Transformation of linezolid during water treatment with chlorine — A kinetic study

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The experimental studies on transformation of emerging contaminant linezolid during water chlorination process have been carried out using UV-Visible spectrometer. The pseudo-first order rate constants of linezolid reaction with free available chlorine (FAC) at 5.0 - 8.8 pH have been determined. The second order rate constants are found to decrease with increase in pH (e.g. apparent second rate constant; \( k''_{app} = 2.88 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) at pH 5.0 and \( k''_{app} = 0.076 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) at pH 8.8 at 298K). Monochlorinated reaction product has been identified by LC/ESI/MS spectra under the experimental conditions. A mechanism involving electrophilic halogenation is proposed based on the kinetic data and LC/ESI/MS spectra. The effect of temperature on the rate of the reaction has been studied at four different temperatures. It is observed that rate constants increase with the increase in temperature and the thermodynamic activation parameters \( E_a \), \( \Delta H^\# \), \( \Delta S^\# \) and \( \Delta G^\# \) are evaluated for the reaction and discussed. The product of the reaction between linezolid and FAC retains the antibacterial activity. The geometry optimization of the reactants and the products has been done using dispersion corrected density functional (DFT-D) method. All the DFT calculations are accomplished using the TurboMole-5.10 package.

**Keywords:** Antibacterial, Chlorination, DFT-D method, Linezolid, Thermodynamic parameters

Numerous drinking water sources are found to be contaminated with pesticides, pharmaceuticals, plasticizers, and anti microbial agents, as well as other potentially harmful chemicals1,2. A large fraction of the total clinically prescribed antibacterial load is discharged into municipal waste water systems, which shows the entry of useful antibacterial agents into natural aquatic environment3. Antibacterial resistant bacteria have been detected in municipal wastewater effluents, sewage-affected surface between chronic water systems, and even drinking water. The medicines, in which antibacterial groups are important, have been identified as emerging environmental contaminants4. Linezolid (LNZ) has 1,3-oxazolidinone moiety containing acetamide sub group at 5th methyl group position. Linezolid has been used extensively for many years in human and veterinary medicines to treat diseases and infections and in feed additives to promote growth rate and weight gain of consumable animals5. Research in recent years has verified the ubiquity of numerous antibacterial compounds in the aquatic environment1. Clinical trials have shown that linezolid is generally well tolerated for up to 28 days. Linezolid has weak basic properties (pka 1.8) and is slightly soluble in water at pH values between 5.0 and 9.0. Solubility increases at a pH value below 3.0 and at higher temperatures6,7. Linezolid is active against a range of bacteria, but its primary clinical role is the treatment of infections caused by aerobic Gram-positive organisms, including resistant strains such as vancomycin and penicillin resistant bacteria8.

The oxazolidinones are entirely new class of antimicrobials and the behavior of oxazolidinones during relevant water treatment process clearly plays a significant role in this regard. Information on chlorine reaction with pharmaceutical compounds is scanty. This investigation has been undertaken with

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the intent of not only quantifying kinetics for reactions of linezolid with free available chlorine (FAC) but also identifying associated transformation, mechanism and to evaluate thermodynamic activation parameters relevant to chlorine based municipal waste water and drinking water disinfection processes.

Experimental Procedure
A stock solution of linezolid (Dr. Reddy Laboratories) was prepared by dissolving appropriate amount of sample in 50% methanol. A stock solution of FAC was prepared by taking appropriate volume of 5% NaOCl (Thomas Baker) in de-ionized water according to the procedure described elsewhere. The stock solution was standardized by iodometry and DPD-FAS titrimetry respectively. 0.02 mol dm$^{-3}$ acetate ($pH$ 4-5), phosphate ($pH$ 6.0-8.5) and borate ($pH$ 8.8) buffers were used to maintain constant $pH$ during experiments conducted in reagent water system. All reagents used were of analytical grade.

Kinetic measurements
All kinetic measurement were performed under pseudo first order condition with FAC in at least ten fold molar excess over LNZ at a constant ionic strength using 0.02 M buffers. The reaction was initiated by mixing thermostatted solutions of free available chlorine (FAC) and LNZ which also contains the necessary volume of buffers. The temperature was uniformly maintained at 25± 0.2°C. Studying the cause of the reaction was followed by monitoring decrease in the absorbance of LNZ as a function of time using a 1 cm path length quartz cell of Cary 50 Bio UV-Visible spectrophotometer.

The application of Beer’s law of LNZ at $\lambda_{\text{max}}$ 252 nm has been verified giving $\varepsilon = 32620$ dm$^3$ mol$^{-1}$ cm$^{-1}$. Pseudo first-order rate constants, ($k'_{\text{obs}}$) were evaluated from the plots of log $[A_t - A_\infty]$ vs time, where $A$ refers to absorbance at any time $t$ and at infinite time $t_\infty$, which excludes the absorbance of any products of LNZ during reaction. The completion of the reaction was checked by LC/ESI/MS.

Instruments used
For kinetic measurements, a CARY 50 Bio UV-Vis Spectrophotometer (Varian BV, The Netherlands) with temperature controller and HPLC system (Agilent 1100 series, USA) was used.

The product analysis was done using Thermo scientific Q-exactive LC/ESI/MS at $pH$ 7. For $pH$ measurements, an Elico $pH$ meter model LI 120 was used.

Product identification method
LNZ was added to 10 mol dm$^3$ phosphate buffer ($pH$ 7) to achieve 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 h and the products were analyzed using the instrument Thermo scientific Q-exactive LC/ESI/MS. MS analysis was conducted using negative mode electro spray ionization (ESI). The observed peak of LC/ESI/MS (Fig.1) is interpreted in accordance with proposed structure of the product. HPLC of LNZ/FAC reaction shows single peak at retention time ($t_R$) 3.86 min and its mass 372 indicates the completion of the reaction and also infers that only one reaction product is formed.

Results and Discussion

Reaction order
The concentration of LNZ was varied in the range from 2×10$^{-6}$ mol dm$^{-3}$ to 2 × 10$^{-5}$ mol dm$^{-3}$ and linearity of plots of log $[A_t - A_\infty]$ vs time ($R^2 \geq 0.9994$) was obtained, which indicates a reaction order of unity in LNZ. This fact is also confirmed by varying [LNZ], which does not result in any change in the pseudo first order rate constants.

The concentration of FAC was varied in the range from 0.5×10$^{-4}$ mol dm$^{-3}$ to 2.5×10$^{-4}$ mol dm$^{-3}$ and the plot of log $k'_{\text{obs}}$ vs log [FAC] was found to be linear with slope close to unity, indicating unit order in FAC. The reaction of LNZ with FAC can be described as a bimolecular, second order reaction LNZ + FAC $\rightarrow$ product.

![Fig. 1—LC/ESI/MS spectra of FAC/LNZ reaction product](image_url)
Effect of pH on reaction

The pH of the reaction mixture was varied from pH 5.0 to pH 8.8 using acetate, phosphate and borate buffers, keeping the other conditions constant throughout the experiment. The rate constants are found to decrease with increase in pH (Table 1). The second order rate constants were also evaluated from the plot of $k'_{\text{obs}}$ vs [FAC] for corresponding pH values (Fig. 2).

Effect of varying ionic strength and dielectric constant

The effect of ionic strength was studied by varying the buffer concentration (pH 7) from 0.002 mol dm$^{-3}$ to 0.02 mol dm$^{-3}$. No significant effect of ionic strength on the rate constant is observed.

The effect of dielectric constant was studied by varying the t-butanol water content in the reaction mixture keeping all other conditions constant. The solvent does not react with the oxidant under experimental conditions. Attempts to measure the relative permittivities are not found to be successful. However, they were computed from the values of pure liquids.$^{11}$ The rate constant $k'_{\text{obs}}$ is found to decrease with decrease in the dielectric constant of the medium. The plot of log $k'_{\text{obs}}$ vs $1/D$ ($R^2 > 0.984$) is found to be linear with negative slope (Fig. 3).

Effects of initially added products

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

Effect of temperature

The rate of the reaction was measured at four different temperatures with varying [FAC]$_0$ to [LNZ]$_0$ ratio from 10:1 to 40:1, keeping other conditions constant. The reaction rate is found to increase with increase in temperature. The second order rate constant $k''_{\text{app}}$ at four different temperatures (25, 30, 35 and 40°C) are obtained. The energy of activation ($E_a$, 14.33 ± 1.5 kJmol$^{-1}$), corresponding to those rate constants, was evaluated from plot of log$k$ vs. 1/T.

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Table 1—Effect of variation in [FAC] and [LNZ] on the chlorination of linezolid in the pH range 5.0 - 8.8 at 25°C

<table>
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<th>pH</th>
<th>[FAC] $\times 10^4$ mol dm$^{-3}$</th>
<th>[LNZ] $\times 10^5$ mol dm$^{-3}$</th>
<th>$k'_{\text{obs}}\times 10^5$ s$^{-1}$</th>
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Fig. 2—Second order plot of $k'_{\text{obs}}$ vs [FAC]
(R² > 0.998) and other activation parameters such as
ΔH° (11.85 ± 1.2 kJ mol⁻¹), ΔS° (-12.4 ± 1.5 Jk⁻¹ mol⁻¹),
ΔG° (15.54 ± 1.6 kJmol⁻¹) with intercept logA (9.8)
were calculated.

Theoretical calculations
The geometry optimization of the reactants and the
products were done using dispersion corrected density
functional (DFT-D) method. For the DFT-D
calculations, Achlrich’s valance triple ζ with one set
of polarization functions per atom (TZVP) was used
in combination with B97-D functional. The initial
structure of the activated complex was made by an
educative guess and then partially optimized. All the
calculations were accomplished using the TurboMole-
5.10 package.

The LNZ reacts with FAC to the formation
of activated complex, so formed activated complex
decomposes to give product. The calculated barrier
height 13.1 kJ mol⁻¹ is in agreement with
experimentally determined activation energy 14.33 kJ
mol⁻¹ (Fig.4).

Kinetics of FAC reactions with LNZ
The reaction of LNZ with FAC is first order with
respect to each reactant, and so can be described by a
second-order rate expression, where kobs is the
observed pseudo-first-order rate constant, and k''app
(in dm³ mol⁻¹ s⁻¹) is the pH -dependent apparent
second-order rate constant for the overall reaction,
which can be calculated from k''app = kobs/[FAC]T.
Kinetic experiments demonstrated a marked
dependence of k''app on pH. As the pKa of LNZ is 1.8
(a strong acid), LNZ dissociates to form anion
[LNZ⁻].

Hypochlorous acid (HOCl) and hypochlorite (OCl⁻)
are the main chlorine species and give free available
chlorine. HOCl is the predominant species below pH
7.5 and above pH 7.5 OCl⁻ is the predominant species.
The decrease in the 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. Hence, HOCl has been considered as the
reactive species.

Linezolid MS fragmentation indicates no
degradation of the molecule under present
experimental conditions. In conclusion, only one
major LNZ chlorination product is identified. The
observed product reveal that there is one plausible
pathway for the linezolid chlorination. The proposed
mechanism for LNZ/FAC reaction is shown in

![Figure 3](image_url)

Fig. 3—Effect of dielectric constant on the chlorination of
linezolid at 25 °C [Ionic strength = 0.02 mol dm⁻³ at 7 pH]

![Figure 4](image_url)

Fig. 4—Energy diagram for LNZ/FAC reaction based on kinetic
data and ESI/MS spectra

![Scheme 1](image_url)

Scheme 1—Proposed mechanism for LNZ/FAC reaction based on
kinetic data and LC-MS spectra
Scheme 1. The rate reaction can be calculated using the following relationships:

\[ \text{Rate} = \frac{d[C]}{dt} = k[C] \]

\[ = k[HOCi]_l[LNZ^-]_l[H^+]_lK \]

\[ = kK[HOCi]_l[LNZ^-]_l[H^+]_l \] ... (1)

\[ [HOCi]_l = [HOCi]_l + [C] \]

\[ = [HOCi]_l + k_i[HOCi]_l[LNZ^-]_l[H^+]_l \]

\[ = [HOCi]_l \{1 + k_i[LNZ^-]_l[H^+]_l\} \]

\[ [HOCl]_l = \frac{[HOCl]_l}{\{1 + K[LNZ^-]_l[H^+]_l\}} \] ... (2)

\[ [LNZ^-]_l = \frac{[LNZ^-]_l}{\{1 + K[HOCi]_l[H^+]_l\}} \] ... (3)

\[ [H^+]_l = \frac{[H^+]_l}{\{1 + K[LNZ^-]_l[HOCi]_l\}} \] ... (4)

Substituting the Eqs (2), (3) and (4) in Eq. (1), we get

\[ \text{Rate} = \frac{kK[HOCi]_l[LNZ^-]_l[H^+]_l}{\{1 + K[LNZ^-]_l[H^+]_l\}} \]

The observed negligible effect of variation in ionic strength on the rate of reaction explains that the reaction is between two neutral species or a neutral and a charged species. This is due to small difference in the rate constants of LNZ and LNZ\(^-\) species with HOCl. The decrease in the magnitude of \( k'_{\text{app}} \) above \( \text{pH} \ 7.5 \) can be attributed to deprotonation of HOCl to yield OCl\(^-\), which is generally a much weaker nucleophile than HOCl\(^+\) while proportion of LNZ anion remains relatively constant. The latter trend also indicates that kinetics of reaction amongst OCl\(^-\) and LNZ is relatively unimportant. The negligible effect of ionic strength on the rate of reaction indicates the reaction between ionic and neutral species.

The effect of dielectric constant on the reaction kinetics has been described in detail in well known monographs. In the present study the rate constant at \( \text{pH} \ 7.0 \) decreases with decrease in dielectric constant of the medium. For a limiting case of zero angle approach between two dipoles or an ion-dipole system, Amis has shown that a plot log \( k'_{\text{obs}} \) vs \( 1/D \) gives a straight line with negative slope for interaction between negative ions and dipole. In the present investigation, the plot of log \( k' \) vs \( 1/D \) is linear with negative slope indicating that the reactions are of negative ion-dipole type. This supports our mechanism as it involves the reaction between [LNZ\(^-\)] and HOCl. The experimental activation energy and theoretical barrier height are in good agreement within 10\% error.

The \( \Delta H^\# \) and \( \Delta S^\# \) values are favorable for electron transfer processes. The favorable enthalpy is due to release of energy on solution changes in the transition state. The low negative value of \( \Delta S^\# \) and the high value of frequency factor indicate that the electrostatic effects are unimportant.

**Conclusion**

LNZ reacts rapidly with FAC at oxidant concentration and \( \text{pH} \) conditions representatives of those likely to be observed in conventional water...
chlorination processes. The second order rate constant values $k''_{\text{app}} = 2.88 \text{ dm}^3\text{ mol}^{-1}\text{ s}^{-1}$ at pH 5.0 and $k''_{\text{app}} = 0.076 \text{ dm}^3\text{ mol}^{-1}\text{ s}^{-1}$ at pH 8.8 indicates that LNZ reacts with FAC during normal water treatment processes.

The reaction of FAC with LNZ involves electrophilic halogenation on acetamide moiety of LNZ leading to the formation of a mono halogenated product. Mechanism for the LNZ/FAC reaction is proposed based on kinetic data and LC/ESI/MS spectra.

The antibacterial activity of Linezolid is due to oxazolidinone and morpholin moieties. The product of LNZ/FAC reaction has both oxazolidinone and morpholin moieties. Hence, the product retains the antibacterial activity.

References
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