Insights into Novel Supramolecular Complexes of Two Solid Forms of Norfloxacin and β-Cyclodextrin

ANA K. CHATTAH,[1] KAMAL H. MROUE,[2] LAURA Y. PFUND,[3] AYYALUSAMY RAMAMOORTHY,[2] MARCELA R. LONGHI,[4] CLAUDIA GARNERO[4]

[1]Facultad de Matemática, Astronomía y Física and IFEG (CONICET), Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, X5000HUA, Argentina

[2]Biophysics and Department of Chemistry, The University of Michigan, Ann Arbor, Michigan, 48109–1055

[3]Department of Chemistry and the Macromolecular Science and Engineering Program, The University of Michigan, Ann Arbor, Michigan, 48109–1055

[4]Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA) CONICET-UNC and Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, X5000HUA, Argentina

Received 31 May 2013; revised 27 June 2013; accepted 10 July 2013

Published online 31 July 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23683

ABSTRACT: The solid-state properties of novel complexes of β-cyclodextrin and two different solid forms of norfloxacin were investigated at the molecular level, in an attempt to obtain promising candidates for the preparation of alternative matrices used in pharmaceutical oral formulations. In order to evaluate the physical properties inherited from the different polymorphs, these supramolecular systems were characterized using a variety of spectroscopic techniques including natural-abundance ^{13}C cross-polarization magic-angle-spinning (CP-MAS) nuclear magnetic resonance (NMR), powder X-ray diffraction, and Fourier transform infrared spectroscopy. The intrinsic proton spin-lattice relaxation times detected in ^{13}C CP-MAS NMR spectra are used to confirm and distinguish the complex formation, as well as to provide better insights into the molecular fragments that are involved in the interaction with β-cyclodextrin. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3717–3724, 2013

Keywords: norfloxacin; polymorphism; cyclodextrins; complexation; characterization; FTIR; solid state NMR; X-ray diffractometry

INTRODUCTION

Multicomponent pharmaceutical solids, in crystalline or amorphous forms, are usually developed to improve the pharmaceutical profile of a single organic molecule in terms of solubility, physicochemical stability, bioavailability, and organoleptic properties.[1,2] A better understanding of the solid-state interactions in these solids can allow for the rational design of active pharmaceutical ingredients (API). Additionally, polymorphism, the ability of a molecule to exist in several different conformations, affects the chemical and physical properties of an API including the solubility, bioavailability, and stability,[3,4] which have a significant impact on pharmaceutical industry.

Norfloxacin (NOR; Figure 1) is a synthetic broad antibacterial fluoroquinolone that has been widely used in the treatment of various infectious diseases, such as gonorrhea, prostate, and urinary tract infections.[5] NOR is very slightly soluble in water, which is associated with the poor biological availability, as only 35%–45% of the orally administered drug is absorbed.[6–8] NOR exists in several solid forms: three anhydrous polymorphs (forms A, B, and C), an amorphous form, a methanol solvate, several hydrate forms, and also salts and cocrystals.[9–11]

An approach to increase the water solubility, stability, and bioavailability of drugs is the formation of complexes with modified starches as cyclodextrins (CDs), calix[n]arenes, and maltodextrins.[12–16] Particularly, solid drug−CD inclusion complexes present challenges for structural analysis. The solid powders can be relatively complex, and multiple amorphous and crystalline phases can be present, including a combination of drug phases.[17,18] In previous reports, CDs have been used to increase the solubility, the dissolution rate, and/or the bioavailability of NOR.[19–21] However, these studies have not considered the different solid forms of this API.

Several solid-state techniques are commonly used to characterize pharmaceutical multicomponent systems and to identify polymorphism.[22] To obtain a complete physical characterization of a product, optical microscopy, thermal analysis, and solid-state spectroscopic techniques are implemented, in particular powder X-ray diffraction (PXRD) and solid-state nuclear magnetic resonance (ssNMR) spectroscopy.[23] Among these techniques, ssNMR, specifically ^{13}C cross-polarization magic-angle spinning (CP-MAS), has proven to be a powerful analytical technique in which heterogeneous samples can be investigated, and amorphous content provides no significant barrier to such studies.[24,25] However, less efforts have been made to measure ^{1}H spin-lattice relaxation times through ^{13}C spectra, which can be very useful in studying the molecular dynamics in complex compounds, and to separate different contributions in mixtures and multicomponents products, using pseudo-2D measurements or in the inverse Laplace transform version.[17,26–28]

Having in mind the improvement on the solubility and dissolution behavior of NOR and to select a proper polymorph for its pharmaceutical use, the aim of the present work was to synthesize and characterize novel supramolecular systems

Correspondence to: Ana K. Chattah (Telephone: +54-351-4334051x369; Fax: +54-351-4334054; E-mail: chattah@famaf.unc.edu.ar)

This article contains supplementary material available from the authors upon request or via the Internet at http://onlinelibrary.wiley.com/.

Journal of Pharmaceutical Sciences, Vol. 102, 3717–3724 (2013)

of various solid forms of NOR with β-cyclodextrin (βCD). The polymorphs of NOR, their physical mixtures with βCD, and the corresponding NOR–βCD complexes were investigated using a variety of spectroscopic techniques: ssNMR, PXRD, and Fourier transform infrared (FT-IR) spectroscopy. Additionally, the measurement of the proton ^{1}H spin-lattice relaxation times (^{1}H T_1) using ^{1}H spin-inversion recovery experiments detected in ^{13}C ramped-amplitude (Ramp)-CP-MAS spectra has enabled us to gain better insights into the complex formation, as well as into the interactions among their individual components at the molecular level.

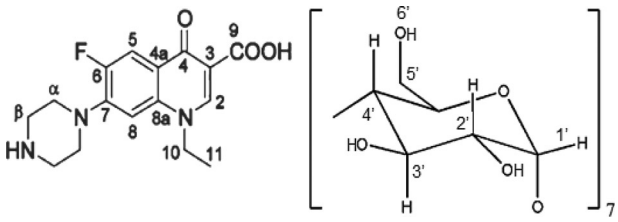

Figure 1. NOR and βCD molecular structures with the carbon numbering used in NMR spectra.

EXPERIMENTAL SECTION

Chemicals and Reagents

Norfloxacin was provided by Parafarm (Buenos Aires, Argentina) and βCD (MW = 1135) was kindly supplied by Ferromet agent (Buenos Aires, Argentina) of Roquette (France). All other chemicals were of analytical grade.

Obtaining the Polymorphic Forms of NOR

The two solid forms of NOR were prepared as reported by Barbas and coworkers.[9–11] NOR C was produced by suspending NOR (marketed as form A) in acetone, heated to reflux, and then allowed to cool slowly to room temperature. In an attempt to produce NOR B, NOR was dissolved in refluxing isopropanol and then cooled slowly to room temperature. However, the product obtained trying to reproduce the experimental conditions was a different solid form; thus, herein we denote this form as NOR B_I.

Solid Samples Preparation

Solid-state systems of NOR B_I or C, in equimolar ratio with the βCD were prepared as follows:

Kneading Method.

The systems NOR B_I:βCD (KN [kneading method] B_I) and NOR C:βCD (KN C) were prepared by accurately weighing appropriate amounts of βCD and then transferring them to a mortar. An acetone–water (50:50, v/v) mixture was added to the βCD powder and the resultant slurry was kneaded for about 10 min. For each system, the corresponding solid form of NOR was added in small portions with the simultaneous addition of solvent in order to maintain a suitable consistency. This slurry was kneaded thoroughly for about 45 min, and the resultant paste was dried in vacuum at 40°C for 48 h, and protected from light.

Physical Mixture.

Physical binary mixtures of NOR B_I:βCD (PM B_I) and NOR C:βCD (PM C) were prepared by simply blending uniformly the corresponding components with a mortar and pestle.

ssNMR Spectroscopy

All ssNMR experiments were performed at 11.75 T on a Agilent/Varian VNMRJ 500 MHz NMR spectrometer operating at a resonance frequency of 125.6 MHz for ^{13}C and 499.44 MHz for ^{1}H, and using a 3.2-mm triple-resonance MAS probe. High-resolution solid-state ^{13}C MAS NMR spectra of NOR B_I and NOR C, as well as the systems KN B_I, KN C, PM B_I, and PM C, were acquired under 10 kHz MAS conditions at ambient temperature using a ramped-amplitude Ramp-CP pulse sequence during an acquisition period of 10 ms with a 2 ms cross-polarization contact time. A 68-kHz SPINAL64[29] pulse scheme was applied to decouple protons during signal acquisition. All spectra were recorded with 2000 transients and a recycle delay of 5 s. Carbon-13 NMR chemical shifts were referenced with respect to Tetramethylsilane at 0 ppm using adamantane as a secondary external standard.

The ^{1}H T_1 were calculated from a series of data collected by ^{1}H spin-inversion recovery experiments detected in ^{13}C Ramp-CP-MAS, in which a ^{1}H π-pulses followed by an inversion recovery delay τ were introduced prior to the Ramp-CP pulse sequence and SPINAL64 decoupling scheme. A 0.2 ms of CP contact time was selected to avoid spin diffusion between protons in order to obtain information of the nearest carbons. Thirteen data points with increasing inversion recovery delays (between 0 and 5 s) were recorded for each sample, with a 5-s recycle delay and 500 transients collected under 10 kHz MAS condition.

Powder X-Ray Diffraction

Powder X-ray diffraction patterns were obtained at ambient temperature using a Bruker D8 Advance diffractometer (Bruker Corporation/ Bruker AXS, Inc., Madison, WI) operating at 40 kV and 40 mA with Cu-Kα radiation (1.5406 Å). All samples were packed into the depression of an indented quartz slide. The powder pattern was collected by scanning 2θ from 3° to 70° with a step size of 0.04° at a rate of 1.5 s/step.

Fourier Transform Infrared

The FT-IR spectra were recorded on a Nicolet 5 SXC FT-IR Spectrophotometer (Madison, Wisconsin). The potassium bromide disks were prepared by compressing the powder.

RESULTS AND DISCUSSION

^{13}C CP-MAS NMR Spectroscopy

The carbon numbering of NOR and βCD molecules is shown in Figure 1. Figure 2 displays the ^{13}C CP-MAS spectra of βCD, NOR B_I, NOR C, the corresponding physical mixtures (PM B_I, PM C), and complexes (KN B_I, KN C). As shown in their individual spectra, both NOR and βCD exhibit different and separate ranges of ^{13}C chemical shifts. The ^{13}C NMR resonances of NOR lie in the 100 –180 ppm region and below 60 ppm, whereas those of βCD lie between 50 and 110 ppm. βCD presents sharp resonances typical of a crystalline system.[16]

The ^{13}C CP-MAS NMR spectrum of NOR C matches well with that previously reported[11] ; however, the ^{13}C CP-MAS spectrum for NOR B_I differs from the reported spectrum for NOR B.[9,10] A number of NOR B_I resonances appear at the same shift positions reported for the form B but with double number

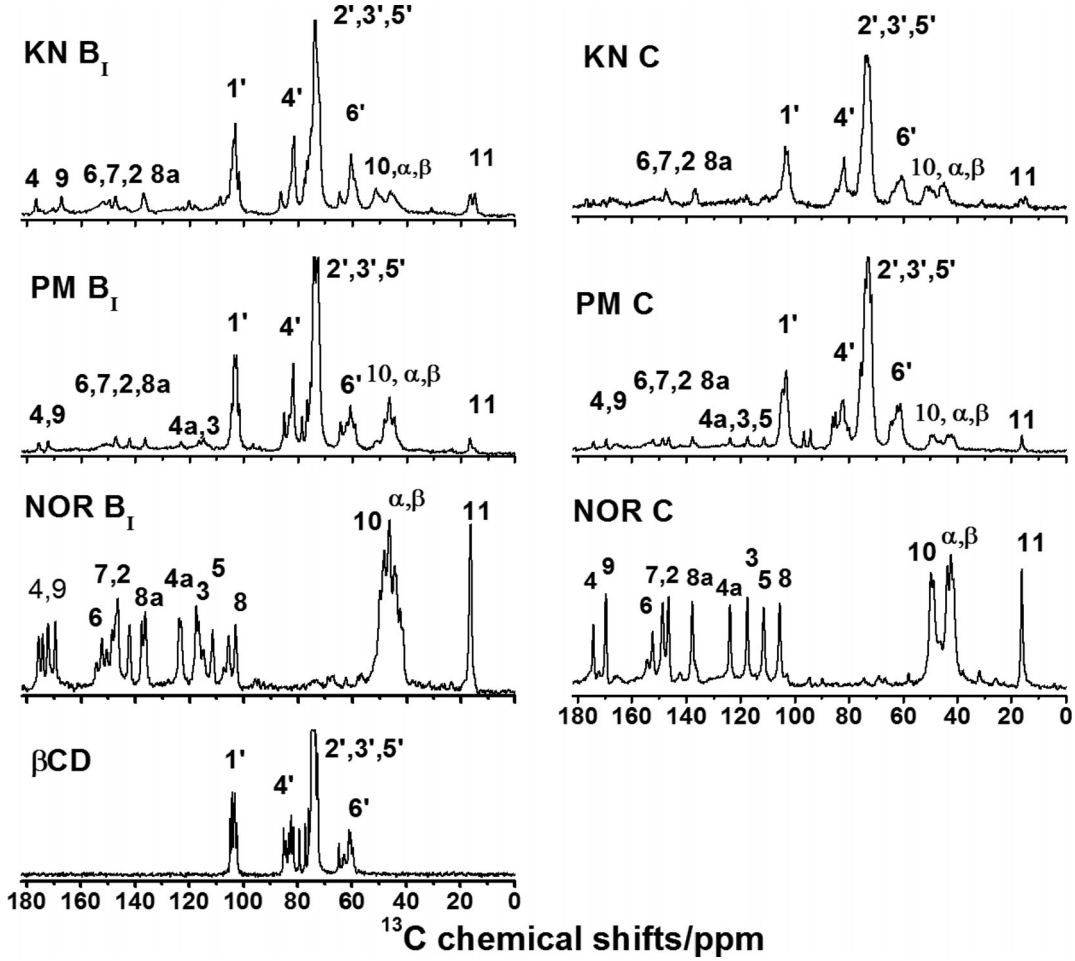

Figure 2. ^{13}C cross-polarization magic-angle-spinning spectra of βCD, NOR B_I and C, PM B_I and C, and the complexes KN B_I and C. Numbering for assignments corresponds to Figure 1. Only signals that are visible have been assigned in the PMs and KNs spectra.

of peaks; the most noticeable is the four peaks corresponding to carbons 4 and 9. Moreover, the spectrum clearly shows that compound NOR B_I is not a mixture of NOR B and C, as the chemical shifts of forms B and C are quite similar. Thus, this result demonstrates that NOR B_I represents a new solid form different than form B, albeit crystallized from the same solvent. Comparing the KN B_I with PM B_I spectra, it is possible to see several differences in all the signals, indicating that complex KN B_I is a completely new solid form. For example, signals corresponding to carbons 1′-6′ of βCD have merged into single and broad peak in the range of 55 –110 ppm; signals corresponding to NOR B_I have shifted in position and are broadened as well. In particular, the 40–50-ppm region corresponding to the piperazine ring (α,β) that in PM B_I maintains the NOR B_I shape but has a completely different feature in the complex. The changes in the signals between 165 and 180 ppm and the signals at 18 ppm (carbon 11), which turned into a doublet, are also remarkable. By comparing the KN C with PM C spectra, differences in the signals belonging to βCD region (between 87 and 57 ppm) were observed, which became broader and the sharp peaks disappeared, for example, for carbons 1′ and 4′. There are also shifts to higher ppm for the group of signals between 55 and 35 ppm. The signal corresponding to carbon 11 (at 16 ppm) appears as a doublet like in KN B_I. KN B_I and KN C can be easily distinguished by observing the signals at 85 and 64 ppm in the KN B_I spectrum that are not present in that of KN C. Note that there are no residues of the precursors in the complexes KNs, as every part of the spectra has changed.

In the PMs, most of the signals appear at the same chemical shift positions as those in the pure compounds, although each spectrum are not the exact addition of the corresponding polymorph of NOR and βCD spectra. For example, the group of signals in the region between 40 and 50 ppm (carbons 10, α, β) has the same feature than the pure NOR polymorph, and the resonances appearing in the region corresponding to βCD are broadened with some remaining sharp peaks. These facts indicate that both compounds (NOR and βCD) are not strongly interacting in the physical mixture. PM B_I and PM C are different compounds, as confirmed by the signals belonging to carbons 10 and α, β of NOR and 4′ and 6′ of βCD.

Proton T_1 Relaxation Analysis

To obtain additional information on the interaction between the pure components in the complexes, ^{1}H T_1 were measured through the carbon spectra. Spin-lattice relaxation times of the different protons in a sample are averaged to a single value by the spin diffusion process. This average is usually complete when protons belong to the same phase domain, whereas relaxation times of different domains in heterogeneous samples

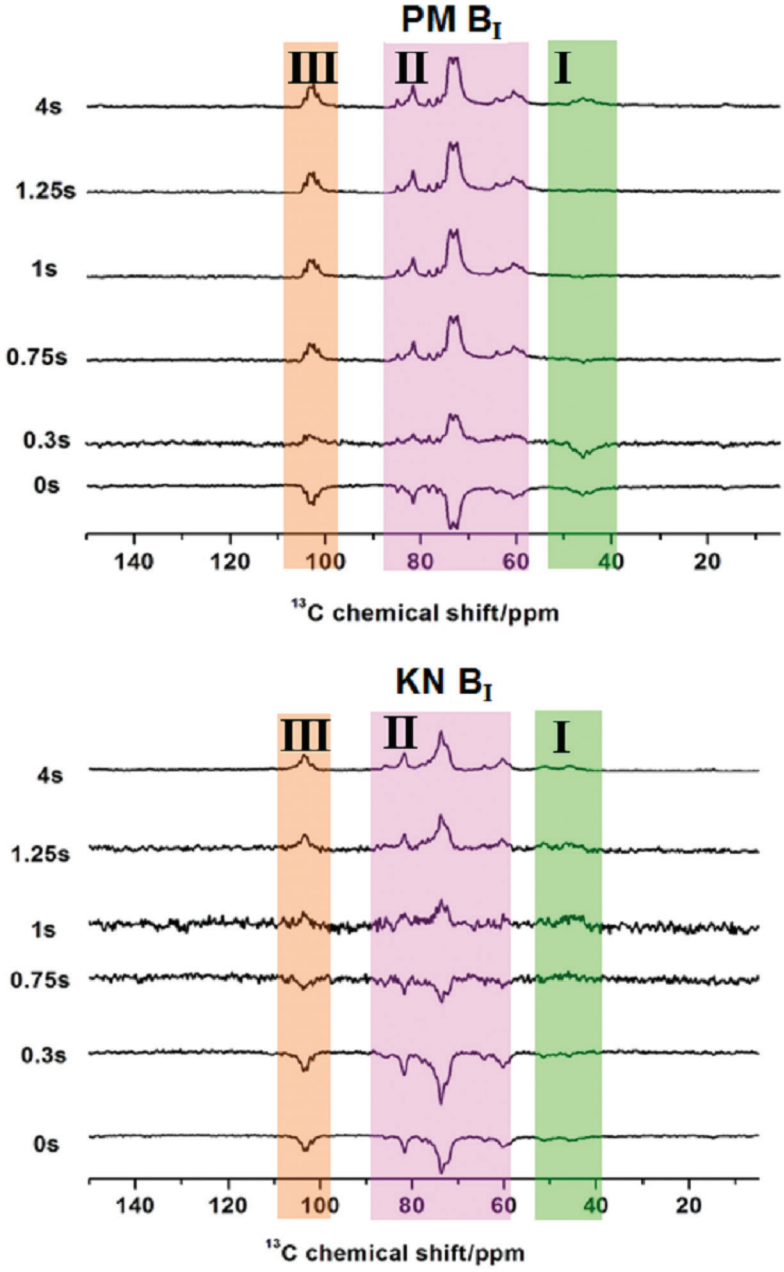

Figure 3. ^{13}C cross-polarization magic-angle-spinning spectra of KN B_I and PM B_I with 200 μs of contact time at different recovery time delays showing the integration regions (I, II, and III) used to calculate spin-lattice relaxation times (T_1) of protons.

can be averaged or not, depending on the dimensions of the different domains and connections between spins.[26]

To determine ^{1}H T_1, signals belonging to different carbon regions in each ^{13}C CP-MAS spectrum were integrated to determine the magnetization as a function of the delay time. Then, the behavior of ^{1}H magnetization as a function of the recovery time was fitted by using one relaxation time in all the cases. It is important to note that with 200 μs of contact time only some signals are visible in the carbon spectra. This short contact time is used to avoid proton spin diffusion during cross-polarization. Thus, the contribution and behavior of protons in very close proximity to specific carbons were obtained.

Figure 3 shows the three different regions that were considered for integration in all the compounds: region I (55 –40 ppm), which is characteristic of carbon 10 and (α, β) of NOR, region II (around 87 –56 ppm), containing signals of carbons (4′, 2′, 3′, 5′, 6′) characteristics to βCD, and region III (around 100 – 110 ppm), where NOR and βCD have signals (8 and 1′). The values of T_1 calculated for these regions are given in Table 1.

In both PMs, the two corresponding components maintain relaxation time values quite similar to those measured for their pure samples: 1.82 s for NOR B_I, 1.91 s for NOR C, and 0.46 s for βCD. Remarkably, all the KN B_I regions present different values in comparison with PM B_I, confirming that KN B_I is a new solid form, homogeneous and different from its precursors (see Table 1). Indeed, Figure 3 shows that regions I and II belonging to PM B_I present null signals at 1.25 and 0.2 s, respectively, whereas in KN B, all the regions recover their positive signals

 DOI 10.1002/jps.23683

Table 1. Values of Spin-Lattice Relaxation Times (T_1) of Protons Corresponding to Each Region of the Carbon Spectra (I, II, or III) in All the Compounds

	^{1}H-T_1 (s)		
	Region I (55–36 ppm)	Region II (87–56 ppm)	Region III (110–100 ppm)
NOR B_I	1.82	–	1.64
PM B_I	1.85	0.46	0.60
KN B_I	**1.55**	**1.45**	**1.38**
NOR C	1.91	–	1.71
PM C	1.80	0.46	0.64
KN C	**1.0**	**0.50**	**0.62**

Errors are within 5%.

at around 0.8 s. Regions inside KN B_I have slightly different values and the longer one corresponds to region I, which corresponds to the piperazine ring of NOR.

In the case of KN C, not all the regions have homogenous T_1 values. The most distinguishable is region I, having a value of 1.80 s in PM C that becomes 1.0 s in KN C (see Table 1). Instead, regions II and III have similar values to that of the physical mixture. Although spectroscopically we can see that PM C and KN C are different, from the point of view of the relaxation behavior there is evidence that KN C has a solid form closer to the physical mixture. The absence of pure drug is also evident from the relaxation time measurements. Again, as occurs in the case of KN B_I, region I has the largest value of T_1.

This different relaxation time values corresponding to region I in both complexes may be attributed to an incomplete spin diffusion averaging, indicating that this value belongs to a different domain that may be involved in an inclusion process. This behavior is in agreement with the results reported by Torres-Labandeira et al.,[20] who reported ^{1}H-NMR studies suggesting that the piperazine group of NOR is bound inside the βCD cavity.

Powder X-Ray Diffraction

The powder X-ray diffractograms for NOR B_I and C and their corresponding PMs and KNs are shown in Figure 4.

The PXRD pattern for NOR C shows excellent agreement with that previously reported in the literature.[11] However, the PXRD pattern for NOR B_I displays reflections distinctly different from those reported previously for NOR B.[9,10] NOR C and NOR B_I are clearly distinguishable by the reflections present in NOR C ($2\theta = 9.2°$, 16.1°, 16.6°, 17.1°, and 18.8°), but absent in NOR B_I. Hence, the solid form NOR B_I obtained is not a mixture of forms C and B, as supported by ^{13}C CP-MAS. Additionally, the PXRD data of NOR B_I show reflections at $2\theta = 10.7°$, 11.4°, 12.6°, and 13.4°, which are in agreement with previous hydrates reported in for NOR A.[30] Then, in order to complete the characterization of this solid, we performed a thermogravimetric analysis (TGA) experiment (Figure 1S in Supporting Information). Our TGA results together with PXRD reveal that the new solid form obtained corresponds to a hydrate of the polymorph B, which is reported for the first time herein. Therefore, the ^{13}C NMR, PXRD, and TGA data suggest that it is difficult to reproduce the experimental conditions for obtaining NOR B.

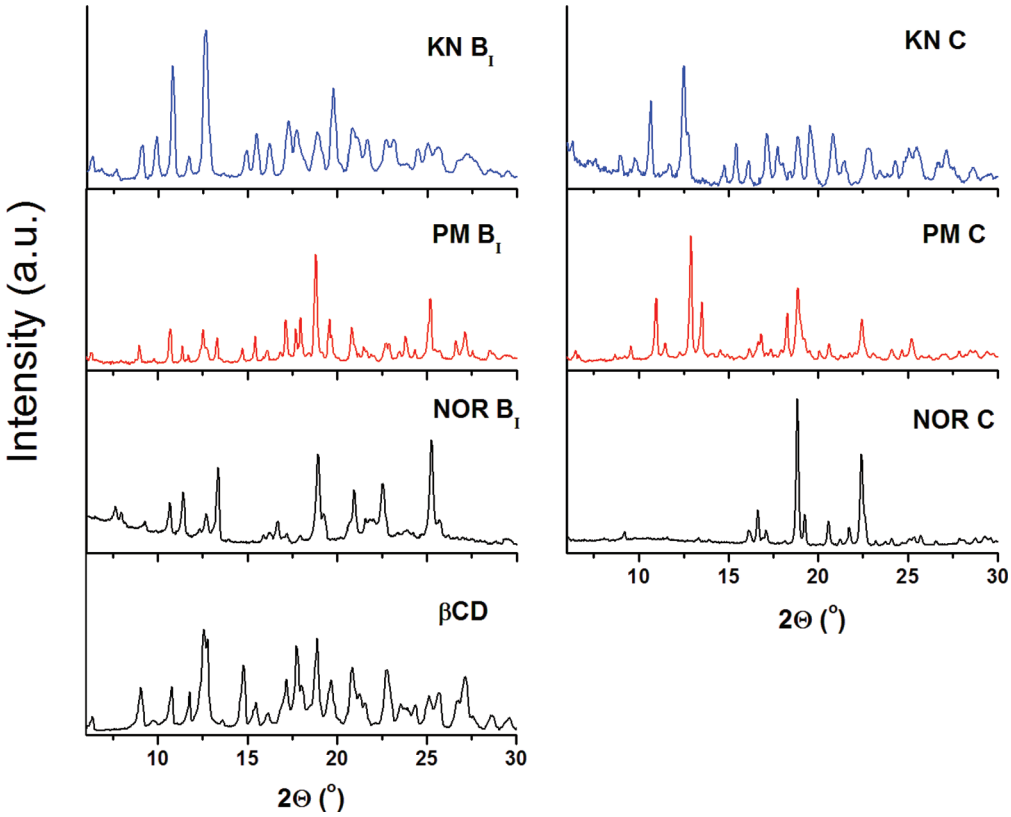

Figure 4. Powder X-ray diffraction patterns of βCD, NOR B_I, NOR C, PM B_I and C, and KN B_I and C.

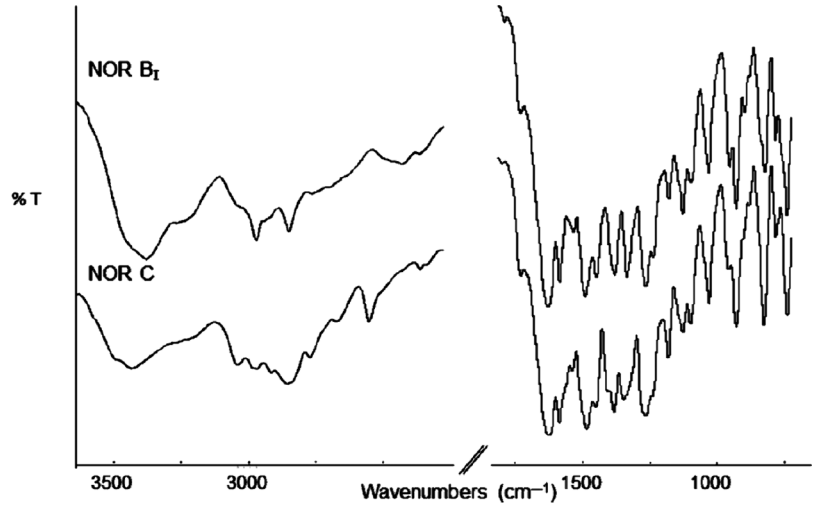

Figure 5. FT-IR spectra of NOR B_I and NOR C.

In the PXRD pattern for PM B_I, characteristic reflections of NOR B_I at $2\theta = 10.7^\circ$ and 13.3° are observable. The PXRD pattern for PM C shows representative reflections of NOR C at $2\theta = 16.1^\circ$, 18.8°, and 22.4°, and also characteristic reflections of βCD at $2\theta = 12.8^\circ$ and 13.5°. Additionally, the PXRD patterns for PM C and PM B_I are distinct, showing that these physical mixtures are not the same.

It is possible to distinguish between the PMs and KNs by examining the PXRD patterns, which show some reflections in each PM diffractogram that are not present in the corresponding KN powder pattern. Furthermore, the PXRD patterns for KN B_I and KN C clearly demonstrate that these are different materials. In the PXRD pattern for KN B_I, there are reflections present at $2\theta = 23.1^\circ$ and 21.0°, which are absent in the PXRD pattern for KN C. These results show that the complexes KN B_I and KN C are distinct crystalline solids having different PXRD patterns from both NOR B_I and NOR C and the two physical mixtures, PM C and PM B_I.

Fourier Transform Infrared

Segments of the FT-IR spectra of NOR B_I and C, βCD, their corresponding PMs, and KNs systems are shown in Figures 5 and 6.

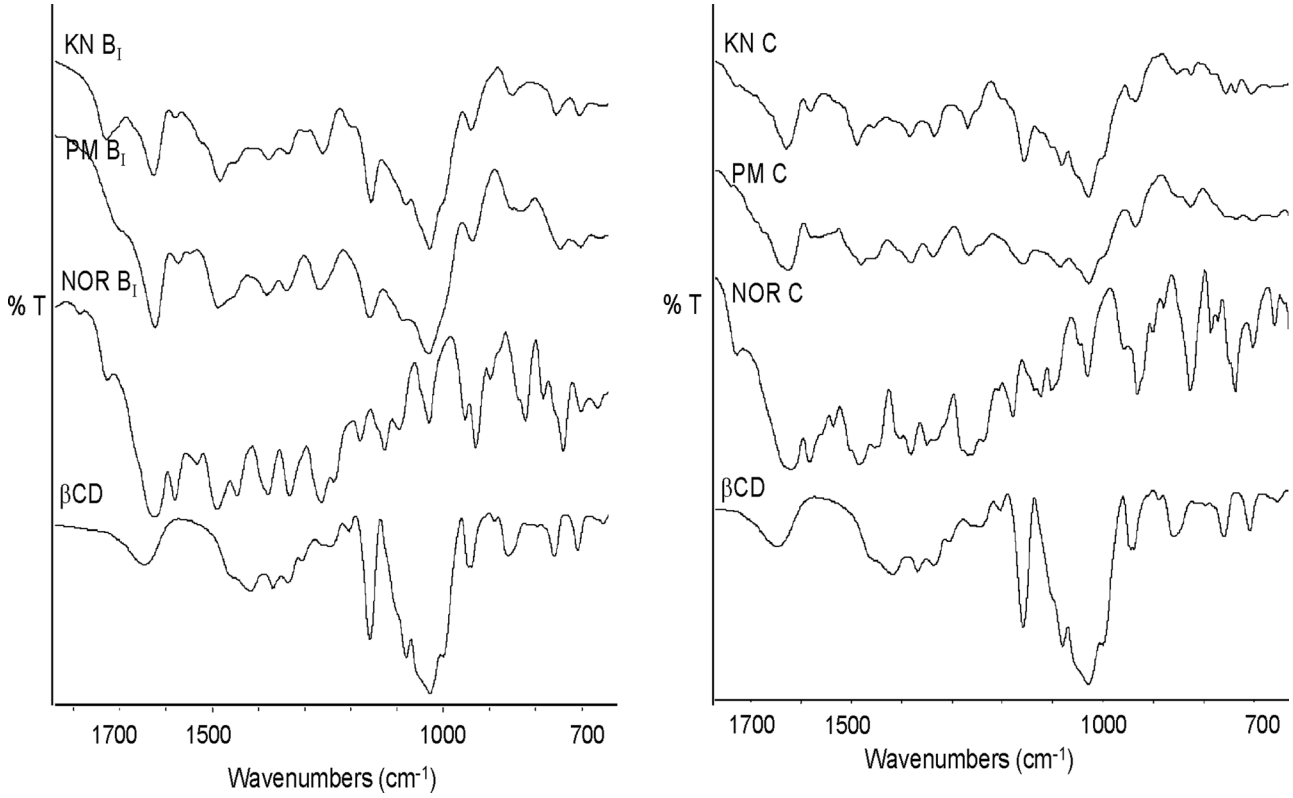

Figure 6. FT-IR spectra of βCD, NOR B_I, PM B_I, KN B_I, NOR C, PM C, and KN C.

 DOI 10.1002/jps.23683

Table 2. Infrared Bands Assignment

Assignment	Wavenumber (cm^{-1})	
	NOR B_I	NOR C
N–H and O–H stretch	3380	3434
Olefinic and aromatic C–H stretch		3042
Asymmetric C–H stretch in methyl and methylene of ethyl and piperazine groups	2972	2915
Symmetric C–H stretch in methyl and ethylene of ethyl and piperazine groups	2852	2769
Hydrogen bonded O–H stretch		2550
Pyridone C=O stretch	1625	1621
N–H deformation	1580	1582
Quinoline ring C–C and C–N stretch	1489	1482
Aromatic C–H deformation	836, 821	827
Rocking vibrations of the alkyl chains	740	737

NOR B_I and NOR C were readily distinguished by the differences in their FT-IR spectra (Figure 5, Table 2). It is important to note that the bands in the region 3500 –2500 cm^{-1}, showing marked differences between both solid forms, have not been previously reported.[9–11] The band between 3600 and 3100 cm^{-1} for NOR B_I, which is more intense than that for NOR C, can be attributed to the overlapping bands of H_2O with amino groups.

As shown in Figure 6, in the FT-IR spectrum of KN B_I, the band assigned to the pyridone C=O stretch (1625 cm^{-1}) was shifted to the higher frequency of 1632 cm^{-1}. In addition, the bands at 1580 and 1264 cm^{-1} were shifted to 1578 and 1269 cm^{-1}, respectively. Also, the band corresponding to the rocking vibrations of the alkyl chains was shifted to 736 cm^{-1}. For KN C (Figure 6), the bands corresponding to the pyridone C=O stretch and to the vibrations of the alkyl chains were shifted to 1628 and 735 cm^{-1}, respectively. In contrast, the spectra of PM B_I and PM C corresponded simply to the superposition of the FT-IR spectra of the single components. From these events, we suggest that the pyridone ring of each polymorph interacted with the βCD in the solid state. Similar results have been reported for NOR/CD complexes, suggesting that the carboxylic portion of the NOR is predominantly included within the apolar cavity of βCD.[31]

CONCLUSIONS

In this work, we have confirmed that solid-state NMR, complemented with other spectroscopic techniques, is a powerful tool for the challenging characterization of different solid forms of an API and its multicomponent pharmaceutical systems with CD.

In particular, we have developed and characterized new supramolecular complexes of two different solid forms of NOR and βCD. Clear evidence of the complexation was observed by variety of spectroscopic techniques (^{13}C CP-MAS ssNMR, PXRD, and FT-IR) and ^1H T_1 measurements.

The ^1H relaxation times confirmed that the complexes are homogeneous samples with absence of free-drug unbound to βCD, and moreover, that they are different to the physical mixtures. A different ^1H T_1 for the piperazine ring and the shifts in the FT-IR spectra of the pyridone ring provided evidence of strong interactions between NOR molecules and βCD. Additionally, it was interesting to note that the solid form denoted as NOR B_I (obtained by reproducing the procedure reported for NOR B) represents a new solid form different from the other reported ones. Our results suggest that the new solid form corresponds to a hydrate of NOR B, not reported in any previous publications. Then, the structural characterization of this new solid form has shown the difficulty in reproducing the experimental conditions for crystallizing NOR B.

We believe that our strategy, based on the interaction between NOR and CD, provides novel complexes that are potential alternatives to optimize insufficient features of drug molecule, such as solubility, stability, or toxic effects. These complexes are valid tools for the preparation of pharmaceutical dosage products, and represent an option to promote the pharmaceutical design for the improvement of physicochemical properties.

ACKNOWLEDGMENTS

We are grateful for financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), the Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT-UNC), and Ministerio de Ciencia y Tecnología (MinCyT) de la Provincia de Córdoba. We also thank Ferromet S.A. (agent of Roquette in Argentina) for their donation of β-cyclodextrin. A. K. C. thanks the Fulbright fellowship to perform a post-doctoral stay at the University of Michigan.

REFERENCES

1. Walsh RDB, Bradner MW, Fleischman S, Morales LA, Moulton B, Rodriguez-Hornedo N, Zaworotko M. 2003. Crystal engineering of the composition of pharmaceutical phases. Chem Commun 2:186–187.
2. Remenar JF, Morissette SL, Peterson ML, Moulton B, MacPhee M, Guzman HR, Almarsson O. 2003. Crystal engineering of novel cocrystals of a triazole drug with 1,4-dicarboxylic acids. J Am Chem Soc 125:8456–8457.
3. Lutker KM, Quiñones R, Xu J, Ramamoorthy A, Matzger AJ. 2011. Polymorphs and hydrates of acyclovir. J Pharm Sci 100:949–963.
4. Hilfiker R, Ed. 2006. Polymorphism in the pharmaceutical industry. Germany: Wiley-VCH Verlag GmbH & Co.
5. Mazuel C. 1991. In Analytical profiles of drug substances; Norfloxacin Florey K, Ed. Vol. 20. San Diego: Academic Press, pp 557–600.
6. Arredondo-García JL, Figueroa-Damián R, Rosas A, Jáuregui A, Corral M, Costa A, Merlos RM, Ríos-Fabra A, Amábile-Cuevas CF, Hernández-Oliva GM, Olguín J, Cardeñosa-Guerra O. 2004. Comparison of short-term treatment regimen of ciprofloxacin versus long-term treatment regimens of trimethoprim/sulfamethoxazole or norfloxacin for uncomplicated lower urinary tract infections: a randomized, multicentre, open-label, prospective study. J Antimicrob Chemother 54:840–843.
7. Lindenberg M, Kopp S, Dressman JB. 2004. Classification of orally administered drugs on the World Health Organization model list of essential medicines according to the biopharmaceutics classification system. Eur J Pharm Biopharm 58:265–278.
8. Olivera ME, Bistoni A, Anun H, Salinas A, Manzo RH. 2004. Ototopical ciprofloxacin in a glycerin vehicle for the treatment of acute external otitis. Ear Nose Throat J 83:625–638.
9. Barbas R, Martí F, Prohens R, Puigjaner C. 2006. Polymorphism of norfloxacin: Evidence of the enantiotropic relationship between polymorphs A and B. Cryst Growth Des 6:1463–1467.
10. Puigjaner C, Barbas R, Portell A, Font-Bardia M, Alcobé X, Prohens R. 2010. Revisiting of the solid state of norfloxacin. Cryst Growth Des 10:2948–2953.

11. Barbas R, Prohens R, Puigjaner C. 2007. A new polymorph of norfloxacin. J Therm Anal Cal 89:687–692.
12. Brewster ME, Loftsson TT. 2007. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Deliv Rev 59:645–666.
13. Corralo Spada J, Zapata Noreña CP, Ferreira Marczak LD, Tessaro IC. 2012. Study on the stability of β-carotene microencapsulated with pinhão (A*raucaria angustifolia* seeds) starch. Carbohyd Polym 89:1166–1173.
14. Munoz-Celaya AL, Ortiz-García M, Vernon-Carter EJ, Jauregui-Rincón J, Galindo E, Serrano-Carreón L. 2012. Spray-drying microencapsulation of T*richoderma harzianum* conidias in carbohydrate polymers matrices. Carbohyd Polym 88:1141–1148.
15. Garnero C, Chattah AK, Longhi M. 2013. Supramolecular complexes of maltodextrin and furosemide polymorphs: A new approach for delivery systems. Carbohyd Polym 94:292–300.
16. Zoppi A, Garnero C, Garro Linck Y, Chattah AK, Monti GA, Longhi M. 2011. Enalapril:β-CD complex: Stability enhancement in solid state. Carbohyd Polym 86:716–721.
17. Pham TN, Watson SA, Edwards AJ, Chavda M, Clawson JS, Strohmeier M, Vogt FG. 2010. Analysis of amorphous solid dispersions using 2D solid-state NMR and ^{1}H T_1 relaxation measurements. Mol Pharm 7:1667–1691.
18. Vogt FG, Strohmeier M. 2012. 2D solid-state NMR analysis of inclusion in drug−cyclodextrin complexes. Mol Pharm 9:3357–3374.
19. Fawaz F, Bonini F, Guyot M, Bildet J, Maury M, Lagueny AM. 1996. Bioavailability of norfloxacin from PEG 6000 solid dispersion and cyclodextrin inclusion complexes in rabbits. Int J Pharm 132:271–275.
20. Torres-Labandeira JJ, de Labra Pinon P, Perez-Marcos B, Alvarez-Lorenzo C, Vila-Jato JL. 1998. Effect of norfloxacin complexation with β-cyclodextrin on the in vitro dissolution behaviour and its interaction with Mg2+ and Al3+. J Thermal Anal Calorim 51:1009–1021.
21. Dua K, Ramana MV, Singh Sara UV, Himaja M, Agrawal A, Garg V, Pabreja K. 2007. Investigation of enhancement of solubility of norfloxacin β-cyclodextrin in presence of acidic solubilizing additives. Curr Drug Deliv 4:21–25.
22. Byrn SR, Pfeiffer RR, Stowell JG, Eds. 1999. Solid state chemistry of drugs. 2nd ed. SSCI, Inc.West Lafayette, Indiana
23. Bugay DE. 2001. Characterization of the solid-state: Spectroscopic techniques. Adv Drug Deliv Rev 48:43–65.
24. Harris RK. 1994. Nuclear magnetic resonance spectroscopy. London: Logman Scientific and Technical.
25. Harris RK. 2007. Applications of solid-state NMR to pharmaceutical polymorphism and related matters. J Pharm Pharmacol 59:225–239.
26. Geppi M, Mollica G, Borsacchi S, Veracini CA. 2008. Solid-state NMR studies of pharmaceutical systems. Appl Spectrosc Rev 43:202–302.
27. Nishiyama Y, Frey MH, Musaka S, Utsumi H. 2010. ^{13}C solid-state NMR chromatography by magic angle spinning ^{1}H T_1 relaxation ordered spectroscopy. J Magn Reson 202:135–139.
28. Mroue KH, MacKinnon N, Xu J, Zhu P, McNerny E, Kohn DH, Morris MD, Ramamoorthy A. 2012. High-resolution structural insights into bone: A solid-state NMR relaxation study utilizing paramagnetic doping. J Phys Chem B 116:11656–11661.
29. Khitrin AK, Fujiwara T, Akutsub H. 2003. Phase-modulated heteronuclear decoupling in NMR of solids. J Magn Reson 162:46–53.
30. Chongcharoen W, Byrn SR, Sutanthavibul N. 2008. Solid state interconversion between anhydrous norfloxacin and its hydrates. J Pharm Sci 97:473–489.
31. Guyot M, Fawaz F, Bildet J, Bonini F, Lagueny AM. 1995. Physicochemical characterization and dissolution of norfloxacin/cyclodextrin inclusion compounds and PEG solid dispersions. Int J Pharm 123:53–63.