Scientific paper

Study of Solution Equilibria Between Gadolinium(III) Ion and Moxifloxacin

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Abstract

The complex formation equilibria between gadolinium(III) ion and moxifloxacin (MOXI) were studied in aqueous solutions. The investigations were performed by glass electrode potentiometric (ionic medium: 0.1 mol dm^{-3} LiCl, 298 K) and UV spectrophotometric measurements. In the concentration range $0.5 \leq [Gd^{3+}] \leq 1.0$; $1.0 \leq [MOXI] \leq 2.0$ mmol dm^{-3} ([MOXI]/[Gd] = 1 : 1 to 5 : 1) and pH between 2.5 and 9.0, gadolinium(III) and moxifloxacin form the complexes of the composition: $Gd(HMOXI)^{3+}$, $Gd(HMOXI)_2^{3+}$, $Gd(HMOXI)_3^{3+}$, $Gd(HMOXI)_2MOXI^{2+}$, $Gd(HMOXI)(MOXI)_2^{+}$, $Gd(MOXI)_3$. The stability constants of the complexes were calculated with the aid of Hyperquad2006 suite of programs, taking into account the hydrolysis of Gd^{3+} ion and protonation of moxifloxacin anion. The possible structure of the complexes, in solution, and their formation mechanism is suggested. The effect of moxifloxacin, and for comparison purpose, DTPA on gadolinium(III) plasma speciation was evaluated by computer simulation.

Keywords: Gadolinium; moxifloxacin; complex formation; solution equilibrium, speciation

1. Introduction

Quinolones are synthetic antibacterial agents widely used in clinical practice for urinary and respiratory infection treatments.[1] Moxifloxacin (MOXI) (1-cyclopropyl-7-[2,8-diazobicyclo(4.3.0)nonane]-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3-quinolone carboxylic acid) (Fig. 1) is a new 8-methoxyquinolone derivate of fluoroquinolones with enhanced activity against Gram-positive bacteria while preserving high activity against Gram-negative bacteria.[2]

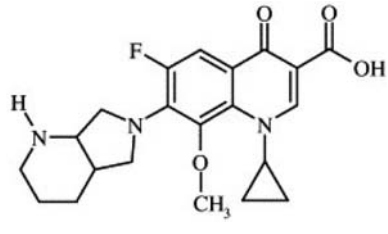

Fig. 1. Structure of moxifloxacin

Fluoroquinolones suppress cell growth by inhibiting activity of bacterial DNA gyrase, an essential bacterial enzyme that maintains superhelical twists in DNA.[3] Some evidence suggests that these drugs interact directly with DNA, blocking the activity of DNA-gyrase repair enzymes.[4]

Recent studies indicate an important role of metal ions in the mechanism of action of these drugs.[5] In the first place, the activity of quinolones is reduced in the presence of certain metal ions by the formation of sparingly soluble metal complexes.[6] On the other hand, it was proposed that metal ions (especially magnesium) are involved in the mechanism of action of these

drugs.[7–10] Structural studies have also been performed on magnesium – norfloxacin[11], magnesium – ciprofloxacin[12–15] and magnesium – ofloxacin and levofloxacin complexes.[16] Metal ions may change the bio-availability of quinolones by changing their solubility or their lipophilicity. The metal complexes of quinolones may have new biological properties in terms of altered minimal inhibitory concentration, antibacterial spectrum, etc.[17] Recently, three novel gadolinium complexes of fluoroquinolone, $Gd(L)_3 \cdot 6H_2O$ {L = Norfloxacin (NFLX), Ofloxacin (OFLX) and Ciprofloxacin (CPLX), respectively}, have been synthesized and inhibitory effect of the ligands and complexes on leukemia HL-60 cell line has been measured by using MTT (Methyl-Thiazol-Tetrozolium) assay method and liver cancer BEL-7402 cell line measured by SRB (Sulphurhodamin B) method.[18] The results indicate that the complex $Gd(OFLX)_3 \cdot 6H_2O$ has strong inhibitory effect on BEL-7402 cell line and $Gd(CPLX)_3 \cdot 6H_2O$ has strong inhibitory effect on HL-60 and BEL-7402 cell lines.

Gadolinium based chelates are widely used as magnetic resonance, or CT scan imaging agents.[19,20] These chelates may interact with quinolones upon concomitant intake (patients already on antibacterial therapy) or release free gadolinium ion in plasma which may interact with plasma or other ligands (ie. drugs). On the other hand quinolone chelates of gadolinium may be candidates for imaging agents.

Therefore, the aim of the present paper is to quantitatively examine the equilibria in moxifloxacin solution in the presence of gadolinium ion to gain better understanding of the identity, stability and speciation in gadolinium and fluoroquinolone family member, moxifloxacin, aqueous solutions. The speciation model derived from such fundamental study should help in pharmacokinetic studies of quinolones in the presence Gd-containing agents and also in the study of toxic effects of Gd-ion upon concomitant intake of Gd-containing compounds and fluoroquinolones.

In this work we studied the complex formation between gadolinium(III) ion and moxifloxacin by using potentiometric and UV spectrophotometric measurements.

2. Experimental

2. 1. Reagents and Analysis

All reagents were of analytical grade purity and were used without further purification. Doubly distilled water was used for preparation of all solutions. Calibrated class A volumetric glassware (relative error in volume measurements less than 1%) was used for analytical work. All mass measurements were made on an electronic balance Ohaus model DV215CD (precision: ± 0.01 mg). The stock solution of gadolinium(III) chloride was prepared by dissolving Gd_2O_3, (p.a., Merck) in HCl ("Suprapure", Merck) and standardized by complexometric titrations using EDTA. The appropriate amount of HCl was added into a stock solution to avoid initial hydrolysis of Gd^{3+} ion. The excess HCl concentration in the gadolinium chloride stock solution was determined potentiometrically using Gran's method, ie., by plotting $(V_0 + V_b) \cdot 10^{E/Q}$ against $V_b \cdot V_0$ is initial volume of the titrated solution, V_b is a volume of added strong base (NaOH), E is a measured emf of the cell and Q is a slope of the glass electrode response. A straight line so obtained, intersects V_b axis at point which is equal to V_e (equivalence volume). The concentration of gadolinium stock solution was 0.0275 mol/dm^3 and HCl, 0.0472 mol dm^{-3} with relative uncertainty better than 1%, as calculated by error propagation formulae. The constancy of the total proton concentration in $GdCl_3$ solution with time was considered as a criterion for the absence of initial Gd^{3+} hydrolysis and was periodically checked by titration against standard NaOH before each series of measurements.

Moxifloxacin hydrochloride, (declared purity > 99%), yellow powder, M_r = 437.9, was obtained from BayerPharma AG (Germany). The standard solution of moxifloxacin (5.35 mmol dm^{-3}) was prepared by direct weighing of the standard substance. The standard solution of HCl was added and its concentration was determined by Gran's method as 6.35 mmol dm^{-3}.

A sodium hydroxide solution was prepared from concentrated volumetric solutions (p.a., Merck, FRG), of nominal concentration 1 mol dm^{-3} (maximum declared error less than 2%) by dilution with freshly boiled doubly distilled water, followed by cooling under a constant flow of purified nitrogen. The alkali concentration was checked by titration against potassium hydrogen phthalate. The prepared titrant had a concentration 0.100 mol dm^{-3}.

A hydrochloric acid solution was made from HCl, ("Suprapure", Merck) and standardized against tris-(hydroxymethyl)aminomethane. The final concentration was 0.104 mol dm^{-3}.

A lithium chloride solution was prepared from LiCl, (p.a., Merck), by dissolving the re-crystallized salt in twice-deionized water. The concentration of this solution was determined by evaporation of a known volume of solution to dryness at 423 K and weighing the residue.

Nitrogen gas, used for stirring solutions and providing an inert atmosphere during the titrations, was purified by passing it through 10% NaOH then 10% H_2SO_4, alkaline solution of pyrogallol, 0.1 mol dm^{-3} solution of KCl and finally distilled water.

2. 2. Apparatus and Procedure

Potentiometric titrations were carried out in a double-walled glass vessel, thermostatted at 298 K. Measurements were made on a Tacussel Isis 20000 pH meter (precision ± 0.1 mV or ± 0.002 pH units) equipped with a Ra-

diometer combined electrode. A Metrohm Dosimat model 665 automatic burette with anti-diffusion tip, was used for delivery of the titrant. The nominal volume of the burette was 5.00 mL. The burette was calibrated in three points. Thus the calculated error in volume was less than ± 10 μL and declared resolution was ± 5 μL. The ionic strength of all test solutions was adjusted to 0.1 mol dm^{-3} with lithium chloride. All measurements were performed under a nitrogen atmosphere.

To reduce the concentration of the hydrogen ion, the alkali was added stepwise from an autoburette in small aliquots (0.005–0.01 cm^3). The potential was monitored after each addition of titrant. The titration protocol was chosen in such a way that the hydrolysis and complexation reactions would proceed in the conditions as close to true equilibrium as possible.[21] Usually stable potential readings were obtained in 3–5 min after addition of the titrant at the beginning of the titration (pH < 3) and in 5–10 min at pH values higher than 3. Potential of the glass electrode is given by the expression: $E = E_0 + Q\ log\ h + E_j$ where h is the concentration of free proton, E_0 is a constant which include standard potential of the glass electrode, Q is the slope of the glass electrode response and E_j is liquid junction potential. The parameters, E_0, Q and E_j were determined by strong acid – strong base titration to check the system suitability. During the titrations of the test solutions the E_0 was determined using the data in the acidic region where no hydrolysis or complexation takes place (so that h is equal to the analytical concentration of proton), by plotting $E - Q\ log\ h$ against h and extrapolating the straight line so obtained to $h = 0$. The free proton concentration was then calculated through the equation: $log\ h = (E - E_0 - E_j)/Q$ which was applied to the whole titration curve. All titrations were carried in duplicate. The agreement between duplicate titration was better than 1%. The water autoprotolysis constant was taken as $pK_w = 13.78 \pm 0.02$.

Spectral measurements were made on double beam UV–Vis spectrophotometer model Lambda 35 (Perkin Elmer, U.S.A.). Operational parameters were: scan speed, 2 nm/s, slit width, 0.3 nm, photometric sensitivity, 0.2 abs. units. Matching pair of 1 cm quartz cuvettes was used for measuring the spectra. Spectral measurements were made on solutions in which the concentration of gadolinium and moxifloxacin were constant (C_{Gd} = 0.072, 0.033 and 0.017 mmol dm^{-3}, C_{MOXI} = 0.051 and 0.035 mmol dm^{-3}) while pH was varied between 3.0 and 9.0 (10 solutions). The pH of the test solutions was measured with glass-calomel electrode couple, which was calibrated as a hydrogen concentration probe according to procedure of Irving *et al.*[22] The pH of each test solution was checked daily, during one week. The stable values, within 0.01 pH and 0.004 absorbance units, were attained after 1 h and remained stable during couple of days. Spectra of the test solutions were recorded in 250–450 nm wavelength interval.

2. 3. Data Treatment

The species formed in the studied systems were characterized by the general equilibrium:

$$p\ Gd + q\ H + r\ MOXI = Gd_pH_q(MOXI)_r \quad (1)$$

and the corresponding constants are given by:

$$\beta_{p,q,r} = \frac{[Gd_pH_q(MOXI)_r]}{[Gd]^p[H]^q[MOXI]^r} \quad (2)$$

where MOXI is the deprotonated molecule of the ligand. Fully protonated moxifloxacin is denoted as H_2MOXI^+.

In this study, the convention has been adopted whereby a complex containing a metal ion, M, proton, H and ligand L, takes the general formula $M_pH_qL_r$, where p, q and r are the stoichiometric indices of the components in the complex. A negative values for q refers to proton removal or hydoxide ion addition during formation of the complex. Thermodynamically these two processes are equivalent and cannot be distinguished potentiometrically. The equilibrium constant for the formation of this complex from its components is then designated by the symbol $\beta_{p,q,r}$. For convenience the species $M_pH_qL_r$ is denoted by the three stoichiometric coefficients (p,q,r) given in the order M, H, L. For simplicity, the charges of these species are omitted.

Three kinds of equilibria should be considered in the present study: (a) protonation of moxifloxacin anion; (b) hydrolysis of Gd^{3+} ion; and (c) general three component equilibria, which include the case $q = 0$, *i.e.* the formation of pure binary complexes of Gd^{3+}. The overall protonation constants of moxifloxacin anion and stability constants of hydrolytic complexes of Gd^{3+} ion were determined in separate experiments. Thus, in evaluation of three component equilibria (c), the binary models (a) and (b) were considered as known. The concentration stability constants of the complexes, $\beta_{p,q,r}$ were calculated with the aid of the suite of computer programs Hyperquad2006.[23] In Hyperquad calculations the identity and stability of complexes which give the best fit to the experimental data, were determined by minimizing the objective function, U:

$$U = (1/N)\sum_{n=1}^{N} w_{ni}(Y_{ni}^o - Y_{ni}^c)^2 \quad (3)$$

where w_{ni} represents a statistical weight assigned to i^{th} residual at n^{th} point of titration curve, and $Y_{ni}{}^o$ and $Y_{ni}{}^c$ refer to observed and calculated either potential or absorbance (o = observed, c = calculated) assuming the specific model and trial constants, respectively. N is the total number of experimental points. Quality of the fit was judged by usual statistical parameters. The weight w_{ni} defined as reciprocal of the variance in the residual $Y_{ni}{}^o - Y_{ni}{}^c$ is calculated using an error propagation formula:

$$w_{ni} = \left(\sum_k \frac{(\partial(Y_{ni}^o - Y_{ni}^c)}{\partial k})^2 \sigma_k^2\right)^{-1} \quad (4)$$

where the summation extends over all parameters, k, for which errors, σ_k, are specified and include titrant volume error (± 2 μL), error in emf readings (± 0.2 mV) and error in absorbance readings (± 0.002 abs units). The standard deviations of the parameters being refined are calculated using the formula:

$$\sigma_i = \left(\frac{U \times G_{ii}}{N-k}\right)^{1/2} \quad (5)$$

where G_{ii} is inverted Hessian used in the Gauss Newton procedure to minimize U. The standard deviations in residuals, s, was calculated as:

$$s = \left\{ewe^T/(N-k)\right\} \quad (6)$$

where e is a vector in residuals either potential or absorbance. Acceptance of the model assumed minimum value of U, random distribution of residuals, standard deviation of parameters (stability constants) less than 30% of the parameter value, standard deviation in residuals less than 3.0 and Pearson's test less than 12.6.

The spectrophotometric data were evaluated with the aid of the program pHAb2006[24] (which also belongs to Hyperquad family but possesses some additional and improved features) and the program Hyperquad which is capable to treat spectral data. Potentiometric and spectrometric data were made consistent by concomitantly evaluating both kind of data with the aid of Hyperquad 2006 suite of programs using the best model obtained in separate treatment.

3. Results and Discussion

In order to study speciation in three-component system Gd^{3+} – H^+ (or OH^-) – moxifloxacin, it is necessary first to characterize the binary equilibria, *i.e.* hydrolysis of gadolinium(III) ion and protonation of moxifloxacin anion, under exactly the same experimental conditions as for complexation study.

3. 1. Hydrolysis of Gadolinium(III) Iion

The emf data of the hydrolysis of 1.0–5.0 mmol dm^{-3} Gd^{3+} ion in a 0.1 mol dm^{-3} LiCl medium are presented in Fig. 2 as the dependence of the hydroxide number of Gd^{3+} on the free hydrogen ion concentration, $-lpg\ h$ (pH). The hydroxide number Z_{Gd} denotes an average number of hydroxide ions reacted per Gd^{3+} ion and was calculated from the analytical concentration of hydrogen ions, H^+, the measured free hydrogen ion concentration, h, and the total concentration of the Gd^{3+} ion, C_{Gd}, according to expression:

$$Z_{Gd} = \frac{h-H}{C_{Gd}} \quad (7)$$

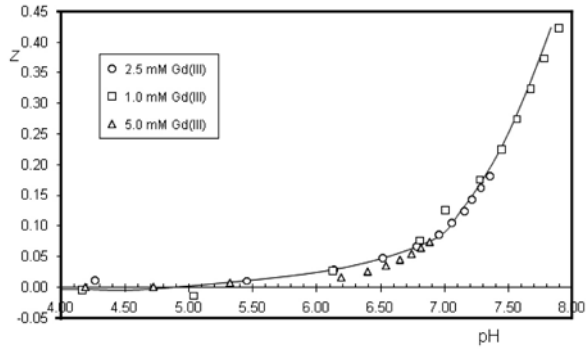

Fig. 2. Hydrolysis of Gd^{3+} ion in a 0.1 mol dm^{-3} LiCl medium, at 298 K, where mM denotes mmol dm^{-3}. Points are experimental data, while the line has been calculated from the composition and stability of the complexes.

Fig. 2 shows that in LiCl medium the hydrolytic curves are superimposed for the different total gadolinium concentrations thus indicating the formation of mononuclear complexes. The maximum value of the hydroxide number reached before the precipitation occured, was between 0.08 and 0.42 depended on gadolinium concentration. The onset of precipitation was indicated by unstable potential readings, steep rise of formation curve and visually, as appearance of slight opacity of solution. At each total concentration of the Gd^{3+} ions, hydrolysis started at about pH 5.

The stability and composition of the complexes formed were determined on the basis of the assumption that the Gd^{3+} ion interacts with water molecules and forms one or more hydrolytic complexes of the general formula $Gd_p(OH)_q^{(3p-q)+}$ according to reaction:

$$pGd^{3+} + qH_2O = Gd_p(OH)_q^{(3p-q)+} + qH^+ \quad (8)$$

where the amount of H^+ produced is equivalent to the quantity of hydroxide ions bound to the Gd^{3+} ion. The overall formation constants, $\beta_{p,q}$ can be defined as:

$$\beta_{p,q} = C_{p,q}[Gd^{3+}]^{-p}[H^+]^{+q} \quad (9)$$

where $C_{p,q}$ denotes the equilibrium concentration of the (p,q) complex and $[Gd^{3+}]$ is the free concentration of Gd^{3+} ion. The general formula $Gd_p(OH)_q^{(3p-q)+}$ is also understood to include an unknown amount of H_2O as solvent and possibly some anions of the medium.

In reaction (8), the hydration of individual ions and complex formation of the Gd^{3+} ion with chloride ions are omitted. The concentration of both chloride ions and water molecules is much higher than the concentration of the Gd^{3+} ion; therefore, it is not possible to determine the changes in concentration of the chloride ions and water molecules. In order to avoid the complex formation effect, the concentration of the medium anion was kept constant (0.1 mol dm^{-3}) and much higher than that of the Gd^{3+} ion. Also, since changes in the mean activity coefficients of

the (p,q) pairs and $\beta_{p,q}$ values can not be simultaneously determined, the constant ionic medium approach[25] was used to keep mean activity coefficients constant.

The composition of the hydrolytic complexes and their stability constants were determined with the aid of program Hyperquad2006.The calculation indicates the formation of only mononuclear complex $Gd(OH)^{2+}$ in the pH range 5.0 to 7.5. The calculated value of the stability constant ($-\log \beta_{1,-1}$) for the complex $Gd(OH)^{2+}$ is 7.96 ± 0.01. This result compares well with literature data (Table 1).

is shown in Fig. 3. Distribution of gadolinium has been calculated by the program Hyss2006.[30] The formation of the complex $Gd(OH)^{2+}$ started at pH 5.7, and with increasing pH, the concentration of this complex increases. The highest concentration of this complex is at pH 7.0. Further increase in pH leads to onset of insoluble $Gd(OH)_3$ whose concentration sharply increases upon increasing pH. We did not detect the formation of any polynuclear hydrolytic species, though scarce literature data indicate the formation of (2,-2) and (3,-4) species.

Table 1. Review of mononuclear hydrolytic species of gadolinium(III) ion in aqueous solutions at 298 K and various ionic media.

Species	-log $\beta_{p,q}$	Ionic medium	Reference
(1,-1)	7.87 ± 0.03	Nitrate, 0.5 M	26
	8.20 ± 0.01	Perchlorate, 3 M	27
	7.3 ± 0.3	Perchlorate, 1 M	28
	7.83 ± 0.05	Perchlorate, 0	29
	7.96 ± 0.01	Chloride, 0.1 M	this work
(1,-2)	13.04 ± 0.03	Nitrate, 0.5 M	26
	14.6 ± 0.5	Perchlorate, 1M	28
(1,-3), solid, log K_{sp}	19.32 ± 0.03	Nitrate, 0.5 M	26
	17.0 ± 0.5	Perchlorate, 1M	28
	17.9 ± 0.1	Chloride, 0.1 M	this work

Table 2. Calculated values of the solubility product of $Gd(OH)_3$(s), $K_{sp}Gd(OH)_3$

$C(Gd^{3+})$, mM	$-\log h_p$	p[Gd^{3+}]	p[OH]	$K_{sp}Gd(OH)_3$
1.00	7.00	3.04	6.78	17.96
2.50	6.85	2.63	6.93	17.92
5.00	6.70	2.32	7.08	17.78

Note. The equilibrium concentrations of Gd^{3+}, [Gd^{3+}], were calculated from $C_{Gd} = [Gd^{3+}] + \beta_{1,-1}\,[Gd^{3+}] \cdot [H^+]^{-1}$.

The formation of insoluble Gd-hydroxide was estimated from experimental titration curves of acidified solutions of gadolinium(III) chloride with sodium hydroxide. When pH ~ 7.5–9 was reached further addition of alkali was stopped since the excess of alkali was not connected with gadolinium hydrolysis. The titration curves were plotted as the dependence of pH on the titration parameter (amount of strong base added per mole of Gd^{3+}). The point of inflexion of pH-metric curve corresponds to the start of formation of hydroxide precipitate. For each total concentration of Gd^{3+} ion the beginning of precipitation was determined ($-\log h_p$). Assuming the formation of $Gd(OH)_3$(s) only, the solubility product $K_{sp} = [Gd^{3+}] \cdot [H^+]^{-3}$ was calculated from the known free concentration of Gd^{3+}. The results are given in Table 2.

Thus, calculated average value of solubility product of $Gd(OH)_3$ is $\log K_{sp} = 17.9 \pm 0.1$. The distribution diagram of the hydrolytic complexes of gadolinium(III) ion

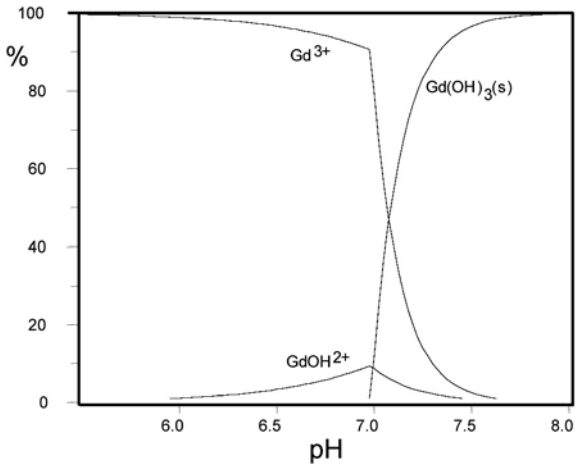

Fig. 3. The distribution of Gd^{3+} hydrolytic species in 0.1 mol dm^{-3} LiCl ionic medium at 298 K. C_{Gd} = 1.00 mmol dm^{-3} .

3. 2. Protonation of Moxifloxacin Anion

Protonation constants, β_n, of the moxifloxacin anion, defined according to the equilibrium:

$$n\mathrm{H}^+ + \mathrm{MOXI}^- = \mathrm{H}_n\mathrm{MOXI};\ \beta_n\ (n = 1, 2) \qquad (10)$$

were determined by glass electrode potentiometric titrations in 0.10 mol dm^{-3} LiCl medium at 298.15 K. Three titrations were carried out with 0.25, 0.50 and 1.10 mmol dm^{-3} total fluoroquinolone concentrations, in the pH range between 3.0 and 10.2. Spectrophotometric measurements were made on solutions in which the concentration of moxifloxacin was the same (0.05 mmol dm^{-3}) while the pH values were varied between 4.0 and 9.4 (15 solutions were used). The calculated values of protonation constants are given in Table 3. Agreement between potentiometrically and spectrophotometrically obtained values was better than 1%. The obtained values are in the range with previously reported data.[31]

Table 3. Potentiometrically and spectrophotometrically determined protonation constants of moxifloxacin (MOXI) defined as:

K_1 = [HMOXI]/[H][MOXI], K_2 = [H_2 MOXI]/[HMOXI][H]

	Potentiometric	Spectrophotometric
log K_1	9.34 ± 0.01	9.30 ± 0.02
log K_2	6.33 ± 0.01	6.27 ± 0.05

3. 3. Complex Formation of Gd^{3+} with Moxifloxacin

Potentiometric Measurements The experimental data obtained by emf measurements in 0.1 mol dm^{-3} LiCl medium at 298 K are shown in Fig. 4.

In order to derive the speciation model for each studied system the experimental data were plotted as the dependence of pH on the titration parameter. The titration parameter, a, was calculated through the formula

$$a = \frac{C_{NaOH}V_{NaOH} - V_0C_{HCl}}{V_0L} \qquad (11)$$

where V_0 and L are the initial volume and concentration of moxifloxacin in the titrated solution. Negative values of a represent the titration of excess of strong acid (HCl). Titration curves of moxifloxacin in the presence of gadolinium ion (Fig. 4) are shifted to the right compared to moxifloxacin alone thus indicating strong complex formation in the system. Since the titration curves of moxifloxacin alone and Gd^{3+} + moxifloxacin do not coincide at low pH values it may be inferred that complexation reaction proceeds even at pH values lower than *ca.* 3. Coincidence of the titration curves of Gd^{3+} + moxifloxacin with different ligand to metal concentration ratios in the pH region around 3 indicates the formation of the 1 : 1 complexes. The titration curve of moxifloxacin alone shows two well separated jumps indicating the titration of two protons from the ligand. In the presence of gadolinium ion these protons are titrated at lower pH values and appearance of two buffer regions on the titration curves points to formation of the complexes with ligand to metal ratio higher than 1 : 1. Thus the formation of complexes with the stoichiometry L/M = 1 : 1; 2 : 1 and 3 : 1 as well as mixed complexes may be expected.

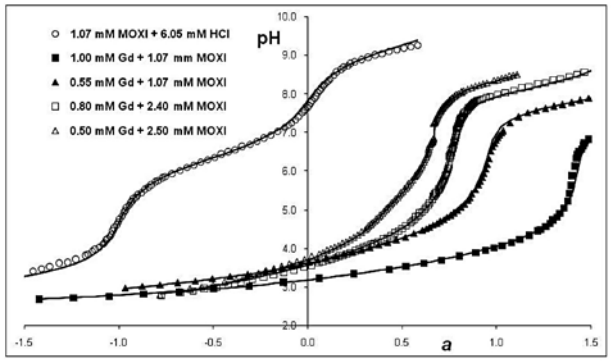

Fig. 4. Potentiometric titration of Gd^{3+} – moxifloxacin solutions with standard NaOH in 0.1 mol dm^{-3} LiCl ionic medium at 298 K. Full lines denote calculated curves. The concentration in mmol dm^{-3} is denoted as mM.

To find the model that gives the best fit to the experimental data, various complexes and combinations thereof were included in Hyperquad2006 calculations up to ligand to metal mole ratio 4:1. During the calculations, the analytical parameters (total metal, ligand and proton concentration) were held constant. The pure hydrolytic complexes and protonated moxifloxacin species were not refined during the calculations. Different strategies were employed in the refinement operations: (i) fixing selected constants to simplify the optimization procedure, (ii) reducing the number of experimental points included in calculations, (iii) "piecewise" fitting of the experimental data. Initially, each titration was treated separately. All the complexes found in this way were included as the starting model for subsequent calculations.

The GdHMOXI and $Gd(HMOXI)_2$ complexes were found at all titrations and concentration ratios. The scatter of the values of their stability constants is within the experimental errors. The complexes Gd(HMOXI)(MOXI) and $Gd(MOXI)_2$ were found at a MOXI:Gd concentration ratio of 2 : 1 and 1 : 1. At the higher concentration ratios (as L/M = 3 : 1 and 5 : 1) a significant improvement of the fit was achieved with the introduction of the complexes (1,3,3), (1,2,3), (1,1,3) and (1,0,3). Mixed hydrolytic complexes are not important even at higher pH values. The complexes with a stoichiometry L/M ≥ 4 were not found.

The preliminary set of complexes obtained in separate calculations is given in Table 4. Statistical parameters which determine the quality of fit are also given.

Table 4. Stability constants of gadolinium – moxifloxacin complexes formed in a 0.1 mol dm^{-3} LiCl ionic medium, at 298 K. L/M denotes ligand to metal mole ratio.

Species (p,q,r)	$\log \beta_{p,q,r} \pm \sigma$ Potentiometric L/M = 0.5–2	Potentiometric L/M = 3–5	Spectrophotometric L/M = 0.5–2	Spectrophotometric L/M = 3–5
(1, 1, 1)	14.72 ± 0.03	14.79 ± 0.06	14.79 ± 0.09	14.75 ± 0.07
(1, 2, 2)	29.65 ± 0.02	29.57 ± 0.08	29.72 ± 0.08	29.67 ± 0.09
(1, 1, 2)	21.20 ± 0.09			
(1, 0, 2)	14.00 ± 0.03		13.8 ± 0.3	
(1, 3, 3)		43.98 ± 0.03		43.95 ± 0.01
(1, 2, 3)		35.18 ± 0.01		
(1, 1, 3)		27.76 ± 0.03		27.80 ± 0.05
(1, 0, 3)		19.00 ± 0.05		19.28 ± 0.08
Statistics	$\chi^2 = 11.82$ $s = 1.2$	$\chi^2 = 15.98$ $s = 1.8$	$\chi^2 = 24.36$ $s = 4.4$	$\chi^2 = 13.65$ $s = 1.3$

The calculated errors (σ) in stability constants reflect the fitting error (due to model) and experimental errors in titrant volume and potential or absorbance readings. Systematic errors on analytical concentrations could not be taken into account; they are rather assumed to be absent. Careful preparation of working solutions, and agreement between replicate titrations ensures the absence of systematic errors thus, it may be assumed that the magnitude of relative errors on concentrations is far less than the errors arising from the choice of model, regression and instrumental errors. It can thus be assumed that uncertainty on stability constants is well represented by calculated standard deviation.

Spectrophotometric Measurements Spectral measurements were performed on Gd^{3+} – moxifloxacin solutions in which the concentration of both, gadolinium ion and moxifloxacin was kept constant while pH was varied by the addition of the standard HCl or NaOH, as appropriate. All UV/Vis spectra show evidence of an intensive band centered at 290 nm and another lower energy broad band appears between 330 and 380. This band shows two well resolved maxima at 340 and 370 nm (Fig. 5).

The high energy band is mainly due to the $\pi \rightarrow \pi^*$ transition in the aromatic ring. The longest wavelength maximum is due to an $n \rightarrow \pi^*$ (HOMO–LUMO) electronic transition[32] and consists of two sub-peaks which are caused by an equilibrium of the moxifloxacin forming an intermolecular hydrogen bond with the solvent molecule water and moxifloxacin forming an intramolecular hydrogen bond of the 4-keto and the 3-carboxylic acid group.[33–35]

Upon increasing the pH from *ca.* 3 to 8 higher energy band shows significant changes in position and maximum intensity (hypsochromic shift). The lower energy band exhibits however, only small changes in a shape, position and intensity (bathochromic shift). Intensity of the band at 340 nm increases upon increasing the pH. Intensity of the band at 370 nm and higher energy band decreases with increasing the pH. In the presence of gadolinium ion, in comparison with the spectrum of moxifloxacin alone, all bands are shifted toward higher wavelengths.

The spectral data were first evaluated with the aid of the pHAb2006 program. In calculations, the molar absorptivities of moxifloxacin anion, H(MOXI) and H_2(MOXI) were known from spectral measurements of moxifloxacin anion protonation and were fixed, while these of gadolinium(III) – aqua ion and pure hydrolytic complexes were set to zero. The calculations were carried out in the following way: the complexes found by potentiometry were included in pHAb calculations and their stability constants were allowed to float. When the best fit of

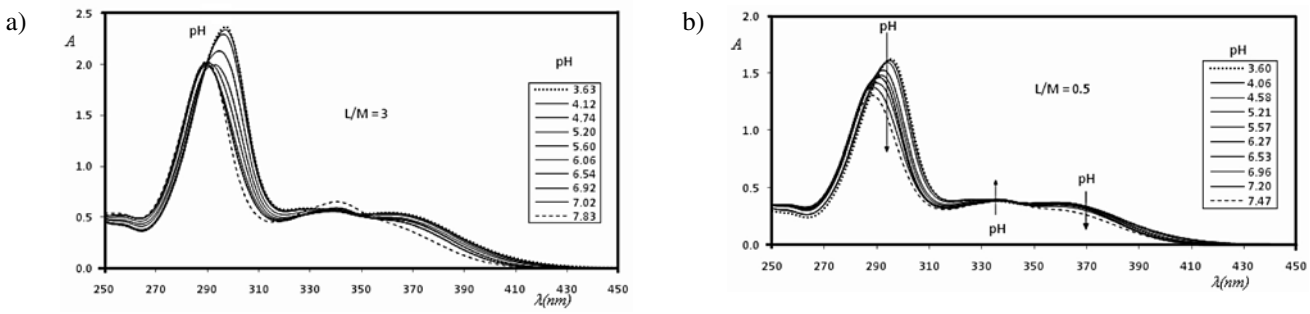

Fig. 5. The UV–Vis spectra of Gd^{3+} – moxifloxacin solutions at different pH values and ligand (L) to metal (M) concentration ratios: (a) L/M = 3, (b) L/M = 0.5

the spectra was achieved the stability constants were varied one at a time simultaneously with variation of molar absorptivities. The accepted results of calculation are given in Table 4.

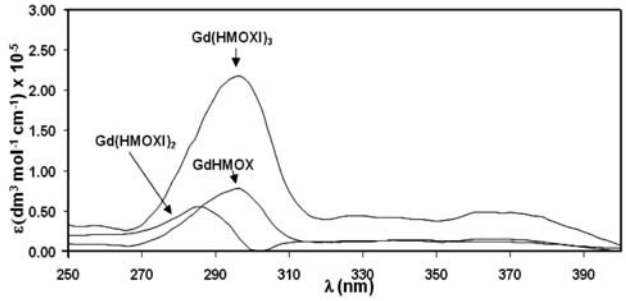

Fig. 6. The calculated spectra of Gd–moxifloxacin species

Along with the stability constants, in spectral calculations, the molar absorptivities of the complexes were calculated. The calculated spectra for Gd–moxifloxacin system are presented in Fig. 6. As seen from Fig. 6 the calculated spectra of $GdHMOXI^{3+}$, $Gd(HMOXI)_2^{3+}$ and $Gd(HMOXI)_3^{3+}$, complexes differs from that of pure HMOXI most significantly in the region of $n \rightarrow p^*$ transition in the 330–370 nm wavelength interval. It is probably caused by breaking the intra- and intermolecular hydrogen bonds due to coordination of both 4-keto and 3-carboxyl oxygens to gadolinium.

From preliminary set of complexes (Table 4) a new calculation cycle was initiated, this time both selected potentiometric and spectrophotometric data were treated concomitantly. On the basis of acceptance criteria the final accepted set of complexes was derived and this set is shown in Table 5.

The distribution diagram of species in the Gd^{3+} – moxifloxacin system, for the concentration ratio [MOXI] /[Gd] = 5 : 1 is shown in Fig 7. As can be seen from Fig. 7, the dominating complex at lower pH values is $Gd(HMOXI)^{3+}$, with the maximum concentration at pH = 4. This complex may be formed *via* the reaction of Gd^{3+} aqua ion and moxifloxacin cation bearing in mind that these species predominate in the 2.0–5.0 pH region.

$$Gd^{3+} + H_2MOXI^+ \rightarrow Gd(HMOXI)^{3+} + H^+ \quad (12)$$

This reaction, from the electrostatic point of view, is not favorable, but one should bear in mind that the carboxyl proton participates in hydrogen bonding both intermolecular with 4-carbonyl oxygen and intramolecular with another moxifloxacin and solvent molecules. This facilitates its release by the action of Gd^{3+} ion. The complex $Gd(HMOXI)^{3+}$ upon increasing the pH, binds another zwitterionic molecule of moxifloxacin and gives the complex $Gd(HMOXI)_2^{3+}$ *via* the reaction path:

$$Gd(HMOXI)^{3+} + HMOXI^{\pm} \rightarrow Gd(HMOXI)_2^{3+} \quad (13)$$

This complex with increasing pH releases protons and gives mixed complex $Gd(HMOXI)MOXI^{2+}$ with a maximum of 10 % concentration at pH 8. As can be seen from Fig. 7, in going from acidic to weakly alkaline medium gradual formation of complexes $GdHMOXI^{3+}$, $Gd(HMOXI)_2^{3+}$ and $Gd(HMOXI)_3^{3+}$ takes place probably by consecutive reactions:

$$Gd^{3+} + H_2MOXI^+ \rightarrow Gd(HMOXI)^{3+} \xrightarrow{+\,HMOXI^{\pm}} \xrightarrow{+\,HMOXI^{\pm}} Gd(HMOXI)_2^{3+} \xrightarrow{+\,HMOXI^{\pm}} Gd(HMOXI)_3^{3+} \quad (14)$$

At pH values higer than 7, protonated moxifloxacin ligands in the complex $Gd(HMOXI)_3^{3+}$ release protons and give neutral complex $Gd(MOXI)_3$. The formation of the complex $Gd(MOXI)_3$ starts at about pH = 8 and with increasing pH, the concentration of this complex increases.

In $Gd(HMOXI)_3^{3+}$ complex moxifloxacin acts as a bidentate O,O- ligand with a probable formation of six-membered ring by 4-keto and 3-carboxyl oxygens (Fig. 8). Gadolinium(III) ion exhibits characteristic coordination numbers 6, 8 and 9.[36] With the most ligands Gd^{3+} takes coordination number 8.[37] Since we did not find any evidence for the formation of L/M = 4 : 1 complex it may be assumed that additional two coordination sites in the gadolinium coordination sphere are filled with water molecules. The similar result was found by Turel et al.[38], in studying the fluorescence properties and structure of Eu^{3+} – ciprofloxacin complex. They found that two bidentate O,O-bonded ciprofloxacine molecules and four aqua ligands are coordinated to the metal. One ciprofloxacin is anionic while the other is zwitterionic. Similar results for lanthanide complexes with ciprofloxacin was found by Pin *et al.*[39] In this work we also found that quinolones may coordinate to metal ion in various states of protonation (ie, zwitterionic, neutral and anionic form). The complex $Gd(HMOXI)_3^{3+}$ is very stable in the 5.0–8.0 pH and is probably formed in plasma, under physiological conditions.

Table 5. Final set of complexes obtained by potentiometric and spectrophotometric measurements in Gd^{3+} – moxifloxacin solutions at 0.1 mol/dm^3 LiCl ionic medium and 298 K.

Species	log $\beta \pm \sigma$
$Gd(HMOXI)^{3+}$	14.78 ± 0.03
$Gd(HMOXI)_2^{3+}$	29.75 ± 0.02
$Gd(HMOXI)_3^{3+}$	43.98 ± 0.03
$Gd(HMOXI)_2MOXI^{2+}$	35.08 ± 0.01
$Gd(HMOXI)(MOXI)_2^{+}$	27.56 ± 0.03
$Gd(MOXI)_3^{0}$	19.20 ± 0.05
Statistics	$\chi^2 = 12.02$
	$s = 2.28$

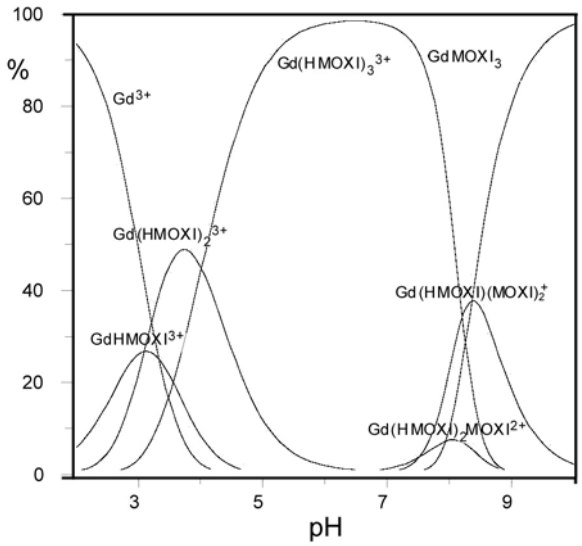

Fig. 7. Distribution diagram of Gd – moxifloxacin species at ligand-to-metal concentration ratio = 5 : 1 and total gadolinium concentration 1.0 mmol dm^{-3}

3. 4. Computer Simulation of the Effect of Moxifloxacin on Gadolinium(III) Distribution in Plasma

The low-molecular weight complex distribution of Gd(III) ion in human blood plasma was first studied by Jackson et al.[40] by computer simulation. Webb et al.[41] studied Gd^{3+} and Cm^{3+} distribution in the gastrointestinal tract and Yue Wang et al.[42] studied Gd^{3+} speciation in human blood plasma taking into account the precipitates and some important mixed complexes. Jinping Wang[43] studied Gd^{3+} speciation in human interstitial fluid. The results of these studies reveal that at lower gadolinium concentration the metal is mainly bound to citrate. At milimolar level of Gd^{3+} concentration and without taking into account the insoluble complexes, transferrin, citrate and glutamate appear as main binders. Introduction of insoluble species into the speciation scheme indicates the predominance of phosphate. DTPA has an effect on Gd^{3+} distribution at concentrations approximately higher than 10^{-5} mol dm^{-3}. We used the simplified model of human blood plasma taking into account only the most important ligands (transferrin, albumin, citrate, phosphate, oxalate, carbonate glutamate and hydroxide) to study the effect of moxifloxacin and DTPA on Gd^{3+} ion distribution between low molecular weight complexes. As competitive the following metal ions were considered: Ca^{2+} and Mg^{2+}. The speciation was calculated for different total concentrations of Gd^{3+} ion at pH = 7.4 using the program Hyss2006. From Fig. 7 it is seen that *tris* complex of moxifloxacin and gadolinium is predominant at physiological range of pH so that this complex only was taken into account in speciation calculations.

The data for stability constants of various complexes were taken from literature.[44] Where more than single data were available the corresponding constants were avera-

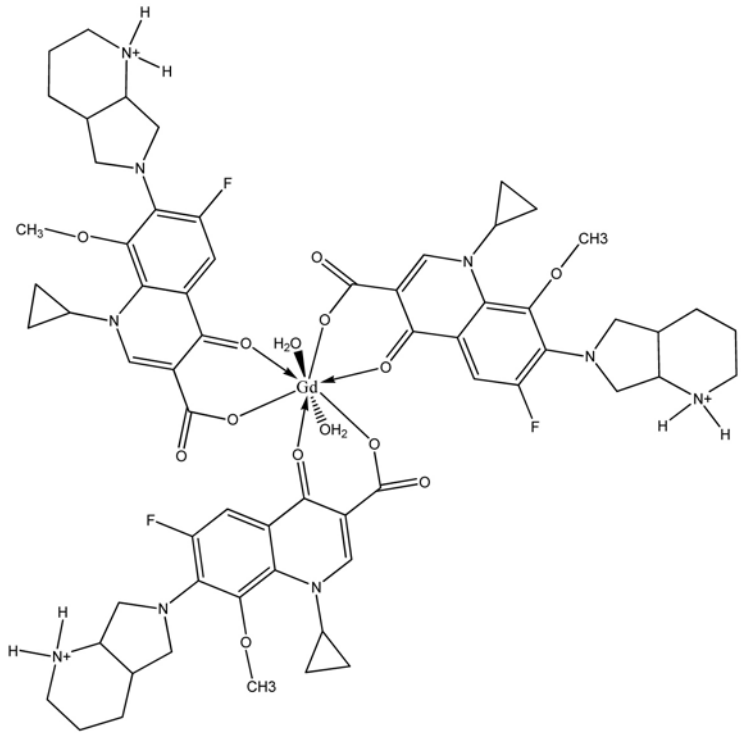

Fig. 8. Possible structure of $Gd(HMOXI)_3^{3+}$ complex in solution.

ged. The plasma concentrations of ligands were taken from reference 45.

The results of calculations indicate that when insoluble phosphate (log ${}^{*}K_{sp0} = -25.62$) was introduced into simulation it is predominant species of all up to the 10^{-2} mol dm^{-3} Gd^{3+}. The relative fraction of $Gd(OH)_{3(s)}$ (log ${}^{*}K_{sp} = 17.9$) increases with increasing total Gd(III) concentration. However, Jackson et al.[40] found that kinetics of the formation of insoluble gadolinium(III) phosphate is very slow so that in considering fast complexation with DTPA and moxifloxacin the formation of phosphate may

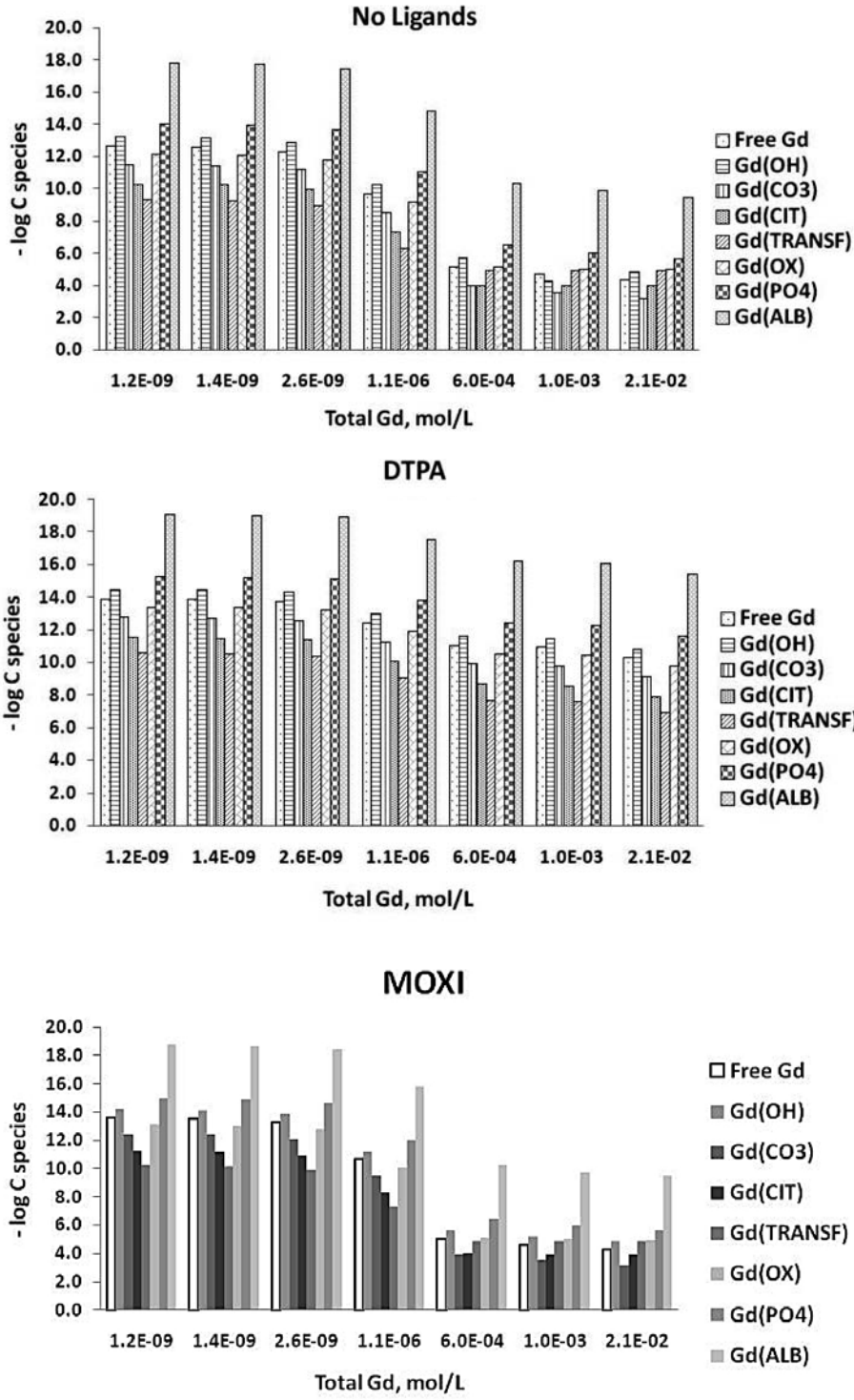

Fig. 9. Calculated distribution of gadolinium species in human plasma at pH = 7.4. CIT = citrate, TRANSF = transferrin, OX = oxalate, ALB = albumin

not be taken into account. Soluble species consist of low molecular weight ligand complexes (carboxylate, amino acid), protein complexes (albumin, transferrin, IgG) free Gd^{3+} and so on. We calculated the distribution only for main binders neglecting amino acid other than glutamic, and ternary complexes. The results are given in Fig. 9 as the distribution of various complexes. Normal Gd^{3+} plasma level is lower than 0.3 μg/L[46] but upon administering Gd(DTPA) for the purpose of MRI, glomerular filtration rate measurements or CT scanning, its concentration may increase up to toxic levels (~ 6 mmol dm^{-3}) followed by its retention in bones.[46] The increase in toxicity was ascribed to facilitated dissociation of the complex in the plasma environment.[19,20] Gd^{3+} toxicity is due to its interference with Ca^{2+} – dependent functions.

DTPA significantly affects the concentration of free Gd^{3+} and the effect is more pronounced with increasing total gadolinium concentration. Moxifloxacin is effective chelator at lower gadolinium total concentration but at milimolar range of total gadolinium concentration distribution of low molecular weight complexes is almost unchanged.

4. Conclusion

Gadolinium(III) ion and moxifloxacin form *in vitro* in aqueous solution, array of complexes of which the *tris* complex $Gd(HMOXI)_3$ predominates at physiological pH values. In accord with literature data for similar complexes, the moxifloxacin is bound to metal ion by carboxylate and 4-carbonyl oxygen. This complex is stable enough to exhaust the normal gadolinium concentration in plasma upon oral intake of one 400 mg dose of moxifloxacin, at low Gd^{3+} concentration (10^{-9}–10^{-7} mol dm^{-3}) so that the presence of moxifloxacin may change Gd^{3+} distribution in plasma. However, at higher Gd^{3+} concentrations moxifloxacin is not competitive chelator with regard to MRI agents (such as DTPA).

5. Acknowledgement

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5. References

1. P. Ball, The Quinolones. History and overview. In: V. T. Andriole (Editor). The Quinolones, Academic Press, San Diego, **2000**, 2–24.
2. M. Donati, M. R. Fermepin, A. Olmo, L. D'Apote and R. Cevenini, *J. Antimicrob. Chemother.*, **1999**, *43*, 825–827.
3. G. Klopman, S. Wang, M.R. Jacobs, J. J. Ellner, *Antimicrob. Agents Chemother.*, **1993**, *37(9)*, 1807–1815.
4. K. Drlica, D. C. Hooper, Mechanism of Quinolone Action, In: D. C. Hopper, J. S. Wolfson, (Editors). Quinolone Antimicrobial Agents, (3rd Edition), American Society for Microbiology, Washington DC, **2003**, 19–41.
5. I. Turel, *Coord. Chem.. Rev.,* **2002**, *232*, 27–47.
6. S. Lober, S. Ziege, M. Rau, G. Schreiber, A. Mignot, P. Koeppe, H. Lode, *Antimicrob. Agents Chemother.,* **1999**, *43(5)*, 1067–1071.
7. G. Palu, S. Valisena, G. Ciarrocchi, B. Gatto, M. Palumbo, *Proc. Natl. Acad. Sci.*, *USA*, **1992**, *89*, 9671–9675.
8. I. Turel, A. Šonc, M. Zupančič, K. Sepčić, T. Turk, *Metal Based Drugs*, **2000**, *7(2)*, 101–104.
9. S. Lecomte, M. H. Baron, M. T. Chenon, C. Coupry, N. J. Moreau, *Antimicrob. Agents Chemother.,* **1994**, *38(12),* 2810–2816.
10. C. Sissi, M. Andreolli, V. Cecchetti, A. Fravolini, B. Gatto, M. Palumbo, *Bioorg. Med. Chem.*, **1998**, *6(9)*, 1555–1561.
11. Z. F. Chen, R. G. Xiong, J. L. Zuo, Z. Guo, X. Z. You, H. K. Fun, *J. Chem. Soc. Dalton Trans.*, **2000**, 4013–4014.
12. M. Župančič, R. C. Korošec, P. Bukovec, *J. Thermal Anal. Calorim.*, **2001**, *63*, 787–795.
13. J. Al-Mustafa, *Acta Chim. Slov.*, **2002**, *49*, 457–466.
14. I. Turel, P. Živec, A. Pevec, S. Tempelaar, G. Psomas, *Eur. J. Inorg. Chem.*, **2008**, *23*, 3718–3727.
15. S. K. Upadhyay, P. Kumar, V. Arora, *J. Struct. Chem.*, **2006,** *47(6)*, 1078–1083.
16. P. Drevenšek, J. Košmrlj, G. Giester, T. Skauge, E. Sletten, K. Sepčić, I. Turel, *J. Inorg. Biochem.,* **2006**, *100*, 1755–1763.
17. L. J. Ming, *Med. Res. Rev.,* **2003**, *23(6)*, 697–762.
18. W. Guo-ping, L. Qun-fang, *J. Zhejiang University (Science edition)*, **2003,** *30(4)*, 417–442. (Google search)
19. P. Hermann, J. Kotek, V. Kubiček, I. Lukeš, *Dalton Trans.,* **2008**, 3027–3047.
20. M. Port, J.-Marc Idee, C. Medina, C. Robic, M. Sabatou, C. Corot, *Biometals*, **2008**, *21*, 469–490.
21. P. Djurdjević, R. Jelić, D. Džajević, M. Cvijović, *Metal Based Drugs*, **2002**, *8*, 235–248.
22. H. M. Irving, M. G. Miles, L. D. Pettit, *Anal. Chim. Acta*, **1967**, *38*, 475–488.
23. P. Gans, A. Sabatini and A. Vacca, *Talanta*, **1996**, *43*, 1739–1753.
24. P. Gans, A. Sabatini and A. Vacca, *Ann. Chim.*, **1999**, *89*, 45–49.
25. L.-O. Ohman, S. Sjöberg, *Coord. Chem. Rev.*, **1996**, *149*, 33–57.
26. L. G. Rodenas, S. J. Liberman, *Talanta*, **1991**, *38(3)*, 313–318.
27. T. Amaya, H. Kakihana, M. Maeda, *Bull. Chem. Soc. Jpn.,* **1973**, *46*, 2889–2890.
28. J. Kragten, L. G. Decnop-Weever, *Talanta*, **1980**, *27(12)*, 1047–1050.
29. G. D. Klungness, R. H. Byrne, *Polyhedron*, **2000**, *19*, 99–107.
30. L. Alderighi, P. Gans, A. Lenco, D. Peters, A. Sabatini, A. Vacca, *Coord. Chem. Rev.*, **1999**, *184*, 311–318.

31. P. Djurdjević, Lj. Joksović, R. Jelić, A. Djurdjević, and M. Jelikić Stankov, *Chem. Pharm. Bull.*, **2007**, *55(12)*, 1689–1699.
32. C. H. Song, H. W. Ryu, J. K. Park, T. S. Ko, *Bull. Kor. Chem. Soc.*, **1999**, *20*, 727–730.
33. D. Gimenez, D. Grasso, L. Sarabia, M. C. Ortiz, *Talanta*, **2004**, *64*, 442–451.
34. U. Neugebauer, A. Szeghalmi, M. Schmitt, W. Kiefer, J. Popp, U. Holzgrabe, *Spectrochim. Acta A*, **2005**, *61*, 1505–1517.
35. H. R. Park, T. H. Kim, K. M. Bark, *Eur. J. Med. Chem.*, **2002**, *37*, 443–460.
36. L. C. Thompson, Complexes, in K. A. Gschneidner Jr, L. Eyring (Eds.), *Handbook on the Physics and Chemistry of Rare Earths,* North Holland Pub. Co., **1979**, 209–297.
37. G. Choppin and E. N. Rizkalla, Solution chemistry of actinides and lanthanides, in K. A. Gschneidner Jr, L. Eyring, G. R. Choppin and G. H. Lander (Eds), *Handbook on the Physics and Chemistry of Rare Earths,* Elsevier Science B.V., **1994**, 559–590.
38. D. Čurman, P. Živec, I. Leban, I. Turel, A. Polishchuk, K. D. Klika, E. Karaseva, V. Karasev, *Polyhedron*, **2008**, *27*, 1489–1496.
39. L. Jia-Bin, Y. Pin, G. Fei, H. Gao-Yi, Y. Kai-Bei, *Chinese J. Chem.*, **2001**, *19(6)*, 598–605.
40. G. E. Jackson, S. Wynchank, M. Woudenberg, *Magnetic Res. Med.,* **1990**, *16*, 57–66.
41. L. M. Webb, D. M. Taylor, D. R. Williams, *J. Alloy and Compounds*, **1998**, *12*, 271–273.
42. Y. Wang, X. Lu, S. Y. Wang, J. F. Han, K. Y. Yang, C. J. Niu, J. Z. Ni, *Chin. Chem. Lett.,* **2001**, *12(2)*, 161–162.
43. J. Wang, H. Zhang, K. Yang, C. Niu, *Biometals*, **2004**, *17*, 599–603.
44. SC-Database. IUPAC stability constants database. Academic Software. UK. **2005.**
45. C. Burtis, E. Ashwood, D. Bruns, B. Sawyer (editors), *Tietz Fundamentals of Clinical Chemistry*, 6th edition, Saunders – Elsevier, St. Louis, USA. **2008**, 836–873.
46. H. Seiler, A. Sigel, H. Sigel, *Handbook on Metals in Clinical and Analytical Chemistry,* Marcel Dekker, N.Y., **1994**, 365–369.

Povzetek

Raziskovali smo ravnotežja nastanka kompleksov med gadolinijevimi(3+) ioni in moxifloxacinom (MOXI) v vodnih raztopinah. Uporabili smo potenciometrične meritve s stekleno elektrodo (ionski medij: 0,1 mol dm^{-3} LiCl, 298 K) in UV spektrofotometrične meritve. V koncentracijskem območju $0,5 \leq [Gd^{3+}] \leq 1,0$; $1,0 \leq [MOXI] \leq 2,0$ mol dm^{-3} ($[MOXI]/[Gd^{3+}] = 1 : 1$ do $1 : 5$) in pH med 2,5 in 9,0, tvorijo gadolinijevi(3+) ioni s moxifloxacinom komplekse s sestavo: $Gd(HMOXI)^{3+}$, $Gd(HMOXI)_2^{3+}$, $Gd(HMOXI)_3^{3+}$, $Gd(HMOXI)_2MOXI^{2+}$, $Gd(HMOXI)(MOXI)_2^{+}$, $Gd(MOXI)_3$. Konstante stabilnosti so bile izračunane s programom Hyperquad2006 z upoštevanjem hidrolize gadolinijevih(3+) ionov in protonacije aniona moxifloxacina. Napovedali smo možne strukture kompleksov v raztopini in predpostavili mehanizem njihovega nastanka. Z računalniško simulacijo smo ovrednotili vpliv moxifloxacina in ga primerjali z vplivom DTPA na porazdelitev gadolinijevih(3+) zvrsti v krvni plazmi.