Kinetics and Mechanism of Oxidation of Ampicillin by Diperiodatocuprate (III) and Cobalt (III) as Catalyst in Aqueous Alkaline Medium

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ABSTRACT

The kinetics and mechanism of Co(III) catalyzed oxidation of Ampicillin by diperiodatocuprate (DPC (III)) in alkaline medium was studied spectrophotometrically at 298 K and at ionic strength of 0.10 mol dm^{-3}. The reaction between DPC and Ampicillin in alkaline medium exhibited (AMP:DPC) 1:4 stoichiometry. The oxidation products were identified by spot test, FT-IR and LC-ESI-MS spectral studies. The reaction exhibited pseudo first-order with respect to DPC and fractional order with respect to ampicillin. Addition of alkali retarded the rate of reaction while Co(III) enhanced the rate and periodate had no effect on rate. DPC(III) was found to be the main active species in alkaline medium in the form of $[Cu(H_3IO_6)_2]^-$. Activation parameters were computed. The plausible mechanism consistent with experimental results was proposed and discussed in detail.

Highlights

- A mechanism for the Co(III) catalyzed oxidation of Ampicillin by DPC (III) is proposed based on kinetic results.
- Among various species of DPC (III) complex, $[Cu(H_3IO_6)_2]^-$ is considered as the most active species for the title work.
- In carrying the reaction, the role of pH is crucial.
- Activation parameters were computed and discussed.
- The overall sequences described are consistent with the present kinetic studies.

Keywords: Kinetics, Mechanism, Oxidation, Diperiodatocuprate (III), Ampicillin.

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INTRODUCTION

Ampicillin (AMP) is a semi-synthetic and broad spectrum antibiotic with melting point of 207°C (lit.208°C) [**1, 2**]. It consists of a heterocyclic group linked to a five member thiazolidine ring including one sulphur atom fused with a four membered beta lactam ring. It is a bacterio-static drug

useful for the treatment of various infections or diseases caused by gram positive and gram negative bacteria [3]. It is widely used in chemotherapy because of its semi-stability in acid [4], low toxicity, efficient absorption and low inhibitory concentration against bacteria [5]. This stability may be due to incorporation of electron withdrawing amino group with a decrease in nucleophilicity side chain carbonyl group [6-8]. Ampicillin can polymerize through nucleophilic attack by its side chain amino group in one molecule upon beta lactam carbonyl group of next molecule [9, 10]. It acts by inhibiting protein synthesis of bacterial cell wall. Different complexes of ampicillin with several transition metals have been studied [11].

Several oxidation reactions of DPC (III) in alkaline medium have been described in earlier literatures [12]. Previous literature states that ampicillin is not stable in aqueous   medium and such stability is of great essential in order to study the structure, reactivity as well as activation parameters. Since the rate of this reaction increased by more than 10 times in the presence of Co (III) catalyst, oxidation of ampicillin by DPC (III) in alkaline medium is important.

Copper (III) has been noticed as an intermediate in Cu(II) catalyzed oxidation of amino acids by peroxydisulphate [13]. Transition metals, in their higher oxidation states, can be stabilized by chelating with polydentate ligands. Some of good oxidants are Diperiodatocuprate (DPC(III)) [14], diperiodatonickelate (DPN (IV)) [15], Diperiodatoargentate (DPA(III)) [16], diperiodatotellurate (DPT-(III)) [17] in alkaline medium within fixed pH range.DPC is a versatile one electron oxidant with specific analytical use[18, 19].Copper complexes have a major role in oxidation process due to their abundances and relevance in analytical and biological chemistry [20]. The uncatalyzed and Osmium (VIII) as well as Ruthenium catalyzed oxidation of 6-aminopenicillanic acid by DPC have been reported in literatures [21]. Soluble periodate complexes of Copper can exist in three forms like monoperiodatocuprate (MPC), Diperiodatocuprate (DPC) and triperiodatocuperate (TPC) in which the most stable form are DPC as $[Cu(H_2IO_6).(H_3IO_6)]^{-2}$ or $[Cu(H_2IO_6)_2.(H_2O)_2]$ as reported in previous literatures [22, 23]. Copper (II)-copper (I) couple has been reported in earlier review literatures [24-27]. It becomes quite interesting to identify the most active species of periodatocuperate as active oxidant. Potassium periodate possesses retarding effect and alkali shows positive effect generally on DPC oxidation as reported in earlier reviews [28]. In the present work, entirely different kinetics measurements are noticed which is necessary to know the mechanistic profile of ampicillin in redox reaction that can develop an insight into interaction of Cu^{3+} and Co (III) ions with ampicillin and its mode of action in biological system. The literature survey reveals that no research work on kinetic studies for alkaline oxidation of ampicillin by DPC (III) in the presence of cobalt (III) catalyst has been carried out. The present research work is aimed to investigate the kinetics and mechanism of ampicillin in alkaline medium by DPC (III) in the presence of catalyst Co (III) and hence to arrive at plausible mechanism including determination of activation properties of this catalyzed oxidation reaction.

MATERIALS AND METHODS

All chemicals used were of A R grade and double distilled water was used throughout the work. Melting point 204°C (literature 207°C) was measured to check the purity of ampicillin (Sigma Aldrich) and then stock solution of ampicillin (0.01 mol dm^{-3}) was prepared by dissolving 0.3494 g of recrystallized ampicillinin 100 mL double distilled water. Potassium periodate solution was prepared by dissolving 0.023 g of (0.01 mol dm^{-3}) KIO$_4$ (Sigma Aldrich) in 100 mL double distilled hot water and   the solution was used only after 24 h. The concentration of potassium periodate solution was determined by iodometrically [29]. The Copper (III) diperiodate DPC (III) was prepared [30, 31] 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 round bottomed flask. The whole mixture was frequently shaken thoroughly and heated on a hot plate for about 2 h. During this period, the mixture turned to intense red, the flask was heated further for 20 min to remove potassium persulphate completely from the mixture by decomposing persulphate. After completion of 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 (sodium thiosulphate, starch, potassium iodide and potassium dihydrogen phosphate) by thiocyanate method and its exact concentration was ascertained. Existence of DPC (III) was verified by UV-Visible spectrophotometer that showed an absorption band with maximum peak at 415 nm. However, accurate concentration of DPC was calculated by UV-Visible spectrophotometer. DPC (III) has a square planar geometry, dsp^2 hybridization and diamagnetism by nature. Similarly, KOH (BDH) and Cobalt chloride (Sigma Aldrich) solutions were prepared and stored safely. 10 mL of ampicillin solution (0.132 mol dm^{-3}) taken in 100 mL RB flask in which 10 mL of DPC (III) (0.528 mol dm^{-3}) was mixed with stoichiometry of 1:4 along with 1.0 mL of each KNO_3, KIO_4, $CoCl_3$ and 2.0 mL of KOH solution of fixed molarities and stirred on metal hot plate for 24 h followed by re- stirring during refluxing with condensation for 24 h. Then the mixture was cooled naturally for 3 days and filtered by whatsman no.1. The products were purified and re-crystallized in ethanol till whole solvent evaporated leaving behind crystals only. Appearance of peaks in UV-Visible spectrophotometer showed the formation of complex. The possible structures of MPC and DPC are given below.
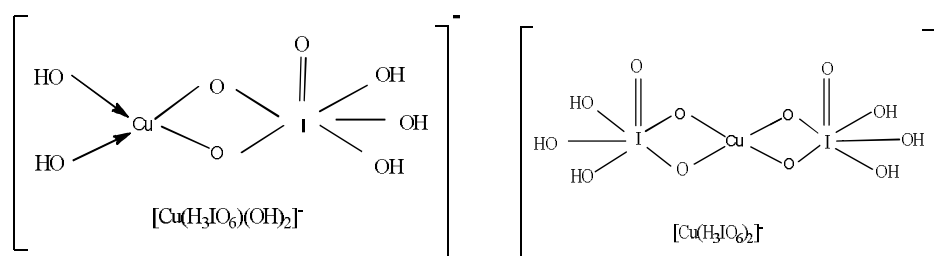
Monoperiodatocuprate (MPC-III) and Diperiodatocuprate (DPC-III).

Instruments used: For accurate pH value, ELICO   LI 613 pH meter was used. For UV data, Peltier Accessory (Temperature control) attached Varian CARY 5000 Bio UV-Vis spectrophotometer within range of 175-3300 nm was used. For FT –IR spectra, Thermo Nicolet, Avatar 370 by Perkin Elimer spectrum II in the range of 4000-400 cm^{-1} that was run as KBr disc from STIC, Cochin, and India. For LC- ESI-MS spectrum, (UPLC-TQD) Mass spectrometer in positive mode by water in the range of (0– 1000) m/z equipped with electron spray ionization source was run in CDIR-CSIR, SAIF, Lucknow.

Kinetic Measurements: As this type of reaction is always very fast in nature, its absorbance were taken quite rapidly along with progress of reaction by following pseudo-first order state when active mass of AMP was greater than that of DPC at 20°C, 25°C, 30°C and 35°C ± 0.1°C unless specified. The reaction was initiated by mixing required quantities of previously thermostatted solutions of AMP into DPC (III) which already contained fixed concentration of KIO_4 along with KNO_3, KOH in the presence of catalyst$CoCl_3$. Data were obtained from UV-Visible spectrophotometer at pH (10-11) and 415 nm wavelength due to DPC by monitoring the decrease in absorbance at molar extinction coefficient (Є) of 6238 ± 20 dm^3 mol^{-1} cm^{-1}. The UV-Visible spectrophotometer was run upto 95% reaction wherein initially added products and dielectric constant didn't exhibit any interference in the reaction.

There was no effect of ubiquitous contamination of initially added carbonate in the reaction. Fresh solutions were nevertheless used to carry out each kinetic run. Regression analysis of experimental data to obtain regression coefficient *r* and standard deviation *S* of points from regression line was completed with the help of Origin 9.6 (2017) software. Plots of log absorbance versus time gave a straight line and hence tend to be of pseudo-first order reaction from slope. A constant concentration of periodate was mixed into reaction mixture all the times. Finally, total concentration of KIO_4 and KOH were determined by assuming the amount present in DPC and added additionally. To check the

effect of periodate, ionic strength, dissolved oxygen etc, kinetics were also conducted into N_2 atmosphere wherein no significant changes were observed. Added carbonate and periodadte dielectric constant etc didn't show any effect.

The application of Beer-Lambert's law was verified from figure 1(a) and found that negligible interference was entertained in the reaction. The maximum wavelength of DPC (III) was noticed at 415 nm figure 1(b).
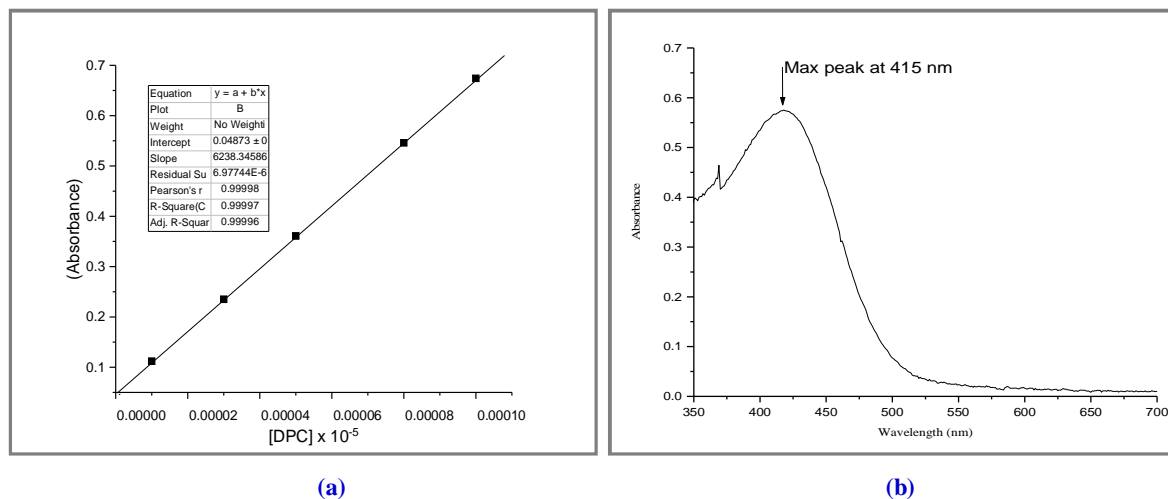

(a)   **(b)**

Figure 1(a). Plot of absorbance vs. [DPC] at 25°C (b) Plot of absorbance vs. wavelength for DPC.

The rate constant was determined from the rate law equation (**1**) for first order reaction as:

$$\ln(Abs)_t = -k_c t + \ln(Abs)_o$$

$$2.303\log(Abs)_t = -k_c t + 2.303\log(Abs)_o$$

$$k_c t = 2.303\log(Abs)_o - 2.303\log(Abs)_t$$

$$k_c = \frac{2.303}{t}\left[\log\frac{(Abs)_o}{(Abs)_t}\right] \qquad ...(1)$$

Here, $(Abs)_o$=Initial absorbance at time 0 sec or just before mixing and $(Abs)_t$ stands for absorbance at any time *t* sec.

RESULTS AND DISCUSSION

Stoichiometry and product analysis: Several sets of reaction mixtures with varying ratio of DPC to ampicillin in presence of constant amounts of KOH and KNO_3 in the presence of Co (III) catalyst were kept for 2.5 h in closed vessel under N_2 atmosphere and the remaining concentration of DPC was analyzed to confirm the accurate stoichiometry by Job's method which was confirmed to be 1:4 for AMP:DPC. When ampicillin reacts with DPC in the presence of Cobalt (III) chloride catalyst in alkaline medium, 2-formyl, 5-5 dimethylthialozidine 4-carboxyllic acid and oxo-phenyl acetic acid were formed as main product which were recrystallized from ethanol, separated by Column Chromatography over neutral alumina by using 80% benzene and 20% chloroform as eluent. The reaction between ampicillin and diperiodatocuprate (III) in alkaline medium can be given as:

A = Ampicillin, B = 2-Formyl 5,5-dimethyl-thiazolidine-4 carboxylic acid, C = oxo-phenyl acetic acid
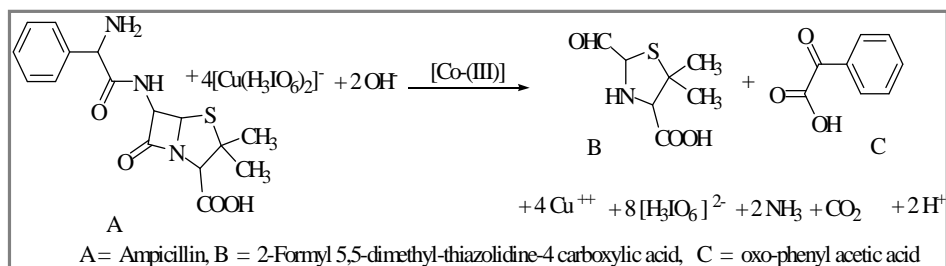
The oxidation product oxo-phenyl acetic acid was identified by FT-IR spectrum that showed a sharp absorption peak at 1744.4 cm^{-1} due to ketone C=O stretch) ,1648.2cm^{-1} due to due to carboxylic acid C=O stretch) and a broad peak at 3340.5 cm^{-1} due to carboxylic acid OH. The product was characterized by LC-ESI-MS that gives m/z at 149 as (m-1) with 42% yield. Elemental analysis for $C_8H_8O_3$ showed 64.05% C (64.11): 04.03 %H (03.95). Appearance of red precipitate with 2, 4-DNP confirmed the α-keto acid presence. Another oxidation product 2-formyl-5,5-dimethylthiozidine-4-carboxylic acid was identified by FT-IR spectrum shows a sharp absorption peak at 1742.3cm^{-1} (due to aldehyde C=O stretch) ,1668.4 cm^{-}(due to carboxylic acid C=O stretch),1384.3cm^{-1}(due to –CH$_3$ stretch), 3437.1 cm^{-1} (due to N-H stretch) and a broad peak at 3352.4 cm^{-1} due to carboxylic acid OH. The product was characterized by LC-ESI-MS that gives m/z at 190.2 as (m +1).along with 28% yield. Elemental analysis for $C_7H_{11}NO_3S$ showed 44.43%C (44.51): 5.86 %H (05.81):07.4%N (07.35):16.95%S (16.89). Both main products were re-identified by formation of 2,4-DNP derivatives. Side products were identified as ammonia by Nesseler's reagent. 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.

Reaction orders: Since the DPC oxidation of AMP in alkaline medium proceeds with measurable rate in the absence of Co (III) catalyst, the catalyzed reaction is understood to occur in parallel paths with

Table 1. Effect of variation of [DPC], [AMP],[KOH] and [Co(III)] on the oxidation of Ampicillin by Diperiodatocuprate (III) in aqueous alkaline medium at 25°C and I = 0.10 mol dm^{-3}

[DPC]x10^5	[AMP]x10^4	[OH$^-$]x10^2	[IO$_4^-$]x10^5	[CoCl$_3$]x10^7	k_U x 10^4 (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	1.99	2.13	1.93
3.0	5.0	0.8	1.0	5.0	1.94	2.03	1.84
5.0	5.0	0.8	1.0	5.0	**1.86**	**1.97**	**1.78**
8.0	5.0	0.8	1.0	5.0	1.89	2.00	1.81
10.0	5.0	0.8	1.0	5.0	1.93	2.19	1.99
5.0	**1.0**	0.8	1.0	5.0	0.64	0.75	0.69
5.0	**3.0**	0.8	1.0	5.0	0.93	1.10	1.01
5.0	**5.0**	0.8	1.0	5.0	**1.86**	**1.97**	**1.78**
5.0	**8.0**	0.8	1.0	5.0	2.66	2.82	2.55
5.0	**10.0**	0.8	1.0	5.0	3.29	3.47	3.14
5.0	5.0	**0.2**	1.0	5.0	2.85	3.36	3.07
5.0	5.0	**0.4**	1.0	5.0	2.40	2.78	2.54
5.0	5.0	**0.6**	1.0	5.0	1.95	2.41	2.21
5.0	5.0	**0.8**	1.0	5.0	**1.86**	**1.97**	**1.78**
5.0	5.0	**1.0**	1.0	5.0	1.60	1.75	1.60
5.0	5.0	0.8	1.0	**1.0**	1.86	0.49	0.31
5.0	5.0	0.8	1.0	**3.0**	1.86	0.98	0.79
5.0	5.0	0.8	1.0	**5.0**	1.86	**1.97**	**1.78**
5.0	5.0	0.8	1.0	**8.0**	1.86	2.34	2.15
5.0	5.0	0.8	1.0	**10.0**	1.86	2.76	2.52

*All concentrations are in mol dm^{-3}

contributions from both the uncatalyzed and catalyzed paths. Thus the total rate constant (k_T) is equal to the sum of rate constants of uncatalyzed reaction (k_U) and that of catalyzed reaction (k_C) so that $k_C = k_T - k_U$. Hence the orders of reaction were determined from slope of $\log k_c$ vs. log (concentration) plots as given in figure 2 and table 1 by varying concentrations of ampicillin, KIO_4, KOH, and $CoCl_3$ while keeping the other parameters constant except concentration of DPC.

Effect of [DPC(III)]: DPC concentration was varied within the range of 1.0×10^{-5} to 1.0×10^{-4} mol dm^{-3}. The linearity and almost parallelism upto 80% completion of the reaction as well as constant values of rate constant with variable concentrations of DPC (Table 1 and Figure 2) are in the support of pseudo first order reaction with respect to DPC.
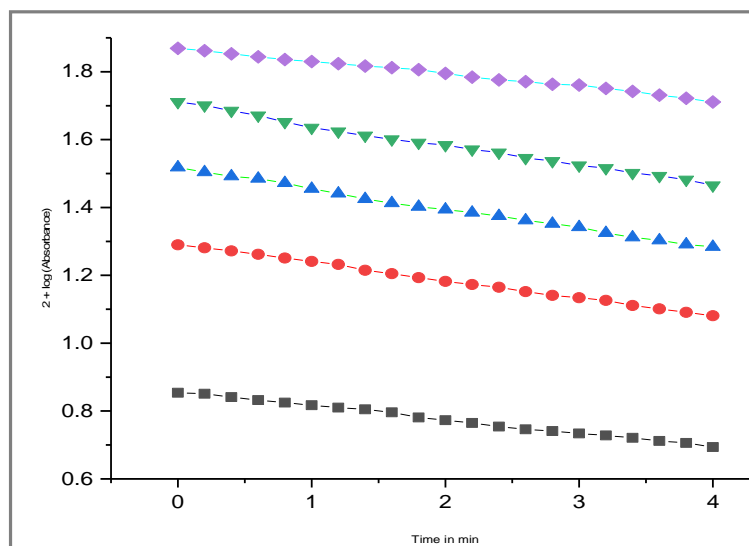
Figure 2. Plot of log (abs) vs. time (■-1.0×10^{-5}, ●- 3.0×10^{-5}, ▲- 5.0×10^{-5}, ▼-8.0×10^{-5}, ◆-10.0×10^{-5}).

Effect of [AMP]: The effect of [AMP] was studied within the range of 1×10^{-4} to 1×10^{-3} mol dm^{-3}. The rate constants (k_c), increased with increase in [AMP] and order with respect to ampicillin was found to be less than 1 (Table 1) (r ≥0.998 ,s ≤ 0.0198) which was also confirmed from the plot of $2 + \log k_c$ vs. $4 + \log$ [AMP] (Figure 3).
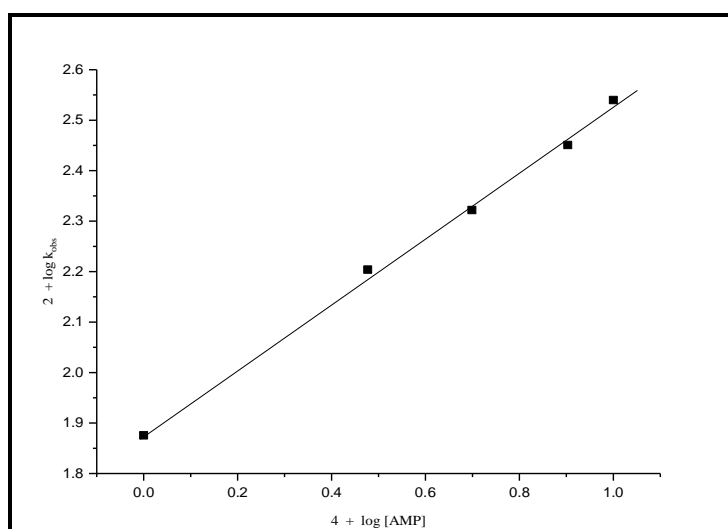

Figure 3. Plot of $2 + \log k_c$ vs. $4 + \log$ [AMP].

Effect of [alkali]: The alkali concentration was varied within the range of 0.02 to 0.1 mol dm^{-3} to study its effect on rate. Rate constant (k_c) decreased with increase in [alkali] and hence showed retarding effect. Order of reaction with respect to alkali was found to be negatively fractional (Table 1) as determined from plot of ($2 + \log k_c$ vs. $4 + \log$ [KOH]) (Figure 4).
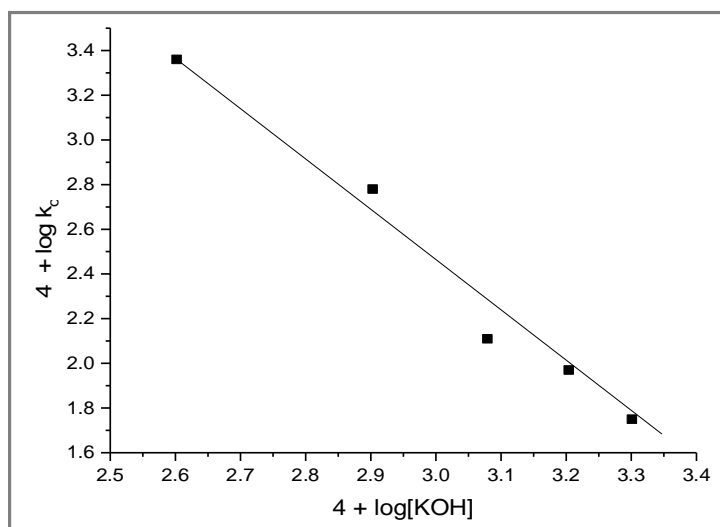

Figure 4. Plot of ($4 + \log k_c$) vs. $4 + \log$[KOH].

Effect of [periodate]: The effect of [KIO$_4$] was studied within the range of 1.0×10^{-5} to 1.0×10^{-4} mol dm^{-3} remaining other active masses constant. It was found that added periodate had negligible effect with increase in [periodate] on the rate of reaction.

Effect of ionic strength (I) and dielectric constant (D): Ionic strength is applied to know the participation of specific species in the reaction like ion-dipole, ion-ion, dipole-dipole with same or opposite charge etc. The effect of ionic strength was studied by varying the concentration of KNO$_3$ in the range of 0.1-0.2 M by keeping concentration of DPC, AMP, KOH constant. Ionic strength of the medium did not have any significant effect on the rate of reaction, as found from the experiment. Dielectric constant of the medium **(D)** can be studied by varying tertiary butyl alcohol at constant concentration of DPC, AMP, KOH, KNO$_3$ by using the equation $D = D_1V_1 + D_2V_2$ where D_1 and D_2 are dielectric constant of water and tert-butyl alcohol and V_1 and V_2 are volume fractions respectively. It showed no significant effect on the rate of reaction.

Effect of initially added products: One of the product, Cu (II)(CuSO$_4$)in the concentration range of $1.0 \times 10^{-5} - 1.0 \times 10^{-4}$ mol dm^{-3} with all other reactant concentrations and condition kept constant, did not have any significant effect on the rate of reaction.

Polymerization study: A known quantity of acrylonitrile monomer was initially added to the reaction mixture and allowed to remain in inert atmosphere for 2 h. The mixture gave a white precipitate on dilution with methanol indicating the presence of free radicals.

Effect of temperature: The influence of temperature on the rate of reaction was studied at four different temperatures (20°C, 25°C, 30°C and 35°C) under constant concentration of AMP, KOH, DPC, CoCl$_3$ keeping other conditions constant. The rate constants were found to increase with increase in temperature. Slope obtained from plot of ($\log k_c$ vs. $1/T$) helped to calculate activation parameter and energy of activation and thence computed in table 2, figure 5.
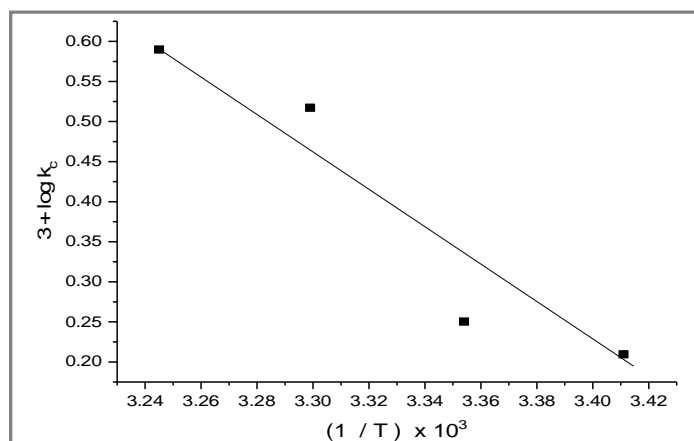
Figure 5. Plot of (3+ log k_c) vs 1/T

The energy of activation for the present reaction was calculated by,

$$E_a = -2.303 \, R \, slope$$

The Arrhenius factor 'A' was calculated by,

$$\log A = \log k_c + \frac{E_a}{2.303 \, RT}$$

The entropy of activation was calculated by,

$$\frac{\Delta S^{\#}}{4.576} = \log k_c - 10.753 - \log T + \frac{E_a}{4.576T}$$

Where, k is in sec^{-1}, temperature in Kelvin and E_a in calories.

The enthalpy of activation was calculated by,

$$\Delta H^{\#} = E_a - RT$$

The free energy of activation was calculated by,

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#}$$

Table 2. Activation parameters for Co (III) catalyzed oxidation of ampicillin by alkaline DPC (III) (A) Effect of temperature on rate constants (k_c)

Temp (K)	(1/T) x 10^3	k_c x 10^3	3 + logk_c
293	3.411	1.62	0.21
298	3.355	1.78	0.25
303	3.3	3.29	0.52
308	3.245	3.89	0.59

(B). Activation parameters

Parameters	Values
Ea (kJ mol^{-1})	48.7
$\Delta H^{\neq}$ (kJ mol^{-1})	46 ± 2
$\Delta S^{\neq}$ (J/K mol^{-1})	-140 ± 4
$\Delta G^{\neq}$ (kJ mol^{-1})	88 ± 3
LogA	05 ± 0.8

Catalytic Constant: Moelwyn- Hughes [**32**] found that both the uncatalyzed and catalyzed reactions always occur simultaneously, so that,

$$k_T = k_u + K_c x [Catalyst]^x$$

Here, k_T is the pseudo-first order rate constant in the presence of Co (III) catalyst, k_U is that for the absence of catalyst, K_c is the catalytic constant and x is the order with respect to [Co(III)] .As temperature raises up, rate of reaction gets enhanced and hence rate constant as well as catalytic constant also increase accordingly. In the present study, the value of x is unity (ie x=1). Thus, catalytic constant can be calculated as:-

$$Kc = \frac{(k_T - k_U)}{[Catalyst]^x} \quad ,$$

Since x =1,so,

$$K_c = \frac{k_T - k_U}{[Co(III)]} = \frac{k_c}{[Co(III)]}$$

The values of K_c were calculated at different temperatures and found to increase with increase in temperatures. Further, plot of log K_c vs. 1/T was linear (Figure 6) and the values of energy of activation and other activation parameters with reference to catalytic constant were calculated and results are given in table 3.
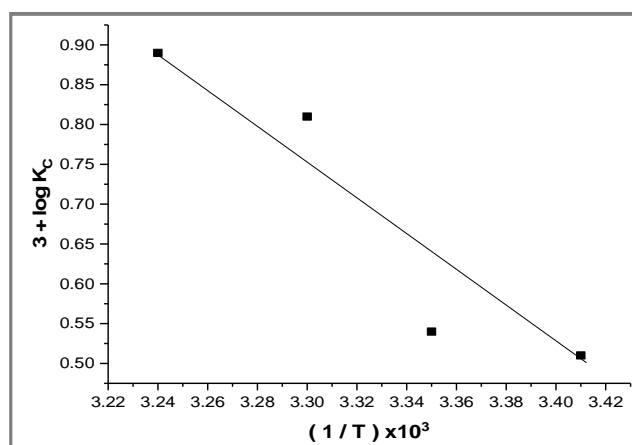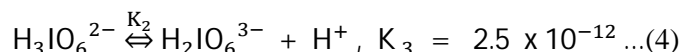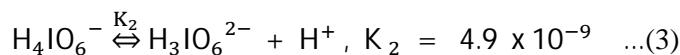

Figure 6. Plot of logK_c vs.1/T.
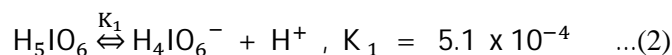
Table 3(A). Effect of temperature on Catalytic constants (K_c)

Temp (K)	(1/T) x 10³	K_c x 10³	3 + log K_c
293	3.41	3.25	0.51
298	3.35	3.47	0.54
303	3.30	6.41	0.81
308	3.24	7.76	0.89

(B). Activation parameters with respect to catalytic constant

Parameters	Values
Ea (kJ mol⁻¹)	47.6 ± 1.5
$\Delta H^{\neq}$ (kJ mol⁻¹)	45 ± 2
$\Delta S^{\neq}$ (J K⁻¹ mol⁻¹)	-82 ± 3
$\Delta G^{\neq}$ (kJ mol⁻¹)	69 ± 2
LogA	8.8 ± 0.2

Since DPC is chelating as well as oxidizing agent, oxidation of different beta lactam antibiotics have been carried out in alkaline medium. Activity of DPC is a function of pH and is capable of subtle control. DPC (II) is water soluble oxidizing reagent that exists as $[Cu(HIO_6)_2(OH)_2]^{7-}$. However in an aqueous alkaline medium and at high pH range as employed in this study periodate is unlikely to exist as HIO_6^{4-} as present in the complex as is evident from its involvement in the multiple equilibria (equation 2-4) [33] depending on pH of the solution.

$$H_5IO_6 \overset{K_1}{\Leftrightarrow} H_4IO_6^- + H^+ , \; K_1 = 5.1 \times 10^{-4} \quad ...(2)$$

$$H_4IO_6^- \overset{K_2}{\Leftrightarrow} H_3IO_6^{2-} + H^+ , \; K_2 = 4.9 \times 10^{-9} \quad ...(3)$$

$$H_3IO_6^{2-} \overset{K_2}{\Leftrightarrow} H_2IO_6^{3-} + H^+ , \; K_3 = 2.5 \times 10^{-12} \; ...(4)$$

In fact, periodic acid exists as H_5IO_6 in acid medium and $H_4IO_6^-$ at pH 7. Thus under the alkaline medium employed in this study the main species are expected to be $H_3IO_6^{2-}$ or $H_2IO_6^{3-}$. At higher concentrations periodate also tends to dimerize but formation of these species is negligible under conditions studied. Hence at the pH employed in this study the soluble Cu(III) complex $[Cu(H_3IO_6)_2]^-$ as reported in earlier literatures.

Mechanism of reaction: The reaction between DPC and ampicillin exhibits 1:4 stoichiometry and confirms pseudo-first order reaction with respect to DPC ,fractional order with respect to ampicillin but alkali showed inverse or retarding effect and negligible effect of periodate. Based on these experimental evidences, a suitable mechanism is proposed along with proper involvement of all species. Fractional order with respect to ampicillin presumably results due to formation of complex between ampicillin and Co(III) catalyst. The complex interacts with DPC to release Co (III) along with active intermediates (I and II). One active intermediate reacts with fresh protonated DPC to yield oxo-phenyl acetic acid in a fast way after amide hydrolysis. In this case, presence of excess of alkali enhances nucleophilic attack at beta-lactam carbonyl carbon atom and helps to open the unstable four member beta lactam ring. The oxidative deamination of amino acid is described in earlier literatures. Another free radical of ampicillin reacts with fresh protonated DPC in a fast way through decarboxylation and deamination to produce carboxylic aldehyde along with formation of Cu^{++} and periodate species. The probable mechanism is given in scheme 1.

Spectroscopic evidence for the complex formation between catalyst and substrate was obtained from UV-Visible spectra by maintaining (5.0 x 10^{-7} M) Co(III), (5.0 x 10^{-4}M) AMP, (0.12M) KOH and a mixture of all. A bathochromic shift was obtained. The Michaelis–Menten plot is in great support for complex formation (Figure 7).
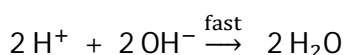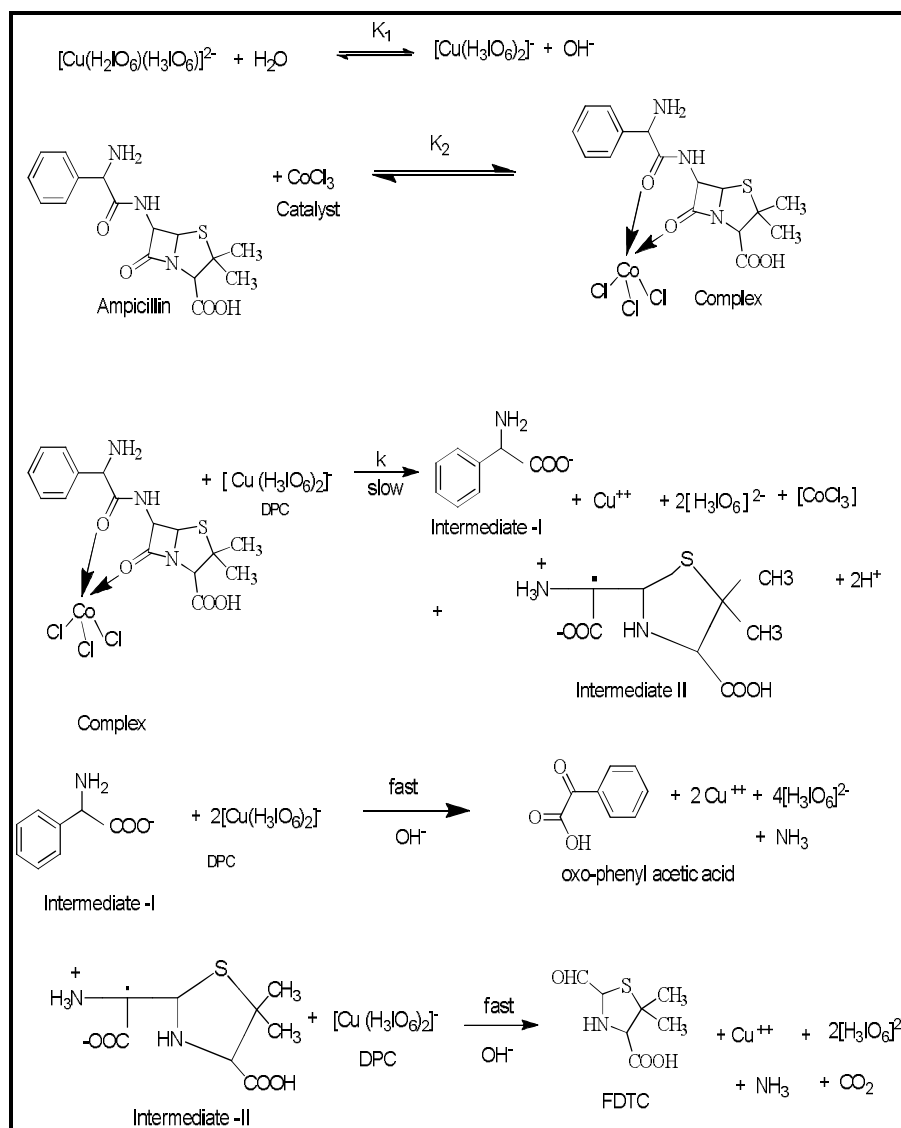
Scheme 1 leads to the rate law equation (6) as -

$$\text{rate} = -\frac{[DPC]}{dt} = k(\text{Complex})[Cu(H_3IO_6)_2]^- \quad ...(5)$$

$$\text{Rate} = kK_1K_2 \frac{[AMP][Co(III)][DPC]}{[OH^-]}$$

$$\frac{\text{rate}}{[DPC]} = k_c = \frac{kK_1K_2[AMP][Co(III)]}{[OH^-]+K_1+K_2[OH^-][AMP]+K_1K_2[AMP]} \quad ...(6)$$

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.

Scheme 1. Detailed scheme for Co(III)catalyzed oxidation of AMP by DPC

$$\frac{[\text{Co(III)}]}{k_c} = \frac{[\text{OH}^-]}{kK_1K_2[\text{AMP}]} + \frac{1}{kK_2[\text{AMP}]} + \frac{[\text{OH}^-]}{kK_2} + \frac{1}{k} \quad ...(7)$$

According to eq^n (7), other conditions being constant, the plots of [Co (III)]/k_c vs. [OH]$^-$ (r ≥ 0.998, ≤ s 0.0025) and [Co (III)]/k_c vs. 1/[AMP] (r ≥ 0.9991, ≤ s 0.0025) should be linear and are found to be so as in figure 7 and 8. From the slopes and intercepts of such plots (Figure 7 and 8), the constants K_1, K_2 and k were calculated as 3.29x10^{-2} mol dm^{-3}, 2.03 x10^3 dm^3 mol^{-1} and 2.63 x 10^4 dm^3 mol^{-1} sec^{-1}, respectively. The value of K_1 is in good agreement with previous literatures.

The effect of ionic strength and dielectric constant of the medium indicates the possibility of involvement of neutral species in the reaction as seen in scheme 1. The modest values of both enthalpy and entropy of activation are favorable for electron transfer reaction. Entropy of activation value is within the range of electron pairing and unpairing process for the loss of degree of freedom and rigid transition state. The higher negative value of $\Delta S^{\neq}$ suggests that intermediate complex is more ordered

than the reactants. Observed modest enthalpy of activation and higher rate constant for slow step indicates that the oxidation presumably occurs via an inner sphere mechanism.
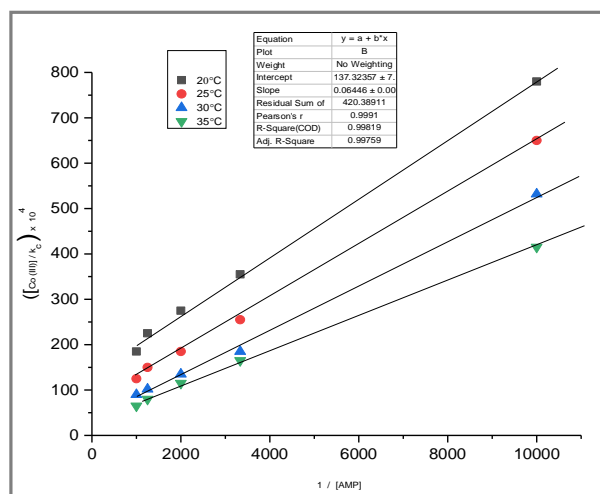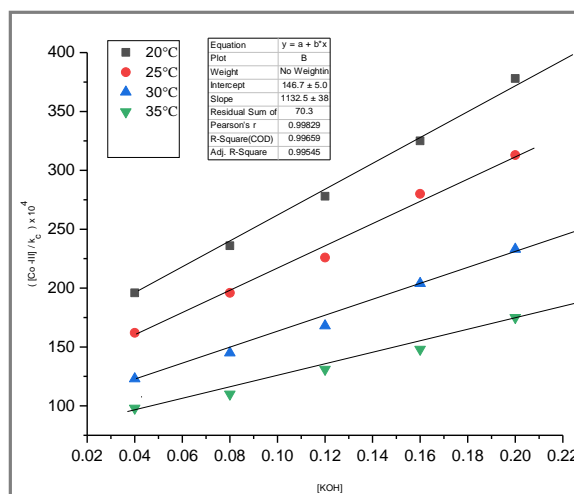

Figure 7. Plot of $[Co(III)]/k_c$ vs.1/[AMP].

Figure 8. Plot of $[Co(III)]/kc$ vs [KOH].

Rate law derivation

From scheme 1,

$$\text{rate} = -\frac{[DPC]}{dt} = k[\text{Complex}][Cu(H_3IO_6)_2]^-$$

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

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

$$\text{rate} = kK_1[C]\frac{[DPC]}{[OH^-]}$$

$$\text{Or,} K_2 = \frac{[C]}{[AMP][Co(III)]}$$

$$\text{Or,} C = K_2[AMP][Co(III)]$$

$$\text{Rate} = kK_1K_2\frac{[AMP]_f[Co(III)]_f[DPC]_f}{[OH^-]} \quad \dots\text{(A-1)}$$

The total concentration of DPC can be given as

$$[DPC]_T = [DPC]_f + +[Cu(H_3IO_6)_2]^{2-} \dots\text{(A-2)}$$

$$= [DPC]_f + K_1\frac{[DPC]}{[OH^-]}$$

$$= [DPC]_f[1 + \frac{K_1}{[OH^-]}]$$

$$= [DPC]_f \left[\frac{[OH^-] + K_1}{[OH^-]} \right]$$

So, $\quad [DPC]_f = \dfrac{[DPC]_T [OH^-]}{[OH^-] + K_1}$ ...(A-3)

Similarly,

$$[AMP]_T = [AMP]_f + C$$
$$[AMP]_T = [AMP]_f + K_2 [AMP]_f [Co(III)]$$

$$[AMP]_T = [AMP]_f [1 + K_2 [Co(III)]$$

So, $\quad [AMP]_f = \dfrac{[AMP]_T}{1 + K_2 [Co(III)]}$

Where, 'T' and 'f' represent total and free concentration respectively.

In view of very low concentration of AMP and Co (III) used,

$$[AMP]_T = [AMP]_f \qquad ...(A-4)$$

Similarly,

$$[Co(III)]_T = [Co(III)]_f + C$$

$$= [Co(III)]_f + K_2 [AMP][Co(III)]_f$$

$$[Co(III)]_f = \frac{[Co(III)]_T}{1 + K_2 [AMP]} \qquad ... (A-5)$$

Substituting values of eq(A-3),(A-4) and (A-5) in (A-1) and omitting T and f subscripts, we get

$$rate = - \frac{d[DPC]}{dt}$$

$$= \frac{kK_1 K_2 [AMP][DPC]_T [Co(III)][OH^-]}{[OH^-]([OH^-] + K_1)(1 + K_2 [AMP]}$$

$$\frac{[Co(III)]}{k_c}$$

$$= \frac{kK_1 K_2 [AMP][DPC][Co(III)]}{[OH^-] + K_1 + K_2 [OH^-][AMP] + K_1 K_2 [AMP]}$$

$$\frac{rate}{[DPC]} = k_c = \frac{kK_1 K_2 [AMP][Co(III)]}{[OH^-] + K_1 + K_2 [OH^-][AMP] + K_1 K_2 [AMP]} \qquad ...(A-6)$$

After rearrangement, we get

$$\frac{[Co(III)]}{k_c} = \frac{[OH^-]}{kK_1 K_2 [AMP]} + \frac{1}{kK_2 [AMP]} + \frac{[OH^-]}{kK_2} + \frac{1}{k} \qquad ...(A-7)$$

APPLICATION

Hundreds of beta lactam antibiotics are common in medical profession in our everyday life. These antibiotics are non-degradable and hence are continuously accumulating in our ecosystem that creates

lots of hazardous effect on our health as well as various type of pollution. Such research work may innovate a new technique for degradation of such chemicals in forthcoming generation. This work helps to analyze relative degradation rate for particular antibiotics. It has wide application in pharmaceuticals industry with a view to modify these chemicals. Hence understanding the mechanism of title work is of great importance to explore new derivatives.

CONCLUSION

The Co(III) catalyzed oxidation of Ampicillin by diperiodatocuprate (III)was studied experimentally. Diperiodatocuprate (DPC-III) $[Cu\ (H_3IO_6)_2]^-$, was considered to be the active species in alkaline medium for the present work. Activation parameters at different temperature were computed. Overall sequences described here are consistent with all experimental evidences including product, spectral analysis, mechanistic and kinetics studies.

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