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A NEW POLYMORPH OF NORFLOXACIN
Complete characterization and relative stability of its trimorphic system

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A new polymorphic form of Norfloxacin has been identified and fully characterized by a variety of methods including powder X-ray diffraction, vibrational spectroscopy (IR and Raman), thermal analysis (DSC and TG), SEM and solid-state NMR spectroscopy. The relationship between the new form C and the previously known forms A and B have been studied. Moreover, the crystal structure of the known form A has been solved by single-crystal methods.

Keywords: *crystal structure, fluoroquinolone, Norfloxacin, polymorphism, solvent mediated transformation, thermal analysis, thermodynamics*

Introduction

Polymorphism is the ability of a substance to crystallize in different crystal modifications, each of them having the same chemical structure but different arrangements or conformations of the molecules in the crystal lattice. Different polymorphs exhibit different physicochemical properties such as solubility, dissolution rate, bioavailability and chemical and physical stabilities. So, polymorphism has become a topic of great interest for both academic research and pharmaceutical industries as it has the potential to significantly affect the physical properties of a compound [1].

Many drugs may appear in more than one crystalline structure, either because of a different arrangement of molecules in the lattice (packing polymorphism) or because of a different conformation of the molecules in the lattice (conformational polymorphism). Sometimes the most stable polymorph is difficult to produce or a metastable form has favorable properties. Regardless of which form is chosen for development, it is of greatest importance for the pharmaceutical industry to ensure reliable and robust processes. The ability of a particular polymorph to crystallize is usually determined by both thermodynamic and kinetic factors. These factors must be well understood in order to explore and to control the polymorphic behaviour of a substance. Hence, it is important, and even a regulatory requirement, to identify the possible polymorphic forms of a product and to know whether polymorphic modifications can transform reversibly (enantiotropy) or irreversibly (monotropy) at atmospheric pressure. When an organic compound exhibits polymorphism of an enantiotropic type, the knowledge of the different domains of thermodynamic stability for every form is essential in order to obtain the desired form by a robust crystallization process and to define the appropriate storage conditions [2].

Norfloxacin, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid is a synthetic broad antibacterial compound discovered in the late nineteen-seventies, belonging to the group of fluoroquinolones. In vitro, it is active against a broad spectrum of gram-positive and gram-negative aerobic bacteria. It is used in the treatment of gonorrhea, prostate and urinary tract infections [3].

Norfloxacin exists in several solid forms: two anhydrous polymorphs (form A and form B) and an amorphous form [4], a methanol solvate [5] and several hydrated forms [3, 6, 7]. In a recent study [8], we updated and corrected the previous knowledge of this drug which was erroneous due to a wrong identification of the system A and B as a monotropic type. The results of solvent mediated transformation experiments were in concordance with the endothermic solid-solid transition observed by DSC. These evidences were enough to prove the enantiotropic relationship between both polymorphs. This is important because many commercial samples of Norfloxacin are provided as the metastable form at room temperature and then, undesirable transformations could occur.

In the present study we describe the crystal structure of the metastable form A by single-crystal X-ray diffraction, as well as the discovery of a new

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polymorphic form of Norfloxacin. Thermal analytical methods, powder X-ray diffraction, vibrational spectroscopy (IR and Raman), SEM and solid-state NMR spectroscopy have been applied in order to characterize the new solid form C. The relative thermodynamic stability of the three anhydrous forms has been determined.

Experimental

Materials

Norfloxacin

Norfloxacin was purchased from Sigma-Aldrich (Ref. N9890).

Preparation of form C of Norfloxacin

Norfloxacin (100 mg) was suspended in 10 mL of acetone. The suspension was heated to reflux. Once the solid was dissolved, it was allowed to slowly cool to room temperature. The solid obtained was isolated by vacuum filtration and washed with cold pentane. Yield=51%.

General methods

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was carried out by means of a Mettler-Toledo DSC-822e calorimeter. Experimental conditions: aluminum crucibles of 40 μL volume, atmosphere of dry nitrogen with 50 mL min^{-1} flow rate, heating rate of 10°C min^{-1}. The calorimeter was calibrated with indium of 99.99% purity.

Thermogravimetry

Thermogravimetric analyses were performed on a Mettler-Toledo TGA-851e thermobalance. Experimental conditions: alumina crucibles of 70 μL volume, atmosphere of dry nitrogen with 50 mL min^{-1} flow rate, heating rate of 10°C min^{-1}.

Powder X-ray diffraction (PXRD)

XRD patterns were obtained on two equipments: A) Panalytical X'Pert PRO powder diffractometer, equipped with a CuK_α source (λ=1.54056 Å) and a X'Celerator Detector, operating at 45 kV and 40 mA. Each sample was scanned between 2 and 50° in 2θ, with a step size of 0.017° and a scan rate of 300 s/step. B) Debye-Scherrer INEL CPS-120 diffractometer, equipped with a CuK_α source (λ=1.54056 Å) and a 120° curved position sensitive detector, operating at 40 kV and 30 mA. Each sample was scanned between 0 and 115° in 2θ, with a step size of 0.029° and a scan rate of 3600 s/step.

X-ray structure determination

Single-crystal X-ray diffraction data were collected on a Bruker Smart CCD area detector with Oxford Cryosystems low temperature system. Cell parameters were refined from the setting angles of 5749 reflections (θ range 1.52<25.00°). Reflections were measured from a hemisphere of data collected of frames each covering 0.3 degrees in omega. Of the 58519 reflections measured, all of which were corrected for Lorentz and polarization effects and for absorption by semi-empirical methods based on symmetry-equivalent and repeated reflections (minimum and maximum transmission coefficients 0.9627 and 0.9713), 4101 independent reflections exceeded the significance level $|F|/\sigma(|F|)>4.0$. The structure was solved by direct methods and refined by full matrix least squares methods on F2. Hydrogen atoms were placed geometrically and refined with a riding model (including torsional freedom for methyl groups) and with U_{iso} constrained to be 1.2 (1.5 for methyl groups) times U_{eq} of the carrier atom. Refinement converged at a final R=0.0572 (wR_2=0.1515, for all 5632 data, 415 parameters, mean and maximum δ/σ 0.000, 0.000) with allowance for the thermal anisotropy of all non-hydrogen atoms. Minimum and maximum final electron density –0.482 and –0.919 e.$Å^{-3}$. A weighting scheme $w=1/[\sigma^2(Fo^2)+(0.0710*P)^2+1.4691*P]$ where $P=(Fo^2+2*Fc^2)/3$ was used in the latter stages of refinement. Complex scattering factors were taken from the program package SHELXTLY as implemented on the Pentium computer.

Raman spectroscopy

Raman spectra were collected using a Jobin Yvon T64000 instrument, with NIR excitation radiation at 514 nm and a liquid-nitrogen-cooled bidimensional CCD detector.

Infrared spectroscopy

FTIR spectra were recorded on a Bomem MB-120 IR spectrophotometer in KBr pellets, at 1 cm^{-1} resolution, from 350 to 5000 cm^{-1}.

Solid state ^{13}C NMR spectroscopy

Solid state ^{13}C NMR spectra were collected on a Varian Unity spectrometer operating at 75.4 MHz. The samples were spun in a 7 mm zirconia rotor and registered at room temperature (22°C). High resolution spectra were recorded using the CP/MAS method (cross-polarization/ magic angle spinning) at 4000 rpm. A 2.5 s contact time and 3 s relaxation time was used. The resulting FIDs were processed with a

line broadening of 1 Hz. Samples were referenced to hexamethylbenzene (methyl: 17.3 ppm).

Solvent mediated transformation experiments

The general procedure consisted in suspending a mixture of both solid forms (10–20 mg of each form) in 1.5 mL of the selected solvent and stirring with a magnetic bar under nitrogen atmosphere, during three days at the temperature of study. The solid obtained was investigated by XRPD and/or DSC analysis.

Results and discussion

Syngle crystal structure of form A

Here we report the first crystal structure solved for an anhydrous form of Norfloxacin. Although no anhydrous crystal structures are available in the literature, crystal structures are available for a dihydrate [7], a methanolate [5], a dihydrochloride monohydrate [9], a $2DCl{\cdot}D_2O$ form [10] and some metal complexes [9, 11]. Single crystals of the metastable form A were grown by vapor diffusion of MTBE into a DMF solution of Norfloxacin. The crystal structure of form A was determined by single crystal X-ray diffraction. Form A crystallizes in monoclinic centrosymmetric space group $P2_1/c$. The ORTEP diagram and atomic numbering are given in Fig. 1. Crystallographic data are summarized in Table 1.

The structure shows the molecule as a zwitterionic form which crystallizes as a dimer (Fig. 2). Specific intermolecular interactions (H-bonding) serve to stabilize this dimer as Norfloxacin has a single hydrogen bond donor (protonated amine), and two good acceptors (carbonylic oxygens).

Efforts to obtain single crystals of polymorphs B and C suitable for an X-ray structure determination were unsuccessful.

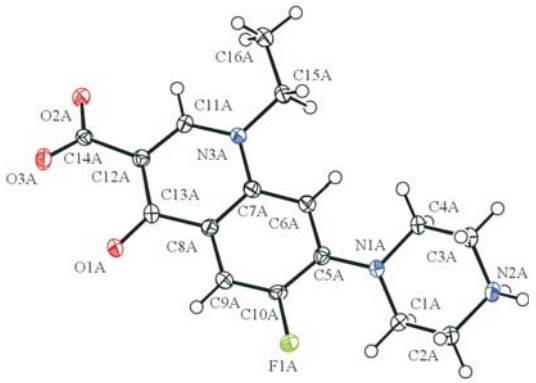


Fig. 1 ORTEP diagram and atomic numbering of the molecule in form A of Norfloxacin

Table 1 Crystallographic data of form A

	Form A
Empirical formula	$C_{16}H_{18}FN_3O_3$
Formula weight	319.33
Radiation	Mo–K_α
Wavelength/Å	0.71073
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	
a/Å	8.5532(4)
b/Å	22.2552(10)
c/Å	17.1680(8)
α/°	90
β/°	102.100(2)
γ/°	90
Volume//Å^3	3195.4(3)
Z	8
Density/g cm^{-3}	1.328
Abs. coeff/mm^{-1}	0.101
F000	1344
Crystal size (mm)	0.38·0.35·0.29
θ range/°	1.52–25.00
Goodness of fit on F^2	1.080
R_1/%	0.0572
R_w/%	0.1407

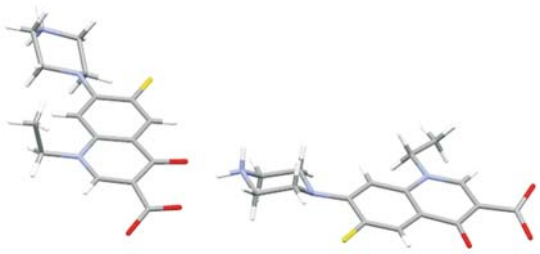


Fig. 2 Molecular structure of Norfloxacin observed in form A which crystallizes as a dimer

Preparation and characterization of form C of Norfloxacin

The new polymorph C of Norfloxacin was obtained by crystallization in acetone and slowly cooling to room temperature. Its observed morphology (SEM) is shown in Fig. 3 together with the observed morphologies of forms A and B.

The DSC curve of this sample shows an endothermic phenomenon at 207°C with a heat of fusion of 130.1 J g^{-1}, as shown in Fig. 4. Thermogravimetric analysis of this sample shows no mass loss from room temperature to 230°C, showing that this product is neither a solvated, nor a hydrated form of Norfloxacin.

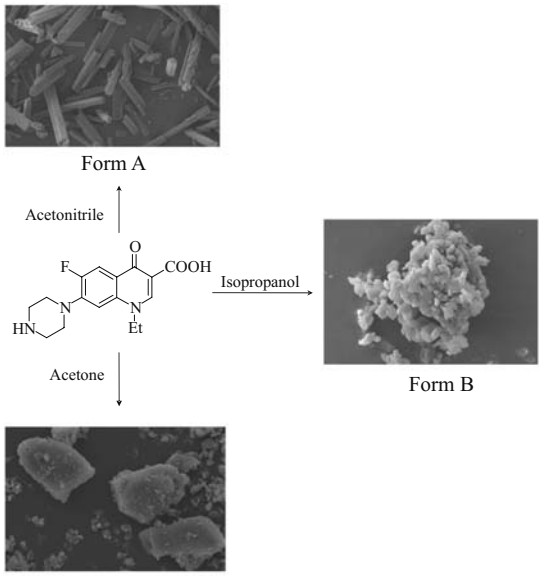


Fig. 3 Observed morphologies (SEM) of the different crystal forms obtained by crystallization

The powder XRD pattern of polymorph C is different from the ones described for forms A and B and allows a clear and fast identification of this polymorph (Fig. 5). The most important positions and relative intensities for the three modifications are listed in Table 2.

Moreover, form C of Norfloxacin has been characterized by means of FTIR and Raman spectroscopy (not shown). Only slight differences can be detected between polymorphs B and C, whereas significant differences between the polymorphic forms A and C can be seen in the whole spectral region. The most striking differences can be found in the valence vibrations of the carboxylic group (A: 1731 cm^{-1}; C: 1715 cm^{-1}), the molecular vibrations (A: 1477 and 1249 cm^{-1}; C: 1484 and 1254 cm^{-1}) and the rocking vibrations of the alkyl chains (A: 749 cm^{-1}; C: 737 cm^{-1}) [12].

The ^{13}C CP/MAS NMR peak assignment of polymorph C together with resonances of forms A and B are shown in Table 3.

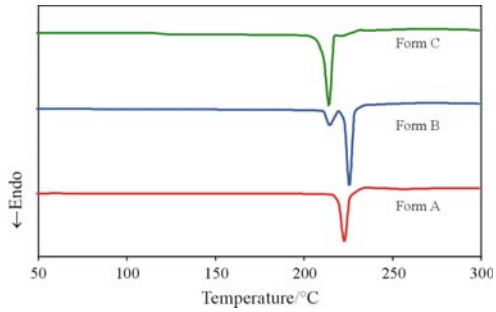


Fig. 4 DSC curves of forms A, B and C of Norfloxacin carried out at the heating rate of 10°C min^{-1}

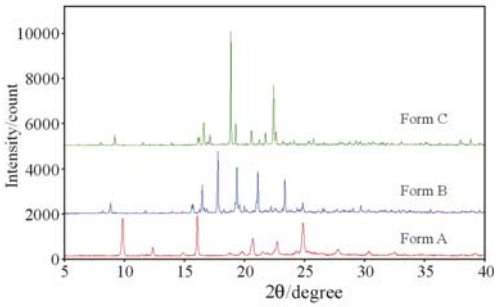


Fig. 5 X-ray powder diffraction patterns of forms A, B and C of Norfloxacin

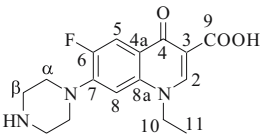
The chemical shifts in the solid state NMR spectra of forms B and C are very similar. However, some differences are observed when comparing resonances of polymorph A with B or C. The main difference is the aromatic carbon (C3) α to the carboxylic group, which is shifted 9 ppm downfield in forms B and C relative to form A. As we reported in a previous study [8], this shift can be attributed to the different orientation of the carboxylic group respect to the quinoline plane. Form A was supposed to have an out of plane orientation, which has been confirmed in the present paper by its crystal structure (23° torsion angle, Fig. 2). Therefore, form C is likely to present an in plane conformation as form B does.

It is important to note that only X-ray diffraction and DSC analysis permit to distinguish between mod-

Table 2 Most important two theta positions (2θ/°) and relative intensities (*I*) of the powder X-ray diffraction patterns of Norfloxacin crystal forms

Form A		Form B		Form C	
2θ/°	*I*/%	2θ/°	*I*/%	2θ/°	*I*/%
9.841	94.46	8.841	16.81	16.585	19.31
12.349	20.92	16.473	44.46	18.852	100.00
16.063	100.00	17.770	100.00	19.263	19.58
20.523	19.88	19.360	71.41	20.561	13.30
20.707	37.02	20.975	16.73	21.734	11.99
22.668	31.23	21.105	63.63	22.412	53.36
24.881	77.09	23.348	52.46	22.621	12.05

Table 3 Solid state ^{13}C NMR peak assignments for Norfloxacin crystal forms

	^{13}C Chemical shift δ/ppm		
	Form A	Form B	Form C
C2, C6, C7	149.8, 147.4	148.8, 151.5, 146.4	148.4, 151.3, 146.3
C3	108.9	117.7	117.3
C4	176.8	174.3	174.1
C4a	120.4	124.1	123.7
C5	110.2	111.4	111.2
C8	106.2	105.6	105.2
C8a	137.1	137.7	137.5
C9	167.5	169.6	169.4
C10	51.5	49.6	49.2
C11	15.0	16.1	15.9
Cα,β	44.2–49.0	40.0–45.3	42.0

ifications B and C of Norfloxacin, whereas all aforementioned techniques are useful to differentiate between forms A and C. So, the method of first choice for a clear distinction of the three crystal forms is DSC or XRPD.

Relative stability of the different modifications

Norfloxacin exists at least in three polymorphic forms. It is of practical interest to know the relative thermodynamic stability of all forms. The main questions to solve are whether two polymorphs are monotropically (one form is more stable than the other at any temperature) or enantiotropically (a transition temperature exists, below and above which the stability order is reversed) related, and for an enantiotropic system, where transition temperature lies.

Table 4 summarizes the physicochemical data of the three modifications of Norfloxacin, obtained from the thermal analysis experiments. According to the Heat of Fusion Rule of Burger and Ramberger [14], forms B and C are enantiotropically related to form A due to the lower enthalpy of fusion and the larger melting point of this last form. Concerning the pair A and B, this relationship was also confirmed by the endothermic transition of form B to form A at about 196°C observed in a DSC analysis [8]. Also on the basis of the Heat of Fusion Rule, modifications B and C appear to be monotropically related since the higher melting form B has the higher enthalpy of fusion. Thus, form B is thermodynamically more stable than form C at all temperatures up to the melting points of either polymorph.

As mentioned above, polymorphs B and C are enantiotropically related to form A. In the DSC experiments we could only determine the experimental

Table 4 Physicochemical data obtained from the thermal analysis experiments

	T_{fus}/°C	ΔH_{fus}/J g^{-1}
Form A	219	122.5
Form B	212	147.2[a)]
Form C	207	130.1

[a]A value for the heat of fusion of form B was estimated, even though single melting DSC endotherms were not obtained [13]

transition temperature of form B to form A. Therefore, we have estimated the transition temperatures between the two enantiotropic pairs by using the treatment of Yu [15]. This method needs the temperatures and enthalpies of fusion to calculate the Gibbs free energy difference at the melting temperature of the lower melting form and to extrapolate to other temperatures.

$$T_{trs} = \frac{\Delta H_{fus,2} - \Delta H_{fus,1} + k\Delta H_{fus,1}(T_{fus,1} - T_{fus,2})}{\frac{\Delta H_{fus,2}}{T_{fus,2}} - \frac{\Delta H_{fus,1}}{T_{fus,1}} + k\Delta H_{fus,1} \ln\left(\frac{T_{fus,1}}{T_{fus,2}}\right)} \quad (1)$$

A value of 0.003 was used for the factor k, which was empirically determined and allows a good approximation of the heat capacity differences in the majority of cases [15]. Using this equation we calculated values of 113°C for the pair C–A and 183°C for the pair B–A.

In order to confirm the enantiotropic transition temperature between polymorphs C and A, we have used the so-called solvent mediated transformation method [16]. This method is based on the relationship between solubility and stability of crystal forms, i.e. the less stable form will also be the most soluble at

Table 5 Experimental conditions and results of the solvent mediated transformation experiments between A and C forms

Solvent	Temperature/°C	Resulting form
DMF	25	C
Xylene	105	C
Xylene	110	A
Xylene	125	A

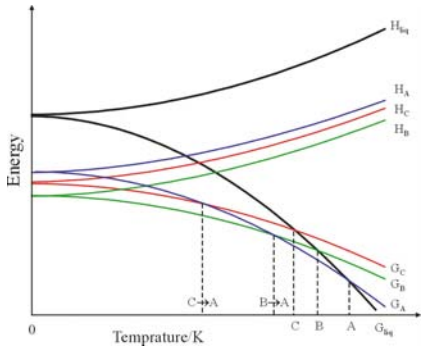


Fig. 6 Semi-schematic energy/temperature diagram of Norfloxacin, showing fusion temperatures of the three forms and solid-solid transitions

given conditions of temperature and pressure. If crystals of both forms are mixed with a saturated solution of the product, the most stable form will grow at the expense of the less stable one. Mixtures of the two modifications were suspended and stirred in a solvent at different temperatures. Table 5 summarizes the experimental conditions and the results obtained in each slurry. Below 105°C the mixtures resulted in pure C form whereas form A was obtained at 110°C and higher temperatures. From these results we can conclude that transition point lies between 105 and 110°C, which matches with the calculated transition temperature using the above-mentioned equation.

Given that the heat of fusion rule points out a monotropic relationship between forms B and C, some solvent mediated transformation experiments in xylene at different temperatures (25–125°C) were performed. As we expected, form B was obtained in all cases.

Energy diagrams such as those proposed by Burger and Ramberger [14] give considerable insight into polymorphic systems. Based on physicochemical data, a semi-schematic energy/temperature diagram was constructed in order to display the thermodynamic relationship of the polymorphs at different temperatures (Fig. 6).

Conclusions

The description of the polymorphic system of Norfloxacin has been completed with the discovery of the new form C. Moreover, the single crystal structure of form A has been solved, showing a dimeric arrangement of the molecule as a zwitterionic form, stabilized by intermolecular hydrogen bonding. The relative thermodynamic stability of the three forms has been fully determined by means of thermal analysis and solvent mediated transformation experiments. The data show an enantiotropic relationship between A and C forms, as well as a monotropic relationship between B and C modifications. New form C shows a high similarity with form B regarding their molecular spectroscopy properties (IR, Raman and NMR) but not with form A, suggesting conformational and spatial distribution differences in the crystal lattice of form A compared to forms B and C. Unfortunately, it has not been possible to grow single crystals of B and C forms suitable for X-ray structure determination to confirm this assumption.

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