ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
19 (1999) 501–510

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Study of solution equilibria between aluminum(III) ion and ofloxacin

Predrag T. Djurdjevic [a,*], Milena Jelikic-Stankov [b]

[a] *Faculty of Science, PO Box 60, 340000 Kragujevac, Yugoslavia*
[b] *Faculty of Pharmacy, PO Box 164, 11001 Belgrade, Yugoslavia*

Received 24 February 1998; received in revised form 6 May 1998; accepted 24 May 1998

Abstract

The complex formation equilibria between aluminium(III) ion and ofloxacin in 0.1 mol l^{-1} ionic medium at 298 K were studied by glass electrode pH-metric and UV spectrophotometric measurements. Within ofloxacin to aluminium mole ratio ranging from 2:1 to 25:1 and in pH interval from 2.5 to 10.5, the obtained experimental results were explained by the formation of the following complexes: Al(Hoflo) (log $\beta_{1,1,1} = 15.93 \pm 0.03$), $Al(oflo)_2$ (log $\beta_{1,2,0} = 14.84 \pm 0.07$), Al(oflo) (log $\beta_{1,1,0} = 10.20 \pm 0.04$) as well as several other mixed and pure hydrolytic complexes. The structure and mechanism of the formation of the complexes and their possible implications on aluminum toxicity were discussed.

Keywords: Ofloxacin; Aluminum complexes; Solution equilibria

1. Introduction

Ofloxacin (9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido-(1,2,3-de)1,4-benzoxazine-6-carboxylic acid), belongs to the class of fluorinated 4-quinolone antibiotics which primarily finds use in the treatment of urinary and respiratory infections. It exhibits strong activity against Gram-negative and some Gram-positive bacteria, though many anaerobic strains are resistant. After oral administration of a single dose of ofloxacin tablet (200 mg) good penetration into body tissues and fluids is observed, with maximal concentrations varying between 1.0 and 8.0 mg l^{-1} [1,2]; Scheme 1.

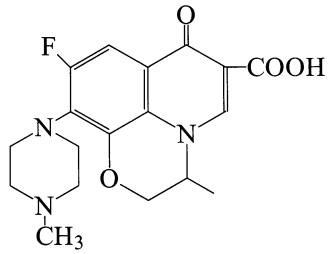
Clinical investigations have shown that concomitant intake of ofloxacin and aluminum-containing antacids results in reduced maximal serum concentration accompanied by the decrease in AUC. Both effects lead to the decreased bioavailability of the drug, down to 30% [3,4]. The explanation for this interaction, bearing in mind the previous findings concerning some other quinolone-metal ions systems [5], is probably chelation between Al^{3+} ion and the 3-carboxyl and 4-oxo functional groups of the ofloxacin.

Experimental data accumulated so far, showed that Al^{3+} ion itself is highly toxic [6]. Upon penetration through the gastrointestinal barrier it

* Corresponding author. Fax: +381-34-335-040; e-mail: preki@knez.uis.kg.ac.yu.

0731-7085/99/$ - see front matter
PII: S0731-7085(98)00246-5

may cause many health disorders noticeably encephalopathia and osteomalacia to the patients on a long-term dialysis [7]. Aluminum toxicity is also observed in patients on a chronic ambulatory dialysis and on a long-term parentheral nutrition. Aluminum-containing drugs, which are given orally to patients with renal failure to control serum phosphate concentration, may be the important source of toxic level of aluminum in some patients [8]. Another source of toxic levels of aluminum may be various types of vaccines in which aluminum-hydroxide is used as adjuvant (e.g. Tick-Borne Encephalitis Virus Vaccine). The presence of ofloxacin may influence Al absorption through two particular mechanisms: (1) Al-complexes with ofloxacin may be stable enough to produce a sufficient decrease in free Al^{3+} concentration so that a significant amount of the poorly soluble aluminum compounds ($Al(OH)_3$, aluminum hydroxo-carbonates or $AlPO_4$) can dissolve; and (2) ofloxacin may form electrically neutral complexes with Al which may, somewhat freely (depending on their lipophilic properties) cross gastrointestinal membrane [9]. Even if neutral complexes do not form, other Al-ofloxacin complexes can release free aluminum ions, which can be coordinated into neutral forms by other nutrients normally present in gastrointestinal tract (citric, tartaric, malic acid, etc.). Hence, the primary aim of this study was to provide reliable data relating to identity and stability of the species formed in aluminum(III) + ofloxacin solutions in vitro so that they could be used in modeling studies of the interactions between Al^{3+} ion and ofloxacin, in vivo. Bearing in mind high toxicity of aluminum, it would be of interest to search for sequestering agent, which would be able to extract aluminum from the body tissues in which it is mostly, accumulated. Ofloxacin does not penetrate into brain tissue but it has excellent penetration into most other tissues (lungs, kidneys, bones) so that the results of the present study should address the problem of aluminum sequestering from these tissues.The study of Al^{3+}-ofloxacin equilibria is complicated by the pronounced hydrolysis of aluminum(III) ion which makes the characterization of the species in equilibrium very difficult [10]. The hydrolysis of aluminum is influenced by many factors: the concentration and nature of the ionic medium, type and rate of addition of the base used to force hydrolysis, temperature, presence of other substances, etc. Hence, the hydrolysis of aluminum must be studied under exactly the same experimental conditions as the complexation reaction. Therefore, in the present study the hydrolytic species, determined in our previous work, under the same experimental conditions, were used [11]. The hydrolytic model comprised the species: $Al(OH)^{2+}$, $Al(OH)_2^{+}$, $Al_3(OH)_4^{5+}$, $Al_{13}(OH)_{32}^{7+}$. To this set of complexes the aluminate, $Al(OH)_4^{-}$ was added, since it is the dominating species at $pH > 6.2$ [12].



OFLOXACIN

Scheme 1.

The literature data on fluoroqinolones complexation with aluminum ion are scarce. Alkayasi et al. [13] have studied the interactions between norfloxacin and Al^{3+}, Mg^{2+} and Ca^{2+} ions, by spectrophotometry. Norfloxacin is structural analog of ofloxacin. Norfloxacin and aluminum form the binary complexes with norfloxacin to aluminum mole ratios 2:1 and 3:1. In the same time they observed that complexation with aluminum enhances the water solubility of norfloxacin. In our previous work [14] we found that norfloxacin with aluminum forms the complexes Al(Hnor) and Al(nor) (nor = norfloxacin) and in contrast to Alkayasi et al. we did not find formation of bis-complex. Other quantitative data on complexation between aluminum ion and fluoroquinolones can not be found in the available literature. Thus, the results obtained in the present work should contribute to the better understanding of interactions between fluoro-

quinolones and aluminum ion or aluminum containing drugs.

2. Experimental

2.1. Reagents and analysis

The stock solution of aluminum(III) chloride was prepared by dissolving doubly recrystallized salt $AlCl_3$ $6H_2O$ p.a. (Merck) in twice distilled water. The appropriate amount of HCl was added to avoid initial hydrolysis of Al^{3+} ion. The aluminum content was determined gravimetrically by the precipitation with 8-hydroxyquinoline and ammonia. Both methods gave the same results within 0.3%. The concentration of the free acid was determined potentiometrically using the Gran plot. The constancy of total proton concentration with time was considered as a criterion for the absence of initial aluminum(III) hydrolysis and was periodically checked by titration against standard NaOH before each series of measurements.

Ofloxacin abbreviated as H(oflo), purity 100%, was from Hoechst (Frankfurt am Main, F.R.G.). Standardization was performed by potentiometric titration against standard NaOH. Sodium hydroxide solution was prepared from concentrated volumetric solutions p.a. (Merck) by diluting with freshly boiled doubly distilled water, cooled under constant flow of purified nitrogen. The alkali concentration was checked by titration against potassium hydrogenphthalate. Hydrochloric acid solution was made from HCl 'Suprapure' (Merck) and standardized against tris(hydroxymethyl) aminomethane. The solution of lithium chloride was prepared form LiCl, p.a (Merck) by dissolving recrystallized salt in twice deionized water. The concentration was determined by evaporation of a known volume of solution to dryness at 573 K and weighing the residue.

2.2. Equipment

Potentiometric measurements were carried out using a Tacussel Isis 20000 digital pH-meter with a precision ± 0.1 mV or ± 0.001 pH units (in some measurements extended scale was used with a precision ± 0.01 mV). The pH meter was equipped with a Tacussel TC-100 combined electrode. Titrant was delivered from a Metrohm Dosimat model 665. The constant temperature was maintained with VEB Prufgerate model E3E circulating ultrathermostat. Spectrophotometric measurements were made with Varian model SuperScan 3 UV-Vis double beam spectrophotometer. Quartz cells (matching pair) with 1 cm pathlength were used; the reference cell being filled with 0.1 mol dm^{-3} LiCl in protonation measurements or, in complexation measurements, with the solution containing all components with exception of aluminum.

2.3. Procedure

All titrations were performed in a double mantled, thermostated glass vessel closed with Teflon cork. The constant temperature, to (298.0 ± 0.1) K was maintained by circulating the thermostated water through the jacket. Purified and oxygen free nitrogen gas was bubbled through the solution for providing an inert atmosphere and stirring. Additional stirring of solution was achieved with magnetic stirrer.

The electrochemical cell used for potentiometric measurements may be represented as RE/test solution (TS)/GE where RE and GE denote reference and glass electrode, respectively. The general composition of the test solution was: TS = M Al^{3+}, H H^+, L oflo, 0.1 mol dm^{-3} Cl^-, where M, H and L denote total molar concentrations of corresponding species.

The 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 includes the standard potential of the glass electrode, Q is the slope of the glass electrode response and E_j is a liquid junction potential whose contribution to E was found to be negligible. The E_0 was determined both, before and during each titration of the test solution, according to previously described procedure [14]. When the difference between two E_0 values was higher than 1.0 mV, the titration was rejected.

To reduce the concentration of the hydrogen ion, the alkali was added stepwise from an autoburette in small aliquots (0.005–0.01 ml). The potential was monitored after each addition of a titrant. The titration protocol was chosen in such way that complexation reactions could proceed in the conditions as close to the true equilibrium as possible. To achieve this the potential readings were taken every 5 min until steady values to ± 0.1 mV min^{-1} were obtained. At some points titration was stopped and solution was left with no further addition of base for the next 2–3 h, with continuos monitoring of potential. The titration was resumed if only the potential did not change more than ± 0.2 mV. Consequently, each titration lasted several (3–4) days. No back titrations were performed. Instead, agreement between duplicate titrations (better than 1%) served as criterion for reversibility of the reaction.

2.4. Data treatment

Three kinds of equilibria should be considered in the present study: (a) protonation of ofloxacinate ion; (b) hydrolysis of aluminum(III) ion; and (c) general three component equilibria,

$$p\mathrm{Al}^{3+} + q\mathrm{H}^{+} + r\mathrm{oflo} \leftrightarrow [\mathrm{Al}_p\mathrm{H}_q(\mathrm{oflo})_r]^{(3p+q-r)+};\ \beta_{p,q,r}$$

which include the case $q = 0$, i.e. the formation of pure binary complexes of Al^{3+}. Negative values of q denote hydroxo complexes. The overall protonation constants of ofloxacinate and stability constants of hydrolytic complexes of aluminum(III) 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 mathematical analysis of the experimental data was performed with the aid of general least-squares program Superquad [15]. In Superquad calculations the identity and stability of complexes which give the best fit to the experimental data, were determined by minimizing the error-squares sum of the potentials, U:

$$U = \sum w_i (E_{\mathrm{obs}} - E_{\mathrm{calc}})^2$$

where w_i represents a statistical weight assigned to each point of titration curve, E_{obs} and E_{calc} refer to the measured potential of the cell and the calculated one assuming the specific model and trial constants, respectively. The best model was chosen using these criteria: (a) the lowest value of U; (b) standard deviation in calculated stability constants less than 0.15 log units; (c) standard deviations in potential residuals, defined as:

$$s = \{\boldsymbol{e}w\boldsymbol{e}^T/(N-k)\}$$

where $\boldsymbol{e}$ is a vector in potential residuals ($E_{\mathrm{obs}} - E_{\mathrm{calc}}$), w is a weighting matrix, N is the number of observations and k is the number of refinable parameters, with standard deviation in volume readings 0.0005 cm^3 and standard deviation in potential readings 0.1 mV, should be less than 3.0. (d) goodness-of-fit statistics, χ (Pearson's test) at 95% confidence level, with 6 degree of freedom, less than 12.6 and (e) reasonably random scatter of potential residuals without any significant systematic trends. Along with Superquad the program Best [16] was also used in calculations.

The spectrophotometric data were evaluated with the aid of the program Squad [17]. In Squad calculations, the composition, stability and molar absorptivities, $\varepsilon_{p,q,r}$ of complexes, were determined by minimizing the sum, S, defined as:

$$S = \sum (A_{\mathrm{obs}} - A_{\mathrm{calc}})^2$$

where A_{obs} and A_{calc} refer to measured absorbance and that calculated according to equation:

$$A_{\mathrm{calc}} = \sum \beta_{p,q,r}[\mathrm{Al}]^p[\mathrm{H}]^q[\mathrm{oflo}]^r\varepsilon_{p,q,r}$$

For Squad calculations the spectra were digitized at 3 nm intervals. Acceptance criteria for each particular model were: (a) S lower than 1.0×10^{-2}; (b) standard deviation of the fit of the spectrum (SD) less than 1.0×10^{-2}; (c) standard deviation in calculated stability constants less than 0.08 log units. All the calculations were performed on an IBM PC PentiumPro/200 compatible computer.

Table 1
Summary of potentiometric data obtained in ofloxacin + Al^{3+} ion system in 0.1 mol dm^{-3} LiCl ionic medium, at 298 K[a]

Run number	C_{Al}	C_{HCl}	C_{oflo}	pH range	L/M
1	0.100	0.010	2.50	6.943–10.241	25.0
2	0.199	0.019	2.50	6.830–10.442	12.5
3	0.498	0.050	2.50	6.626–10.348	5.0
4	0.995	0.093	2.50	5.096–9.805	2.5
5	1.990	0.186	2.45	3.943–10.423	2.0
6	0.990	2.480	2.75	2.873–9.355	2.5
7	1.490	2.530	2.76	2.690–7.902	2.0
8	1.990	2.570	2.83	3.031–7.321	1.5

[a] All concentrations are expressed in mmol dm^{-3}. L/M denote initial ofloxacin to aluminum concentration ratio.

3. Results and discussion

In order to study speciation in three-component system Al^{3+}–H^+(or OH^-)–ofloxacin, it is necessary first to characterize the binary equilibria, i.e. hydrolysis of aluminum(III) ion and protonation of ofloxacin anion, under exactly the same experimental conditions as for complexation study. Hydrolysis of aluminum was studied in our previous work [11] so the results obtained there were used in this work.

3.1. Protonation of ofloxacin anion

Protonation constants, β_n, of ofloxacin anion, defined according to equilibrium:

$$n\mathrm{H}^+ + \mathrm{oflo}^- \leftrightarrow \mathrm{H}_n\mathrm{oflo} \quad (n = 1, 2)$$

were determined by glass electrode potentiometric titrations in 0.1 mol dm^{-3} LiCl medium at 298 K. Three titrations were carried out with 0.5, 1.2 and 2.45 mmol dm^{-3} total ofloxacin concentrations, in the pH range between 3.1 and 10.2. The experimental data were treated by using Superquad program. In total 290 points were included in calculations. Spectrophotometric measurements were made on solutions in which the concentration of ofloxacin was the same (2.48 mmol dm^{-3}) while the pH values were varied between 4.0 and 9.4 (22 solutions were used). The pH of the solutions was adjusted by the addition of standard alkali or HCl (as appropriate) and measured using glass | calomel electrode couple which was calibrated according to Irving et al. [18]. The spectra were taken in 220–450 nm wavelength interval. For the purpose of Squad calculations the obtained spectra were digitized at every 3 nm. The calculated values of protonation constants were: $\log \beta_1 = 8.212 \pm 0.002$ and $\log \beta_2 = 14.240 \pm 0.006$. Agreement between potentiometrically and spectrophotometrically obtained values was better than 1%.

3.2. The aluminum(III)-ofloxacin system

3.2.1. Potentiometric measurements

The experimental data obtained by emf measurements in 0.1 mol dm^{-3} LiCl medium at 298 K are summarized in Table 1. In the pH range studied (3.0–9.5) the maximum apparent ligand number reached was ca. 1.2. The highest concentration ratio of ofloxacin to Al^{3+} was 25:1. Beyond pH 9.0, solutions became turbid and drifting potential readings were obtained. No higher concentration ratios of ofloxacin to Al were used because they would seriously change constancy of the medium and in addition, the buffering effect of ofloxacin will hinder the reliable potentiometric measurements.

The analysis of the formation curves, plotted as the dependence of average ligand number, Z_c, on-log [oflo], indicate the formation of polynuclear complexes. From the maximal Z_c values attained it can be seen that either the complex $Al(oflo)_2$ or some mixed complexes, may be important. Titration curves of Al^{3+} + oflo show two weak buffer regions which are steeper than these of ofloxacin, thus indicating the titration of addi-

tional protons. The position of buffer regions depends upon concentration ratio of ofloxacin to aluminum, thus suggesting the formation of polynuclear species at lower [oflo]/[Al] values.

The equilibria in oflo + Al^{3+} system may be represented in a general form:

$$p\mathrm{Al}^{3+} + q\mathrm{H}^{+} + r\mathrm{oflo}^{-} \leftrightarrow \mathrm{Al}_p\mathrm{H}_q(\mathrm{Glu})_r$$

The stability constants of various (p, q, r) species formed in the above reaction, may be defined as:

$$\beta_{p,q,r} = C_{p,q,r} m^{-p} h^{-q} a^{-r}$$

where $C_{p,q,r}$ denotes the equilibrium concentration of the complex, m, h and a denote free concentrations of aluminum(III), proton and ofloxacin, respectively. Negative values of q represent hydroxo complexes. To determine the composition and stability constants of the species formed, the titration data were analyzed using the programs Best and Superquad. The following complexes were selected to find the model which best fit the experimental data: (1, 0, 1); (1, 0, 2); (1, 1, 1); (1, 2, 1); (1, 1, 2); (1, −1, 1); (1, −2, 1); (1, −3, 1); (1, −1, 2); (1, −2, 2); (1, −2, 3) and polymers (2, 1, 1); (2, 2, 1); (2, 1, 2); (2, −1, 1); (2, −2, 1); (2, −2, 2); (2, −3, 1); (2, −3, 2); (3, −1, 1); (3, −2, 1); (3, −1, 2); (3, −2, 2). More than 20 various models were tested. During the calculations analytical parameters (M_0, H_0 and L_0) were held constant while E_0 values were allowed to float. The hydrolytic complexes and protonated species of ofloxacinate were not refined during the calculations. First, each titration curve was treated separately using the program Best. Complexes were added in the model one at a time until the lowest value of σ_{fit} was achieved (usually less than 0.003). These complexes were then used as the starting model for the Superquad calculations. The following complexes were included: (1, 0, 1); (1, 0, 2); (1, −1, 1); (2, −1, 1) and (2, −2, 2). Then the data belonging to all titration curves, referred to one particular ofloxacin to aluminum concentration ratio, were treated together. As the additional criterion for model selection, served the refined values of E_0. If they were different from experimental ones for more than 0.5 mV, the model was considered as inadequate. The results of calculations indicate that the main species in aluminum–ofloxacin solutions are the binary complexes $Al(oflo)_2$ and Al(oflo) as well as the mixed protonated complex Al(Hoflo). Mixed hydrolytic polynuclear complexes are important at higher pH values. Calculated values of the stability constants are presented in Table 2 together with the calculated set of statistical parameters.

The distribution of various complexes in solution is shown in Fig. 1. Concentration of pure hydrolytic species, with exception of (13, −32) and (1, −4), is small, less than 5%, so they were not shown in Fig. 1. The dominating complex, at pH values between 3.5 and 5.5 is Al(Hoflo) with a maximum concentration at pH 3.5. Bearing in mind pH range in which it forms, one may suppose that its formation proceeds according to reaction:

$$\mathrm{Al(OH)}^{2+} + \mathrm{H_2oflo}^{+} \Leftrightarrow \mathrm{Al(Hoflo)}^{3+} + \mathrm{H_2O}$$

Isoelectric point of ofloxacin is at pH 7.1 so that at pH values lower than 5.0 most ofloxacin exists in the cationic form. Reactive species of aluminum at pH between 3.0 and 3.6 is monohy-

Table 2
Results of nonlinear least squares potentiometric data treatment of complexation equilibria between ofloxacin and Al^{3+} ion in 0.1 mol dm^{-3} LiCl ionic medium at 298 K[c]

Species	log ($\beta_{p,q,r} \pm \sigma$)	
	Superquad	Best
$Al(OH)^{2+}$	−5.62[a]	–
$Al(OH)_2^{+}$	−9.76[a]	–
$Al_{13}(OH)_{32}^{7+}$	−106.2[a]	–
$Al_2(OH)_2^{4+}$	−7.15[a]	–
$Al(OH)_4^{-}$	−23.46[b]	–
$Al_3(OH)_4^{5+}$	−13.73[a]	–
$Al(Hoflo)^{3+}$	15.93 ± 0.03	15.98 ± 0.08
$Al(oflo)_2$	14.84 ± 0.07	14.85 ± 0.02
Al(oflo)	10.20 ± 0.04	10.28 ± 0.08
Al(OH)oflo	3.04 ± 0.05	3.20 ± 0.10
$Al_2(OH)_2oflo^{3+}$	6.4 ± 0.1	6.38 ± 0.06
χ^2	21.4	–
S	3.40	–

[a] Ref. [11].
[b] Ref. [14].
[c] Statistical parameters, χ^2, s and σ are defined in the text.

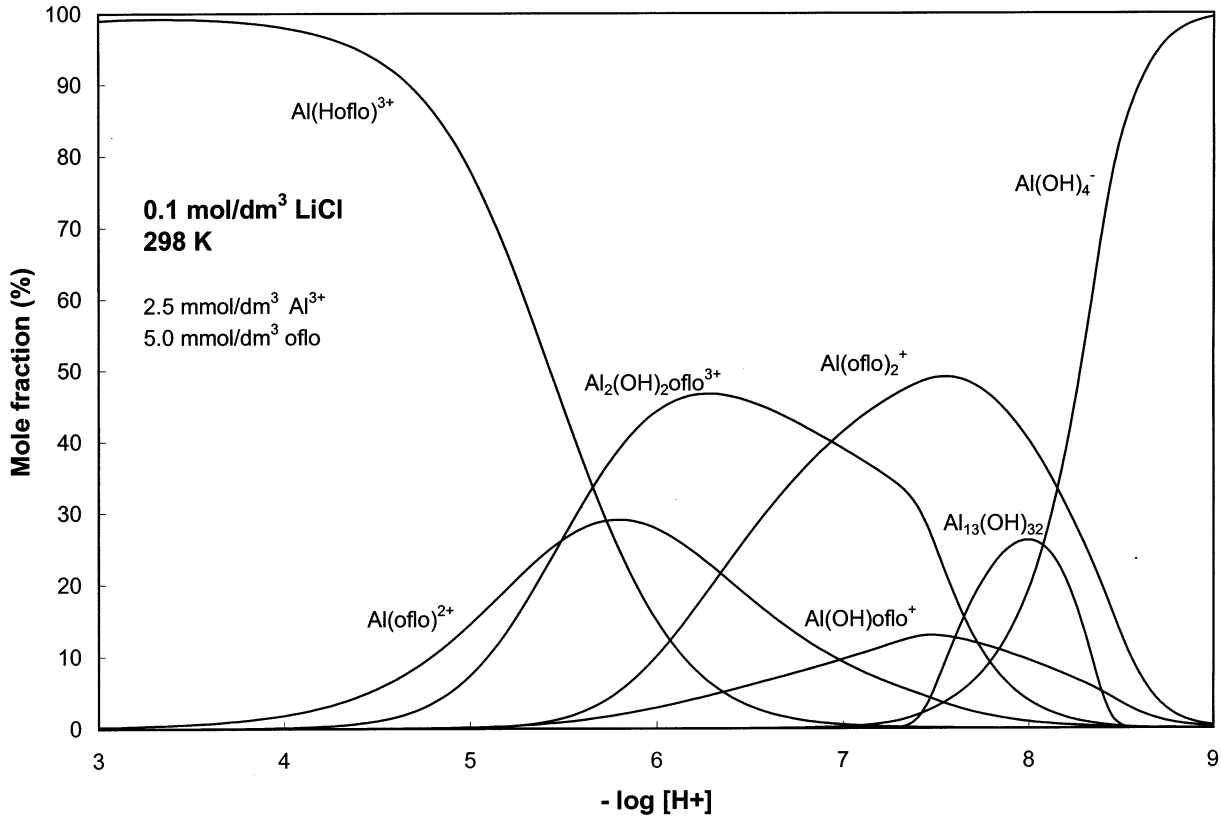


Fig. 1. Distribution of species in ofloxacin + Al^{3+} solutions.

droxo complex $Al(OH)^{2+}$ so that above reaction should be more probable than the one in which aqua-aluminum reacts with neutral ofloxacin:

$$Al^{3+} + Hoflo \Leftrightarrow Al(Hoflo)^{3+}$$

The binary complex $Al(oflo)^{2+}$ may be formed according to reaction:

$$Al(OH)^{2+} + Hoflo \Leftrightarrow Al(oflo)^{2+} + H_2O$$

Upon increasing the pH complex Al(oflo) begins to hydrolyze to Al(OH)oflo complex and to polymerize. The mixed dimer may be formed either by direct attaching of ofloxacin anion to aluminum hydrolytic dimer:

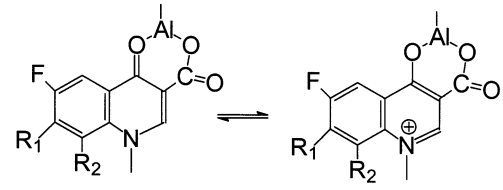
$$Al_2(OH)_2^{4+} + oflo^- \Leftrightarrow Al_2(OH)_2oflo^{3+}$$

The second molecule of ofloxacin may be directly bound to Al(oflo) or it may react with mixed hydrolytic complex to produce bis-complex:

$$Al(OH)oflo + Hoflo \Leftrightarrow Al(oflo)_2 + H_2O$$

It seems, judging by Fig. 1, that both reactions may take place simultaneously. Aluminate is the dominating complex at higher pH values. It is probably, precursor of the species which eventually precipitate from the solution.

In Al(oflo) complex 3-carboxyl and 4-carbonyl groups are involved in coordination owing to high affinity of aluminum toward oxygen. Concerning the structure of the complex we believe that equilibrium between two forms exists (Scheme 2).



Scheme 2.

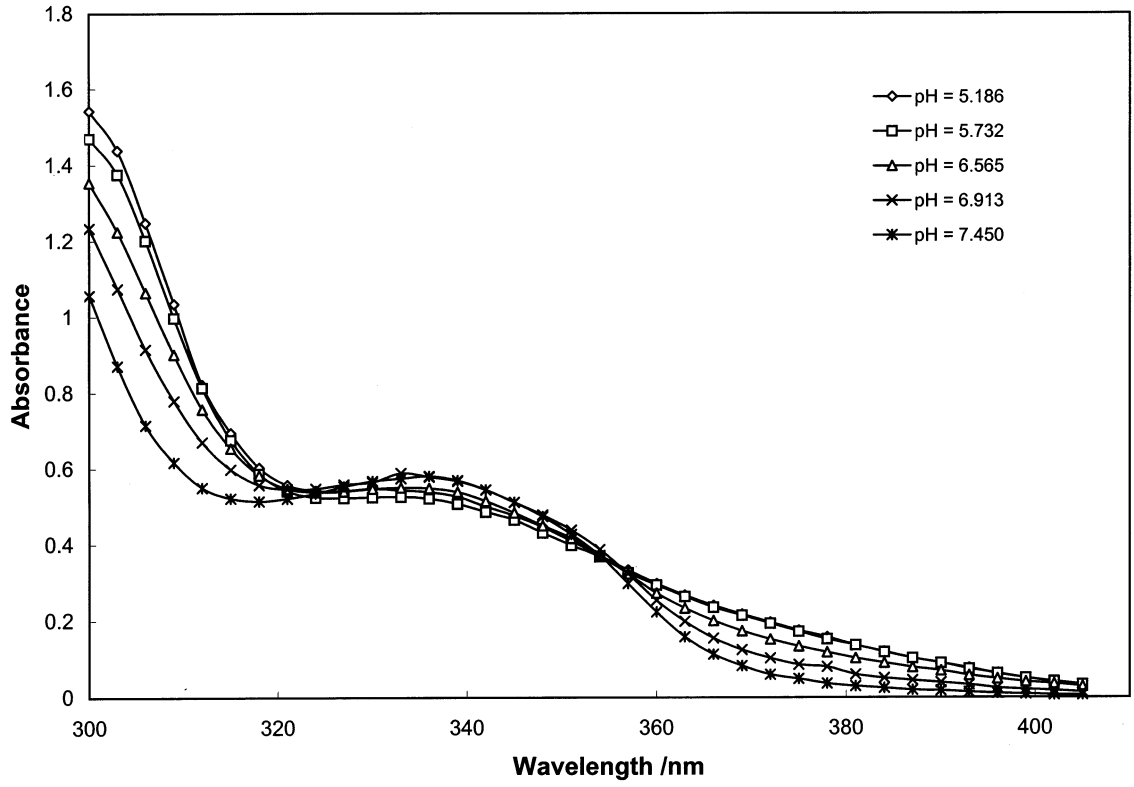


Fig. 2. Absorption spectra of aluminum(III) + ofloxacin solutions at different pH values. $[Al^{3+}] = 2.2 \times 10^{-5}$ mol dm^{-3}; [oflo] $= 5.5 \times 10^{-5}$ mol dm^{-3}.

The equilibrium is however, shifted to the left because of higher resonance stability of the structure in which double carbonyl bond is not broken.

3.3. Spectrophotometric measurements

Spectral measurements were made on solutions in which the concentration of aluminum and ofloxacin were constant ($C_{Al} = 2{,}18 \times 10^{-5}$ mol dm^{-3}, $C_{oflo} = 5.51 \times 10^{-5}$ mol dm^{-3}) while pH was varied between 4.0 and 9.0 (16 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. [18]. The pH of each test solution was checked daily, during one month. The stable values, within 0.01 pH and 0.004 absorbance units, were attained after 1 h and remained stable during couple of weeks. Solutions with pH values higher than 8.0 required longer time for equilibration, approximately, 1 week. Spectra of the test solutions were recorded in 220–450 nm wavelength interval. The experimentally obtained spectra are shown in Fig. 2. The spectra were not corrected for ofloxacin absorption.

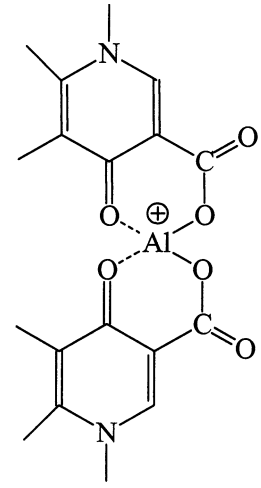
The spectra corrected for absorption of ofloxacin, exhibit an intensive band in 260–320 nm region with a well defined maximum at 303 nm, the position of which depends on pH. Another broad band appears between 350 and 410 nm. The band shows satisfactorily well defined maximum at 372 nm. In comparison with the spectrum of ofloxacin alone, all bands are shifted toward higher wavelengths (batochromic shift) for ca. 10 nm. In pH region 4.0–9.0 only the band centered at 303 nm showed significant dependence of absorptivity on pH, while the lower energy

band did not considerably change upon changing the pH. This indicates the formation of binary complexes in solution.

The spectral data were evaluated with the aid of the Squad program. In calculations, the molar absorbtivities of ofloxacinate ion, H(oflo) and H_2(oflo) were known from spectral measurements of ofloxacinate protonation and were fixed, while these of aluminum(III)-aqua ion and pure hydrolytic complex $(3,-4)$ were set to zero. To make the potentiometric and spectrophotometric measurements self-consistent, the calculations were carried out in the following way: first, all the complexes found in appropriate potentiometric titrations were included in Squad calculations and their stability constants were fixed while the molar absorptivities were allowed to float. The Squad multiple regression option was used for these calculations. In case the negative values for molar absorptivities were obtained, stability constants of the complexes were varied too, one at a time. Second, the complexes were added or excluded from the initial model also, one at a time. When the best possible fit was achieved (lowest S and SD values) the non-negative non-linear least squares option of Squad was used in a final calculation cycle. With the obtained set of complexes and their stability constants, Superquad calculations of potentiometric titrations were repeated and statistical parameters of the fit re-determined. This cycling was repeated until the best possible agreement between potentiometric and spectrophotometric models was achieved. Results of calculation showed that the complexes Al(Hoflo), $Al(oflo)_2$, Al(oflo), Al(OH)oflo and $Al_2(OH)_2$oflo were accepted with the stability constants: $\log \beta_{1,1,1} = 15.70 \pm 0.02$; $\log \beta_{1,0,2} = 14.83 \pm 0.05$; $\log \beta_{1,0,1} = 11.15 \pm 0.09$; $\log \beta_{1,-1,1} = 3.10 \pm 0.05$; and $\log \beta_{2,-2,1} = 6.10 \pm 0.09$, respectively, with (average) statistics, $SD = 1.2 \times 10^{-3}$ and $S = 1.0 \times 10^{-3}$. These results are in good agreement with potentiometric ones (Table 2). Calculated spectrum of Al(Hoflo), possesses two bands: the higher energy band centered at 280 nm and a broad band with absorption maximum near 340 nm. Comparison with the spectrum of pure ofloxacin indicates that the band centered at 280 nm belongs to quinolone nucleus, while the broad band at 330–360 nm may be attributed to overlapping Al-carbonyl and Al-carboxyl bonds absorption.

The results obtained in this study by potentiometric and spectrophotometric measurements in Al + ofloxacin system, indicate that at lower pH values ofloxacin forms strong complexes with aluminum ion, more stable than those with citric acid [19]. It means that ofloxacin may, upon concomitant intake, ameliorate gastrointestinal apsorption of aluminum ion *via* chelate formation. Bis–chelate complex encircles aluminum in hydrophobic environment (Scheme 3), so despite of +1 charge of this complex it probably, may cross gastrointestinal mucosa.

On the other hand, upon penetration of ofloxacin into tissues, it may extract aluminum ion from the cells through the formation of mixed or binary complex which could leave the cell somewhat freely. However, it does not seem possible that ofloxacin could extract aluminum from blood plasma, since in this case aluminum is tightly bound to transferrin [20]. The situation may be different in case of toxic levels of aluminum in plasma when ofloxacin would be able to



STRUCTURE OF Al(OFLO)2 COMPLEX

Scheme 3.

partly bind aluminum though chelation. This chelation is not that effective as with specially design chelate agents [21]. Further computer speciation simulations are needed to elucidate this aspect of ofloxacin action. Another possible site of ofloxacin action are kidneys. It is known that fraction of aluminum not bound to transferrin is excreted in kidneys or it may form deposits in kidney tissue [8]. Ofloxacin is also excreted in kidneys, mostly unchanged, and could in principle, mobilize aluminum from its deposits. The complexes formed could then be reabsorbed back into circulation via cortical veins. Thus, toxic level of aluminum in plasma may increase.

Acknowledgements

The financial support from the Ministry of Science and Technology of Serbia is gratefully acknowledged.

References

[1] V. Andriole, The Quinolones, Academic Press, London, 1988.
[2] M. Neuman, Clin. Pharmocokinetics 14 (1988) 96–121; T. Bergan, Quinolones, in: P.K. Peterson, J. Verhoef (Eds.), Antimicrobial Agents, Annual 2, Elsevier, Amsterdam, 1987, pp. 161–183
[3] K.M. Deppermann, H. Lode, Drugs 45 (3) (1993) 65–72.
[4] S.C. Wallis, B.G. Charles, L.R. Gahan, L.J. Filippich, M.D. Bredhauer, P.A. Duckworth, J. Pharm. Sci. 85 (1996) 803–809; HC. Neu, Am. J. Med. 87 (1989) 25–95; H. Shishido, K. Matsumoto, T. Nagatake, S. Tabuchi, Chemotherapy 36 (1988) 256–264
[5] J.M. Sultan, E.O.F. Suliman, Analyst 117 (1992) 1523–1526; D.S. Lee, H.J. Han, K. Kim, W.B. Park, J.K. Cho, J.H. Kim, J. Pharm. Biomed. Anal. 12 (1994) 157–164; L. Fratini, E.E.S. Schapoval, Int. J. Pharm. 127 (1996) 279–282
[6] R.B. Martin, Clin. Chem. 32 (1986) 1797–1806; M.R. Wills, J. Savory, in: S.S. Brown, J. Savory (Eds.), Chemical toxicology and chemistry of metals, Academic Press, New York, 1983, pp. 303–316
[7] D.N.S. Kerr and M.K. Ward, Aluminum intoxication: history of its clinical recognition and management, in: H. Sigel, A. Sigel (Eds.), Metal ions in biological systems, Vol. 24, Aluminum and its role in biology. Marcel Dekker, NY, 1988, pp. 217–258
[8] M.R. Wills, J. Savory, Aluminum toxicity and chronic renal failure, in: H. Sigel, A. Sigel (Eds.), Metal ions in biological systems. Vol. 24, Aluminum and its role in biology, Marcel Dekker, NY, 1988, pp. 315–345; M.J. Strong, R.M. Garruto, J.G. Joshi, W.R. Mundy, T.J. Shafer, J. Toxicol. Environ. Health 48 (1966) 599–613; N.A. Partridge, F.E. Regnier, J.L. White, S.L. Hem, Kidney Int. 35 (1989) 1413–1417
[9] R.W. Harris, in: F.R. Dintzis, J.A. Laslo (Eds.), Mineral absorption from the monogastric GI tract: chemical, nutritional and physiological aspects, Plenum Press, NY, 1989, pp. 67–93; G. Berthon, M. Venturini, J. Inorg. Biochem. 37 (1989) 69–90; G. Berthon, S. Dayde, J. Am. Coll. Nutr. 11 (1992) 340–348
[10] C.F. Baes, R.E. Mesmer, The hydrolysis of cations, Wiley, NY, 1976; J.W. Akitt, Prog. Nucl. Mag. Res. Spectr. 21 (1989) 1–149; C. Orvig, The aqueous chemistry of aluminum, in: G.H. Robinson (Ed.), Coordination chemistry of aluminum, VCH, Weinheim, 1993; W.R. Harris, G. Berthon, J. Philip Day, C. Exley, T.P. Flaten, W.F. Forbes, C. Orvig, P.F. Zatta, J. Toxicol. Environ. Health 48 (1996) 543–568
[11] P. Djurdjevic and R. Jelic, Main Group Met. Chem. 21 (1998) 331–346
[12] L.O. Ohman, Inorg. Chem. 27 (1988) 2565–2570.
[13] H.N. Alkayashi, M.H. Abdelhay, M.S. Salem, M.A. Garaibeh, T.E. Nawas, Int. J. Pharm. 87 (1992) 73–77.
[14] P. Djurdjevic, M. Jelikic-Stankov, Anal. Chim. Acta 300 (1995) 253–259.
[15] P. Gans, A. Sabatini, A. Vacca, J. Chem. Soc. Dalton Trans. (1985) 1195–1200
[16] A.E. Martell, R.J. Motekaitis, Determination and use of stability constants, VCH, Weinheim, 1988.
[17] D.J. Legget (Ed.), Computational methods for the determination of formation constants, Plenum Press, NY, 1985, pp. 159–220
[18] H.M. Irving, M.G. Miles, L.D. Pettit, Anal. Chim. Acta 38 (1967) 475–488.
[19] P. Slanina, W. Frech, L.G. Ekstrom, L. Loof, S. Slorach, A. Cedergren, Clin. Chem. 32 (1986) 539–541; R.B. Martin, J. Inorg. Biochem. 28 (1986) 181–187
[20] W.R. Harris, J. Sheldon, Inorg. Chem. 29 (1990) 119–124.
[21] D.J. Clevette, C. Orvig, Polyhedron 9 (1990) 151–161.