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PAPER

Multinuclear solid state NMR investigation of two polymorphic forms of Ciprofloxacin-saccharinate

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Two polymorphic forms of a novel pharmaceutical compound, ciprofloxacin-saccharinate (CIP-SAC), are analyzed using one dimensional (1D) and two dimensional (2D) ^{1}H nuclear magnetic resonance (NMR) at fast magic angle spinning (MAS). Additionally ^{15}N spectroscopy and ^{1}H–^{13}C correlation experiments were performed to complement our conclusions. The 1D ^{1}H NMR spectra of CIP and complexes reveal valuable information about the ionic bonding between ciprofloxacin and saccharine. Additionally, these spectra allow us to perform a clear characterization of each solid form, giving the number of molecules per unit cell in one of the polymorphs. From 2D ^{1}H–^{1}H spectra obtained through double quantum correlations we can arrive at important conclusions about the hydrogen bonding, conformation, and intra and inter-molecular interactions present in these compounds. Comparing and contrasting the ^{1}H–^{1}H correlation data obtained for both polymorphic forms and taking into account the single crystal structure data existing for the solid form CIP-SAC (II) was possible to extract some conclusions on the polymorph CIP-SAC (I) where no single crystal information is available. ^{1}H MAS NMR is shown to be an important tool in the field of polymorphism and for the characterization of multicomponent pharmaceutical compounds.

Introduction

Multicomponent crystalline pharmaceutical solids, as for example ionic complexes or salts, are usually developed to improve the pharmaceutical profile of a single organic molecule in terms of solubility, stability, bioavailability and organoleptic properties.[1,2] A better understanding of the solid-state interactions in these complexes may lead to a rational design of crystalline active pharmaceutical ingredients (API) to rapidly advance a drug candidate through development to the launch of a product. On the other hand, the phenomenon of polymorphism and its influence on the chemical and physical properties of molecular crystals is well known.[3] This is especially true for pharmaceutical compounds, where polymorphic changes in the APIs can lead to significant effects on bioavailability. Identification and characterization of polymorphs is therefore essential during all stages of the development and manufacture of pharmaceuticals. Several solid state techniques are commonly used to characterize multicomponent systems and to identify polymorphism.[4] Among them solid state NMR spectroscopy, especially ^{13}C cross-polarization (CP) magic-angle spinning (MAS), has proven itself as a powerful experiment for this purpose.[5–7] However, less use has been made of ^{1}H solid state NMR spectroscopy[8–10] due to the large broadening of the signals that results from extensive dipolar-coupled proton networks. Recent advances such as fast MAS and improved homo-nuclear decoupling techniques have led to increased resolution for organic compounds, making this technique very attractive for the direct observation of ^{1}H interactions which usually determine conformation and functionality in pharmaceutical products.[11–14]

The present multicomponent compound is a new ciprofloxacin saccharinate recently derived.[15] Ciprofloxacin (CIP) is a widely prescribed, broad-spectrum oral fluoroquinolone antibiotic approved for the treatment of several types of infections. Interestingly, ciprofloxacin saccharinate (CIP-SAC) can exist in two different polymorphic forms, CIP-SAC (I) and CIP-SAC (II). This multicomponent system is a complex molecule containing more than 20 carbon atoms. Previously, the identification of each solid form of CIP-SAC as well as the determination of some interactions and structures for these compounds has been done using several solid state techniques, such as single-crystal and powder X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, infrared,

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and ^{13}C and ^{1}H–^{13}C solid state nuclear magnetic resonance spectroscopy.[16]

In the present work we investigate the intra- and intermolecular interactions present in CIP-SAC (I) and CIP-SAC (II) using one dimensional and double quantum (DQ) ^{1}H MAS NMR spectra. From ^{1}H spectroscopy it is possible to extract through-bond interactions, ionic interactions and proximity between aromatic rings (π–π interactions). Also changes in the couplings and conformation of the polymorphs relative to CIP are determined. ^{1}H NMR interactions data are contrasted and compared with the complete crystallographic structure data available for CIP-SAC (II), obtained from single crystal X-ray diffraction experiments. These experiments also provide predictions about interactions in CIP-SAC (I), for which no single crystal structure is available. Additionally, natural abundance ^{15}N CPMAS NMR experiments and ^{1}H–^{13}C correlation experiments (REPT-HSQC) contribute to support our conclusions.

Experimental details

Samples

Samples were provided by Dr M.E. Olivera from the Department of Pharmacy, Universidad Nacional de Cordoba. CIP was obtained by neutralization of the hydrochloride salt (Ciprofloxacin hydrochloride (CIP·HCl, USP, Neuland Laboratories Ltd®), pharmaceutical grade). SAC was obtained by neutralization of the sodium salt (SAC-Na, Parafarm, China) and recrystallized from water. CIP-SAC (I) was prepared as reported.[15] CIP-SAC (II) was obtained by a slight modification of patent application P-060105581.[17] Fig. 1 shows the molecular structure of CIP-SAC together with the numbering used in the text.

NMR experiments

Solid state ^{1}H MAS NMR experiments and ^{1}H–^{1}H DQ MAS experiments were performed on a Bruker Avance III spectrometer at a ^{1}H Larmor frequency of 850 MHz. One-dimensional ^{1}H MAS spectra at 60 kHz MAS rate were recorded using a double-resonance MAS probe supporting rotors of 1.3 mm outer diameter.

One dimensional ^{15}N CP-MAS NMR spectra were collected using ^{1}H–^{15}N cross-polarization magic angle spinning (CP-MAS) on a Bruker Avance II operating at 300 MHz proton Larmor frequency equipped with a 4 mm MAS probe. The ^{15}N Larmor frequency was 30.42 MHz. Ammonium chloride was used as an external reference for the ^{15}N spectra and glycine was used to adjust the Hartman–Hahn matching condition for the cross-polarization experiments. The MAS spinning rate was 10 kHz. Different numbers of transients were recorded for each compound in order to obtain an adequate signal to noise ratio. The recycling time was 15 s and the CP contact time was 6 ms. The TPPM sequence was used for heteronuclear decoupling during acquisition with a proton field H_{1H} satisfying $\omega_{1H}/2\pi = \gamma_{1H}H_{1H}/2\pi = 50$ kHz.[18]

For the dipolar-dephasing pulse sequence,[19] a dephasing time of 200 μs was used.

The ^{1}H–^{13}C REPT-HSQC[20] experiments were carried out on the Bruker Avance III spectrometer. A commercial 2.5 mm MAS double-resonance probe was used, at 25 kHz MAS spinning frequency with 90° pulses of 2.5 μs on both channels and the same field strength for the dipolar decoupling. TPPM dipolar decoupling was employed, using approximately 160° pulses and a phase-modulation angle of 30°.

Two-dimensional ^{1}H DQ MAS spectra were recorded using the back-to-back (BaBa) pulse sequence described in ref. 21 with excitation and reconversion times of one rotor period (at 59 524 Hz MAS, $\tau_R = 16.8$ μs). Phase-sensitive two-dimensional DQ experiments have been recorded with 128 rotor synchronized t_1 increments using the States-TPPI method.

Results and discussion

One dimensional ^{1}H NMR spectra

Table 1 shows the ^{1}H chemical shift for the MAS NMR solid state spectra for all the samples. Tentative assignments were done taking into account the results obtained from heteronuclear correlation experiments ^{1}H–^{13}C (see below), homonuclear correlation ^{1}H–^{1}H DQ MAS experiments (see below) and solution ^{1}H spectra.[16] Aromatic protons (H(2), H(5) and H(8)) appear unresolved in the spectra of the pure drug, as can be seen in the spectra in Fig. 2(a). Signals from methylene groups (H(1b) and H(1c)) are resolved but are observed at negative ppm. These negative chemical shift values can be attributed to strong interactions between methylene groups and neighboring aromatic moieties.[22] Previous studies proved the zwitterionic character of

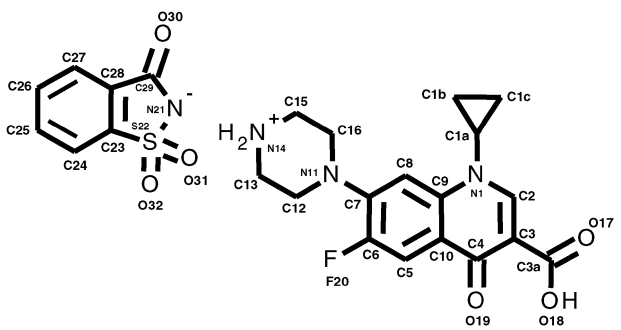

Fig. 1 Chemical structure of CIP-SAC showing the labels used in this work.

Table 1 ^{1}H chemical shifts corresponding to the MAS spectra. Some values have been extracted from the DQ-MAS spectra

^{1}H	CIP (ppm)	CIP-SAC (I) (ppm)	CIP-SAC (II) (ppm)
H(1a)	1.4	3.3	3.3
H(1c)	−2.2	−2.2, 1.1	1.1
H(1b)	−0.3	−0.2, 0.3, 2.2	1.9
Piperazine (Pip)	3.3	3.3	3.3
H(2)	6.0–7.3	6.6	6.6
H(8)	6.0–7.3	6.0	6.0
H(5)	6.0–7.3	7.2	7.2
NH	15.0	11.1	11.4
OH	—	13.1, 14.3	13.0
Saccharine (SAC)	—	7.7	7.7

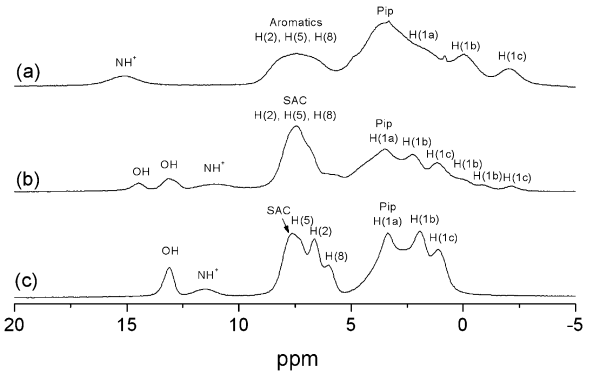

Fig. 2 ^{1}H MAS spectra at 60 kHz of (a) CIP, (b) CIP-SAC (I) and (c) CIP-SAC (II).

CIP,[23] therefore the resonance present at 15 ppm is assigned to the HN(14)$^{+}$ protons.

This signal is observed only at very high MAS rates (60 kHz.). Usually in the solution spectra this signal is not present due to the fast exchange rate of protons.

In the ^{1}H solid state spectra of CIP-SAC (I) (Fig. 2(b)) we can observe three signals in the range from 10–15 ppm, two of them correspond to the carboxyl proton, a clear evidence of the existence of at least two molecules in the asymmetric unit in this polymorph.[15]

This assignment is supported by the results of the 2D NMR experiment in CIP-SAC (I) (see below). The broader signal is assigned to the HN(14)$^{+}$ protons. As already observed in the spectrum of the pure drug, methylene protons in CIP-SAC I are detected at negative ppm values under the presence of ring currents from aromatic moieties in close spatial proximity. Multiplicity is also observed for other signals in this polymorph confirming the existence of two molecules in the asymmetric unit, see for example the protons of the cyclopropyl group.

CIP-SAC (II) presents fewer resonances than the former sample as can be seen in the ^{1}H NMR spectrum displayed in Fig. 2(c). In the range of 10–15 ppm only two signals are observed, one of them corresponding to the carboxyl proton and the other to the HN(14)$^{+}$ protons. No shifts to negative ppm values can be seen in this compound, indicating a substantial difference in the crystal structure compared to the other polymorph. Aromatic protons as well as the protons from the cyclopropyl group are well resolved. We would like to point out that ^{1}H NMR spectra at fast MAS could be an important tool for distinguishing and identifying polymorphic forms.[10]

Note that the NH(14)$^{+}$ signals in the two polymorphs are shifted towards lower frequency values relative to that in the uncomplexed drug. This is indicative of the salt formation. In contrast, because of the zwitterionic character of CIP, on formation of the salt, protons from the piperazine (Pip) group do not exhibit significant changes. This demonstrates that the carboxylic group of CIP is deprotonated and the hydrogen atom of this group is relocated close to the nitrogen in the piperazine group.

^{1}H–^{13}C heteronuclear correlation experiments

HETCOR FSLG and REPT-HSQC experiments for CIP-SAC (II) were performed to obtain short and long range

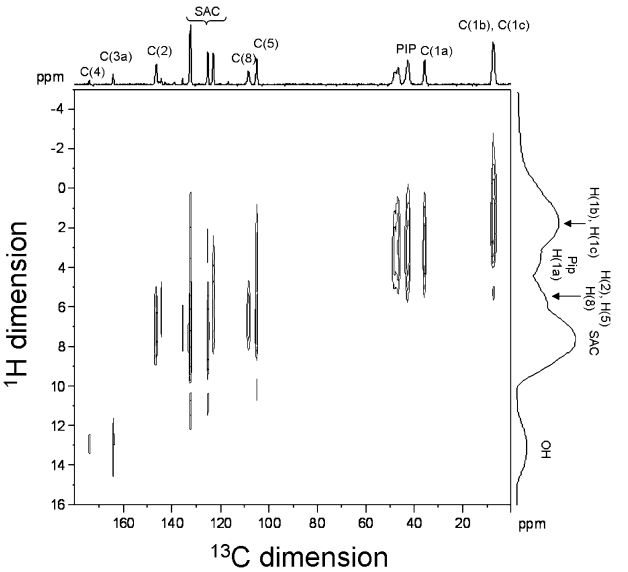

Fig. 3 ^{1}H–^{13}C HETCOR FSLG experiment for CIP-SAC (II).

heteronuclear correlations. The HETCOR FSLG experiments for CIP and CIP-SAC (I) were reported in a previous work.[15] The 2D spectra reported in ref. 15 reveal clear and well resolved correlations between carbons and their bonded protons. In the ^{1}H projection corresponding to CIP, the proton belonging to the carboxyl group is not observed which is in agreement with the zwitterionic character of this compound. On the other hand, the carboxylate proton is detected in CIP-SAC (I) and also in CIP-SAC (II) in the ^{1}H projection (see Fig. 3), confirming that the zwitterion was reformed by salt formation.

Moreover in the ^{1}H projection of the HETCOR FSLG experiment of CIP-SAC (I) two resonances can be identified corresponding to the acid proton confirming the assignments for those resonances in the 1D ^{1}H spectra of this compound.

The polar environment in the piperazine ring of the zwitterionic CIP is not significantly affected by the salt formation. This fact is supported by minor changes observed in the protons positions of the saccharinates spectra. Besides, protons in the aromatic rings H(2), H(5) and H(8) clearly show shifts in both polymorphs suggesting that new interactions are occurring in the saccharinates. A similar behavior was observed by other authors and attributed to changes in hydrogen bonds or π–π interactions.[24]

In a REPT-HSQC experiment the dipolar recoupling periods are responsible for the polarization transfer from ^{1}H to ^{13}C. The heteronuclear correlations explored here are based on dipole–dipole couplings between these two types of nuclei. This experiment was performed following the pulse sequence presented in ref. 20. Dipolar recoupling times of one and two rotor periods were used to obtain short and longer distance correlations respectively (see Fig. 4). Fig. 4(a) shows correlations between carbons and chemically bonded protons. This allows us to determine the range of ^{1}H chemical shifts for different groups in the molecule. Saccharine protons for example are in the range of 7.6 ppm–8.0 ppm. Aromatic protons can be identified between 5.6 ppm and 7.0 ppm. Additionally aromatic protons are resolved in the ^{1}H dimension with an uncertainty of ±0.5 ppm.

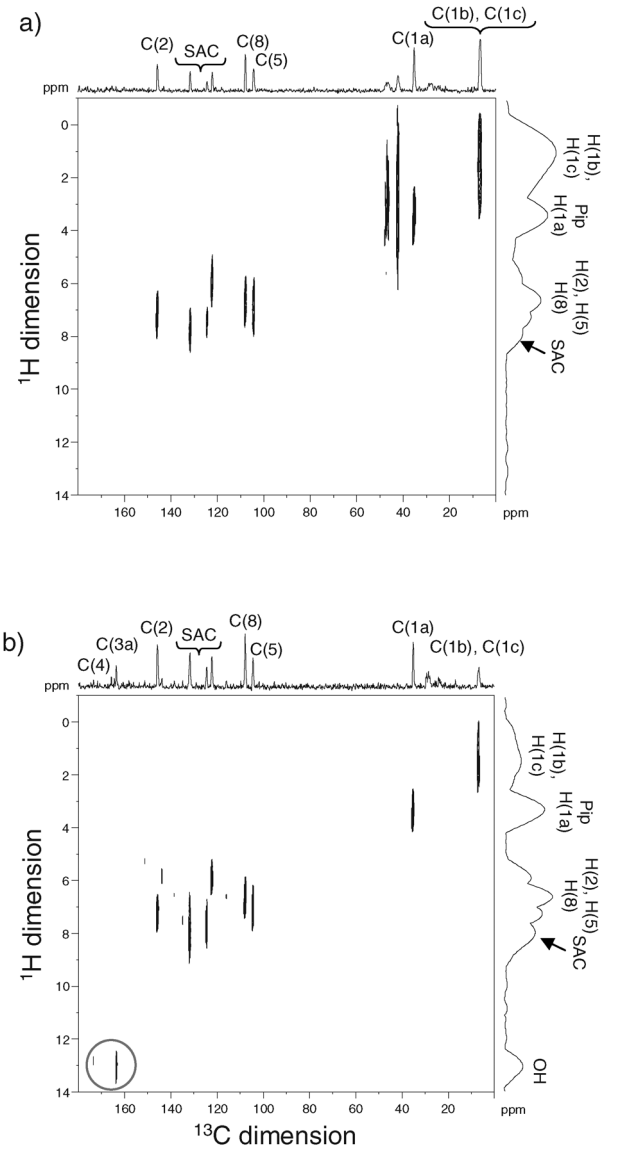

Fig. 4 ^{1}H–^{13}C REPT-HSQC experiment for CIP-SAC (II). (a) One rotor recoupling period. (b) Two rotor recoupling periods.

Some additional important interactions are clearly seen when using a longer recoupling time of two rotor periods. Note, firstly, that the OH proton is now observed in the ^{1}H dimension in Fig. 4(b) and, secondly, that the correlation between C(4) and C(3a) shows that there is a hydrogen bond between O(18) and O(19), consistent with XRD data.[16]

^{1}H–^{1}H DQMAS experiments

Fig. 5(a) shows the ^{1}H DQ MAS spectrum of CIP-SAC (II) and Table 2 displays the corresponding correlations. In the DQ dimension, correlations are located at the sum of chemical shift frequencies of the two nuclei involved. Peaks on the diagonal are due to dipolar couplings between protons with similar chemical shifts, whereas other coherences appear symmetric to this diagonal[25] Since an X-ray single-crystal structure is available for CIP-SAC (II), this study offers the opportunity to verify ^{1}H estimation from X-ray diffraction data and in this particular case to obtain further information

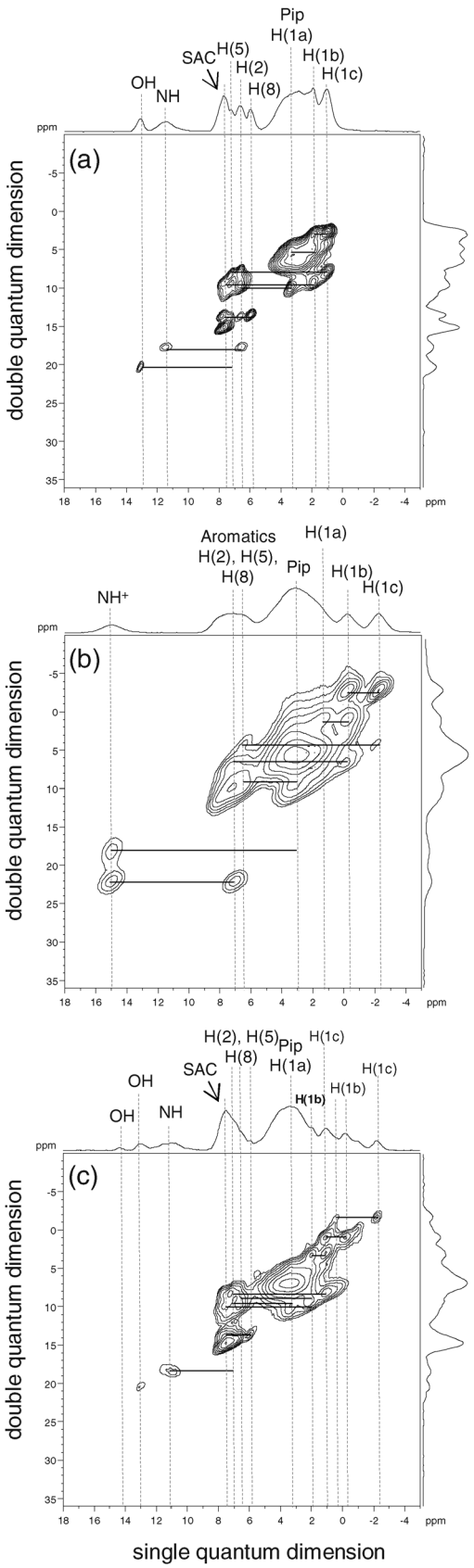

Fig. 5 ^{1}H–^{1}H DQ MAS NMR spectrum of: (a) CIP-SAC (II), (b) CIP and (c) CIP-SAC (I).

Table 2 DQMAS correlations for CIP-SAC (II)

Correlation	CIP-SAC (II) $\omega_A + \omega_B$ (ppm)
H(1b)–H(1c)	3.0
H(1b)–H(1a)	5.2
H(1c)–H(2)	7.7
H(1a)–H(2)	9.9
H(1b)–SAC	9.6
H(5)–H(2)	13.8
SAC–H(8)	13.7
NH–H(2)	18.0
OH–H(5)	20.2
Pip–Pip	6.6
SAC–SAC	15.4

about the polymorph (I) where no diffraction data are available. As a rule of thumb, valid for typical organic solids, the presence of a correlation in a ^{1}H DQ solid state NMR spectrum recorded with very short DQ excitaction times indicates a spatial ^{1}H–^{1}H separation of less than 3 Å. Thus, correlations in these experiments can also give an insight about the presence of ionic interactions and $\pi\cdots\pi$ interactions.

The ^{1}H–^{1}H DQ MAS correlation spectrum of CIP-SAC (II) shows strong autocorrelation peaks at ω_{DQ} = 3.3 + 3.3 = 6.6 ppm and ω_{DQ} = 7.7 + 7.7 = 15.4 ppm, corresponding to Pip–Pip and saccharine–saccharine correlations respectively. The assignment of the latter correlation signal is based on hetero nuclear correlation spectra, which showed that the signal of aromatic protons is observed in the chemical shift range of 6.0 ppm–7.2 ppm. Three distinct signals of aromatic protons at 6.0, 6.6 and 7.2 ppm are resolved. In the DQ spectrum of CIP-SAC (II) a correlation between an aromatic proton at 7.2 ppm and the proton located at the carboxyl group at 13.0 ppm is found at ω_{DQ} = 13.0 + 7.2 = 20.2 ppm. Based on the known crystal structure of CIP-SAC (II) the aromatic proton at 7.2 ppm involved in this correlation can be assigned to the proton site H(5). This gives evidence of a hydrogen bond between O(18)–H(18)$\cdots$(19) leading to the formation of an extra six membered cycle involving O(19), C(4), C(3), C(3a), O(18)–H(18) in the structure, confirming the single crystal X-ray data.[16]

As observed by X-ray single crystal experiments, CIP-SAC (II) forms a columnar structure, in which the columns approach each other with interleaved rings pertaining to the CIP unit, in a typical $\pi\cdots\pi$ interaction.[23] The close spatial proximity of aromatic proton sites from neighboring molecules can be directly observed in the 1H–1H DQ correlation spectrum of CIP-SAC (II). The DQ signal observed at ω_{DQ} = 13.8 ppm = 7.2 + 6.6 ppm results from a DQ coherence between the aromatic proton sites H(5) and H(2). The intramolecular distance, however, is by far too large to excite a DQ coherence between these two sites, providing direct evidence for a molecular packing with close proximities between aromatic moieties of neighboring molecules. This kind of interaction is a key element of the complex molecular organization in CIP-SAC (II).

Another intermolecular correlation in this compound is observed at ω_{DQ} = 9.6 ppm between SAC protons and protons of the cyclopropyl group. The latter are in close spatial proximity (H(1a), H(1b) and H(1c)) to each other, with distances of aproximately 2.5 Å between them, leading to strong correlation signals at ω_{DQ} = 3.0 (H(1b)–H(1c)) ppm and ω_{DQ} = 5.2 ppm (H(1a)–H(1b)). Correlation signals at ω_{DQ} = 7.7 ppm (H(1c)–H(2)) and ω_{DQ} = 9.9 ppm (H(1a)–H(2)), however, indicate the close spatial proximity of cyclopropyl and aromatic protons, which may result from the inclination of the cyclopropyl group in the crystal structure with respect to the molecular plane defined by the aromatic rings.[16]

Table 3 DQMAS correlations for ciprofloxacin

Correlation	CIP $\omega_A + \omega_B$ (ppm)
H(1b)–H(1c)	−2.5
H(1c)–aromatic	4.1
H(1b)–H(1a)	1.1
H(1b)–aromatic	6.3
Pip–aromatic	9.0
NH–Pip	18.2
NH–aromatic	22.0
Pip–Pip	6.6

Fig. 5(b) shows the ^{1}H–^{1}H DQ NMR correlation spectrum of CIP. A strong autocorrelation signal is present at ω_{DQ} = 3.3 + 3.3 = 6.6 ppm between piperazine protons, allowing the assignment of these proton sites. The cyclopropyl proton sites are well resolved and show correlation signals with protons of the aromatic groups (see correlations at ω_{DQ} = 6.3 ppm (H(1b)–H(Aromatic)) and ω_{DQ} = 4.1 ppm (H(1c)–H(Aromatic)). The different acidity of the three different nitrogen sites (N(1), N(11) and N(14)) in CIP leads to the protonation of N(14) and N(1) which is confirmed by the correlation signals between N(14)H and the Pip group (ω_{DQ} = 18.2 ppm) and the N(1)H–Aromatics correlation signal at ω_{DQ} = 22.0 ppm may occur in CIP, leading to a N(1) bonded to a hydrogen (Table 3).

Fig. 5(c) shows the ^{1}H–^{1}H DQ MAS spectrum of CIP-SAC (I). CIP-SAC (I) shows an even more complex DQ correlation spectrum. Multiplicity of sites is easiest seen at the high frequency signals in the range from 10–16 ppm. In spite of the complexity, the DQ correlation pattern of CIP-SAC (I) yields features of both, the CIP-SAC (II) DQ correlation spectrum as well as the CIP DQ correlation spectrum (see Table 4). Correlation signals between cyclopropyl protons and the aromatic ^{1}H sites are found at ω_{DQ} = 8.3 ppm, ω_{DQ} = 8.8 ppm and ω_{DQ} = 9.3 ppm. And the intermolecular correlation at ω_{DQ} = 9.9 ppm between SAC and cyclopropyl ^{1}H sites indicates a close spatial proximity between those groups in CIP-SAC (I).

Note that in both cases CIP-SAC (II) and CIP-SAC (I) the absence of the NH–Pip correlation may be attributed to the salt formation which is based on proton transference from the SAC amide position to the CIP.

^{15}N NMR spectra

Natural abundance ^{15}N CP-MAS spectra recorded for all the samples are displayed in Fig. 6. Ciprofloxacin and its complexes contain three and four different nitrogen sites respectively. Some of those nitrogen sites are directly involved in the ionic interaction present between saccharine and ciprofloxacin molecules. Moreover, the number of ^{15}N MAS NMR signals provides direct evidence for the presence of more than one

Table 4 DQMAS correlations for CIP-SAC (I)

Correlation	CIP-SAC (I) $\omega_A + \omega_B$ (ppm)
H(1b)–H(1c)	−1.9, 0.9, 3.3
H(1c)–aromatics	8.3
H(1b)–aromatics	8.8
H(1a)–aromatics	9.3
H(1b)–SAC	9.9
SAC–aromatics	13.5
NH–aromatic	18.2
Pip–Pip	6.6
SAC–SAC	15.4

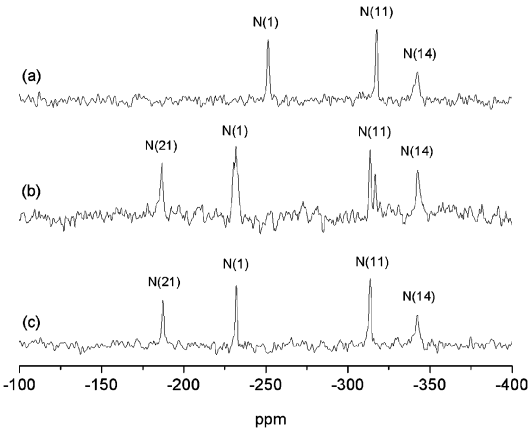

Fig. 6 CPMAS ^{15}N NMR spectra of (a) CIP, (b) CIP-SAC (I) and (c) CIP-SAC (II).

molecule per unit cell, as stated in previous sections and supported by other 1D and 2D NMR experiments. This kind of evidence demonstrates the importance of ^{15}N MAS NMR studies of this kind of samples.

In the ^{15}N CP-MAS spectrum of CIP, three nitrogen resonances are observed (see Fig. 6(a)). The ^{15}N spectral assignment for Ciprofloxacin in solution was reported in ref. 26. Based on chemical shifts of CIP in solution, the spectral assignments for CIP and its saccharine co-crystals CIP-SAC (I) (Fig. 6(b)) and CIP-SAC (II) (Fig. 6(c)) in the solid state were derived with the help of additional spectral editing experiments. The chemical shifts for all the samples are displayed in Table 5.

Note that the chemical shift assigned to the site N(14) is basically unchanged going from the pure drug to the saccharinates co-crystals. This fact supports the zwitterionic character of CIP as already pointed out in the discussion of 1D ^{1}H NMR spectra. The ^{15}N signal at −317.7 ppm in CIP assigned to N(11) shows a minor change in chemical shift in the polymorphs, and a splitting in the case of CIP-SAC (I) (−313.7 ppm in CIP-SAC (II) and −313.7 ppm, −316.9 ppm in CIP-SAC (I)).

The NMR signal assigned to N(1), which is observed at −251.5 ppm in the pure drug, is significantly shifted in the polymorphs by ∼20 ppm towards higher frequency values. In the ^{15}N NMR spectrum of CIP-SAC (II) the N(1) signal is located at −232.0 ppm, while it is split in the case of CIP-SAC (I) (−230.4 ppm and −232.0 ppm). The splitting of the signal in CIP-SAC (I) is a direct indication for more than one molecule per unit cell. The changes observed in the signal assigned to N(1) suggest that this region of the molecule is strongly affected by salt formation. All nitrogen resonances belonging to CIP-SAC (II) can be seen in the CIP-SAC (I) spectrum (see Table 5).

Table 5 ^{15}N chemical shifts from CP/MAS spectra of CIP, CIP-SAC (I) and (II)

^{15}N	CIP (ppm)	CIP-SAC (I) (ppm)	CIP-SAC (II) (ppm)
N(1)	−251.5	−232.0, −230.4	−232.0
N(11)	−317.7	−316.9, −313.7	−313.7
N(14)	−342.3	−342.3	−342.3
N(21)	np	−186.8	−187.2

np, not present.

Conclusions

In this paper we have applied a variety of NMR experiments to characterize the local packing in two polymorphic forms of a new pharmaceutical compound, ciprofloxacin-saccharinate.

In particular, ^{1}H MAS and ^{15}N CP-MAS NMR spectra proved to be suitable tools to identify the two polymorphs and to detect the number of molecules in the unit cell of CIP-SAC (I).

In the ^{1}H MAS spectra of the saccharinate co-crystals the signal of the NH proton shifts towards lower frequency, indicating the presence of ionic bonds between CIP and saccharine molecules. This is further supported by the absence of a correlation signal between NH and piperazine proton spins in the DQ spectra. Moreover, the piperazine protons and the piperazine nitrogen N(14) are not affected by the ionic bond due to the zwitterionic character of CIP, *i.e.* the carboxylic group of CIP is deprotonated and the hydrogen atom of this group is located close to the nitrogen in the piperazine group. The zwitterionic character is reverted by salt formation originated from proton transference from SAC amide to CIP.

The predictions from XRD data for CIP-SAC (II) are in good agreement with the results of the ^{1}H–^{1}H DQ correlation spectrum and the REPT-HSQC experiments. Moreover, solid state NMR experiments provided experimental evidence for intramolecular hydrogen bonds, intermolecular proximities, and π–π interactions . The hydrogen bond between O(18) and O(19) was verified by the REPT-HSQC experiments. Protons of the cyclopropyl group are observed at negative ppm in the ^{1}H NMR spectra of CIP and CIP-SAC (I). However this is not the case in CIP-SAC (II), indicating changes in the π···π interactions due to different local packing arrangements in the different compounds.

Comparing the three compounds it is seen, that the local packing of CIP and CIP-SAC (II) is clearly distinguished in the 2D DQ correlations spectra, whereas CIP-SAC (I) exhibits features from both packing arrangements, as well as new correlations. The shift of proton signals assigned to the cyclopropyl group towards lower frequency is also indicative for substantial changes in the local packing in this region of the molecule.

To conclude, CIP-SAC (I) and CIP-SAC (II) exhibit similarities in the molecular conformation. The unknown crystal

packing of CIP-SAC (I) presents a molecular site with a packing similar to CIP-SAC (II) and a site with an arrangement closer to that in the pure drug.

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