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RESEARCH PAPER

Transformation of levofloxacin during water chlorination process: kinetics and pathways

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ABSTRACT

The kinetics and mechanism of removal of the fluoroquinolone antibacterial levofloxacin (LFC) by free available chlorine (FAC) during water chlorination processes have been investigated for the first time between pH values 4.2 and 8.5. The pH-dependent second-order rate constants were found to decrease with increase in pH (*e.g.* apparent second order rate constant; $k''_{app} = 20\,dm^3\ mol^{-1}\ s^{-1}$ at pH 4.2 and $k''_{app} = 1\,dm^3\ mol^{-1}\ s^{-1}$ at pH 8.5 and at 25°C). The products of the reaction were determined by liquid chromatography and high resolution mass spectrometry. There are two plausible pathways for the LFC chlorination. The major channel is electrophilic halodecarboxylation of the quinolone moiety in which HOCl reacts at tertiary N(4) amine to form a reactive chlorammonium intermediate ($R_3N(4)Cl^+$) that can catalytically halogenate LFC, and the minor channel is chlorination at the piperazinyl moiety in which HOCl reacts at tertiary N(4) amine to form a reactive chlorammonium intermediate ($R_3N(4)Cl^+$) followed by intermediate degradation both at the piperazinyl and quinolone moieties with successive chlorination. The effect of temperature on the rate of the reaction was studied at four different temperatures and rate constants were found to increase with increase in temperature and the thermodynamic activation parameters E_a, $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta G^{\neq}$ were evaluated and discussed.

KEYWORDS: kinetics, chlorination, levofloxacin, emerging contaminant, fluoroquinolone, pathways

1. INTRODUCTION

Levofloxacin (LFC) belongs to the fluoroquinolone class of antibacterials which are among the most important compounds used in medicine. Although antibiotics have been applied in large quantities for some decades, the existence of these substances in the environment has attracted little attention until recently. Pharmaceuticals, of which antibacterial groups are important, have been identified as emerging environmental contaminants [1,2].

Fluoroquinolones are broad-spectrum antibacterial compounds used in human and veterinary application [3]. Because of the incomplete metabolism of these compounds within the human body [4], a large fraction of the total clinically prescribed fluoroquinolone load is discharged into municipal waste water systems [5], which represent a principal avenue for entry of such human-use antibacterial agents into the natural aquatic environment [6,7]. Fluoroquinolones have been detected at different concentrations ranging from $\mu g\ dm^{-3}$ to $ng\ dm^{-3}$ in municipal waste water and effluents from sewage treatment plants [8,9].

Continuous exposure of bacterial communities to growth inhibitory concentrations (or the lowest concentration at which bacterial growth is measurably inhibited) of antibacterial agents (roughly 2 to $5\,\mu g\ dm^{-3}$) can promote bacterial resistance to these antibiotics [10,11]. Possible induction of fluoroquinolone resistance in bacteria residing within affected aquatic system could therefore be of significance to human health. The behaviour of fluoroquinolones during relevant water treatment processes clearly plays a significant role in this regard.

Information on reaction of chlorine with pharmaceutical compounds is scarce. This investigation was undertaken with the intent of not only quantifying the kinetics of reactions of levofloxacin (LFC) with free available chlorine (FAC) but also identifying associated transformation, mechanisms, degradation products, and to evaluate the thermodynamic activation parameters relevant to chlorine-based municipal waste water and drinking water disinfection processes.

2. EXPERIMENTAL

2.1 Materials and methods

A stock solution of levofloxacin (Dr Reddy Laboratories) was prepared by dissolving appropriate amount of sample in 20 – 50% methanol. A stock solution of FAC was prepared by taking an appropriate volume of 5% NaOCl (Thomas

Baker) in deionised water according to the literature procedure [12]. The stock solution was standardized by iodometry and DPD-FAS titrimetry respectively [13]. 0.01 mol dm^{-3} acetate (pH 4 – 5), phosphate (pH 6 – 8.5), and borate (pH 9 – 11) buffers were used to maintain constant pH during experiments conducted in reagent water system. All reagents used were of analytical grade.

(i) For kinetic measurements, a CARY 50 Bio UV-Vis Spectrophotometer (Varian BV, The Netherlands) with temperature controller and HPLC system (Agilent 1100 series, USA) were used.
(ii) For product analysis, LC/MSD Trap system (Agilent 1200 series, USA) and for pH measurements, an Elico pH meter model LI 120 were used.

2.2 Kinetic measurements

All kinetic measurement were performed under pseudo-first order condition with FAC in at least 10-fold molar excess over LFC at a constant ionic strength using 0.01 mol dm^{-3} buffers. The reaction was initiated by mixing thermostated solutions of FAC and LFC which also contained the necessary volume of buffers. The temperature was uniformly maintained at $25 \pm 0.1\,^{\circ}C$. The course of reaction was followed by monitoring the decrease in the absorbance of LFC as a function of time in a 1 cm path length quartz cell of the Cary 50 Bio UV-Vis spectrophotometer.

The application of Beer's law of LFC at λ_{max} 295 nm was verified giving $\varepsilon = 59475\ dm^3\ mol^{-1}\ cm^{-1}$. Pseudo first-order rate constants, k'_{obs}, were evaluated from the plots of log $(A_t - A_\infty)$ *versus* time, where 'A' refers to absorbance at any time t and t_∞ is at infinite time which excludes the absorbance of any products of LFC during the reaction. The first-order plots in almost all the cases were linear up to 80% completion of the reaction and k'_{obs} values were reproducible within $\pm$ 7% (Table 1). The parent compound loss was also monitored by HPLC system (Agilent 1100 series, USA) with RX-C18 column (4.6 mm × 250 mm, 5 µm) with UV diode array detector. The observed rate constants from the HPLC method were in good agreement with UV-visible methods. FAC concentration was measured by DPD-colorimetry or DPD-FAS titrimetry [12] at the conclusion of each kinetic experiment.

Table 1 Effect of variation of [FAC] and [LFC] on the rate of chlorination of LFC at 25°C. Ionic strength = 0.01 mol dm^{-3} at 25°C

pH	10^4 [FAC] (mol dm^{-3})	10^5 [LFC] (mol dm^{-3})	10^4 k_{obs} (s^{-1})	
			Found	Calculated
4.2	0.4	0.4	8.3	7.9
	0.7	0.4	14.3	13.8
	1.0	0.4	19.0	19.6
	1.3	0.4	27.0	25.7
	1.6	0.4	32.0	31.5
5.6	0.4	0.4	5.9	5.7
	0.7	0.4	10.4	9.9
	1.0	0.4	14.2	14.2
	1.3	0.4	19.1	18.5
	1.6	0.4	23.4	22.7
6.2	0.4	0.4	4.6	4.4
	0.7	0.4	8.1	7.7
	1.0	0.4	10.6	11.0
	1.3	0.4	14.5	14.3
	1.6	0.4	17.9	17.6
6.7	0.4	0.4	3.5	3.2
	0.7	0.4	6.2	5.6
	1.0	0.4	8.4	8.0
	1.3	0.4	10.6	10.5
	1.6	0.4	13.7	12.9
7.0	0.4	0.4	2.8	2.6
	0.7	0.4	5.2	4.6
	1.0	0.4	6.9	6.5
	1.3	0.4	8.5	8.5
	1.6	0.4	11.3	10.5
	1.0	0.2	7.0	6.5
	1.0	0.4	6.9	6.5
	1.0	0.6	6.9	6.5
	1.0	0.8	7.0	6.5
	1.0	1.0	6.9	6.5
7.4	0.4	0.4	2.0	1.9
	0.7	0.4	3.3	3.3
	1.0	0.4	4.6	4.7
	1.3	0.4	5.8	6.2
	1.6	0.4	7.7	7.6
7.8	0.4	0.4	1.1	1.2
	0.7	0.4	1.9	2.2
	1.0	0.4	2.7	3.0
	1.3	0.4	3.5	3.8
	1.6	0.4	4.2	4.6
8.5	0.4	0.4	0.4	0.4
	0.7	0.4	0.8	0.8
	1.0	0.4	1.0	1.1
	1.3	0.4	1.4	1.4
	1.6	0.4	1.6	1.7

Error ± 7%.

3. RESULTS AND DISCUSSION

3.1 Product analysis

Levofloxacin was added to 0.01 mol dm^{-3} pH 7 phosphate buffer to achieve a starting concentration of 100 mg dm^{-3}. FAC solution was subsequently added to initiate reactions at oxidant: substrate molar ratios ranging from 1 : 2 to 5 : 1. The reaction mixture was kept for 12 hrs and the products were analysed using the LC/MSD Trap system (Agilent 1200 series, USA) MS analyses were conducted using positive mode electrospray ionization (ESI^+), over a mass scan range of 50 – 1000 m/z. The observed peaks of the LC/MS spectra (Figures 4a and b) were interpreted in accordance with the proposed structure of the products. The identified products are listed in Table 2.

3.2 Reaction order

A plot of $\log k'_{obs}$ *versus* $\log [FAC]_o$ with 5 points was linear with a slope of 0.968 and $r^2 \geq 0.992$, indicating that this reaction can be treated as first order with respect to FAC.

3.3 Effect of pH on the reaction

The pH of the reaction mixture was varied from pH = 4.2 – 8.5 by using acetate, phosphate and borate buffers, keeping other conditions constant throughout the experiment. The rate was found to decrease with increase in pH. The graph of apparent second-order rate constant, k''_{app} *versus* pH is shown in Figure 1.

3.4 Effect of varying ionic strength and dielectric constant

The effect of ionic strength (I) was studied by varying the buffer concentration (pH = 7) from 0.005 to 0.05 mol dm^{-3}. No significant effect of ionic strength on the rate constant was observed.

The effect of dielectric constant (D) was studied by varying the *t*-butanol water content in the reaction mixture with all other conditions being maintained constant. The solvent did not react with the oxidant under experimental conditions. The rate constant k'_{obs} decreased with decrease in the dielectric constant of the medium. The plot of $\log k'_{obs}$ *versus* $1/D$ with nine points was linear with a negative slope of 183.3 and $r^2 \geq 0.991$.

Table 2 Identified degradation products of LFC during chlorination by LC/MS analysis

LFC products	t_R (min)[a]	$M+H^+$ measured	Theoretical mass (Da)	Molecular formula	Difference in measured and theoretical mass	Degradation site moiety
LFC_P1	7.1	352.4	351.9 (Major Product)	$C_{17}H_{19}O_2N_3FCl$	+0.5	Quinolonic
LFC_P2	7.5	338.5	335.0	$C_{16}H_{18}O_4N_3F$	−2.5	Piperazinyl
LFC_P5	7.5	341.0	340.0	$C_{15}H_{15}O_3N_3FCl$	0.0	Quinolonic and piperazinyl
LFC_P4	8.6	354.3	354.0	$C_{16}H_{17}O_3N_3FCl$	+0.7	Quinolonic and piperazinyl
LFC_P3	11.8	372.5	370.5	$C_{16}H_{18}O_4N_3FCl$	−1.0	Piperazinyl
LFC_P6	12.5	241.3	241.5	$C_{11}H_9O_2NFCl$	+1.2	Quinolonic and piperazinyl

[a]Retention time.

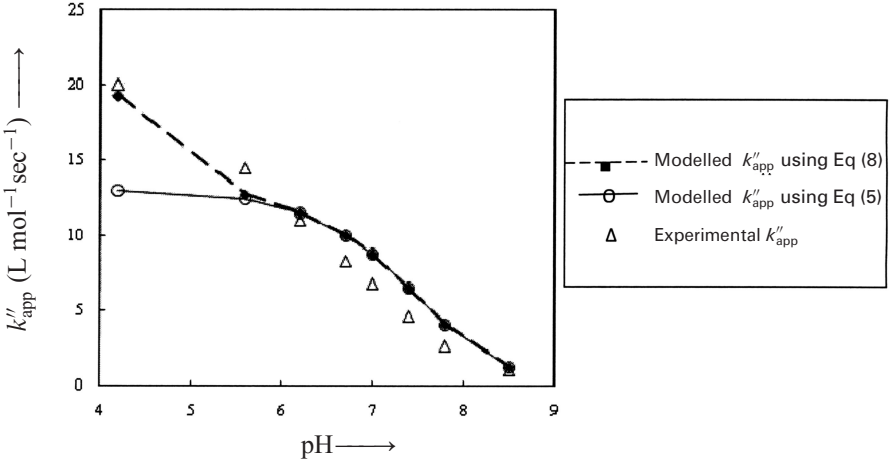


Figure 1 Effect of pH on FAC/LFC reaction and kinetic model fits to the k''_{app} values.

3.5 Effects of initially added products

Initially added products, such as chloramines or combined chlorine (CC), did not have any significant effect on the rate of reaction.

3.6 Effect of temperature

The rate of the reaction was measured at four different temperatures with varying 10 : 1 to 40 : 1 $[FAC]_o$ to $[LFC]_o$ ratios by keeping other conditions constant; the rate was found to increase with increase in temperature. The second-order rate constants k''_{app} at four different temperatures 20, 25, 30 and 35°C were obtained. The energy of activation, E_a, (28.6 ± 3.0 kJ mol^{-1}) corresponding to these rate constants was evaluated from plot of $\log k''_{app}$ *versus* $1/T$ ($r^2 > 0.984$) and other activation parameters $\Delta H^{\neq}$ (26.1 ± 2.7 kJ mol^{-1}), $\Delta S^{\neq}$ (-12.4 ± 1.5 J K^{-1} mol^{-1}), $\Delta G^{\neq}$ (29.8 ± 3.0 kJ mol^{-1}) were calculated.

3.7 Kinetic modelling

The reaction of LFC with FAC is first order with respect to each reactant and so can be described by a second-order rate expression.

$$\frac{d[LFC]_T}{dt} = -k'_{obs}[LFC]_T = -k''_{app}[FAC]_T[LFC]_T \quad (1)$$

where k'_{obs} is the observed pseudo-first order rate constant, T represents the sum of all acid base species for a given reactant and k''_{app} ($dm^3\,mol^{-1}\,s^{-1}$) is the pH-dependent apparent second-order rate constant for the overall reaction, which can be calculated from $k''_{app} = (k'_{obs}/[FAC]_T)$. The variation in k''_{app} from pH 4.2 to 8.5 can be attributed to the varying importance of specific reaction amongst the individual acid–base speciations of LFC and FAC. The acid–base speciation of FAC and LFC can be modelled by

$$HOC1 \overset{K_a}{\rightleftharpoons} OC1^- + H^+$$

$$[FAC]_T = [HOC1] + [OC1^-] = \sum_{i=1,2} \alpha_i [FAC]_T \tag{2}$$

$$[LFC]_T = [LFC^+] + [LFC] + [LFC^-] = \sum_{j=1,2,3} \beta_j [LFC]_T \tag{3}$$

Here,

$$\alpha_1 = \frac{[H^+]}{[H^+] + K_{a,HOC1}} \text{ for HOC1}$$

$$\alpha_2 = \frac{K_{a,HOC1}}{[H^+] + K_{a,HOC1}} \text{ for } OC1^- \text{ (with } K_{a,HOC1} = 10^{-7.5}) \text{ [14].}$$

$$\beta_1 = \frac{[H^+]^2}{[H^+]^2 + K_{a_1}[H^+] + K_{a_1}K_{a_2}} \text{ for } LFC^+$$

$$\beta_2 = \frac{K_{a_1}[H^+]}{[H^+]^2 + K_{a_1}[H^+] + K_{a_1}K_{a_2}} \text{ for LFC}$$

$$\beta_3 = \frac{K_{a_1}K_{a_2}}{[H^+]^2 + K_{a_1}[H^+] + K_{a_1}K_{a_2}} \text{ for } LFC^-$$

where, K_{a_1} and K_{a_2} represent acid dissociation constants of LFC neutral to LFC anion, and LFC cation to LFC neutral species, respectively.

Incorporating Eqns (2) and (3) into Eqn (1) yields

$$\frac{d[LFC]_T}{dt} = -\sum_{\substack{i=1,2 \\ j=1,2,3}} k_{ij}\alpha_i [FAC]_T \beta_j [LFC]_T \tag{4}$$

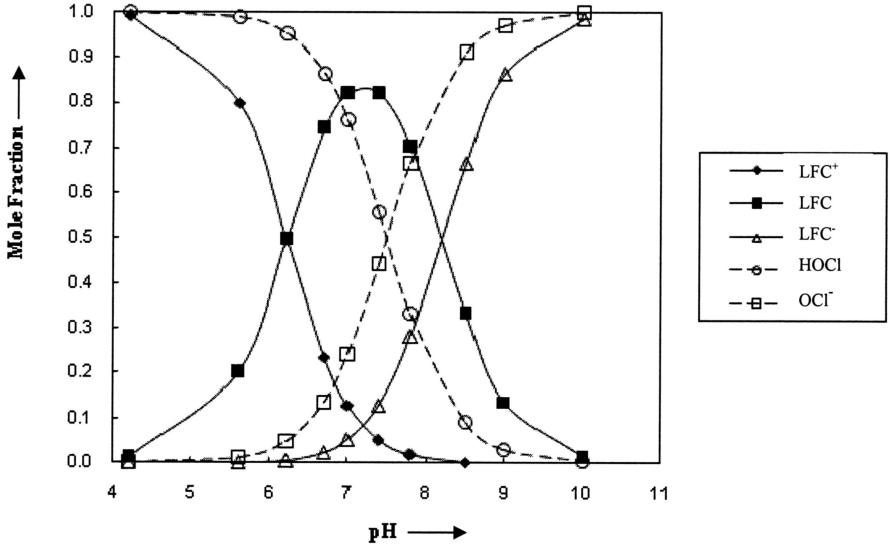


Figure 2 Mole fractions of LFC and FAC species at different pH.

$$k''_{app} = -\sum_{\substack{i=1,2\\ j=1,2,3}} k_{ij}\alpha_i\beta_j \tag{5}$$

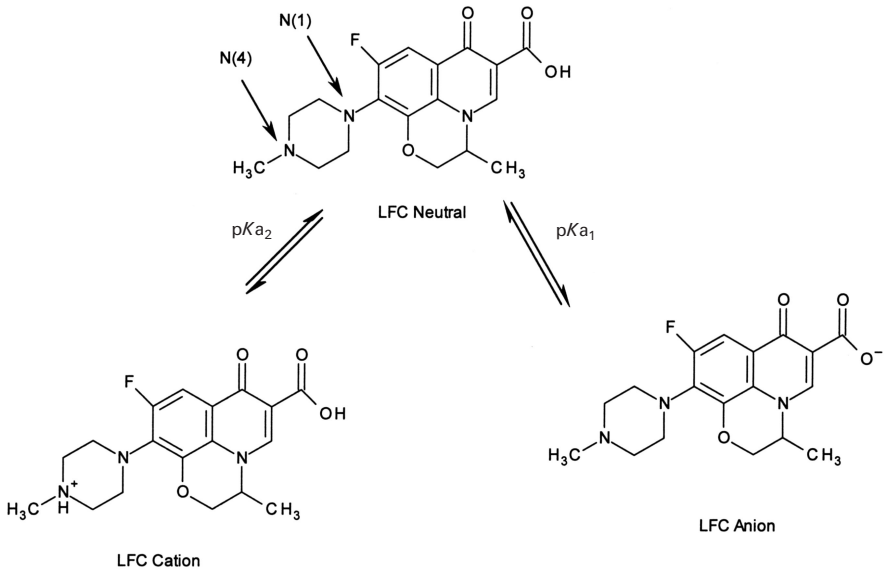
where, k_{ij} represents specific second-order rate constants for the reactions of each oxidant species i with each substrate species j.

It is evident that the LFC neutral species is dominant between pH 6 and 8; LFC^+ species dominant below pH = 6; LFC^- species is dominant above pH 8 (Figure 2).

The decrease in magnitude of k''_{app} above pH 7.5 can be attributed to deprotonation of HOCl to yield OCl^-, which is generally a much weaker electrophile than HOCl [15], while the proportion of neutral LFC remains relatively constant. The latter trend also indicates that the kinetics of reaction amongst OCl^- and various LFC species are relatively unimportant and can be omitted from Eqn (5).

The substantial increase in the apparent second-order rate constant with decrease in pH below 5 (Figure 1) may be attributed to an acid-catalysed reaction between LFC and HOCl, Eqn (6), in parallel to trends observed for various phenols, methoxybenzenes [16,17] and trimethoprim [18,19]

$$LFC + HOC1 + H^+ \xrightarrow{k_{H^+}} \text{products} \tag{6}$$



p$Ka_1 = 6.2$ and a$Ka_2 = 8.2$

Figure 3 Levofloxacin speciation pattern.

where, k_{H^+} represents the third-order rate constant for such a reaction in $dm^6\ mol^{-2}\ s^{-1}$. Including an H^+ catalysis terms in Eqns (4) and (5) yields Eqns (7) and (8)

$$\frac{d[FAC]_T}{dt} = -k''_{app}[FAC]_T[FAC]_T$$

$$= -(k_{H^+}[H^+] + k_{11}\alpha_1\beta_1 + k_{12}\alpha_1\beta_2 + k_{13}\alpha_1\beta_3)[FAC]_T[LFC]_T \quad (7)$$

$$k''_{app} = (k_{H^+}[H^+] + k_{11}\alpha_1\beta_1 + k_{12}\alpha_1\beta_2 + k_{13}\alpha_1\beta_3) \quad (8)$$

The magnitudes of k_{H^+} and each k_{ij} term were calculated by non linear regression of the k''_{app} values at different pH.

From Eqns (5) and (8) the specific second-order rate constants for the reaction of HOCl with LFC^+, LFC and LFC^- species in the pH range 4.2–8.5 were obtained as $k_{11} = 16.0 \pm 1.6\ dm^3\ mol^{-1}\ s^{-1}$, $k_{12} = 7.0 \pm 0.7\ dm^3\ mol^{-1}\ s^{-1}$ and $k_{13} = 15.0 \pm 1.5\ dm^3\ mol^{-1}\ s^{-1}$ respectively. The third-order H^+ catalysis rate constant for the reaction of HOCl with LFC was found to be $k_{H^+} = 6.0\ (\pm 0.3) \times 10^4\ dm^6\ mol^{-2}\ s^{-1}$.

Levofloxacin is an amphoteric compound that exhibit the acid-base speciation ($pK_{a_1} = 6.2$ and $pK_{a_2} = 8.2$) [20] shown in Figure 3. A second-order

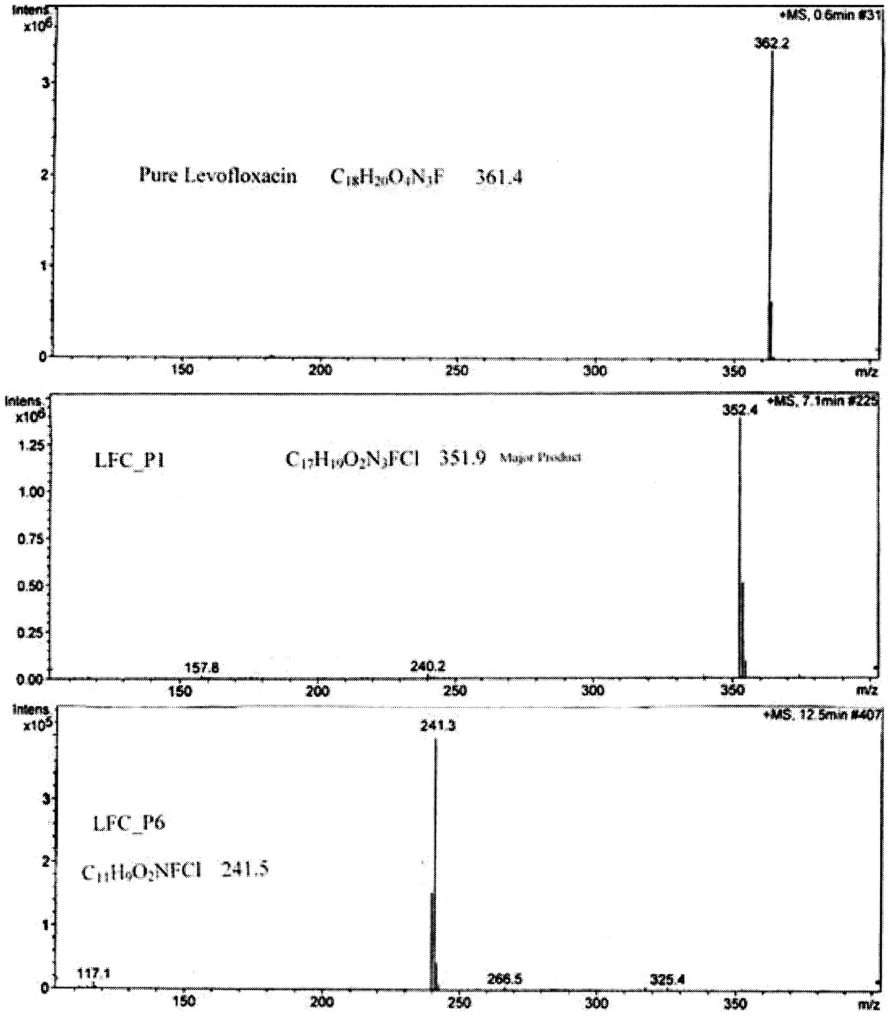


Figure 4a LC/MS spectra of levofloxacin and levofloxacin chlorination products.

kinetic reaction model that accounts for these speciation patterns [18,16,12,21] was used to identify levofloxacin's most reactive species and to achieve preliminary identification of the individual functional moieties participating in reactions with FAC.

The MS fragmentation spectra of LFC (Figures 4a and 4b) indicate degradation at the piperazinyl and quinolone substituent instead of the oxazinyl group. In conclusion, one major and five minor LFC chlorination products were

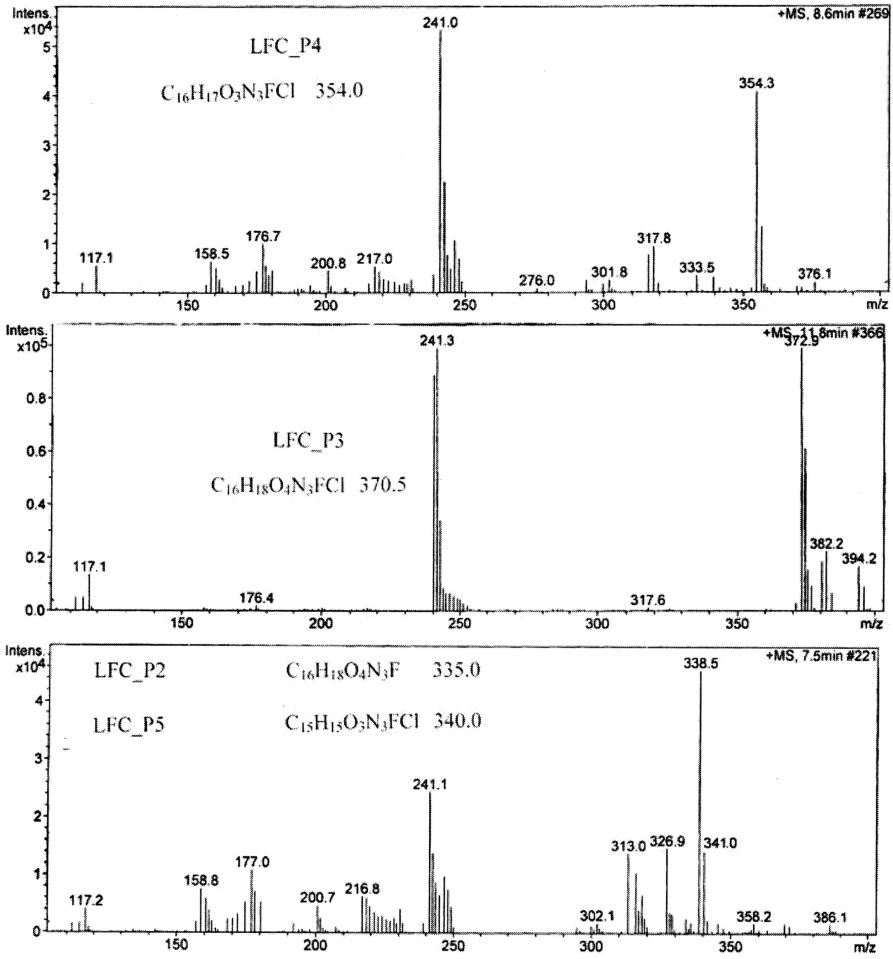
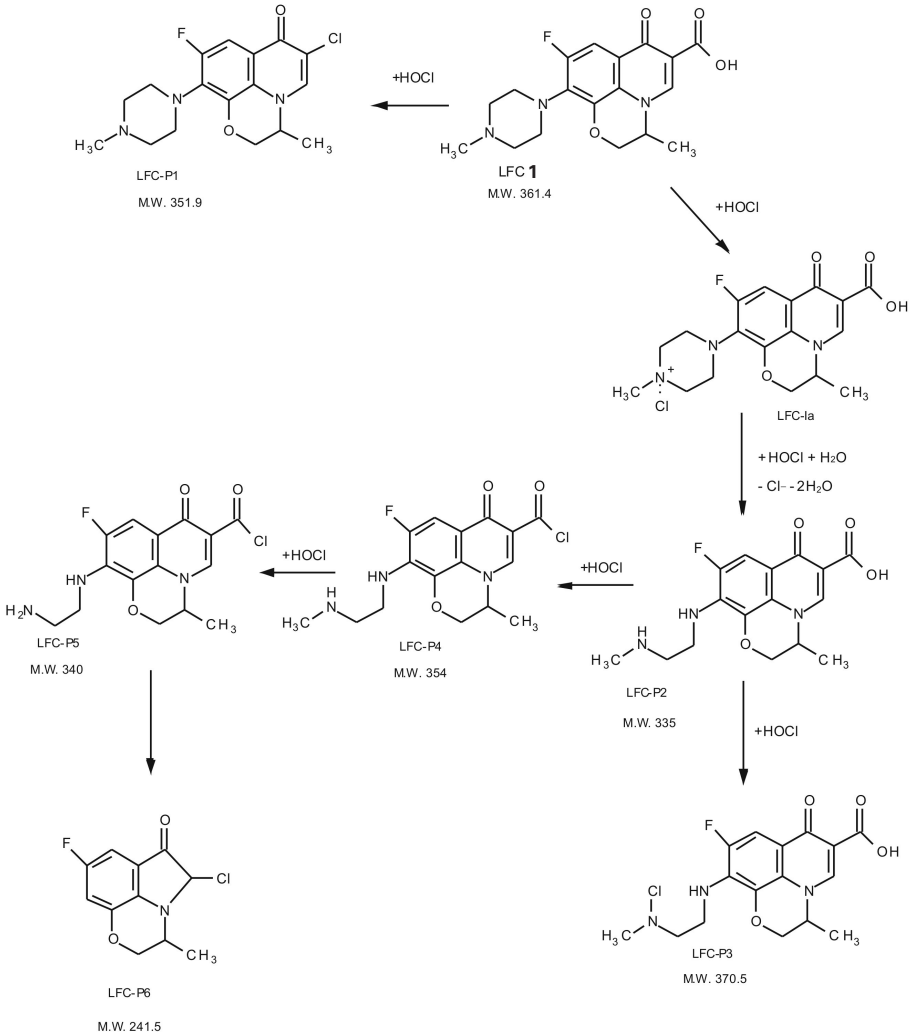


Figure 4b LC/MS spectra of levofloxacin chlorination products.

identified, indicating degradation at the quinolone substituent and also at the piperazinyl moiety (Table 2). No degradation products were found corresponding to degradation at the oxazinyl group.

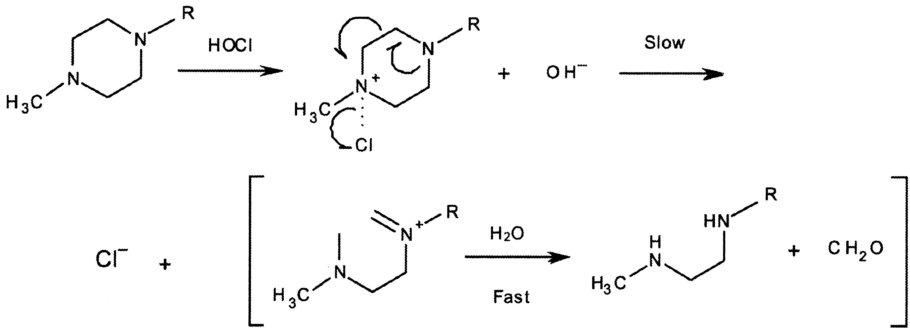
The observed products reveal that there are two plausible pathways for the chlorination of levofloxacin, *i.e.* chlorination at the piperazinyl ring and electrophilic halodecarboxylation of the quinolone ring as shown in Scheme 1.

The major product identified by LC/MS spectrum was LFC_P1, which has an LFC structure, in which the quinolone carboxylic group is displaced by a Cl atom. Decarboxylation (resulting in a mass loss of 45 daltons) and monochlorina-



Scheme 1 Proposed pathways for LFC/FAC reaction based on LC/MS.

tion (leading to mass gain of 35 daltons) would yield a net loss of 10 daltons relative to the parent LFC molecule, in accord with the mass difference between LFC and LFC_P1. Electrophilic halodecaboxylation is suggested for enrofloxacin (EF) in its reaction with FAC [22] and this process is relatively well documented with respect to bromination and chlorination reactions [23–25]. but only for specific types of substrate [26,27]. Various mono and dihydroxy benzoic acid species have been shown to undergo halodecaroxylation during treatment with



aqueous bromine or chlorine species. Prior investigation also suggests that HOCl can halodecaroxylate aliphatic β-keto acids (*via* halogenation of their enol tautomers) [26,27]. Each of these reactions can be attributed to the directing effects of activating carbon atoms in the hydroxyl tautomer β to the carboxylic carbon.

The minor products LFC_P2, LFC_P3, LFC_P4, LFC_P5 and LFC_P6 indicate that the initial reaction of LFC with FAC appears to proceed in a manner analogous to that of ciprofloxacin (CF) [23], *i.e.* (*via* chlorination of N(4) atom). LFC reacts with HOCl to form an unstable intermediate, LFC-Ia, which subsequently decays to the product LFC-P2. The rate-determining step of this mechanism is fragmentation/imine formation, which is followed by rapid imine hydrolysis to yield an aldehyde or ketone and the corresponding free amine.

Various experimental evidence indicates that in the minor channel, the initial reaction of FAC with LFC occurs at the N(4) amine nitrogen of the piperazine ring similar to the reactions of ciprofloxacin (CF). This suggest that FAC reacts with LFC primarily at the piperazine moiety, because the N(1) atom ($pKa = 5.2$) [20] of the piperazine ring is acidic and should be much less reactive towards electrophilic FAC than the N(4) atom. Hence, the latter amine should represent the specific site at which FAC reacts.

A comparison of the pH-dependence of the second-order rate constants for reaction of chlorine with LFC, ciprofloxacin (CF) and enrofloxacin (EF) is shown in Figure 5. As it evident from the $\log k''_{app}$ *versus* pH plots, the chlorination of LFC is slow compared to CF and EF. The N(4) atom belongs to a tertiary amine group whereas ciprofloxacin has a secondary amine group at its piperazinyl substituent, and tertiary amines have lower chlorination rates than secondary amines. Differences between tertiary and secondary amines are in agreement with Abia *et al.* [28], who found rate constants of $8.9 \times 10^3\ dm^3\ mol^{-1}\ s^{-1}$ for

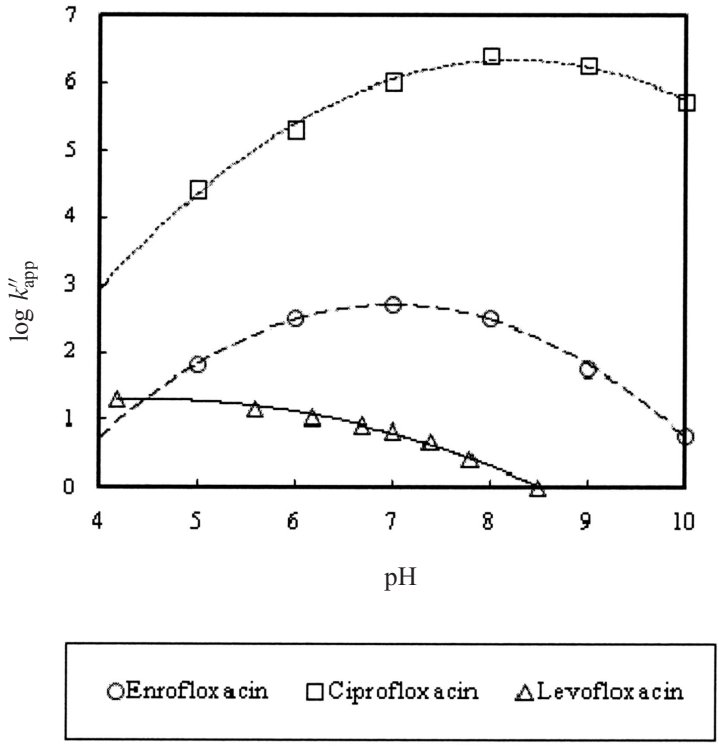


Figure 5 pH-dependence of the apparent second-order rate constants for reaction of chlorine with fluoroquinolones at 25°C.

dimethylamine *versus* $6.9 \times 10^1\ dm^3\ mol^{-1}\ s^{-1}$ for triethylamine. Also, the presence of the stable oxazinyl group explains the slow chlorination rate compared to enrofloxacin and ciprofloxacin.

The observed negligible effect of variation of ionic strength on the rate of reaction indicates the reaction is either between two neutral species or a neutral and a charged species [29]. This is due to the small difference in the rate constants of LFC, LFC^+ and LFC^- species with HOCl. The effect of LFC^+ is nullified by the effect of LFC^-.

The effect of solvent on the reaction kinetics has been described in detail in a well-known monograph [30]. For the limiting case of zero angle approach between two dipoles or an ion-dipole system, Amis has shown that a plot $\log k'_{obs}$ *versus* $1/D$ gives a straight line with negative slope for the interaction between negative ions and dipole or two dipoles. In the present study the rate constant at pH = 7 decreased with decrease in the dielectric constant of the medium.

The positive value of $\Delta H^{\neq}$ and $\Delta G^{\neq}$ indicate that the transition state is highly solvated, increasing the size of the transition state. The moderate values of $\Delta H^{\neq}$ and $\Delta G^{\neq}$ are both favourable for electron-transfer processes [31]. The negative value of $\Delta S^{\neq}$ indicates that the transition state is more ordered than the reactants.

3.8 Environmental implications of fluoroquinolone degradation products

Fluoroquinolones have the ability to inhibit bacterial DNA replication which is believed to be linked to their quinolone moieties [32], suggesting that piperazine and quinolone fragmentation may lead to elimination of antibacterial activity. The product of the major channel involves halodecarboxylation of the quinolone moiety and it may retain its antibacterial activity like ciprofloxacin and enrofloxacin, but the products of the minor channel involve degradation at both piperazine and quinolone moieties, suggesting a loss of antibacterial activity.

4. CONCLUSIONS

Levofloxacin reacts rapidly with FAC at an oxidant concentration and pH conditions representative of those likely to be observed in conventional water chlorination processes.

The observed products reveal that there are two plausible pathways for chlorination of LFC, the minor channel involves chlorination at the piperazinyl moiety and the major channel involves electrophilic halodecarboxylation of the quinolone moiety.

The product of the major channel involves halodecarboxylation of the quinolone moiety and hence, it may retain antibacterial activity as observed in ciprofloxacin and enrofloxacin after chlorination.

Further toxicological studies on the degradation products of LFC are required to understand the environmental implications of the reaction of LFC with FAC.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

[1] Halling-Sorensen, B., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Holten Lutzoft, H.C. and Jorgensen, S.E. (1998) *Chemosphere*, **36**, 357.

[2] Kummerer, K. (2004) *Pharmaceuticals in the environment*, 2nd edn, p. 55. Springer-Verlag, Heidelberg, Berlin.

[3] Nicolle, L.E. (1996) In: Lorian, V. (ed.),*Antibiotics in laboratory medicine*, 4th edn, pp. 793 – 812. Williams and Wilkins, Baltimore.

[4] National Research Council (US) (1999) *National Academic Press, Washington D.C.*

[5] Hartmann, A., Alder, A.C., Koller, T. and Widmer, R.M. (1998) *Environ. Toxicol. Chem*., **17**, 377.

[6] Hirsch, R., Ternes, T.A., Haberer, K. and Kratz, K.L. (1999) *Sci. Total Environ*., **225**, 109.

[7] Golet, E.M., Xifra, I., Siegrist, H., Alder, A.C. and Giger, W. (2003) *Environ. Sci. Techn*., **37**, 3243.

[8] Miao, X.S., Bishay, F., Chen, M. and Metcalfe, C.D. (2004) *Environ. Sci. Techn*., **38**, 3533.

[9] Kummerer, K. (2010) *Annu. Rev. Environ. Resource*, **35**, 57.

[10] Levy, S.B., Marshall, B., Schluerderberg, S., Rowse, D. and Davis, J. (1988) *Antimicrobial Agents Chemother*., **32**, 1801.

[11] Levy, S.B. and Marshall, B. (2004) *Nat. Med*., **10**, S122.

[12] Dodd, M.C. and Huang, C.H. (2004) *Environ. Sci. Techn*., **38**, 5607.

[13] APHA (American Public Health Association), AWWA (American Water Works Association). and WPCF (Water Pollution Control Facility), (1998) *Standard methods for the examination of water and wastewater*, 20th edn. Washington DC, USA.

[14] Morris, J.C. (1966) *J. Phys. Chem*., **10**, 3798.

[15] Gerritsen, C.M. and Margerum, D.W. (1990) *Inorg. Chem*., **29**, 2757.

[16] Gallard, H. and Von Gunten, U. (2002) *Environ. Sci. Techn*., **36**, 884.

[17] Pinkston, K.E. and Sedlak, D.L. (2004) *Environ. Sci. Techn*., **38**, 4019.

[18] Rebenne, L.M., Gonzalez, A.C. and Olson, T.M. (1996) *Environ. Sci. Techn*., **30**, 2235.

[19] Dodd, M.C. and Huang, C.H. (2007) *Water Res*., **41**, 647.

[20] Lin, C.E., Deng Y.J., Liao, W.S., Sun, S.W., Lin, W.Y. and Chen, C.C. (2004) *J. Chromatogr*., **1051**, 283.

[21] Qiang, Z. and Adams, C.D. (2004) *Environ. Sci. Techn*., **38**, 1435.

[22] Dodd, M.C., Shah, A.D., von Gunten, U. and Huang, C.H. (2005) *Environ. Sci. Techn*., **39**, 7065.

[23] Farinholt, L.H., Stuart, A.P. and Twiss, D. (1940) *J. Am. Chem. Soc*., **62**, 1237.

[24] Grovenstein, E. and Henderson, U.V. (1956) *J. Am. Chem. Soc*., **78**, 569.

[25] Larson, R.A. and Rockwell, A.L. (1979) *Environ. Sci. Techn*., **13**, 325.

[26] Boyce, S.D. and Hornig, J.F. (1983) *Environ. Sci. Techn*., **17**, 202.

[27] Itoh, S.I., Naito, S. and Unemoto, T. (1985) *Water Res*., **19**, 1305.

[28] Abia, L., Armesto, X.L., Canle, L.M., Garcia, M.V. and Santaballa, J.A. (1998) *Tetrahedron*, **54**, 521.

[29] Laidler, K.J. (2004) *Chemical Kinetics*, 3rd edn. Vol. 183, p. 198. Pearson Education, (Singapore) Pte. Ltd, Indian Branch, Delhi, India.

[30] Amis, E.S. (1966) *Solvent effects on reaction rates and mechanisms*, Academic Press, New York.

[31] Pauling, L. (1969) *The nature of the chemical bond*, 3rd edn. p. 496. Oxford and IBH publishers. New Delhi.

[32] Shen, L.L., Mitscher, L.A., Sharma, P.N., O'Donnel, T.J., Chu, D.W.T and Cooper, C.S. (1989) *Biochemistry*, **28**, 3886.