Role of Ru(III) as an inhibitor in oxidation of lactose by [Cu(bipy)$_2$]$^{2+}$ in alkaline medium: Spectrophotometric and kinetic studies

Ashok Kumar Singh*, Manjula Singh, Jaya Srivastava & Shahla Rahmani
Department of Chemistry, University of Allahabad, Allahabad 211 002, India
Email: ashokeks@rediffmail.com/ ashok.au2@yahoo.co.in

Received 21 September 2012; revised and accepted 17 April 2013

Kinetics of oxidation of lactose by [Cu(bipy)$_2$]$^{2+}$ in alkaline medium using Ru(III) as an inhibitor has been studied spectrophotometrically at 40 °C. The studies show that the rate of the reaction is zero order with respect to [Cu(bipy)$_2$]$^{2+}$ and first order with respect to [lactose]. The order of reaction is found to be two at low concentrations of OH$^-$ (from 1.48×10$^{-5}$ to 3.47×10$^{-5}$ M) and less than two at its high concentrations (from 4.27×10$^{-5}$ to 6.31×10$^{-5}$ M). There is a substantial decrease in the pseudo-zero order rate constant with increase in the concentration of Ru(III) chloride, indicating the role of Ru(III) chloride as an inhibitor. Decrease in the rate with increase in dielectric constant of the medium is observed, while ionic strength of the medium and bipyridyl concentration has no influence on the rate. Based on kinetic data and spectrophotometric evidences, a suitable mechanism is proposed for the studied reaction.

Keywords: Kinetics, Reaction mechanisms, Oxidations, Copper-bipyridyl complex, Ruthenium, Lactose

The use of inorganic oxidants such as Cu(II), ammoniacal Ag(I) and Nessler’s reagents in the uncatalyzed oxidation of reducing sugars in alkaline medium have been reported$^{1-3}$. It is also reported that Cu(II) along with various complexing agents has been used for the oxidation of several reducing sugars in alkaline medium$^{4-7}$. Being a transition metal, copper is involved in various biological processes, viz., embryonic development, mitochondrial respiration, regulation of hemoglobin levels as well as hepatocyte and neuronal functions$^8$. Copper also serves as a cofactor for several enzymes like Cu, Zn-superoxide dismutase, cytochrome oxidase, lysyl oxidase and ceruloplasmin$^9-10$. Copper complexes are reported to have potential use as antimicrobial, antiviral and antitumor agents$^{11}$. In a recent study, it is reported that copper complexes with pyridoxal semicarbazone (PLSC) as ligand showed anticancer activity$^{12}$. Among various transition metals, the use of platinum group metal ions especially Ru(III) Os(VIII), Ir(III), Rh(III) and Pd(II) as catalyst due to their strong catalytic properties is reported$^{13}$. Literature reveals that Ru(III) complexes are frequently used as catalyst for the oxidation of various organic compounds like sugars, alcohol, ketone, etc., in acidic and alkaline medium$^{14-20}$. It is also reported that Ru(III) complex shows antibacterial activity by inhibiting the growth of bacteria$^{21}$. The antimicrobial activity of this complex is presumably affected by the cleavage of the chromosomal DNA of the bacteria. Ru(III) complexes are also reported to have outstanding anticancer properties$^{22,23}$.

In view of the antibacterial, anticancer and antimicrobial activity of Ru(III) complexes and also in view of chemical and biological activity of Cu(II), the present study on the oxidation of lactose in alkaline medium has been undertaken. In this investigation, our main aim was to ascertain whether Cu(II) as an oxidant in alkaline medium behaves in the same way as potassium iodate$^{14}$ and sodium metaperiodate$^{15}$ in the earlier reported Ru(III)-catalyzed oxidation of reducing sugars in alkaline medium. The role of Ru(III) chloride in alkaline medium and reducing sugar in the reaction under investigation is also studied. The possibility of formation of a complex between the reactive species of Ru(III) chloride and the reactive species of Cu(II) in alkaline medium and the formation of an activated complex by the interaction of two oppositely charged species are also investigated.

Materials and Methods

Standard solutions of CuSO$_4$ (Qualigens, AR grade) and bipyridyl (AR grade) were prepared by dissolving their requisite amounts in doubly distilled water separately. In the present kinetics study,
bipyridyl was used as complexing agent so that the reaction mixture remains homogeneous throughout the course of reaction. The standard solution of reducing sugar, i.e., lactose (AR grade, CDH) was prepared daily by weighing the requisite amount and dissolving it in doubly distilled water. Ruthenium(III) chloride (1 g, Unichem) was dissolved in 1000 mL HCl (0.01 M) to get a stock solution of Ru(III) chloride as $7.63 \times 10^{-3}$ M. Throughout the study the medium of the reaction was maintained alkaline with the help of sodium carbonate solution. Standard solution of sodium carbonate (Merck) was prepared by dissolving the appropriate amount in doubly distilled water. Concentration of hydroxyl ions was calculated by measuring the $p$H of the reaction mixture at room temperature. Standard solution of potassium chloride (E. Merck) was prepared by dissolving its weighed amount in doubly distilled water. Potassium chloride was used for maintaining constant ionic strength of the medium. For kinetic measurements, Varian Carry Win UV-vis spectrophotometer connected with Peltier accessory and computer was used. For $p$H measurements, EUTECH Instrument pH 510 was used.

**Kinetic studies**

Copper compounds in both oxidation states, i.e., Cu(II) and Cu(I) absorb in the visible region and hence oxidation of lactose by $[\text{Cu(bipy)}_2]^{2+}$ using Ru(III) as an inhibitor in alkaline medium was studied spectrophotometrically. The absorption spectra of $[\text{Cu(bipy)}_2]^+$ solutions were recorded at 380–800 nm and absorbance maximum was found at 425 nm with molar absorptivity $5.11 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. At 425 nm, the product, $[\text{Cu(bipy)}_2]^+$, absorbs maximum while oxidant $[\text{Cu(bipy)}_2]^{2+}$ absorbs minimum.

One set of reaction mixture containing the reactants, i.e., copper sulphate, sodium carbonate, ruthenium(III) chloride, potassium chloride and bipyridyl and another set having same reactants except copper sulphate, were taken in two separate conical flasks and placed in a thermostatic water bath at constant temperature of 40 °C with an accuracy of ±0.1 °C. When the reaction mixture had attained the required temperature, the calculated amount of sugar solution was added in both sets of the reaction mixture and immediately transferred to quartz cell of 1 cm width and placed in the sample compartment of the spectrophotometer. The mixture containing the solution of CuSO$_4$ was the absorbing sample while the mixture without CuSO$_4$ solution was the reference sample. The progress of the reaction was monitored spectrophotometrically by measuring the increase in absorbance at 425 nm. The absorbance versus time plot over a defined time range was used to calculate the value of rate constants.

The zero order rate constants for various kinetic runs were calculated based on least squares method by $A = kt + A_0$, where $A$ is the absorbance at time $t$ in min, $A_0$ is the absorbance at zero time and $k$ is pseudo-zero order rate constant and $t$ is the time in minute.

The order for various species was determined from the slope of the plot of log $k$ versus log(conc.) of the respective species.

**Stoichiometry and products analysis**

Different sets of experiments were performed with different $[\text{Cu(II)}]$:[$\text{lactose}$] ratios under the condition $[\text{Cu(II)}] >> [\text{lactose}]$. The estimation of un Consumed Cu(II) showed that one mole of lactose was oxidized by four moles of $[\text{Cu(bipy)}_2]^{2+}$. On the basis of equivalence and kinetic studies and also spot test$^{24}$ and thin layer chromatography$^{25}$ for the confirmation of sodium salt of formic acid, it was inferred that sodium salts of arabinonic acid and lyxonic acid are the other oxidation products of the reaction under investigation.

**Results and Discussion**

**Kinetic studies**

Kinetics of oxidation of lactose by $[\text{Cu(bipy)}_2]^{2+}$ in alkaline medium using Ru(III) as an inhibitor has been investigated at 40 °C. Throughout the study, the concentration of Cu(II) was kept very low as compared to that of lactose. Progress of the reaction was followed by monitoring the absorbance of $[\text{Cu(bipy)}_2]^+$ at different intervals of time. For the determination of order of reaction with respect to Cu(II), the concentration of Cu(II) was varied from $1.00 \times 10^{-4}$ to $10.00 \times 10^{-4}$ M (Fig. 1). The concentration of all other reactants was kept constant throughout at constant temperature of 40 °C. The observed values of pseudo-zero order rate constant, $k$, for various kinetic runs did not show much change with the change in the concentration of Cu(II) (Table 1), indicating the order of reaction with respect to [Cu(II)] to be zero. To study the effect of lactose concentration on the rate of oxidation, its concentration was varied from $1.00 \times 10^{-2}$ M to $10.00 \times 10^{-2}$ M at constant concentrations of all other reactants and at constant
Fig. 1 – Zero order plots between absorbance of [Cu(bipy)]$_2^{2+}$ and time at 40 °C. [bipy] = 40.00×10$^{-4}$ M; [Lactose] = 1.00×10$^{-2}$ M; [Cu(II)] = 7.60×10$^{-3}$ M; [Na$_2$CO$_3$] = 1.00×10$^{-2}$ M; $\mu$ = 30.0×10$^{-2}$ M [Cu(II)] = 1.00×10$^{-2}$ M (curve 1); 2.00×10$^{-2}$ M (curve 2); 4.00×10$^{-2}$ M (curve 3); 6.00×10$^{-2}$ M (curve 4); 8.00×10$^{-2}$ M (curve 5); 10.00×10$^{-2}$ M (curve 6)).
In order to study the effect of [OH\(^-\)] on the rate of oxidation, the concentration of OH\(^-\) was varied from 1.48×10\(^{-5}\) – 6.31×10\(^{-5}\) M at constant concentrations of all other reactants and at constant temperature 40 °C. From the values of pseudo-zero order rate constants, it may be concluded that order of reaction with respect to OH\(^-\) at its low concentration (from 1.48×10\(^{-5}\) – 3.47×10\(^{-5}\) M) is two and becomes less than two at its higher concentration (from 4.27×10\(^{-5}\) – 6.31×10\(^{-5}\) M). A plot of log \(k\) versus log [OH\(^-\)] was linear with a positive intercept on y-axis (Supplementary Data, Fig. S2). From the slope of linear plot, the order of reaction with respect to OH\(^-\) was found to be 1.72. The change in order with respect to [OH\(^-\)] from two to one \((n = 1.72)\) was further verified by the plot of \(k/[OH^-]\) versus [OH\(^-\)] (Fig. 2), where the first order kinetics observed at low concentration of OH\(^-\) (from 1.48×10\(^{-5}\) – 3.47×10\(^{-5}\) M) tends towards zero order at its higher concentration (from 4.27×10\(^{-5}\) – 6.31×10\(^{-5}\) M). This result is also supported by simple as well as multiple regression analysis. The correlation coefficients in the oxidation of lactose for the effects of [sugar], [Ru(III)] and [OH\(^-\)] the pseudo-zero order-constant \((k_1)\) were calculated by the help of STATGRAPHICS CENTURIAN and found to be 0.99 in each case. There is no change in pseudo-zero order rate constant values with the change in [bipyridyl]. The reaction has also been studied at four different temperatures, i.e., 35 °C, 40 °C, 45 °C and 50 °C and the observed values of pseudo-zero order rate constant were utilized to calculate various activation parameters (Table 1).

Cu(II) has been used as an oxidant in various oxidation processes either in acidic or in alkaline medium\(^{26,27}\). In the present study, Cu(II) has been used with bipyridyl as complexing agent in alkaline medium. On the basis of kinetic results obtained and spectrophotometric evidence collected, it is believed that the reactive species of Cu(II) is [Cu(bipy)]\(^{2+}\) instead of free Cu(II). Formation of the complex, [Cu(bipy)]\(^{2+}\), is confirmed by its absorption at \(\lambda_{max}\) 670 nm with molar extinction coefficient 0.59×10\(^{-2}\) dm\(^3\) mol\(^{-1}\) cm\(^{1}\), which is also supported by literature\(^{28}\).

Using electrochemical and spectrophotometric techniques Taqui Khan and co-workers\(^{29}\) have investigated the chloro-complexes of ruthenium(III) in 0.1 M KCl at pH 0.4, 1.0 and 2.0 and 25 °C. They observed that the \([\text{RuCl}_3(\text{H}_2\text{O})_2]^{+}\) species is the most stable species present in solution under experimental conditions at pH 2 and is stabilized in its hydrolyzed form according to the equilibrium shown below:

\[
\text{[RuCl}_3(\text{H}_2\text{O})_2]^{+} + \text{H}_2\text{O} \leftrightarrow \text{[RuCl}_2(\text{H}_2\text{O})_2(\text{OH})_2]^{-} + \text{H}_3\text{O}^{+}
\]

In the present study, Ru(II) in each kinetic run was from a solution of Ru(III) chloride prepared in 0.01 M HCl (pH = 2.0). In view of the above, the starting species may be assumed to be \([\text{RuCl}_3(\text{H}_2\text{O})_2]^{-}\) in the present investigation. Further, since the medium of the reaction was maintained alkaline throughout the study, it is necessary to take into account the effect of [OH\(^-\)] on the rate of oxidation. Considering the positive effect of OH\(^-\) concentration on pseudo-zero order rate constant, the existence of the following equilibrium in the reaction may be assumed:

\[
\text{[RuCl}_3(\text{H}_2\text{O})_2]^{-} + \text{OH}^{-} \leftrightarrow \text{[RuCl}_2(\text{H}_2\text{O})_2(\text{OH})_2]^{-} + \text{H}_2\text{O}
\]

Of the two species, i.e., \([\text{RuCl}_3(\text{H}_2\text{O})_2]^{-}\) and \([\text{RuCl}_2(\text{H}_2\text{O})_2(\text{OH})_2]^{-}\), \([\text{RuCl}_3(\text{H}_2\text{O})_2]^{+}\) can be considered as the reactive species of Ru(III) chloride in alkaline medium because only with this species as the reactive species the positive effect of [OH\(^-\)] on the rate of oxidation can be explained. This also finds support from the spectra observed for Ru(III) chloride alone and Ru(III) chloride with varying concentrations of OH\(^-\), where increase in absorbance with the increasing [OH\(^-\)] is observed (Fig. 3).
In the presence of alkali, the reducing sugars undergo a tautomeric change resulting in the formation of enediol anion and enediol. The formation of enediol anion and enediol in presence of alkali is also supported by Isbell and co-workers. In the present investigation, on the basis of the observed kinetic data, it may be concluded that it is the enediol anion which is the reactive species of the reducing sugar.

**Complex formation**

On the basis of the observed kinetic data, an effort was made to find out the possibility of formation of a complex between the reactive form of Cu(II) and also the reactive form of the reducing sugar molecule. Spectra of solutions containing \([\text{Cu(bipy)}]^{2+}\) and \(\text{Na}_2\text{CO}_3\) and also \([\text{Cu(bipy)}]^{2+}\), \(\text{Na}_2\text{CO}_3\) and three different concentrations of reducing sugar were recorded (Supplementary Data, Fig. S3). It is apparent that with the increase in sugar concentration from \(1 \times 10^{-2} - 2.0 \times 10^{-2}\) M and \(4 \times 10^{-2}\) M, there is an increase in absorbance from 0.31 to 0.34 and 0.43, indicating formation of a complex between \([\text{Cu(bipy)}]^{2+}\) and reducing sugar molecule (Scheme 1).

Attempt was also made to ascertain the possibility of formation of a complex between reactive species of Ru(III) chloride in alkaline medium, i.e., \([\text{RuCl}_2(\text{H}_2\text{O})_2(\text{OH})_2]^-\) and the complex \((C_1)\). UV spectra of the solution of \([\text{Cu(bipy)}]^{2+}\), \(\text{Na}_2\text{CO}_3\) and reactive form of reducing sugar as well as for solutions containing \([\text{Cu(bipy)}]^{2+}\), \(\text{Na}_2\text{CO}_3\), reactive form of reducing sugar and two different concentrations of Ru(III) chloride were recorded. The increase in absorbance from 0.18 to 0.46 and 0.60 indicates the formation of a complex between the species \([\text{RuCl}_2(\text{H}_2\text{O})_2(\text{OH})_2]^-\) and the reactive complex \((C_1)\) as shown in Scheme 2.

**Mechanism and derivation of rate law**

On the basis of the observed kinetic orders with respect to the reactants, the spectrophotometric evidence collected for the formation of complexes, the effect of dielectric constant of the medium on the rate of oxidation (discussed later) and the positive entropy of activation (discussed later), the most probable reaction path way is proposed for the oxidation of lactose by \([\text{Cu(bipy)}]^{2+}\) in alkaline medium using Ru(III) as an inhibitor (Scheme 3).
\[
\text{(I)}
\]

\[
\begin{align*}
\text{(II)} & \quad \text{(Cu(II))} \\
\end{align*}
\]

\[
\text{(III)} \quad \text{inactive complex (C}_2^*\text{)}
\]

\[
\text{(IV)} \\
\text{(V)} \\
\text{(VI)}
\]

where \( R \) stands for \( \text{C}_4\text{H}_6\text{O}_4 \)

\[
\text{Scheme 3}
\]
The same process may be repeated for galactose, the other unit of lactose.

On the basis of Scheme 3, the rate in terms of formation of product i.e., \([\text{Cu(bipy)}_2]^{2+}\) is shown in Eq. (1).

\[
\text{Rate} = \frac{d[\text{Cu(I)}^*]}{dt} = 4k_4 \ [C_1] \ [\text{OH}^-] \quad \ldots(1)
\]

where 4 indicates that one mole of lactose is oxidized by 4 moles of Cu(II).

On applying steady state approximation to the concentrations of \(E^-\) and \(C_1\), we have Eqs (2) and (3), respectively

\[
\frac{d[E^-]}{dt} = 0 = k_1 [S][\text{OH}^-] - k_{-1} \ [E^-] + k_2 \ [E^-][\text{Cu(II)}^*]
\]

or \( [E^-] = \frac{k_1 [S][\text{OH}^-]}{k_{-1} + k_2 [\text{Cu(II)}^*]} \quad \ldots(2) \)

and

\[
\frac{d[C_1]}{dt} = 0 = k_2 \ [\text{Cu(II)}^*][E^-] - k_{3} \ [\text{Ru(III)}^*] \ [C_1] - k_{4} [C_1][\text{OH}^-]
\]

or \( [C_1] = \frac{k_2 [\text{Cu(II)}^*][E^-]}{k_{3} [\text{Ru(III)}^*] + k_{4} [\text{OH}^-]} \quad \ldots(3) \)

On substituting the value of \(E^-\) from Eq. (2) in Eq. (3), we have Eq. (4)

\[
[C_1] = \frac{k_1 k_2 [\text{Cu(II)}^*][S][\text{OH}^-]}{k_{-1} + k_2 [\text{Cu(II)}^*] \left\{k_{3} [\text{Ru(III)}^*] + k_{4} [\text{OH}^-]\right\}} \quad \ldots(4)
\]

From Eqs. (1) and (4), we can write Eq. (5).

\[
\text{Rate} = \frac{4k_1 k_2 k_4 [\text{Cu(II)}^*][S][\text{OH}^-]^2}{\left\{k_{-1} + k_2 [\text{Cu(II)}^*] \left\{k_{3} [\text{Ru(III)}^*] + k_{4} [\text{OH}^-]\right\}\right\}} \quad \ldots(5)
\]

Since \(k_2\) is the rate constant for the fast reaction, the inequality \(k_2 [\text{Cu(II)}^*] >> k_{-1}\), can be assumed as valid and under this condition, Eq. (5A) will be reduced to Eq. (6).

\[
\text{Rate} = \frac{4k_1 k_4 [S][\text{OH}^-]^2}{k_{3} [\text{Ru(III)}^*] + k_{4} [\text{OH}^-]} \quad \ldots(6)
\]

Equation (6) is the final rate law, which explains the above experimental findings regarding order in \([\text{Ru(III)}], [\text{sugar}]\) and \([\text{OH}^-]\).

Further, since \(k_2\) is the rate constant for the step (III) and \(k_4\) is the rate constant for the slow and rate determining step (IV), hence at low concentrations of \(\text{OH}^-\) the inequality \(k_2 [\text{OH}^-] << k_{3} [\text{Ru(III)}]_T\) can be assumed as valid and under this condition Eq. (6) will be reduced to Eq. (7).

\[
\text{Rate} = \frac{d[\text{Cu(I)}^*]}{dt} = k' \ [\text{Ru(III)}]_T^{-1} [S][\text{OH}^-]^2 \quad \ldots(7)
\]

where \(k' = \frac{4k_1 k_4}{k_3}\)

Equation (7) is the rate law valid at low concentration of \(\text{OH}^-\), where order with respect to \([\text{OH}^-]\) is two.

In the case of zero order reaction, since the rate is actually equal to zero order rate constant \((k)\), the rate Eq. (6) can also be expressed as

\[
\text{Rate} = \frac{d[\text{Cu(I)}^*]}{dt} = k = \frac{4k_1 k_4 [S][\text{OH}^-]^2}{k_{3} [\text{Ru(III)}]_T + k_{4} [\text{OH}^-]} \quad \ldots(8)
\]

or \( \frac{1}{k} = \frac{k_{3} [\text{Ru(III)}]_T}{4k_1 k_4 [S][\text{OH}^-]^2} + \frac{1}{4k_1 [S][\text{OH}^-]} \quad \ldots(9) \)

It is evident from Eq. (9) that a plot of \(1/k\) versus \([\text{Ru(III)}]_T\), will be linear with an intercept on the y-axis (Fig. 4). This proves the validity of rate law (6) or (8) and hence the proposed reaction mechanism. From the intercept and the slope of the straight line, the values of \(k_1\) and \(k_2/k_4\) were calculated and found to be 1.49 mol\(^{-1}\) dm\(^3\) s\(^{-1}\) and 1.46\times10\(^{2}\) mol\(^{-2}\) dm\(^6\), respectively. Utilizing these values of \(k_1\) and \(k_2/k_4\) and rate Eq. (8), the values of \(k\) for \(\text{Ru(III)}\) and \(\text{OH}^-\) variations were calculated and are presented in Table 2. There is a close similarity between the observed and calculated values of \(k\) obtained at 40 °C for the variation of \([\text{Ru(III)}]\) and \([\text{OH}^-]\) in the oxidation of lactose by \([\text{Cu(bipy)}_2]^{2+}\) in alkaline medium. This further proves the validity of the rate law (6) or (8) and hence the reaction mechanism proposed in Scheme 3.
It is a well known that for reaction between ions of opposite charge there is generally an entropy increase in going from reactants to activated complex and for ions of like charge there is an entropy decrease\(^{31}\). In the present study, the observed positive entropy of activation provides support for the formation of the neutral complex in the rate determining step by the interaction of two oppositely charged species, \((C_1)\), and \(\text{OH}^-\). In this case the activated state or transition state will be less polar than the initial state.

Kinetic runs were conducted at constant temperature 40 °C, with different ionic strength of the medium under uniform reaction conditions. There was almost no change in \(k\) with the change in ionic strength of the medium.

Studies were carried out with varying dielectric constant of the medium under uniform reaction conditions. The temperature was maintained constant at 40 °C. The change in dielectric constant of the medium was ensured by the addition of ethyl alcohol (5 - 20 %) to the reaction mixture. It was observed that with the decrease in the dielectric constant of the medium, there was an increase in pseudo-zero order rate constant, \(k\). This is an additional support for the formation of a neutral complex, \((C_1)\), in the rate determining step (IV) by the interaction of two oppositely charged species.

From the slope of the linear plot of \(\log k\) versus \(1/D\), the value of size of the activated complex \((d_{AB})\) may be calculated as

\[
d_{AB} = \frac{Z_AZ_Be^2N/(2.303\times4\pi\epsilon_0)RT \times \text{slope}}
\]

and \(Z_A\) and \(Z_B\) are the charges of the reacting ions. The value of \(d_{AB}\) was found to be 3.05 Å in the present case.

When the results of the present investigation were compared with the results reported earlier for the oxidation of reducing sugars by alkaline potassium iodate\(^{14}\) and sodium metaperiodate\(^{15}\) using Ru(III) chloroide as homogeneous catalyst, it was found that pseudo-zero order kinetics in Cu(II) concentration is contrary to first to zero order kinetics observed with \([\text{IO}_3^-]\) and \([\text{IO}_4^-]\). In the earlier reported cases \(\text{IO}_3^-\) and \(\text{IO}_4^-\) were taken as the reactive species of potassium iodate\(^{14}\) and sodium metaperiodate\(^{15}\) respectively. However, in the present investigation, the soluble \([\text{Cu(bipy)}_2]^{2+}\) complex was found to be the reactive species of Cu(II) in alkaline medium. In the present case the role of Ru(III) was as an inhibitor in \([\text{Cu(bipy)}_2]^{2+}\) oxidation of lactose, whereas Ru(III) was found to be a positive catalyst in iodate\(^{14}\) and periodate\(^{15}\) oxidation of reducing sugars in alkaline medium. As a positive catalyst, Ru(III) combined with the reactive species of \(\text{KIO}_3\)\(^{14}\) and \(\text{NaIO}_4\)\(^{15}\) to form complex species of the type \([\text{RuCl}_3(\text{H}_2\text{O})(\text{OH})_2(\text{IO}_3)^-]\) and \([\text{RuCl}_3(\text{H}_2\text{O})(\text{OH})_2(\text{IO}_4)^-]\), respectively and enhanced the oxidative capacity of oxidants. However, in the present investigation, Ru(III) in the form of \([\text{RuCl}_3(\text{H}_2\text{O})_2(\text{OH})_2]^+\) combined with \((C_1)\) to form the inactive complex, \((C_2)\), and played the role of an inhibitor. The observed order of reaction with respect to \(\text{OH}^-\) at its low (from \(1.48\times10^2\) \(M\) - \(3.47\times10^2\) \(M\)) concentration being two, which becomes less than two at its high concentration (\(4.27\times10^2\) - \(6.31\times10^5\) \(M\)) distinguishes the present

<table>
<thead>
<tr>
<th>[Ru(III)](\times10^7) (M)</th>
<th>[OH(^-)](\times10^3) (M)</th>
<th>(k\times10^7) (mol dm(^{-3}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calc.</td>
<td>Expt.</td>
<td></td>
</tr>
<tr>
<td>3.80</td>
<td>2.57</td>
<td>4.87</td>
</tr>
<tr>
<td>7.60</td>
<td>2.57</td>
<td>2.89</td>
</tr>
<tr>
<td>15.20</td>
<td>2.57</td>
<td>1.60</td>
</tr>
<tr>
<td>22.80</td>
<td>2.57</td>
<td>1.09</td>
</tr>
<tr>
<td>30.40</td>
<td>2.57</td>
<td>0.84</td>
</tr>
<tr>
<td>38.00</td>
<td>2.57</td>
<td>0.68</td>
</tr>
<tr>
<td>7.60</td>
<td>1.48</td>
<td>1.90</td>
</tr>
<tr>
<td>7.60</td>
<td>2.04</td>
<td>1.10</td>
</tr>
<tr>
<td>7.60</td>
<td>2.57</td>
<td>2.89</td>
</tr>
<tr>
<td>7.60</td>
<td>3.47</td>
<td>4.95</td>
</tr>
<tr>
<td>7.60</td>
<td>4.27</td>
<td>7.09</td>
</tr>
<tr>
<td>7.60</td>
<td>5.62</td>
<td>11.32</td>
</tr>
<tr>
<td>7.60</td>
<td>6.31</td>
<td>13.53</td>
</tr>
</tbody>
</table>

\([\text{Cu(bipy)}_2]^{2+} = 6.00\times10^4\ M; [\text{bipy}] = 4.00\times10^4\ M; [\text{Lactose}] = 1.00\times10^{-2}\ M; [\text{Na}_2\text{CO}_3] = 1.00\times10^{-2}\ M; \mu = 30.00\times10^{-2}\ M\).
study from the earlier reported\textsuperscript{14,15} studies, where first order kinetics observed at low concentrations of hydroxyl ions tends to zero order at its higher concentrations. The observed positive entropy of activation in the present study provides support to the rate determining step where the interaction takes place between two oppositely charged species whereas in earlier reported studies\textsuperscript{14,15} the interaction between two negatively charged species in the rate determining step is supported by the observed negative entropy of activation.

Conclusions
The present studies shows that in the oxidation of lactose by $[\text{Cu(bipy)}_2]^{2+}$ using Ru(III) chloride as an inhibitor in alkaline medium, Cu(II) in the form of $[\text{Cu(bipy)}_2]^{2+}$ participates in the reaction and forms a reactive complex with enediol anion (E\textsuperscript{-}) in the fast step. The species, $[\text{RuCl}_3(\text{H}_2\text{O})_2(\text{OH})_2]^{+}$, proposed as the reactive species of Ru(III) chloride in alkaline medium plays the role of an inhibitor in the oxidation of lactose. An inactive complex, (C\textsubscript{1}), is formed between the species $[\text{RuCl}_3(\text{H}_2\text{O})_2(\text{OH})_2]^{+}$ and the reactive complex, (C\textsubscript{2}), in a step prior to the rate determining step. This unreactive complex is responsible for the role of Ru(III) chloride as an inhibitor in the $[\text{Cu(bipy)}_2]^{2+}$ oxidation of lactose in alkaline medium. The interaction between a charged species, (C\textsubscript{1}), and a negatively charged OH\textsuperscript{-} ion is well supported by the observed positive entropy of activation and also by the effect of dielectric constant of the medium on the rate of oxidation.

Supplementary Data
Supplementary data associated with this article, i.e., Figs S1-S3, are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_52A(05)599-607_SupplData.pdf.

References