------------------|
| syst | orthorhombic | orthorhombic | orthorhombic | orthorhombic |
| space group | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ |
| a (Å) | 18.099(5) → | 14.9942 (4) | 18.569(5) → | 14.681 (4) |
| b (Å) | 15.926(6) → | 15.5338(4) | 13.185(5) → | 17.452(4) |
| c (Å) | 7.768(1) → | 7.8420(2) | 7.833(3) → | 7.978 (2) |
| V (Å ³) | 2239.08 | 1826.53 | 1917.77 | 2044.11 |

tetrahydrate form induces shrinkage of the unit cell in two directions and small expansion in the third direction (Table 4).

By dehydrating the naloxone hydrochloride dihydrate, the water disappears from the net again without breaking of the symmetry but without lowering of the dimensionality of the H-bond network. In contrast to naltrexone hydrochloride tetrahydrate, the water molecules in the naloxone dihydrate are isolated and therefore are not mutually attached by H-bonds. The DSC measurements indicate that the dehydration of the naloxone hydrochloride dihydrate takes place in two steps, but at the same time, hot-humidity stage XRPD could only record the powder patterns corresponding to the dihydrate and anhydrate forms. The structural data together with the DSC and XRPD data suggest the formation of an intermediate hydrate form isostructural to the dihydrate. The two water molecules in naloxone hydrochloride dihydrate form different H-bonds. One of the water molecules forms H-bonds with the $-OH$ group attached to C(3) and C(14). The second water molecule forms H-bonds with the carboxyl group and with the Cl^- anion. The H-bonds formed by the water molecule with the $-OH$ groups are significantly shorter ($L_1 = 1.731$ and $L_2 = 1.864$) than those formed by the second water molecule ($L_3 = 2.882$ and $L_4 = 2.184$). Therefore, the first dehydration event noticed in the DSC data probably corresponds to the loss of the water molecules that are involved in H-bonding with the carboxyl group and Cl^- anion. The same loosely bonded water molecules are probably also responsible for loss of water noticed in the microphotographs that were taken at room temperature. Figure 18 presents the two water molecules involved in H-bonds with different lengths, together with the symmetry-related water molecules.

The dehydration process of the dihydrate form induces shrinkage of the unit cell in one direction and expansion in two directions (Table 4).

The hot-humidity stage XRPD data showed that the anhydrate form of naloxone hydrochloride converts (at similar conditions) faster to the dihydrate than the anhydrate form of naltrexone hydrochloride converts to the tetrahydrate form. In both cases, the conversions to the hydrated forms of naloxone and naltrexone hydrochloride are taking place via an intermediate form. Naloxone hydrochloride dihydrate is obtained via an amorphous form and the naltrexone hydrochloride tetrahydrate via an unknown crystalline hydrate. The principal packing of the molecules in the naloxone hydrochloride anhydrate is considerably changed in comparison with the dihydrate, leaving voids in the crystal structure (Figure 19) that are not present in the anhydrate of naltrexone hydrochloride. This could be a reason that the hydration process of naloxone is faster compared to naltrexone.

Another significant structural difference is that the N atom prefers to form H-bonds with the carboxylic group in the naltrexone hydrochloride forms, and with the Cl^- anion in the naloxone hydrochloride forms.

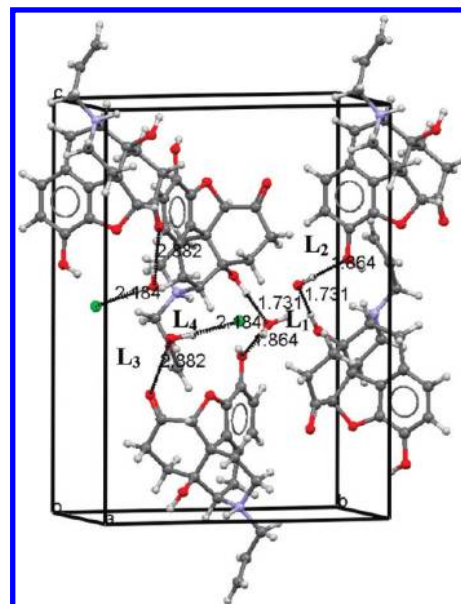


Figure 18. H-bonds and their length formed by the two water molecules present in naloxone hydrochloride dihydrate, together with the symmetry related water molecules.

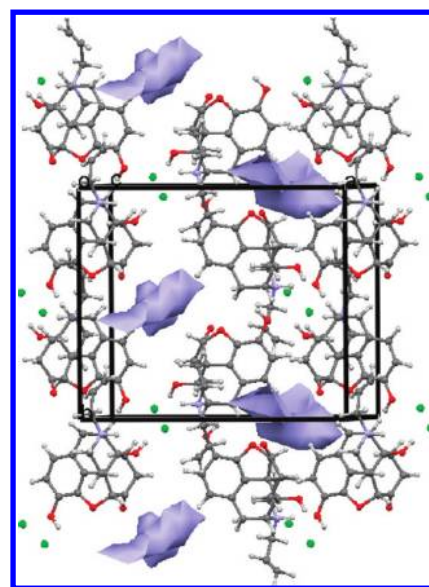


Figure 19. Naloxone hydrochloride anhydrate and the voids created after dehydration, depicted in blue.

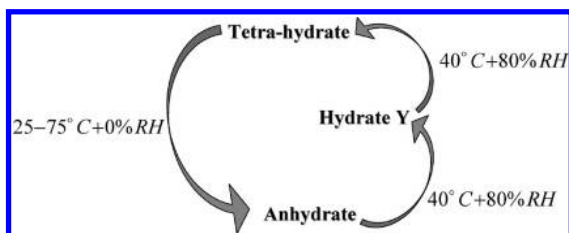
Moreover, dehydration of naltrexone hydrochloride tetrahydrate takes place with shrinkage of the volume of the unit cell with 18.42%, whereas naloxone hydrochloride dihydrate shows an expansion of the unit cell with 6.18% during dehydration.

Despite naltrexone and naloxone hydrochloride being chemically and structurally related and showing some similarities in their behavior, the overall hydration and dehydration process is fundamentally different.

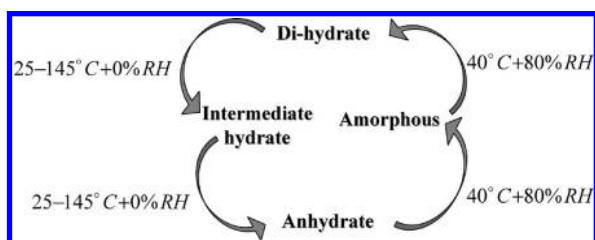
4. Conclusions

The crystal structure of naltrexone hydrochloride anhydrate was determined successfully from X-ray powder diffraction data collected in transmission capillary geometry and using the DASH software for structure determination and Topas software for the final Rietveld refinement.

The combination of the applied techniques (DSC, TGA, SSNMR, hot-humidity XRPD) together with the structural data show consistent results and yield a detailed conversion scheme for naltrexone hydrochloride as well as for the naloxone hydrochloride. Hot-humidity stage X-ray powder diffraction showed complete and reversible conversion between the tetrahydrate and the anhydrate forms of naltrexone hydrochloride, respectively between the dihydrate and the anhydrate forms of naloxone hydrochloride. The conversion of naltrexone hydrochloride anhydrate to the tetrahydrate form takes place via an intermediate hydrate form. The conversion of the naloxone hydrochloride anhydrate to the dihydrate form takes place via an amorphous form. The existence of a intermediate hydrate isostructural to naloxone hydrochloride dihydrate is suggested from the combination of DSC, XRPD, and SSNMR measurements. The conversions can be summarized in the following scheme for naltrexone hydrochloride:



and for naloxone hydrochloride:



SSNMR confirmed the existence of another hydrate form for naltrexone hydrochloride and excludes the existence of a mixture of the anhydrate and tetrahydrate forms. In the case of naloxone hydrochloride, the existence of the amorphous form was also confirmed. The impact of dehydration and hydration on the H-bonding networks is clearly reflected in the solid-state NMR C^{13} spectra.

Comparison of the crystal structures of the hydrated and the anhydrate forms of naltrexone and naloxone hydrochloride reveals some similarities but also significant differences. The investigated forms of the two antagonists crystallize all in the

orthorhombic space group $P2_12_12_1$. The dehydration processes for naltrexone hydrochloride tetrahydrate and naloxone hydrochloride dihydrate induces different changes in the unit cells of the hydrated forms in order to form the anhydrated forms. The difference in the H-bonding pattern showed by the two opioids seems to be a footprint for the crystal structures and for the hydration/dehydration behavior of the two antagonists. Remarkably, dehydration of naltrexone hydrochloride tetrahydrate takes place with shrinkage of the volume of the unit cell with 18.42%, whereas naloxone hydrochloride dihydrate shows an expansion of the unit cell with 6.18% during dehydration. Despite the fact that naltrexone and naloxone hydrochloride are chemically and structurally related and show some similarities in their behavior, the overall hydration and dehydration processes are fundamentally different.

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References

- (1) Karle, I. L. *Acta Crystallogr., Sect. B* **1974**, *30*, 1682–1686.
- (2) Pert, C. B.; Snyder, S. H. *Mol. Pharm.* **1974**, *10*, 868–879.
- (3) Ginzburg, H. M.; Glass, W. J. *J. Clin. Psychiatry* **1984**, *45*, 4–6.
- (4) Le Dain, A. C.; Madsen, B. W.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1992**, *45*, 635–640.
- (5) Sime, R. L.; Forehand, R.; Sime, R. J. *Acta Crystallogr., Sect. B* **1975**, *31*, 2326–2330.
- (6) Guguta, C.; Peters, Th. P. J.; de Gelder, R. *Cryst. Growth Des.* **2008**, *8* (11), 4150–4158.
- (7) Howard, C. J.; Kisi, E. H. *J. Appl. Crystallogr.* **2000**, *33*, 1434–1435.
- (8) Dollase, W. A. *J. Appl. Crystallogr.* **1986**, *19*, 267–272.
- (9) Boulif, A.; Louër, D. *J. Appl. Crystallogr.* **1991**, *24*, 987–993.
- (10) Pawley, G. S. *J. Appl. Crystallogr.* **1981**, *14*, 357–361.
- (11) David, W. I. F.; Shankland, K.; Van de Streek, J.; Pidcock, E.; Motherwell, S. *DASH version 3.0*; Cambridge Crystallographic Data Centre: Cambridge, U.K., 2004.
- (12) Cheary, R. W.; Coelho, A. A. *J. Appl. Crystallogr.* **1992**, *25*, 109–121.
- (13) Gerbaud, G.; Ziarelli, F.; Caldarelli, S. *Chem. Phys. Lett.* **2003**, *377*, 1.
- (14) Song, Z.; Antzutkin, O. N.; Feng, X.; Levitt, M. H. *Solid State Nucl. Magn. Reson.* **1993**, *2*, 143–146.
- (15) Young, R. A. *The Rietveld Refinement*; Oxford University Press: Oxford, U.K., 1996.

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