Structural Characterization of Anhydrous Naloxone- and Naltrexone Hydrochloride by High Resolution Laboratory X-Ray Powder Diffraction and Thermal Analysis

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ABSTRACT: The crystal structures of the analgesic compounds anhydrous naloxone and naltrexone hydrochloride were determined *ab initio* from high resolution laboratory X-ray powder diffraction data. Both compounds crystallize in the orthorhombic space group $P2_12_12_1$ with lattice parameters of $a = 14.6588(10)$ Å, $b = 17.4363(9)$ Å, $c = 7.96200(22)$ Å, and $V = 2035.06(23)$ Å^3 for naloxone hydrochloride and $a = 15.4560(5)$ Å, $b = 14.9809(4)$ Å, $c = 7.84121(18)$ Å, and $V = 1815.58(11)$ Å^3 for naltrexone hydrochloride. The crystal structure of anhydrous naloxone hydrochloride forms one-dimensional chains through hydrogen bonds. In the crystal structure of anhydrous naltrexone hydrochloride, two-dimensional sheets are formed by hydrogen bonds. The dehydration processes of naloxone hydrochloride dehydrate and naltrexone hydrochloride tetrahydrate was analyzed by DTA, DSC, TG, and MG. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 96:3316–3323, 2007

Keywords: Crystal structure; X-ray powder diffractometry; Dehydration; Thermal analysis; Transition

INTRODUCTION

Analgesia is an important area of medicine. The reduction of the perceived physical pain is needed in several procedures or treatments. Physicians have some choices when prescribing analgesic agents. The main two groups of the compounds commonly used are anti-inflammatory drugs and opiates. The anti-inflammatory drug group contains aspirin, indomethacin, ibuprophen, tolmetin, diclofenac, ketorolac, naproxen, piroxicam, *etc*. The opiate group contains codeine, hydromorphone, oxymorphone, hydrocodone, oxycodone, butorphanol, propoxyphen, fentanyl, morphine and others.[1] The opiate analgesics work by binding to and activating opioid receptors (μ, κ, δ). When overdosed or abused opiates can cause addiction[2] or death.[3,4]

Naloxone and naltrexone are both used to treat overdose of opioids. Both compounds are μ-opiod receptor competitive agonists. Naloxone is often used intravenously for faster action and naltrexone is more commonly used in long-term treatment of overdose and addiction. Comparing with naloxone, naltrexone is a much stronger competitive agonist at κ-receptor. It is also used to treat alcohol addiction.[5]

Both compounds are manufactured as hydrochloride salts in hydrated form by several companies. The crystal structures of naloxone hydrochloride dihydrate ($C_{19}H_{26}ClNO_6$) and naltrexone hydrochloride tetrahydrate ($C_{20}H_{32}ClNO_8$) were determined from single crystal diffraction data.[6,7]

In a crude approximation the therapeutic effectiveness of a dose of a drug may be affected by its solubility.[8] The solubility of salts may be

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limited by a common ion effect. This is especially the case when hydrochloride salts are a concern since gastric fluid contains chlorine ions. Further the solubility is a function of the crystal structure of a solid form. They vary depending on a polymorph used. They also vary between hydrated and anhydrous species. The common belief that the anhydrous species dissolve more than the hydrated ones may not always be true.[9] The relative solubility of the forms depends on the location of the transition temperature for the enantiotropic system. The ease of transition from less stable to a more stable form at chosen temperature and a subsequent change in solubility will depend on the characteristics of the crystal structure.

In this paper we will report the previously unknown crystal structures of anhydrous naloxone and naltrexone hydrochlorides and evaluate their dehydration mechanisms.

EXPERIMENTAL

Sample preparation

Powder samples of naloxone hydrochloride dihydrate and naltrexone hydrochloride tetrahydrate were purchased from Fluka (>99%) and Sigma (>99%). In order to determine the crystal structures of the anhydrous forms, small amounts of gently ground naloxone hydrochloride dihydrate and naltrexone hydrochloride tetrahydrate were kept for 3 days under high vacuum in a Schlenk tube with dried P_2O_5 at a temperature of $T =$ 100°C. Subsequently, the powders were sealed in glass capillaries of 0.5 mm diameter (Hilgenberg glass No. 50) in the glove box under argon gas using a hot wire.

Thermal Analysis

Thermal analyses of naloxone hydrochloride dihydrate and naltrexone hydrochloride tetrahydrate were conducted using DSC- as well as simultaneous DTA/TGA/MS-techniques. DSC measurements were carried out with a DSC 404 calorimeter (Netzsch, Selb) at a heating rate of 5 K/min in vacuum with the samples sealed in aluminum boxes (Fig. 1). For DTA/TGA/MS investigations a Simultaneous-Thermal-Analysator STA 409 (Netzsch, Selb) was used with the samples in alumina crucibles in an argon flow of 100 mL/min at a heating rate of 5 K/min and,

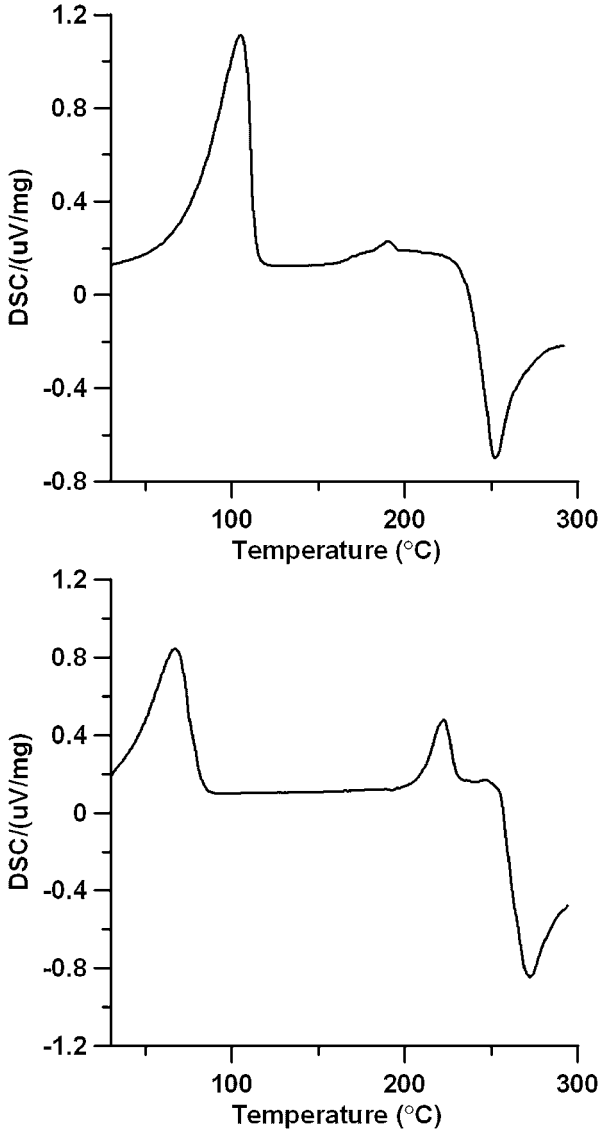

Figure 1. DSC trace of naloxone hydrochloride dihydrate (top) and naltrexone hydrochloride tetrahydrate (bottom) obtained with a heating rate 5°C/min. The sample mass was 13.2 mg for naloxone hydrochloride dehydrate and 18.2 mg for naltrexone hydrochloride tetrahydrate.

respectively (Fig. 2). All temperatures given are the onset values of the corresponding peak. The dehydration peak and endothermic peak were observed in all runs.

X-ray Powder Diffraction

X-ray powder diffraction patterns of anhydrous naloxone and naltrexone hydrochloride were

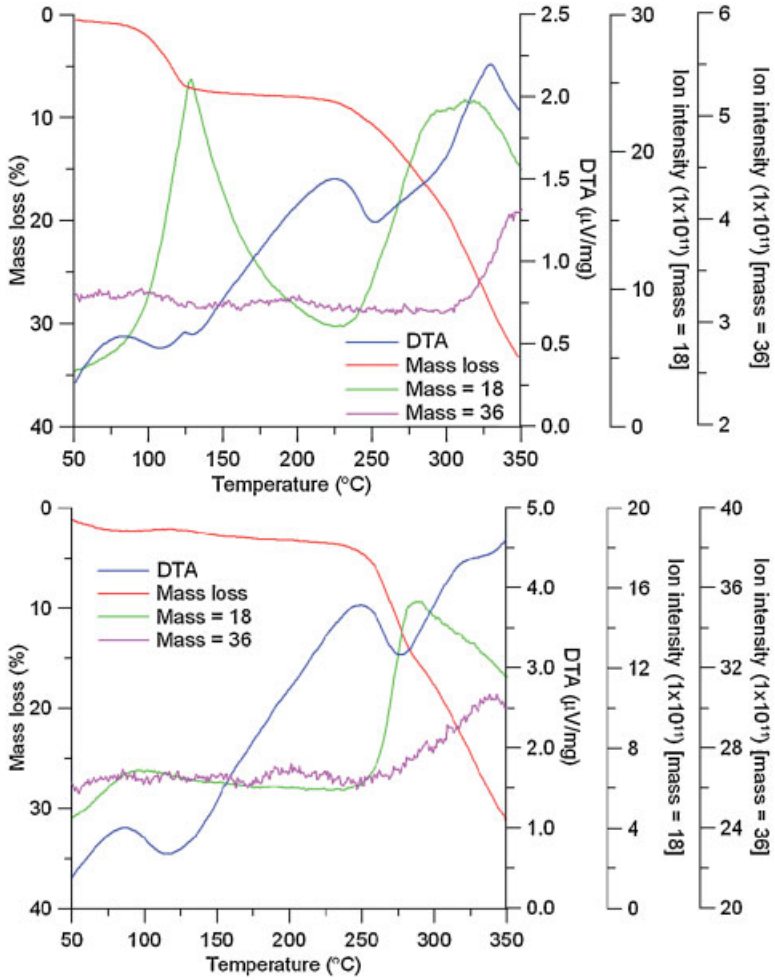

Figure 2. DTA trace and TG/MS profile of naloxone hydrochloride dihydrate (top) and naltrexone hydrochloride tetrahydrate (bottom) obtained with a heating rate 5°C/min. The sample mass was 13.8 mg for naloxone hydrochloride dehydrate and 6.8 mg for naltrexone hydrochloride tetrahydrate. The DTA (solid line, the mass loss (dashed line)) and the ion current for mass 18 (chain dotted line) are shown.

recorded at room temperature on a high resolution laboratory X-ray powder diffractometer (D8 ADVANCE, Bruker, Cu $K\alpha_1$ radiation from primary Ge(111)-Johanson-type monochromator; Våntag-1 position sensitive detector (PSD) in Debye–Scherrer geometry). These data were taken in steps of 0.0172° (2θ) for 0.05°/min with a 6° opening of the PSD, which converts to 20.64 s per step. The samples were spun during measurement for better particle statistics.

Crystal Structure Determination

Indexing of the powder diffraction patterns of anhydrous naloxone and naltrexone hydrochloride was carried out using the program ITO.[10] The space group of both compounds could be unambiguously determined as $P2_12_12_1$ from the observed extinction rules. The cell volumes of 2035.06 Å^3 for naloxone hydrochloride and 1815.58 Å^3 for naltrexone hydrochloride and the maximum number of formula units of $Z = 4$ in $P2_12_12_1$ suggested one molecule of these complexes in the asymmetric units. The determinations of the crystal structures of naloxone- and naltrexone hydrochloride were performed as described in detail in chapter 9 of the book Solid State Characterization of Pharmaceuticals.[11] The global optimization program DASH (CCDC) program[12] using the method of simulated annealing was used for structure determination. Molecular connectivity models for naloxone and naltrexone hydrochloride were taken from the Cambridge

Structural Database (NALOXC for naloxone hydrochloride and PABCEA for naltrexone hydrochloride). All intramolecular distances and angles were kept during the simulated annealing runs. For the naloxone and naltrexone molecules, 8 internal degrees of freedom each were introduced to global optimization: 3 translations, 3 rotations and 2 torsion angles (Fig. 3). In case of naloxone, the torsion angles determine the orientation of the allyl chain, whereas in case of naltrexone the torsion angles describe the orientation of the plane bisecting the piperidine ring. Three additional translations for the chlorine atom of the hydrochloride molecule were added. Convergence was typically reached after *ca.* 10 million trials, corresponding to several hours on a Pentium 2 GHz personal computer.

High quality Rietveld refinements were carried out using the program GSAS[13] using slack soft constraints for bond lengths, angles and planar groups. Maximum residuals in the electron density map between −0.376 and 0.462 for anhydrous naloxone hydrochloride and between −0.367 and 0.355 for anhydrous naltrexone hydrochloride were obtained. No inversion center for both compounds was found, confirming $P2_12_12_1$ as the correct space group. The final Rietveld plots are given in Figure 4. Crystallographic data and agreement factors of the Rietveld refinements are listed in Table 1. CCDC 600172 and 600173 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

RESULTS AND DISCUSSION

Crystal Structure

A comparison of the molecular structures of naloxone and naltrexone from this work and from literature is not possible, because of the limited information in the powder patterns. However, it is possible to compare the molecular packings imposed by these crystal structures. The conformation of the crystal structures of naloxone hydrochloride dihydrate[6] and naltrexone hydrochloride tetrahydrate[7] can be readily described as T-shape, similar to the geometries of other rigid opiates of morphine[14] and codeine[15] (Figs. 5 and 6). In the crystal structure of naloxone hydrochloride dihydrate, the naloxone-, the hydrochlorine-, and the water-molecules form a Three-dimensional network stabilized by hydrogen bonding. On contrary, in the anhydrous phase, one-dimensional chains are formed by hydrogen bonds between the protonated naloxone and the chloride ions, leading to a channel structure with the channels running along *c*-axis. Upon dehydration, the unit cell volume increase by 4.9% while the density decreases by 13.3% reflecting the increased "porosity" and the fact that the strength of hydrogen bonding for naloxone hydrochloride anhydrate is much smaller than that of naloxone hydrochloride dihydrate in the crystal structure (Fig. 5). In the crystal structure of naltrexone hydrochloride tetrahydrate, a similar type of Three-dimensional network stabilized by hydrogen bonding as found for naloxone hydrochloride dihydrate is formed by the naltrexone-, the hydrochlorine- and the water-molecules. The anhydrous phase of naltrexone hydrochloride is different from that of naloxone hydrochloride in a sense that two-dimensional sheets are formed by the naltrexone and the hydrochlorine molecules which are interconnected by hydrogen bonds. Upon dehydration, the unit cell volume decreases by 18.9% while the density increases by 3.52%. Similar density but much smaller volume of the anhydrous phase leads to the conclusion that the strength of hydrogen bonding for anhydrous naltrexone hydrochloride is similar to that of naltrexone hydrochloride tetrahydrate in the crystal structure.

Water molecules seem to stabilize naloxone hydrochloride dihydrate, its unit cell shrinks but the symmetry remains unchanged when compared to that of the anhydrous form. There are different locations of water molecules in naloxone hydrochloride dihydrate. One water molecule is less attached to the naloxone molecules and chlorine atoms and resides in the channels. The other water molecule is an integral and supporting of the structure.

Thermal Analysis

Both compounds show complex dehydration behavior. From DSC (Fig. 1) it follows that naloxone hydrochloride dihydrate has a broad dehydration endotherm starting at $T = 40^{\circ}C$ and lasting till $T = 110^{\circ}C$ and naltrexone hydrochloride tetrahydrate begins dehydration immediately on heating and ends at $T = 80^{\circ}C$.

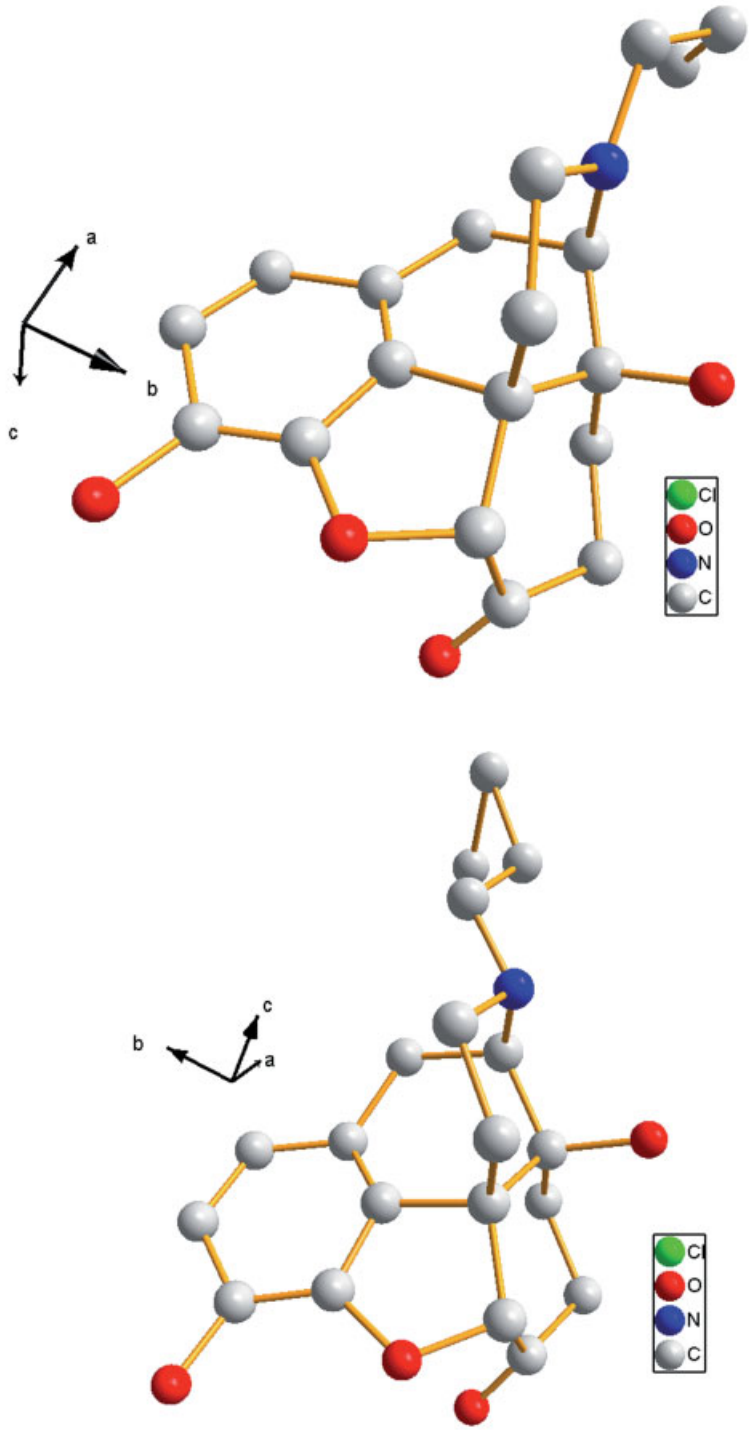

Figure 3. Idealized molecular conformations of naloxone (top) and naltrexone (bottom) molecules. The legend shows Cl. The figure doesn't have it.

Naloxone hydrochloride dihydrate seems to be loosing water from two distinct sites. A shoulder on the slope of dehydration endotherm is demonstrated in the inset to Figure 1 (top). Following dehydration, naloxone hydrochloride dihydrate shows two other small endotherms. The very small endotherm at $T = 172^{\circ}C$ and an endotherm at $T = 192^{\circ}C$ are likely related to solid–solid phase transition and as such deserve investigation by itself. We will be reporting on this in a separate

Table 1. Crystallographic Data for Naloxone and Naltrexone Hydrochloride

Compound	Naloxone Hydrochloride Anhydrate	Naloxone Hydrochloride Dihydrate	Naltrexone Hydrochloride Anhydrate	Naltrexone Hydrochloride Tetrahydrate
Formula	$C_{19}H_{24}ClNO_5$	$C_{19}H_{26}ClNO_6$	$C_{20}H_{24}ClNO_4$	$C_{20}H_{32}ClNO_8$
Formula weight (in g/mol)	363.84	399.87	377.87	449.92
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Z	4	4	4	4
a (in Å)	14.6588(10)	13.293(3)	15.4560(5)	18.099(5)
b (in Å)	17.4363(9)	18.592(15)	14.9809(4)	15.926(6)
c (in Å)	7.9620(2)	7.852(2)	7.8412(2)	7.768(11)
V (in Å^3)	2035.0(2)	1940.6(17)	1815.5(2)	2239(3)
D-calc (in g/cm^3)	1.187	1.369	1.382	1.335
Wavelength (in Å)	1.54059	—	1.54059	—
R-p (in %)[a]	5.59	—	5.23	—
R-wp (in %))[a]	7.51	—	6.70	—
R-F^2 (in %)[a]	15.29	—	13.50	—
Starting angle (in ° 2θ)	6.0000	—	7.203	—
Final angle (in ° 2θ)	59.9908	—	64.995	—
Step width (in ° 2θ)	0.0172	—	0.0172	—
Time/step (in s)	20.64	—	20.64	—
No. of reflections	3139	—	3360	—
No. of variables	78	—	95	—
Reference	—	Klein et al.[6]	—	Le Dain et al.[7]

[a]R-p, R-wp, and R-F^2 as defined in GSAS.[15]

paper. The compound shows an exothermic transition with an onset at $T = 236$°C which is likely related to decomposition.

Naltrexone hydrochloride tetrahydrate also appears to be loosing water in a complex way from selected sites up to $T = 80$°C. Thus, to obtain anhydrous samples it appeared sufficient to heat the samples to $T = 100$°C and to apply a small driving force in form of vacuum to the remaining in channels water molecules. The dehydration is followed by an endothermic transition with an onset at $T = 205$°C which is likely related to a solid–solid phase transition and an exothermic event likely related to decomposition at $T = 255$°C.

The conclusions derived from DSC measurements regarding water sites were tested by performing thermogravimetric analysis coupled with mass spectrometry (TG/MS). The mass loss curves for both hydrates are shown in Figure 2. For naloxone hydrochloride dihydrate, there is a weight loss of approximately 7% which corresponds to a maximum for the ion current for the mass 18, which is likely correlated to water loss. The stoichiometric content of water for naloxone dihydrate is 9%. The experimental weight loss varies depending on the time spend while attempting to reach equilibration in the stream of argon gas. This suggests that water molecules can leave the crystal lattice easily, responding to water vapor pressure difference in the sample and in the dry argon gas. The experimentally determined weight loss indicates likely that water sites were partially depopulated. A phenomenon like this often occurs in case of channel hydrates when water molecules populate or depopulate channels as a function of a change in relative humidity.[11] Lack of the weight loss at $T = 172$°C and $T = 192$°C suggests that these two small endotherms observed in the DSC (Fig. 1) are related to solid–solid phase transitions. The DTA curve also indicates two maxima in the dehydration region of the temperature and other two maxima in the decomposition area confirming this conclusion.

The weight loss curve for naltrexone hydrochloride tetrahydrate demonstrated in Figure 2 corresponds to a well equilibrated sample, i.e. the one that spent considerable amount of time in dry argon gas. The weight loss below $T = 100$°C is small. However, it can be bigger providing that the measurement starts without equilibration period. The DTA curve shows one maximum, irregardless of measurement delay time, in the dehydration region, indicating that the three molecules are bound to the crystal lattice in a

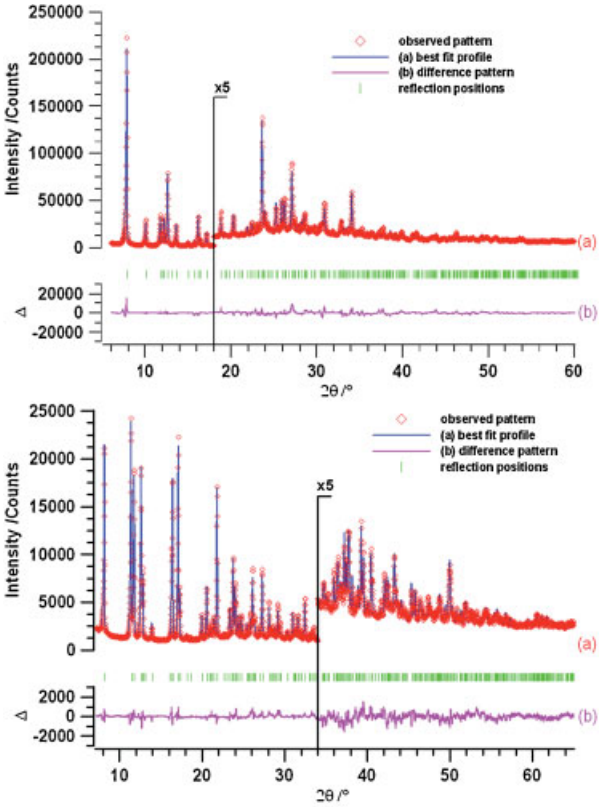

Figure 4. Scattered X-ray intensity of naloxone (top) naltrexone hydrochloride anhydrate (bottom) at ambient conditions as a function of diffraction angle 2θ. The observed patterns (plus symbols) and the best Rietveld fit profiles (line) and the difference curves between observed and calculated profile are shown. The wavelength was $\lambda = 1.54059$ Å (Cu $K\alpha_1$): The R-values are R-wp = 7.51% for naloxone hydrochloride anhydrate and R-wp = 6.70% for naltrexone hydrochloride anhydrate. The high-angle parts are enlarged by a factor of 5, starting at 18° (2θ) for naloxone hydrochloride anhydrate and at 34° (2θ) for naltrexone hydrochloride anhydrate.

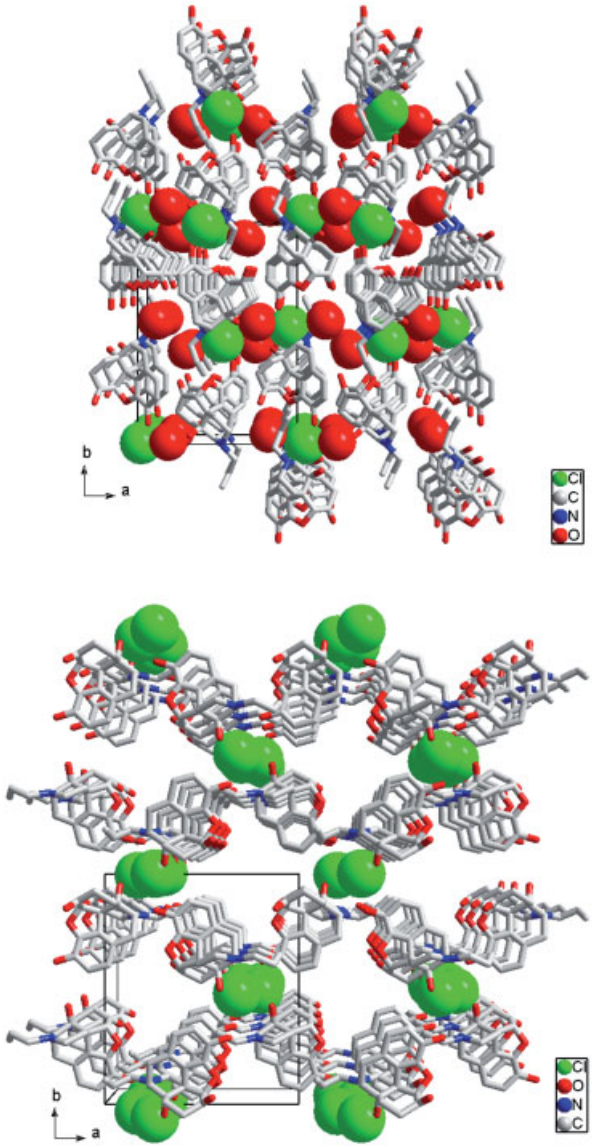

Figure 5. Representations of the packing of the molecules of naloxone hydrochloride dihydrate[6] and naloxone hydrochloride anhydrous in a projection along c-axis (blue: Oatoms, green N-, or Cl-(enlarged) atoms, black: C-atoms) using the coordinates as derived from the simulated annealing procedure. Hydrogen atoms are omitted for clarity.

similar fashion. There is also one maximum in ion current for the mass 18 in the dehydration region further supporting the concept of energetical equivalency of the water molecules.

CONCLUSION

Both, naloxone hydrochloride dihydrate and naltrexone hydrochloride tetrahydrate have the affinity to loose water due to a reduced water vapor pressure and their composition is variable depending on the surroundings. The ease of water loss is different, however, for naloxone and naltrexone hydrochloride hydrates. The difference appears to be related to crystal packing for both hydrates. Traditional methods of water content determination will likely indicate variable results, which will depend on sample preparation, leading to a conclusion of a variable hydrate. This in fact will be the case and the water content of a sample will be responding to relative humidity of the surroundings. Laboratory based X-ray powder

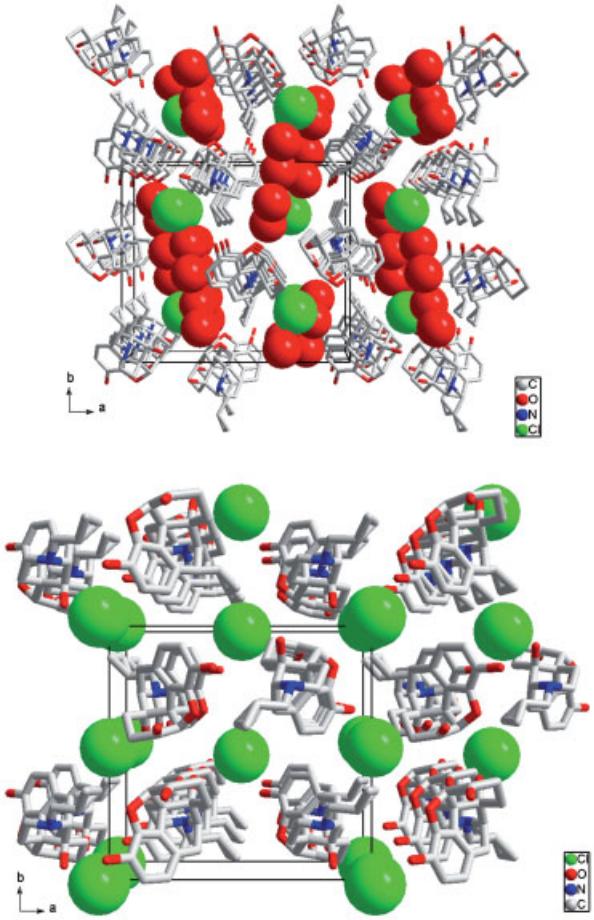

Figure 6. Representations of the packing of the molecules of naltrexone hydrochloride tetrahydrate[7] and naltrexone hydrochloride anhydrate in a projection along c-axis (blue: O-atoms, green N-, or Cl-(enlarged) atoms, black: C-atoms) using the coordinates as derived from the simulated annealing procedure. Hydrogen atoms are omitted for clarity.

diffraction crystallographic analysis can help visualizing water environment in the crystal structure and find differences in water-crystal structure association, which otherwise may be difficult to envisage.

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