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Crystal structure of ciprofloxacin hexahydrate and its characterization

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Abstract

Ciprofloxacin hexahydrate has been prepared and its crystal structure has been determined by X-ray crystallography. The molecule is in the zwitterionic form in solid state, the carboxylic proton being present at terminal piperazine nitrogen atom (N(74)) which is in contrast to the previously reported structures of quinolones. Water molecules are involved in a very complicated hydrogen bonding network. Different other techniques (IR, Raman spectroscopy, thermal and elemental analysis) were used to characterize the title compound and to compare its properties with similar compounds. © 1997 Elsevier Science B.V.

Keywords: Quinolone; Ciprofloxacin; Synthesis of a hydrate; Crystal structure; Zwitter ion; Characterization

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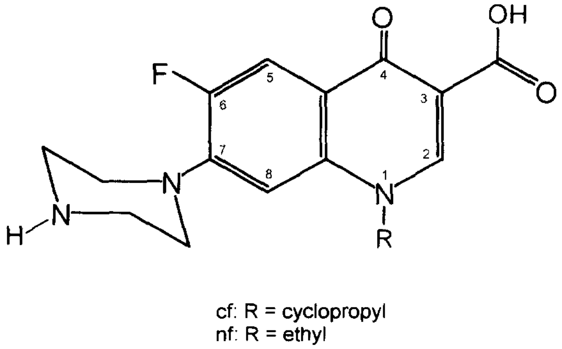
1. Introduction

Ciprofloxacin (cf = 1-cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-1,4-dihydroquinoline-3-carboxylic acid) (Scheme 1) is a synthetic antibacterial fluoroquinolone related to nalidixic acid. It is one of the most active fluoroquinolones with a wide spectrum of activity including Enterobacteriaceae, *Pseudomonas aeruginosa*, *Haemophilus* and *Neisseria spp.* and also against staphylococci and some other Gram-positive bacteria (Reynolds, 1993).



Scheme 1. cf = 1-cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-1,4-dihydroquinoline-3-carboxylic acid.

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Table 1
Crystal data and structure refinement for cf·$6H_2O$.

Empirical formula	$C_{17}H_{30}FN_3O_9$	
Formula weight	439.44	
Temperature	293(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	$P\bar{1}$	
Unit cell dimensions	$a = 9.5273(10)$ Å	$\alpha = 94.794$ (13)°
	$b = 9.972(2)$ Å	$\alpha = 100.304$ (10)°
	$c = 11.249(2)$ Å	$\gamma = 91.354$ (11)°
Volume	1047.0(3) Å^3	
Z	2	
Density (calculated)	1.394 Mg m^{-3}	
Absorption coefficient	0.118 mm^{-1}	
F(000)	468	
Θ range for data collection	1.85–29.89°	
Limiting indices	$-1 \le h \le 11$, $-13 \le k \le 11$, $-13 \le l \le 13$	
Reflections collected	4479	
Independent reflections	3715 [R(int) = 0.0223]	
Refinement method	Full-matrix least squares on F^2	
Data/restraints/parameters	3714/12/307	
Goodness-of-fit on F^2	1.028	
R indices [$I > 2\sigma(I)$]	$R_1 = 0.0455$, $wR_2 = 0.1011$	
R indices (all data)	$R_1 = 0.0793$, $wR_2 = 0.1184$	
Largest diff. peak and hole	0.162 and -0.194 e·Å^{-3}	

It has already been established that norfloxacin (nf) (Scheme 1), an analogue of cf bearing an ethyl group at position 1, forms different hydrates under ambient conditions. This was proposed on the base of powder patterns and crystal structure determination (Mazuel, 1991; Golič et al., 1992). Additionally, polymorphism of nf was studied. Two different crystalline forms as well as the amorphous substance can be prepared under different conditions (Šuštar et al., 1993). All these factors could contribute to different properties of the compounds and it is therefore important to evaluate them.

Our aim was to determine the crystal structure of a newly prepared hydrated form of cf and to compare its properties with those already reported for anhydrous cf.

2. Materials and methods

2.1. Materials

Ciprofloxacin and ciprofloxacin hydrochloride hydrate (laboratory samples) were obtained from Krka, Pharmaceutical factory (Slovenia). Ammonia solution (25%) was supplied by Kemika, Zagreb and cesium carbonate was supplied by Merck, Darmstadt.

2.2. Instrumentation

FT-IR spectra were recorded using KBr pellets or in Nujol and poly(chlorotrifluoroethylene) oil mulls between CsI windows on a Perkin Elmer 2000 FT-IR spectrometer or Digilab FTS-80 Fourier Spectrometer. Conditions: range, 4000–400 (220) cm^{-1}; resolution, 2–8 cm^{-1}; 10–60 scans.

The Raman spectra were recorded on a Perkin Elmer FT-Raman System 2000.

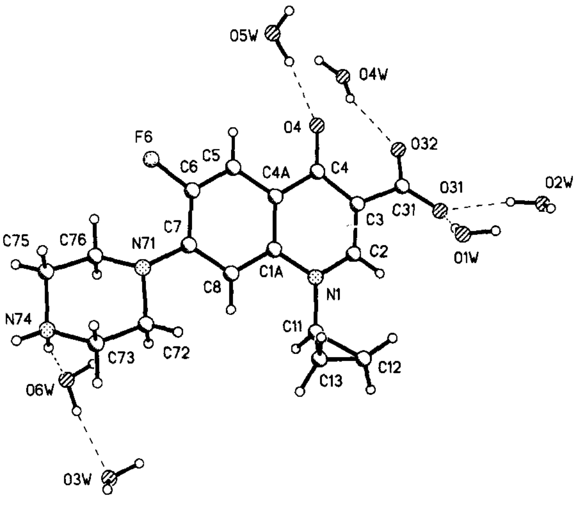

Fig. 1. View of cf·$6H_2O$ entity with atomic labeling.

Table 2
Atomic coordinates (× 10^4) and equivalent isotropic displacement parameters ($Å^2 x10^4$) for cf·$6H_2O$·U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor

	x	*y*	*z*	*U* (eq)
N(1)	1704(2)	3687(2)	5164(2)	261(4)
C(11)	2346(2)	3320(2)	4102(2)	296(5)
C(12)	3918(3)	3173(3)	4283(3)	441(7)
C(13)	2930(3)	1957(2)	3964(2)	395(6)
C(1A)	398(2)	3095(2)	5284(2)	241(5)
C(2)	2338(2)	4656(2)	6002(2)	272(5)
C(3)	1815(2)	5137(2)	7004(2)	267(5)
C(31)	2703(3)	6208(2)	7851(2)	296(5)
O(31)	4022(2)	6225(2)	7878(2)	412(4)
O(32)	2073(2)	7030(2)	8457(2)	428(5)
C(4)	478(2)	4601(2)	7205(2)	288(5)
O(4)	−88(2)	4980(2)	8090(2)	466(5)
C(4A)	−206(2)	3533(2)	6298(2)	258(5)
C(5)	−1497(2)	2903(2)	6425(2)	303(5)
C(6)	−2121(2)	1906(2)	5592(2)	293(5)
F(6)	−3322(2)	1268(1)	5792(1)	428(4)
C(7)	−1567(2)	1468(2)	4549(2)	253(5)
N(71)	−2274(2)	411(2)	3738(2)	270(4)
C(72)	−1500(2)	−129(2)	2805(2)	290(5)
C(73)	−2250(2)	−1412(2)	2148(2)	331(6)
N(74)	−3759(2)	−1169(2)	1616(2)	320(5)
C(75)	−4517(2)	−618(2)	2581(2)	329(5)
C(76)	−3770(2)	661(2)	3206(2)	304(5)
C(8)	−295(2)	2088(2)	4419(2)	264(5)
O(1W)	5140(3)	3645(2)	8106(3)	886(9)
O(2W)	6677(3)	7048(2)	9366(2)	744(7)
O(3W)	−1250(3)	624(3)	−1261(2)	744(7)
O(4W)	−500(3)	7945(2)	9102(2)	718(7)
O(5W)	−2135(2)	4468(3)	9414(2)	673(7)
O(6W)	−3500(2)	888(2)	74(2)	433(5)

Data for the X-ray study were taken in a Siemens P4 diffractometer. Crystallographic and procedural data are listed in Table 1. The structure was solved by direct methods and refined by full-matrix least-squares on F^2, by means of the program package SHELXTL PLUS for PC (Sheldrick, 1994), also used for making the drawing. All hydrogen atoms were clearly seen in the ΔF maps. Those of the organic ligand were nevertheless idealized, whereas those of water molecules were refined with fixed O–H distances (0.86 Å).

Table 3
Bond lengths [Å] and bond angles [°] for cf·$6H_2O$

N(1)–C(2)	1.343(3)	C(4)–C(4A)	1.465(3)
C(4A)–C(5)	1.403(3)	N(1)–C(1A)	1.398(3)
C(5)–C(6)	1.356(3)	N(1)–C(11)	1.460(3)
C(6)–F(6)	1.359(2)	C(11)–C(12)	1.487(3)
C(6)–C(7)	1.411(3)	C(11)–C(13)	1.488(3)
C(7)–C(8)	1.384(3)	C(12)–C(13)	1.496(3)
C(7)–N(71)	1.409(3)	C(1A)–C(8)	1.402(3)
N(71)–C(72)	1.462(3)	C(1A)–C(4A)	1.410(3)
N(71)–C(76)	1.479(3)	C(2)–C(3)	1.368(3)
C(72)–C(73)	1.518(3)	C(3)–C(4)	1.432(3)
C(73)–N(74)	1.489(3)	C(3)–C(31)	1.504(3)
N(74)–C(75)	1.484(3)	C(31)–O(31)	1.251(3)
C(75)–C(76)	1.501(3)	C(31)–O(32)	1.254(3)
C(4)–O(4)	1.251(3)		
C(2)–N(1)–C(1A)	119.6(2)	C(3)–C(4)–C(4A)	115.3(2)
C(2)–N(1)–C(11)	119.6(2)	C(5)–C(4A)–C(1A)	117.7(2)
C(1A)–N(1)–C(11)	120.7(2)	C(5)–C(4A)–C(4)	120.3(2)
N(1)–C(11)–C(12)	118.3(2)	C(1A)–C(4A)–C(4)	122.0(2)
N(1)–C(11)–C(13)	118.7(2)	C(6)–C(5)–C(4A)	120.2(2)
C(12)–C(11)–C(13)	60.4(2)	C(5)–C(6)–F(6)	118.1(2)
C(11)–C(12)–C(13)	59.8(2)	C(5)–C(6)–C(7)	123.6(2)
C(11)–C(13)–C(12)	59.8(2)	F(6)–C(6)–C(7)	118.4(2)
N(1)–C(1A)–C(8)	120.9(2)	C(8)–C(7)–N(71)	123.6(2)
N(1)–C(1A)–C(4A)	118.4(2)	C(8)–C(7)–C(6)	116.5(2)
C(8)–C(1A)–C(4A)	120.7(2)	N(71)–C(7)–C(6)	119.8(2)
N(1)–C(2)–C(3)	125.4(2)	C(7)–N(71)–C(72)	115.9(2)
C(2)–C(3)–C(4)	119.3(2)	C(7)–N(71)–C(76)	114.8(2)
C(2)–C(3)–C(31)	117.3(2)	C(72)–N(71)–C(76)	111.0(2)
C(4)–C(3)–C(31)	123.4(2)	N(71)–C(72)–C(73)	110.2(2)
O(31)–C(31)–O(32)	124.9(2)	N(74)–C(73)–C(72)	110.9(2)

Table 3 (continued)

O(31)–C(31)–C(3)	117.0(2)	C(75)–N(74)–C(73)	109.9(2)
O(32)–C(31)–C(3)	118.1(2)	N(74)–C(75)–C(76)	110.1(2)
O(4)–C(4)–C(3)	124.0(2)	N(71)–C(76)–C(75)	110.4(2)
O(4)–C(4)–C(4A)	120.7(2)	C(7)–C(8)–C(1A)	121.3(2)

2.3. Preparation of the compound

Anhydrous cf was dissolved in the solution of ammonia. After few days pale brown-yellow needles were formed. The crystals were filtered off and dried in air. Different concentrations of ammonia were used for the syntheses but the same product was always isolated. The only difference was the amount of water molecules involved in the product which varied from four to six molecules (see Section 3 for explanation).

It is interesting to note that the title compound was also prepared by another method. Ciprofloxacin was dissolved in the water solution of Cs_2CO_3 (mole ratio 1:1). Few drops of 2 M sodium hydroxide solution were added until the solution was clear and in few days crystals were grown.

3. Results and discussion

The crystal structure determination showed that the cell contained 2 units of cf·$6H_2O$. The view of the compound with atomic numbering is given in Fig. 1. Final relevant atomic positions and values of bond distances and angles are listed in Table 2 and Table 3, respectively.

Table 4
The comparison of C–O bond lengths in some of the quinolone molecules found in the literature (see text for the references). Note that the numbering of the atoms was adjusted to fit with the title compound numbering

	C(4)–O(4)	C(31)–O(32)	C(31)–O(31)
nal	1.261(8)	1.323(9)	1.230(10)
nf	1.248(1)	1.260(2)	1.246(2)
nf.2HCl	1.321(5)	1.238(5)	1.305(5)
cf–HNO_3	1.297	1.339	1.314
lm	1.246(2)	1.236(2)	1.262(3)
cx	1.248(3)	1.316(3)	1.211(3)

Table 5
Distances in hydrogen bonds A–H...B [Å]

	d(A.. B)	d(H.. B)
N(74)–O(6W)	2.828(3)	1.942(3)
N(74)–O(1W)[c]	2.837(3)	2.132(3)
O(1W)–O(31)	2.821(3)	1.95(3)
O(1W)–O(5W)[d]	2.812(3)	1.98(3)
O(2W)–O(31)	2.834(3)	1.97(3)
O(2W)–O(5W)[d]	2.835(4)	1.97(3)
O(3W)–O(4W)[e]	2.826(4)	1.94(3)
O(3W)–O(4W)[a]	2.928(4)	2.08(3)
O(4W)–O(32)	2.829(3)	2.05(3)
O(4W)–O(2W)[f]	2.888(3)	2.05(3)
O(5W)–O(4)	2.721(3)	1.86(2)
O(5W)–O(32)[b]	2.919(3)	2.16(3)
O(6W)–O(32)[a]	2.716(2)	1.83(2)
O(6W)–O(3W)	2.835(3)	1.97(2)

Symmetry transformations used to generate equivalent atoms: [a] −x,−y+1,−z+1; [b] −x,−y+1,−z+2; [c] −x,−y,−z+1; [d] x+1,y,z; [e] x,y−1,z−1; [f] x−1,y,z.

It was reported before that not all water molecules are equivalently bound in some fluoroquinolone-metal complexes and that some loss of water occurs readily, either by drying the compound in vacuo or even by prolonged exposure of the compound to air. The consequence was that the number of water molecules determined from crystal structure analysis was not in total agreement with elemental or thermal analyzes (Baenzinger et al., 1986; Turel et al., 1994). Thus it was not surprising that the same effect was also observed by analyzing different samples of the title compound.

The crystal structure of cf·$6H_2O$ has revealed an important fact that the molecule of cf is clearly in a zwitterionic form. Carboxylic group is deprotonated, as can be seen in the virtually identical distances C(31)–O(31) and C(32)–O(32), whereas 2 hydrogen atoms are attached to N(74), which are clearly seen in the ΔF maps, both forming donor hydrogen bonds to water molecules.

It is known that quinolones could exist in zwitterionic form in water solution (Lee et al., 1994), but as follows from the literature review and

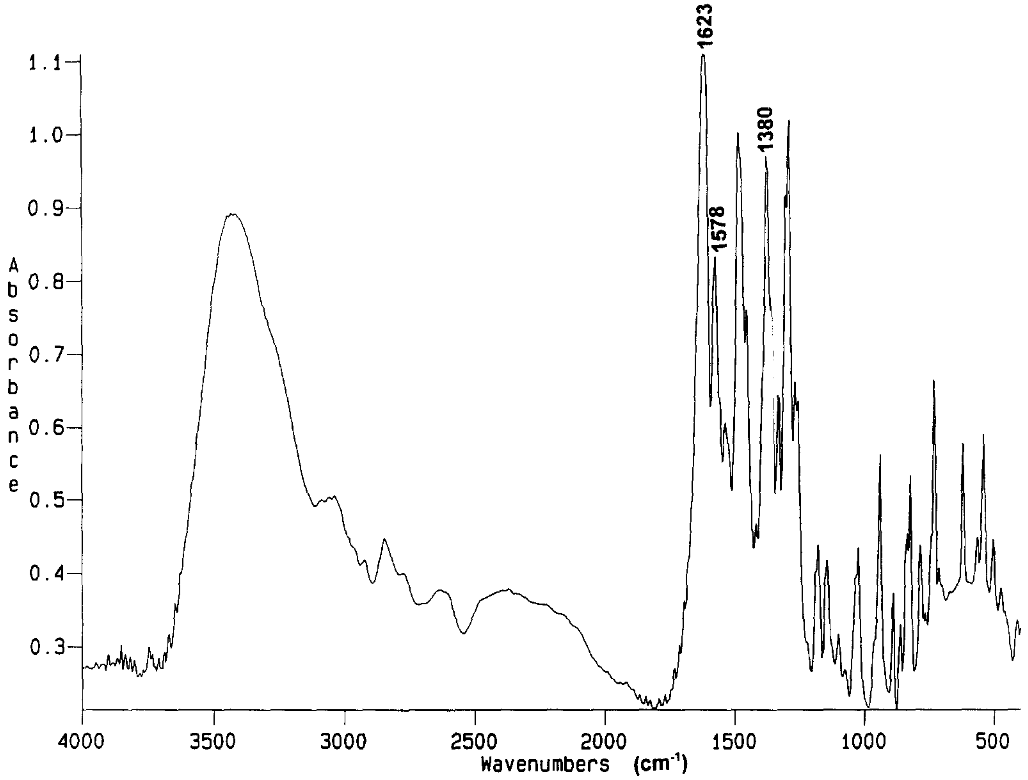

Fig. 2. IR spectrum of cf.$6H_2O$.

Cambridge Structural Database System, 1996 search, this is the first such example in solid state in the quinolone family described so far.

In order to confirm this assumption it was necessary to compare C–O distances (Table 4) and the appearance of the carboxyl and ring carbonyl groups in the solved crystal structures of some related compounds. The carboxylic proton of nalidixic acid (nal) (Achari and Neidle, 1976) interacts with the carbonyl oxygen to form an intramolecular hydrogen bond. Similar hydrogen bonding was observed in the structure of cinoxacin (cx) (Rosales et al., 1985). In marked contrast the hydrogen atom of the carboxylic acid in lomefloxacin (lm) (Riley et al., 1993) was found to be hydrogen bonded to the terminal nitrogen atom of an adjacent piperazine ring system. In the crystal structure of norfloxacin trihydrate (nf) (Golič et al., 1992) the organic ligands and the water molecules build a hydrogen bonded network. The carbonyl oxygen of nf·2HCl salt (Turel et al., 1996) is protonated. This proton is bonded via intramolecular hydrogen bond to the oxygen O(32) of the carboxylic group. There is a double bond between C(31) and O(32) atoms, whereas C(31)–O(31) is a clear single bond.

Table 6

Comparison of selected IR and Raman (R) bands of cf hexahydrate, cf and cf hydrochloride in the 1650–1340 cm^{-1} region with possible assignments

	$\nu(C=O)_P$	$\nu(O{-}C{-}O)_A$	$\nu(O{-}C{-}O)_s$
cf.$6H_2O$, IR	1623 vs	1578 m	1380 s
R	1613 s	1578 m	1382 vs
cf, IR	1618 vs	1589 s	1376 s
R	1615 s	1589 s	1380 vs
cf.HCl.H_2O, IR	1625 vs	—	1384 s
R	1622 vs	—	1382 s

v = very; s = strong; m = medium; P = pyridone; A = asymmetric; S = symmetric.

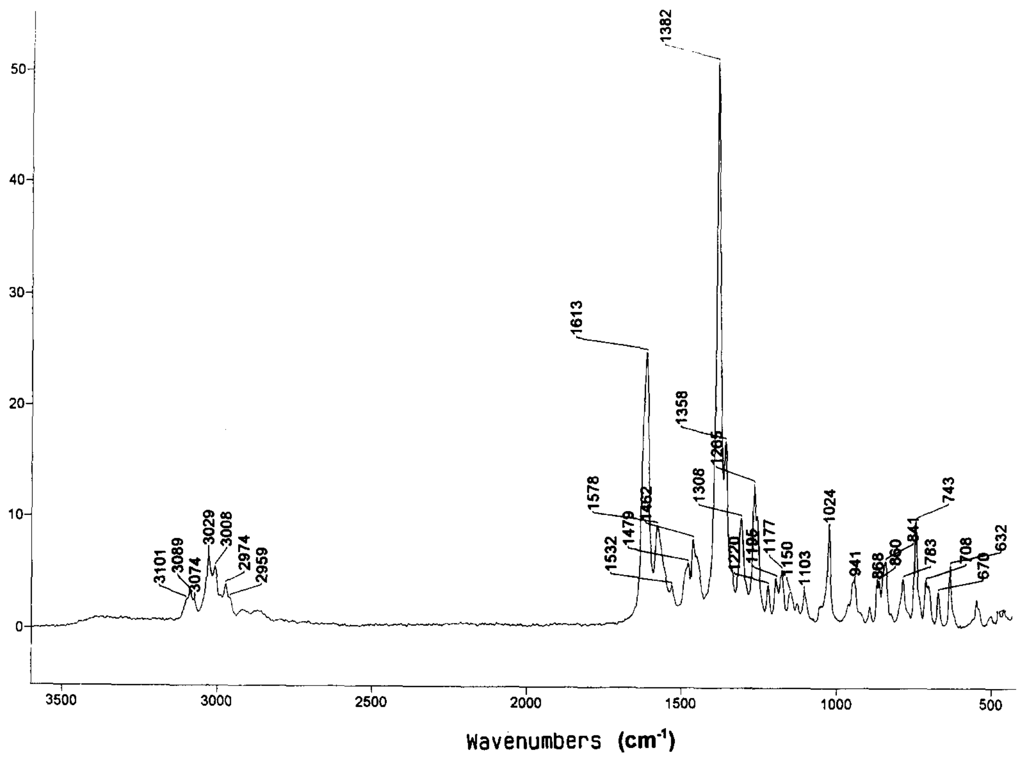

Fig. 3. Raman spectrum of cf.6H_2O.

It is important to stress distinctive differences in these distances between cf·6H_2O and the cf-HNO_3 adduct (Macías Sánchez et al., 1994). In the title compound the C–O distances are much shorter indicating that the role of carboxylic group is not the same in both compounds. There is no detailed explanation of the interaction between the carboxylic group and ring carbonyl group in the cf-HNO_3 adduct, but according to the acidic medium used in the synthesis it could be proposed that the carbonyl group is protonated, as in nf·2HCl (Turel et al., 1996).

The C=O distance of the ring carbonyl group is in the range from 1.246 to 1.261 Å in the majority of compounds (Table 4), the exceptions are nf·2HCl and cf-HNO_3 adduct with distances 1.321 and 1.297 Å respectively, which could again be the proof that ring carbonyl oxygen is protonated in the later.

The distances and angles between the atoms of the rigid quinolone ring system, as well as those of piperazine ring, are similar to those found in reported structures (Turel et al., 1994; 1996).

Hydrogen bonds build a very complicated network, all possible donor hydrogens participating actively linking water molecules among them and with the protonated amino group, the carboxylate group and the carbonyl at position 4 (Table 5).

3.1. IR and Raman spectra

The IR spectrum of hydrated cf is very similar to that of the anhydrous variety, the majority of bands below 1700 cm^{-1} being shifted for 5–11 cm^{-1} due to the bonding of water molecules in the structure (Fig. 2, Table 6).

It was found before (Mazuel, 1991; Turel and Bukovec, 1996) that some quinolone spectra exhibit a strong ν(COOH) band above 1700 cm^{-1}, whereas there is no such peak in others. The absence of this peak in cf spectrum could now be explained by deprotonation of –COOH group confirmed by its crystal structure. It is known that ionic carboxylates (Deacon and Phillips, 1980) show no carbonyl band at about 1700 cm^{-1}, but depict 2 characteristic bands in the range 1650-1510 cm^{-1} and 1400-1280 cm^{-1} that could be assigned as ν(O-C-O) asymmetric and symmetric stretching vibrations. It is however very difficult to assign these vibrations in the spectra of cf unequivocally due to the numerous bands present in these regions, but the combination of IR and Raman spectroscopies enabled us to propose some possible assignments. The IR band at 1578 cm^{-1} in the spectrum of cf·$6H_2O$ sample and the corresponding band at 1589 cm in anhydrous cf could be assigned as ν(O-C-O) asymmetric stretching vibration. There is no such band in the IR spectrum of cf·HCl which is in agreement with this proposal. The IR band at 1380 cm^{-1} in the spectrum of cf·$6H_20$ sample and at 1376 cm^{-1} in cf could be assigned as ν(O-C-O) symmetric stretching vibration. The corresponding bands in the Raman spectra (Fig. 3, Table 6) at 1382 and 1380 cm^{-1} are namely much stronger than the IR bands.

We have also found that after the heating the sample of hydrated cf to higher temperatures (80 and 280°C) a peak at 1727 cm^{-1} appeared in the spectrum which is similar to the anhydrous cf studied before (Turel and Bukovec, 1996). It seems that the role of -COOH group in the structure is changed during heating though the mechanism of this phenomenon is not explained yet.

Acknowledgements

We are grateful to Krka-Pharmaceutical factory (Slovenia) for the gift of cf samples and to Professor B. Orel for IR and Raman spectra. Supplementary material available on request from the authors. Table SI: Anisotropic Thermal Parameters. Table SII: Hydrogen-atom positions. Table SIII: Calculated and observed structure factors.

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