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Kinetics and Mechanism of Oxidation of Dicloxacillin by Copper (III) Diperiodate Complex in Aqueous Alkaline Medium

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ABSTRACT

The kinetics and mechanism of oxidation of Dicloxacillin by diperiodatocuprate [DPC (III)] in aqueous alkaline medium was studied spectrophotometrically at 298 K and ionic strength of 0.10 mol dm^{-3}. The reaction between DPC (III) and Dicloxacillin (DCLX) in alkaline medium showed (DCLX: DPC-III) 1:4 stoichiometry. The reaction products were identified by spot test, elemental analysis, FT-IR and LC-MS spectral studies. The reaction was of pseudo-first order with respect to DPC (III) and fractional order with respect to Dicloxacillin as well as alkali but periodate showed retarding fractional order. Monoperiodatocuprate (MPC-III) was found to be the main active species in the aqueous alkaline medium in the form of [Cu (H_2IO_6) $(H_2O)_2$]. Activation and thermodynamic parameters with respect to uncatalyzed rate constant (k_U), slow step rate constant (k) and equilibrium constants were determined. The plausible mechanism consistent with experimental results was proposed and discussed in detail.

Keywords: Kinetics, Diperiodatocuprate (III), Oxidation, Dicloxacillin, Mechanism

INTRODUCTION

Penicillanic Acid Derivatives (PADs) are composed of beta lactam ring fused with a thiazolidine ring. Among various PADs, beta-lactam antibiotics are widely applied by human and animals against different diseases due to narrow/broad spectrum anti-bacterial potency with a huge demand in hospitals, households, sewages and veterinary applications [1]. PADs, after application, do not degrade and get emitted into the aquatic environment and contaminate drinking water, regional discharge or waste samples, surface water, ground water, rivers, lakes and coastal waters [2,3]. For the degradation of PADs in aqueous solution, modern oxidation processes are to be developed as most of intermediates, hence formed, can be definitely mineralized into CO_2, water and mineral species. Effluents from the drug manufacturing industries [4]. Accumulate in wastewater and cosmetic wastewater treatment [5]. Plants and can pollute natural water reservoirs and such contamination can induce bacterial resistance even at environmental concentrations. Hence personal care products (PCPs) and antibiotic resistance represents a serious health problem and different advanced oxidation processes (AOPs) have to be developed for the degradation of such emergent chemical pollutants [6-11].

Dicloxacillin, discovered in 1961 and introduced in 1968, is one of the chlorinated PADs with narrow spectrum potency which is widely effective against gram positive bacteria or beta lactamases [6] producing organisms like ***Staphylococcus aureus***. It acts by inhibiting the synthesis of bacterial cell wall or cross linkage between linear peptidoglycon polymer chains which is quite essential component of cell wall of gram-positive bacteria [12,13]. Isoxazolyl group, present on the side chain of penicillin nucleus, supports the action of beta lactamases resistant as these are intolerant of side chain steric hindrance. Hence it binds penicillin-binding proteins and inhibits peptidoglycon cross-linkage[d]. Its molecular formula, molar mass and IUPAC name are $C_{19}H_{17}Cl_2N_3O_5S$, 470.327g mol^{-1} and (2S,5R,6R)-6-{[3-(2,6-dichlorophenyl)-5-methyl-oxazole-4-carbonyl]amino}-3,3-dimethy-7-oxo-4-thia-l-azabicyclo[3.2.0]heptanes-2-carboxylic acid respectively. Its structure is given in **Figure 1**.

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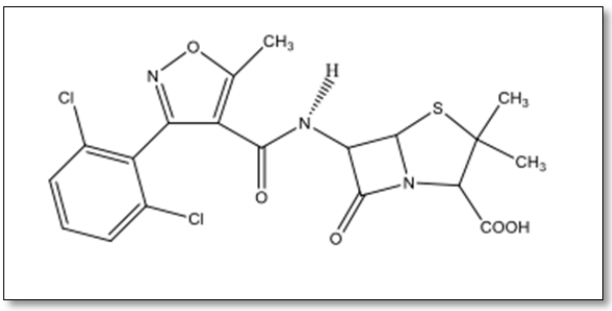


Figure 1. Structure of dicloxacillin.

Diperiodatocuprate (DPC-III) [14], Diperiodatoargentate (DPA-III) [15], Diperiodatonickelate (DPN-IV) [16], etc. are common polydentate ligands as well as oxidizing agents which can form stable complexes with transition metals. Malatesta [17,18] had initially synthesized diperiodatocuprate (III) more than a half-century ago. Many research works have been reported on the synthesis, structural determination stability, nature and analytical applications of this complex [19,20]. Diperiodatocuprate (III) has a flexible one electron-donating nature [21] and DPC (III) has a square planar geometry with dsp^2 hybridization and diamagnetic nature. It acts as an analytical reagent and hence used in many biological and analytical electron transfer reactions [22,23].

Literature regarding to dicloxacillin is scanty and hence a limited report on oxidation of dicloxacillin in acid as well as in alkaline medium could have been encountered in earlier literatures. Abdelrahman MM et al. (2018) [24] have described the oxidation of dicloxacillin by Chromatographic methods for quantitative determination of ampicillin, dicloxacillin and their impurity 6-aminopenicillanic acid. Kumar et al. (2015) [25] have reported the kinetics and mechanism of oxidation of dicloxacillin sodium [DXS] by chloramine-T [CAT] in [HCl] medium. Similarly, Bhinge and Malipatil (2015) [26] have reported the development and validation of a stability indicating method for the simultaneous estimation of cefixime and dicloxacillin using the RP-HPLC method**.** Stage et al. (2018) [27] have described that dicloxacillin induces CYP2C- and CYP3A-mediated drug metabolism - *in vivo* and *in vitro*. Guzman et al. (2015) [28] have explained the evaluation of water matrix effects, experimental parameters, and the degradation pathway during the TiO_2 photo catalytical treatment of the antibiotic dicloxacillin. Acharya et al. (2013) [29] have reported the development and validation of RP-HPLC method simultaneous estimation of amoxicillin and dicloxacillin in bulk drug and capsule.

We have already investigated the oxidation of different PADs like ampicillin, amoxicillin, catalyzed dicloxacillin and carbenicillin along with publications in different reputed journals [30]. Now, the present research work is aimed to investigate the kinetics and mechanism of oxidation of dicloxacillin in the absence of catalyst and hence to arrive at plausible mechanisms including determination of both activation and thermodynamic properties as well as calculation of uncatalyzed rate constant (k_U), slow step rate constant (k) and equilibrium constants at different temperatures.

EXPERIMENTAL PART

Reagents and chemicals

All chemicals of Analytical Reagent (AR) grade and double distilled water were used throughout the work. The stock solution of dicloxacillin (0.01 mol dm^{-3}) was prepared by dissolving 0.470 g of recrystallized dicloxacillin in 100 ml double distilled water. Potassium periodate solution was prepared by dissolving 0.023 g (0.01mol dm^{-3}) of KIO_4 (Sigma Aldrich) in 100 ml double distilled hot water and the solution was used only after 24 h. Iodometric method was used to determine the concentration of potassium periodate solution [31].

Instrumentation

ELICO LI 613 pH meter was used to measure the pH of the solution. The electronic absorption spectra were recorded on Varian CARY 5000 UV-VIS spectrophotometer in the range of 200-1000 nm. The infra-red spectra of the complexes were recorded on Thermo Nicolet, Avatar 370 FT-IR spectrometer in the range of 4000-400 cm^{-1} that was run as KBr disc. The mass spectrum of the products was recorded on the UPLC-TQD Mass spectrometer in positive mode in the range of 0 – 1000 m/z.

Synthesis of reagent

Copper (III) diperiodate complex was prepared [32,33]. by mixing copper sulphate (3.54 g), potassium periodate (6.80 g), potassium persulphate (2.20 g) and potassium hydroxide (9.0 g) in a 250 ml double distilled water in a round bottomed flask, shaken frequently thoroughly and heated on a hot plate for about 2 h. During this period, the mixture turned to intense red and the flask was heated further for 20 min to ensure the removal of excess potassium persulphate completely from the mixture upon degradation of persulphate. After completion of the reaction, the mixture was cooled and filtered through sintered glass crucible G-4 and the dark red-brown solution (filtrate) was diluted to 250 ml by adding double-distilled water. The aqueous solution of DPC (III) was standardized by iodometric titration ($Na_2S_2O_3$, starch, KI and KH_2PO_4) by thiocyanate method and its exact concentration was ascertained. The existence of DPC (III) was verified by UV-visible spectrophotometer that showed an absorption band with a maximum peak at 415 nm. Finally, the accurate concentration of DPC (III) was calculated by UV-visible spectrophotometer.

Synthesis of complex

10 ml of dicloxacillin solution (0.132 mol dm^{-3}) was taken in a 100 ml RB flask. To this 10 ml DPC(III) (0.528 mol

dm^{-3}) was mixed in 1:4 stoichiometric ratio along with 1.0 ml of each KNO_3 (Himedia), KIO_4 and 2.0 ml of KOH solution of fixed molarities and stirred on metal hot plate for 24 hours followed by re-stirring during re-fluxing with condensation for 24 hours. Then the mixture was cooled naturally for 3 days and filtered by Whatsman no.1. The products were purified and recrystallized in ethanol till the whole solvent evaporated leaving behind crystals only. The formation of DPC (III) complex was confirmed by the appearance of peaks in UV-Visible spectrophotometer. The possible structures of DPC (III) and MPC (III) are given in **Figure 2**.

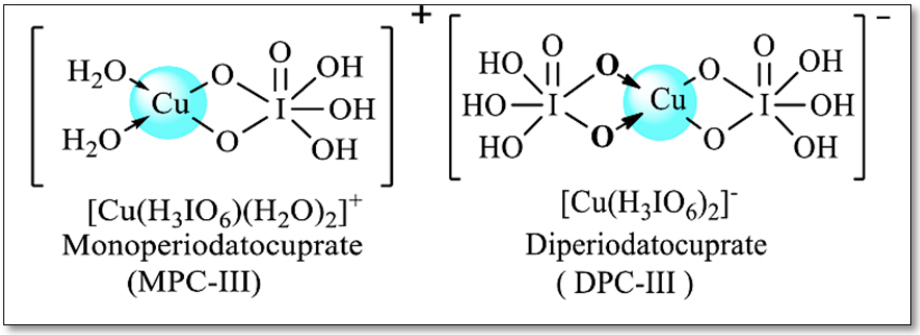


Figure 2. Possible structures of MPC (III) & DPC (III).

KINETIC MEASUREMENTS

The reaction is very fast in nature, its absorbance was taken rapidly along with the progress of the reaction when the active mass of dicloxacillin was greater than that of DPC (III) at 20□, 25□, 30□ and 35□ ± 0.5□ unless specified. The reaction was conducted by mixing required quantities of previously thermo-stated solutions of dicloxacillin into DPC (III) which already contained a fixed concentration of KIO_4 along with KNO_3 and KOH. Data were recorded from UV-Visible spectrophotometer at pH (9.2-10) and 415 nm wavelength by monitoring the decrease in absorbance at the molar extinction coefficient (**€**) of 6242 ± 50 $dm^3\ mol^{-1}cm^{-1}$. The UV visible spectrophotometer was run up to 87 % reaction.

Regression analysis of experimental data to obtain regression coefficient (r) and standard deviation (s) of points from the regression line was completed with the help of Origin 9.6 (2017) software. Plots of log (abs) versus time gave a straight line and hence rate constants (k_U) were calculated from slopes. Those k_U values agreed within ± 5% error and were the average of at least three independent kinetic runs. A constant concentration of periodate and nitrate were mixed into reaction mixture frequently in each time. Finally, the total concentration of KIO_4 and KOH were determined by assuming the amount present in DPC (III) and added additionally. To check the effect of periodate, ionic strength, dissolved oxygen, etc, kinetics were also conducted into the N_2 atmosphere wherein no significant changes were observed. Added carbonate and periodate dielectric constant etc didn't show any effect. The application of Beer-Lambert's law was verified from **Figure 3** and found that negligible interference was entertained in the reaction. The maximum wavelength of DPC (III) was noticed at 415 nm.

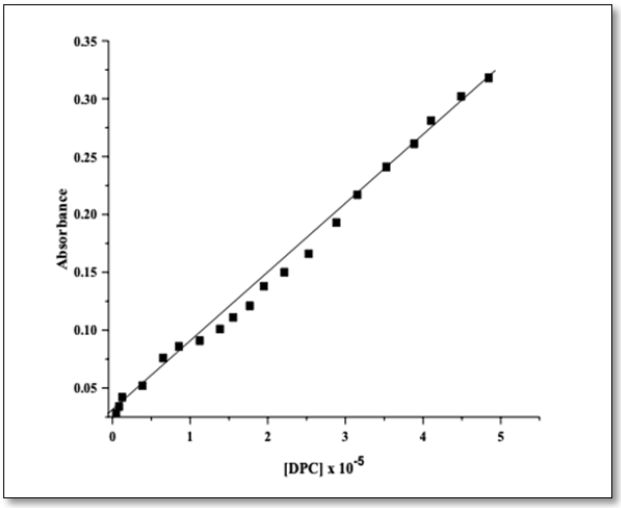


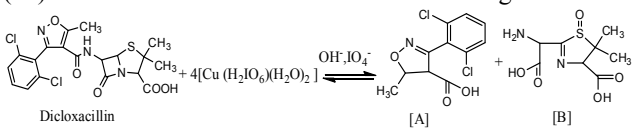
Figure 3. Plot of absorbance vs. [DPC] at 25°C.

RESULTS AND DISCUSSION

Stoichiometry and product analysis

Different sets of reaction mixtures with varying ratio of DPC (III) to dicloxacillin in presence of constant amounts of KOH and KNO_3 were kept for 3 h in a closed vessel under N_2 atmosphere and the remaining concentration of DPC (III) was analyzed to confirm the accurate stoichiometry by ***Job's method*** which was confirmed to be ***1:4*** for DCLX: DPC (III). When dicloxacillin reacts with DPC (III) in aqueous alkaline medium, 2, 6-dichlorophenyl)-5-methyl-4, 5-dihydroisoxazole-4-carboxylic acid ($C_{11}H_9Cl_2NO_3$) and 3-(2-(amino (carboxy) methyl)-5, 5-dimethyl-4, 5-dihydrothiazole-4-carboxylic acid-1-oxide were formed as the main product which was recrystallized from ethanol, separated by Column Chromatography over neutral alumina by using 80% benzene and 20% chloroform as eluent. Side product CO_2 was qualitatively detected by bubbling N_2 gas through the acidified reaction mixture and passing the gas liberated through the tube filled with lime water.

The reaction between dicloxacillin and Diperiodatocuprate (III) in alkaline medium is given as:



$$+4\ Cu^{++} + 4\ H_2IO_6^{3-} + 4\ H^+ + 5\ H_2O$$

where A = (2,6)-dichlorophenyl-5methyl-4-dihydroisoxazole-4-carboxylic acid & B = 3-(2-(amino (carboxyl) methyl-(5, 5)-dimethyl-(4,5)-dihydrothiazole-4-carboxylic acid-1-oxide

(Scheme 1: Reaction between uncatalyzed dicloxacillin and Copper (III) diperiodate)

Both DCLX- DPC (III) complex and products were characterized by LC-MS, which gave m/z at 792 (m-2) for complex ($C_{19}H_{23}Cl_2N_3CuIO_{13}S$), the first product (2, 6-dichlorophenyl)-5-methyl-4, 5-dihydroisoxazole-4-carboxylic acid) gave m/z at 248 and the second product (3-(2-(amino (carboxy) methyl)-5, 5-dimethyl-4, 5-dihydrothiazole-4-carboxylic acid-1-oxide) at 273 (m+1) respectively. A sharp absorption peak at 1633.4cm^{-1} (due to ketonic / carboxylic C=O stretch) ,1388.5 & 1118.5 cm^{-1} (due to CH_3 stretch) and 3448.2 cm^{-1} (due to N-H stretching) and a broad peak at 2917.9 cm^{-1} (due to carboxylic OH group). The first product, 2, 6-dichlorophenyl)-5-methyl-4, 5-dihydroisoxazole-4-carboxylic acid ($C_{11}H_9Cl_2NO_3$) showed C- 48.20(48.35), H-3.31(3.25), Cl-25.87(25.72), N-5.11(5.04) besides oxygen. The second product 3-(2-(amino (carboxy) methyl)-5, 5-dimethyl-4, 5-dihydrothiazole-4-carboxylic acid-1-oxide ($C_8H_{12}N_2O_5S$) showed C- 41.37(41.43), H-5.21(5.34) N-12.06(11.83) and S –13.81(13.95) besides oxygen. Both LC-MS and FT-IR spectrum are presented in **Figure 3** and **Figure 4** respectively.

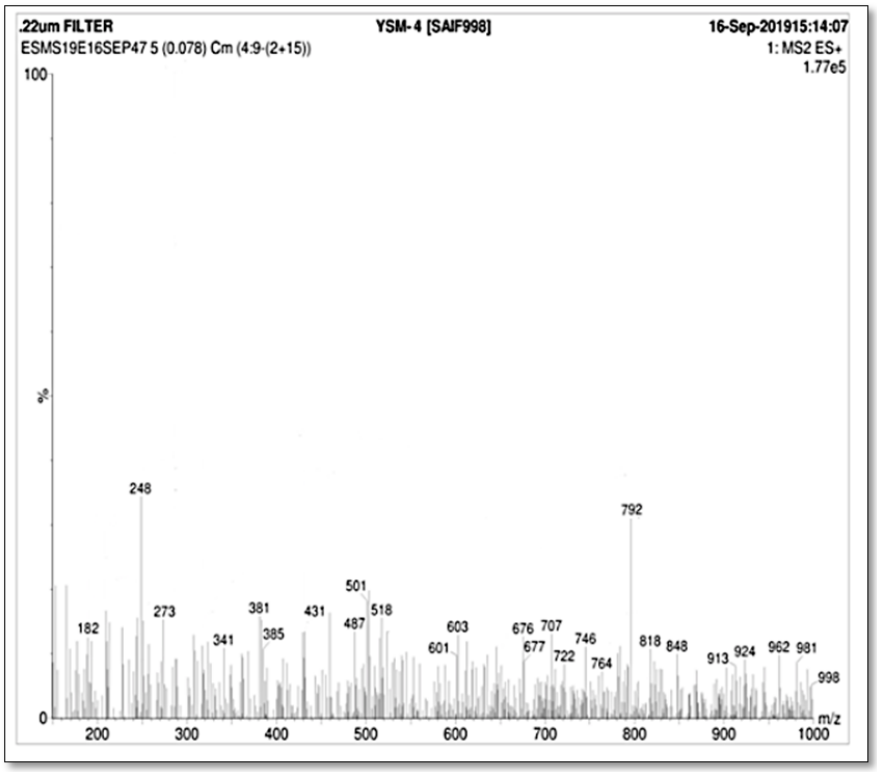


Figure 3. LC-MS of Complex & product formation for Oxidation of DCLX by DPC (III).

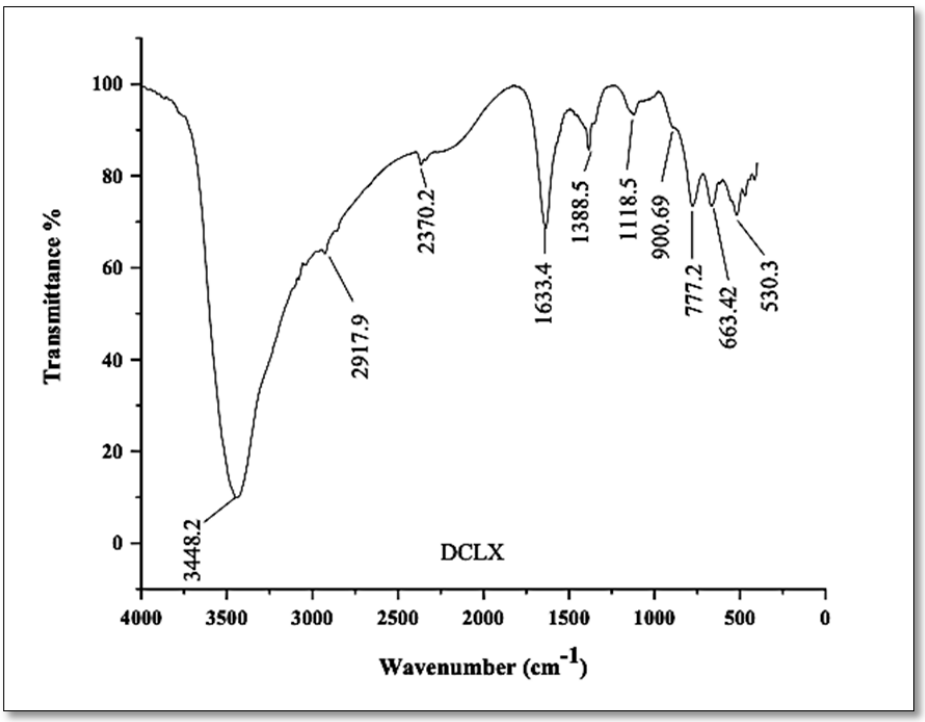


Figure 4. FT-IR of products formation for Oxidation of DCLX by DPC (III).

Reaction orders

Reaction orders were determined from the slope of log (absorbance) versus time plots as given in **Figure 5** and **Table 1** by varying concentrations of dicloxacillin, KIO_4 and KOH remaining other parameters constant except the concentration of DPC (III).

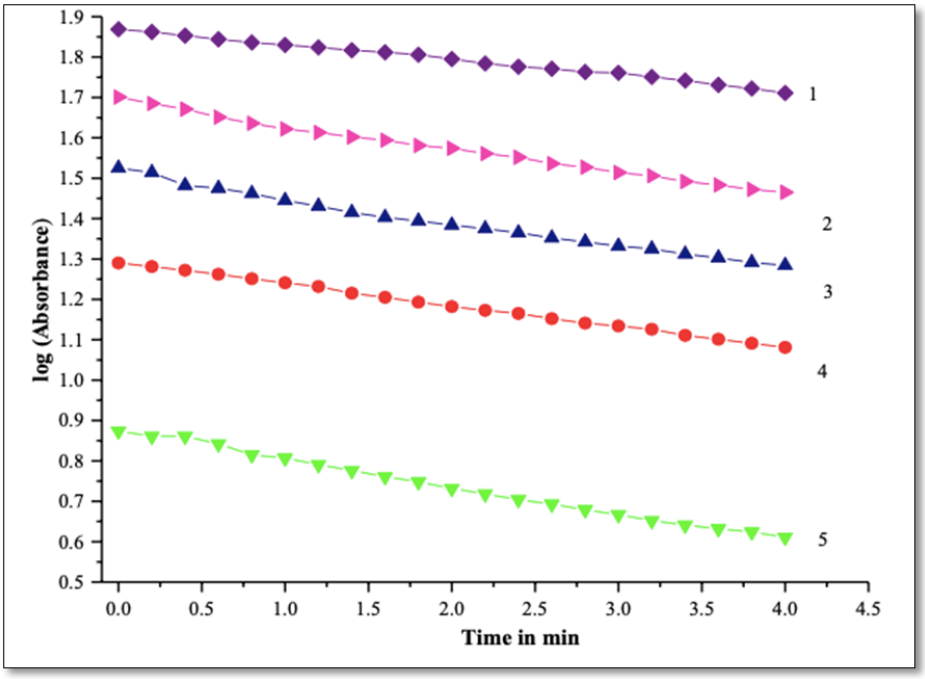


Figure 5. Order Plot of log (absorbance) vs. time for the oxidation of DCLX by DPC (III).

Table 1. Effect of variation of [DPC], DCLX], [KIO_4] and [KOH] on the oxidation of dicloxacillin by Diperiodatocuprate (III) in aqueous alkaline medium at 298 K and I = 0.10 / mol dm^{-3}.

*[DPC] x 10^5	*[DCLX] x 10^4	*[OH^-] x 10^2	*[IO_4^-] x 10^5	**k_U x 10^{-4} (s^{-1})	Order
1.0	5.0	0.8	1.0	1.80	
3.0	5.0	0.8	1.0	1.85	
5.0	5.0	0.8	1.0	**1.86**	
8.0	5.0	0.8	1.0	1.82	
10.0	5.0	0.8	1.0	1.88	
5.0	**1.0**	0.8	1.0	0.68	
5.0	**3.0**	0.8	1.0	1.35	
5.0	**5.0**	0.8	1.0	**1.86**	**0.75**
5.0	**8.0**	0.8	1.0	2.55	
5.0	**10.0**	0.8	1.0	3.24	
5.0	5.0	**0.2**	1.0	0.75	
5.0	5.0	**0.4**	1.0	1.12	
5.0	5.0	**0.6**	1.0	1.57	
5.0	5.0	**0.8**	1.0	**1.86**	**0.531**
5.0	5.0	**1.0**	1.0	2.31	
5.0	5.0	0.8	**1.0**	**1.86**	**-0.203**
5.0	5.0	0.8	**3.0**	1.55	
5.0	5.0	0.8	**5.0**	1.31	
5.0	5.0	0.8	**8.0**	0.86	
5.0	5.0	0.8	**10.0**	0.53	

*****Concentrations are expressed in mol dm^{-3}. ** k_U denotes uncatalyzed rate constant*

Effect of [DPC (III)]

DPC (III) concentration was varied within the range of 1.0 x 10^{-5} to 1.0 x 10^{-4} mol dm^{-3}.The linearity and almost parallelism plots of log absorbance versus time up to 87% completion of the reaction by keeping other concentrations remaining constant indicated first order reaction in DPC (III). **Table 1** and **Figure 2** are in good support of pseudo first-order reaction with respect to DPC (III).

Effect of [DCLX]

The effect of [DCLX] was studied by varying [DCLX] within a range of 1 x 10^{-4} to 1 x 10^{-3}mol dm^{-3}. Rate constants (k_U) increased with increase in [DCLX] and order with respect to dicloxacillin was found to be 0.75 ($r \geq 0.994$, $s \leq 0.004$), as confirmed from linear plot of (5 + log k_U) vs 4 + log [DCLX], **(Figure 6** and **Table 1)**.

Effect of [Alkali]

The effect of alkali was studied by varying [OH^-] within the range of 0.02 to 0.1 mol dm^{-3}. Rate constants (k_U) increased with increase in [alkali] and order of reaction with respect to alkali was found to be 0.531 ($r \geq 0.997$, $s \leq 0.0003$), as confirmed by the linear plot of (5 + log k_c) vs. 4 + log [KOH]), **(Figure 7** and **Table 1)**.

Effect of [Periodate]

The effect of [KIO_4] in case of DCLX was observed by varying the [KIO_4] within the range of 1.0 x 10^{-5} to 1.0 x 10^{-4} mol dm^{-3}

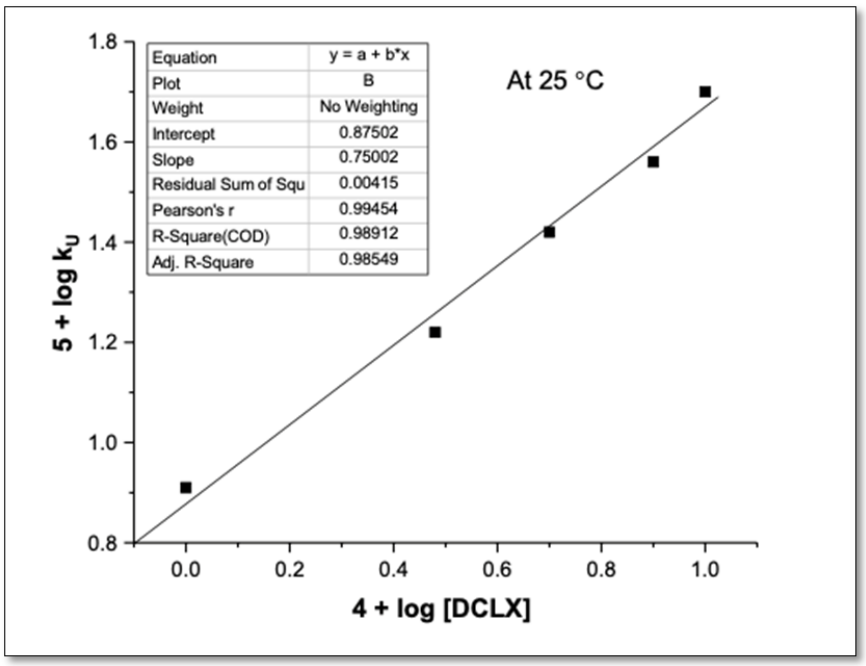


Figure 6. Plot of (5 + log k_U) vs. 4 + log [DCLX].

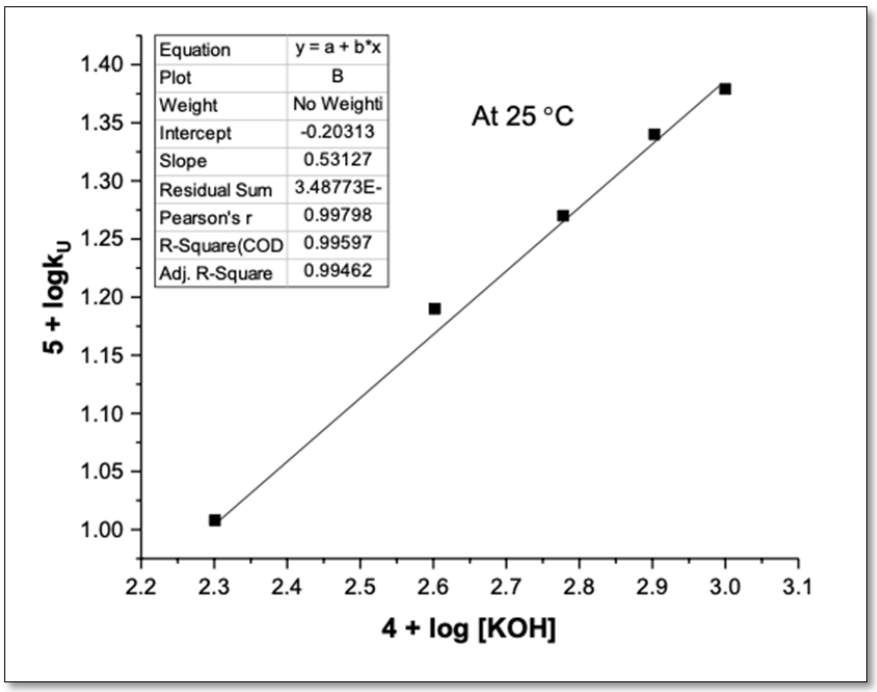


Figure 7. Plot of (5 + log k_U) vs. 4 + log[KOH].

remaining other active masses and constant. Rate constants decreased with an increase in [IO_4^-] and the order of reaction was - 0.203 ($r \geq 0.998$, $s \leq 0.009$), as confirmed by the linear plot of (4 + log k_U) vs. 5 + log [KOI_4]), as computed in **Figure 8** and **Table 1**.

Effect of ionic strength (I) and dielectric constant (D)

Increase in ionic strength did not have any significant effect on the rate of reaction. There was no effect of dielectric constant on the rate of the catalyzed reaction.

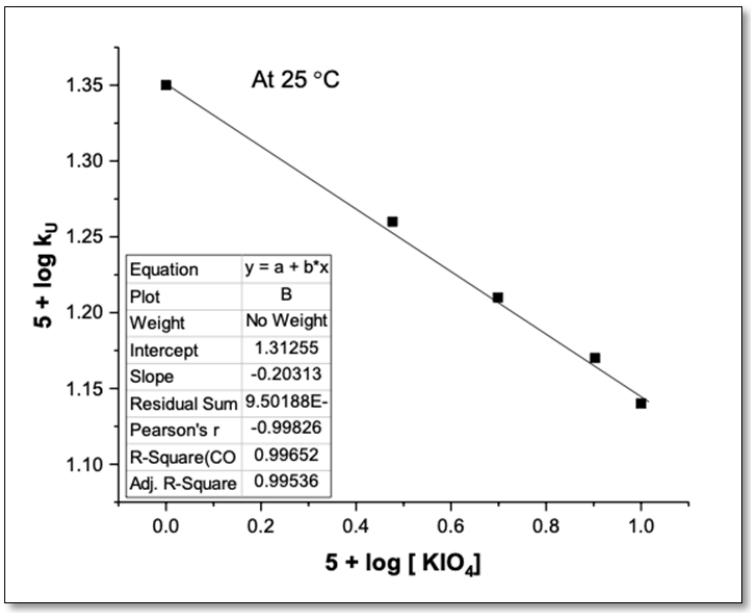


Figure 8. Plot of 5 + log [KIO_4] vs. 4 + log k_U.

Effect of initially added products and polymerization study

Initially added product ($CuSO_4$ -II)) did not exhibit any significant effect on the rate of reaction. A known quantity of acrylonitrile [34] monomer was initially added to the reaction mixture and allowed to remain in the inert atmosphere for 3.0 h. No precipitate was obtained from the mixture on dilution with methanol indicating the absence of free radicals.

Effect of temperature

Effect of temperature on the rate of oxidation reaction was studied at four different temperatures under the constant concentration of DCLX, KOH, and DPC (III) keeping other conditions constant. The rate constants increased with the rise in temperature. Slopes obtained from the plot of uncatalyzed rate constant ($\mathbf{k_U}$) and slow step rate constant (**k**) helped to calculate activation as well as thermodynamic parameters and thence computed in **(Table 2** and **3**) and **(Figure 9a** and **9b)**. Similarly, slopes and intercepts obtained from the plot of equilibrium constant versus reciprocal of temperature helped to calculate activation as well as thermodynamic parameters and thence computed in **Figure 10a, 10b** and **10c** and **Table 4a** and **4b.**

Table 2. Activation parameters from uncatalyzed rate constant ($\mathbf{k_U}$) for DCLX.

Parameters	**Values**
Ea (k $Jmol^{-1}$)	50.0
$\Delta H^{\neq}$ (k $Jmol^{-1}$)	47.0 ± 2
$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	-152.0 ± 3
$\Delta G^{\neq}$ (kJ mol^{-1})	92.0 ± 4
LogA	4.92 ± 0.04

Table 3. Activation parameters from slow step rate constant (**k**) for DCLX.

Parameters	**Values**
Ea (k $Jmol^{-1}$)	68.85
$\Delta H^{\neq}$ (k $Jmol^{-1}$)	66 ±2
$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	-88 ± 4
$\Delta G^{\neq}$ (k J mol^{-1})	94 ± 3
LogA	8 ± 1.0

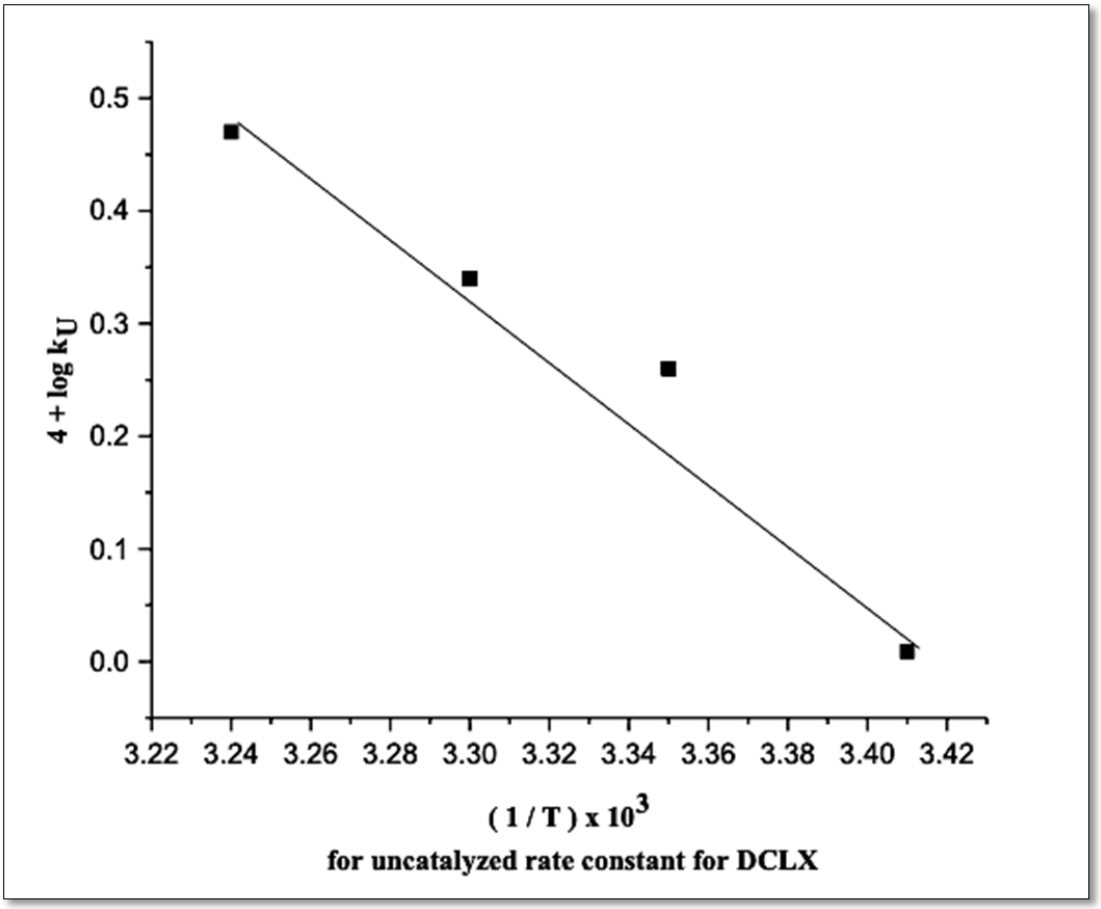


Figure 9a. Plot of 4 + log k_U vs. 1/ T.

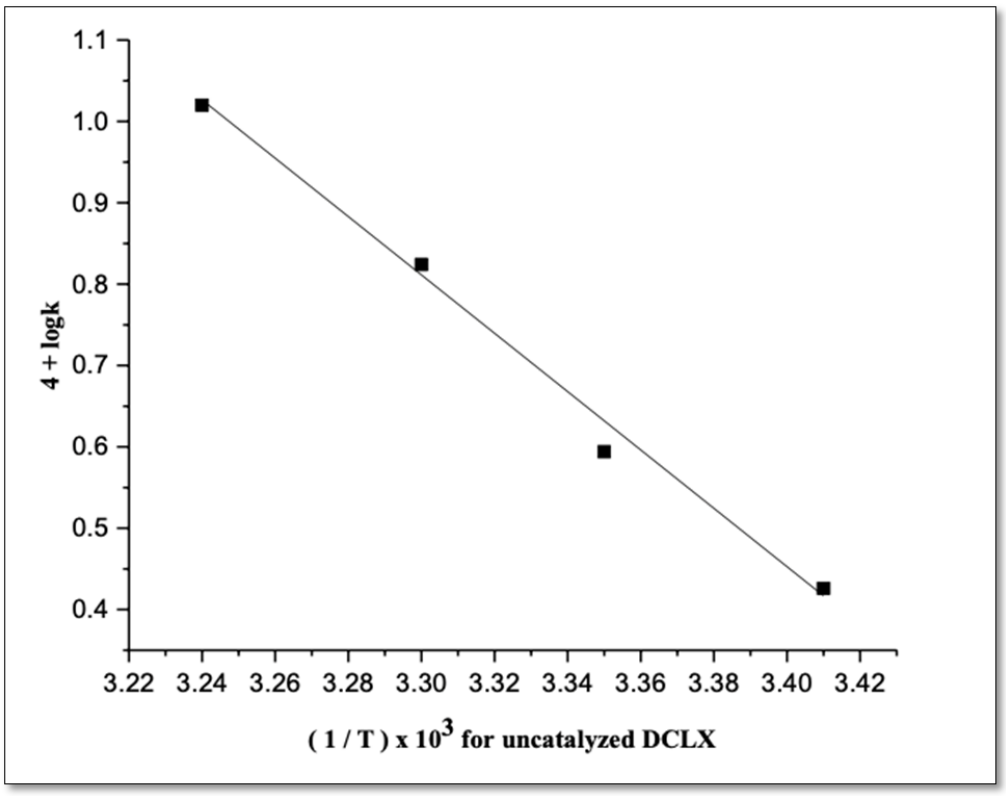


Figure 9b. Plot of (4 + log k) vs 1/T.

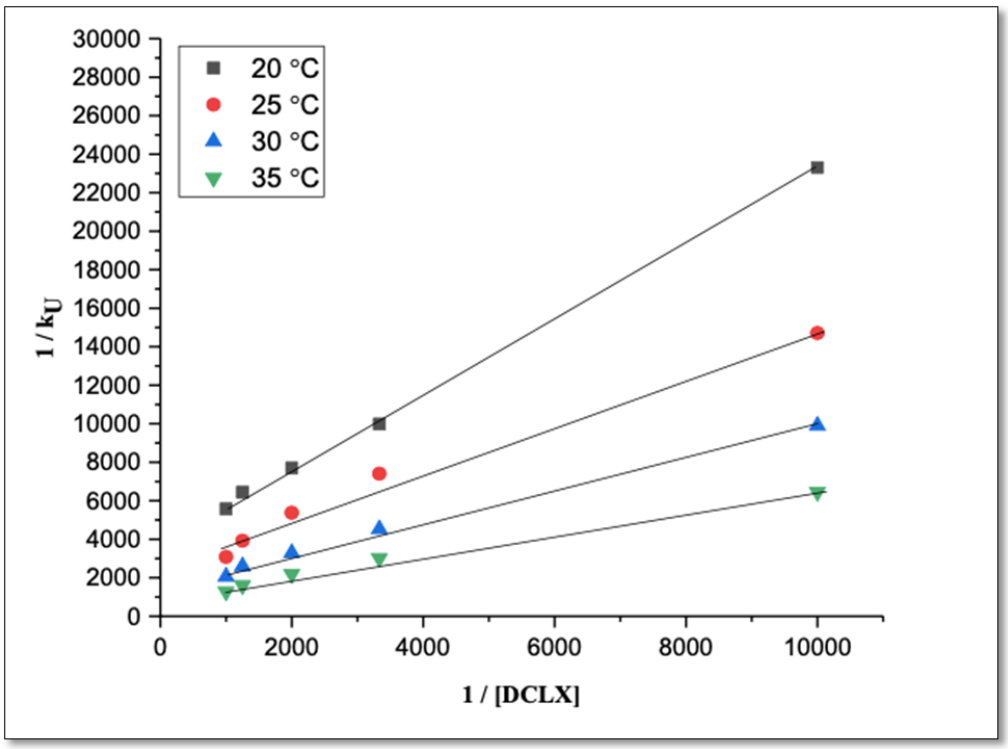


Figure 10a. Plot of { (1 / [DCLX] vs [1 / k_U]}.

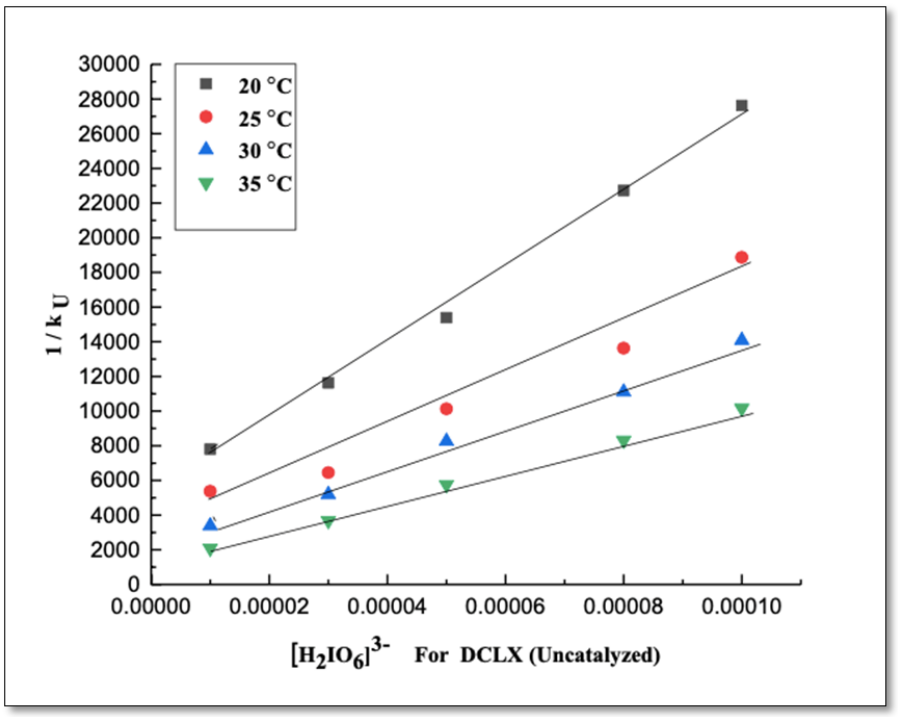


Figure 10b. Plot of { $[H_2IO_6]^{3-}$ vs $[1 / k_U]$} for DCLX.

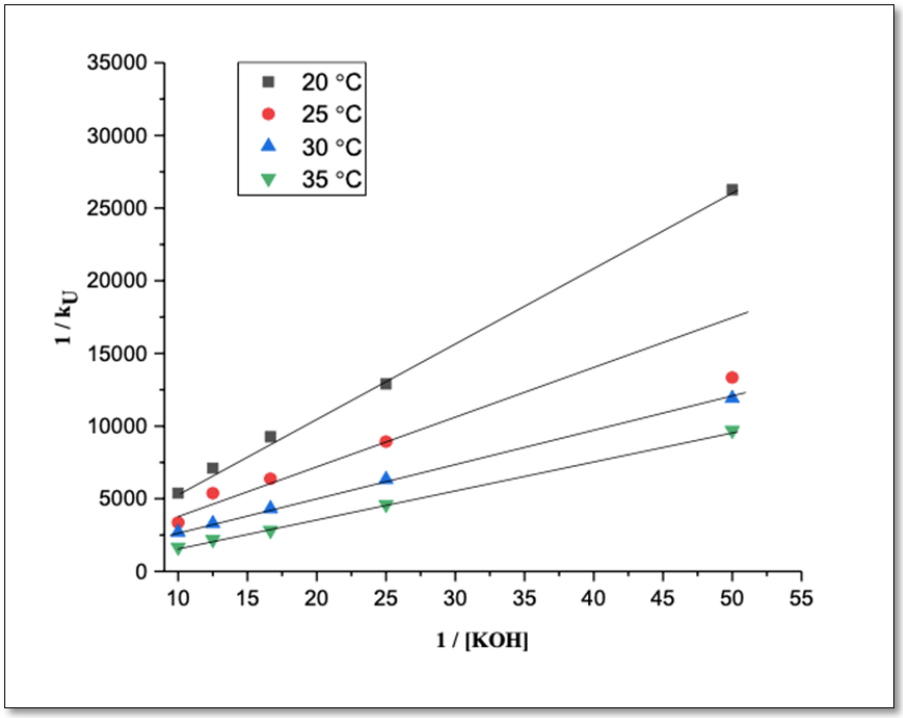


Figure 10c. Plot of {(1 / [KOH] vs $[1 / k_U]$} for DCLX.

Figure 10a, 10b and **10c** represent verification plots for uncatalyzed oxidation of DCLX by DPC (III) in alkaline medium. According to equation (7), remaining other conditions being constant, the plots of $[1 / k_U$ vs. 1/[DCLX] ($r \geq 0.999, \leq s\ 0.009$) , $[1 / k_U$ vs. $[H_2IO_6]^{3-}$ ($r \geq 0.998, \leq s$ 0.0001) and $[1 / k_U$ vs. 1 / [KOH] ($r \geq 0.999, \leq s\ 0.008$) should be linear and are found to be so as in **Figure 10a, 10b** and **10c**.

Table 4a. Equilibrium constants and slow step rate constant for DCLX.

Equilibrium Constants ↓	**Absolute Temperatures**			
Temperature →	20 □	25 □	30□	35 □
k (Slow step rate constant) x 10^{-4}	2.67	3.93	6.68	10.49
K_1	1.22	1.84	2.36	2.95
K_2 x 10^{-5}	5.61	7.8	8.21	12.53
K_3	10609.37	7661.47	5818.38	1848.65

Table 4b. Thermodynamic parameters from equilibrium constants for DCLX.

Thermodynamic Quantities	**Values from K_1**	**Values from K_2**	**Values from K_3**
ΔH°_{298} (k J mol^{-1})	44.96	36.89	-82.64
ΔS°_{298} (J K^{-1} mol^{-1})	155.33	140.21	-203.03
ΔG°_{298} (k J mol^{-1})	-1.33	-4.89	-22.14

Since DPC (III) is a strong oxidant as well as chelating agent, oxidation of different β-lactam antibiotics has been carried out in an alkaline medium. The activity of DPC (III) is a function of pH and is capable of subtle control. DPC (III) is water-soluble oxidizing reagent that exists as $[Cu(HIO_6)_2(OH)_2]^{7-}$ as well as $[HIO_6]^{4-}$ under higher pH condition. It has been evident that it can also exist as $[Cu(H_3IO_6)_2]^{-}$ or $[Cu(H_2IO_6)(OH)_2]^{2-}$ or $[Cu(H_2IO_6)(H_2O)_2]$ or $[Cu(H_3IO_6)(H_2O)_2]^{+}$ in aqueous alkaline medium. Periodic acid exists as H_5IO_6 in acid medium. The main species most active for the title work is $[Cu(H_2IO_6)(H_2O)_2]$ as reported in earlier literature. At higher alkali concentration, periodate ion tends to dimerize.

$$H_5IO_6 \rightleftharpoons H_4IO_6^- + H^+, \quad K_1 = 5.1 \times 10^{-4}$$

$$H_4IO_6^- \rightleftharpoons H_3IO_6^{2-} + H^+, \quad K_2 = 4.9 \times 10^{-9}$$

$$H_3IO_6^{2-} \rightleftharpoons H_2IO_6^{3-} + H^+, \quad K_3 = 2.5 \times 10^{-12}$$

Probable mechanism of reaction

Reaction between DPC (III) and dicloxacillin exhibits 1:4 stoichiometry and confirms pseudo-first order reaction with respect to DPC (III) while fractional orders with respect to DCLX, alkali and periodate. Based on these experimental evidences, a suitable mechanism is proposed along with proper involvement of all species. In the first step, DPC (III) reacts with hydroxide ion to form the de-protonated form of DPC (III) which yields MPC (III) and free periodate in the presence of water. Occurrence of fractional order with respect to DCLX presumably results due to formation of complex by reaction between DCLX and DPC (III). This complex interacts with fresh one mole of MPC (III) to yield an intermediate (A) along with regeneration of oxidant (DPC-III). In the second step, the active intermediate (A) reacts with fresh mole of MPC (III) to form another intermediate B that interacts with another two moles of MPC (III) to yield the final products as 2, 6-dichlorophenyl)-5-methyl-4, 5-dihydroisoxazole-4-carboxylic acid and 3-(2-(amino (carboxy) methyl)-5, 5-dimethyl-4, 5-dihydrothiazole-4-carboxylic acid-1-oxide, as represented in the mechanism correspondingly below by scheme 1 **(Figure 12)**. The complete probable structure of **Complex C** is presented in **Figure 13**.

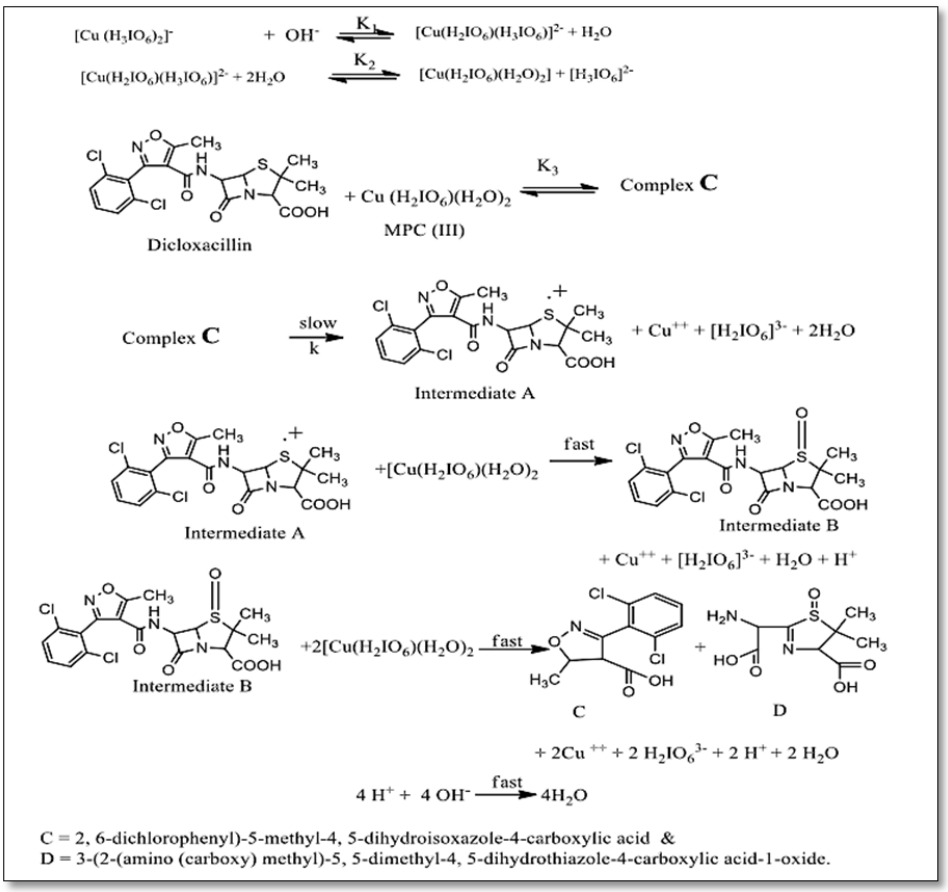


Figure 12. Scheme 1: Detailed Scheme for catalyzed oxidation of DCLX by DPC (III).

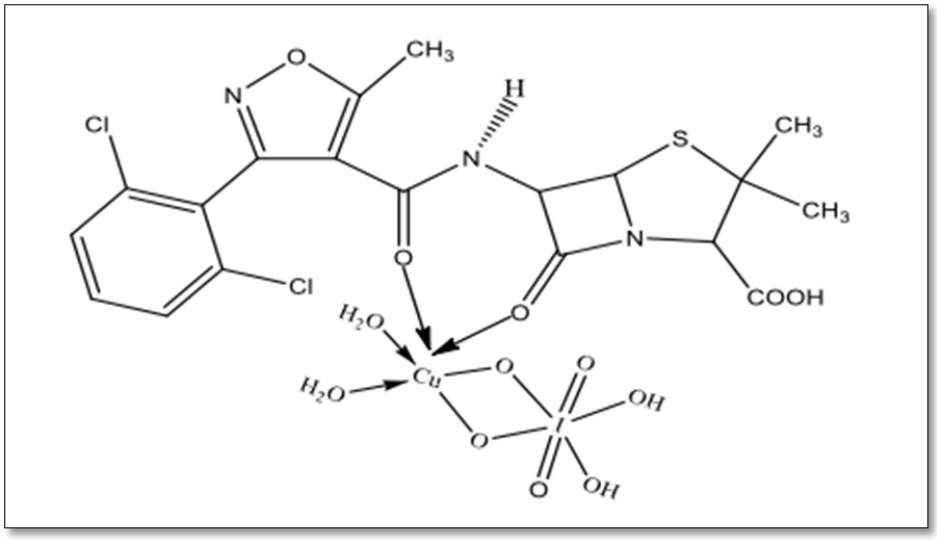


Figure 13. Probable structure of **Complex C.**

Spectroscopic evidence for the complex formation between reagent DPC (III) and substrate (DCLX) was obtained from UV –visible spectra by resisting (5.0×10^{-4} M) AMX, (0.12 M) KOH and a mixture of all. A bathochromic shift was obtained. The Michaelis – Menten plot is in great support for complex formation, **(Figure 5)**.

Scheme 1 leads to the rate law equation (6) as -

$$\text{rate} = -\frac{d[DPC]}{dt} = k[C] \qquad [5]$$

$$k_{obs} = \frac{kK_1K_2K_3[DPC][DCLX][OH^-]}{[H_3IO_6^{2-}] + K_1[OH^-][H_3IO_6^{2-}] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][DCLX]} \qquad [6]$$

This equation (6) describes all kinetic orders observed for different species. The rate law equation (6) can be rearranged into equation (7) that suits for verification.

$$\frac{1}{k_{obs}} = \frac{[H_3IO_6^{2-}]}{kK_1K_2K_3[DCLX][OH^-]} + \frac{[H_3IO_6^{2-}]}{kK_2K_3[DCLX]} + \frac{1}{kK_3[DCLX]} + \frac{1}{k} \qquad [7]$$

(k_{obs} is equivalent to k_U and stands for uncatalyzed rate constant.)

DERIVATION OF RATE LAW

From Scheme 1,

$$\text{Rate} = -\frac{d[DPC]}{dt} = k[\text{Complex}] = k[C] \qquad \textbf{[A-1]}$$

From the law of mass action, the third equilibrium constant can be given by,

$$K_3 = \frac{[C]}{[Cu(H_2IO_6)(H_2O)_2[DCLX]}$$

After rearrangement, we get,

$$[C] = K_3[Cu(H_2IO_6)(H_2O)_2[DCLX] \qquad \textbf{[A-2]}$$

Substituting the value of C from eq. [A-2], we get,

$$\text{Rate} = -\frac{d[DPC]}{dt} = K_1K_3[Cu(H_2IO_6)(H_2O)_2[DCLX] \qquad \textbf{[A-3]}$$

The second equilibrium constant can be given by

$$K_2 = \frac{[Cu(H_2IO_6)(H_2O)_2[H_3IO_6^{2-}]}{[Cu(H_2IO_6)(H_3IO_6]^{2-}}$$

This can be rearranged into

$$[Cu(H_2IO_6)(H_2O)_2] = \frac{K_2\,[Cu(H_2IO_6)(H_3IO_6]^{2-}}{[H_3IO_6]^{2-}} \qquad \textbf{[A-4]}$$

The first equilibrium constant can be given by:

$$K_1 = \frac{[Cu(H_2IO_6)(H_3IO_6]^{2-}}{[Cu(H_3IO_6)_2]^-[OH^-]}$$

This can be rearranged into

$$[Cu(H_2IO_6)(H_3IO_6]^{2-} = K_1\,[Cu(H_3IO_6)_2]^-[OH^- \qquad \textbf{[A-5]}$$

Substituting eq. [A-4] to [A-5] in eq. [A-3], we get,

$$\text{Rate} = -\frac{d[DPC]}{dt} = \frac{kK_1K_2K_3[DCLX]_f[DPC]_f[OH^-]_f}{[H_3IO_6)_2]^-} \qquad \textbf{[A-6]}$$

The total concentration of [DPC] can be given as

$$[DPC]_T = [DPC]_f + [Cu(H_2IO_6)(H_3IO_6]^{2-} + [Cu(H_2IO_6)(H_2O)_2] + [C] \qquad \textbf{[A-7]}$$

where T and f denote total and free concentrations

$$= [DPC]_f + K_1[Cu(H_2IO_6)_2]^-[OH^-] + \frac{K_1K_2[Cu(H_2IO_6)_2]^-[OH^-]}{[H_3IO_6^{2-}]} + \frac{K_1K_2K_3[Cu(H_3IO_6)_2]^-[OH^-][DCLX]}{[H_3IO_6]^{2-}}$$

$$[DPC]_T = [DPC]_f + K_1[DPC]_f[OH^-] + \frac{K_1K_2[DPC]_f[OH^-]}{[H_3IO_6^{2-}]} + \frac{K_1K_2K_3[DPC]_f[OH^-][DCLX]}{[H_3IO_6^{2-}]}$$

$$[DPC]_f = \frac{[DPC]_T[H_3IO_6^{2-}]}{[H_3IO_6^{2-}] + K_1[OH^-][H_3IO_6^{2-}] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][DCLX]} \qquad \textbf{[A-8]}$$

The total concentration of $[OH^-]$ can be given by

$$[OH^-]_T = [OH^-]_f + [Cu(H_2IO_6)(H_3IO_6]^{2-} + [Cu(H_2IO_6)(H_2O)_2] + [C]$$

$$[OH^-]_T = [OH^-]_f + K_1[DPC][OH^-]_f + \frac{K_1K_2[DPC][OH^-]_f}{[H_3IO_6]^{2-}} + \frac{K_1K_2K_3[DPC][OH^-]_f[DCLX]}{[H_3IO_6]^{2-}} \qquad [OH^-]_T = [OH^-]_f\{1 + K_1\,[DPC] + \frac{K_1K_2[DPC]}{[H_3IO_6]^{2-}} + \frac{K_1K_2K_3[DPC][DCLX]}{[H_3IO_6]^{2-}}\}$$

In view of low concentrations of DPC and $H_3IO_6^{2-}$ used, last three terms inside bracket can be neglected in comparison with unity.

$$[OH^-]_T = [OH^-]_f \qquad \textbf{[A-9]}$$

Similarly, in case of low concentrations of DPC and $H_3IO_6^{2-}$ used

$$[DCLX]_T = [DCLX]_f \qquad \textbf{[A-10]}$$

Putting these values of $[DPC]_f$ from eq^n. [A-8], $[OH^-]_f$ from eq^n. [A-9] and $[DCLX]_f$

from eq^n. [A-10] in eq^n. [A-6] after omitting subscripts T and f, we get,

$$\text{Rate} = -\frac{d[DPC]}{dt} = \frac{kK_1K_2K_3[DCLX][DPC][OH^-]}{[H_3IO_6^{2-}] + K_1[OH^-][H_3IO_6^{2-}] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][DCLX]}$$

Or, $$\frac{1}{k_{obs}} = \frac{[H_3IO_6^{2-}]}{kK_1K_2K_3[DCLX][OH^-]} + \frac{[H_3IO_6^{2-}}{k\,K_2K_3[DCLX]} + \frac{1}{kK_3[DCLX]} + \frac{1}{k} \qquad \textbf{[A-11]}$$

Scheme 1 clarifies the participation of neutral species in the reaction due to invariable ionic strength and dielectric constant. The modest values of both enthalpy and entropy of activation, within the range of electron pairing and unpairing process for the loss of degree of freedom and rigid transition state, are favourable for electron transfer reaction.

The higher negative value of $\Delta S^{\neq}$ suggests that the intermediate complex is probably highly ordered than the reacting species. The above results, evidences and lower rate constant for slow steps indicate that the oxidation presumably occurs via an inner-sphere mechanism. The reducing property of the substrate is, probably, reduced in the absence of catalyst and the path of the uncatalyzed oxidation is extended by increasing the activation energy.

CONCLUSION

Oxidation of dicloxacillin by DPC (III) was studied experimentally in aqueous alkaline medium. (MPC-III) [Cu (H_2IO_6) $(H_2O)_2$] was considered to be the main active species for the present work. Activation and thermodynamic parameters with respect to uncatalyzed rate constant **(k_U)**, slow step rate constant (**k**) as well as equilibrium constants (**K_1, K_2** and **K_3)** at different temperatures were determined. Overall sequences described here are inconsistent with all experimental evidences including products, spectral analysis, mechanistic and kinetics studies in the favour of pseudo-first order reaction.

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