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Short communication

Dissolution rates of ciprofloxacin and its cocrystal with resorcinol

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Abstract

The synthesis of cocrystals is presented as an alternative to improve the properties of active pharmaceutical ingredients, especially those related to solubility and dissolution rate. In this work the dissolution rate of ciprofloxacin, a zwitterionic fluoroquinolone antibiotic, has been compared to its cocrystal with resorcinol. To this end, dissolution rate has been determined at several biorelevant pH values, and also in two simulated gastrointestinal fluids (FeSSIF and FaSSIF). Results show that both, ciprofloxacin and the cocrystal, dissolve more slowly as pH increases (from 2.0 to 7.4), as ionization degree of ciprofloxacin decreases. In addition, dissolution is not enhanced by the components of the gastrointestinal fluids.

Keywords

Dissolution rate; cocrystal; ciprofloxacin; solid state; FaSSIF; FeSSIF; Simulated gastrointestinal fluid

Introduction

The dissolution rate plays an important role in drugs with limited solubility. In fact, for drugs with very poor aqueous solubility, the rate at which they dissolve is often the slowest step, and it exerts a rate-limiting effect on drug bioavailability. Currently about 60 % of the drugs coming from synthesis are poorly soluble and hence have a low oral bioavailability [1]. Diverse strategies have been developed to improve solubility, dissolution rate and subsequently the bioavailability of these drugs. These include formation of salts, inclusion complexes with cyclodextrines, amorphization, formulation of solid dispersions with hydrophilic polymers, changing the crystal form (polymorphs, hydrates, etc.) [2-5]. A promising strategy is the crystallisation of the drug with a suitable coformer to obtain a cocrystal, which may have different physical properties compared to the drug, such as melting point, moisture sorption, compressibility, solubility, and dissolution rate, without altering the pharmacological effect of the drug candidate [4-7].

A cocrystal can be defined as a stoichiometric multi-component system connected by non-covalent interactions where all the components present are solid under ambient conditions [5]. A pharmaceutical cocrystal is composed of an active pharmaceutical ingredient (API) and a suitable and pharmaceutically

accepted molecule called a coformer. Cocrystals are constructed from intermolecular interactions such as van der Waals contact forces, π-π interactions, and especially hydrogen bonding. There are several methods to obtain cocrystals in the literature, such as slow solvent evaporation, slurry crystallization, solid state grinding, and melting [8-13].

The API selected in this study is ciprofloxacin (CIP), a poorly soluble fluoroquinolone antibiotic active against both Gram-positive and Gram-negative bacteria, which is mainly used in the treatment of lower respiratory and urinary tract infections. The aim of this study is to compare the dissolution rate of CIP in different media and pH values to that of the prepared cocrystal with resorcinol. In addition, possible changes in the solid state of the drug and its cocrystal during the dissolution process are also investigated.

Materials and methods

Instruments

Dissolution rate assays were carried out using a GLpKa titrator from Sirius Analytical Instruments Ltd (Forest Row, UK), equipped with a Sirius D-PAS spectrometer, a bifurcated fibre-optic dip probe from Hellma Analytics (Müllheim, Germany) with path length of 1 cm, and a two channels solvent degasser from SMI-LabHut Ltd. (Churcham, UK). The apparatus was controlled from a computer running the RefinementPro2 software. Acidity constant determinations were done with the same instrument as described elsewhere [14].

The powder X-ray diffraction (PXRD) characterization was performed using a PANalytical X'Pert PRO MPD θ/θ powder diffractometer of 240 mm radius equipped with a PIXcel detector from PANalytical B.V. (Almelo, The Netherlands). The apparatus was set in a configuration of convergent beam with a focalizing mirror and a transmission geometry, with flat samples sandwiched between low absorbing films. The detector active length was 3.347º. Work power was 45 kV – 40 mA with a defined beam height of 0.4 mm. Five repeated scans were done from 2 to 60 2θº with a step size of 0.026 2θº and a measuring time of 40 seconds per step.

Calorimetric analysis of the samples was performed on differential scanning calorimetry (DSC) (Mettler Toledo DSC822e, Switzerland). Samples equivalent to 2-5 mg of the drug or cocrystal were loaded into aluminum crucibles. The thermal behavior of each sample was investigated in a temperature range of 30-300 °C with 10 °C/min heating rate under a continuous flow of dry nitrogen at 50 mL/min.

Reagents

Ciprofloxacin (>98 %), resorcinol (>99 %), toluene (99.9 %), and potassium chloride (>99 %) were from Sigma-Aldrich (St. Louis, MO, USA). Sodium acetate anhydrous (>99 %), potassium dihydrogen phosphate (>99.5 %), and 0.5 M NaOH standard solution (Titrisol®) were for Merck (Darmstad, Germany). FaSSIF (Fasted State Simulated Intestinal Fluid) and FeSSIF (Fed State Simulated Intestinal Fluid) powders were from Biorelevant.com (London, UK). The simulated gastrointestinal solutions of FaSSIF and FeSSIF were prepared as specified by the manufacturer. Water was purified by a Milli-Q plus system from Millipore (Bedford, MA, USA) with resistivity of 18.2MΩ cm.

Methods

Ciprofloxacin cocrystal was prepared using slurry crystallization method. 100 mg of ciprofloxacin was

mixed with 42 mg of resorcinol in 2 mL of toluene. The suspension was kept under stirring overnight and solid was collected next day by filtration under vacuum for 30 min. Then, it was characterized by DSC and powder X-ray diffraction (PXRD) (Figure 1). It can be observed that the signals corresponding to the cocrystal are different than the ones of the pure components. The formed cocrystal had 1:1 stoichiometry.

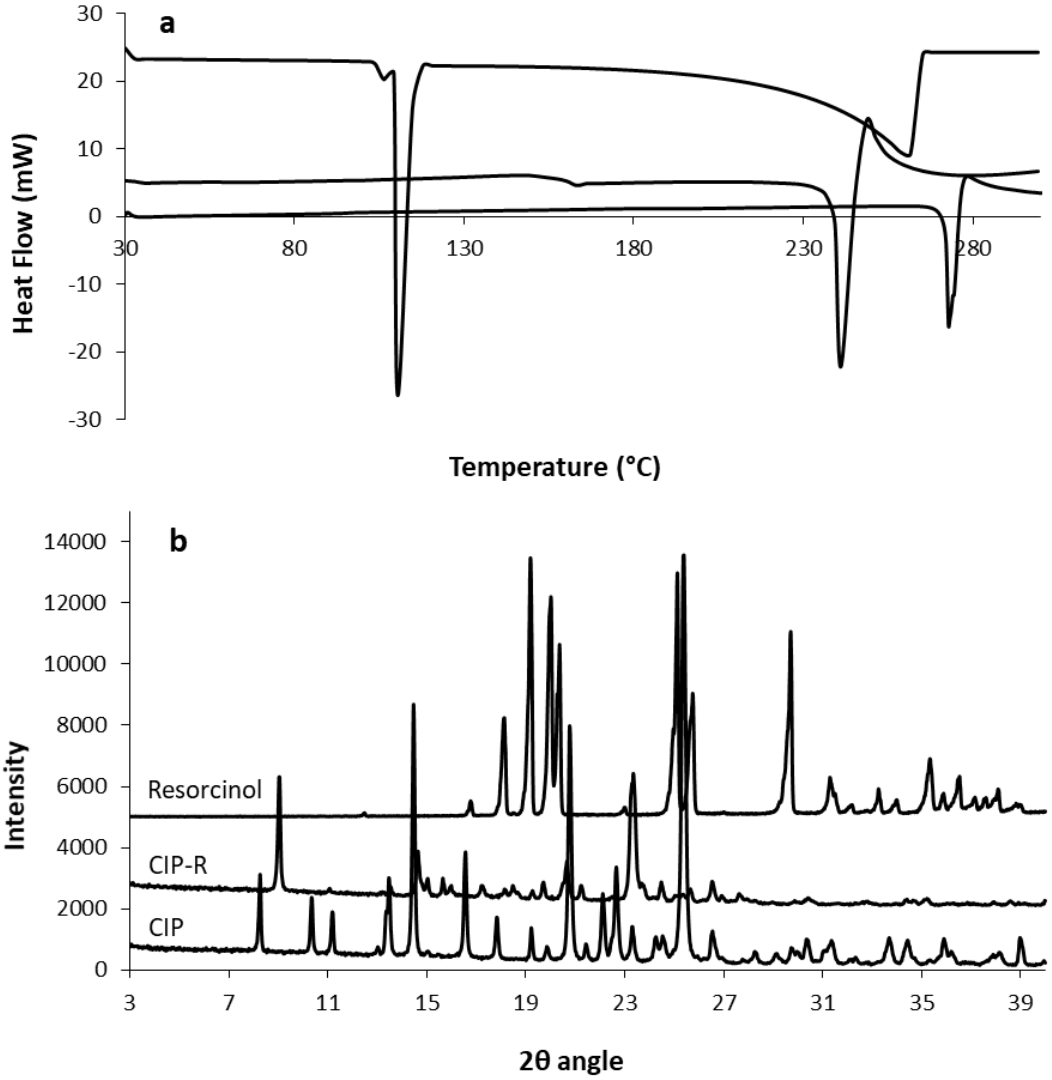

Figure 1: (a) DSC curve for resorcinol, the cocrystal, and CIP. (b) PXRD diffractogram for resorcinol, the cocrystal, and CIP.

For dissolution rate measurements the "GI dissolution method" has been followed [15]. Briefly, disks of 3 mm diameter containing 8-10 mg of drug or cocrystal (8 mg of cocrystal containing around 5 mg of CIP, according to a 1:1 stoichiometry) were prepared by applying a constant pressure of 0.1 ton. A Sirius GLpKa system was used to perform the measurements. Temperature was 25±1 °C and ionic strength was kept constant at 0.15 M. Study was performed at different pH sectors (2.0, 4.0, 5.5, and 7.4) which are typically encountered in the GI tract. To perform experiments 1.5 mL of a 0.125 M acetate and 0.125 M phosphate buffer solution at pH = 1.6 was introduced into the sample vial. Then, the instrument added 13.5 mL of 0.15 M aqueous KCl solution, which raised initial pH around 2. Dissolution started and the medium was stirred at a constant rate throughout the experiment. After 30 minutes, 0.5 M KOH solution was automatically dispensed to adjust to the next pH, and so on. In all experiments UV-vis spectra were recorded at fixed intervals, normally 30 s. Dissolution rate was determined individually at each pH sector, and then experiments were repeated in a full sequence of pHs (from 2.0 to 7.4) staying 30 minutes at each pH. Additionally, dissolution rates were also determined in the acetate/phosphate buffer at pH 5.0 and

6.5, and in 15 mL of two simulated gastrointestinal fluids (FeSSIF, pH 5.0 and FaSSIF, pH 6.5). The concentration of sample in solution at each time point is determined from the spectroscopic data in the 320-410 nm wavelength range, using previously determined molar extinction coefficients. In this range solutions of FaSSIF, FeSSIF and resorcinol present a minimum absorbance so that they don't interfere in the quantification of ciprofloxacin. Data corresponding to saturated signals were excluded for the calculation. Equation 1 was used to calculate the dissolution rate of the compounds,

$$[X]_t = S\left(1\text{-}e^{-K_d(t-t_0)}\right) \quad (1)$$

where $[X]_t$ is the weight (in grams) of the compound in solution at time t (min), S is the extrapolated solubility (g) of the drug, K_d is the rate constant for dissolution (min^{-1}), and t_0 is a term allowing for a temporal offset. The dissolution rate (g min^{-1}) is given by the product $K_d \cdot S$.

After the dissolution rate experiments, the solid state of the remaining disks was analysed using PXRD.

Results and Discussion

Dissolution behaviour of ionisable compounds is strongly dependent on pH value of the surrounding medium. As drug moves throughout the gastrointestinal tract (GIT) it is exposed to different pH environments that affect its degree of ionization, solubility, and thus dissolution or precipitation, according to the drug ionization constants. CIP is a zwitterionic compound with pK_{a1} = 6.20±0.04 and pK_{a2} = 8.56±0.06 (values provided at 25 °C and 0.15 M ionic strength) [16]. However, CIP exists in four different microspecies (X^-, $XH^{\pm}$, XH^0, and XH_2^+) in solution. Völgyi et al. [17] studied the acid base equilibria of CIP and determined its protonation macroconstants and microconstants. Figure 2 shows the species distribution curve of this compound according to the values provided in the study. The zwitterionic species is always predominant relative to the non-charged species independently of the pH of the solution. CIP is positively charged at low pH values, and its ionization degree decreases as pH increases. The maximum percentage of zwitterionic form and neutral forms exist at pH 7.3, where CIP has a log S_0 around -3.7 (-3.62 [18], -3.72 [19], and -3.76 measured in this work by the shake-flask method according to [20]). At higher pH values, ionization degree rises again due to the deprotonation of the basic group. On the contrary, resorcinol is a highly soluble very weak diacid which remains non-ionized (neutral) in the whole pH range between 2 and 7.4.

Figure 3 shows the aqueous dissolution profile at separate pH sectors for ciprofloxacin and its cocrystal with resorcinol (CIP-R). The dissolution rate reached a maximum value at pH 2, where both CIP and CIP-R totally dissolve. As pH of the medium increases, the percentage of CIP dissolved and its dissolution rate start to decrease, as stated in Table 1.

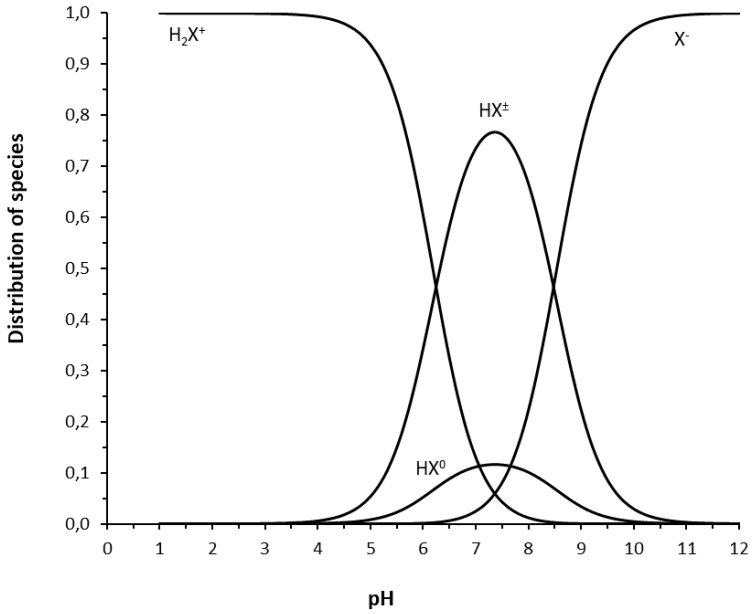

Figure 2: species distribution diagram for CIP, according to the acidity macroconstant and microconstantvalues provided in [17].

Table 1: Percentage of CIP dissolved, and dissolution rate of CIP and CIP-R in the dissolution experiments at different pH sectors.

pH	% CIP Dissolved		Dissolution Rate (mg/min)	
	Ciprofloxacin	Cocrystal	Ciprofloxacin	Cocrystal
2.01 ± 0.04	97.5 ± 0.1	100.0 ± 0.3	1.2 ± 0.2	0.56 ± 0.06
4.01 ± 0.05	41 ± 3	98.0 ± 0.5	0.13 ± 0.01	0.23 ± 0.9
5.41 ± 0.03	7.1 ± 0.7	11 ± 1	0.017 ± 0.006	0.021 ± 0.001
7.41 ± 0.08	3.7 ± 0.2	6 ± 1	0.0033 ± 0.0004	0.003 ± 0.002

This is the expected behavior according to the ionization state of CIP. The percentage of dissolved CIP decreases dramatically when changing pH from 2 to 4, with only 41% dissolved after 30 minutes (Table 1), and drops to around 6 and 4% at pH 5.5 and 7.4, respectively. Instead, the cocrystal dissolves totally after 25 minutes at pH 4, but its dissolution also decreases significantly at pH 5.5 and 7.4 (11 and 6% of CIP dissolved) respectively. Dissolution rate is directly related to the slope of the dissolution curve. Comparison of dissolution rates between CIP and its CIP-R evidences that at very low pH values (pH 2) the dissolution rate of CIP is more than twice the one of the cocrystal, probably due to the effect of resorcinol (neutral at this pH), which slows dissolution of CIP-R down. However, as pH increases the dissolution rate of CIP and CIP-R tend to be the same, reaching a minimum value of 0.003 mg min^{-1} at pH 7.4.

On the contrary, the full pH experiment (Figure 4) does not reflect the actual dissolution rate of the compound at each pH, as both CIP and CIP-R dissolve almost totally at the first sector (pH 2). Then solutions stay supersaturated at the following pH sectors (4.0, 5.5, and 7.4). In case of CIP there is evidence of precipitation at pH 7.4, since the signal falls to zero. Although this kind of experiment does not allow knowing the dissolution behavior of the compounds at a given pH because each sector is influenced by the previous one, it can simulate better the processes that compounds experience when moving along the gastrointestinal tract, where different pH environments are encountered.

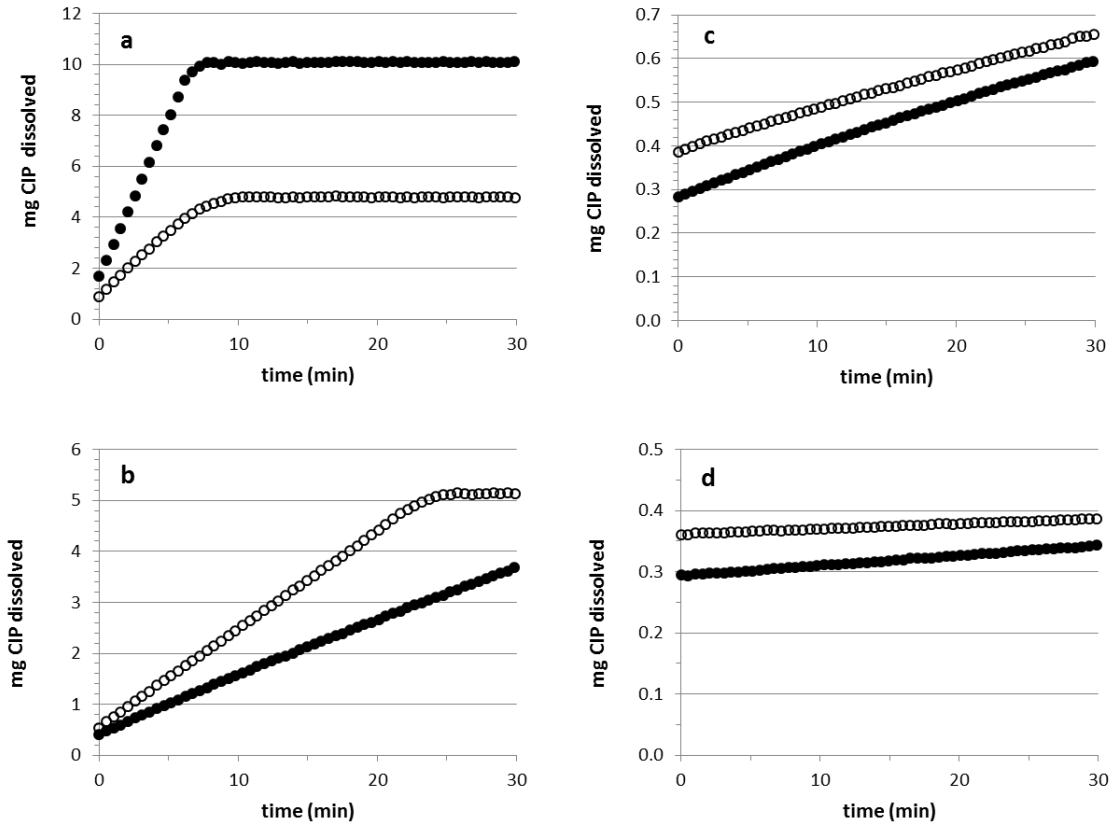

Figure 3: Dissolution profile at separate pH sectors for CIP (●) and its CIP-R (○): (a) pH = 2; (b) pH = 4; (c) pH = 5.5; (d) pH = 7.4.

X-ray analysis of the surface of the solid remaining in the disks indicates that approximately 50% of the solid remaining in CIP tablets is transformed to CIP·3H_2O, whatever the working pH. Meanwhile, the surface of the cocrystal tablets contain about 90% of the original cocrystal, and only 10% of CIP·3H_2O. These transformations in the tablet surface may modify, to some extent, the dissolution of the compounds, especially for CIP where the percentage of transformation to the trihydrated form is higher. In general, the appearance of hydrated forms slows the dissolution process down because of the lower ion-dipole interaction energy liberated on its dissolution [21]. Only at pH 4.0 a slightly decrease in the dissolution rate of CIP compared to CIP-R was observed. Nevertheless, there are not enough evidences to know the reasons behind CIP-R higher dissolution rate. On one hand the cocrystal itself could dissolve faster than the pure API, and on the other hand there could be a decrease in the dissolution of the API caused by the transformation into CIP·3H_2O on the surface of the tablet.

Results of dissolution rate determinations in simulated gastrointestinal media are shown in Table 2 and Figure 5. Experiments have been done not only in the two gastrointestinal fluids, but also in acetate/phosphate buffer at the same pH. In this way it is possible to evaluate the real effect of the gastric media components (mainly bile salts and lecithin) to dissolution behavior of the compounds, independently of pH. Table 2 and Figure 5a show a different behavior of CIP and CIP-R at pH 5. The cocrystal dissolves faster than the pure API. However the differences cannot be attributed to FeSSIF components since in both cases dissolution rates are practically the same in FeSSIF and in the plain buffer. Again, the reason can be due to a faster dissolution of the cocrystal, or due to the transformation of the API into the trihydrated form, as stated by PXRD. In fact, X-ray analysis of the remaining tablets indicated that the surface of the cocrystals were hardly modified (90% cocrystal and 10% CIP·3H_2O) in these two media, whereas the tables of CIP alone were strongly modified (10% CIP and 90% CIP·3H_2O). As expected,

dissolution rate values of CIP and CIP-R at pH 5 are in between the ones determined at pH 4.0 and 5.5 (Table 1).

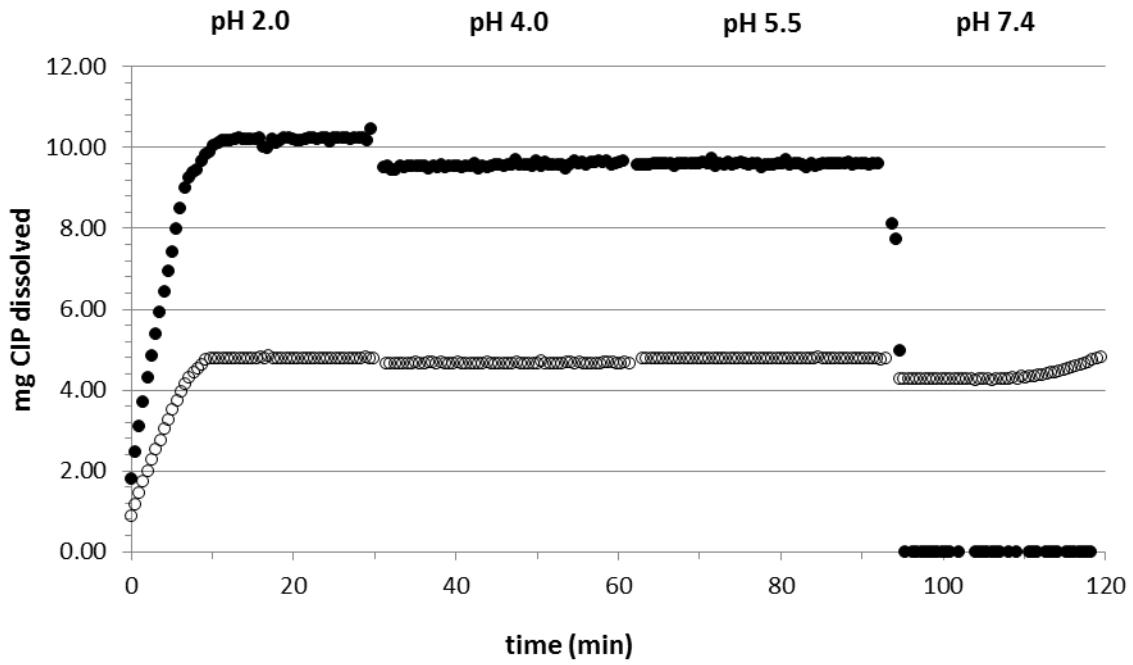

Figure 4: Full pH dissolution experiment for CIP (●) and CIP-R (○).

Results in FaSSIF and the corresponding plain buffer (pH 6.4) (Figure 3b) show that either dissolution rate or percentage of CIP dissolved for CIP and CIP-R are equivalent. Again, these results are located in between the results at pH 5.5 and 7.4 in Table 1 for both compounds. Thus, apparently there is no effect of FaSSIF or FeSSIF components on the dissolution rate; only the ionization degree seems to play an important role in their dissolution. The zwitterionic nature of CIP may be one of the reasons that gastrointestinal simulated fluids have no solubilizing effect on CIP, since other studies [22-24] point out that the micelles formed in these fluids have negatively charged surface, what means attraction for cations, repulsion for anions, and may be no effect on zwitterions.

Table 2: Percentage of CIP dissolved, and dissolution rate of CIP and CIP-R; in FeSSIF, FaSSIF and in standard buffer at the same pH (5.0 and 6.5 respectively).

pH	% CIP Dissolved		Dissolution Rate (mg/min)	
	Ciprofloxacin	Cocrystal	Ciprofloxacin	Cocrystal
FeSSIF (pH = 4.94 ± 0.05)	23 ± 4	94 ± 2	0.07 ± 0.01	0.25 ± 0.04
Buffer pH = 4.94 ± 0.05	26 ± 1	105 ± 4	0.067 ± 0.001	0.29 ± 0.1
FaSSIF (pH = 6.37 ± 0.05)	1.7 ± 0.1	2.5 ± 0.5	0.0038± 0.0004	0.005 ± 0.002
Buffer pH = 6.43 ± 0.04	2.2 ± 0.2	2.10 ± 0.07	0.005 ± 0.001	0.003 ± 0.001

Conclusions

Dissolution rate of ciprofloxacin and its cocrystal with resorcinol have been determined at several pH values of pharmaceutical interest in an acetate-phosphate buffer. Results show that dissolution rate of ciprofloxacin decreases according to ionization state of ciprofloxacin, i.e., it is maximum at pH 2 and has the minimum value at pH 7.4, where ciprofloxacin is mainly in its zwitterionic form. The same behavior is

observed with the cocrystal. However, ciprofloxacin dissolves faster than the cocrystal at pH 2, but the cocrystal dissolves faster than ciprofloxacin at intermediate pH values (4.0, 5.0 and 5.5). The reason can be attributed either to a better dissolution of the cocrystal itself, or a decrease in the dissolution of ciprofloxacin due to the formation of the more insoluble trihydrated form in the surface of the tablets, as stated by PXRD. Gastric fluid components such as lecithin and bile salts have not shown any improvement in the dissolution rate of the studied compounds.

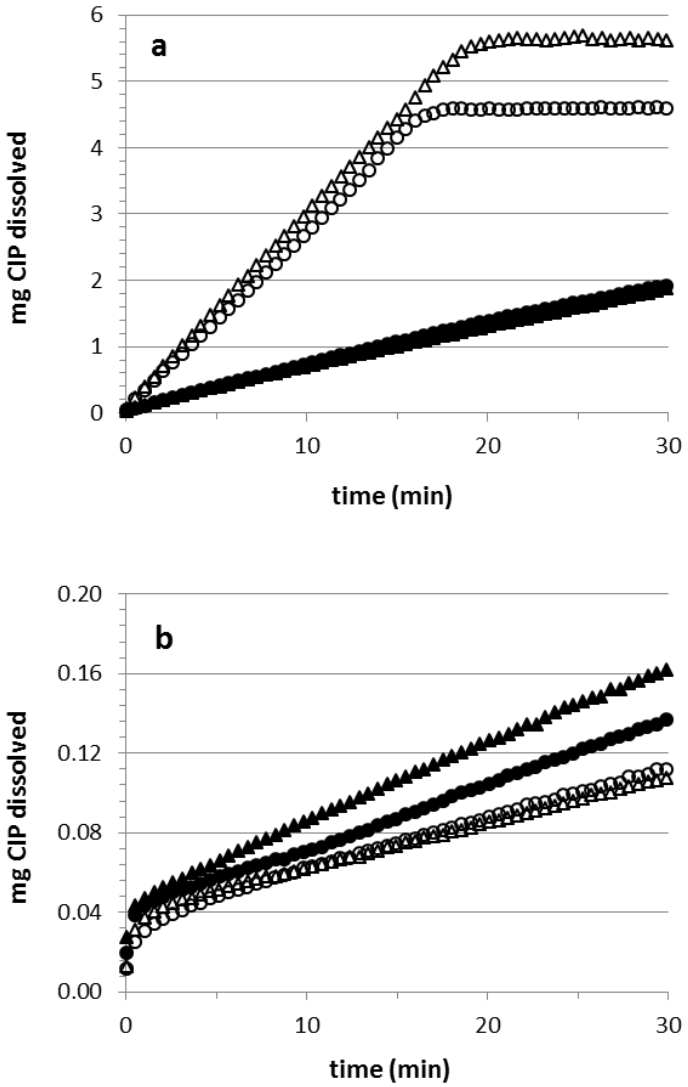

Figure 5: Dissolution profile in simulated gastrointestinal fluids and buffer at the same pH. (a) CIP in FeSSIF (●), CIP in buffer at pH 5 (▲), CIP-R in FeSSIF (○), CIP-R in buffer at pH 5 (Δ); (b) CIP in FaSSIF (●), CIP in buffer at pH 6.5 (▲), CIP-R in FaSSIF (○), CIP-R in buffer at pH 6.5 (Δ).

Acknowledgements

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