In vitro simulation of drug interaction: ciprofloxacin/zinc chloride

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In vitro dissolution testing has long been used as a tool in drug product development and quality control, however, its potential for drug/food and drug/drug interactions has not yet been fully exploited. Ciprofloxacin absorption in vivo may be reduced when co-administered with different metallic compounds. In the present study, in vitro ciprofloxacin solubility and drug dissolution from tablets were performed in the reactive media containing zinc chloride in order to simulate ciprofloxacin/zinc interaction observed in vivo. The precipitates collected from dissolution vessel and from mixture containing ciprofloxacin-hydrochloride and zinc-chloride were investigated using XRPD, TGA, DCS, FTIR. Ciprofloxacin-hydrochloride solubility and drug dissolution from tablet were reduced in aqueous media containing increasing amounts of zinc-chloride. Complex with probable chemical structure $[cfH_2]_2 \cdot [ZnCl_4] \cdot 2H_2O$ *was generated in the presence of high concentrations of ciprofloxacin-hydrochloride and zinc-chloride, indicating that small volume dissolution experiments can be useful in biopharmaceutical characterisation of drug interaction studies.*

Key words: Drug interaction – Dissolution – Solubility – Characterisation of the precipitates – Ciprofloxacin.

In vitro dissolution testing has long been used as a tool in drug product development and quality control. Although there is an emerging interest for *in vitro* simulation of drug/food and drug/drug interaction studies, the potential of dissolution testing has not yet been fully exploited in this area. Ciprofloxacin is fluoroquinolone antibiotic which is characterised by pH dependent solubility and variable permeability along the gastrointestinal tract [1]. Ciprofloxacin is rapidly absorbed from the proximal parts of the gastrointestinal tract with absolute bioavailability ranging between 60 and 80 % of the administered dose [2-4]. Pharmacokinetic studies have shown reduced bioavailability of ciprofloxacin after simultaneous administration with metal cation (iron, zinc, calcium, aluminium) containing preparations [5-7]. Generally, formation of nonabsorbable complex has been postulated as the interaction mechanism [8-10] although some authors commented that other physicochemical factors, such as solubility, may also play a role [6]. It has been reported that ciprofloxacin hydrochloride tablet dissolution is retarded in the presence of metallic compounds indicating that it might be used as an *in vitro* tool for drug interaction simulation [9, 11, 12]. Numerous ciprofloxacin-zinc complexes have been isolated and described in the literature [13-15] but the biopharmaceutical relevance of that studies is unknown as the complexes described were isolated, mainly, from non-aqueous media. On the other hand, Zupančič *et al*. [16] synthesised two types of ciprofloxacin-zinc complexes in aqueous media and suggest that complexation between ciprofloxacin and zinc salts (i.e chloride or sulfate) is highly pH dependent. It has been shown in a previous study [11] that *in vitro* solubility/dissolution studies together with characterisation of the precipitates formed can be used in biopharmaceutical characterisation of physicochemical ciprofloxacin-iron interaction. Previously, interaction between ciprofloxacin hydrochloride and ferrous sulfate was observed in mini paddle apparatus and small volume of media [11]. Bearing in mind that there is no literature data related to biopharmaceutical characterisation of ciprofloxacin-zinc interaction using mini paddle apparatus, thus ciprofloxacin-zinc interaction was simulated *in vitro* by performing dissolution studies of ciprofloxacin hydrochloride in the reactive small volume media containing different amounts of zinc chloride. Solubility studies of ciprofloxacin hydrochloride in the presence of increasing amounts of zinc chloride were also performed. The precipitates collected from the dissolution vessel and mixture of ciprofloxacin hydrochloride and zinc chloride solutions were investigated for their solid state properties in order to elucidate the potential interaction mechanism involved.

I. MATERIALS AND METHODS

1. Materials

Ciprofloxacin tablets (Marocen 500 mg, Hemofarm-Stada, Serbia) were purchased commercially. Marocen tablets contain 500 mg of ciprofloxacin as the active ingredient and the other ingredients are: microcrystalline cellulose, crospovidone, hydroxypropyl methylcellulose, macrogol 4000, magnesium stearate, maize starch, colloidal silicon dioxide, titanium dioxide (E 171). Ciprofloxacin hydrochloride crystalline powder was kindly donated from Hemofarm-Stada, Serbia. The investigated compouds zinc chloride (Centrohem, Serbia) was of analytical grade and used for the interaction study.

2. Solubility study

Ciprofloxacin hydrochloride crystalline powder solubility in water and the reactive media containing different amounts of zinc chloride was determined. All determinations were performed at ambient temperature (25 °C). Samples were continuously shaken on a laboratory shaker (Unimax 1010, Heidolph, Schwabach, Germany) for 6 h and, after centrifugation and filtration through the membrane filter (pore size 0.45 μm), appropriately diluted and assayed UV spectrophotometrically (UV-Vis spectrophotometer Evolution 300, Thermo Fisher Scientific, Madison, United States) at 276 nm. Samples pH values were monitored (pH meter-Hanna 9321, United States).

3. Complex formation

In order to investigate the potential for complex formation, ciprofloxacin hydrochloride and zinc chloride solutions in water (0.05 and 0.2 M, respectively) were mixed in different ratios (9:1, 7:3, 5:5, 3:7 and 1:9) and examined for precipitate formation. The sample, where a precipitate was formed was collected after centrifugation and filtration through membrane filter, dried at room temperature and investigated for its solid state properties.

4. Dissolution studies

Dissolution studies were performed in the mini paddle dissolution apparatus (Erweka DT 700, Heusenstamm, Germany) at 50 rpm using 50 mL of water as dissolution media. To evaluate the effect of zinc on ciprofloxacin dissolution, relevant amounts of zinc chloride were added to each vessel concomitantly with ciprofloxacin tablet. Dissolution samples (0.5 mL) were withdrawn at the predefined time intervals, filtrated through the membrane filter (pore size 0.45 μm),

appropriately diluted and assayed UV spectrophotometrically (UV-Vis spectrophotometer Evolution 300, Thermo Fisher Scientific, Madison, United States) at 276 nm. The experiments were performed in triplicate. Where a precipitate was formed during the dissolution studies, it was collected at the end of dissolution run and dried at room temperature for further characterisation. Sample pH values were monitored (pH meter-Hanna 9321, United States) at the end of dissolution run.

5. Characterisation of the precipitates formed

Characterisation of the precipitates formed was performed using differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), powder X-ray powder diffraction (XRPD) and Fourier transform infrared analysis (FTIR).

DSC experiments were performed using a Mettler Toledo DSC 821 with a refrigerated cooling system LabPlant RP-100. The sample weight was 5 mg or more and sample volume was sufficient to provide proper contact between the powder and the bottom of the pan. DSC measurements were carried out at a heating/cooling rate of 10 °C/min. The DSC system was controlled by Mettler Toledo Star software (version 6.10) working on a Windows NT operating system. The unit was calibrated with indium and zinc standards [17].

TGA measurements have been performed by a Mettler TG 50 module connected with a Mettler MT5 balance and monitored by Mettler Toledo STAR software (version 6.10). All the measurements have been performed while heating the samples from 20 to 250 °C at the rate of 10 °C/min in the nitrogen. Mass of the samples was about 5-12 mg [17].

XRPD analysis was conducted using a Miniflex II Desktop X-ray diffractometer Rigaku with Ilaskris cooling unit with Cu Kβ radiation. The scanning angle ranged from 5 to 40° of 2θ scale at a step size of 0.05°/s in each case [17].

FTIR spectra were recorded on a Fourier Transform Infrared Nicolet Magna IR 560 E.S.p spectrophotometer coupled with a MCT/A detector. The data were acquired with the Nicolet software Omnic (version 4.1). Sixty-four scans of symmetrical interferograms were averaged and the spectrum was calculated from 650 to 4000 cm^{-1} at 2 cm^{-1} spectral resolution. A KBr disk method was used with a 1 % sample loading. KBr disks were prepared by direct compression under 8 bar pressure for 1 min [17].

II. RESULTS

1. Solubility study

Concentrations of ciprofloxacin dissolved in the reactive media containing different amounts of zinc chloride are presented in *Figure 1*. Experimentally obtained aqueous solubility of ciprofloxacin hydrochloride was determined to be 42 mg/mL (i.e 109 mM, final pH 4.04) and it decreased ten-fold in the presence of zinc chloride. The effect observed can not be attributed to the pH dependent ciprofloxacin solubility where ciprofloxacin solubility in water is high at pH values below 5 and above 10, and low near the isoelectric point, which is close to neutral [18]. Also, it was postulated that both common ion effect and formation of new ionic species may contribute.

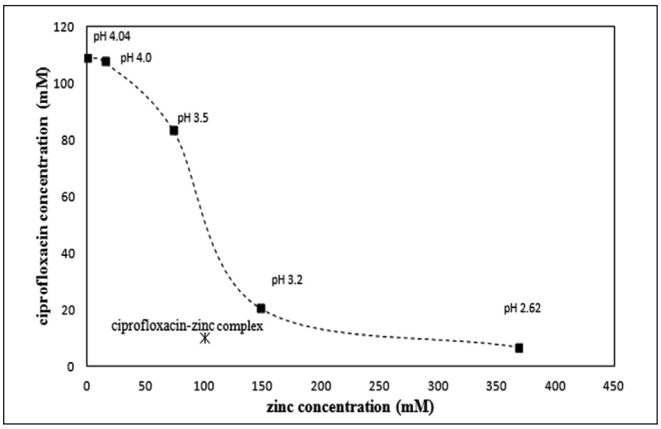

Figure 1 - Concentration of ciprofloxacin hydrochloride dissolved in reactive media containing different concentration of zinc chloride. Point × refers to experimentally obtained ciprofloxacin concentration in the solution above the precipitate collected from mixture of ciprofloxacin hydrochloride and zinc chloride solutions (i.e. ciprofloxacin-zinc complex).

2. Complex formation

When clear solutions of ciprofloxacin hydrochloride and zinc chloride were mixed in different volumetric ratios, the immediate and the most abundant precipitation, indicating potential complex formation, was observed in the mixture containing 25 mM ciprofloxacin hydrochloride and 100 mM zinc chloride (1:4 molar ratio). Ciprofloxacin concentration in the solution above the precipitate was found to be 3.8 mg/mL (~ 9.9 mM; pH 3.24).

3. Dissolution study

Ciprofloxacin tablet dissolution profiles obtained in water without/with different amounts of zinc chloride added are presented in *Figure 2*. Ciprofloxacin tablet dissolution in water (i.e control) was rapid and almost complete. Addition of 10 and 14 mg/mL (i.e 74 and 103 mM, respectively) zinc chloride slightly decreased ciprofloxacin dissolution, but the total amount dissolved was not affected. In the presence of 20 mg/mL zinc chloride (ciprofloxacin to zinc molar ratio ~ 1:5), ciprofloxacin tablet dissolution was markedly reduced, with total of 54 % dose dissolved. The concentration of ciprofloxacin dissolved was lower when compared to its equilibrium solubility in media containing the same amount of zinc chloride (i.e. 5.4 vs 7.9 mg/mL), indicating the presence of non-sink conditions. Final media pH values were 4.04, 3.40, 3.24 and 3.21 in pure media (control) and media containing 10, 14 and 20 mg/mL zinc chloride, respectively, indicating that the effects observed could not be attributed solely to the media pH value. In addition, excipients present in Marocen tablet containing 500 mg ciprofloxacin hydrochloride have no influence on ciprofloxacin dissolution behaviour.

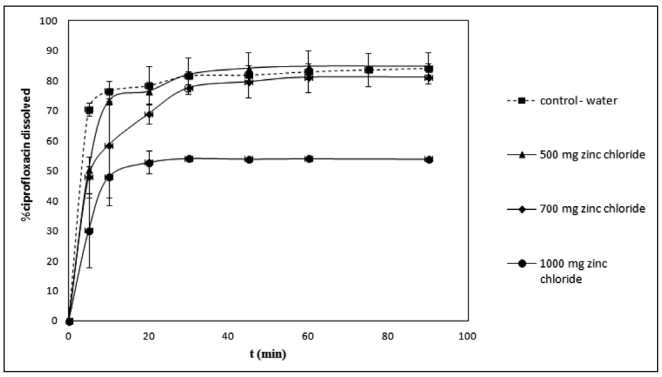

Figure 2 - Ciprofloxacin tablet dissolution in water without (control) and with addition of different amounts of zinc chloride.

4. Characterisation of the precipitates formed

X-ray powder diffractograms (XRPD) of the different solid phases investigated are presented in *Figure 3*. In contrast to the ciprofloxacin hydrochloride and ciprofloxacin betaine (base) diffractograms, prominent peaks at 11.35, 12.60 and 17.15 2θ can be observed in the diffractogram of the precipitate formed from the mixture of ciprofloxacin hydrochloride and zinc chloride solutions. Similar pattern can be observed in the diffractogram of the precipitate collected from the dissolution study, although the peak intensities were somewhat lower. It may be postulated that the observed decrease in peak intensities may be due to a reduction in the crystalline fraction of the precipitate caused by the presence of tablet excipients.