Research Article

Kinetics and Mechanism of Oxidation of Dicloxacillin by Copper (III) diperiodate Complex and Co (III) Catalyst in Alkaline Medium

Yuv Raj Sahu[1], Parashuram Mishra[1*]

[1,1*]Bio-inorganic and Materials Chemistry Research Laboratory, Department of Chemistry, Mahendra Morang Adarsha Multiple Campus, Biratnagar, (Tribhuwan University) Nepal.

***Corresponding author's E-mail:** prmmishra@rediffmail.com

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ABSTRACT

Dicloxacillin was oxidized by copper (III) diperiodate complex) spectrophotometrically at 298 K and an ionic strength of 0.10 mol dm-3 by Cobalt Chloride (III) catalyst in alkaline medium to study its kinetics and mechanism. A stoichiometry of 1:4 (DCLX: DPC-III) was confirmed for this oxidation. Evidences obtained from elemental analysis, LC-MS and FT-IR spectral studies favored in the identification of reaction products. Pseudo-first order with respect to DPC (III) and fractional order with respect to Dicloxacillin as well as alkali were confirmed while periodate showed negative fractional or retarding order. Monoperiodatocuprate (MPC-III) was identified as the main active species as [Cu (H2IO6) (H2O)2]. Thermodynamic and activation parameters were determined from rate constants, catalytic and equilibrium constants. A plausible mechanism was proposed consistent with experimental results.

Keywords: Copper (III) diperiodate complex, Dicloxacillin, Kinetics, Mechanism, Rate constant.

INTRODUCTION

Penicillanic Acid Derivatives (PADs) are essential part of research due to their high detection frequency in the environment and the increasing bacterial resistance formation. Among various PADs, beta-lactam antibiotics are of extreme interest, since some of them are broad spectrum anti-bacterial with a growing demand in hospitals, households, sewages and veterinary applications. There is major concern that PADs emitted into the aquatic environment can influence drinking water. Contamination with PADs has been widely disclosed all around the world in various aquatic forms including discharge or waste samples, surface water and ground water. For the degradation of PADs in aqueous solution, interesting remedy processes are desired because under oxidation–degradation process most of composed intermediates can be mineralized into CO_2, water, and mineral species. Thus, the present investigation is oxidative in nature, mostly drug transformations under the natural environment are most likely to follow oxidation path between useful oxidants with few of the most frequently used PADs antibacterial agents. The proposed work will disclose a novel application in the field of pharmaceuticals as well as kinetics. Nano sized colloidal manganese dioxide, hexacyanoferrate (III), diperiodatocuprate (III) used as an effective oxidant for oxidative degradation of different PADs antibacterial agents in aqueous alkaline medium. So, this study will be adequately used in waste-water treatment at the sites polluted by PADs antibacterial agents. Effluents from the drug manufacturing industries accumulate in wastewater treatment plants and can pollute natural water reservoirs and such contamination can lead to the development of antibacterial resistance in indirect way. Hence personal care products (PCPs) and antibiotic resistance represents a serious health problem and different advanced oxidation processes (AOPs) have to be applied in the degradation of such emergent chemical pollutants[1-4].

Also regarded as Diclocil (BMB)2, it is a narrow spectrum chlorinated beta lactam antibiotic belonging to penicillin grade 1 which is widely effective against gram positive bacteria or beta lactamases[5] producing organisms like Staphylococcus aureus. It was discovered in 1961 and introduced in 1968.It is used to lower the effectiveness of birth control pills and enter into breast milk. 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. 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. It has less intrinsic antibacterial activity and a narrower spectrum than benzyl penicillin. It is not active against methicillin-resistant Staphylococcus aureus, enterococcal species and gram-negative bacilli. 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. Dicloxacillin is 95% to 99% bound to serum proteins, mainly albumin. It is believed to have lower incidence of severe hepatic adverse effects than flucloxacillin but a higher incidence of renal adverse effects, should be used with caution due to risk of cholestanic hepatitis.[6]. Its structure is given in Figure 1.

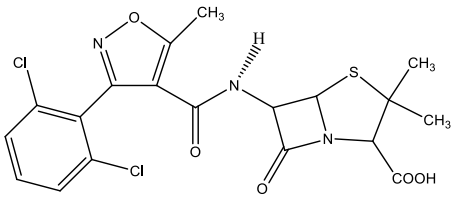


Figure 1: Dicloxacillin

Transition metals can form stable complexes with polydentate ligands like diperiodatocuprate (DPC-III)[7], Diperiodatoargentate (DPA-III)[8], diperiodatonickelate (DPN-IV)[9]. These oxidants are used for the analysis of different organic oxidation reactions[10]. Diperiodatocuprate (III) was first synthesized by Malatesta[11, 12] 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[13, 14]. Diperiodatocuprate (III) has a flexible one electron-donating nature[15]. It acts as an analytical reagent and hence used in many biological and analytical electron transfer reactions[16, 17]. Since Cu (III) is generally involved as an active intermediate species appearing in many electron transfer reactions, it becomes quite essential to know the role of Cu (III) / Cu (II) couple, as described in earlier literature[18]. Several oxidation methods of dicloxacillin in acid as well as in alkaline medium have been described in earlier literature like oxidation of dicloxacillin by Chromatographic methods for quantitative determination of ampicillin, dicloxacillin and their impurity 6-aminopenicillanic acid[19], kinetics and mechanism of oxidation of dicloxacillin sodium[DXS] by chloramine-T [CAT] in [HCl] medium[20] development and validation of a stability indicating method for the simultaneous estimation of cefixime and dicloxacillin using the RP-HPLC method[21], dicloxacillin induces CYP2C- and CYP3A- mediated drug metabolism – in vivo and in vitro[22],evaluation of water matrix effects , experimental parameters, and the degradation pathway during the TiO_2 photocatalytical treatment of the antibiotic dicloxacillin[23], Development and validation of RP-HPLC method simultaneous estimation of amoxicillin and dicloxacillin in bulk drug and capsule[24] etc.

Now, we have undertaken the present research work to investigate the kinetics and mechanism of oxidation of dicloxacillin in aqueous alkaline medium in the presence of Co (III) catalyst and hence to arrive at plausible mechanisms including determination of both activation and thermodynamic properties as well as calculation of catalyzed rate constant, catalytic constant (efficiency), slow step rate constant and equilibrium constants at different temperatures.

MATERIALS AND METHODS

Reagents and Chemicals

Chemicals used were of Analytical Reagent (AR) grade and double distilled water was 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 hours. Iodometric method was used to determine the concentration of potassium periodate solution[25].

Instrumentation

The pH of the solution was measured by ELICO LI 613 pH meter. 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

The Copper (III) diperiodate (DPC-III) was prepared[26,27] 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. The whole mixture was frequently shaken thoroughly and heated on a hot plate for about 2 hours. During this period, the mixture turned to intense red and the flask was heated further for 20 minutes to remove potassium persulphate completely from the mixture by decomposing persulphate. After completion of the reaction, the mixture was cooled and filtered through sintered glass crucible G-4 and the dark red-brown solution 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. However, the accurate concentration of DPC (III) was calculated by UV-visible spectrophotometer. DPC (III) has a square planar geometry with dsp^2 hybridization and diamagnetic nature. Similarly, KOH (BDH) and the other required solutions were prepared and stored safely.

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 appearance of peaks in UV-Visible spectrophotometer showed the formation of the complex. The possible structures of DPC (III) and MPC (III) are given in Supplementary file (SF 1).

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 $CoCl_3$ (III), KIO_4 along with KNO_3 and KOH. Data were obtained from UV-Visible spectrophotometer at pH (9.2-10) and 415 nm wavelength due to DPC (III) 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 85% reaction wherein initially added products and dielectric constant didn't exhibit any interference in the reaction.

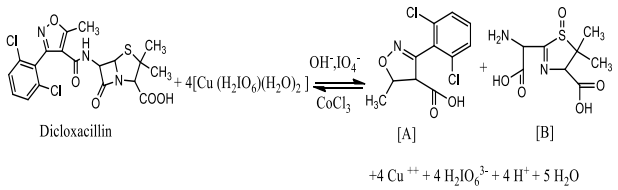
Regression analysis of experimental data to obtain regression coefficient (r) and standard deviation (s) of points from the regression line was completed with the help of Origin 9.6 (2017) software. Plots of log (abs) versus time gave a straight line and hence rate constants (k_{obs}) were calculated from slopes. The k_c values agreed within ± 5% error and were the average of at least three independent kinetic runs. A constant concentration of periodate was mixed into reaction mixture all the 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 was 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 (**SF-2**) and found that negligible interference was entertained in the reaction. The maximum wavelength of DPC (III) was noticed at 415 nm.

RESULTS AND DISCUSSION

Stoichiometry and Product Analysis

Several sets of reaction mixtures with varying ratio of DPC (III) to dicloxacillin in presence of constant amounts of $CoCl_3$, KOH and KNO_3 were kept for 2.5 hrs 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 1:2 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:



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 catalyzed dicloxacillin and Copper (III) diperiodate complex

Both complex and products were characterized by LC-MS, which gave m/z at 635 for complex ($C_{19}H_{17}Cl_5CoN_3O_5S$), 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) at273 (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 Co (III) -DCLX complex ($C_{19}H_{17}Cl_5CoN_3O_5S$) showed % elemental analysis as C-35.9 (35.45), H-2.70(2.48), Cl–27.89(27.54), Co-9.27(9.21), N-6.61(6.40) and S-5.04(5.08) besides oxygen. 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 supplementary file SF-3 and SF-4 respectively.

Reaction Orders

The orders of reaction were determined from the slope of log k_{abs} versus log (concentration) from different time plots as given in Figure SF-5 and Table-1 by varying concentrations of dicloxacillin, $CoCl_3$, KIO_4 and KOH while keeping the other parameters constant except the concentration of DPC (III).

Table 1: Effect of variation of [DPC]*, [DCLX], [$CoCl_3$ (III)], [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^-] x10^2	[IO_4^-] x10^5	[$CoCl_3$]x10^7	k_Ux10^4 (s^{-1})	k_T x 10^3(s^{-1})	k_C x 10^3 (s^{-1})
1.0	5.0	0.8	1.0	5.0	1.80	2.25	2.07
3.0	5.0	0.8	1.0	5.0	1.85	2.27	2.08
5.0	5.0	0.8	1.0	5.0	**1.86**	**2.32**	**2.13**
8.0	5.0	0.8	1.0	5.0	1.82	2.28	2.09
10.0	5.0	0.8	1.0	5.0	1.88	2.30	2.11
5.0	**1.0**	0.8	1.0	5.0	0.68	1.05	0.98
5.0	**3.0**	0.8	1.0	5.0	1.35	1.65	1.51
5.0	**5.0**	0.8	1.0	5.0	**1.86**	**2.32**	**2.13**
5.0	**8.0**	0.8	1.0	5.0	2.55	2.98	2.72
5.0	**10.0**	0.8	1.0	5.0	3.24	3.48	3.15
5.0	5.0	**0.2**	1.0	5.0	0.75	1.12	1.04
5.0	5.0	**0.4**	1.0	5.0	1.12	1.41	1.51
5.0	5.0	**0.6**	1.0	5.0	1.57	1.85	1.85
5.0	5.0	**0.8**	1.0	5.0	**1.86**	**2.32**	**2.13**
5.0	5.0	**1.0**	1.0	5.0	2.31	2.98	2.54
5.0	5.0	0.8	**1.0**	5.0	**1.86**	**2.32**	**2.13**
5.0	5.0	0.8	**3.0**	5.0	1.55	1.73	1.24
5.0	5.0	0.8	**5.0**	5.0	1.31	1.11	1.02
5.0	5.0	0.8	**8.0**	5.0	0.86	0.85	0.80
5.0	5.0	0.8	**10.0**	5.0	0.53	0.64	0.61
5.0	5.0	0.8	1.0	**1.0**	1.86	1.15	0.96
5.0	5.0	0.8	1.0	**3.0**	1.86	1.34	1.15
5.0	5.0	0.8	1.0	**5.0**	**1.86**	**2.32**	**2.13**
5.0	5.0	0.8	1.0	**8.0**	1.86	2.95	2.76
5.0	5.0	0.8	1.0	**10.0**	1.86	4.67	4.48

*Concentrations are expressed in mol dm^{-3}.

Effect of [DPC (III)]

The DPC (III) concentration was varied in 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 85% completion of the reaction by keeping other concentrations remaining constant indicated first order reaction in DPC (III). Table 1 and Figure (SF 5) are in the support of pseudo first-order reaction with respect to DPC (III).

Effect of [DCLX]

The effect of [DCLX] was studied within a range of 1 x 10^{-4} to 1 x 10^{-3}mol dm^{-3}. The rate constants (k_c) increased with increase in [DCLX] and order with respect to dicloxacillin was found to be 0.625 ($r \geq 0.996$, $s \leq 0.001$) which was also confirmed from the plot of (4+ log k_c) vs 4+ log [DCLX], Figure 2 and Table 1.

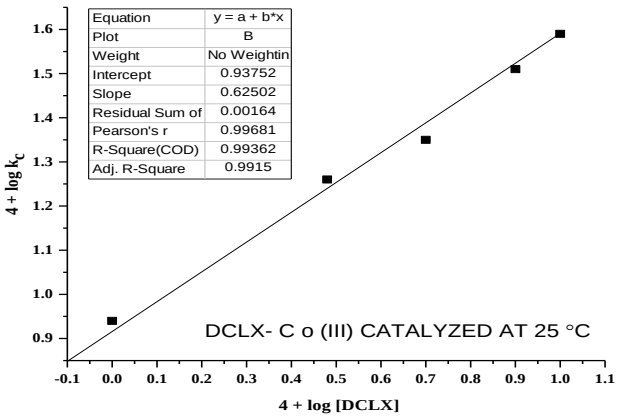


Figure 2: Plot of (4 + log k_c) vs. 4 + log [DCLX]

Effect of [Alkali]

The effect of alkali was studied by varying [OH^-] in the range of 0.04 to 0.2 mol dm^{-3} DPC (III), $CoCl_3$ (III), DCLX, as well as ionic strength. Rate constant (k_c) increased with

increase in [alkali] and order of reaction with respect to alkali was found to be 0.50 (r ≥ 0.997, s ≤ 0.004), confirmed by the linear plot of (4 + log k_c) vs. 4 + log [KOH]), Figure 3 and Table 1.

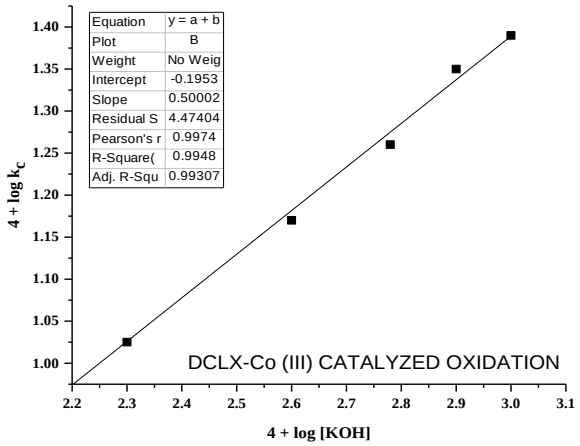


Figure 3: Plot of (4 + log k_c) vs. 4 + log[KOH]

Effect of [Periodate]

Effect of [Periodate]: The effect of [KIO_4] in case of DCLX was observed by varying the concentration range from 1.0 x 10^{-5} to 1.0 x 10^{-4} mol dm^{-3} remaining other active masses and conditions were constant. It was observed that rate constants decreased with an increase in [IO_4^-] and the order of reaction was - 0.484 (r ≥ 0.996, s ≤ 0.001), confirmed by the linear plot of (4 + log k_c) vs. 5 + log [KOI_4]), as computed in Figure 4, Table 1.

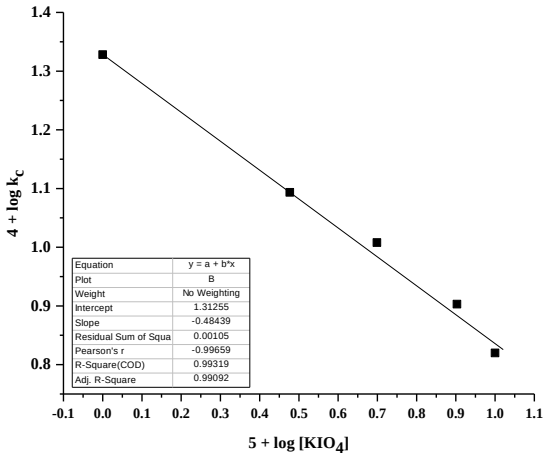


Figure 4: Plot of 5 + log [KIO_4] vs. 4 + log k_c

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.

Effect of Initially Added Products and Polymerization Study

Initially added product ($CuSO_4$ (II)) didn't show any significant effect on the rate of reaction. A known quantity of acrylonitrile[28] monomer was initially added to the reaction mixture and allowed to remain in the inert atmosphere for 3.0 hours. The mixture gave no precipitate on dilution with methanol indicating the absence of free radicals.

Effect of Temperature

The effect of temperature on the rate of oxidation reaction was studied at four different temperatures under the constant concentration of DCLX, $CoCl_3$ (III), KOH, and DPC (III) keeping other conditions constant. The rate constants increased with the rise in temperature. Slope obtained from the plot of catalyzed rate constant (3 + log k_c vs. 1 / T) helped to calculate the both activation as well as thermodynamic parameter and thence computed in Table 2, Figure SF-6. Similarly, slope obtained from the plot of catalytic constant (log Kc vs. 1 / T) helped to calculate the both activation as well as thermodynamic parameter and thence computed in Table 3, Figure SF-7.

Table 2: Activation parameters from catalyzed rate constant for DCLX

Parameters	Values
Ea (k $Jmol^{-1}$)	36.12
$\Delta H^{\neq}$ (k $Jmol^{-1}$)	33 ± 2
$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	-184 ± 2
$\Delta G^{\neq}$ (k J mol^{-1})	91 ± 2
LogA	3.5 ± 0.2

Table 3: Activation parameters from catalytic constant for DCLX

Parameters	Values
Ea (k $Jmol^{-1}$)	36.79
$\Delta H^{\neq}$ (k $Jmol^{-1}$)	34 ± 1
$\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$)	-181 ± 0.6
$\Delta G^{\neq}$ (k J mol^{-1})	89 ± 3
LogA	3.79 ± 0.2

Activation parameters with respect to slow step rate constant (k) of DCLX

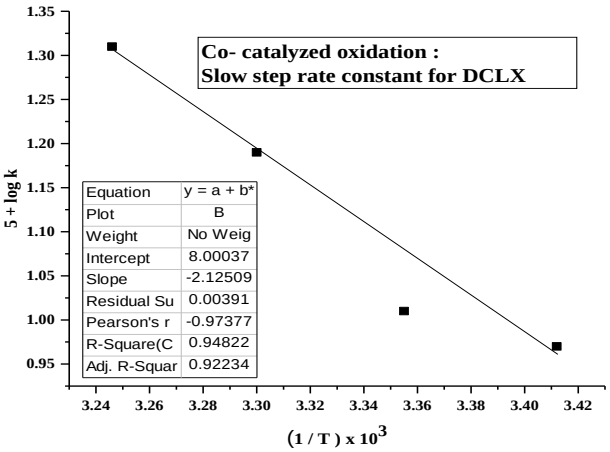


Figure 5: Plot of (5 + log k) + 1 / T for DCLX

Table 4: Activation parameters from slow step rate constant for DCLX

Parameters	Values
Ea (k Jmol^{-1})	41.61
$\Delta H^{\neq}$ (k Jmol^{-1})	39 ± 2
$\Delta S^{\neq}$ (JK^{-1}mol^{-1})	-191 ± 2
$\Delta G^{\neq}$ (k J mol^{-1})	96 ± 2
LogA	3.2 ± 0.4

Since DPC (III) is chelating as well as the oxidizing agent, oxidation of different β-lactam antibiotics has been carried out in an alkaline medium. The activity of DPC 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.

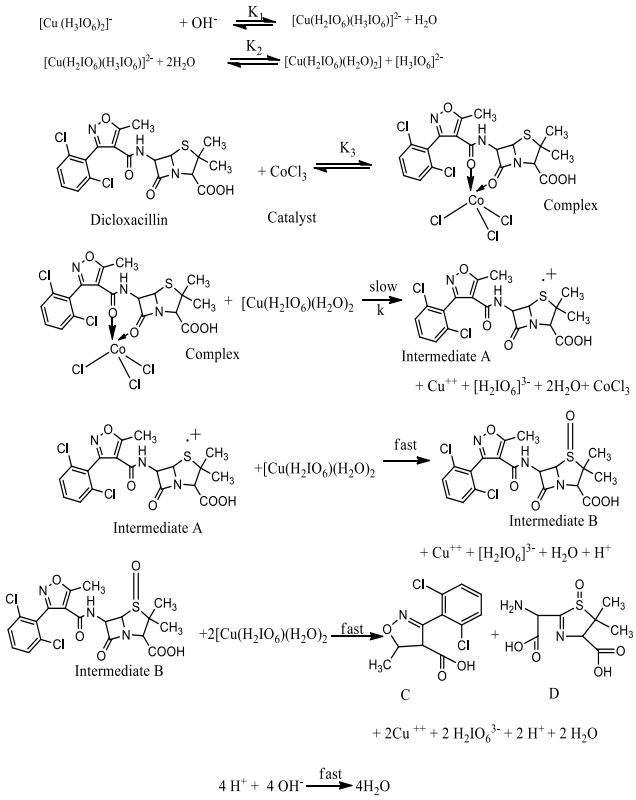
$$H_5IO_6 \rightleftharpoons H_4IO_6^- + H^+, \quad K_1 = 5.1 \times 10^{-4}$$

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

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

Probable Mechanism of Reaction

The reaction between DPC (III) and dicloxacillin exhibits 1:4 stoichiometry and confirms pseudo-first order reaction with respect to DPC (III), fractional order with respect to dicloxacillin and alkali but periodate showed retarding effect and negatively fractional order. 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 Co (III) catalyst. This complex interacts with fresh one mole of MPC (III) to yield an intermediate (A) along with regeneration of catalyst, Co (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 above by Scheme 1.



Scheme 1: Detailed Scheme for catalyzed oxidation of DCLX by DPC (III) and Co (III)

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.

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

Complete rate law equation has been presented in supplementary file SF-8.

Activation and thermodynamic parameters from Equilibrium Constants of DCLX

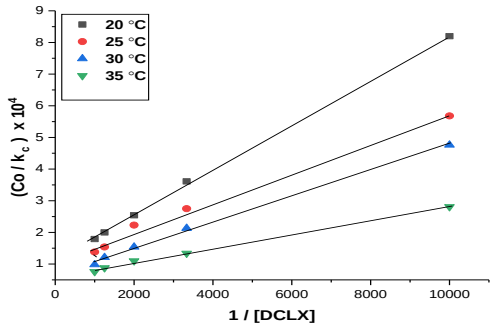


Figure 8 (a): Plot of { (1 / [DCLX] vs [Co / k_c]} for DCLX

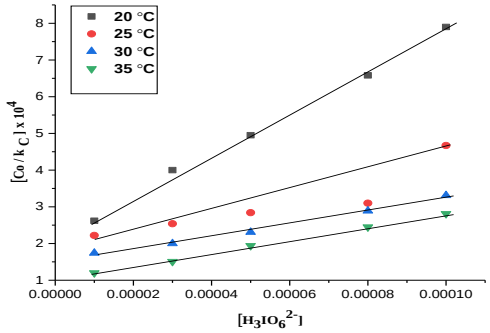


Figure 8 (b): Plot of { $[H_2IO_6]^{3-}$ vs [Co / k_c]} for DCLX

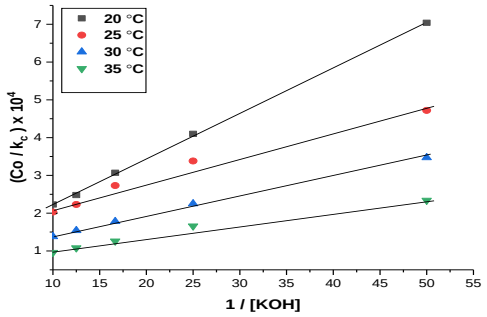


Figure 8 (c): Plot of { (1 / [KOH] vs [Co / k_c]} for DCLX

Figure 8 (a, b, c) represent verification plots for catalyzed oxidation of DCLX by DPC (III) in alkaline medium. According to equation (7), remaining other conditions being constant, the plots of [Co (III) / k_c vs.1 / [KOH] ($r \geq 0.995, \leq s\ 0.00356$), [Co (III) / k_c vs. 1/[DCLX] ($r \geq 0.997, \leq s\ 0.0045$) and [Co (III) / k_c vs. $[H_2IO_6]^{3-}$ ($r \geq 0.998, \leq s\ 0.00324$) should be linear and are found to be so as in Figure 8 (a), 8 (b) and 8 (c).

Table 5(a): Equilibrium constants and slow step rate constant for DCLX

Equilibrium Constants ↓	Absolute Temperatures			
Temperature →	**20 ℃**	**25 ℃**	**30℃**	**35 ℃**
k (Slow step rate constant)	0.93 x 10^{-4}	1.02 x 10^{-4}	1.57 x 10^{-4}	2.06 x 10^{-4}
K_1	2.63 x 10^{6}	2.81 x 10^{6}	3.23 x 10^{6}	3.63 x 10^{6}
K_2	3.715	3.17	3.019	2.344
K_3	1318.25	1548.81	1659.59	2137.96

Table 5(b): 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})	16.4 ± 2	21.45 ± 1	22.77 ± 1
ΔS°_{298} (J K^{-1} mol^{-1})	176.35 ± 4	82.75 ± 2	138.5 ± 3
ΔG°_{298} (k J mol^{-1})	-36.941 ± 0.6	-2.87 ± 0.4	-18.27 ± 0.5

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 favorable 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 reaction is extended by increasing the activation energy.

CONCLUSIONS

The Co (III) catalyzed oxidation of dicloxacillin by DPC (III) was studied experimentally in an alkaline medium. (MPC-III) [Cu (H_2IO_6) (H_2O)$_2$] was considered to be the active species for the present work. Both activation and thermodynamic parameters with respect to catalyzed rate constant (**k_c**), catalytic constant (**K_c**), slow step rate constant (**k**) as well as equilibrium constants (**K_1, K_2** and **K_3**) at different temperatures were computed. Overall sequences described here are inconsistent with all experimental evidences including product, spectral analysis, mechanistic and kinetics studies.

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