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Chromatographic and permeation analysis of ciprofloxacin metal complexes

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Abstract

Aqueous solutions of ciprofloxacin and its metal complexes with Mg, Cu, Bi and Fe were investigated with the aim to characterize their retention and permeability properties with consequent complex stability evaluation. For this purpose HPLC coupled with UV detector for ciprofloxacin and with atomic absorption spectrometer for metal ions detection was used. Determination of lipid barrier permeability was carried out using a Sartorius absorption apparatus with the lipid-type of membrane. Under the applied experimental conditions, no significant differences in retention and permeability properties were found, indicating the low stability of studied ciprofloxacin complexes in aqueous solutions. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ciprofloxacin metal complexes; Chromatography; Diffusion

1. Introduction

Ciprofloxacin is the member of broad-spectrum fluoroquinolones, synthetic antibiotics introduced into clinical practice some ten years ago mainly for the treatment of infections of internal organs. It is one of the most commonly prescribed fluoroquinolones, being especially active against Gram-negative organisms including many organisms resistant to penicillins, cephalosporins and aminoglycosides (Rang et al., 1995). Although in general, the fluoroquinolones are well absorbed after oral ingestion, ciprofloxacin absorption varies in the range between 50 and 70% (Neuman, 1988). Additionally, numerous pharmacokinetic studies have shown reduced bioavailability of fluoroquinolones after simultaneous administration of metal ions containing preparations (Sahai et al., 1993; Marshall and Piddock, 1994; Kozjek et al., 1996). Drugs like antacids and mineral-vitamin preparations as well as dairy products can

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therefore lower plasma concentrations of fluoroquinolones resulting in diminution of efficacy or even inactivation (Lomaestro and Bailie, 1991; Hart et al., 1991), raising the risk of resistance development as well. One probable explanation for reduced bioavailability is complex formation between fluoroquinolone molecule and the metal cation. The resulting complex could be then too large to be effectively transferred through the membrane lipopolysaccharide layer. The fluoroquinolone molecule can provide two groups of attachment to the central metal atom, namely 3-carboxyl and 4-oxo, which are also known to be essential for pharmacologic activity of the drug (Vincent et al., 1981; Polk, 1989; Lomaestro and Bailie, 1991). These groups can therefore form bonds of ionic or coordinate covalent character to the central cation, creating a chelate-type complex. Data on complex formation and its role in vivo reported in literature are, however, divergent. In spite of different experimental approaches and conclusions, it is clear that the stability of the complex is greatly dependent on the nature of a quinolone, a metal ion involved and environmental conditions. In the case of ciprofloxacin, for example, complexes of stability order $Fe^{3+} \approx Al^{3+} > Cu^{2+} > Fe^{2+} \approx Zn^{2+} > Mg^{2+} > Ca^{2+}$ were demonstrated by potentiometric titrations. In the same study a very good correlation between formation constants of norfloxacin complexes and reduced bioavailability in dogs was found (Wallis et al., 1996). The oxidation of Fe^{2+} to Fe^{3+} at pH of small intestine and complex formation with ciprofloxacin was established in another study (Kara et al., 1991). On the other hand, the investigation of ciprofloxacin interactions with Al^{3+}, Mg^{2+}, Ca^{2+} and Fe^{2+} salts in aqueous solutions showed that ciprofloxacin does not form complexes with these ions (Sanchez et al., 1994). Some kind of molecular associates were found, suggesting that these could be the reason of reduced absorption of ciprofloxacin as well.

The aim of present study was the characterization of ciprofloxacin and its complexes with Mg, Cu, Bi and Fe using distribution and permeability measurements. Distribution between two immiscible solvents and solubility are among several physicochemical properties that can be used for evaluation of complex formation and stability. As partition chromatography in many instances proved to be useful for comparison of distribution characteristics inside a specified group of compounds, reversed phase HPLC with UV detection was applied for this purpose. Elution of metal ions as determined by HPLC coupled with atomic absorption spectrometry was monitored with the aim to support obtained distribution data. Determination of diffusion rate constants using Sartorius absorption model was the basis for the lipid

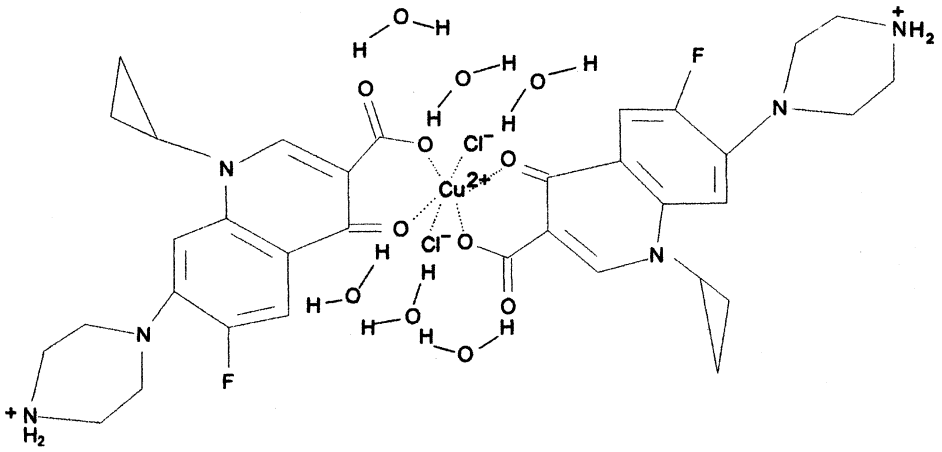

Fig. 1. Structure of Cu–ciprofloxacin complex as determined by X-ray diffraction analysis indicating the possible sites of metal chelation. Similar spatial arrangements can be found for other complexes used in this study as well.

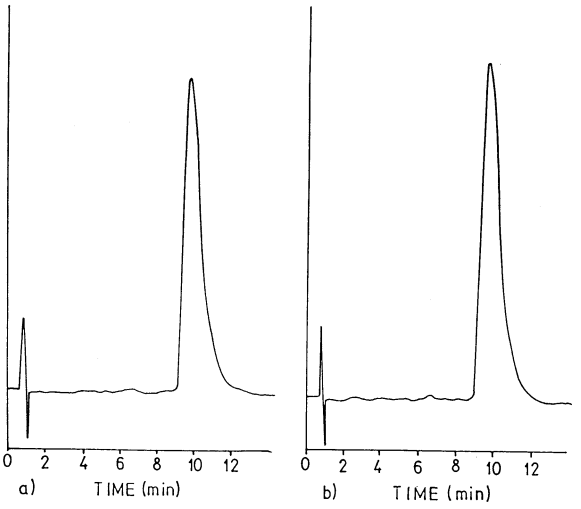

Fig. 2. Chromatograms of (a) ciprofloxacin and (b) Fe–ciprofloxacin complex aqueous solution.

barrier permeability evaluation of ciprofloxacin and its complexes.

2. Materials and methods

2.1. Chemicals and solutions

Ciprofloxacin (Cf) was obtained from Krka Pharmaceuticals (Novo mesto, Slovenia); complexes of ciprofloxacin (Fig. 1): $Fe(Cf)_3 \cdot 4H_2O$, $Bi(Cf)_3Cl \cdot 3H_2O$, $Cu(Cf)_2Cl_2 \cdot 6H_2O$ and $Mg(Cf)_2 \cdot 4H_2O$ were supplied by Faculty of Chemistry and Chemical Technology, University of Ljubljana (Turel, 1995; Zupančič and Bukovec, 1996). HPLC grade methanol (Merck, Darmstadt, Germany), glass distilled deionized water, analytical grade potassium dihydrogenphosphate and sodium hydrogenphosphate were used for preparation of mobile phases. Sample solutions for HPLC were prepared by dissolving approximately 10 mg of each compound in 100 ml of deionized water, stirred for 3 h and filtered through cellulose filter. 3 ml of obtained stock solution were further diluted with phosphate buffer pH 7.0 up to 10 ml. Sample solutions for diffusion experiment were prepared by ten times dilution of stock solution with deionized water. The membrane and the lipid components needed for diffusion experiment were obtained from Sartorius AG (Göttingen, Germany).

2.2. HPLC experiment

The liquid chromatograph comprised a Knauer 364 solvent delivery pump, a Rheodyne 7125 sample injector with a 20-μl loop, a Knauer strip chart recorder or HP 3395 integrator. In one set of experiments the liquid chromatograph was equipped with a Knauer variable wavelength UV detector set at 275 nm. For another set of experiments the atomic absorption spectrometer Perkin Elmer AAS 2280 was coupled with liquid chromatograph using thermospray sample introduction system. The 120×4 mm I.D. stainless steel column packed with 5 μm packing of Nucleosil

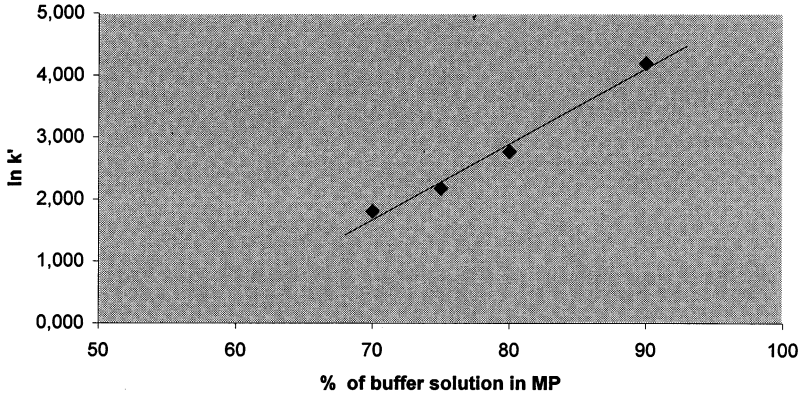

Fig. 3. The relationship between $\ln k'$ (capacity factor) and percent of buffer solution in mobile phase.

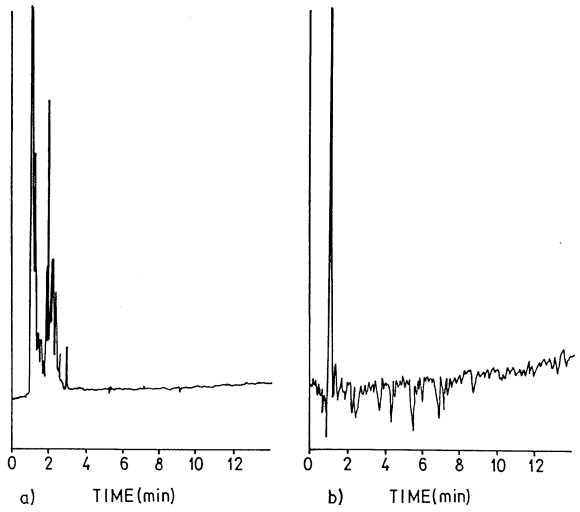

Fig. 4. Chromatogram of (a) Cu–ciprofloxacin complex and (b) Mg–ciprofloxacin complex aqueous solution as recorded by an AAS detection.

100 C18 was used. The chromatography was carried out at room temperature with a mobile phase consisting of methanol and 0.02 M phosphate buffer solution at pH 7.0 (25:75, v/v). The flow rate was 1 ml/min. After equilibrating the chromatographic system, samples of each individual compound as well as mixtures of them were injected into the system and their respective chromatograms recorded.

2.3. Diffusion experiment

Diffusion testing was performed at room temperature using Sartorius absorption apparatus SM-16750 consisting of two containers separated with an artificial membrane (Stricker, 1971). The membrane of type SM-RS (cellulose nitrate) was impregnated with the mixture of lipid components N (1-dodecanol) and S1 (sodium dioctylsulfosuccinate) in the ratio 4.20:0.10. A peristaltic pump was used to achieve the uniform circulation of solutions at the membrane, enabling faster equilibration of the system as well. The 100-ml aqueous solutions of ciprofloxacin or its metal complexes in concentration of approximately 10 mg/l were put in the container I and 100 ml of distilled water in the container II. After starting a peristaltic pump, 2 ml of sample solutions were taken from both containers at time zero, after 30 min and then after every 60 min for 7 h. The withdrawn samples were analyzed spectrophotometrically and afterwards returned into the system. The amounts of compound diffused after different time intervals were used for diffusion rate constant calculation according to Stricker's equation (Stricker, 1971):

$$K_d = \frac{(Cm_2 - Cm_1)V_0}{(t_2 - t_1)C_0F}$$

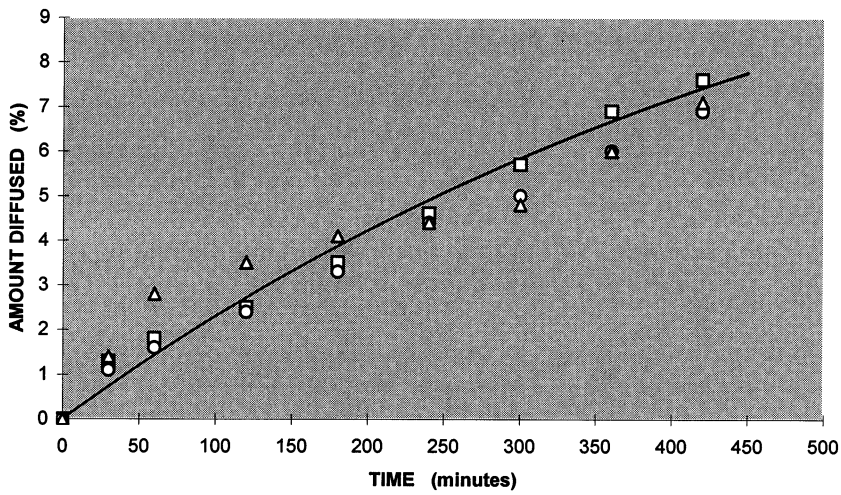

Fig. 5. Amount of ciprofloxacin diffused through an artificial lipid membrane from ciprofloxacin (□), Fe–ciprofloxacin (Δ), and Bi–ciprofloxacin complex (○) aqueous solutions.

Table 1
Mean diffusion rate constants as determined according to Stricker equation (cm/min) for ciprofloxacin (Cf), Fe–ciprofloxacin complex (Fe–Cf), and Bi–ciprofloxacin complex (Bi–Cf) showing also that the difference between the means is not significant (NS) at a probability of 95%

Compound	Experiment 1	Experiment 2	Experiment 3	Mean value	S.D.	ANOVA
Cf	0.00031	0.00043	0.00039	0.00038	0.00006	NS
Fe–Cf	0.00036	0.00027	0.00035	0.00033	0.00005	NS
Bi–Cf	0.00038	0.00035	0.00029	0.00034	0.00005	NS

where K_d is diffusion rate constant of a particular compound, t is time, C_0 is an initial concentration of ciprofloxacin or its metal complex in container I, V_0 is an initial volume of solution in container II (100 ml), F is a membrane area (40 cm^2), Cm_2 and Cm_1 are concentrations of particular compound in container II at time t_2 and t_1 respectively. The diffusion rate constants were compared statistically with the aim to detect a potential significant difference in diffusion properties among studied compounds.

3. Results and discussion

Distribution characteristics of complexes are in general distinguishable from those of the parent molecule. Reversed phase liquid chromatography was used for such comparative evaluation of distribution behaviour of ciprofloxacin and its complexes with Fe^{3+}, Bi^{3+}, Cu^{2+} and Mg^{2+}. The chromatographic method used was similar to several published analytical procedures for ciprofloxacin using ODS column. In our case, however, instead of acidic conditions more suitable for separation purposes, the phosphate buffer solution at pH 7.0 was used. The purpose of avoiding acidic conditions during analysis was to use conditions more favorable for retainment of complex integrity. Although, as expected, resulting chromatograms showed increased peak tailing, the performance of the method was still adequate for the purpose of this study. Within day precisions were 4.2% for peak area measurements and 0.4% for retention time measurements, while between day precisions were 4.5 and 1.8% for peak area and retention time measurements, respectively. The linearity of the method was also demonstrated in the range between 50 and 150% of studied concentration with correlation coefficients higher than 0.980. Concerning experimental conditions and performance, the developed method proved to be suitable for our purposes and was applied afterwards for the determination of ciprofloxacin and its complexes in aqueous solutions.

Chromatograms obtained after injections of ciprofloxacin, its individual complexes or their mixtures showed very similar pattern without additional broadening or even splitting of peaks and with the same retention times at about 9.3 min for all compounds (Fig. 2). It is obvious that only ciprofloxacin has been retained by the stationary phase thus proving the dissociation of complexes in sample solutions. Using mobile phases with different contents of buffer solution no separation between ciprofloxacin and its complexes was achieved as well, confirming previous results. With linear extrapolation of ln k' (capacity factor) values to 100% buffer content in mobile phase (Fig. 3), a high value of 5.32 was obtained indicating high lipophilicity of ciprofloxacin, explaining also low permeability and high membrane adsorption determined in Sartorius diffusion experiment.

Magnesium and copper complexes were used for chromatographic retention determination of metal ions. As it is reported that these ions and especially those of copper form quite stable complexes with fluoroquinolones in the pH range between 5.5 and 7.5, some retainment of ions could be expected. Atomic absorption spectrometer coupled with HPLC was used to monitor elution of studied cations. At retention times of

ciprofloxacin no metal ions were detected (Fig. 4), indicating that neither of these cations is coeluted with ciprofloxacin. From chromatograms shown it is evident that Mg^{2+} ions are eluted within solvent peak, whereas a slight retention can be noticed in the case of Cu^{2+} ions. This retainment, however, can not be used as an evidence for complex presence, as this would change the retention time and the shape of ciprofloxacin peak as well. Rather this observation can be attributed, in accordance with known high affinity of Cu^{2+} ions for polar groups, to interactions of these cations with free silanolic groups present on the stationary phase.

Diffusion experiment was performed in triplicate with ciprofloxacin and its complexes with Bi^{3+} and Fe^{3+}. Average amounts of compounds diffused are shown in Fig. 5 together with the simulated concentration time profile. At several time points some differences can be noticed among studied compounds, showing however a very similar diffusion pattern. Statistical evaluation of diffusion rate constants obtained leads to the same conclusion. Using ANOVA a significant difference between the means can not be proved at a probability level of 95% (Table 1). In addition, all three compounds are absorbed/adsorbed onto the lipophilic membrane in a similar large extent. From these results it can be concluded that only ciprofloxacin as such, whose high lipophilic character is due to zwitter ion formation at experimental conditions, is participating in diffusion process.

From our experiments based on partition chromatography and diffusion experiment no evidence for existence of stable ciprofloxacin complex with Fe^{3+}, Bi^{3+}, Mg^{2+} and Cu^{2+} in aqueous solutions was found. Organic solvents in the case of chromatography and dynamic nature of both processes can in the course of an experiment actually influence the equilibrium conditions of complexes studied. The situation in vivo is, however, also dynamic and heterogeneous. Experimental results obtained in our study therefore strongly suggest that not the complex formation, but rather some other processes influenced by the presence of metal ions are responsible for impaired ciprofloxacin absorption. For this reason, adsorption on coadministered substances, influence on paracellular route of absorption and effects on gastrointestinal motility seem to be more probable cause of reduced bioavailability of ciprofloxacin in man and animals.

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