

The Effect of Surfactants on Equilibria in Aluminium(III) Ion + Ofloxacin Solution and Adsorption of Ofloxacin on Aluminium-Oxide

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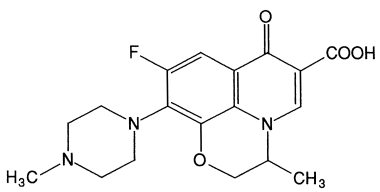
The protonation and complex formation equilibria in aluminium(III) + ofloxacin (Hoflo) solutions in the presence of either cetyltrimethylammonium bromide (CTAB, 5.0 mmol L^{-1}), cetylpyridinium chloride (CPCL, 2.0 mmol L^{-1}) or polyethylene glycol tert-octylphenyl ether (triton X-100, 1.0 mmol L^{-1}) have been studied by glass-electrode potentiometric measurements in a 0.1 mol L^{-1} LiCl ionic medium at 298 K. In the concentration range $0.4 \leq C_{Al} \leq 1.0$ mmol L^{-1}, with a ligand to metal ratio of 1:1 to 5:1, and $3.5 \leq -\log h \leq 7.0$, a non-linear least squares treatment of the data indicate that in all studied systems the complex $Al(oflo)^{2+}$ forms as the dominating one. Its overall stability constant (log β) is in the range 10.37–11.90 (depending on the type of surfactant), which is about 1 log unit higher than in the absence of surfactants. The formation of *bis*(ofloxacinato) and mixed protonated or hydrolytic complexes is largely suppressed in the presence of surfactants. The adsorption of ofloxacin on aluminium oxide was studied in neutral, acidic (0.1 mol L^{-1} HCl) and alkaline (0.001 mol L^{-1} NaOH) media; in absence and presence of ionic surfactants; sodium dodecylsulfate (SDS, 10 mmol L^{-1}); or CTAB (5.0 mmol L^{-1}). The adsorption is of the Freundlich type and is higher in neutral media with no presence of surfactants, while in an acidic medium it is significantly enhanced in the presence of SDS. Both surfactants increase the adsorption in an alkaline medium. The observed phenomena were explained based on the hydrophilic/lipophilic properties of the surfactants.

In the previous work[1] we studied the complex formation equilibria between Al^{3+} ion and ofloxacin (Hoflo) in a 0.1 mol L^{-1} LiCl ionic medium at 298 K. At ligand-to-metal concentration ratios between 2:1 to 10:1 the main complexes found were $Al(oflo)_2^{+}$, $Al(Hoflo)^{3+}$ and $Al(oflo)^{2+}$ with several mixed hydrolytic complexes, in the pH range 3.0–8.0. In the presence of sodium dodecylsulfate (SDS), hydrolysis was favored, and only the formation of the $Al(oflo)^{2+}$ complex was detected at higher ligand-to-metal concentration ratios (> 3).[2] Since the presence of SDS was unfavorable for complexation, in the present work we used cationic, cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPCL) and a neutral surfactant, polyethylene glycol *tert*-octyl phenyl ether (triton X-100), with a ligand-to-metal concentration ratio of up to 5:1 to study their effect on identity and stability of species formed in the Al^{3+} + ofloxacin system.

Ofloxacin (9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7*H*-pyrido(1,2,3-*de*)1,4-benzoxazine-6-carboxylic acid), H(oflo), belongs to the class of fluorinated 4-quinolone antibiotics, which finds use in the treatment of systemic urinary and respiratory infections.[3,4] From the chemical structure (Scheme 1) it may be seen that ofloxacin belongs to a class of heterocyclic amino acids, which in solution may exist in cationic, zwitterionic, neutral and anionic forms. The neutral form (Hoflo) presented in Scheme 1, actually, for the difference of naturrally occurring amino acids may exist in solution, since the protonation constant of carboxylate group and



Scheme 1. Structure of ofloxacin.

tertiary nitrogen are not separated too much apart.[5,6] Thus, the number of forms as well as the vicinity of the carbonyl and carboxyl groups make ofloxacin a suitable ligand for hard acid metal ions, particularly for aluminium(III) and iron(III) ions. Aluminium is generally regarded as a toxic or detrimental element.[7,8] Nevertheless, its compounds are extensively used pharmaceutically as antacids, phosphate binders or adjuvants in various protein-based vaccines. Environmental sources of aluminium include food, food additives, drinks, aerosols, etc. Normally, despite an oral intake ranging from 5 to 10 mg daily (ingestion of food, aluminium-based drugs and drink, atmospheric dust), aluminium is very little absorbed in serum and tissues.[9] Normal serum levels are 0.07 to 0.30 μmol L^{-1}. However, high levels of aluminium may accumulate in the tissues of patients who have renal insufficiency or kidney failure, and are treated by dialysis fluids that contain aluminium, or are given aluminium hydroxide gels to control a high plasma phosphate level.[10] These patients may develop blood, bone or

brain diseases, which may linked to excess aluminum.[11] Clinical investigations[12,13] have shown that concomitant intake of ofloxacin and aluminum-based drugs results in a reduced maximal serum concentration of ofloxacin accompanied by decrease in AUC (area under the "concentration vs time" curve), thus leading to a decreased bio-availability of the durug, down to 30%. An explanation for this interaction may be chelation between aluminium and ofloxacin and/or the adsorption of ofloxacin on the surface of aluminium-hydroxide/aluminium phosphate. These interactions may result in an increased availability of soluble aluminium. As already pointed out by several authors,[14,15,16] dietary carboxylic acids (especially citric) may form stable complexes with Al, giving rise to shift of the dissolution equilibrium of poorly soluble $Al(OH)_3$ or $AlPO_4$ to the right, and thus to increase their solubility. In this way, released aluminium ions may be complexed into neutral forms either by dietary acids or by other substances present in gastrointestinal (gi) tract. These forms could then pass through the gi membrane.[17] Therefore, it may be expected that the simultaneous ingestion of ofloxacin and $Al(OH)_3$ can lead to the formation of soluble Al-complexes that can be absorbed by the gi tract. Various surface-active substances, normally present in the gi tract,[17] will influence the aluminium–ofloxacin complex formation. Also, the cellular uptake of aluminium–ofloxacin complexes is dependent on their interaction with membrane phospholipids so as to mimic their function; the effect of surfactants (or, surface-active agents, SAA), CTAB, CPCL and triton X-100 on complex formation between Al^{3+} and ofloxacin was studied. These substances alter the properties of solution interfaces (solution–vapor, solution–solid, etc.).[18] In this way, SAA may influence the processes taking place at or inside the interfaces. The SAA are generally classified as either anionic, cationic or nonionic (neutral) according to the charge of their hydrophilic head group upon dissolution in water.[19] If sufficient SAA is added to an aqueous solution, aggregation of its molecules occurs, giving rise to ordered structures called micelles. Micelles are often spherical in shape, but at a larger concentration of SAA, they can take other, more distorted forms.[20] The threshold concentration of SAA at which micelles begin to form is termed the critical micelle concentration (CMC).[21] The CMC values[22] are for SDS, 8.2 for CTAB 0.70–0.98 for CPCL 0.58–0.62 and for triton X-100 0.24–0.90 mmol L^{-1}. The CMC values vary with the composition and concentration of the supporting ionic medium; therefore, in this work we used significantly higher concentrations of SAA than their CMC. Other substances present in solution could partition into the interior of the micelle, thereby increasing the total aqueous solubility of the substance by a process referred to as micellar solubilization.[23] Thus, the surfactant would have the effect to solubilize both the quinolone and its chelate complex with aluminum, to exclude water molecules from the complexation reaction sphere and to prevent the hydrolysis of Al–ofloxacin complexes.

The primary aim of the present paper was to provide reliable data concerning speciation in aluminum(III) + ofloxacin + surfactant solutions so that they could be used in modeling studies of aluminum-based drugs and ofloxacin interactions in vivo. Adsorption phenomena play important roles in hydrolyzed aluminium solutions. Aluminum hydroxide and various hydrolytic polymers are surface active and can easily adsorb changed species from a solution. It is therefore of interest to study adsorption in a model system, Al_2O_3 + Hoflo, in order to gain a better understanding of speciation in Al^{3+} + ofloxacin solutions. The adsorption of ofloxacin on aluminium oxide and the effect of anionic surfactant, sodium dodecylsulfate (SDS, 10.0 mmol L^{-1}) and a cationic one, CTAB (5.0 mmol L^{-1}), were investigated to derive a model for ofloxacin bioavailability upon a concomitant intake of aluminium-antacids and ofloxacin.

The solution equilibria between the aluminum(III) ion and ofloxacin in the presence of CTAB, CPCL or triton X-100 were studied by potentiometry in the 2:1 to 5:1 concentration range of ofloxacin to aluminium. The protonation equilibria of the ofloxacin anion as well as the hydrolysis of aluminum (in the presence of CPCL) were investigated in separate experiments.

Experimental

Reagents and Analysis. A stock solution of aluminum(III) chloride was prepared by dissolving doubly recrystallized $AlCl_3 \cdot 6H_2O$ p.a. (Merck) in twice-distilled water. The appropriate amount of HCl was added to avoid an initial hydrolysis of the Al^{3+} ion. The aluminum content was determined gravimetrically by precipitation with either 8-quinolinol or ammonia. Both methods gave the same results within 0.3%. The concentration of free acid was determined potentiometrically using a Gran plot. The constancy of the total proton concentration with time was considered to be 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, purity 100% (M_r = 361.4) was from Hoechst (Frankfurt am Main, F.R.G.). Standardization was performed by potentiometric titration against standard NaOH. Sodium *n*-dodecyl sulfate (SDS), $CH_3(CH_2)_{11}OSO_3Na$, M_r = 288.4, cetyltrimethyl ammonium bromide (CTAB), $[C_{16}H_{33}N^+(CH_3)_3]Br^-$, M_r = 364.5, and cetylpyridinium chloride were products of Sigma (USA) while polyethylene glycol *tert*-octylphenyl ether (triton X-100), M_r = 647, was a product of Fluka (Austria). Before use SDS was purified by washing with ether and 95% ethanol and subsequently dried in a desiccator containing P_2O_5 CTAB amd CPCL were washed with ethanol and recrystallized from water. The traces of poly(ethyleneglycoil)s in triton were removed by extraction with *n*-buthanole. A purity of surfactants was checked with TLC and spectrophotometry according to recommended procedure[24,25] and by a measurement of the pH of their water solution. No basic impurities were detecteed.

A sodium hydroxide solution was prepared from concentrated volumetric solutions p.a. (Merck) by diluting with freshly boiled doubly distilled water, and cooled under a constant flow of purified nitrogen. The alkali concentration was cheked by titration against potassium hydrogen phthalate. The hydrochloric acid solution was made from HCl "Suprapure" (Merck) and standardized against tris(hydroxymethyl)aminomethane. A solution of lithium chloride was prepared from 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.

Equipment. Potentiometric measurements were carried out using a Tacussel Isis 20000 digital pH-meter with a resolution

±0.1 mV (in some measurements an extended scale was used with a resolution ±0.01 mV). The pH meter was equipped with a Tacussel TC-100 combined electrode. Titrant was delivered from a Metrohm Dosimat model 665. A constant temperature was maintained with a VEB Prufgerate model E3E circulating ultrathermostat. Spectral measurements were made on single-beam Pye Unicam SP5-600 spectrophotometer.

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

An 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^+, X SAA, L oflo, 0.1 mol L^{-1} Cl^-, where M, H, X, and L denote the total molar concentrations of the 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(s), 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 neigligible. E_0 was determined as described previously.[2] Experimental data obtained in strong acid–strong base titration were analyzed with the aid of the Magec[26] program. The calculated values were Q = 59.1 mV and self-protolysis constant of water, pK_W = 13.50(2). In the presence of surfactants the obtained values for pK_W were 13.12 ± 0.02 (CTAB), 13.01 + 0.03 (CPCL) and 13.40 ± 0.06 (triton).

To reduce the concentration of 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 titrant. The titration protocol was chosen in such a way that the hydrolysis and complexation reactions would proceed under conditions as close to true equilibrium as possible. To achieve this, potential readings were taken every 5 min until steady values of ±0.1 mV min^{-1} were obtained. Hence, the average equilibration time for each point was 5–10 min at the beginning of titration and 20–30 min when complexation or hydrolysis occurred. If stabilization of potential readings could not be achieved within this time interval, the addition of a new aliquot of titrant was initiated, and corresponding point was excluded from the calculations. No back titrations were performed. Instead, agreement between duplicate titrations (better than 1%) served as a criterion for reversibility of the reaction. The titrations were terminated when drifted potential readings were obtained and turbidity of the solutions was observed.

Adsorption of Ofloxacin on Aluminium Oxide. Prior to use, aluminium-oxide (Merck, p.a.) was heated to 1373 K for three hours, and afterwards cooled to room temperature in a desiccator containing CaO. A series of test tubes with grounded stoppers were prepared by washing in concentrated hot HNO_3. 0.1 g of aluminium oxide was weighed (with accuracy ±0.1 mg) into each of the test tubes. Varying volumes of the stock solution of ofloxacin (0.1–0.5 mL) were transferred into tubes using an Eppendorf micro-pipette. Then, three series were prepared by the further addition of either, distilled water, 0.1 mol L^{-1} HCl or 0.001 mol L^{-1} NaOH, so as to make the final volume of the solution 5.0 mL. Other series were prepared in the same way, but with the addition of either SDS (10.0 mmol L^{-1}) or CTAB (5.0 mmol L^{-1}). Tubes were stoppered, clamped into a thermostated (298 K) shaker, and energetically shaken for 3 hours. The concentration of ofloxacin was determined spectrophotometrically at 270 nm. The calibration curve was taken in the concentration range of ofloxacin between 1–30 μg mL^{-1}.

Data Treatment. Three kinds of equilibria should be considered in the present study: (a) protonation of the ofloxacinate ion, (b) hydrolysis of the aquaaluminium(III) ion, and (c) general three-component equilibria,

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

which include the case q = 0, i.e. the formation of pure binary complexes of Al^{3+}. Negative values of q represent hydroxo complexes. The overall protonation constants of ofloxacinate were determined in separate experiments. The hydrolysis of aquaaluminium(III) ion was taken into account based on our previous work.[27] Thus, in evaluating the three component equilibria (c), the binary models (a) and (b) were considered as being known.

A mathematical analysis of the experimental data was performed with the aid of a general least-squares program, Superquad.[28] 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 the 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 the following criteria: (a) the lowest value of U, (b) standard deviation in calculated stability constants less than 0.15 log units (ie. about 10%, of the corresponding stability constant value), (c) standard deviations in potential residuals, defined as:

$$s = \{ewe^T/(N-k)\},$$

where 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 a standard deviation in volume readings of 0.005 mL and a standard deviation in potential readings of 0.1 mV should be less than 3.0. (d) goodness-of-fit statistics, χ^2 (Pearson's test) at the 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[29] was also used for calculations.

Results and Discussion

The Effect of CPCL on Hydrolysis of the Aquaaluminium(III) Ion. The hydrolysis of aquaaluminum in the absence of surfactants[1] and in the presence of either SDS, CTAB, tween 20 or triton X-100,[30] in 0.1 mol L^{-1} LiCl, at 298 K, was studied in our earlier work so the results obtained there were used in this work. The main hydrolytic species were $Al(OH)^{2+}$, $Al_3(OH)_4{}^{5+}$ and $Al_{13}(OH)_{32}{}^{7+}$. In addition to this

Table 1. Summary of Potentiometric Experimental Data Obtained in 0.1 mol L^{-1} LiCl(S) Ionic Medium at 298 K CTAB = cetyltrimethylammonium bromide, CPCL = cetylpyridinium chloride. C_X denotes concentration of corresponding species in mmol L^{-1}.

Run	C_{Al}	$C_{ofloxacin}$	C_{HCl}	S + CTAB	S + CPCL	S + Triton	pH range	Z_{max}
1	5.0	—	3.0	—	2.0	—	2.587–4.231	0.127
2	2.0	—	3.0	—	2.0	—	2.593–4.526	0.329
3	1.0	—	3.0	—	2.0	—	2.533–4.485	0.500
4	0.5	2.5	3.0	—	2.0	—	2.853–7.091	1.80
5	1.0	2.5	3.0	—	2.0	—	2.737–6.616	1.90
6	2.0	2.5	3.0	—	2.0	—	2.624–6.192	1.17
7	1.0	2.4	3.0	5.0	—	—	2.640–6.712	1.40
8	0.6	1.0	2.0	5.0	—	—	3.082–6.988	1.58
9	0.4	1.2	2.0	5.0	—	—	3.025–7.123	1.86
10	1.0	2.4	3.0	—	—	1.0	2.714–6.100	1.22
11	0.6	1.0	2.0	—	—	1.0	2.799–6.153	1.59
12	0.4	1.2	2.0	—	—	1.0	2.939–7.498	2.0

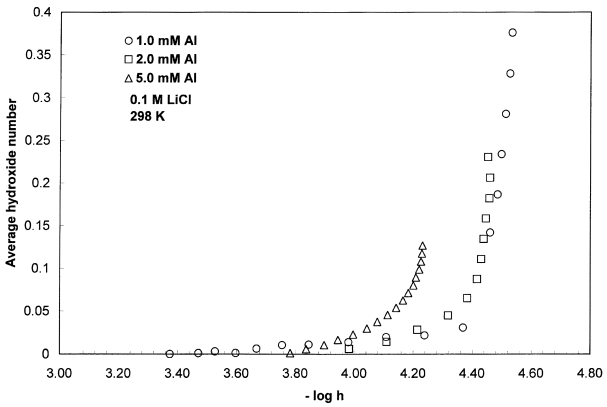


Fig. 1. Hydrolysis of Al^{3+} in 0.1 mol L^{-1} LiCl medium, at 298 K in the presence of 2.0 mmol L^{-1} cetylpyridinium-chloride.

primary model, soluble $Al(OH)_3$ and solid $Al(OH)_3$ were added. Since the effect of CPCL on hydrolysis was not studied previously in this work, it was investigated under the same experimental conditions. The obtained experimental data are summarized in Table 1. Under the chosen experimental conditions the aquaaluminum(III) ion hydrolyzes between pH 3.5 and 4.5, depending on its concentration. The maximum value reached for the average hydroxide number, Z, defined as: $Z = (h - H)/M$, was ca. 0.4. The data show that, for each concentration of aluminum studied, separate titration curves, $Z = Z(-\log h)$, were obtained (Fig. 1). This indicates the formation of polynuclear complexes. The pH region in which hydrolysis occurs depends upon the total concentration of aluminium. Thus, as the concentration of Al increases, the beginning of the hydrolysis shifts toward lower pH values, while at the same time the degree of the hydrolysis decreases. Possible complexation of the aluminium ion with the chloride ion from the ionic medium should appear as a constant effect because of the relatively high concentration of the medium; therefore, is should not affect the number of hydroxide ions bound to aluminium. Though the titrations were performed over a wide pH range, for the purpose of calculations, some reduction of the number of points was necessary. Points at pH values lower than 3.0, where the hydrolysis is neglible, and at pH's higher than 4.5, where solutions became turbid and colloid formation may take place, were excluded from the calculations.

Reacting with water molecules the aluminium ion forms one or more hydrolytic complexes of the general composition $Al_p(OH)_q^{(3p-q)^+}$ [further abbreviated as (p, q)] whose overall formation constants, $\beta_{p,q}$, may be defined as

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

where $C_{p,q}$ denotes the equilibrium concentration of the (p, q) complex and m is the concentration of the free aluminium ion. First, each titration curve was processed separately using the program Best. Complexes from the initial set comprising (1, −1), (1, −2), (1, −3), (2, −1), (2, −2), (2, −3), (2, −4), (2, −6), (3, −3), (3, −4), (6, −12), (6, −15), (6, −18), (8, −12) and (13, −32) were introduced one at a time until the minimum vasue of σ_{fit} was obtained. During the calculations, all of the titration parameters (M_0, H_0) were kept constant while the pH values in a repeated cycle of calculations were adjusted until the best possible value of σ_{fit} was obtained. The calculations indicate that the hydrolytic curves can be fitted with the complexes: $Al(OH)^{2+}$, $Al(OH)_3(aq)$, $Al_3(OH)_4{}^{5+}$ and $Al_{13}(OH)_{32}{}^{7+}$. All of the titration curves were then processed together, this time using the program Superquad. In Superquad calculations the E_0 values were allowed to float, while all analytical parameters were held constant. All of the complexes found in the Best calculations were accepted, except of tridecamer. A stepwise introduction of other complexes from the initial set lead either to their rejection or to a much worse set of statistical parameters determining the goodness of the fit. The higher hydrolytic polymers were not accepted as well. To decide which set of complexes gave the best fit, all afore-mentioned complexes were refined together. No acceptable fit was obtained. Therefore, in the next calculation cycle the stability constant of the monomer $Al(OH)^{2+}$ was held constant (fixed) while these of $Al(OH)_3$ and $Al_3(OH)_4{}^{5+}$ were varied. Both complexes were accepted with the values for log β being slightly higher than in a previous calculation. Thus, the results of the calculation show that the experimental data can be fitted

Table 2. The Stability Constants of Hydrolytic Complexes of Aluminium in 0.1 mol L^{-1} LiCl Ionic Medium, at 298 K, in the Presence of Cetylpyridinium-Chloride
The constants ($\beta_{p,q}$) are defined for the equilibrium: $pAl^{3+} + qH_2O \rightleftarrows Al_p(OH)_q^{(3p-q)^+}$. K_S is a solubility product defined for equilibrium: $Al(OH)_{3(s)} + 3H^+ \rightleftarrows Al^{3+} + 3H_2O$.

Species	$Al(OH)^{2+}$	$Al_3(OH)_4{}^{3+}$	$Al(OH)_3$(aq)	Al(OH)3(s)
$-\log \beta_{p,q}$	4.81 ± 0.06	13.82 ± 0.02	14.17 ± 0.08	pK_S = 10.38 ± 0.02

Statistical parameters of the fit: χ^2 = 12.0–12.5; s = 1.3–1.6

with the complexes $Al(OH)^{2+}$, $Al(OH)_3$(aq) and $Al_3(OH)_4{}^{5+}$. Their respective stability constants ($\log \beta_{p,q}$) are given in Table 2. It can be seen that the calculated statistical parameters of the fit satisfy all of the acceptance criteria, thus confirming the existence of the (1, −1), (1, −3) and (3, −4) hydrolytic complexes in the presence of CPCL with high probability. The distribution diagram of the formed hydrolytic complexes was calculated using the program Solgaswater,[30] and is given in Fig. 2. The complex $Al_3(OH)_4{}^{5+}$ is important only at higher concentrations of aluminium. Insoluble hydroxide forms between pH 4.0 and 4.5, depending on the total aluminium concentration. Because its relative quantity sharply increases with increasing the pH of the solution, part of the measurements were actually performed in a micro-heterogeneous system. It seems that the formation of the tridecamer, characteristic of the hydrolysis of the Al^{3+} aqua ion in the presence of other SAA, is suppressed in the presence of CPCL.

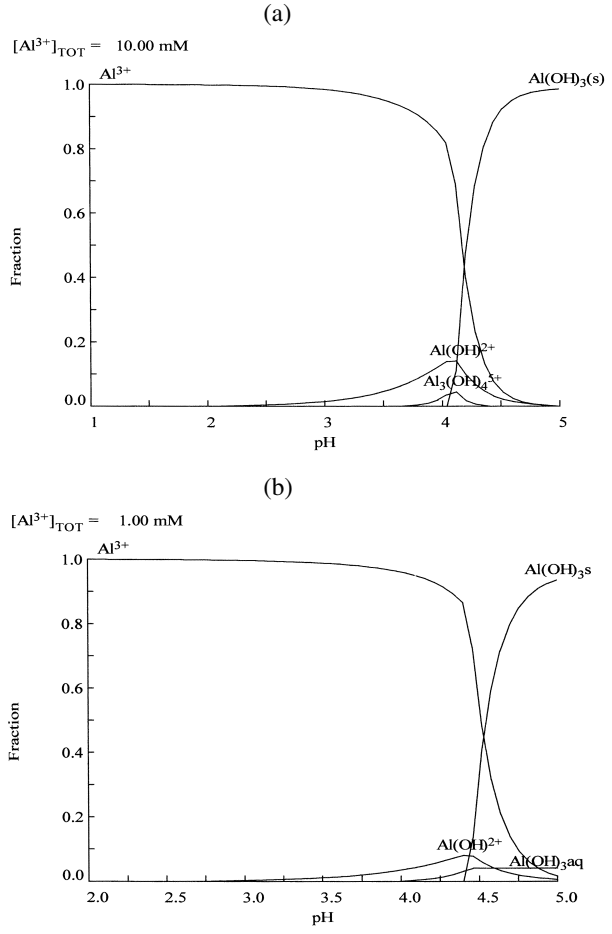


Fig. 2. Distrubution diagram of Al^{3+} hydrolytic products: (a), $[Al^{3+}]_{tot}$ = 10.0 mmol L^{-1}; (b), $[Al^{3+}]_{tot}$ = 1.0 mmol L^{-1} (0.1 mol L^{-1} LiCl + 2.0 mmol L^{-1} cetylpyridinium chloride, 298 K).

Determination of the Solubility Product of $Al(OH)_3$. The solubility product of aluminium hydroxide in the presence of CPCL was determined from the hydrolytic curves (Fig. 1) employing a procedure described in previous work.[27] For the reaction:

$$Al(OH)_3(s) + 3H^+ \rightleftarrows Al^{3+} + 3H_2O$$

the equilibrium constant, K_S, is defined as

$$K_s = \frac{[Al^{3+}]}{[H^+]_p{}^3}.$$

The concentration of hydrogen ion, at which precipitation (or dissolution) of aluminium hydroxide begins, $[H^+]_p$, was determined by extrapolation of higher, nearly linear part of the hydrolytic curve to intercept with the $-\log h$ axes. Form the point of intercept, pH_{ext}, the concentration of hydrogen ion was calculated as

$$[H^+]_p = \frac{1}{\gamma_+} 10^{-pH_{ext}}$$

where the activity coefficient for hydrogen ion, γ_+, was calculated from the extended Debye-Huckel equation.[31] The free concentration of aluminium ion, $[Al^{3+}]$, was calculated from the equation

$$C_{Al} = [Al^{3+}] + \sum p\beta_{p,q}[Al^{3+}]^p[H^+]^{-q} \quad ,$$

which, for total aluminum concentrations, 1.0, 2.0 or 5.0 mmol L^{-1}, was solved for free aluminium concentrations using the program Species.[32] The average value of K_S was pK_S = 10.38 ± 0.02, which is slightly higher than that in pure ionic medium, pK_S = 10.32 ± 0.04.[27]

Protonation of the Ofloxacinate Ion. The overall protonation constants, β_n, of the ofloxacin anion, defined according to equilibrium

$$nH^+ + oflo^- \rightleftarrows H_noflo;\ \beta_n\ (n = 1,2),$$

were determined by glass electrode potentiometric titrations in a 0.1 mol L^{-1} LiCl medium at 298 K. Three titrations were carried out with 0.5; 1.2 and 2.45 mmol L^{-1} total ofloxacin

Table 3. Calculated Protonation Constants of Ofloxacin Anion in 0.1 mol L^{-1} LiCl ionic Medium (S), in the Presence or Surfactants, at 298 K

SDS = sodium dodecylsulfate, CTAB = cetyltrimethylammonium bromide, CPCL = cetylpyridinium chloride. β_n denotes overall protonation constant for equilibrium: $nH^+ + oflo^- \rightarrow H_noflo$ ($n = 1, 2$) while K_i denotes successive protonation constant defined for equilibrium: $H^+ + H_{i-n+1}$ oflo $\rightarrow H_ioflo$ ($n = 2$, $i = 1, 2$).

Stability constant	S	S + CPCL	S + CTAB	S + SDS	S + Triton
Log β_1	8.212 ± 0.003	8.21 ± 0.04	8.42 ± 0.04	9.03 ± 0.01	9.24 ± 0.02
Log β_2	14.24 ± 0.01	14.51 ± 0.07	16.17 ± 0.05	16.98 ± 0.1	16.32 ± 0.3
Log K_1	8.212	8.21	8.42	9.03	9.24
Log K_2	6.03	6.30	7.75	7.96	7.08

concentrations, with the addition of either, CTAB (5.0 mmol L^{-1}), CPCL (2.0 mmol L^{-1}) or triton X-100 (1.0 mmol L^{-1}), in the pH range between 2.4 to 10.2. The experimental data were treated by using the Superquad program. In total, 110 points were included in the calculations. The results are given in Table 3 together with statistical parameters from Superquad calculations (χ^2 and s). As can be seen from Table 3, protonation constants of the ofloxacin anion are considerably different in the presence of surfactants than in a pure ionic medium. Data in a pure ionic medium and in the presence of SDS are taken from previous studies.[1,2] The first successive protonation constant (log K_1) refers to the protonation of tertiary 4′-piperazinyl nitrogen, while the second one (log K_2) refers to protonation of the carbonylate group.[5,6] The stabilizing effect on the first protonation increases in the order S ≅ CPCL < CTAB < SDS < triton, while on the second protonation the order is S < CPCL < triton < CTAB < SDS. Thus, triton exerts highest influence on a first protonation, while SDS and CTAB show greater influence on the second protonation. Since the first protonation leads to the formation of the zwitterionic form of ofloxacin, according to the equation

$$H^+ + oflo^- \rightleftarrows Hoflo^{\pm},$$

which may easily partition into the interior of neutral surfactant triton X-100, the equilibrium is shifted to the right, thus giving rise to a higher value of the protonation constant. The second protonation gives cationic form of the oloxacin with positively charged 4′-nitrogen; therefore, anionic SDS by surrounding the H_2oflo^+ molecule prevents its dissociation.

The Aluminium(III)–Ofloxacin System in the Presence of Surfactants. The experimental data obtained by *emf* measurements in a 0.1 mol L^{-1} LiCl medium 298 K and in the presence of CTAB (5.0 mmol L^{-1}), CPCL (2.0 mmol L^{-1}) and triton X-100 (1.0 mmol L^{-1}) are summarized in Table 1. In the studied pH range (2.5–7.5) the maximum apparent ligand number reached was ca. 2.2. The highest concentration ratio of ofloxacin to Al^{3+} was 5:1. Beyond pH 7.0, solutions became turbid and drifting potential readings were obtained. No higher concentration ratios of ofloxacin to Al were used due to the low solubility of ofloxacin and because they would seriously change the constancy of the medium. In addition, the buffering effect of ofloxacin may hinder the reliable potentiometric measurements. Part of the obtained titration curves is shown in Fig. 3 as the dependence of pH on the titration parameter, *a*. The distinct separation of Al^{3+} + oflo titration curves in the presence of surfactants from these of ofloxacin itself, and Al^{3+} + ofloxacin without the addition of surfactants, indicates the formation of strong complexes between aluminium and ofloxacin.

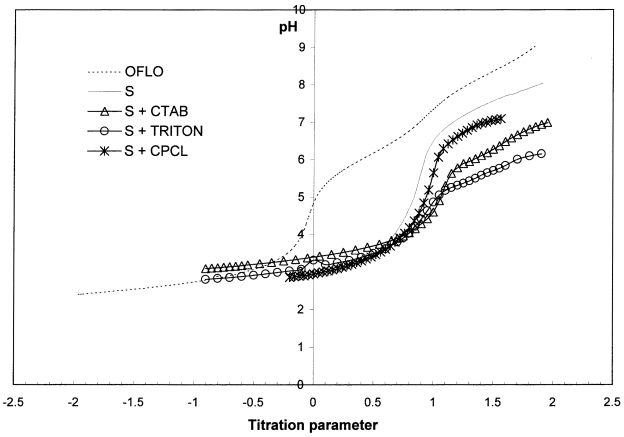


Fig. 3. Titration curves pH = *f*(*a*), for Al^{3+} + ofloxacin solutions in 0.1 mol L^{-1} LiCl + surfactants medium, at 298 K. Titration curves for ofloxacin and Al^{3+} + ofloxacin were taken form Ref. 1.

The establishment of equilibrium in solutions was moderately slow, especially at the pH values higher than 6.0. High turbidity of solutions was observed at pH values near to 7.5.

In order to derive a speciation model for each studied system, the experimental data were plotted as the dependence of the average ligand number, Z_{Al} on −log [oflo]. First, the titration parameter, *a*, was calculated using the formula

$$a = \frac{BV_B + V_0L - V_0[HCl]}{V_0L}$$

where *B* and V_B denote the concentration and volume of a strong base (NaOH), respectively, while V_0 and *L* are the initial volume and concentration (oflo) of the titrated solution. Thus, the titration parameter was set to zero at the beginning of titration of the cationic form of ofloxacin. Negative values of *a* represent the titration of an excess of strong acid (HCl). The concentration of free ligand was calculated from the formula

$$l = [oflo] = \frac{(2-a) - h + [OH^-]}{\beta_1 h + 2\beta_2 h^2},$$

Table 4. Calculated Stability Constants of Al^{3+}–Ofloxacin Complexes in 0.1 mol L^{-1} LiCl(S) Ionic Medium, in the Presence of Surfactants, at 298 K
Concentrations of surfactants were: SDS, 10.0 mmol L^{-1}; Triton, 1.0 mmol L^{-1}; CTAB, 5.0 mmol L^{-1} and CPCL, 2.0 mmol L^{-1}.

Species	S[a)]	S + SDS[b)]	S + triton	S + CTAB	S + CPCL
Al(Hoflo)	15.93 ± 0.03	—	16.40 ± 0.08	—	—
$Al(oflo)_2$	14.84 ± 0.07	—	—	—	—
Al(oflo)	10.20 ± 0.04	10.28 ± 0.08	10.37 ± 0.08	11.56 ± 0.02	11.90 ± 0.08
Al(OH)oflo	4.21 ± 0.05	3.04 ± 0.1	—	—	—
$Al_2(OH)_2oflo$	6.4 ± 0.1	4.56 ± 0.06	—	3.6 ± 0.1	3.4 ± 0.1
χ_2					
S					

a) Data taken from Ref. 1. b) Data taken from Ref. 2.

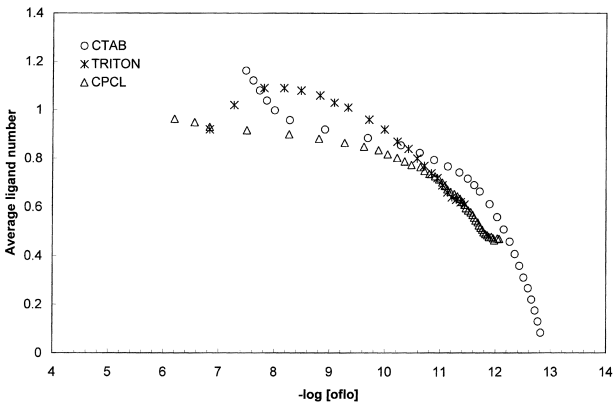


Fig. 4. Formation curves in Al^{3+} + ofloxacin system, in the presence of either, CTAB, CPCL or triton X-100.

The expression for the average ligand number was then

$$Z_{Al} = \frac{C_L - l \cdot \alpha_{L(H)}}{C_{Al}}$$

where $\alpha_{L(H)}$ is defined as $\alpha_{L(H)} = 1 + \beta_1 h + \beta_2 h^2$.

An analysis of the formation curves (Fig. 4) indicates the formation of mononuclear complexes over a relatively wide range of ligand concentrations, corresponding to pH values between 3.5 and 5.5. Sinze Z_{Al} approaches a plateau at ca. 1.0, it means that either the complex $Al(oflo)^{2+}$ or some mixed mononuclear complexes may be important. Two buffer regions could be seen on the titration curves. The first buffer region is seen at a pH of about 3.5, and the other one at pH 5.5 to 7.0 (depending on the type of surfactant).

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

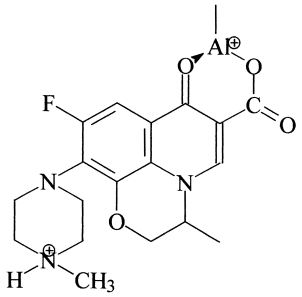
$$pAl^{3+} + qH^+ + r\text{oflo}^- \rightleftarrows Al_pH_q(oflo)_r.$$

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

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

where $C_{p,q,r}$ denotes the equilibrium concentration of the complex; m, h and l denote free concentrations of aluminium(III), proton and ofloxacin respectively. To determine the composition and stability constants of the formed species, 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, the analytical parameters (M_0, H_0 and L_0) were held constant, while the 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 to 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. Then, the data belonging to all titration curves, referred to one particular surfactant, were treated together. The refined values of E_0 served as an additional criterion for model selection. If they were different from experimental ones for more than 0.5 mV, the model was considered to be inadequate. The finally accepted complexes are given in Table 4. As can be seen from the Table 4, in the presence of surfactants speciation in the Al^{3+} + ofloxacin system considerably changes in comparison with that in their absence. In the presence of cationic SAA (CTAB and CPCL) the dominating complex is $Al(oflo)^{2+}$ with a significantly higher value of the stability constant than in a pure ionic medium. The presence of neutral SAA, triton X-100, suppresses the formation of hydrolytic complexes and favors the protonated one. The probable structure of a protonated complex is presented in Scheme 2. It may have three or two positively charged sites, depending on whether 4-carbonyl oxygen is bound to Al by a covalent or coordinative bond, respectively. Because the triply-positive structure is not probable, the structure in Scheme 2 is the dominant one in solution. Neutral SAA prevents an attack of water on the Al–O bond by intercalating the $Al(Hoflo)^{3+}$ molecule into the interior of a neutral micelle. In this way, additional stabilization of protonated complex occurs.

The effect of cationic SAA on Al–oflo complexation originates from their influence on the hydrolysis of aluminium and the dissociation of ofloxacin. A change in the reactive species



Scheme 2. Possible structure of the Al–oflo complex.

leads to a change in the identity of the formed complex species. Cationic SAA favor the formation of $Al(OH)_3$, which may be regarded as a soluble colloidal micelle, and at the same time lower the concentration of the zwitterionic form of ofloxacin. Thus, the main reaction in the system should be

$$Al(OH)_3{}^0(aq) + H_2oflo^+ \rightleftarrows Al(oflo)^{2+} + 2H_2O + OH^-.$$

Alkalization of the solution may lead to increasing of oflo concentration

$$Hoflo + OH^- \rightleftarrows oflo^- + H_2O.$$

The aluminium hydroxide micelle is positively charged due to aluminium-ion adsorption on the surface, and may be represented as[33]

$$\{pAl(OH)_3 \cdot qAl^{3+} \cdot 3(q - z)Cl^-\} \cdot 3zCl^-.$$

Due to an electrostatic repulsion, this micelle is not protected by cationic SAA, and is additionally prone to an attack of negatively charged ofloxacin.

A distribution diagram of the Al-ofloxacin species in the presence of triton X-100 is shown in Fig. 5a. At a total Al concentration of 1.0 mmol L^{-1} and ligand-to-metal ratio 2.5:1 the maximum formation of the $Al(Hoflo)^{3+}$ complex takes place in the pH interval 3–5. The formation of solid $Al(OH)_3$ begins at pH 5.3 and it is a shift of 1.2 pH units toward a more aciedic medium in comparison with the Al–oflo system with no presence of SAA (Fig. 5b). Effectively, it means, since the pH interval in which soluble species of Al exist, is narrowed, that in the presence of triton, dissolution of $Al(OH)_{3(s)}$, under the action of ofloxacin, will be hindered. Probably, solid $Al(OH)_3$ becomes coated with neutral SAA and thus, protected. The distribution diagram in the presence of CTAB (Fig. 5c) shows that the dominating complex is $Al(oflo)^{2+}$, which forms in the pH interval 3–6. The beginning of precipitation of $Al(OH)_3$ is at pH 5.7. This wider range of pH values in which soluble aluminum species exist means that CTAB enhances the solubility of $Al(OH)_3$ in comparison with triton X-100. A similar effect is exerted by CPCL. If distribution diagrams in the presence of SAA are compared with that in their absence it may be noted that the pH of the beginning of $Al(OH)_{3(s)}$ precipitation in the absence of SAA is at 6.5, and it is a shift of about 2 pH units in comparison with the beginning of precipitation in purely hydrolyzed aluminium solutions (pH 4.5).[1] Thus, in the gi tract

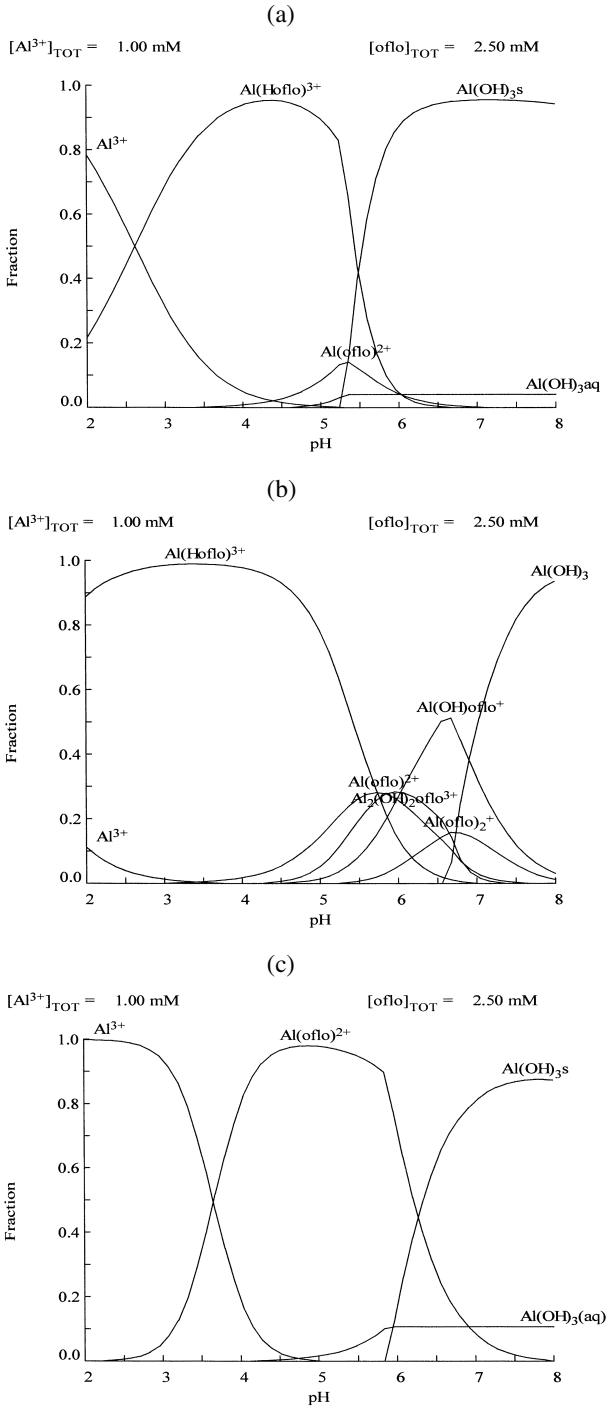


Fig. 5. Distribution diagram of Al^{3+} species in Al^{3+} + ofloxacin systems. (a) in the presence of 1.0 mmol L^{-1} triton X-100, (b) with no addition of surfactants (0.1 mol L^{-1} LiCl, 298 K, Ref. 1), (c) in the presence of 5.0 mmol L^{-1} CTAB.

ofloxacin may enhance the availability of soluble aluminium. Since, however the absorption mainly takes place in the proximal small intestine,[34] the extent of possible absorption will be determined by the presence of bioligands which can bind aluminium in the form of neutral, liposoluble, complexes and pH (6.5–7) in this section of the small intestine.[35] The most im-

portant ligand is phosphate. For constructing the distribution diagram in the presence of phosphate the data presented by Berthon et al.[36] were used. The distribution diagram in the presence of phosphate (Fig. 6), in absence of ternary complex formation, shows that ofloxacin will have no influence on solubilized aluminium absorption in the intestine. Most solubilized aluminium in the stomach, will be excreted with bowel mucus in the form of insoluble phosphate, in feces, while there exist the possibility that one small fraction may be absorbed in the form of a neutral complex,[37] $Al_2(OH)_3PO_4$. Anionic aluminate may play an important role in absorption only in some pathological cases of increased pH values, reduced phosphate concentration and prolonged contact of digestion products with epithelial cells of the small intestine. In blood plasma, however distribution diagrams[38] suggest that ofloxacin may enhance aluminium (not bound to transferrin) excretion in urine, since it forms stable, positively charged complexes. Surfactants ameliorate the formation of $Al(oflo)^{2+}$ complex, and may thus favor urinary excretion of serum aluminium.

Absorption of Ofloxacin on Aluminium Oxide. In order to quantify the bio-availability of ofloxacin in the presence of Al-based antacids, as a model system, we examined the adsorption of ofloxacin on aluminum oxide in neutral, acidic and alkaline media, in both the absence and presence of ionic SAA, SDS and CTAB. The concentration range of ofloxacin was 7–50 μg mL^{-1}. The adsorption isotherms are given in Figs. 7a, 7b and 7c, as the dependence of the extent of adsorption (mmoles of ofloxacine adsorbed per gram of aluminum-oxide) on the concentration of ofloxacin. It can be seen that the isotherms are of Freundlich type

$$\frac{n}{m} = kc^{1/x},$$

where n denote moles of adsorbate, m mass of adsorbens, k and n are constants while c stands for concentration. The adsorption in neutral medium (Fig. 7a) is much greater in absence of SAA, and significantly decreases in their presence. Adsorbens, aluminium oxide tends to have a positively charged surface by the adsorption of either hydrogen or Al^{3+} ions.[33] Since their concentration is extremely low, it adsorbs ofloxacin from the solution in which it mainly exists as zwitterions. In the presence SAA, competition for adsorption sites between ionic surfactants and ofloxacin occurs, resulting in decreasing ofloxacin adsorption.

In acidic medium adsorption is greatly enhanced in the presence of SDS (Fig. 7b). This may be explained by taking into account an electrostatic attraction between a positively charged surface of aluminum and a negative OSO_3^- group of SDS.

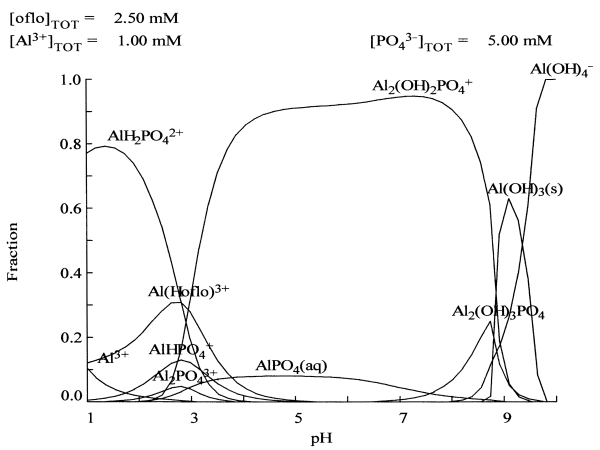


Fig. 6. Distribution diagram of Al^{3+} species in Al^{3+} + ofloxacin + phosphate solution in the presence of triton X-100. Data for stability of phosphate species are taken from Ref. 36.

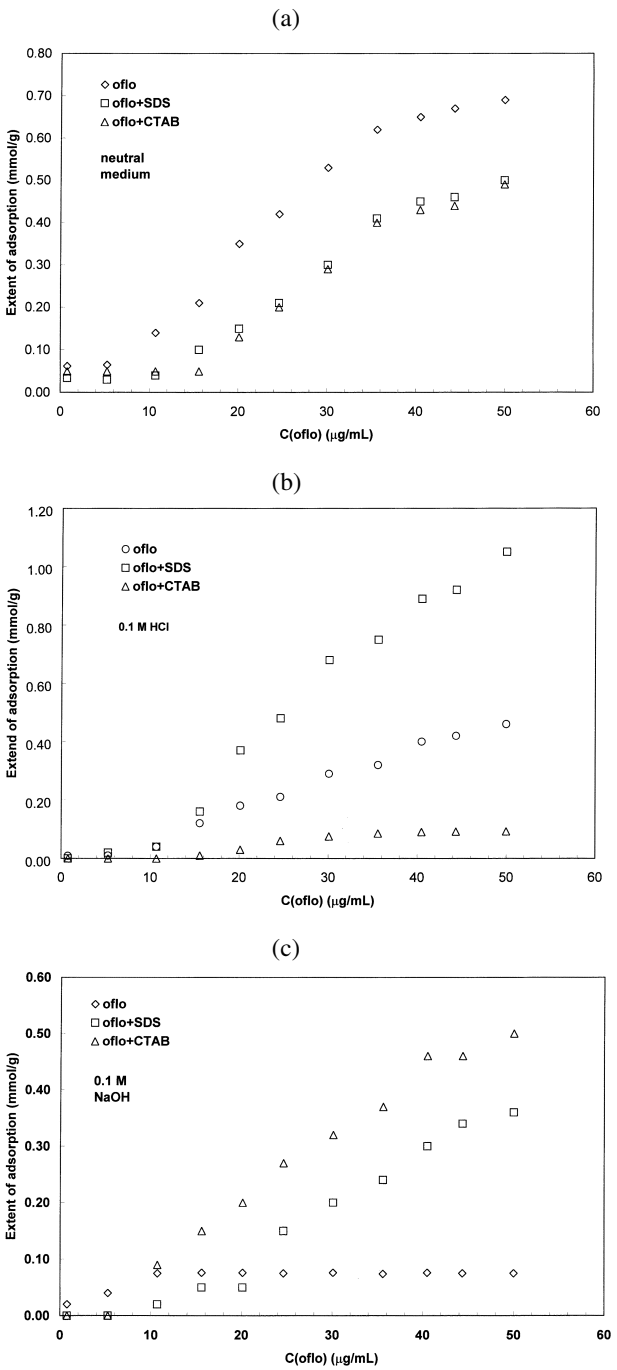


Fig. 7. Adsorption of ofloxacin on aluminum-oxide. (a) in neutral medium, (b) in 0.1 mol L^{-1} HCl, (c) in 0.001 mol L^{-1} NaOH.

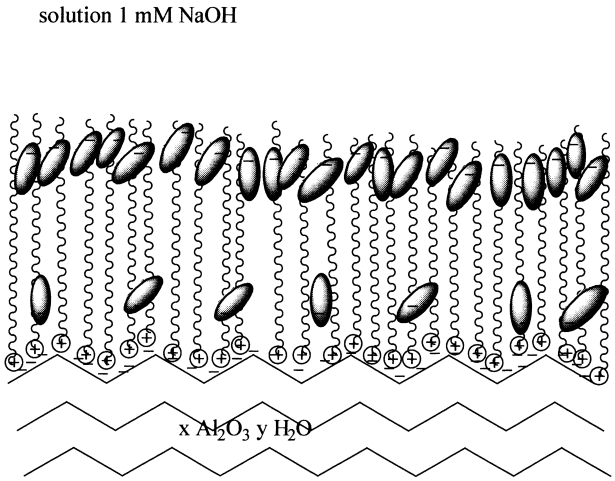


Fig. 8. Adsorption of ofloxacin on aluminium-oxide in the presence of CTAB in 0.001 mol L^{-1} NaOH.

However, such an arrangement is unfavorable for the hydrocarbon tail of SDS, which becomes exposed to water.[39] Since ofloxacin is an amphiphilic molecule, its hydrophobic part may be intercalated into hydrocarbon tails of SDS, while the polar positively charged part (protonated nitrogen) becomes reverted to water. In the absence of SAA, the adsorption of the cationic form of ofloxacin decreases due to competition with hydrogen ions. Positively charged CTAB obviously completely hinders the adsorption of ofloxacin.

In an alkaline medium, aluminum surface becomes negative due to the adsorption of hydroxide ions from the solution (Fig. 7c). Thus, in the same way as SDS in acidic medium, CTAB promotes adsorption of ofloxacin anion. SDS decreases adsorption because of competitive adsorption while in absence of SAA ofloxacin anion is adsorbed in small extent due to competition with hydroxide ion. The model, which may explain the obtained data in the presence of CTAB is depicted in Fig. 8. Ofloxacin anion possesses an amphiphilic character with the hyhdrophobic part involving. A quinolone nucleus and the substituted piperazine and hydrophilic one consisting of carboxylate and carbonyl groups. CTAB is adsorbed on an Al_2O_3 negatively charged (from OH^-) surface, while the ofloxacin anion is intercalated into the hydrophobic part of CTAB, so that its negatively charged end is turned toward water. Some ofloxacin molecules may approach the surface of the adsorbent, in which case its negative end is turned to surface due to electrostatic forces. Molecules remaining in solution are probably solubilized by CTAB micelles. S-shaped isotherms, characteristic for adsorption of ionic SAA on polar adsorbens[40] were not detected in the presence of ofloxacin. This means that bi-layer adsorption of SAA is hindered in ofloxacin solution.

References

1 P. Djurdjevic and M. Jelikic-Stankov, *J. Pharm. Biomed. Anal.*, **19**, 501 (1999).

2 I. Lazarevic, M. Jelikic-Stankov, and P. Djurdjevic, *Main Group Met. Chem.*, **21**, 609 (1998).

3 V. Andriole, "The Quinolones," Academic Press, London (1988).

4 M. Neuman, *Clin. Pharmocolinet.*, **14**, 96 (1988); T. Bergan, Quinolones in "Antimicrobial Agents, Annual 2," ed by P. K. Peterson and J. Verhoef, Elsevier, Amsterdam (1987), pp. 161–183.

5 D. S. Lee, H. J. Han, K. Kim, W. B. Park, J. K. Cho, and J. H. Kim, *J. Pharm. Biomed. Anal.*, **12**, 157 (1994).

6 M. Jelikic, D. Veselinovic, and P. Djurdjevic, *Talanta*, **39**, 665 (1992).

7 A.C. Alfrey, Toxicity of detrimental metal ions: aluminum. In "Handbook of metal-ligand interactions in biological fluids. Bioinorganic medicine," Vol 2, ed by G. Berthon, Marcell Dekker (1995), pp. 735–742; G. Berthon, *Coord. Chem. Rev.*, **149**, 241 (1996).

8 R. B. Martin, *Clin. Chem.*, **32**, 1797 (1986); W. R. Harris, G. Berthon, J. Philip Day, C. Exley, T. P. Flaten, W. Forbes, T. Kiss, C. Orvig, and P. Zatta, *J. Toxicol. Environ. Health*, **48**, 543 (1996).

9 C. Exley, E. Burgess, J. Philip Day, E. H. Jeffery, S. Melethil, and R. A. Yokel, *J. Toxicol. Environ. Health*, **48**, 569 (1996); S. S. Buys, J. P. Kushner, Hematologic effects of aluminum toxicity, in "Aluminum and health: A critical review," ed by H. Gitelman, Marcel Dekker, N.Y. (1989), pp. 235–256; "Handbook of toxicity of inorganic compounds," ed by R. L. Bertholf, M. R. Wills, J. Savory, H. G. Seiler, H. Sigel, and A. Sigel, Marcel Dekker, N.Y. (1988), pp. 55–64.

10 "Aluminum in biology and medicine," Ciba Foundation Symposium. Vol. 169, ed by D. J. Chadwick, J. Whelan, Wiley, Chichester (1992); B. Corain, G. G. Bombi, A. Tapparo, M. Perazzolo, and P. Zatta, *Coord. Chem. Rev.*, **149**, 11 (1996).

11 J. Savory, C. Exley, W. F. Forbes, Y. Huang, J. G. Joshi, T. Kruck, D. R. C. McLachlan, and I. Wakayama, *J. Toxicol. Environ. Health*, **48**, 615 (1996); G. D. Fasman and C. D. Moore, *Proc. Natl. Acad. Sci. USA*, **91**, 11232 (1994); T. Kiss, *Archives Gerontol. Geriatrics*, **21**, 99 (1995); "Aluminum in chemistry, biology and medicine," ed by R. B. Martin, Aluminum in biological systems, in M. Nicolini, P. F. Zatta, and B. Corain, Cortina International, Verona, Raven Press, New York (1991), pp. 3–20.

12 K. M. Deppermann and H. Lode, *Drugs*, **45(3)**, 65 (1993).

13 S. C. Wallis, B. G. Charles, L. R. Gahan, L. J. Filippich, M. D. Bredhauer, and P. A. Duckworth, *J. Pharm. Sci.*, **85**, 803 (1996); H. C. Neu, *Am. J. Med.*, **87**, 25 (1989); H. Shishido, K. Matsumoto, T. Nagatake, and S. Tabuchi, *Chemotherapy*, **36**, 256 (1988).

14 M. Venturini Soriano and G. Berthon, *J. Inorg. Biochem.*, **69**, 1 (1998), N. Alliey, M. Venturini Soriano, and G. Berthon, *Annals Clin. Lab. Sci.*, **26**, 122 (1996), G. Berthon and S. Dayde, *J. Am. Coll. Nmutr.*, **11**, 340 (1992).

15 J. L. Domingo, M. Gomez, and J. M. Llobet, J. Corbella, *Kidney Int.*, **39**, 598 (1991); J. L. Domingo, M. Gomez, D. J. Sanchez, and J. M. Llobet, J. Corbella, *Res. Com. Chem. Pathol. Pharmacol.*, **79**, 377 (1993).

16 N. A. Partridge, F. E. Regnier, J. L. White, and S. L. Hem, *Kidney Int.*, **35**, 1413 (1989); P. Slanina, W. Frech, L. G. Edtrom, L. Loof, S. Slorach, and A. Cedergren, *Clin. Chem.*, **32**, 539 (1986).

17 J. J. Powel and R. P. Thompson, *Proc. Nutr. Soc.*, **52**, 241 (1993); N. J. Birch, Intestinal absorption and metal ligand interactions, in "Handbook of metal-ligand interactions in biological fluids: Bioinorganic Chemistry," Vol. 2, ed by G. Berthon, Mercell Dekker, N.Y., pp. 773–780; G. B. van der Voet, "Intestinal absorption of aluminum," in "Aluminum in biology and medicine," Ciba Foundation Symposium, Vol. 169. Chichester, J. Wiley (1992),

pp. 109–122.

18 B. Kueper, M. Pitts, T. Simpkin, and T. Sale, "Technology Practices Manual for Surfactants and Cosolvents," Rice Univ., Houston (1997), Chapter 4; A. Abramzon, "Poverhnostno-aktivnye veschestva (Surface Active Substances)," Khimiya, Leningrad (1981).

19 "Surfactant-Enhanced Subsurface Remediation," ACS Symposium Series 594, ed by D. A. Sabatini, R. C. Knox, and J. H. Harwell, American Chemical Society, Washington (1995).

20 A. I. Serdyuk, R. V. Kucher, "Micellyarnye perehody v rastvorah poverhnostno alktivnyh veschestv (Micellar transitions in solutions of surface active substances)," Naukova Dumka, Kiev (1987).

21 M. J. Rosen, "Surfactants and Interfacial Phenomena," 2nd ed, J. Wiley and Sons, New York (1989).

22 S. B. Savvin, R. K. Tshernova, and S. N. Shtykov, "Aanaliticheskie Reagenty. Poverhnostno-aktivnye veschestva (Analytical Reagents. Surface Active Substances)," Nauka, Moskva (1991); P. Mukerjee, K. J. Mysels, "Critical micelle concentration of aqueous surfactant systems," US Dept. Commerce. Nat. Bur. Stand. Nat. Stand. Ref. Data Ser. No. 36, Washington D.C. (1971).

23 P. H. Elworthy, A. T. Florence, and C. B. MacFarlane, "Solubilization by Surface Active Agents," Chapman and Hall, London (1968).

24 K. Toel and S. Motomizu, T. Umano, *Talanta*, **29**, 103 (1982)

25 W. Selig and Z. Fresenius, *Anal. Chem.*, **300**, 183 (1980).

26 P. W. Linder, R. G. Torington, and D. R. Williams, "Analysis Using Glass Electrode," Open University Press, Milton Keynes (1984).

27 P. Djurdjevic, R. Jelic, and D. Dzajevic, *Main Group Met. Chem.*, **23**, 409 (2000).

28 P. Gans, A. Sabatini, and A. Vacca, *J. Chem. Soc., Dalton Trans.*, **1985**, 1195.

29 A. E. Martell and R. M. Motekaitis,"Determination and Use of Stability Constants," VCH, Weinheim (1988).

30 G. Eriksson, *Anal. Chim. Acta*, **112**, 375 (1979).

31 A. L. Horvath, "Handbook of aqueous electrolyte solutions," Ellis Horwood Ltd., Chichester (1985), pp 206–232; D. Dobos, "Electrochemical data," Akademiai Kiado, Budapest (1978).

32 L. D. Pettit (University of Leeds), personal communication.

33 J. Y. Bottero, J. L Berisllon, Aluminium and Iron(III) Chemistry: Some implications for organic substances removal in I. H. Suffet, P. MacCarthy (eds.), "Aquatic Humic Substances—Influence on Fate and Treatment of Pollutants," Advances in Chemistry Series 219, American Chemical Society, Washington (1989), pp. 425–442; W. Stumm and J. J. Morgan, "Aquatic Chemistry," Wiley Intersci., N.Y. (1970), pp. 445–512.

34 C. Guyton, "Textbook of Medical Physiology," 8th Edition, W. B. Saunders Comp., Harcourt Brace Jovanovic, Philadelphia (1991).

35 W. D. Kaehny, A. P. Hegg, A. C. Alfrey, *New Eng. J. Med.*, **296**, 1389 (1977); D. P. Froment, B. A. Molitoris, B. Buddington, N. Miller, and A. C. Alfrey, *Kidney Int.*, **36**, 978 (1989).

36 S. Dayde, M. Fillella, and G. Berthon, *J. Inorg. Biochem.*, **38**, 241 (1990); N. Alliey, F. Biron, M. Venturini Soriano, and G. Berthon, *J. Inorg. Biochem.*, **59**, 241 (1995).

37 C. Orvig, G. Berthon, Speciation studies in relation to aluminum bioavailability, in G. Berthon (ed), "Handbook of metal-ligand interactions in biological fluids: Bioinorganic chemistry," Vol. 2, Mercel Dekker, N.Y. (1995), pp. 1266–1280.

38 W. R. Harris, *Clin. Chem.*, **40**, 1809 (1994); P. E. Gardiner, M. Stoeppler, and H. W. Nurnberg, The speciation of aluminum in human blood serum, in P. Bratter, P. Schramel (ed), "Trace element analytical chemistry in medicine and biology," Gruyter & Co., Berlin (1984), pp. 299–310.

39 D. Attwood, A. T. Florence, "Surfactant Systems," Chapman and Hall, London (1983); "Micellization, Solubilization and Microemulsions," ed by K. L. Mittal, Plenum Press, N.Y. (1977); Internet WEB, http://www.surfactants.net.

40 G. D. Parfitt and C. H. Rochester, "Adsorption from solution at the solid/liquid interface," Academic Press, London (1983).