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Structural Investigations of Hydrate, Anhydrate, Free Base, and Hydrochloride Forms of Morphine and Naloxone

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ABSTRACT: For the structurally related agonist morphine and the antagonist naloxone, hydrates and anhydrates exist for both the free bases and the hydrochloride salts. We present five new crystal structures, determined from X-ray powder diffraction data, for morphine and naloxone: four anhydrates and one hydrate. The structures were solved by the DASH and FIDDLE programs and were refined with TOPAS. These new structures, together with already known structures from the Cambridge Structural Database, enabled us to investigate the influence of the subtle molecular differences between these agonists and antagonists, the role of water, and the effect of the chloride counterion on structural properties of morphine and naloxone in the solid state. All structures, except one, crystallize in the orthorhombic space group $P2_12_12_1$, although the crystal packings are quite different. Naloxone anhydrate crystallizes in the monoclinic space group $P2_1$. In all cases dehydration causes an anisotropic shrinkage of the unit cell but only for the free base of naloxone this also induces a breaking of the symmetry. For morphine dehydration causes a lowering of the dimensionality of the hydrogen-bonding network, but for naloxone this is not the case since the dimensionality of the network is not dominated by the water molecules. When comparing the structures of the free bases and the hydrochloride salts, it is clear that the chloride ion always takes part in the hydrogen bonding networks and that it prefers to bridge to water instead of nitrogen. These results suggest that the introduction of water or counterions such as chlorine generate structures with higher dimensional hydrogen bonding networks than the corresponding anhydrate or free base structures.

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 to 1000 times more potent enhancing receptor binding than their corresponding agonists.[2] The agonist morphine, (5α,6α)-7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol, a prototypical opioid, is a highly potent opiate analgesic drug and is one of the main active agents in opium. The chemical structure of morphine was established by Gates & Tschudi in 1952,[3] who succeeded in synthesizing the complete molecule. In 1925, Gulland & Robinson[4] had already proposed exactly the same structure but were unable to confirm it. The molecular structure of morphine was finally determined and verified from two projections of hydroiodide dihydrate by MacKay & Hodgkin[5] in 1955, and therefore the chemical structure was verified. In Figure 1 the molecular structure of morphine is presented. It has been observed that when a suitable substituent is introduced on the nitrogen atom of a nor-morphine derivate, the resulting compounds are not addicting and are actually narcotic antagonists. The antagonist naloxone (NLX) (5α)-17-(allyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one is a drug used to counter the effects of opioid overdose, for example, morphine or heroin. Although differing only slightly from narcotics, the antagonist naloxone can completely block the analgesic and euphoric effects of agonists, and currently it is used as emergency treatment for overdose of narcotics and/or alcohol.

NLX is marketed as naloxone hydrochloride due to its solubility in water. The molecular structure of the naloxone is presented in Figure 1.

For the structurally related agonist morphine and the antagonist naloxone, hydrates and anhydrates exist for both the free bases and the hydrochloride salts. The hydrochloride trihydrate form of morphine (refcode MORPHC) was determined by Gylbert in 1973[6] and crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters: $a = 6.941$ Å, $b = 13.019$ Å, $c = 20.750$ Å, $V = 1875.071$ Å^3. The crystal structure of the hydrochloride anhydrate form is not described in literature. The crystal structure of the morphine monohydrate (refcode MORPHM01) was determined in 1976 by Bye.[7] It crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters: $a = 7.438(1)$ Å, $b = 13.751(3)$ Å, $c = 14.901(3)$ Å, $V = 1524.073$ Å^3. The morphine anhydrate (refcode MORPIN) was determined for the first time by Duchamp, but 3D coordinates are not available in the CSD.[8] The authors reported that the morphine anhydrate crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters: $a = 7.408$ Å, $b = 13.713$ Å, $c = 14.781$ Å, $V = 1501.541$ Å^3. Making a comparison between the unit cell parameters of the monohydrate and anhydrate forms of morphine, the values reported for the unit cell parameters are doubtful. The unit cell parameters of the morphine anhydrate are very close to the cell parameters of the morphine monohydrate. Therefore, it is unlikely that the reported unit cell is related to the crystal structure of the morphine anhydrate. Crystallographic data of the morphine hydroiodide di-hydrate (refcode MORPHI) and the morphine hydrobromide dihydrate (refcode ZZZRHC) are also available from the CSD indicating that the two forms are isostructuraly related, crystallizing in the orthor-

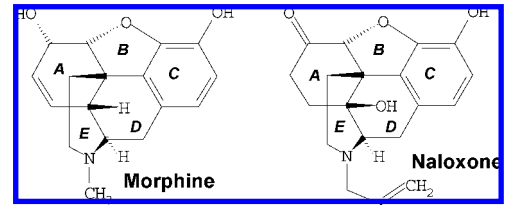

Figure 1. The molecular structures of morphine and naloxone.

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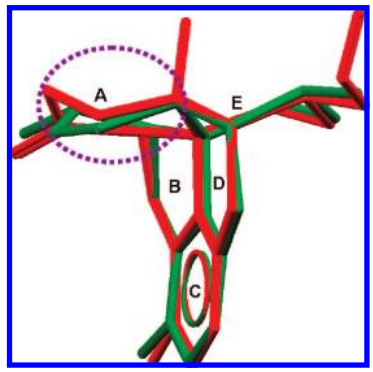

Figure 2. The T-shaped five-ring system depicting with green the molecule of morphine and with red the molecule of naloxone (the dashed circle points to the different A ring conformation).

Table 1. Instrumental and Data Collection Parameters

Typical Instrument Settings	
sample holder	0.5 mm glass capillary tube
system	Bruker AXS D8 Advance $\theta/2\theta$
generator	40 kV, 40 mA
measuring circle (mm)	435
radiation (Å)	Cu $K_{\alpha1}$, $\lambda = 1.54056$ Å
monochromator	primary, focusing curved Ge 111
geometry	transmission capillary configuration
detector	VÅNTEC-1
Typical Measuring Conditions	
range (° 2θ)	5−50 (60)
step size (° 2θ)	0.0084696
step time (s)	50
total data collection time (h)	ca. 75 (92)
spinning (rpm)	15

hombic system with space group $P2_12_12_1$.[5] The hydrobromide and hydroiodide forms of morphine are not subjected to the present study due to their toxic properties and are therefore less important for the pharmaceutical industry.

Naloxone hydrochloride exists in different crystal forms, but just one of them is described in the literature and available from the CSD database: the dihydrate form (refcode NALOXC02). The cell parameters were described for the first time in 1974,[1] but the crystal structure was determined later, in 1975 and redetermined in 1987. It crystallizes in the orthorhombic space group $P2_12_12_1$ with cell parameters: $a = 7.769(3)$ Å, $b = 13.234(5)$ Å, $c = 18.492(7)$ Å, $V = 1901.254$ Å^3.[9] The monohydrate form of the naloxone crystallizes in the orthorhombic system with space group $P2_12_12_1$ and cell parameters: $a = 13.903(5)$ Å, $b = 7.257(7)$ Å, $c = 16.641(1)$ Å, $V = 1678.99$ Å^3.[10]

Comparing the molecular conformation of the agonist (i.e., morphine) and the antagonist (i.e., naloxone) opioids the well-known T-shape is observed. 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 (Figure 2). Furthermore, there are no remarkable differences in the bond lengths and angles. The B and D rings take an envelope conformation, while E ring has a chair conformation and C is a flat ring. The A ring has a twisted boat conformation in the already known morphine forms, while, within the known naloxone forms, the A ring has a twisted chair conformation.

The overall molecular conformation of the agonist and antagonist opioids shows minor differences although chemically they are quite different. The effects of the longer substituent on the nitrogen atom or the existence of a counter ion is not affecting in a dramatic way the molecular structure of the two opioids.

Comparing the existent structural data in the CSD shows that all morphine and naloxone forms crystallize in the orthorhombic space group $P2_12_12_1$. From a crystallographic point of view it is interesting to determine how substituents, water, and counterion influence the chemical crystallography of the agonist and antagonist under subject. In this study the crystal structures of the naloxone monohydrate and four new anhydrate forms of morphine, naloxone, and their hydrochloride salts are determined from X-ray powder diffraction data. Knowledge of the structural crystallographic data enables us to investigate the influence of the subtle molecular differences between this agonist and antagonist, the role of water, and the effect of the chloride counterion on structural properties of morphine and naloxone in the solid state.

2. Experimental Procedures

Material. Morphine hydrochloride and morphine were obtained from Campro Scientific commercial supply. Naloxone hydrochloride was obtained from Diosynth commercial supply. The preparation procedures of the individual hydrate/anhydrate forms for morphine hydrochloride, morphine, naloxone hydrochloride and naloxone are described below.

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

Preparation of Morphine Anhydrate. Morphine anhydrate was obtained by dehydrating the monohydrate at 150 °C, under vacuum (10^{-2} mbar). Before and after the experiment, argon gas was flushed into the reaction vessel. The sample was stored in a glove box under nitrogen atmosphere.

Preparation of Naloxone Hydrochloride Dihydrate. The dihydrate form of naloxone hydrochloride was prepared from a diluted aqueous HCl solution of starting material at room temperature (1 g of NLX/100 mL of H_2O). After the 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 vacuum (10^{-2} mbar) and in the presence of P_2O_5 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. Attempts to grow crystals suitable for single-crystal diffraction failed.

Preparation of Naloxone Monohydrate. A solution of sodium bicarbonate (0.25 g of $NaHCO_3$/100 mL of H_2O) was added to an aqueous solution of naloxone hydrochloride (1 g of naloxone hydrochloride/100 mL of H_2O). After complete addition, the reaction mixture was stirred for 1 h at room temperature to stimulate CO_2 release. The formed naloxone free base was extracted from the NaCl solution with chloroform. After the total removal of the chloroform the monohydrate form of naloxone was obtained.

Preparation of Naloxone Anhydrate. The previously obtained white powder of naloxone monohydrate was heated at 140°C, under vacuum (10^{-2} mbar). Before and after the experiment, argon gas was flushed into the reaction vessel. White needle-shaped crystals of naloxone anhydrate sublimated on the reaction vessel. The sample was stored in a glove box under nitrogen atmosphere.

X-ray Powder Diffraction (XRPD). X-ray powder diffraction measurements for crystal structure determination were performed using a Bruker AXS D8 Advance X-ray diffractometer. The D8 was equipped with a Johansson type monochromator with a focusing curved Ge 111 crystal. A VÅNTEC-1 detector was used with an effective angular region of 2°. The data was collected in transmission capillary geometry using monochromatic Cu $K_{\alpha1}$ radiation. The anhydrate samples were lightly ground, using an agate mortar with pestle and mounted in 0.5 mm glass capillary tubes. The capillary tubes were filled with the materials and sealed in a glove box under nitrogen atmosphere. The capillary tubes were spun at 15 rpm during data collection in order to

Table 2. Lattice Parameters and Pawley Refinement Results

lattice parameters	morphine hydrochloride anhydrate	morphine anhydrate	naloxone hydrochloride anhydrate	naloxone anhydrate
system	orthorhombic	orthorhombic	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
a (Å)	16.179	13.861	17.453	8.540
b (Å)	12.816	12.770	14.684	12.679
c (Å)	7.430	7.690	7.978	7.652
β (°)				97.081
V (Å^3)	1540.882	1362.059	2044.816	822.330
zero point correction	0.084	0.032	0.057	0.030
Pawley refinement results				
no. of reflections	134	249	173	254
data points	4738	6862	4612	6515
Pawley χ^2	9.306	7.056	22.460	4.880

reduce preferred orientation effects[11] and to minimize instrumental and sample packing aberrations.[12]

The most important instrumental and data collection parameters are presented in Table 1.

Data Analysis for Crystal Structure Determination. All diffraction patterns (except the one for naloxone monohydrate) were indexed using DICVOL91[13] to obtain lattice parameters that were subsequently refined in a Pawley fit.[14] *Z*-matrices describing the molecular topology of the fragments in the compound were generated automatically by DASH[15] from the structural data of the investigated compounds available in the CSD and 20 runs with 10^7 simulated annealing (SA) 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 Topas software.[16]

The crystal structure of naloxone monohydrate was determined using the FIDDLE program (2008). A final full Rietveld refinement was performed on the naloxone monohydrate structure using the Topas software.

Solid-State NMR Spectroscopy (SSNMR). 100.6 MHz solid-state ^{13}C cross-polarization/magic angle spinning (CP/MAS) spectra were obtained using a Bruker AXS 400 MHz spectrometer. The samples were packed into a 4 mm rotor and spun with a rate of 12 kHz. The ^{13}C spectra were collected using decay with 90° excitation pulse, 6.5 ms relaxation delay, and 2.5 ms cross polarization time.

3. Results

Crystal Structure Determination of the Four Anhydrate Forms. Indexing, Pawley Refinement and Simulated Annealing Process. The 2θ ranges used for indexing, Pawley refinement, and SA were from 5−45° for the anhydrate forms of morphine hydrochloride and naloxone hydrochloride with a spatial resolution of 2.0129 Å, and from 5−60° for the anhydrate forms of morphine and naloxone, with a spatial resolution of 1.5406 Å.

Twenty reflections with low and high intensity were introduced into DICVOL91 and an orthorhombic cell was found for the anhydrates of morphine hydrochloride, morphine and naloxone hydrochloride. Naloxone anhydrate crystallizes in the monoclinic system. Space group determination in DASH resulted in $P2_12_12_1$ for morphine hydrochloride, morphine and naloxone hydrochloride, and $P2_1$ for the naloxone anhydrate. The volume and space group $P2_12_12_1$, respectively, $P2_1$ correspond to $Z' = 1$ (molecules per asymmetric unit). ^{13}C solid-state NMR confirmed the number of molecules per asymmetric unit to be equal to one.

The lattice parameters obtained after indexing were subsequently refined along with background, zero point, peak shape parameters and reflection intensities using Pawley refinement. The characteristic parameters of the orthorhombic cells and the monoclinic one obtained after Pawley refinements 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:

$$\sigma^2 = \sigma_1^2 \sec^2\theta + \sigma_2^2 \tan^2\theta \quad (1)$$

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

$$\gamma = \gamma_1 \sec\theta + \gamma_2 \tan\theta \quad (2)$$

The two other asymmetry parameters are fully defined by the peak fitting procedure and cannot be refined by Pawley fitting. The investigated samples gave sharp diffraction lines, for the first eight fitted peaks, with a mean FWHM = 0.178° for morphine hydrochloride anhydrate, 0.085° for morphine anhydrate, 0.092° for naloxone hydrochloride anhydrate and 0.076° for naloxone anhydrate, and a minimum value FWHM = 0.131° for morphine hydrochloride anhydrate, 0.072° for morphine anhydrate, 0.078° for naloxone hydrochloride anhydrate and 0.0704° for naloxone anhydrate.

The profile Pawley χ^2 is described in DASH as

$$\chi^2 = \{\Sigma_i^N w_i[y_i(\text{obs}) - y_i(\text{calc})]^2\}/(N - P + C) \quad (3)$$

where y_i(obs) is the observed intensity at the ith step in the powder diffraction pattern; y_i(calc) 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, where N is the number of data points, P

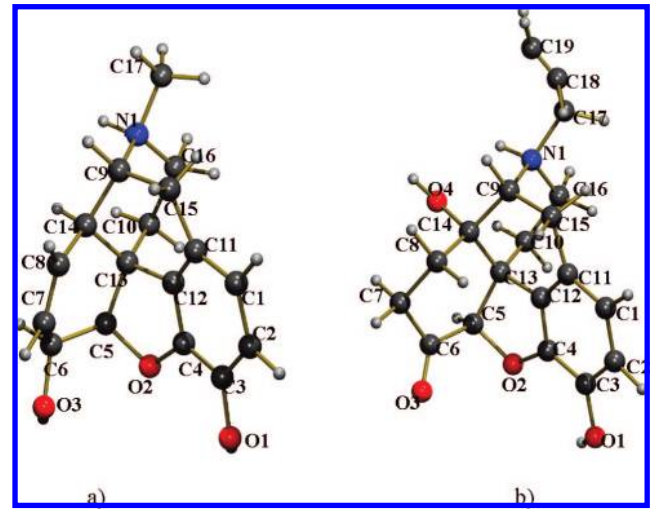

Figure 3. The molecular conformations used for the *Z*-matrices of (a) morphine; (b) naloxone.

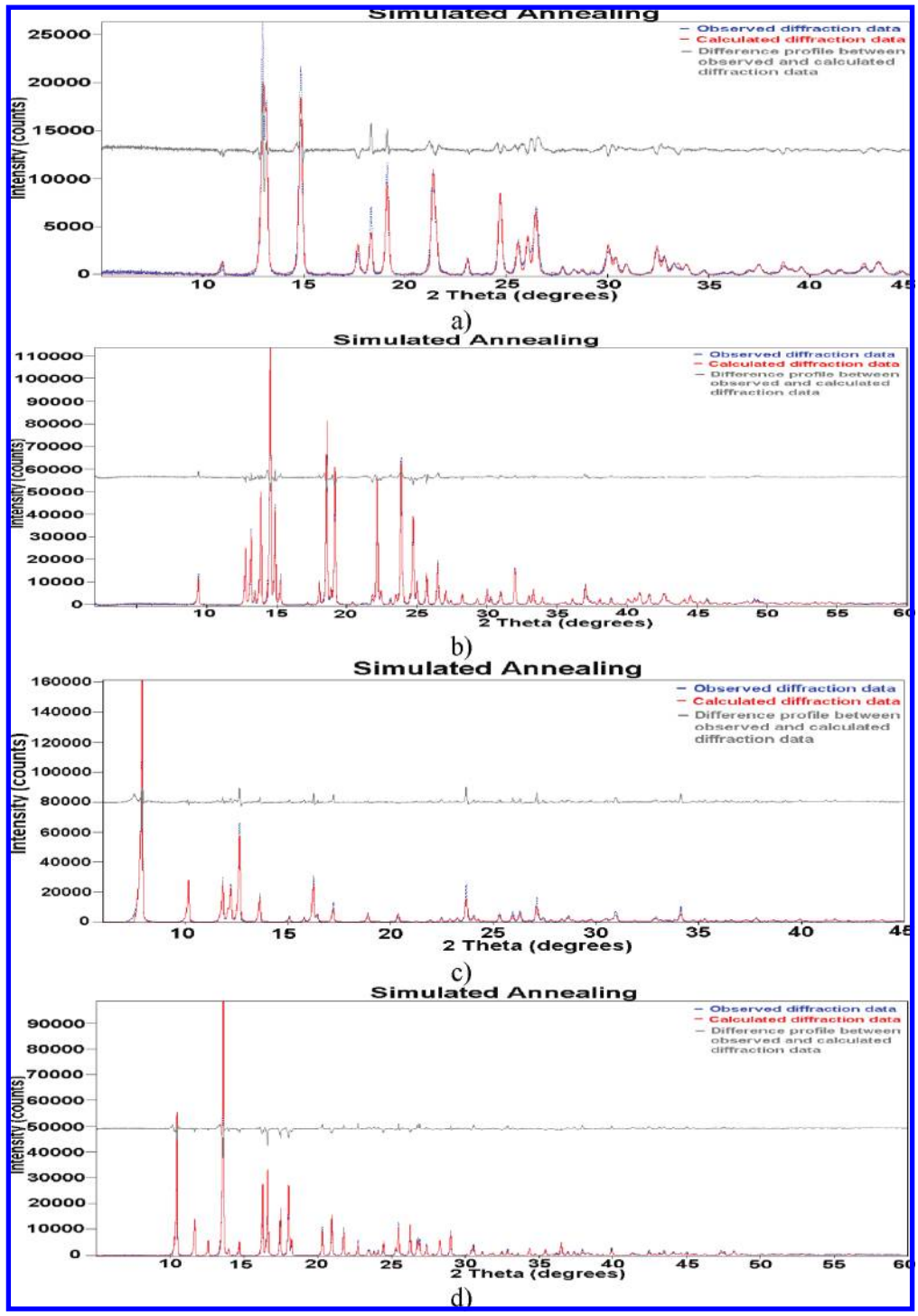

Figure 4. The fit to the diffraction data returned by the SA processes for the anhydrate forms of (a) morphine hydrochloride anhydrate; (b) morphine anhydrate; (c) naloxone hydrochloride anhydrate; (d) naloxone anhydrate.

is the number of parameters, and C is the number of parameter constraints.

The molecular conformations of the Z-matrices describing the molecular geometry of the investigated forms are shown in Figure 3.

Morphine does not have free routable torsion angles. The 20 solutions obtained after the simulated annealing processes showed χ^2 profile values of 18.66 for morphine hydrochloride anhydrate and 19.17 for morphine anhydrate.

Naloxone has two routable torsion angles: C18:C17:N1:C9 and C19:C18:C17:N1, optimized during the SA process. The SA process ended up with 20 solutions having a χ^2 profile values of 40.87 for naloxone hydrochloride anhydrate and 28.62 for naloxone anhydrate.

The fit of the observed and calculated diffraction data for the best solutions found after simulated annealing are shown in Figure 4 for all four investigated forms.

Rietveld Refinement. After structure solution, full Rietveld refinements were applied to the best solutions returned by the SA processes.[17] To the crystal structure of naloxone monohydrate previously determined with FIDDLE software, a full Rietveld refinement was also applied. Almost all

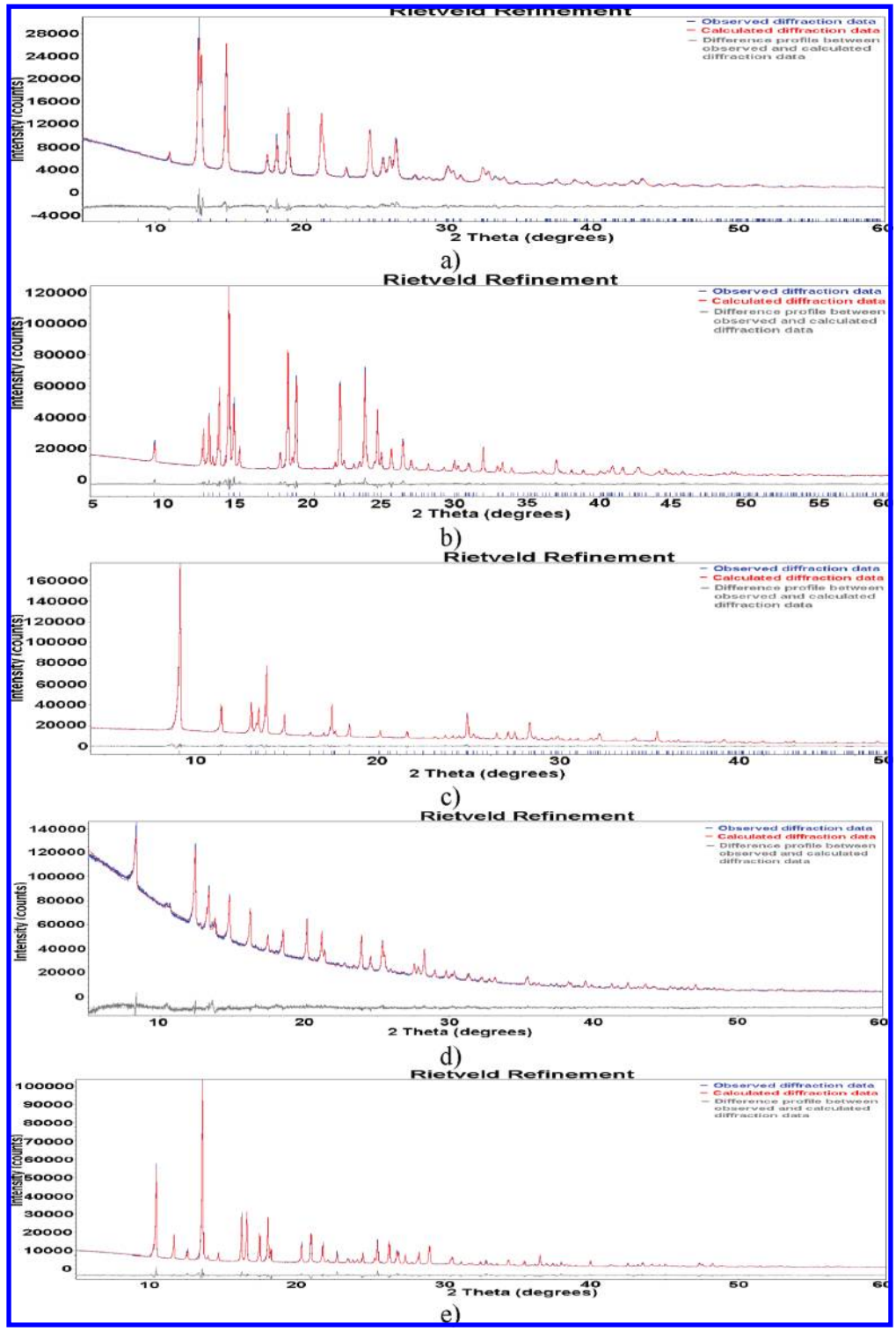

Figure 5. The fit after the final Rietveld refinement for (a) morphine hydrochloride anhydrate; (b) morphine anhydrate; (c) naloxone hydrochloride anhydrate; (d) naloxone monohydrate; (e) naloxone anhydrate.

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 full Rietveld refinements proceeded smoothly to reach a minimum characterized by an excellent fit to the diffraction profiles presented in Figure 5 for all five investigated forms. The crystallographic data of the refined crystal structures is presented in Table 3.

4. Discussion

Many pharmaceutical compounds are available on the market in crystalline form mainly due to reasons of stability during the time between the manufacture of the active ingredient and when it used by the patient. Most of the crystalline drugs are able to form solvates or hydrates. Hydrates are solid adducts containing the parent compound (e.g., the anhydrate of a drug) and water. Because of its small molecular volume, high ability

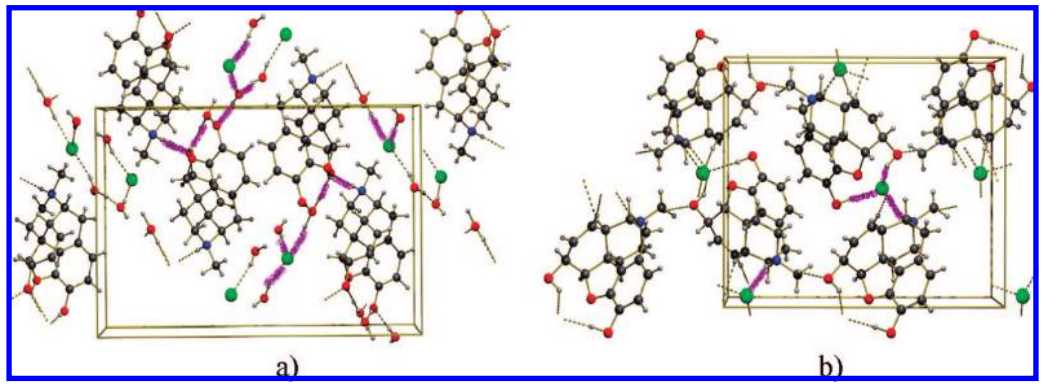

Figure 6. The packing of the morphine: (a) hydrochloride trihydrate, (b) hydrochloride anhydrate (characteristic hydrogen-bonds are depicted in purple).

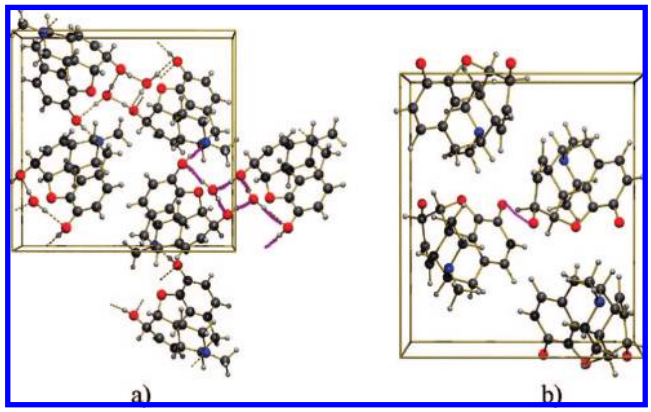

Figure 7. The packing of the morphine: (a) monohydrate, (b) anhydrate (characteristic hydrogen-bonds are depicted in purple).

to form hydrogen-bonds and nontoxic properties, water is an ideal solvent for linking drug molecules into stable crystalline forms. The environment of the water molecules may exhibit various defined packing patterns, which will reflect in the drug behavior. The presence of water molecules influences the intermolecular interactions (affecting the internal energy and enthalpy) and the crystalline disorder (entropy), and hence influences the free energy, the thermodynamic activity, solubility, dissolution rate, stability, and bioavailability.[18] Therefore, we will further discuss various structural features and in particular typical hydrogen-bonds formed by morphine, naloxone, and their hydrochloride forms in order to evaluate the influence of the subtle molecular differences between these two agonist and antagonist opioids, the role of water, and the effect of the chloride counterion on structural properties of morphine and naloxone in the solid state.

In morphine hydrochloride trihydrate, the morphine molecules are packed in a zigzag head-to-tail fashion. Hydrogen-bonds are formed between the -OH group attached to C(6) and the N atom from another morphine molecule. With the aid of the water molecules, the zigzag layers in morphine hydrochloride trihydrate are assembled in a three-dimensional network. The three-dimensional network is formed by hydrogen-bonds between the -OH groups attached to C(3), respectively C(6) and water molecules. The Cl^- is involved in hydrogen-bonding with the water molecules as well. Hydrogen-bonds are also observed between different water molecules. The anhydrate form of morphine hydrochloride has the zigzag packing pattern like in the trihydrate, but this time the morphine molecules are bonded in a head-to-Cl^--to-tail fashion forming a one-dimensional network between the N atom, the Cl^- and -OH groups attached to C(3) and C(6). The packing of the morphine molecules in morphine hydrochloride trihydrate and hydrochloride anhydrate is presented in Figure 6.

The molecules of morphine in morphine monohydrate are hydrogen-bonded in a head-to-tail fashion forming layers assembled together by the water molecules into a three-dimensional network. The hydrogen-bonds are formed between the -OH group attached to C(3) and the N atom. The water molecules are also involved in hydrogen-bonding on one hand with the -OH groups attached to C(3) and C(6) and on the other hand with other water molecules. In the absence of water molecules the morphine molecules are packed in a head-to-head fashion, forming a one-dimensional network. The -OH group attached to C(3) of a morphine molecule is then involved in hydrogen-bonding with the -OH group attached to C(6) of another morphine molecule. The packing of the morphine molecules in morphine monohydrate and anhydrate is presented in Figure 7.

Table 3. Crystallographic Data of the Refined Forms after Full Rietveld Refinement

cell parameters	morphine hydrochloride anhydrate	morphine anhydrate	naloxone hydrochloride anhydrate	naloxone monohydrate	naloxone anhydrate
system	orthorhombic	orthorhombic	orthorhombic	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
a (Å)	16.079(4)	13.861(7)	17.452(4)	13.903(5)	8.539(2)
b (Å)	12.874(6)	12.777(1)	14.681 (4)	7.257(7)	12.678 (4)
c (Å)	7.494(6)	7.690(4)	7.978 (2)	16.641(1)	7.652(2)
β (°)					97.078
V (Å^3)	1551.50	1362.06	2044.10	1678.99	822.16
Final profiles					
R_{wp}	4.161	4.157	2.650	2.901	5.212
R_p	3.034	3.016	1.850	2.211	3.492
GOOF	**2.373**	**3.643**	**2.600**	**1.527**	**3.344**

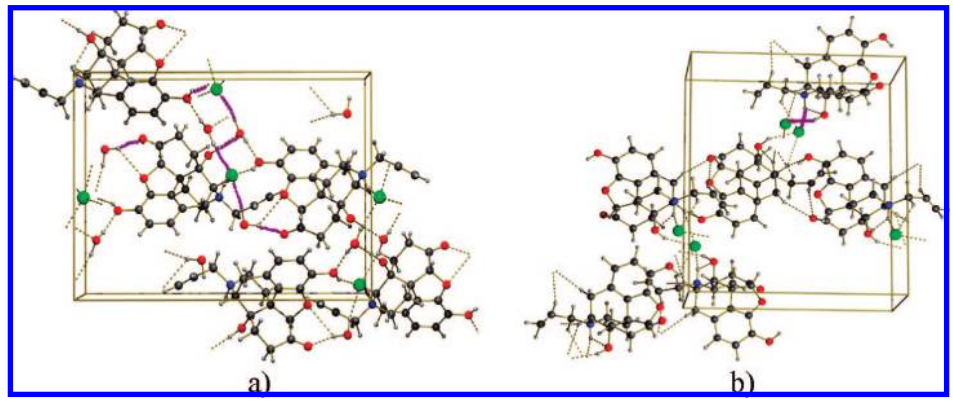

Figure 8. The packing of the naloxone: (a) hydrochloride dihydrate, (b) hydrochloride anhydrate (characteristic hydrogen-bonds are depicted in purple).

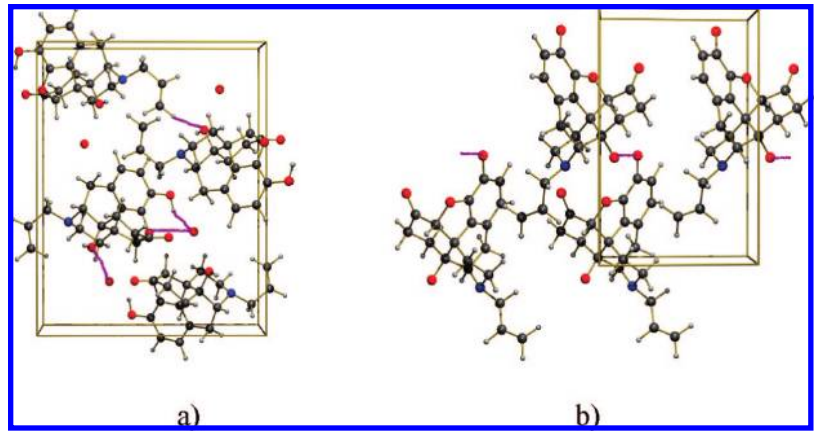

Figure 9. The packing of the naloxone: (a) monohydrate, (b) anhydrate (characteristic hydrogen-bonds are depicted in purple).

Table 4. Characteristic Structural Data of the Eight Investigated Forms

opioids	H-bond network	packing pattern of the molecules	characteristic H-bond
morphine hydrochloride trihydrate	**3D**	**head-to-tail**	**C(6)−OH···N** **C(3)−OH···O$_{(water)}$** **C(6)−OH···O$_{(water)}$** **OH$_{(water)}$···Cl$^-$** **OH$_{(water)}$···O$_{(water)}$**
morphine hydrochloride anhydrate	**1D**	**head-to-Cl$^-$-to-tail**	**N−H···Cl$^-$** **C(3)−OH···Cl$^-$** **C(6)−OH···Cl$^-$**
morphine monohydrate	3D	head-to-tail	C(3)−OH···N C(3)−OH···O$_{(water)}$ C(6)−OH···O$_{(water)}$
morphine anhydrate	1D	head-to-head	C(3)−OH···O−C(6)
naloxone hydrochloride dihydrate	**3D**	**head-to-tail**	**C(3)−OH···O$_{(water)}$** **C(14)−OH···O$_{(water)}$** **OH$_{(water)}$···Cl$^-$** **OH$_{(water)}$···O−C(6)**
naloxone hydrochloride anhydrate	**3D**	**head-to-tail**	**N−H···Cl$^-$** **C(3)−OH···Cl$^-$** **C(14)−OH···Cl$^-$**
naloxone monohydrate	1D	head-to-allyl	C(3)−OH···O(water) OH(water)···O−C(14) OH(water)···O(2) C(19)−H···O−C(14)
naloxone anhydrate	1D	head-to-tail	C(3)−OH···O−C(14)

The naloxone molecules in naloxone hydrochloride dihydrate are also packed in a three-dimensional network with the aid of water molecules. Hydrogen-bonds are formed between the -OH group attached to C(3), respectively C(14) and water molecules. The carboxyl group and the Cl$^-$ are also involved in hydrogen-bonding with the water molecules. As expected, hydrogen-bonds between different water molecules are also observed. Obviously, the water molecules play a stabilizing role in the network formation of naloxone hydrochloride dihydrate. The naloxone molecules in naloxone hydrochloride anhydrate are also assembled into a three-dimensional network, but this time with the aid of the Cl$^-$ anion. Cl$^-$ forms hydrogen-bonds with the

N atom and with the (-OH) groups attached to C(3), and C(14). The packing of the naloxone molecules in naloxone hydrochloride dihydrate and hydrochloride anhydrate is presented in Figure 8.

Naloxone monohydrate and anhydrate form one-dimensional chains of naloxone molecules hydrogen-bonded in a zigzag fashion. The -OH groups attached to C(3), respectively C(14) are involved in hydrogen-bonding in both forms. Thus, in the monohydrate form the hydrogen-bonds are formed between the -OH groups of the naloxone molecules and the water molecules. In the anhydrate, intermolecular hydrogen-bonds between the -OH groups from different naloxone molecules are observed. The allylic tail of the naloxone molecule is also involved in hydrogen-bonding. The -OH group attached to C(14) forms hydrogen-bonds with the allylic tail. The packing of the naloxone molecules in naloxone monohydrate and anhydrate is presented in Figure 9.

The presence of water molecules leads to the formation of three-dimensional networks in the hydrated forms of the agonists' morphine, morphine hydrochloride and the antagonist naloxone hydrochloride. On the other hand, the anhydrate forms of morphine, morphine hydrochloride and naloxone are able to form only one-dimensional networks due to the absence of water molecules and their involvement in hydrogen-bonding.

Dehydration of the hydrate forms of both the salt and the free base of morphine induces a change in the hydrogen-bond network dimensionality from 3D to 1D. For naloxone, the dehydration takes place without any change in the hydrogen-bond network dimensionality.

The salt forms of naloxone show a higher dimensionality than the free base forms due to the involvement of Cl^- in additional hydrogen-bonding. Naloxone hydrochloride anhydrate forms a three-dimensional network due to the favorable position of the Cl^- in the crystal structure. Morphine hydrochloride anhydrate is only able to form a one-dimensional network due to a different position of the Cl^- in the crystal structure.

All the hydrate forms of the investigated opioids form 3D hydrogen-bond networks, exception naloxone monohydrate that forms a 1D hydrogen-bond network. Naloxone monohydrate dehydrates to form the anhydrate with breaking of the orthorhombic symmetry, ending up in a monoclinic one with space group $P2_1$. Apparently, in this case, the water molecules play a role in stabilizing the orthorhombic symmetry. By dehydrating the monohydrate water molecules disappear from the net, however, without disturbing the one-dimensional network of naloxone.

In the anhydrate forms of the investigated opioids, the counterion Cl^- usually takes over the role of the water molecules. Cl^- can form hydrogen-bonds with the N atom as well, but when water molecules are present in the crystal structure, Cl^- prefers the water molecules instead of the N atom. When Cl^- and water molecules are not present (morphine anhydrate and naloxone anhydrate), intermolecular hydrogen-bonds are formed between the -OH groups, taking over the role of the counterion and/or water molecules. While in the monohydrate form of naloxone the allylic group is involved in hydrogen-bonding, but in the anhydrate form the allylic group is no longer involved.

The characteristic structural features of the hydrate and anhydrate forms of naloxone, naloxone hydrochloride, morphine, and morphine hydrochloride are presented in Table 4.

Besides having a different substituent on the N atom, the OH group attached to C(6) in morphine is substituted with a carboxyl group in naloxone. But on the other hand, in naloxone another OH group is attached to C(14). In the general hydrogen-bonding scheme of morphine, the two OH groups attached to C(3) and respectively to C(6) are always involved in hydrogen-bonding. For naloxone this also holds for the two OH groups attached to C(3) and C(14). The carboxyl group attached to C(6) is only involved in hydrogen-bonding with water molecules in naloxone hydrochloride dihydrate. Although the OH groups are differently positioned in morphine and naloxone, they are equally involved in the general hydrogen-bonding scheme.

Incorporation of water into the crystal lattice produces a new unit cell different from that of the anhydrate and, consequently, the physical properties and the behavior of the solvate may differ from those of the anhydrate.[19] In all cases, the dehydration processes of morphine hydrochloride trihydrate, morphine monohydrate, naloxone hydrochloride dihydrate and naloxone monohydrate to form the anhydrates induce only an anisotropic shrinkage of the unit cells.

4. Conclusions

The crystal structures of the anhydrate forms of morphine hydrochloride, morphine, naloxone hydrochloride and naloxone were successfully determined from X-ray powder diffraction data collected in transmission capillary geometry. The DASH software was used for structure determination and Topas software for Rietveld refinement. The crystal structure of naloxone monohydrate was determined by FIDDLE software and successfully refined by Topas software.

Comparison of the crystal structures of the hydrated and the anhydrate forms of morphine hydrochloride, morphine, naloxone hydrochloride and naloxone reveals similarities. The hydrate and anhydrate forms of morphine hydrochloride, morphine, naloxone hydrochloride and naloxone crystallize all, except one, in the orthorhombic space group $P2_12_12_1$. The dehydration processes of morphine hydrochloride trihydrate, morphine monohydrate, naloxone hydrochloride dihydrate, and naloxone monohydrate induces only an anisotropic shrinkage of the unit cells of the hydrated forms.

The dehydration process of the hydrate forms of the investigated opioids takes place without any change in symmetry except for naloxone monohydrate, which shows a lowering of the orthorhombic symmetry to the monoclinic one during dehydration. The hydrogen-bond dimensionality reduces to lower values for the anhydrate forms of the agonist opioid, while for the antagonist there is no change in dimensionality.

The counterion Cl^- takes over the role of the water molecules in the anhydrates forming hydrogen-bonds with the N atom as well. When water molecules are present, Cl^- prefers the water molecules instead of the N atom. If water molecules and the counterion are not present, intermolecular hydrogen-bonds are formed between different -OH groups. The allylic group of the naloxone molecule takes over the role of the counterion Cl^- only in its absence. For the rest, the N atom can act as a donor as well as an acceptor.

For morphine and naloxone, the introduction of water or counterions such as chlorine generates structures with higher dimensional hydrogen bonding networks than the corresponding anhydrate or free base structures.

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