J Therm Anal Calorim (2015) 119:989–994
DOI 10.1007/s10973-014-4233-1

Grinding effect on levofloxacin hemihydrate

Rafael Nicolay Pereira · Cinira Fandaruff · Manoela Klüppel Riekes · Gustavo A. Monti · Carlos Eduardo Maduro de Campos · Silvia Lucia Cuffini · Marcos Antonio Segatto Silva

Received: 23 November 2013 / Accepted: 5 October 2014 / Published online: 19 October 2014

Abstract The grinding techniques were used in different pharmacotechnical process. The control of the effect of grinding in solid state properties of drugs is very important, mainly in hydrated drugs. Levofloxacin hemihydrate (LVF) is a good example of this type of compounds and a broad spectrum antibiotic of the fluoroquinolone drug class. The samples of LVF with and without grinding were studied using different characterization techniques such as thermogravimetry, differential scanning calorimetry, fourier-transformed infrared, X-ray powder diffraction, and hot stage microscopy. The purpose of the present study was to evaluate the effects of grinding in the dehydration and rehydration processes in levofloxacin hemihydrate. After heating, the samples lost water molecules and the rehydration process was modified depending on defects due to the grinding. At room temperature, the complete transformation to the hemihydrate form was detected only for the sample without grinding. On the other hand, the milled sample showed two phases, hydrate and anhydrate forms. Therefore, the defects in the crystalline structure would cause the irreversible transformation.

Keywords Levofloxacin · Hemihydrate · Grinding · Rehydration

R. N. Pereira (✉) · C. Fandaruff · M. K. Riekes · S. L. Cuffini · M. A. S. Silva
PGFAR, University Federal of Santa Catarina, Florianópolis, SC 88040-970, Brazil
e-mail: rafael_nicolay@yahoo.com.br

G. A. Monti
Faculty of Mathematics, Astronomy and Physics (FAMAF), University National of Córdoba, Córdoba, Argentina

C. E. M. de Campos
University Federal of Santa Catarina, Florianópolis, SC 88040-970, Brazil

S. L. Cuffini
Instituto de Ciência e Tecnologia, Universidade Federal de São Paulo, São Paulo, Brazil

Introduction

The study of the different crystal structure of anhydrous and solvate compounds and the correlation with solid-state properties are the highlights in the performance and processing of drugs [1, 2]. The most common solvate in pharmaceutical compounds is the hydrate. The water molecules in the solid can be present either on stoichiometric or in nonstoichiometric ratio depending on the crystal lattice arrangement [3]. The water molecules can also modify intermolecular interactions, crystalline disorder, and changes in free energy of the drug as well as other properties such as the thermodynamic activity, mechanical processing, solubility, dissolution rate, stability, and bioavailability [4].

Due to a great amount of hydrate pharmaceutical drugs, this type of compounds has become the object of intensive research over the last decade [5]. Levofloxacin hemihydrate is a broad spectrum antibiotic of the fluoroquinolone drug class, and the levo isomer of its predecessor ofloxacin. Its spectrum of activity includes most strains of pathogenic bacteria responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections [6]. Two crystalline forms of levofloxacin, monohydrate and hemihydrate, are known which present different stability. The hemihydrate levofloxacin is the most used as active pharmaceutical ingredient (API) and the crystal structure alteration, depending on the manufacturing process, was reported [7, 8].

Here, we present the impact of the grinding method in the levofloxacin hemihydrate before and after the dehydration and rehydration processes.

Materials and methods

Materials

Levofloxacin hemihydrate samples were obtained from Zhejiang East-Asia Pharm. The samples were named as LVF rm (raw material) and LVF g40 (grinding). The LVF g40 was prepared by grinding for 40 min in a porcelain mortar.

Methods

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) curves of LVF were obtained in a DSC-60 cell (Shimadzu) using open aluminum crucibles at a heat rate of 10 K min^{-1}, 2 mg of sample, and dynamic N_2 atmosphere of 50 mL min^{-1}. The DSC analysis was carried out through a heating cycle (firstly heated from 298 to 413 K; after, cooled until 298 K). The DSC was previously calibrated with in ($\Delta H = 28.54$ J g^{-1} K^{-1}) and Zn (692.6 K) patterns.

Thermogravimetric analysis (TG)

The TG curves were obtained using a thermobalance TGA-50 (Shimadzu), dynamic N_2 atmosphere of 50 mL min^{-1}, 4 mg of sample, and heating rate of 10 K min^{-1}. The TG analysis was carried out through a heating cycle (firstly heated from 298 to 413 K; after, cooled until 298 K).

X-ray powder diffraction (XRPD)

X-ray powder diffraction (XPRD) patterns were recorded on a XPERT PANalytical diffractometer, equipped with X' Celerator detector, using Ni-filtered k_α radiation of a Cu tube operating with 45 kV and 40 mA, 2θ range from 5° to 60°, scan step size of 0.033°, and scan step time of 45 s. The Soller, divergent, and anti-scattering slits used were 0.04 rad, 0.25°, and 0.5°, respectively.

Scanning electronic microscopy

LVF rm and LVF g40 samples were analyzed using a scanning electron microscopy (Philips, Model XL 30) at an intensity of 10 kV, using magnification of 300× and 1,000×.

Hot stage microscopy

LVF rm and LVF g40 samples were analyzed using an Olympus BX50 microscope equipped with a Mettler Toledo FP-82 hot stage. The same heating condition rates of DSC analysis were applied.

Results and discussion

Thermal behavior

In Fig. 1, the TG curves show the heating cycle for the samples LVF rm and LVF g40. Both samples were heated from 298 to 353 K at a rate of 10 K min^{-1}. After that, the samples were cooled to 313 K. The dehydration of the sample LVF rm and LVF g40 can be observed in the first heating cycle. Both samples LVF rm and LVF g40 lost water mass of 2.557 and 2.367 %, respectively (Table 1).

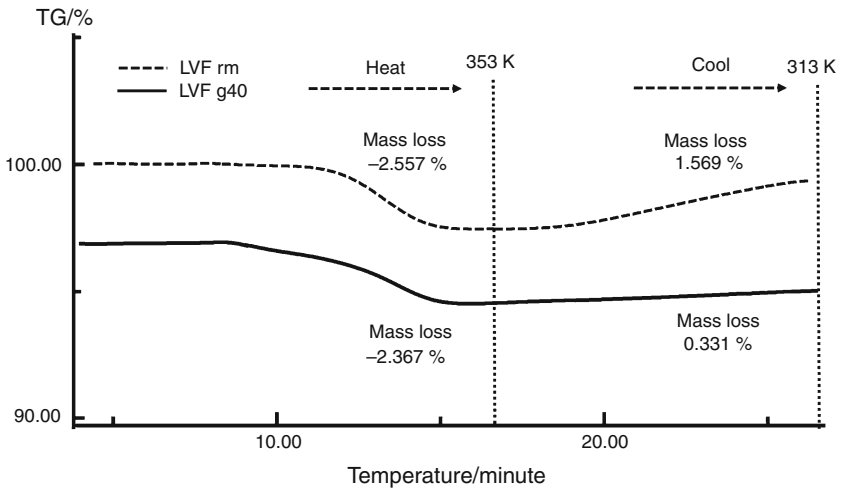

Fig. 1 TG curves of LVF rm and LVF g40. Both samples were carried out with heating/cooling cycles

Table 1 LVF rm and LVF g40 mass variation versus temperature in TG curves

	Temperature/K	353	313
LVF rm	Mass loss/%	−2.557	1.569
LVF g40	Mass loss/%	−2.367	0.331

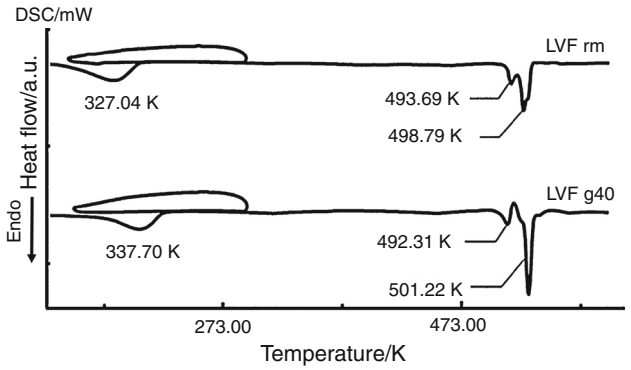

Fig. 2 DSC curves of LVF rm and LVF g40. Both samples were carried out with heating/cooling cycles

Table 2 LVF rm and LVF g40 endothermal events of DSC curves

	Event 1	Event 2	Event 3
LVF rm			
Temperature/K	327.04	493.69	498.79
ΔH/J g^{-1} K^{-1}	−70.06	−18.38	−84.55
LVF g40			
Temperature/K	337.70	492.31	501.22
ΔH/J g^{-1} K^{-1}	−77.97	−1.23	−13.84

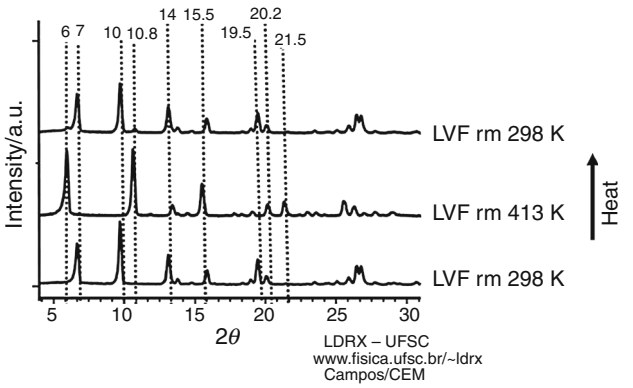

Fig. 4 X-ray powder diffraction of LVF rm carried out with heating/cooling cycles. LVF rm 298 K (room temperature, at 298 K); LVF rm 413 K (heating at 413 K); LVF rm 298 K (cooling at 298 K after heating at 413 K)

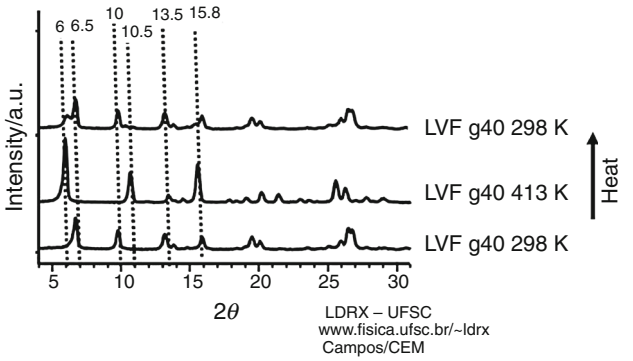

Fig. 5 X-ray powder diffraction of LVF g40 carried out with heating/cooling cycle. LVF g40 298 K (room temperature, at 298 K); LVF g40 413 K (heating at 413 K); LVF g40 298 K (cooling at 298 K after heating at 413 K)

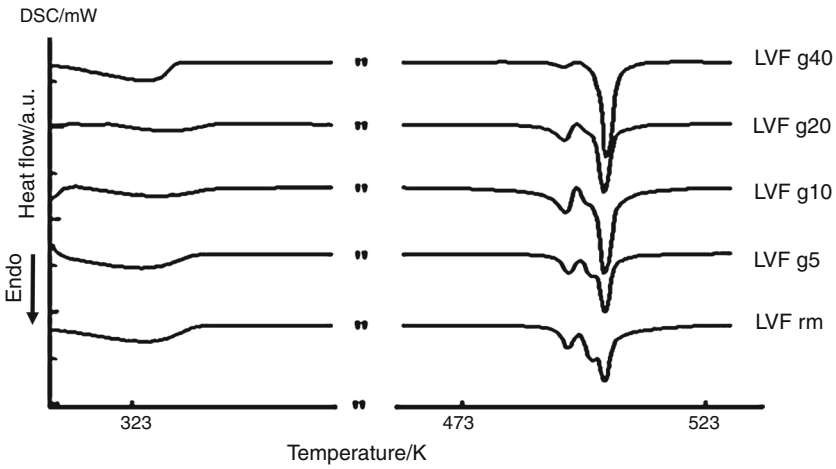

Fig. 3 DSC curves for LVF rm grinding at different times (5, 10, 20, and 40 min)

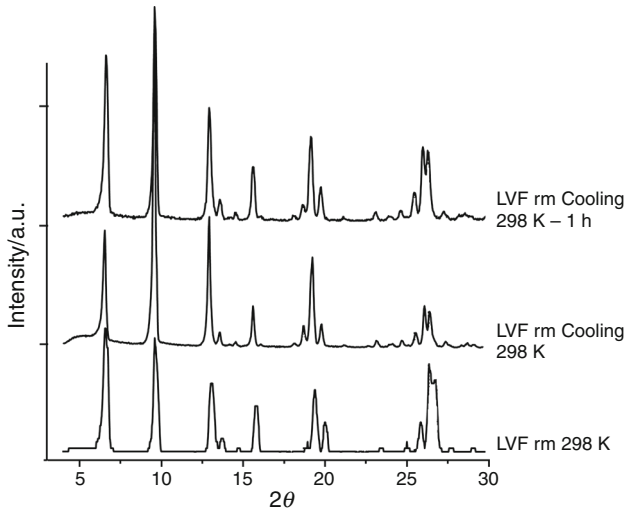

Fig. 6 Analysis of stability of LVF rm by X-ray powder diffraction with heating/cooling cycle. The samples were evaluated at room temperature (298 K), just after the heating/cooling cycle (298–413–298 K) and 1 h after finished the cooling cycle

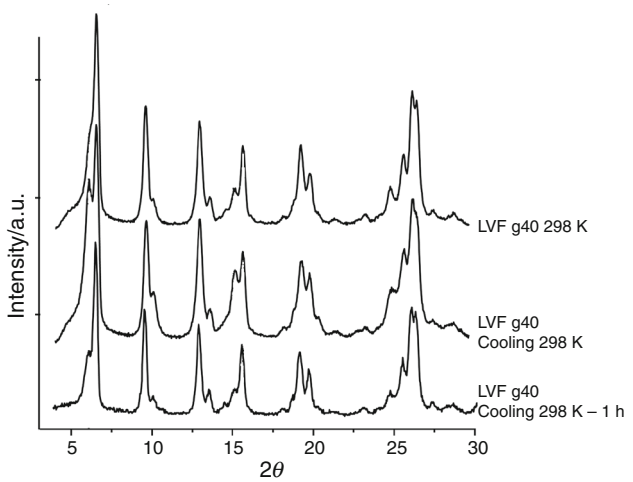

Fig. 7 Analysis of stability of LVF g40 by X-ray powder diffraction with heating/cooling cycle. The samples were evaluated at room temperature (298 K), just after the heating/cooling cycle (298–413–298 K) and 1 h after finished the cooling cycle

The DSC curves of the samples of LVF rm and LVF g40 are shown in Fig. 2. Water molecule loss was not observed after the first heating cycle. Additionally, the DSC curve (Fig. 2) of LVF rm showed three different endothermic events. The first event at 327.04 K represents the loss of water and the second and third events (493.69 and 498.79 K, respectively) were reported by Kitaoka et al. 1995 [7] as the melting of α, β, and γ levofloxacin polymorphs. On the other hand, the LVF g40 showed the loss of water about 10 K higher than the LVF rm. The second and third events occurred at 492.31 K with -1.23 J g^{-1} K^{-1} of enthalpy and at 501.22 K with -13.84 J g^{-1} K^{-1} of enthalpy, respectively (Table 2).

DSC curves were performed to evaluate the effect of grinding LVF rm at different times (5, 10, 20, and 40 min). Figure 3 shows time grinding effects on the TG/DSC results. It was observed that melting endotherm reported as β and γ polymorph were modified depending on time grinding.

X-ray powder diffraction (XRPD)

Figure 4 shows the diffraction pattern of the LVF rm at heating/cooling cycles between the temperatures of 298–413–298 K. During the first heating cycle from 298 to 413 K of LVF rm, the phase transition from hemihydrate to the anhydrate form was observed as previously demonstrated by Reddy et al. (2004) [8]. After cooling LVF rm until 298 K, the original anhydrous phase was detected. This result is in agreement with the thermoanalytical data (Figs. 1, 2) which evidence the presence of the pure anhydrate form of levofloxacin at room temperature (second heating cycle).

The X-ray diffraction patterns of the LVF g40 as a function of the temperature are presented in Fig. 5. When the LVF g40 sample was heated until 413 K, it was also observed the phase transition due to the anhydrous form. However, the cooling of LVF g40 sample produced a mixture of two solid forms, anhydrous and hemihydrate. Therefore, the rehydration process was irreversible since the hydration was incomplete.

The samples were evaluated at room temperature (298 K), just 1 h after the heating/cooling cycle (298–413–298 K) (Figs. 6, 7). The LVF rm sample transformed reversibly to the original anhydrous structure after the heating/cooling cycle. Therefore, the explanation would be that the water molecules flow freely through the crystal structure. In Fig. 7, the diffraction patterns for LVF g40 show that the mixture of hemihydrate and anhydrous forms was stable during 1 h after the heating cycle. In this case, the permanent defects in the crystalline solid due to grinding process would interfere with the water molecule mobility.

Scanning electronic microscopy (SEM) and hot stage microscopy (HSM)

The scanning electronic microscopy studies were used to confirm that the grinding process induced a reduction on the particle sizes. Figure 8 shows the bigger particles in the

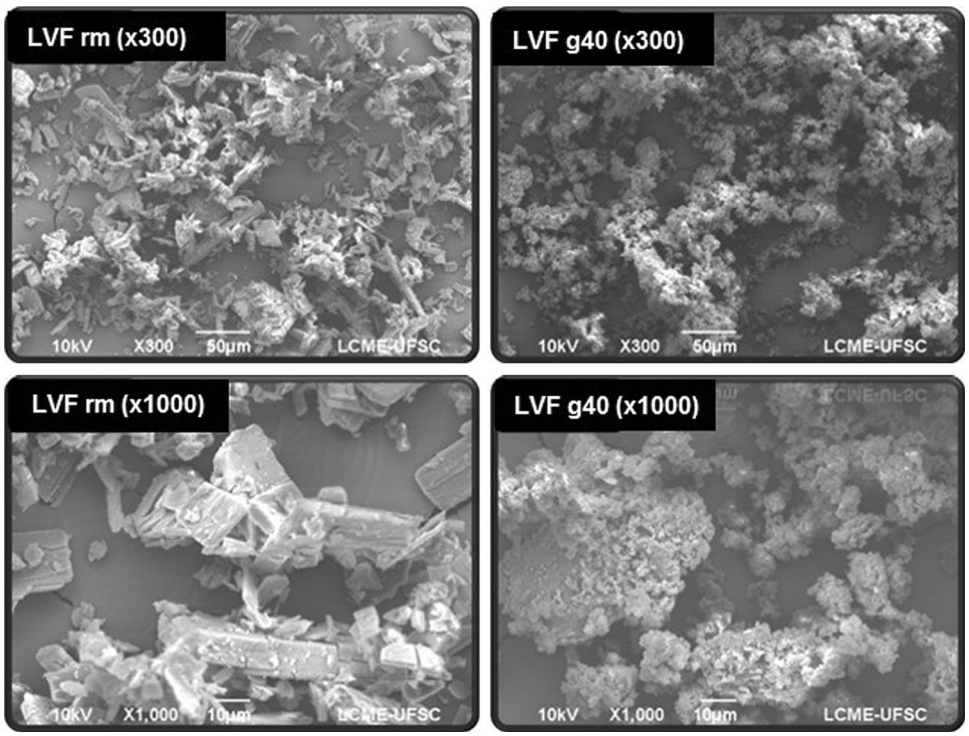

Fig. 8 SEM of LVF rm and LVF g40

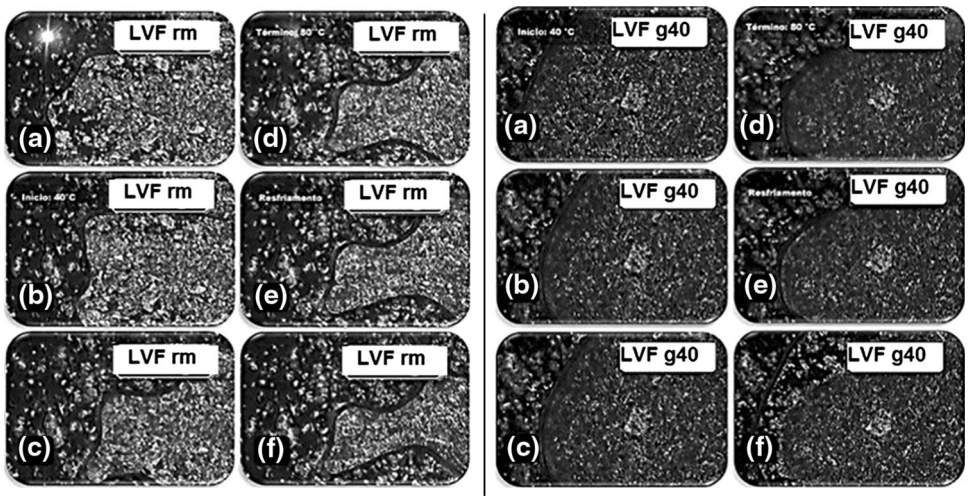

Fig. 9 Optical microscopy equipped with heating system (hot stage) of LVF rm and LVF g40. Steps in the release of the water molecule of the samples (LVF rm and LVF g40)

LVF rm than LVF g40 ones. Therefore, the grinding process caused important differences in particle sizes when comparing both samples LVF rm and LVF g40 (magnification 1,000×). In general, bigger particles present more crystalline order and fewer defects.

The hot stage microscopy studies were performed (Fig. 9) and the samples were heated at 10 K m^{-1} in until 413 K and cooled at 10 K m^{-1} in until 298 K. The release and absorption of water molecules were observed and these results were in agreement with thermoanalytical technique data. Therefore, also the HSM data showed that the defects impact in the water mobility in the crystalline structure.

Conclusions

Levofloxacin hemihydrate is a broad spectrum antibiotic and the grinding process was studied in samples with and without grinding. In order to understand the effects of the grinding in the hydrate compounds, the dehydration and rehydration processes were studied using different solid-state techniques. The heating and cooling methods showed different final solid-state forms depending on the grinding process. The rehydration of the crystalline samples was reversible; however, the milled sample showed an irreversible transition since both anhydrous and hemihydrate

forms were detected until 1 h after cooling. Therefore, the defects in the crystalline structure, caused by grinding process, would interfere in the water mobility; avoiding a completely reversible rehydration transition.

Acknowledgements The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support, *Pós-graduação em Farmácia* (PGFAR—UFSC) and Pró-reitoria de Pós-graduação (PRPG). The XRPD measurements were carried out at Laboratório de Difração de Raios-X of Universidade Federal de Santa Catarina.

References

1. Bernstein J. Polymorphism in molecular crystals. 14th ed. Oxford: IUCr, Oxford; 2013.
2. Vippagunta SR, Brittain HG, Grants DJW. Crystalline solids. Adv Drug Deliv Rev. 2001;48:3–26.
3. Jeffery GA. Water structure in organic hydrates. Acc Chem Res. 1969;2:344–52.
4. Khankari RK, Grant DJW. Pharmaceutical hydrates. Thermochim Acta. 1995;248:61–79.
5. Brittain HG. Polymorphism in pharmaceutical solids. In: Morris KR, editor. Structural aspects of hydrates and solvates. Milford: Discovery Laboratories, Inc; 2006.
6. Nelson JM, Chiller TM, Powers JH, Angulo FJ. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. Clin Infect Dis. 2007;7:977–80.
7. Kitaoka H, Wada C, Moroi R, Hakusui H. Effect of dehydration on the formation of levofloxacin pseudopolymorphs. Chem Pharm Bull. 1995;43(4):649–53.
8. Reddy MS, Eswaraiah S, Reddy KR, Reddy SR, Prakash PJ. Novel anhydrous crystalline form of levofloxacin and process for preparation there of levofloxacin. United States Patent Application Publication, US 2004/0152701 A1, 2004.