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Stability of furosemide polymorphs and the effects of complex formation with β-cyclodextrin and maltodextrin

Claudia Garnero[a,*], Ana Karina Chattah[b], Marcela Longhi[a]

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

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ABSTRACT

The effect of the formation of supramolecular binary complexes with β-cyclodextrin and maltodextrin on the chemical and physical stability of the polymorphs I and II of furosemide was evaluated in solid state. The solid samples were placed under accelerated storage conditions and exposed to daylight into a stability chamber for a 6-month. Chemical stability was monitored by high performance liquid chromatography, while the physical stability was studied by solid state nuclear magnetic resonance, powder X-ray diffraction and scanning electron microscopy. Changes in the physical appearance of the samples were evaluated. The studies showed a significant stabilizing effect of β-cyclodextrin on furosemide form II. Our results suggest that the complex formation is a useful tool for improving the stability of furosemide polymorphs. These new complexes are promising candidates that can be used in the pharmaceutical industry for the preparation of alternative matrices that improve physicochemical properties

1. Introduction

Recently, much attention has been focused on the investigation of polymorphism of active pharmaceutical ingredients (API) due to its importance for the pharmaceutical technology industry. The presence of polymorphs often creates stability problems since they may have very different physical, chemical and mechanical properties such as melting points, solubility, dissolution rates, particle morphology, optical properties, chemical reactivity and physical stability (Bernstein, 2008; Braga, Grepioni, Maini, & Polito, 2009; Lee, Erdemir, & Myerson, 2011). The consequence is that, for example, at given conditions only one form is stable, and the other forms are metastable or definitely unstable. In addition, polymorphic changes from one form to another can strongly affect the bioavailability and therapeutic properties of an API, as well as the side effects of the product (Aaltonen et al., 2009). As these facts have a strong impact on the pharmaceutical development, the selection of the right solid state form is crucial for the production of reliable and effective pharmaceutical products.

The stability of APIs is in particular a matter of great concern because it affects the safety and efficacy of the drug product. The formation of degradation impurities may cause a loss of the efficacy of some APIs and initiate possible adverse effects. Therefore, the investigation of the chemical and physical stability of APIs is essential to ensure their quality and safety and applies a scientific and intelligent approach to formulation development.

Furosemide (FUR) is one of the most commonly used diuretic with rapid action that is normally administered as tablets or intravenous and intramuscular injectable products. It is a potent loop diuretic frequently used in the treatment of congestive heart failure, chronic renal failure, edemas, and hypertension (Prandota, 2002). FUR, which has seven polymorphic forms, contains a secondary amine group, and is therefore susceptible to acid catalyzed hydrolysis. At high temperatures, it hydrolyzes to 4-chloro-5-sulphamoylanthranillic acid and furfuryl alcohol which is quickly converted to levulinic acid. The photochemical degradation of FUR has been extensively reported. Several authors found that FUR exhibits photooxidation, photohydrolysis and photodechlorination (Bundgaard, Norgaard, & Nielsen, 1988; Chen & Burka, 2007; Kurmi, Kumar, Singh, & Singh, 2014; Vargas et al., 1998). According to the Biopharmaceutical Classification System (Custodio, Wu, & Benet, 2008), FUR is a class IV substance due to its low solubility and permeability. As a consequence, FUR has a poor oral bioavailability

* Corresponding author.
E-mail addresses: garneroc@fcq.unc.edu.ar (C. Garnero), chattah@famaf.unc.edu.ar (A.K. Chattah), mrlcor@fcq.unc.edu.ar (M. Longhi).

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since it is preferentially absorbed in the gastric mucosa and upper intestine where it shows the lowest solubility.

In our recent studies, we reported useful strategies to enhance the drug bioavailability of FUR. In particular, new supramolecular complexes of forms I and II of FUR with maltodextrin (MD) and β-cyclodextrin (βCD) (Garnero, Chattah, & Longhi, 2013, 2014) improved the solubility and dissolution rate of the drug. We demonstrated that the binary complexes resulted in more effective drug delivery systems, providing an alternative to the preparation of matrices that enhance the oral bioavailability of FUR. However, no detailed evaluation of the stability of FUR polymorphs and the binary complexes in solid state is available.

In this work, we focused our study on polymorphs I and II of FUR in solid state. We evaluated the effect of the supramolecular binary complexes with βCD and MD on their chemical and physical stability, in particular, on the photochemical degradation processes of FUR. To investigate stability, the solid samples were placed under accelerated storage conditions and exposed to daylight into a stability chamber over a 6-month period. Chemical stability was monitored by high performance liquid chromatography (HPLC), while physical stability was studied by using solid state nuclear magnetic resonance (ssNMR), powder X-ray diffraction (PXRD) and scanning electron microscopy studies (SEM). In addition, the hygroscopicity was determined.

2. Experimental

2.1. Materials

Furosemide was provided by Parafarm (Argentina); β-cyclodextrin (MW = 1135) was kindly supplied by Ferromet agent of Roquette (France) and Maltodextrin (DE17) was given by Todo Droga (Argentina). All other chemicals used were of analytical grade and the solvents were of HPLC grade. A Millipore Milli Q Water Purification System (Millipore, Bedford, MA, USA) generated the water used in these studies.

2.2. Preparation of solid samples

2.2.1. Furosemide polymorphic forms

The two solid forms of FUR, I and II, were obtained as described in our previous reports (Garnero et al., 2013). Form I was recrystallized from a hot saturated solution of FUR in methanol, while Form II was obtained by evaporation under reduced pressure, from an acetone solution at 25 °C.

2.2.2. Binary systems

Solid-state binary systems of FUR polymorphs I and II in equimolar ratio with the ligands βCD and MD were prepared as previously reported (Garnero et al., 2013, 2014), and are summarized as follows:

2.2.2.1. Kneading method (KN). The systems FUR I:βCD (KN I_{CD}), FUR II:βCD (KN II_{CD}), FUR I:MD (KN I_{MD}) and FUR II:MD (KN II_{MD}) were prepared by accurately weighing appropriate amounts of the ligand and then transferring them to a mortar. An ethanol-water (50:50, v/v) mixture was added to the powder and the resultant slurry was kneaded for about 10 min. For each system, the corresponding solid form of FUR was added in small portions simultaneously with the solvent in order to maintain a suitable consistency. This slurry was kneaded thoroughly for about 30 min, and the resultant paste was dried in vacuum at 40 °C for 48 h, and protected from light.

2.2.2.2. Physical mixture (PM). Physical binary mixtures of FUR I:βCD (PM I_{CD}), FUR II:βCD (PM II_{CD}), FUR I:MD (PM I_{MD}) and FUR II:MD (PM II_{MD}) were prepared by simply blending uniformly the corresponding components with a mortar and pestle.

2.3. Content determination

For the determination of FUR content in the powders of each binary system, an amount of powder containing 10 mg of FUR was dissolved in a methanol-water (50:50, v/v) mixture. After appropriate dilution with mobile phase, the samples were analyzed with HPLC-UV, using a validated procedure described below. Each content determination was performed in triplicate and the average and standard deviations were calculated.

2.4. Stability design

To investigate the effect of complexation on the photodegradation processes of FUR polymorphs under accelerated storage conditions, the tests were executed following the requirements of the International Conference on Harmonization guidelines (ICH Q1A(R2), 2003). In order to perform the stability study, each FUR polymorph, the supramolecular complexes prepared using KN and their PMs were stored in triplicate in glass vials at 40 °C and 75% relative humidity (RH), and exposed to daylight into a stability chamber for 6 months.

2.4.1. Chemical stability study

To assess the chemical stability of the samples, the content of FUR was measured at established times of storage, initial time ($t = 0$), after three months ($t = 3$) and after six months ($t = 6$). The solid samples were dissolved and analyzed applying an HPLC stability-indicating method. The HPLC system was an Agilent 1100 (Agilent, Waldbronn, Germany). The HPLC experiments were performed under isocratic conditions. The samples were prepared in duplicate and the results were expressed as means of the three determinations in each one. *Chromatographic conditions*: the column used was a Phenomenex Gemini C18 250 mm × 4.6 mm i.d. filled with 5 μm particles, and with a precolumn (guard cartridge SecurityGuard C18 4 mm × 3.0 mm i.d.) supplied by Phenomenex (Torrance, CA, USA); the mobile phase was prepared with phosphate buffer(0.01 M KH_2PO_4 adjusted to pH 3.0)-acetonitrile 60:40 (v/v), filtered through a 0.45 μm Millipore membrane and degassed prior to use; the optimum flow rate was 1.5 mL min^{-1}; the column temperature was 25 °C, and the injection volume was 20 μL. The detection wavelength was set at 276 nm. The experimental conditions were set up to avoid interferences from the degradation products.

2.4.2. Physical stability study

In order to evaluate possible solid phase transformations, the physical stability of the samples FUR I, FUR II, KN I_{CD} and KN II_{CD} was analyzed by using the following techniques: Solid-state NMR spectroscopy (ssNMR), Powder X-ray Diffraction (PXRD) and Scanning electron microscopy (SEM) at the initial time ($t = 0$) after three months ($t = 3$) and after six months ($t = 6$) of storage.

High-resolution solid-state ^{13}C spectra of samples were recorded using the ramp cross polarization/magic angle spinning (CP-MAS) sequence with proton decoupling during acquisition (Harris, 1994). All ssNMR experiments were performed at room temperature in a Bruker Avance II spectrometer equipped with a 4 mm MAS probe, operating at 300.13 MHz for protons. The operating frequency for carbons was 75.46 MHz. Glycine was used as external reference for the ^{13}C spectra and to set up the Hartmann–Hahn matching condition in the cross-polarization experiments. All the spectra were recorded with 1600 scans, a contact time of 2 ms during CP and a recycling time of 5 s. The spinning rate for all the samples was 10 kHz. ^{1}H spin-lattice relaxation

Table 1
Recovery percentages for FUR obtained by HPLC in the samples studied for 6 months.

	FUR I		FUR II	
Polymorph	64.8 ± 0.9		79.6 ± 0.4	
Supramolecular systems	CD	MD	CD	MD
Kneading method (KN)	78 ± 2	86 ± 3	94 ± 3	95 ± 2
Physical mixture (PM)	55 ± 1	52 ± 2	72.7 ± 0.1	86 ± 3

times in the laboratory frame ($^1H\,T_1$) were measured for the same samples under static conditions with a saturation recovery pulse sequence. In this experiment, the initial ^1H magnetization was saturated by a train of 40 $\pi/2$ pulses fora period of 160 μs and then allowed to recover along the *z*-axis during a time between 10 μs and 300 s. The recycling delay in these experiments was 5 s.

Powder X-ray diffraction patterns were obtained at room temperature using a Philips PW1800 diffractometer, operating at 40 kV and 30 mA with Cu-Kα radiation. The powder patterns were collected by scanning 2θ from 2° to 40° with a step size of 0.02° at a scanning rate of 4 s/step.

The microscopic shape and morphology of the samples were investigated and photographed using a Carl Zeiss Sigma scanning electron microscope. The samples were fixed on a brass stub using a double-sided aluminum tape and then gold-coated under vacuum by employing a sputter coater Quorum 150 to improve their conductivity.

2.5. Hygroscopicity study

The solid samples of FUR polymorphs, the supramolecular complexes prepared using KN and their PMs were accurately weighed before storage. The samples were withdrawn at pre-determined intervals (every 30 days) to monitor their weight changes. All these experiments were carried out in triplicate.

3. Results and discussion

3.1. Chemical stability

In order to achieve information on the kinetics of the degradation process of each FUR form, the drug content in the samples was measured during storage using an HPLC stability-indicating method. Then Fig. 1 displays the degradation curves (Ln C_t/C_0 vs. time) of the samples after exposure to daylight at 40 °C and 75% RH.

The photodegradation of free FUR forms I and II in solid state was compared to their photochemical degradation in samples containing ligands (βCD and MD) under identical experimental conditions. Table 1 shows that the amount of FUR in the different solid systems stored at 40 °C and 75% RH decreases with time with respect to the initial value. As seen in Fig. 1, the drug in the systems KN I_{CD}, KN II_{CD}, KN I_{MD} and KN II_{MD} appear to be much more stable than in its corresponding physical mixtures (PM). Particularly, the higher content of FUR in the systems KN II_{CD} and KN II_{MD} shows that the kneading complexes allow the increase in the stability of FUR II. In addition, it can be observed that for polymorph I KN I_{MD} is more stable than KN I_{CD}. As demonstrated by the results, the decrease in the amount of FUR in the supramolecular systems as a function of time is lower than in their PMs upon storage under the applied conditions. Thus, the supramolecular systems decrease the chemical reactivity of FUR.

Furthermore, the degradation curves show linear relationships for FUR, forms I and II, the supramolecular complexes and their PMs, indicating that the chemical photodegradation in solid state follow an apparent first-order kinetics. The kinetic parameters (Table 2), which are important from the pharmaceutical point of view, were determined with a model of first-order reaction and a linear regression analysis.This kinetic model can be expressed by the following equation:

Table 2
Kinetic parameters that characterize the effect of supramolecular complexes on the photodegradation process of FUR polymorphs in solid state.

	k_0 (day^{-1})	k_{obs} (day^{-1})	t_{90} (day)	t_{50} (day)	k_0/k_{obs}
FUR I	$(2.7 \pm 0.1)10^{-3}$		39	257	
KN I_{CD}		$(0.84 \pm 0.04)10^{-3}$	125	825	3.2
KN I_{MD}		$(1.02 \pm 0.09)10^{-3}$	103	679	2.6
PM I_{CD}		$(3.7 \pm 0.3)10^{-3}$	28	187	0.7
PM I_{MD}		$(3.5 \pm 0.2)10^{-3}$	30	198	0.8
FUR II	$(1.38 \pm 0.04)10^{-3}$		76	502	
KN II_{CD}		$(0.32 \pm 0.02)10^{-3}$	328	2166	4.3
KN II_{MD}		$(0.41 \pm 0.03)10^{-3}$	256	1690	3.3
PM II_{CD}		$(1.81 \pm 0.09)10^{-3}$	58	383	0.8
PM II_{MD}		$(0.69 \pm 0.08)10^{-3}$	152	1004	2

Table 3
Gain of weight at the end of 6 months of storage (expressed as g of adsorbed moisture per 100 g of dry solid)

	FUR I		FUR II	
Polymorph	0		1.85	
Supramolecular systems	CD	MD	CD	MD
Kneading method (KN)	1.67	31.25	2.46	27.54
Physical mixture (PM)	2.37	22.67	1.73	19.67

$$\mathrm{Ln}\,C_t = \mathrm{Ln}\,C_0 - kt \quad (1)$$

where C_t and C_0 are the concentration of FUR at different reaction times and at initial time, respectively, k is the degradation rate constant and t is time. Thus, k can be calculated from the slope of fitted lines.

Therefore, the intrinsic rate constant of photodegradation of the free FUR polymorphs (k_0), the observed rate constant of photodegradation of FUR polymorphs in the presence of ligands (k_{obs}), the half-life (t_{50}) and the time of 10% degradation of the drug (t_{90}), were estimated. The kinetic results demonstrated that the photodegradation rate of FUR I was higher than that of FUR II, revealing a higher chemical reactivity of the groups present on the surface of the molecule of FUR I. Additionally, the complexes obtained by the kneading method had a stabilizing effect on each FUR form with respect to the photodegradation of the free polymorphs. In particular, the evaluation of the relations k_0/k_{obs} showed that the KN II_{CD} had the slowest photodegradation rate.

3.2. Hygroscopicity study

Table 3 displays the increase of weight under storage as a function of time. The increase of weight of each FUR polymorph sample, and the supramolecular complexes prepared by KN and their PMs stored at 40 °C and 75% RH is expressed as g of adsorbed moisture per 100 g of dry solids.

Our results showed a slight gain of weight for both polymorphs and the systems with βCD, corresponding to a minor change of 2.5% in the weight under storage (i.e. in an atmosphere up to 75% of RH at 40 °C). It is particularly important in countries with tropical climate, where the RH is relatively higher than 80% most of the year. In contrast, the samples obtained with MD exhibited a gradual increase in weight due to water vapor adsorption under the same conditions. The increase in weight was above 5%, indicating that these samples were hygroscopic. Additionally, it was observed that the binary systems obtained by the kneading method were more hygroscopic than their respective PMs.

Also, the physical appearance of the samples was evaluated. All samples were white powders at $t = 0$. In particular, changes were observed within three months. The systems that were obtained

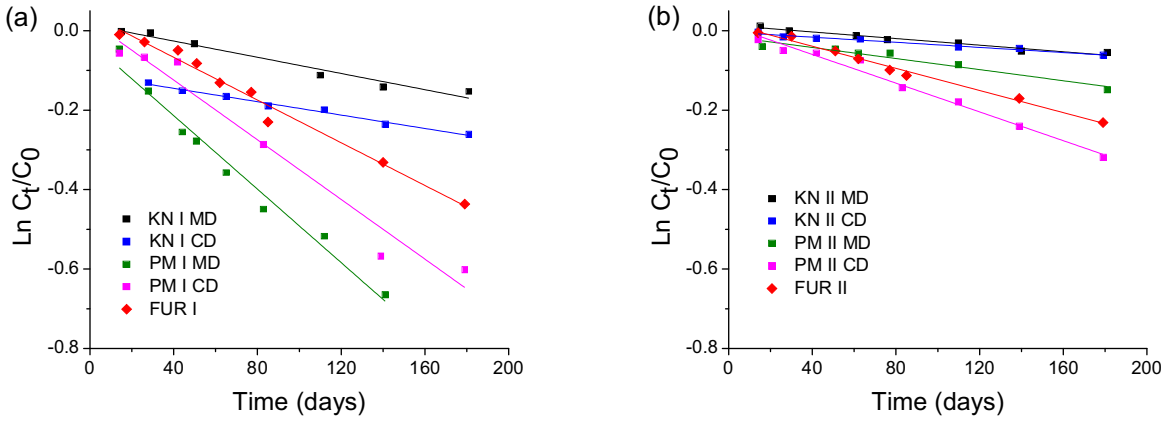

Fig. 1. Remaining (a) FUR I and (b) FUR II in each sample, after exposure to daylight at 40 °C and 75% RH in the presence of βCD and MD.

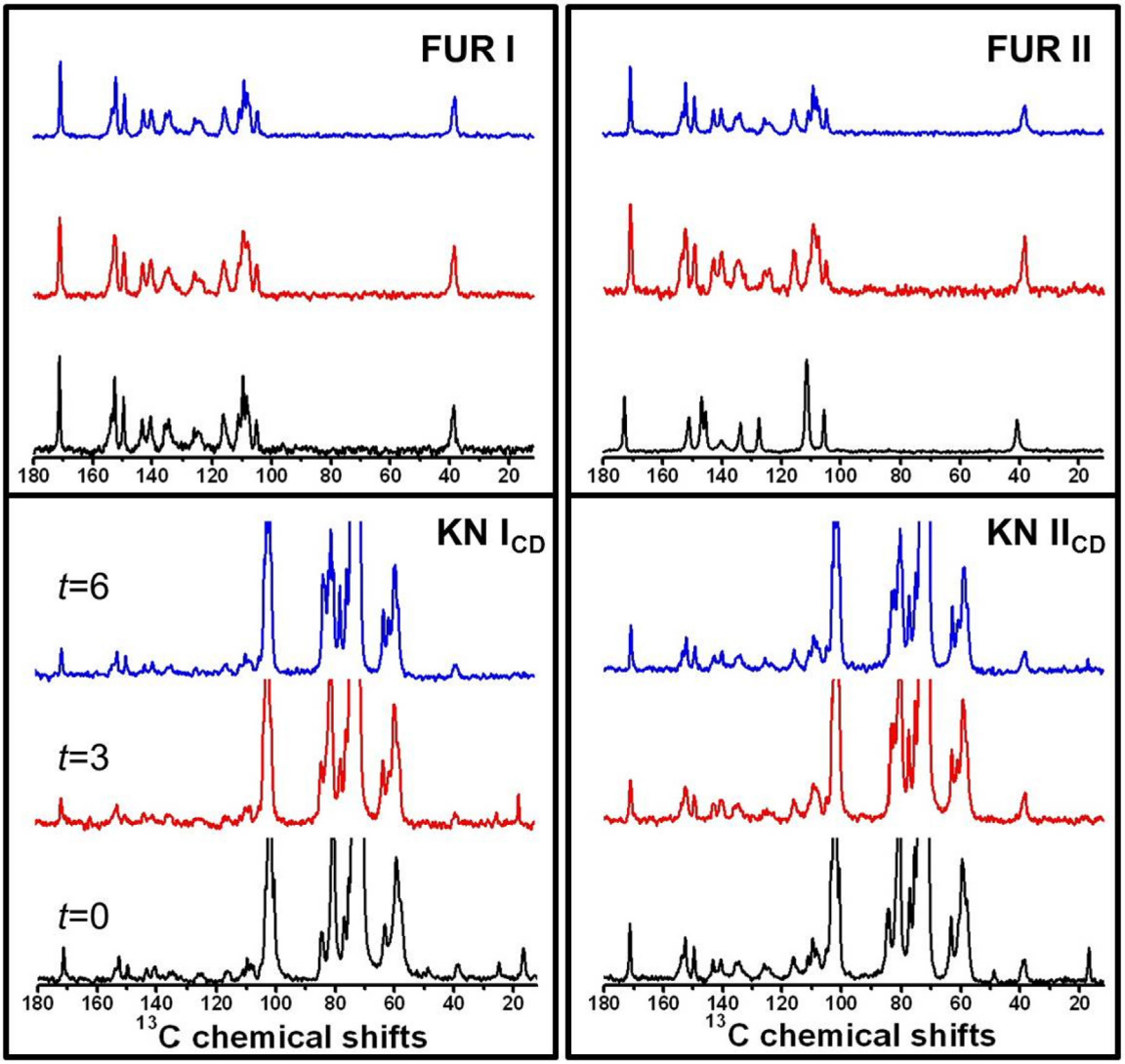

Fig. 2. ^{13}C CP-MAS spectra of FUR I, FUR II, KN I_{CD} and KN II_{CD}, for the three times under study from bottom to top: $t = 0$ (black line), $t = 3$ (red line), and $t = 6$ (blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

with MD and had the appearance of moistened powders showed signs of hydration. A change of color was also observed. FUR II, KN II_{CD}, PM II_{CD}, KN II_{MD} and PM II_{MD} became yellowish.

3.3. Physical stability

Temperature and humidity are important factors responsible for the physical instability of drugs.This instability may promote

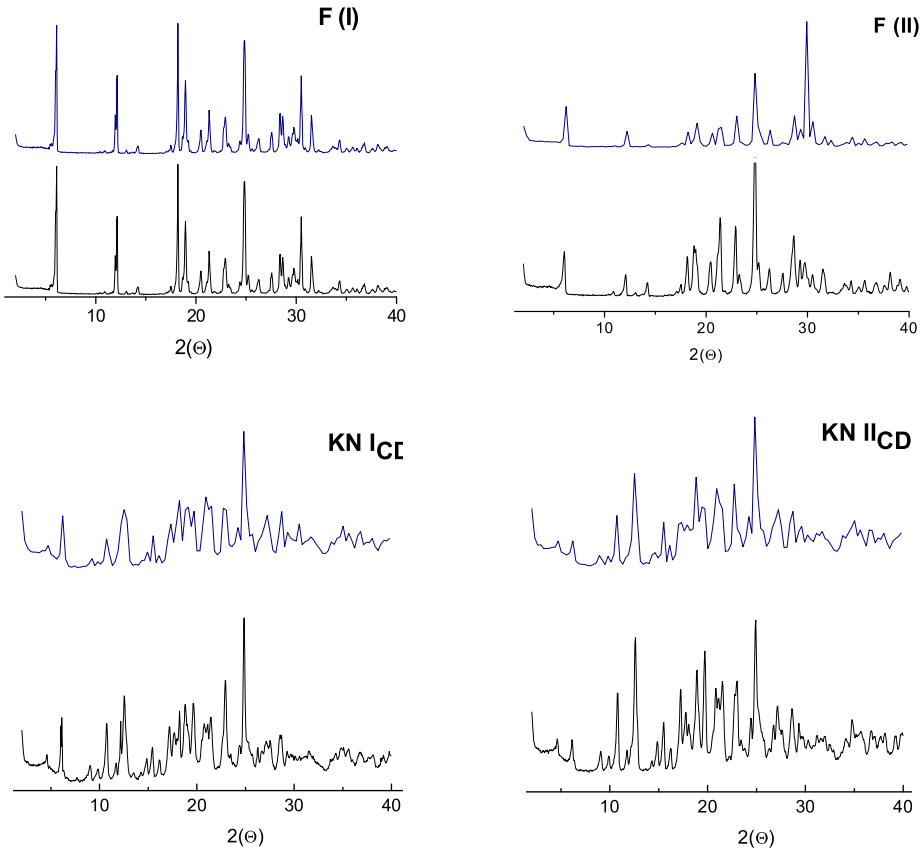

Fig. 3. Comparison of PXRD diffraction patterns of FUR I, FUR II, KN I_{CD} and KN II_{CD} at $t = 0$ (black) and $t = 6$ (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the reorganization of molecules in the crystal during storage. Thus, samples of FUR I, FUR II, KN I_{CD} and KN II_{CD} were analyzed by ssNMR, PXRD and SEM at determined intervals ($t = 0$, $t = 3$ and $t = 6$ months) to evaluate their physical stability and observe possible polymorphic transformations. It is important to mention that the other samples under study were not followed because they became moistened powders due to their high hygroscopicity.

3.3.1. Solid-state NMR spectroscopy (ssNMR)

Solid-state NMR provides a direct way to monitor changes in drug-CD solid complexes and can be applied to identify polymorphic changes under accelerated storage conditions (Monti, Chattah, & Garro Linck, 2014; Paradowska & Wawer, 2014; Zoppi et al., 2011).

Fig. 2 displays the ^{13}C CP-MAS of FUR I and II, KN I_{CD} and KN II_{CD}, for the three times of storage ($t = 0$, $t = 3$ and $t = 6$). The FUR polymorphs I and II (Garnero et al., 2013) and the formation of the binary complexes with MD and βCD previously reported (Garnero et al., 2013, 2014) were characterized using ssNMR.

It can be seen that in the spectra of FUR I there are not significant changes in the chemical shifts or intensity of the signals for the three times of storage. In contrast, FUR II clearly shows a phase transformation to form I at $t = 3$, that is not reversed at $t = 6$. This transformation was coincident with the color change of the sample.

In contrast to the pure FUR polymorphs, the spectra of KN I and II do not display major changes during storage. Indeed, it is possible to observe that the KN I_{CD} and KN II_{CD} spectra for $t = 3$ and $t = 6$ are globally similar to those corresponding to $t = 0$, respectively.

Table 4
Proton spin lattice relaxation times T_1 for FUR I and II and the KN complexes, measured at the times $t = 0$, 3, 6. Errors are below 10%. It only displays the times with major proportion.

	T_1 (s)		
	t = 0	t = 3	t = 6
FUR I	46	50	47
KN I_{CD}	1.3	1.1	1.0
FUR II	35	21	21
KN II_{CD}	1.1	1.0	1.2

Besides, some minor differences can be observed. For example, by analyzing the spectrum of KN I_{CD} for $t = 6$ in comparison with that at t = 0, the feature of the signals around 80 ppm changes while there is absence of the signals around 17 and 25 ppm. In KN II_{CD}, slight changes can be observed in the spectrum at $t = 3$, with different intensities of the signal at 82 ppm, a shift in the signal at 78 ppm was less than 1 ppm, and the absence of the signal at 17 ppm, compared with the spectrum at $t = 0$.

To obtain additional information on the changes and transformations of FUR polymorphs and binary systems under storage, we carried out ^1H T_1 relaxation-time experiments. To determine the ^1H T_1 values from the saturation-recovery experiments for each compound, the broad ^1H spectrum was integrated to obtain the magnetization. Then, the behavior of ^1H magnetization as a function of the recovery time was fitted using one or two relaxation times. The resulting values of T_1 having a large proportion in each sample are shown in Table 4. It is interesting to note that FUR I

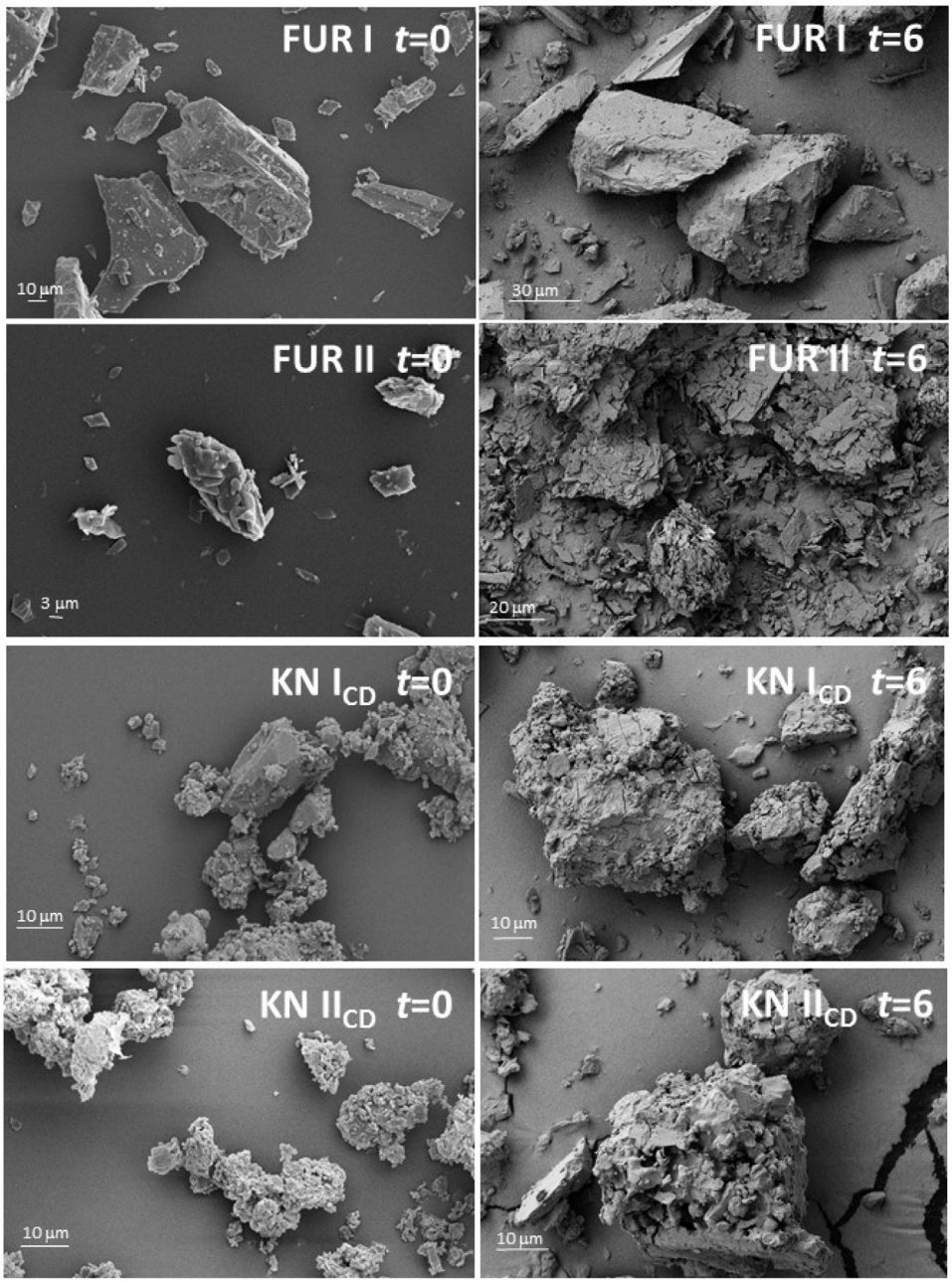

Fig. 4. SEM microphotographs of FUR I, FUR II, KN I_{CD} and KN II_{CD}, for $t = 0$ and $t = 6$.

maintains its structure under storage, displaying the same relaxation time throughout 6 months. In contrast, T_1 values at t = 3 and t = 6 for FUR II are different from those at $t = 0$. This difference in the relaxation times is due to changes in the FUR II structure occurring under storage, then confirming the information given by the solid state spectra.

In contrast to the behavior of FUR polymorphs, KN I_{CD} and KN II_{CD} maintained their T_1 values during the 6 months of storage. This confirms that, in the scale of the spin diffusion processes (10–1000 Å), the KN systems are stable under storage.

3.3.2. Powder X-ray diffraction (PXRD)

Fig. 3 shows the PXRD patterns of FUR I and II, KN I_{CD} and KN II_{CD} obtained at times of storage $t = 0$ and $t = 6$. As mentioned above, the formation of the binary systems KN I_{CD} and KN II_{CD} has been previously reported (Garnero et al., 2014).

Different recrystallization behaviors between FUR I and FUR II can be observed upon storage at 40 °C and 75% RH. After 6 months of storage, FUR I exhibits unmodified PXRD pattern, showing no phase transformations with respect to $t = 0$. However, in the PXRD pattern for FUR II, it is possible to observe the characteristic peaks of FUR II at 12.1°, 14.3°, 21.44°, 22.9°, and 28.6° (2θ), and diffraction peaks located at 18.2°, 29.3°, 29.7°, 30.5° and 31.6° (2θ), which are consistent with the characteristic peaks of FUR I. Therefore, our PXRD results suggest some amounts of FUR II recrystallizes as FUR I, revealing that a phase transformation occurs.

On the other hand, the samples KN I_{CD} and KN II_{CD} shows unmodified XRD patterns throughout the six months, meaning that they maintain their physical characteristics. Because the recrystallization of the drug did not occured, we concluded that are physically stable.

3.3.3. Scanning electron microscopy studies (SEM)

Additionally, morphological stability was studied by SEM because humidity and temperature may promote the organization of adsorbed molecules in crystal nucleus. Fig. 4 shows the structure of FUR I and II, KN I_{CD} and KN II_{CD} for the times of storage $t = 0$ and $t = 6$. The FUR polymorphs I and II, KN I_{CD} and KN II_{CD} have been previously characterized and reported (Garnero et al., 2013, 2014).

After 6 months, under accelerated storage conditions, it was possible to observe that FUR I maintained its characteristic hexagonal morphology, while FUR II exhibited predominantly agglomerated structures of fine prisms which could be due to the effect of humidity. However, the polymorphic transformation of FUR II into FUR I did not show a visible change in the morphology of its particles. On the other hand, KN I_{CD} and KN II_{CD} exhibited a change in their morphology and irregular arrangements of their particles where block structures with small particles were adhered to the surface.

4. Conclusions

The stability studies proposed in this work clearly demonstrated that FUR polymorphs differ significantly from their chemical and physical properties. This is probably because the groups present on the surface of the molecules have different reactivities.

The results obtained from the chemical studies confirmed that the complexes with the βCD and MD ligands obtained by the kneading method are able to significantly reduce the chemical photodegradation process of polymorphs I and II. Particularly, the solid complexes exhibited a high degree of protection, with photodegradation rates always lower than those of the free polymorphs under the same experimental conditions. However, the systems PM I_{MD}, PM I_{CD} and PM II_{CD} showed higher rates in comparison with the free polymorphs.

The physical studies performed on the free polymorphs using the high resolution ^{13}C NMR spectra in solid state, PXRD patterns and the physical appearance mainly showed a phase conversion of FUR II after three months of storage. These facts demonstrated that the form II of FUR is the most susceptible to suffer phase transformation under stress conditions. Additionally, the stabilizer effect of βCD on FUR II was determined with these techniques.

Our results demonstrated that the physical stability of FUR II can be improved under accelerated conditions in the presence of βCD. The physical stability was made possible by the occurrence of the interactions between FUR II and βCD in the complexation that physically stabilized the drug, and improved the chemical stability decreasing the solid-state chemical reactivity. On the other hand, our investigation confirmed that FUR I, KN I_{CD} and KN II_{CD} systems are chemically and physically stable under accelerated storage conditions, displaying minor changes only at microscopic scale. In conclusion, our results clearly indicate that complex formation is a useful tool for improving the stability of FUR polymorphs. Also, they represent a great development towards novel applications in the pharmaceutical fields of scientific research and industry. In particular, the system KN II_{CD} can be categorized as a suitable candidate for solid dosage.

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