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Communication

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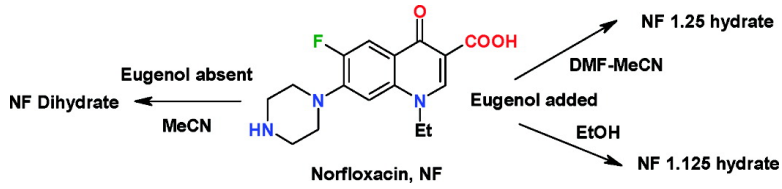

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Crystal Structures of Norfloxacin Hydrates

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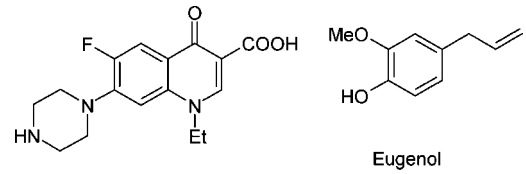
ABSTRACT: Two novel hydrate forms of norfloxacin (NF) were serendipitously obtained during cocrystallization with eugenol. NF 1.25 hydrate and 1.125 hydrate are isomorphous crystal structures in the $P2_1/c$ space group, each with two symmetry-independent drug molecules, two full water molecules, and a third water of 0.5 and 0.25 partial occupancy, respectively. Water promoted proton transfer results in a shift from neutral to ionic hydrogen bonding between norfloxacin molecules in hydrate structures. The presence of eugenol additive gave novel NF hydrates of lower water stoichiometry, whereas crystallization in its absence gave the known NF dihydrate.

Norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, NF hereafter) is a broad spectrum fluoroquinolone antibacterial agent used to treat urinary tract infections.[1] The drug molecule attacks bacterial DNA by making a complex with DNA-gyrase.[2] Hydration plays an important role in altering the solubility and stability of drugs.[3] About one-third of drugs are able to form hydrates, which often changes their pharmacological property, dissolution rate profile, and stability of the formulation. Normally, anhydrate drugs are more water soluble than their hydrate forms for thermodynamic reasons. Norfloxacin is an exception in that its hydrates are more soluble than the anhydrate.[4] Different solid-state forms of NF reported in the literature are three polymorphs (A, B, C),[5a,b] an amorphous form,[5a] and a methanol hydrate.[5c] Interconversion between NF hydrates[6] was studied by powder X-ray diffraction (PXRD) and IR spectroscopy. Two X-ray crystal structures of NF labeled form A[7] are reported in the triclinic and monoclinic crystal systems.[8] Among the numerous NF hydrates (such as 1.5, 2.0, 2.5, 3.0, 5.0),[6] only the dihydrate[9] is characterized by single crystal X-ray diffraction, and water stoichiometry below 1.5 in NF hydrates is not reported to our knowledge.

Norfloxacin and eugenol[10] (Scheme 1) were cocrystallized with the idea that the OH group of eugenol and NH and COOH groups of the drug would form N−H···O, O−H···O hydrogen bonds and further assisted by $\pi-\pi$ stacking[11] will promote cocrystal formation.[12,13] We report serendipitous crystallization of two novel hydrate forms of norfloxacin during these experiments. Crystallization of a 1:1 molar NF-eugenol cogrinded solid from MeCN-DMF gave a 1.25 hydrate, whereas dissolution of the same starting materials in hot EtOH gave single crystals of a closely related hydrate that was assigned 1.125 water content by X-ray diffraction (see Supporting Information for experimental details). These novel NF hydrate crystal structures (1.25, 1.125) and the known dihydrate[9] adopt the same space group $P2_1/c$, but their unit cell parameters are quite different (Table 1). The dihydrate structure has one norfloxacin and two water molecules in the asymmetric unit.

NF 1.25 hydrate crystal structure was satisfactorily solved and refined to a good R-factor of 0.0484 with two crystallographically unique norfloxacin molecules (A and B), two water molecules (O1W, O2W), and a third water (O3W) of 0.50 site occupancy (see ORTEP in Figure S1a, data AN_847(I), Supporting Information). Reflections on a different single crystal assigned as 1.125 hydrate were not strong enough to give immediate structure solution/refinement. The presence of two NF molecules, two waters, and a third water (O3W) of 0.25 sof could be inferred (ORTEP in Figure S1b, data AN_848(II) in Supporting Information). However, $(\Delta/\sigma)_{max}$ was high (0.488) due to a set of weak reflections from a

Scheme 1. Norfloxacin and Eugenol Used for Cocrystallization Provided Novel NF Hydrates



Norfloxacin, NF

Eugenol

Table 1. X-ray Crystallographic Parameters of NF Hydrates

	dihydrate $C_{16}H_{18}FN_3O_3 \cdot 2H_2O$	1.25 hydrate $C_{16}H_{18}FN_3O_3 \cdot 1.25H_2O$	1.125 hydrate $C_{16}H_{18}FN_3O_3 \cdot 1.125H_2O$
data no.		AN_847(I)	AN_848(II)
space group	$P2_1/c$	$P2_1/c$	$P2_1/c$
a/Å	8.265(3)	17.5341(11)	17.4911(16)
b/Å	21.698(4)	8.9942(6)	8.9542(8)
c/Å	9.5250(17)	19.9186(13)	19.7990(18)
α/°	90	90	90
β/°	110.794(19)	90.411(1)	90.252(2)
γ/°	90	90	90
T/K	123(2)	100(2)	100(2)
V/Å^3	1596.9(6)	3141.2(4)	3100.9(5)
Z, Z'	4, 1	8, 2	8, 2
R-factor	0.053	0.0484	0.1122
$(\Delta/\sigma)_{max}$	<0.001	0.001	0.488
S	0.962	1.049	1.183
reference	ref 9	this paper	this paper

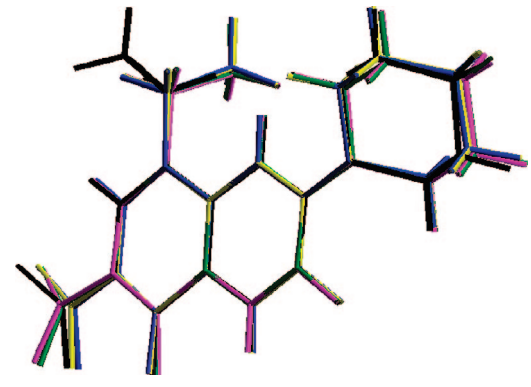


Figure 1. Overlay of norfloxacin molecules in 1.25 hydrate (green and yellow, A and B), 1.125 hydrate (magenta and blue, A and B), and dihydrate (black) crystal structures. COO group torsions in 1.25/1.125 hydrate = C3−C2−C1−O2 = 18.9/−24.1°, C19−C18−C17−O5 = −6.8/10.0°.

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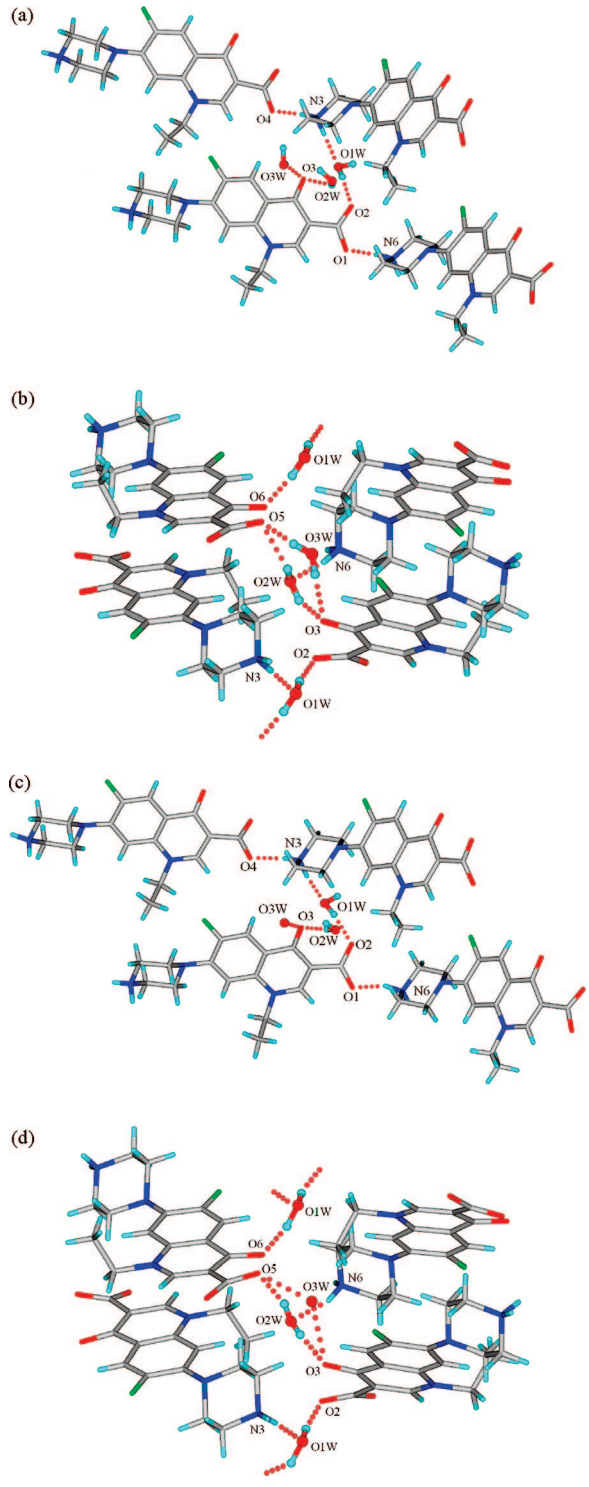

Figure 2. Hydrogen bonding in NF 1.25 hydrate (a) and (b), and 1.125 hydrate (c) and (d). Molecular packing and hydrogen bonding in these isomorphous hydrates is identical.

moderate quality crystal, which was the best one after many trials. The unit cell volume of 1.25 hydrate is slightly larger than 1.125 hydrate at 100 K (3141.2(4) vs 3100.9(5) Å^3). When the same data set was indexed in RLATT[14] using strong reflections only, a satisfactory refinement emerged with one NF, one water, and a second water (O2W) of 0.13 sof (data AN_848(III) in Supporting Information, *R*-factor 0.1079, acceptable $(\Delta/\sigma)_{max} < 0.001$). Structure solution/refinement of 1.125 hydrate in unit cell of half the volume by using strong reflections only means that one O atom of the COO moiety has slightly elongated ellipsoids (Figure S1c,

Table 2. Hydrogen Bond Parameters in NF 1.25 and 1.125 Hydrates (Neutron-Normalized N−H and O−H Distances)

D−H···A	H···A/Å	D···A/Å	∠D−H···A/°
NF 1.25 Hydrate			
N6−H6B···O2W	1.77	2.766(2)	170.5
N3−H3A···O1W	1.80	2.8058(19)	177.9
N6−H6A···O1	1.62	2.6148(19)	167.6
N3−H3B···O4	1.66	2.6658(19)	171.3
O1W−H1B···O6	1.84	2.7832(17)	159.8
O1W−H1A···O2	2.01	2.9837(18)	169.6
O3W−H3A···O5	1.70	2.676(3)	170.5
O2W−H2A···O2	2.24	3.118(2)	147.6
O2W−H2A···O3	2.12	2.868(2)	131.4
O2W−H2B···O3W	1.80	2.617(3)	138.8
O2W−H2B···O6	2.44	3.093(2)	123.5
O3W−H3B···O3	1.78	2.597(3)	138.3
NF 1.125 Hydrate			
N6−H6B···O2W	1.79	2.770(5)	161.5
N3−H3A···O1W	1.80	2.803(6)	172.0
N6−H6A···O1	1.63	2.600(5)	159.0
N3−H3B···O4	1.67	2.658(6)	164.5
O1W−H1B···O6	1.89	2.804(5)	152.9
O1W−H1A···O2	1.96	2.940(5)	170.7
O2W−H2A···O3	1.79	2.768(5)	172.2
O2W−H2B···O6	1.96	2.890(6)	156.2
O3W[a]···O5		2.636(16)	
O3W[a]···O6		2.911(18)	
O3W[a]···O3		2.489(18)	
O3W[a]···O2W		2.609(17)	

[a] H atoms could not be located on O3W of 0.25 s.o.f.

Supporting Information). We selected crystal data AN_847(I) and AN_848(II) (Table 1) for further discussion. The justification for assigning two NF hydrates of slightly different water stoichiometry and the reasons for the choice of final unit cells are given in the Supporting Information.[15]

The tricyclic framework of norfloxacin molecules has a similar conformation, but the ethyl group adopts a different orientation in 1.25 and 1.125 hydrates compared to the dihydrate structure (Figure 1). The two conformers in the same crystal structure differ by a small change in COO group orientation only.[16] Norfloxacin and its hydrates exhibit conformational polymorphism[17] by displacement in flexible alkyl group orientations. The piperazine ring in all five NF molecules is N-protonated (1.25 hydrate $Z' = 2$, 1.125 hydrate $Z' = 2$, and dihydrate $Z' = 1$)[18] and has a similar conformation within 5° torsion angle variation. NF 1.25 hydrate structure is sustained by $N^+-H\cdots O_{water}$ (1.80 Å, 177.9°; 1.77 Å, 170.5°) and $N^+-H\cdots O_{carboxyl}$ (1.66 Å, 171.3°; 1.62 Å, 167.6°). The two symmetry-independent water molecules engage in O−H···O bonding with carbonyl (1.84 Å, 159.8°) and carboxylic oxygen (2.01 Å, 169.6°) of norfloxacin and the second water makes a O−H···O bond with the third water molecule of 50% occupancy (1.80 Å, 138.8°). Molecular packing and hydrogen bonding in the isomorphous 1.125 hydrate structure is identical (Figure 2, Figure S2, and Table 2).[19] The lower water content could be a reason for moderate crystal quality of 1.125 hydrate. Stacking of inversion related NF molecules generates hydrophilic galleries that include water molecules connected via O−H···O hydrogen bonds. In contrast to the similarity of 1.25 and 1.125 hydrate structures, the dihydrate has better π-stacking of aromatic molecules (~3.5 Å, Figure S3, Supporting Information) because the water molecules are shifted outside the stacks and this brings the aromatic quinoline rings in close proximity. There are differences in the positioning of water molecules in these channel hydrates (Figure 3).

PXRD of hydrates crystallized using norfloxacin + eugenol from DMF-MeCN showed a concomitant mixture of 1.25, 1.125 and dihydrate NF forms (Figure 4). Since the molecular packing in the crystal lattice of 1.25 and 1.125 hydrates is similar, except for the extra fractional water molecule, their simulated PXRD lines are overlapping. It is difficult to quantify the proportion of 1.25 and

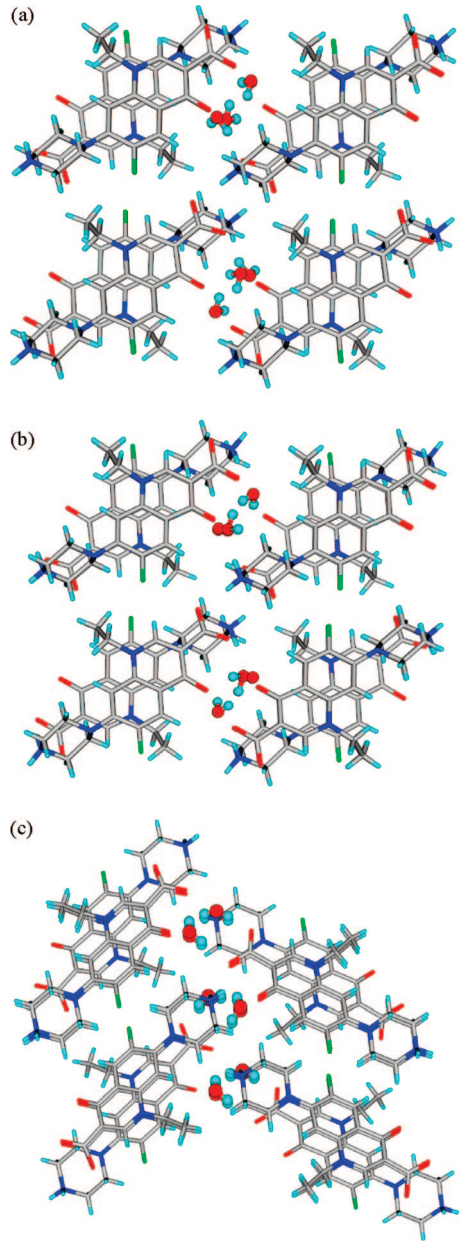

Figure 3. Water molecules in the hydrophilic channels of norfloxacin 1.25 hydrate (a) and 1.125 hydrate (b). In contrast, the dihydrate (c) has two kinds of water molecules, one in hydrophilic channels and the other inside piperazinyl stacks.

1.125 hydrates in the crystalline solid. The powder profile of NF crystallized from DMF-MeCN (without added eugenol) showed a good match with the calculated lines for the pure dihydrate (Figure S4, Supporting Information), and there is no evidence of lower stoichiometry hydrates. In conclusion, *the presence of a cocrystal former gave crystals of novel hydrate forms of Norfloxacin*. A similar observation in the literature is attempted cocrystallization of oxalic acid and benzidine giving polymorphic sesquihydrates of oxalic acid.[20]

Thermogravimetric analysis of the hydrate composition from NF + eugenol crystallization showed a 7.5% weight loss (Figure 5), whereas the pure dihydrate exhibited water loss of 9.7% by weight (Figure S5, Supporting Information) in the temperature range 40−140 °C. The bulk solid characterized by PXRD and TGA in Figures 4 and 5 is a mixture of at least three NF hydrates.

Norfloxacin hydrates of water stoichiometry 1.5 (sesquihydrate), 2 (dihydrate), and higher water content (2.5, 3, 5) are known.[5−7] We discovered two novel hydrate forms of norfloxacin of lower

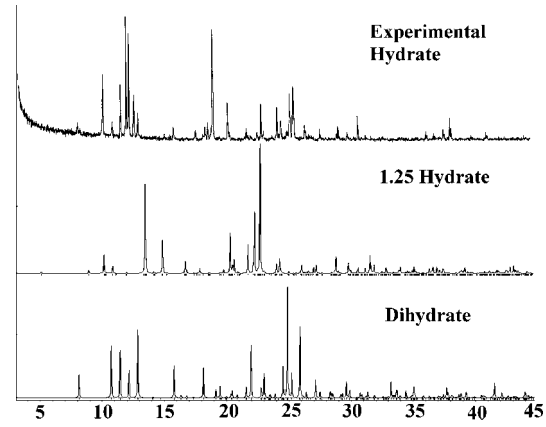

Figure 4. Powder X-ray diffraction (intensity vs 2θ) of norfloxacin hydrates (experimental) crystallized from DMF-MeCN starting from NF + eugenol (top). Simulated powder lines of 1.25 hydrate and dihydrate crystal structure (middle and bottom). The 1.25 hydrate has similar PXRD lines as 1.125 hydrate. The small shift in 2θ values is because single crystal structures were determined at 100−125 K, whereas PXRD was recorded at room temperature.

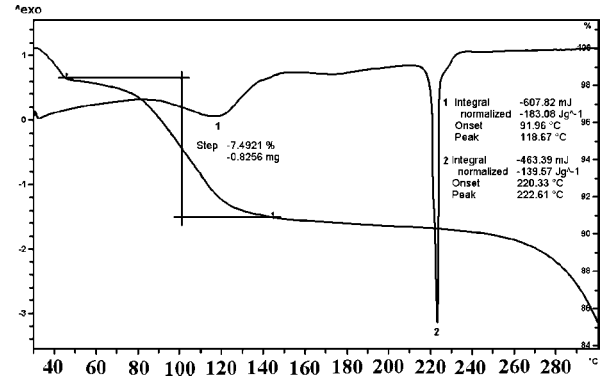

Figure 5. TGA on norfloxacin hydrates crystallized from NF + eugenol showed a weight loss of 7.5% indicating the presence of extra water due to concomitant dihydrate. The calculated weight loss for 1.25, 1.125 hydrates and dihydrate is 6.58, 5.96, 10.13%. DSC endotherms indicate water release and melting.

water content (1.25, 1.125) in the presence of cocrystal former eugenol. A continuum of NF hydration states has implications in the patenting of drugs.[21] The availability of additional X-ray crystal structures confirms the zwitterionic state of NF in all hydrate forms, and this could explain the greater solubility of hydrates compared to the well-known triclinic anhydrate form.[8] Water-induced proton transfer in norfloxacin from anhydrate (neutral) to zwitterionic (hydrates) postulated from IR data[6b] is now validated by X-ray diffraction. The recent appearance of a second monoclinic NF anhydrate polymorph with zwitterionic hydrogen bonding[5b,8] opens leads for further studies on the bioavailability of norfloxacin forms.

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Supporting Information Available: Crystallographic details, packing diagrams, PXRD and DSC/TGA plots, crystal structures of hydrates (.cif,.hkl), and method of structure solution/refinement using RLATT are available via the Internet at http://pubs.acs.org.

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(8) Actually, two single crystal X-ray diffraction structures of anhydrate norfloxacin are known. Form A (triclinic):[7] $P\bar{1}$, $a = 4.324(1)$ Å, $b = 9.635(1)$ Å, $c = 18.089(1)$ Å, $\alpha = 78.51(1)°$, $\beta = 87.12(1)°$, $\gamma = 80.57(1)°$, $V = 728.4(2)$ Å^3, $Z' = 1$, $R_1 = 0.105$. Form A (monoclinic):[5b] $P2_1/c$, $a = 8.5532(4)$ Å, $b = 22.2252(10)$ Å, $c = 17.1680(8)$ Å, $\beta = 102.100(2)°$, $V = 3195.4(3)$ Å^3, $Z' = 2$, $R_1 = 0.0687$. Both authors refer to their structures as form A though the crystal system, unit cell dimensions, molecules in the asymmetric unit ($Z' = 1$, 2), hydrogen bonding (neutral vs. ionic), and overall packing (calculated powder XRD) are visibly different to classify them as triclinic and monoclinic polymorphs. We thank the authors of ref 5b for kindly providing us with the.cif file of the monoclinic form (CCDC deposition number 686995). The triclinic structure[7] is archived as CSD refcode VETVOG.

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(15) Justification for 1.125 hydrate stoichiometry and cell choice ($Z' = 2$) is given in the Supporting Information. X-ray reflections.hkl files are provided for readers.

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