"The way to succeed is to double your error rate.”

~Thomas Watson

List of Publications, Conferences attended &

Papers presented
List of Research Publications

1. Mechanistic investigations on the oxidation of L-valine by Ag(III) periodate complex in alkali media: a kinetic approach

2. Thermodynamic quantities for the different steps involved in the oxidation of ketorolac drug by copper(III) periodate complex in aqueous alkaline medium: A mechanistic approach

3. Osmium(VIII) catalyzed oxidation of DL-ornithine monohydrochloride by a new oxidant, diperiodatoargentate(III) in aqueous alkaline medium

4. Mechanistic aspects of uncatalyzed and ruthenium(III) catalyzed oxidation of DL-ornithine monohydrochloride by silver(III) periodate complex in aqueous alkaline medium

5. Mechanistic investigations of ruthenium(III) catalyzed oxidation of pentoxifylline by copper(III) periodate complex in aqueous alkaline medium
6. Os(VIII)/Ru(III) catalysed oxidation of L-valine by Ag(III) periodate complex in aqueous alkaline medium: A comparative kinetic study


7. Mechanistic aspects of oxidation of loop diuretic drug furosemide by Ag(III) periodate complex in alkali media: a kinetic approach


8. Mechanistic aspects of Os(VIII) catalyzed oxidation of loop diuretic drug furosemide by Ag(III) periodate complex in aqueous alkaline medium


9. Voltammetric oxidation and determination of loop diuretic furosemide at a multi-walled carbon nanotubes paste electrode


10. Voltammetric behavior of theophylline and its determination at multi-wall carbon nanotube paste electrode

11. Electro-oxidation of nimesulide at gold electrode and its determination in pharmaceutical dosage form and human biological fluid

**Shweta J. Malode** and Sharanappa T. Nandibewoor*,


12. Electrochemical oxidation and determination of nimesulide using a carbon paste electrode

**Shweta J. Malode** and Sharanappa T. Nandibewoor*,

_Z. Phys. Chem.,_ (2012) (Communicated) **IF: 1.361** (Oldenbourg)

13. Spectral and mechanistic investigation of osmium(VIII) catalyzed oxidation of 6-aminopenicillanic acid by alkaline copper(III) periodate complex

**Shweta J. Malode** and Sharanappa T. Nandibewoor*,

_Acta Chim. Slov.,_ (2012) (Communicated) **IF: 1.011** (Slovenian Chemical Society)

14. Mechanistic aspects of uncatalyzed and ruthenium(III) catalyzed oxidation of DL-ornithine by copper(III) periodate complex in aqueous alkaline medium: A comparative kinetic study

Jyothi C. Abbar, **Shweta J. Malode** and Sharanappa T. Nandibewoor*,


15. Osmium(VIII) catalyzed and uncatalyzed oxidation of a hemorheologic drug pentoxifylline by alkaline copper(III) periodate complex: A comparative kinetic and mechanistic approach

Jyothi C. Abbar, **Shweta J. Malode** and Sharanappa T. Nandibewoor*,

_Polyhedron, _**29**, 2875-2883 (2010) **IF: 2.033** (Elsevier)
16. Kinetic and mechanistic aspects of osmium(VIII) catalyzed oxidation of DL-ornithine by copper(III) periodate complex in aqueous alkaline medium  

17. Electrochemical determination of a hemorheologic drug, pentoxifylline at a multi-walled carbon nanotube paste electrode  
Jyothi C. Abbar, **Shweta J. Malode** and Sharanappa T. Nandibewoor*, *Bioelectrochemistry,* **83**, 1-7 (2012) **IF: 3.520** (Elsevier)

18. Oxidation of 6-aminopenicillanic acid by an alkaline copper(III) periodate complex in the absence and presence of ruthenium(III) as a homogeneous catalyst  
Nagaraj P. Shetti, **Shweta J. Malode** and Sharanappa T. Nandibewoor*, *Polyhedron,* **30**, 1785-1798 (2011) **IF: 2.033** (Elsevier)

19. Electrochemical behavior of an antiviral drug acyclovir at fullerene-C$_{60}$-modified glassy carbon electrode  
Nagaraj P. Shetti, **Shweta J. Malode** and Sharanappa T. Nandibewoor*, *Bioelectrochemistry,* (2012) (Revised) **IF: 3.520** (Elsevier)

20. Electro-oxidation of captopril at gold electrode and its determination in pharmaceuticals and human fluids  
Conferences Attended and Papers Presented


2. Presented the paper at 29th Annual Conference of Indian Council of Chemists held at Department of Chemistry, Punjab University, Chandigarh on 19th - 21st December 2010 and awarded “Prof. G. Gopala Rao Centenary Commemorative Award” for the best oral presentation

National Seminar Attended

1. Attended UGC sponsored national seminar on “Recent Advances in Chemistry-2010” organized by P. G. Department of Studies in Chemistry, Karnatak University, Dharwad, on 18th March 2010

Young Scientist Award

Received “Prof. G. Gopala Rao Centenary Commemorative Award” for the best oral presentation of paper entitled, “Thermodynamic quantities for the different steps involved in the oxidation of furosemide drug by alkaline Ag(III) periodate complex in absence and presence of osmium(VIII) as a homogeneous catalyst” at 29th Annual Conference of Indian Council of Chemists held at Department of Chemistry, Punjab University, Chandigarh on 19th – 21st December 2010
"Success is not the result of spontaneous combustion. You must first set yourself on fire."

~Fred Shero

Reprints of Published Research Articles
Scientific paper

Mechanistic Investigations on the Oxidation of L-valine by Ag(III) Periodate Complex in Alkali Media: a Kinetic Approach

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Abstract

The oxidation of an amino acid, L-valine (L-val) by diperiodatoargentate(III) (DPA) in alkaline medium at a constant ionic strength of 0.006 mol dm$^{-3}$ was studied spectrophotometrically. The reaction between DPA and L-val in alkaline medium exhibits 1:1 stoichiometry (L-val:DPA). Intervention of free radicals was observed in the reaction. Based on the observed orders and experimental evidences, a mechanism involving the protonated diperiodatoargentate(III) (DPA) as the reactive species of oxidant has been proposed. The products were identified by spot test and characterized by spectral studies. The reaction constants involved in the different steps of the mechanism were calculated. The activation parameters with respect to slow step of the mechanism were computed and discussed. The thermodynamic quantities were determined for different equilibrium steps. Isokinetic temperature was also calculated and found to be 188.9 K.

Keywords: Kinetics, mechanism, oxidation, l-valine, diperiodatoargentate(III).

1. Introduction

Amino acids act not only as the building blocks in protein syntheses but they also play a significant role in metabolism and have been oxidized by a variety of oxidizing agents.\textsuperscript{1} The study of the oxidation of amino acids is of interest because of their biological significance and selectivity towards the oxidant to yield the different products.\textsuperscript{2-4} L-valine is an essential amino acid with hydrocarbon side chains amino acid. It is usually found in the interior of proteins. It has an antagonistic property with structurally similar leucine and isoleucine, and imbalance among these three items results in suspension of growth. Particular symptoms of valine deficiency include loss of balance during locomotion, changes in the ventral horn and susceptibility to irritation allergens. Some of valine derivatives have antibiotic action.

Diperiodatoargentate(III) (DPA) is a powerful oxidizing agent in alkaline medium with the reduction potential,\textsuperscript{5} 1.74 V. It is widely used as a volumetric reagent for the determination of various organic and inorganic species.\textsuperscript{6,7} Jaya Prakash Rao \textit{et al}.\textsuperscript{8} have used DPA as an oxidizing agent for the kinetics of oxidation of various substrates. They normally found that order with respect to both oxidant and substrate was unity and $[\text{OH}^-]$ was found to enhance the rate of reaction. It was also observed that they did not arrive the possible active species of DPA in alkali and on the other hand they proposed mechanisms by generalizing the DPA as $[\text{Ag(HL)L}]^{(x+1)-}$. However, Anil Kumar \textit{et al}.\textsuperscript{9,10} put an effort to give an evidence for the reactive form of DPA in the large scale of alkaline pH.

In the present investigation, we have obtained the evidence for the reactive species for the DPA in alkaline medium. The DPA is a metal complex with Ag in 3+ oxidation state like Cu\textsuperscript{3+} in DPC and Fe\textsuperscript{3+} in hemoglobin. However, former is a single equivalent oxidant, having a structural similarity with DPA and DPC; and latter has structural dissimilarity with DPN.\textsuperscript{11} Since multiple equilibria between different Ag(III) species are involved, it would be interesting to know which of the species is the active oxidant.

Literature survey reveals that there is no report on the oxidative mechanism of L-val by diperiodatoargentate(III). Hence, it was important and interesting for the detailed investigation of oxidation of L-valine by DPA in aqueous alkaline medium. The present study deals with...
the title reaction to investigate the redox chemistry of DPA in alkaline media, to compute the thermodynamic quantities of various steps of Scheme 1 and to arrive at a suitable mechanism.

2. Experimental

2.1. Materials and Reagents

All chemicals used were of reagent grade and millipore water was used throughout the work. A solution of L-valine (S. D. Fine Chem.) was prepared by dissolving an appropriate amount of recrystallised sample in millipore water. The purity of L-valine sample was checked by comparing its melting point 294 °C with literature data [Lit. mp. 296 °C]. The required concentration of L-valine was obtained from its stock solution. A stock solution of the medium in the solution was measured by ELICO (LI 56, Lit. mp. 296 °C) spectrophotometer. The pH of the medium in the solution was maintained by adding appropriate amounts of recrystallised sample in millipore water. The purity of L-valine sample was checked by comparing its melting point 294 °C with literature data [Lit. mp. 296 °C].

2.2. Preparation of DPA

DPA was prepared by oxidizing Ag(I) in presence of KIO₄ as described elsewhere:¹³ the mixture of 28 g of KOH and 23 g of KIO₄ in 100 cm³ of water along with 8.5 g AgNO₃ was heated just to boiling and 20 g of K₂S₂O₈ was added in several lots with stirring and then allowed to cool. It was filtered through a medium porosity fritted glass filter and 40 g of NaOH was added slowly to the filtrate, whereupon a voluminous orange precipitate aggregated. The precipitate is filtered as above and washed three to four times with cold water. The pure crystals were dissolved in 50 cm³ water and warmed to 80 °C with constant stirring thereby some solid was dissolved to give a red solution. The resulting solution was filtered when it was hot and on cooling at room temperature, the orange crystals separated out and were recrystallised from water.

The complex was characterized from its U.V. spectrum, which exhibited three peaks at 216, 255 and 362 nm. These spectral features were identical to those reported earlier for DPA.¹³ The magnetic moment study revealed that the complex is diamagnetic. The compound prepared was analysed for silver and periodate by acidifying a solution of the material with HCl,¹⁴ recovering and weighing the AgCl for Ag and titrating the iodine liberated when excess of KI was added to the filtrate for IO₄⁻.

2.3. Kinetic Measurements

Kinetic measurements were performed on a Varian Cary Bio-50 UV-visible spectrophotometer. The kinetics was followed under pseudo-first order condition where [L-val] > [DPA] at 25 ± 0.1 °C, unless specified. The reaction was initiated by mixing the DPA to L-val solution, which also contained required concentrations of KNO₃, KOH and KIO₄. The progress of reaction was followed spectrophotometrically at 360 nm by monitoring the decrease in absorbance due to DPA with the molar absorptivity index, ‘ε’ to be 13900 ± 10 dm³ mol⁻¹ cm⁻¹. It was verified that there was a negligible interference from other species present in the reaction mixture at this wavelength.

The reaction was followed to more than 90% completion of the reaction. Plots of log (absorbance) versus time lead to the first order rate constants (kₗ). The plots were linear up to 80% completion of reaction and rate constants were reproducible within ±5%. During the kinetics a constant concentration 5.0 × 10⁻⁵ mol dm⁻³ of KIO₄ was used throughout the study unless otherwise stated. Thus, the possibility of oxidation of L-val by periodate was tested and found that there was no significant interference due to KIO₄ under experimental condition. The total concentration of OH⁻ was calculated by considering the amount present in the DPA solution and that additionally added. Kinetics runs were also carried out in N₂ atmosphere in order to understand the effect of dissolved oxygen on the rate of reaction. No significant difference in the results was obtained under a N₂ atmosphere and in the presence of air. In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates.

In view of the modest concentration of alkali used in the reaction medium, attention was also directed to the effect of the reaction vessel surface on the kinetics. Use of polythene/acrylic wares and quartz or polyacrylate cells gave the same results, indicating that the surface did not have any significant effect on the reaction rates.

Regression analysis of experimental data to obtain regression coefficient ‘r’ and the standard deviation ‘S’, of points from the regression line, was performed with the Microsoft office Excel – 2003 programme.

3. Results and Discussion

3.1. Stoichiometry and Product Analysis

Different sets of reaction mixtures containing varying ratios of DPA to L-val in presence of constant amount of OH⁻, KNO₃ were kept for 4 hours in a closed vessel under nitrogen atmosphere. The remaining concentration of DPA was estimated spectrophotometrically at 360 nm. The results indicated a 1:1 stoichiometry as given in Scheme 1.
The main reaction product was identified as isobutyraldehyde by spot test. The nature of aldehyde was confirmed by its IR spectrum, which showed a carbonyl stretch at 1,719 cm\(^{-1}\) and a band at 2,938 cm\(^{-1}\) due to aldehydic C–H stretch and characterized by its \(^1\)H NMR spectrum (singlet at \(\delta\) 9.2 ppm due to –CHO group, multiplet at \(\delta\) 1.8 ppm due to 1H of –CH, a doublet at \(\delta\) 1.0 ppm due to 6H of two equivalent –CH\(_3\) group), thus confirming the presence of isobutyraldehyde. It was further observed that the aldehyde does not undergo further oxidation under the present kinetic conditions. A test for corresponding acid proved negative.

The by-products were identified as ammonia by Nessler’s reagent, the CO\(_2\) was qualitatively detected by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through tube containing lime water. The formation of free Ag\(^+\) in solution was detected by adding KCl solution to the reaction mixture, which produced white turbidity due to the formation of AgCl.

3.2 Reaction Orders

The reaction orders were determined from the slope of log \(k_{\text{obs}}\) versus log (concentration) plots by varying the concentrations of L-val, alkali in turn while keeping all other concentrations and conditions constant.

3.3 Effect of [Diperiodatoargentate(III)]

The oxidant DPA concentration was varied in the range of 1.0 \(\times\) 10\(^{-5}\) to 1.0 \(\times\) 10\(^{-4}\) mol dm\(^{-3}\) and the fairly constant \(k_{\text{obs}}\) values indicate that order with respect to [DPA] was unity (Table 1). This was also confirmed by linearity of the plots of log (Absorbance) versus time (r \(\geq\) 0.998, S \(\leq\) 0.02) up to 80% completion of the reaction (Fig. 1).

3.4 Effect of [L-valine]

The effect of L-valine on the rate of reaction was studied at constant concentrations of alkali, DPA and periodate at a constant ionic strength of 0.006 mol dm\(^{-3}\). The substrate, L-val was varied in the range of 1.0 \(\times\) 10\(^{-4}\) to 1.0 \(\times\) 10\(^{-3}\) mol dm\(^{-3}\). The \(k_{\text{obs}}\) values increased with increase in concentration of L-val. The order with respect to [L-val] was found to be less than unity (Table 1) (r \(\geq\) 0.994, S \(\leq\) 0.004). This was also confirmed by the plots of \(k_{\text{obs}}\) versus [L-val]\(^{0.78}\) which is linear rather than the direct plot of \(k_{\text{obs}}\) versus [L-val] (Fig. 2).
3.5. Effect of [Alkali]

The effect of increase in concentration of alkali on the reaction was studied at constant concentrations of L-valine, DPA and periodate at a constant ionic strength of 0.006 mol dm$^{-3}$ at 25 °C. The rate constants decreased with increase in alkali concentration (Table 1), indicating negative fractional order dependence of rate on alkali concentration ($r \geq 0.964$, $S \leq 0.003$), which is rarely observed in DPA oxidation.

3.6. Effect of [Periodate]

The effect of increasing concentration of periodate was studied by varying the periodate concentration from $1.0 \times 10^{-5}$ to $1.0 \times 10^{-4}$ mol dm$^{-3}$ keeping all other reactant concentrations constant. It was found that the added periodate had negligible effect on the rate of reaction.

3.7. Effect of Ionic Strength (I) and Dielectric Constant of the Medium (D)

The addition of KNO$_3$ at constant [DPA], [L-val], [OH$^-$] and [IO$_4$$^-$$^-$] was found that increasing ionic strength of the reaction medium increases the rate of the reaction (Fig. 3). Varying the t-butyl alcohol and water percentage varied dielectric constant of the medium, ‘D’. The $D$ values were calculated from the equation $D = D_w V_w + D_B V_B$, where $D_w$ and $D_B$ are dielectric constants of pure water and t-butyl alcohol respectively and $V_w$ and $V_B$ are the volume fractions of components water and t-butyl alcohol respectively in the total mixture. The decrease in dielectric constant of the reaction medium decreased the rate of reaction (Fig. 3).

![Figure 3](image-url)

3.8. Effect of Initially Added Products

The externally added products, Ag(I) (AgNO$_3$) and isobutyraldehyde did not have any significant effect on the rate of the reaction.
3.9. Polymerization Study

The intervention of free radicals in the reaction was examined as follows. The reaction mixture, to which a known quantity of acrylonitrile monomer was initially added, was kept for 2 hours in an inert atmosphere. On diluting the reaction mixture with methanol, a white precipitate was formed, which indicated the intervention of free radicals in the reaction.17

3.10. Effect of Temperature

The kinetics was studied at six different temperatures (15, 20, 25, 30, 35 and 40 °C) under varying concentrations of L-valine and alkali keeping other conditions constant. The rate constants were found to increase with an increase in temperature. The rate constants (k) of the slow step of the reaction mechanism were obtained from the slopes and intercepts of 1/kobs versus 1/[L-valine] and 1/kobs versus [OH–] plots at six different temperatures and were used to calculate the activation parameters. The energy of activation corresponding to these constants was evaluated from the Arrhenius plot of log k versus 1/T (r ≥ 0.9988, S ≤ 0.011) and other activation parameters obtained are tabulated in Table 2.

In the later period of 20th century the kinetics of oxidation of various organic and inorganic substrates have been studied by Ag(III) species, which may be due to its strong versatile nature of two electrons oxidant. Among the various species of Ag(III), Ag(OH)4–, diperiodatoargentate(III) and ethylenebis(biguanide), (EBS), silver(III) are of maximum attention to the researcher due to their relative stability.18 The stability of Ag(OH)4– is very sensitive towards traces of dissolved oxygen and other impurities in the reaction medium whereupon it had not drawn much attention. However, the other two forms of Ag(III) are considerably stable,8–14, 19, 20 the DPA is used in highly alkaline medium and EBS is used in highly acidic medium.

It is known that L-val exists in zwitterionic form in aqueous medium.21 In highly acidic medium, it exists in the protonated form, whereas in highly basic medium it is in the fully deprotonated form.21

The literature survey reveals that the water soluble diperiodatoargentate(III) has a formula [Ag(IO6)2]2– with dsp2 configuration of square planar structure, similar to diperiodotocopper(III) complex with two bidentate ligands, periodate to form a planar molecule.13 When the same molecule is used in alkaline medium, it is unlikely to be existed as [Ag(IO6)2]2– as periodate is known to be in various protonated forms depending on pH of the solution as H2IO6– and H2IO62– in pH ≤ 7; H2IO62– and H2IO63– in pH > 7 and dimeric form,22 H2IO64– in alkaline medium with highly concentrated solution. However, H2IO62–, H2IO6 and H2IO64– may be neglected as the reaction medium is alkaline and low [IO42–] used in the study. Hence, the IO4– is existed as either H2IO62–, H2IO63– or both. Therefore, under the present condition, diperiodotoargentate(III), may be depicted as [Ag(H2IO6)2]–. The similar speciation of periodate in alkali was proposed for diperiodotocinnamate(IV).23 On contrary, the authors in their recent past studies have proposed the DPA as [Ag(HL)2]3– in which ‘L’ is a periodate with uncertain number of protons or ‘HL’ is a protonated periodate of uncertain number of protons.8 This can be ruled out by considering the alternative form of IO4– at pH > 7 which is in the form H2IO62– in the pH range 7–12 and H2IO63– of pH ≥ 12.23 Hence, DPA could be as [Ag(H2IO6)2]3– or [Ag(H2IO6)]2– depending on pH of the reaction medium.

The reaction between diperiodotoargentate(III) complex and L-valine in alkaline medium has the stoichiometry 1:1 (DPA:L-val) with a first order dependence on [DPA] and a apparent less than unit order in [substrate], a negative fractional order dependence on [alkali]. No effect of added products was observed. Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [oxidant], [reductant], [OH–] and [IO4–] may be well accommodated.

<table>
<thead>
<tr>
<th>Table 2. Thermodynamic activation parameters for the oxidation of L-valine by DPA in aqueous alkaline medium with respect to the slow step of Scheme 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (K)</td>
</tr>
<tr>
<td>288</td>
</tr>
<tr>
<td>293</td>
</tr>
<tr>
<td>298</td>
</tr>
<tr>
<td>303</td>
</tr>
<tr>
<td>308</td>
</tr>
<tr>
<td>313</td>
</tr>
</tbody>
</table>

(B) Activation Parameters (Scheme 2)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔH° (kJ mol^-1)</td>
<td>52 ± 2</td>
</tr>
<tr>
<td>ΔS° (J K^-1 mol^-1)</td>
<td>-106 ± 4</td>
</tr>
</tbody>
</table>

(C) Effect of temperature to calculate K1 and K2 for the oxidation of L-valine by diperiodotoargentate(III) in alkaline medium.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>10^4K1 (mol dm^-3)</th>
<th>10^4K2 (dm^3 mol^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>288</td>
<td>0.60</td>
<td>2.70</td>
</tr>
<tr>
<td>293</td>
<td>0.87</td>
<td>1.96</td>
</tr>
<tr>
<td>298</td>
<td>1.06</td>
<td>1.55</td>
</tr>
<tr>
<td>303</td>
<td>1.42</td>
<td>1.22</td>
</tr>
<tr>
<td>308</td>
<td>1.72</td>
<td>0.83</td>
</tr>
<tr>
<td>313</td>
<td>2.87</td>
<td>0.66</td>
</tr>
</tbody>
</table>

(D) Thermodynamic quantities using K1 and K2

<table>
<thead>
<tr>
<th>Thermodynamic quantities</th>
<th>Values from K1</th>
<th>Values from K2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔH (kJ mol^-1)</td>
<td>43.4 ± 2.0</td>
<td>-42 ± 2</td>
</tr>
<tr>
<td>ΔS (J K^-1 mol^-1)</td>
<td>67 ± 3</td>
<td>-59 ± 3</td>
</tr>
</tbody>
</table>
It is interesting to note that in most of the reports of DPA oxidation, \( \text{OH}^- \) had an increasing effect on the rate of the reaction, periodate retarded the rate of reaction, MPA was considered as active species of DPA and free radical intervention was observed. However, in the present kinetic study, different kinetic results have been obtained. In this study, increasing the concentration of \( \text{OH}^- \) decreases the rate of the reaction and negligible effect of periodate on rate of reaction; free radical intervention was observed in the reaction and DPA itself was the active species of the reaction. The result of decrease in rate of reaction with increase in alkalinity (Table 1) can be explained in terms of prevailing equilibrium of formation of \( [\text{Ag(H}_3\text{IO}_6\text{)}_2]^- \) from \( [\text{Ag(H}_2\text{IO}_6\text{)(H}_3\text{IO}_6)]^- \) hydrolysis as given in the following equation.

\[
[\text{Ag(H}_2\text{IO}_6\text{)(H}_3\text{IO}_6)]^- + \text{H}_2\text{O} \rightleftharpoons [\text{Ag(H}_3\text{IO}_6\text{)}_2]^+ + \text{OH}^- \tag{1}
\]

Such type of equilibrium (1) has been well noticed in literature. Because of this reaction and the fact that \( k_{\text{obs}} \) values are inverse function of hydroxyl ion concentration with fractional order in \( \text{OH}^- \) concentration, the main oxidant species is likely to be \( [\text{Ag(H}_3\text{IO}_6\text{)}_2]^+ \) and its formation by the above equilibrium is important in the present study. The less than unit order in [L-val] presumably results from formation of a complex (C) between the DPA species and L-val prior to the formation of the products. This complex (C) decomposes in a slow step, to form iso-butyraldehyde, Ag(I) and periodate as given in Scheme 2.

![Scheme 2. Detailed Scheme for the oxidation of L-valine by alkaline diperiodatoargentate (III).](image)

The direct plot of \( k_{\text{obs}} \) versus [L-val] was drawn to know the parallel reaction if any along with interaction of oxidant and reductant. However, the plot of \( k_{\text{obs}} \) versus [L-val] was not linear. Thus, in Scheme 2, the parallel reaction and involvement of two molecules of L-valine in the complex are excluded. The probable structure of the complex (C) is given in Scheme 3.

![Scheme 3. The probable structure of the complex (C).](image)

Spectroscopic evidence for the complex formation between oxidant and substrate was obtained from UV-vis spectra of [L-val] (5 × 10^{-4} mol dm^{-3}), [DPA] (5.0 × 10^{-5} mol dm^{-3}), [OH^-] (0.002 mol dm^{-3}) and mixture of DPA and L-val. A bathochromic shift of about 6 nm from 256 to 262 nm in the spectra of DPA was observed (Fig. 4).

However, the Michaelis-Menten plot proved the complex formation between DPA and L-val, which explains the less than unit order dependence on [L-val]. Such complex between an oxidant and substrate has also been observed in other studies.\(^{25}\)

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Scheme 2 leads to the rate law (3)

\[
\text{rate} = \frac{d[DPA]}{dt} = \frac{k K_1 K_2 [\text{L-val}]}{[\text{OH}^-] + K_1 + K_1 K_2 [\text{L-val}]} \quad (2)
\]

\[
\frac{1}{k_{obs}} = \frac{[\text{DPA}]}{k K_1 K_2 [\text{L-val}]} = \frac{1}{k K_2 [\text{L-val}]} + \frac{1}{k} \quad (3)
\]

This explains all the observed kinetic orders of different species. The rate law (3) can be rearranged in the following form, which is suitable for verification.

\[
\frac{1}{k_{obs}} = \frac{[\text{OH}^-]}{k K_1 K_2 [\text{L-val}]} + \frac{1}{k K_2 [\text{L-val}]} + \frac{1}{k} \quad (4)
\]

According to equation (4), other conditions being constant, plots of \(1/k_{obs}\) versus \([\text{OH}^-]\) (\(r \geq 0.999, S \leq 0.014\)) and \(1/k_{obs}\) versus \(1/[\text{L-val}]\) (\(r \geq 0.999, S \leq 0.016\)) should be linear and are found to be so (Fig. 5). The slopes and intercepts of such plots lead to the values of \(K_1\) and \(K_2\) were calculated at different temperatures and these values are given in Table 2. The vant Hoff’s plots were made for variation of \(K_1\) and \(K_2\) with temperature (\(\log K_1\) versus \(1/T\) (\(r \geq 0.9882, S \leq 0.006\)) and \(\log K_2\) versus \(1/T\) (\(r \geq 0.9971, S \leq 0.007\)) and the values of enthalpy of reaction \(\Delta H\), entropy of reaction \(\Delta S\) and free energy of reaction \(\Delta G\), were calculated for the first and second equilibrium steps. These values are given in Table 2. A comparison of \(\Delta H\) value (43.4 ± 2.0) from \(K_1\) with that of \(\Delta H^\#\) (52 ± 2) of rate determining step supports that the first step of Scheme 2 is fairly fast since it involves low activation energy.27

The thermodynamic quantities for the first and second equilibrium steps of Scheme 2 can be evaluated as follows. \([\text{L-val}]\) and \([\text{OH}^-]\) (as in Table 1) were varied at six different temperatures. The plots of \(1/k_{obs}\) versus \([\text{OH}^-]\) and \(1/k_{obs}\) versus \(1/[\text{L-val}]\) should be linear (Fig. 5). From the slopes and intercepts, the values of \(K_1\) and \(K_2\) were calculated at different temperatures and these values are given in Table 2. The vant Hoff’s plots were made for variation of \(K_1\) and \(K_2\), with temperature (\(\log K_1\) versus \(1/T\) (\(r \geq 0.9882, S \leq 0.006\)) and \(\log K_2\) versus \(1/T\) (\(r \geq 0.9971, S \leq 0.007\)) and the values of enthalpy of reaction \(\Delta H\), entropy of reaction \(\Delta S\) and free energy of reaction \(\Delta G\), were calculated for the first and second equilibrium steps. These values are given in Table 2. A comparison of \(\Delta H\) value (43.4 ± 2.0) from \(K_1\) with that of \(\Delta H^\#\) (52 ± 2) of rate determining step supports that the first step of Scheme 2 is fairly fast since it involves low activation energy.27

Figure 4. Spectroscopic evidence for the complex formation between DPA and L-val. (a) UV-vis spectra of DPA complex (360 nm and 256); (b) UV-vis spectra of mixture of L-val and DPA (360 nm and 262 nm); (c) UV-vis spectra of L-val;

Figure 5. Verification of rate law (4) of oxidation of L-valine by DPA at 25 °C.

The values of \(\Delta H^\#\) and \(\Delta S^\#\) were both favourable for electron transfer processes. The favourable enthalpy was due to release of energy on solutions changes in the transition state. The negative value of \(\Delta S^\#\) suggests that the intermediate complex is more ordered than the reactants.28 The observed modest enthalpy of activation and a higher rate constant for the slow step indicates that the oxidation...
presumably occurs via an inner-sphere mechanism. This conclusion is supported by earlier observations.29

The activation parameters for the oxidations of some amino acids by DPA are summarized in Table 3. The entropy of the activation for the reaction falls within the observed range. Variation in the rate within a reaction series may be caused by change in the enthalpy or entropy of activation. Changes in the rate are caused by changes in both $\Delta H^\#$ and $\Delta S^\#$, but these quantities vary extensively in a parallel fashion. A plot of $\Delta H^\#$ versus $\Delta S^\#$ is linear according to the following equation.

$$\Delta H^\# = \beta \Delta S^\# + \text{constant}$$

$\beta$ is called the isokinetic temperature. It has been asserted that apparently linear correlations of $\Delta H^\#$ with $\Delta S^\#$ are sometimes misleading and the evaluation of $\beta$ by means of the above equation lacks statistical validity.30 Exner advocates an alternative method for the treatment of experimental data.31 If the rates of several reactions in a series have been measured at two temperatures and log $k_2$ (at $T_2$) is linearly related to log $k_1$ (at $T_1$), i.e., log $k_2 = a + b$ log $k_1$, he proposed that $\beta$ can be evaluated from the equation.

$$\beta = \frac{T_1 T_2 (b-1)}{(T_2 b-T_1)}$$

We have calculated the isokinetic temperature to be 188.9 K by plotting log $k$, at 303 K versus log $k_1$ at 298 K ($r \geq 0.9994$, $S \leq 0.006$) in Fig. 6. The value of $\beta$ (188.9 K) is lower than experimental temperature (298 K). This indicates that the rate is governed by the entropy of activation.32 The linearity and the slope of the plot obtained may confirm that the kinetics of these reactions follows a similar mechanism, as previously suggested.

### 4. Conclusion

Among various species of DPA in alkaline medium, protonated DPA i.e., [Ag(H$_2$IO$_6$)$_2$]$^-$ is considered as active species for the title reaction. The results indicate that, the role of pH in the reaction medium is crucial. Rate constant of slow step and other equilibrium constants involved in the mechanism are evaluated and activation parameters with respect to slow step of reaction were computed. The overall mechanistic sequence described here is consistent with product studies, mechanistic and kinetic studies.

### 5. Appendix

According to Scheme 2

$$\text{rate} = -\frac{d [\text{DPA}]}{dt} = k [\text{C}] =$$

$$= \frac{k K_1 K_2 [\text{L-val}] [\text{Ag}(\text{H}_2\text{IO}_6) (\text{H}_3\text{IO}_6)]^2}{[\text{OH}^-]}$$

Where $T$ and $f$ refer to total and free concentrations.

$$[\text{DPA}]_f = \frac{[\text{DPA}]_t}{1 + \frac{K_1}{[\text{OH}^-]} + \frac{K_1 K_2 [\text{L-val}]}{[\text{OH}^-]}}$$

![Figure 6. Plot of log $k_2$ at 303 K versus log $k_1$ at 298 K for isokinetic temperature (Table 3). (1) L-isoleucine; (2) L-alanine; (3) L-proline; (4) L-leucine; (5) L-valine.](image-url)
In view of low concentrations of DPA used, the second term of above eqn. is neglected. Therefore,

\[
[L\text{-val}]_T = [L\text{-val}]_i + [C]
\]

Similarly,

\[
[L\text{-val}]_T = [L\text{-val}]_f \left( \frac{[OH]^\ddagger}{[OH]^\ddagger + K_1 K_2 [DPA]_f} \right)
\]

Substituting Equations (II), (III) and (IV) in (I) and omitting the subscripts \( T \) and \( f \) we get

\[
\text{rate} = \frac{d [DPA]}{dt} = \frac{k K_1 K_2 [L\text{-val}] [DPA]}{[OH]^\ddagger + K_1 K_2 [L\text{-val}]}
\]

5. References

Povzetek

Z UV-Vis spektroskopijo smo raziskovali oksidacijo L-valina s srebrovo (III) soljo perjodove kisline (DPA) v alkalnem mediju pri konstantni ionski moči 0.006. Izkazalo se je, da reakcija med L-valinom in DPA poteče v stehiometričnem razmerju 1:1 ob sodelovanju prostih radikalov. Predpostavili smo mehanizem reakcije, ki kot reaktivno obliko oksidanta privzema protonirano obliko DPA. Produkte smo karakterizirali spektrofotometrično in s “spot” testi. Izračunali smo konstante reakcijske hitrosti za posamezne stopnje reakcije ter parametre aktivacije za posamezne stopnje. Za posamezna ravnotežja smo ocenili tudi termodinamske parametre ter izokinetično temperaturo (188.9 K).
Thermodynamic Quantities for the Different Steps Involved in the Oxidation of the Drug Ketorolac by Copper(III) Periodate Complex in Aqueous Alkaline Medium: A Mechanistic Approach

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Abstract The oxidation of ketorolac (KET) by diperiodatocuprate(III) (DPC) in aqueous alkaline medium at a constant ionic strength of 0.10 mol·dm$^{-3}$ was studied spectrophotometrically at 298 K. The reaction is of first order in [DPC] and has less than unit order in both [KET] and [alkali], and negative fractional order in [periodate]. The oxidation reaction in alkaline medium has been shown to proceed via a DPC-ketorolac complex, which decomposes slowly in a rate determining step followed by other fast steps to give the products. The main products were identified by spot test, IR and GC-MS spectral studies. The reaction constants involved in the different steps of the mechanism were calculated at different temperatures, which yielded thermodynamic quantities for different steps of the reaction scheme. The activation parameters with respect to the slow step of the mechanism were computed and discussed; thermodynamic quantities were also determined.

Keywords Ketorolac · Oxidation · Copper(III) · Kinetics · Mechanism · Thermodynamic studies

1 Introduction

Ketorolac (KET) (5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid) is a member of a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs) in the family of heterocyclic acetic acid derivatives. It is used for treating inflammation and pain. Other drugs in this class include ibuprofen (Motrin) and naproxen (Naprosyn, Aleve), but ketorolac is more effective than other NSAIDs in reducing pain from both inflammatory and non-inflammatory causes. Ketorolac reduces the production of prostaglandins, chemicals that cells of the immune system make that cause the redness, fever, and pain of inflammation and that also are believed to be important in the production of non-inflammatory pain. It does this by blocking the enzymes that cells use to make prostaglandins (cyclooxygenase 1 and 2). As a result, pain as well as inflammation and its signs and symptoms, redness,
swelling, fever, and pain, are reduced. It is used for the short-term treatment of moderate to moderately severe acute pain associated with muscle sprains and strains, dental pain, and pain after surgery or giving birth. An ophthalmic solution of ketorolac is used to treat eye pain and to relieve the itchiness and burning of seasonal allergies [1, 2].

In recent years, the study of highest oxidation state of transition metals has intrigued many researchers. Transition metals in a higher oxidation state can be stabilized by chelation with suitable polydentate ligands. Metal chelates such as diperiodatocuprate(III) [3], diperiodatoargentate(III) [4] and diperiodatonickelate(IV) [5] are good oxidants in a medium with an appropriate pH value. Periodate and tellurate complexes of copper in its trivalent state have been extensively used in the analysis of several organic compounds [6]. Copper(III) is shown to be an intermediate in the copper(II) catalyzed oxidation of amino acids by peroxydisulphate [7]. The oxidation reaction usually involves the copper(II)–copper(I) couple and such aspects are detailed in different reviews [8]. The use of diperiodatocuprate(III) (DPC) as an oxidant in alkaline medium is new and restricted to a few cases due to the fact of its limited solubility and stability in aqueous medium. DPC is a versatile one-electron oxidant for various organic compounds in alkaline medium and its use as an analytical reagent is now well recognized [9]. Copper complexes have occupied a major place in oxidation chemistry due to their abundance and relevance in biological chemistry [10]. They have also been used [11] in the differential titration of organic mixtures, in the estimation of chromium, calcium and magnesium from their ores, and antimony, arsenic and tin from their alloys. Since multiple equilibria between different copper(III) species are involved, it would be interesting to know which of the species is the active oxidant.

In earlier reports [12] on DPC oxidation, both periodate and alkali had a retarding effect in most of the reactions. However in the present study we have made entirely different kinetic observations. A literature survey reveals that there are no reports on the oxidation of ketorolac by any oxidant either in acid or alkaline medium. In view of the pharmaceutical importance of ketorolac, such oxidation studies may throw some light on the mechanism of conversions of the compounds in biological systems. The present study deals with the title reaction to investigate the redox chemistry of DPC in alkaline media, to arrive at a suitable mechanism on the basis of kinetic and spectral results and to compute the thermodynamic quantities of various steps of the reaction.

2 Experimental

2.1 Materials and Reagents

All chemicals used were of reagent grade and doubly distilled water was used throughout the work. The copper(III) periodate complex was prepared [13] and standardized by a literature procedure [14]. The UV–vis spectrum with a maximum absorption at 415 nm verified the existence of the copper(III) complex. The solutions of ketorolac (S.D. Fine Chem.) and copper sulphate (BDH) were prepared by dissolving known amounts of the samples in distilled water. The purity of ketorolac sample was checked by comparing its melting point (438 K) with the literature value (440 K). Periodate solutions were prepared and standardized iodo metrically [15]. The required alkalinity and ionic strength were maintained by KOH (BDH) and KNO₃ (Analar) respectively in the reaction solutions. t-Butyl alcohol (S.D. Fine Chem.) was used to vary the dielectric constant of the medium.
2.2 Kinetic Measurements

The kinetic measurements were performed on a Varian CARY 50 Bio UV–visible spectrophotometer. The kinetics was followed under pseudo-first order condition where \([\text{KET}] \gg [\text{DPC}]\) at 298.2 ± 0.1 K, unless specified. The reaction was initiated by mixing DPC and KET solutions, which also contained the required concentrations of KNO₃, KOH and KIO₄. The progress of reaction was followed spectrophotometrically at 415 nm by monitoring the decrease in absorbance due to DPC with the molar absorbancy index, \(\varepsilon\), equal to 6235 ± 100 dm³·mol⁻¹·cm⁻¹ (literature \(\varepsilon = 6230 [16]\)). It was shown that there is a negligible interference from other species present in the reaction mixture at this wavelength.

The pseudo-first order rate constants, \(k_{\text{obs}}\), were determined from the \(\log_{10} (\text{absorbance})\) versus time plots. The plots were linear up to 85% completion of the reaction over the range of \([\text{OH}^-]\) used. The orders for various species were determined from the slopes of plots of \(\log_{10} k_{\text{obs}}\) versus \(\log_{10} (\text{concentration of species})\) except for [DPC] in which no variation of \(k_{\text{obs}}\) was observed, as expected for the reaction conditions. During the kinetics experiments, a constant concentration of 1.0 \(\times\) 10⁻³ mol·dm⁻³ of KIO₄ was used unless otherwise stated.

Since periodate is present in the excess of DPC, the possibility of oxidation of ketorolac by periodate in alkaline medium at 298 K was tested. The progress of the reaction was followed iodometrically. However, it was found that there was no significant reaction under the experimental conditions employed compared to the DPC oxidation of ketorolac. The total concentration of periodate and OH⁻ were calculated by considering the amount present in the DPC solution and that additionally added. Kinetics runs were also carried out in N₂ atmosphere in order to understand the effect of dissolved oxygen on the rate of reaction. No significant difference in the results was obtained under a N₂ atmosphere and in the presence of air. In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates.

2.3 Instruments Used

The kinetic measurements were performed on a Varian CARY 50 Bio UV–visible spectrophotometer. For product analysis, a QP-2010S Shimadzu gas chromatograph mass spectrometer, Nicolet 5700-FT-IR spectrometer (Thermo, USA), and for pH measurements Elico pH meter model LI 120 were used.

2.4 Stoichiometry and Product Analysis

Different sets of reaction mixtures containing excess of DPC to ketorolac in presence of constant amounts of OH⁻ and KNO₃ were kept for 6 hours in a closed vessel under an inert atmosphere. The remaining DPC concentration was estimated spectrophotometrically at 415 nm. The results indicated 1:6 stoichiometry as given in Scheme 1.

The main product was identified as 5-benzoyl-1-(2-hydroxy-ethyl)-1H-pyrrole-2-carboxylic acid. The nature of the product was confirmed by its IR spectrum: a sharp band at 1720 cm⁻¹ due to carboxylic C=O stretching, a sharp band at 1635 cm⁻¹ due to ketonic C=O stretching, and a broad band at 3428 cm⁻¹ due to H-bonded OH stretching. Further, the product was subjected to GC-MS spectral analysis. GC-MS data was obtained on a QP-2010S Shimadzu gas chromatograph mass spectrometer. The mass spectrum showed a molecular ion peak at 259 amu confirming 5-benzoyl-1-(2-hydroxy-ethyl)-1H-pyrrole-2-carboxylic acid product (Fig. 1). All other peaks observed in GC-MS can be interpreted in
Fig. 1 GC-mass spectrum of 5-benzoyl-1-(2-hydroxy-ethyl)-1H-pyrrole-2-carboxylic acid with its molecular ion peak at 259 amu

accordance with the observed structure of the 5-benzoyl-1-(2-hydroxy-ethyl)-1H-pyrrole-2-carboxylic acid. The product Cu(II) was identified by UV–vis spectra. The reaction products did not undergo further oxidation under the present kinetic conditions.

The byproduct CO₂ was qualitatively detected [12] by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through a tube containing limewater.

Regression analysis of experimental data to obtain the regression coefficient \( r \) and standard deviation \( S \) of the points from the regression line, was performed using the Microsoft Excel-2003 program.

3 Results and Discussion

3.1 Reaction Orders

The reaction orders were determined from the slopes of plots of the logarithm of the rate constant against the logarithm of the concentration of ketorolac, alkali and periodate in turn while keeping all other concentrations and conditions constant.

3.2 Effect of [Diperiodatocuprate(III)]

The oxidant DPC concentration was varied in the range of \( 5.0 \times 10^{-5} \) to \( 5.0 \times 10^{-4} \) mol·dm\(^{-3} \) and the fairly constant \( k_{obs} \) values indicate that the order with respect to [DPC] was unity.
Table 1  Effect of variations of the concentrations of DPC, KET, IO\textsubscript{4} and OH\textsuperscript{−} on the oxidation of ketorolac by diperiodatocuprate(III) in alkaline medium at 298 K, I = 0.10 mol·dm\textsuperscript{−3}  

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<th>[OH\textsuperscript{−}] (10\textsuperscript{2} mol·dm\textsuperscript{−3})</th>
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(Table 1). This was also confirmed by the linearity of the plots of log (absorbance) versus time (r = 0.986, S ≤ 0.014) up to 85% completion of the reaction.

3.3 Effect of Ketorolac Concentration

The effect of ketorolac on the rate of reaction was studied at constant concentrations of alkali, DPC and periodate at a constant ionic strength of 0.10 mol·dm\textsuperscript{−3}. The substrate, ketorolac was varied in the range of 5.0 × 10\textsuperscript{−4} to 5.0 × 10\textsuperscript{−3} mol·dm\textsuperscript{−3}. The k\textsubscript{obs} values increased with increase in concentration of ketorolac (Table 1). The apparent order with respect to [KET] was found to be less than unity (r = 0.9935, S ≤ 0.009) under the concentrations studied. This is also confirmed in the plots of k\textsubscript{obs} versus [KET]\textsuperscript{0.48} which is linear, rather than the direct plot of k\textsubscript{obs} versus [KET] (Fig. 2).

3.4 Effect of Alkali Concentration

The effect of increase in concentration of alkali on the reaction was studied at constant concentrations of ketorolac, DPC and periodate at a constant ionic strength of 0.10 mol·dm\textsuperscript{−3} at 298 K. The rate constants increased with increase in alkali concentration (Table 1), indicating an apparent less than unit order dependence of rate on the alkali concentration (r = 0.9989, S ≤ 0.007).
3.5 Effect of Periodate Concentration

The effect of increasing concentration of periodate was studied by varying the periodate concentration from $5.0 \times 10^{-4}$ to $5.0 \times 10^{-3}$ mol·dm$^{-3}$ keeping all other reactant concentrations constant. It was found that the added periodate had a retarding effect on the rate of reaction. The order with respect to periodate concentration was less than negative unity ($r = 0.9969$, $S \leq 0.004$; Table 1).

3.6 Effect of Ionic Strength ($I$) and Dielectric Constant on the Medium ($D$)

The addition of KNO$_3$ at constant [DPC], [KET], [$\text{IO}_4^-$] and [$\text{OH}^-$], showed that increasing ionic strength had a negligible effect on the rate of the reaction.

Dielectric constant of the medium, $D$ was varied by varying the $t$-butyl alcohol and water percentage. The $D$ values were calculated from the equation, $D = D_W V_W + D_B V_B$, where $D_W$ and $D_B$ are dielectric constants of pure water and $t$-butyl alcohol respectively, and $V_W$ and $V_B$ are the volume fractions of components water and $t$-butyl alcohol, respectively, in the total volume of the mixture. The decrease in dielectric constant of the reaction medium had a negligible effect on the rate of the reaction.

3.7 Effect of Added Products

Addition of the products, 5-benzoyl-1-(2-hydroxy-ethyl)-1H-pyrrole-2-carboxylic acid and copper(II) (CuSO$_4$), did not have any significant effect on the rate of the reaction.

3.8 Polymerization Study

The intervention of free radicals in the reaction was examined as follows. The reaction mixture, to which a known quantity of acrylonitrile monomer was initially added, was kept for 2 hours in an inert atmosphere. On diluting the reaction mixture with methanol, a white precipitate was formed, indicating the intervention of free radicals in the reaction [16]. The blank experiments, with either DPC or ketorolac alone, with acrylonitrile did not give any polymerization under the same conditions as those for reaction mixture. Initially added acrylonitrile decreased the rate of the reaction indicating free radical intervention, which is also the case in earlier work [17].
3.9 Effect of Temperature ($T$)

The kinetics was studied at six different temperatures: 288, 293, 298, 303, 308, and 313 K with varying concentrations of ketorolac, alkali and periodate, keeping other conditions constant. The rate constants were found to increase with increasing temperature. The rate constants ($k$) of the slow step of Scheme 1 were obtained from the slopes and intercepts of $1/k_{obs}$ versus $1/[\text{KET}]$, $1/k_{obs}$ versus $[\text{H}_3\text{IO}_6^-]$ and $1/k_{obs}$ versus $1/[\text{OH}^-]$ plots at six different temperatures and were used to calculate the activation parameters. The energy of activation corresponding to these constants was evaluated from the Arrhenius plot of $\log_{10}k$ versus $1/T$ ($r = 0.9968$, $S \leq 0.009$) and other activation parameters obtained are tabulated in Table 2.

Table 2 Activation parameters for the oxidation of ketorolac by DPC in aqueous alkaline medium with respect to the slow step of Scheme 2: (A) Effect of temperature; (B) Activation parameters; (C) Effect of temperature on $K_4$, $K_5$ and $K_6$ for the oxidation of ketorolac by diperiodatocuprate(III) in alkaline medium; (D) Thermodynamic quantities calculated from $K_4$, $K_5$ and $K_6$

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<th>$K_4$ ($10^{-1}$ dm$^3$·mol$^{-1}$)</th>
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<th>$K_6$ ($10^{-3}$ dm$^3$·mol$^{-1}$)</th>
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<td>$\Delta H$ (kJ·mol$^{-1}$)</td>
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<td>$\Delta S$ (J·K$^{-1}$·mol$^{-1}$)</td>
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$[\text{DPC}] = 1.0 \times 10^{-4}$ mol·dm$^{-3}$, $[\text{KET}] = 1.0 \times 10^{-3}$ mol·dm$^{-3}$, $[\text{IO}_4^-] = 1.0 \times 10^{-3}$ mol·dm$^{-3}$, $[\text{OH}^-] = 0.05$ mol·dm$^{-3}$, and $I = 0.10$ mol·dm$^{-3}$
The water-soluble copper(III) periodate complex is reported [18] to be \( [\text{Cu} (\text{HIO}_6^-)_2 (\text{OH})_2^-] \). However, in an aqueous alkaline medium and in the high pH range employed in the study, periodate is unlikely to exist as \( \text{HIO}_4^- \) (as in the complex) as is evident from its involvement in the multiple equilibria [19] Eqs. 1–3, depending on the pH of the solution.

\[
\text{H}_3\text{IO}_6 \rightleftharpoons K_1 \text{H}_4\text{IO}_6^- + \text{H}^+, \quad K_1 = 5.1 \times 10^{-4} \tag{1}
\]
\[
\text{H}_4\text{IO}_6^- \rightleftharpoons K_2 \text{H}_3\text{IO}_6^- + \text{H}^+, \quad K_2 = 4.9 \times 10^{-9} \tag{2}
\]
\[
\text{H}_3\text{IO}_6^- \rightleftharpoons K_3 \text{H}_2\text{IO}_3^- + \text{H}^+, \quad K_3 = 2.5 \times 10^{-12} \tag{3}
\]

Periodic acid exists as \( \text{H}_3\text{IO}_6^- \) in acid medium and as \( \text{H}_4\text{IO}_6^- \) near \( \text{pH} = 7 \). Hence, under alkaline conditions as employed in this study, the main species are expected to be \( \text{H}_3\text{IO}_5^- \) and \( \text{H}_2\text{IO}_3^- \). Thus, at the pH employed in this study (\( \text{pH} = 12.7 \)), the soluble copper(III) periodate complex might be \( [\text{Cu}(\text{H}_3\text{IO}_6^-)_2^-] \), a conclusion also supported by earlier work [3].

The reaction between the diperiodatocuprate(III) complex and ketorolac in alkaline medium has the stoichiometry 1:6 (KET: DPC) with a first order dependence on [DPC] and an apparent order of less than unity both on the [substrate] and [alkali], a negative fractional order dependence on the [periodate] and no effect of added products was observed. Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [DPC], [KET], [OH\(^-\)] and [IO\(^-\)] may be well accommodated.

In most of the reports [12] on DPC oxidation, both periodate and OH\(^-\) had a decreasing effect on the rate of the reaction. However, in the present kinetic study, different kinetic results have been obtained. In this study OH\(^-\) had increased and periodate retarded the rate of the reaction. The results of increase in rate of reaction with increase in alkalinity (Table 1) can be explained in terms of prevailing equilibrium of formation of \( [\text{Cu}(\text{H}_2\text{IO}_6)(\text{H}_3\text{IO}_6)]^{2-} \) from \( [\text{Cu}(\text{H}_3\text{IO}_6^-)_2^-] \) as given in the following Eq. 4

\[
[Cu(H_3IO_6^-)_2^-] + \text{OH}^- \xrightleftharpoons{K_4} [Cu(H_2IO_6)(H_3IO_6)]^{2-} + \text{H}_2\text{O} \tag{4}
\]

Also the decrease in rate with increase in \( [\text{H}_3\text{IO}_6^-] \) (Table 1) suggests that the displacement of a ligand periodate takes place to give free periodate and the equilibrium of copper(III) periodate complex to form monoperiodatocuprate(III) (MPC) species as given in Eq. 5 is established

\[
[Cu(H_2IO_6)(H_3IO_6)]^{2-} + 2\text{H}_2\text{O} \xrightleftharpoons{K_5} [Cu(H_2IO_6)(H_2O_2)^-] + \text{H}_3\text{IO}_3^- \tag{5}
\]

Such types of equilibria Eqs. 4 and 5 have been well noticed in literature [20]. It may be expected that a lower periodate complex such as monoperiodatocuprate(III) (MPC) is more important in the reaction than the DPC. The inverse fractional order in \( [\text{H}_3\text{IO}_6^-] \) might also be due to this reason. Therefore, MPC might be the main reactive form of the oxidant.

The less than unit order in [KET] presumably results from formation of a complex (C) between the MPC species and ketorolac prior to the formation of the products. This complex (C) decomposes in a slow step to form a free radical derived from ketorolac along with Cu\(^{2+}\), CO\(_2\), and \( \text{H}_2\text{IO}_5^- \). This free radical species further reacts with three molecules of MPC in a fast step to form an intermediate, 5-benzoyl-2,3-dihydro-pyrrolizin-1-one. This intermediate species then reacts with two more molecules of MPC in a further fast step to form the products such as 5-benzoyl-1-(2-hydroxy-ethyl)-1H-pyrrole-2-carboxylic acid, Cu(II) and periodate as given in Scheme 2.
Since Scheme 2 is in accordance with the generally well-accepted principle of non-complementary oxidations taking place in a sequence of one-electron steps, the reaction between the substrate and oxidant would afford a radical intermediate. A free radical scavenging experiment revealed such a possibility. This type of radical intermediate has also been observed in earlier work [12]. The direct plot of $k_{\text{obs}}$ versus [KET] was drawn to know the parallel reaction, if any, along with interaction of oxidant and reductant. However, the plot of $k_{\text{obs}}$ versus [KET] was not linear. Thus, in Scheme 2, the parallel reaction and involvement of two molecules of ketorolac in the complex are excluded. The probable structure of the complex (C) is given in Scheme 3.

Spectroscopic evidence for the complex formation between DPC and KET was obtained from UV–vis spectra of ketorolac ($5.0 \times 10^{-4}$), DPC ($5.0 \times 10^{-5}$), [OH$^-$] = 0.05 mol·dm$^{-3}$ and a mixture of both. A hypsochromic shift of 5 nm from 323 to 318 nm in the spectra of DPC was observed as given in Fig. 3.
Fig. 3 Spectroscopic evidence for the complex formation between DPC and KET. (a) UV–vis spectra of DPC complex (415 nm); (b) UV–vis spectra of mixture of KET and DPC (415 nm and 318 nm); (c) UV–vis spectra of KET (323 nm)

The Michaelis–Menten plot (Fig. 4A) also proved the complex formation between DPC and ketorolac, which explains the less than unit order dependence on [KET]. Such a complex between an oxidant and substrate has been observed in other studies [20].

Scheme 2 leads to the rate law Eq. 6.

\[
\text{rate} = -\frac{d[DPC]}{dt} = \frac{k K_4 K_5 K_6 [\text{KET}][\text{DPC}][\text{OH}^-]}{[\text{H}_3\text{IO}_6^{2-}] + K_4[\text{H}_3\text{IO}_6^{2-}][\text{OH}^-] + K_4 K_5[\text{OH}^-] + K_4 K_5 K_6[\text{KET}][\text{OH}^-]} \tag{6}
\]

\[
\frac{1}{k_{\text{obs}}} = \frac{[\text{H}_3\text{IO}_6^{2-}]}{k K_4 K_5 K_6[\text{KET}][\text{OH}^-]} + \frac{[\text{H}_3\text{IO}_6^{2-}]}{k K_4 K_5[\text{KET}]} + \frac{1}{k K_6[\text{KET}]} + \frac{1}{k} \tag{8}
\]
According to Eq. 8, other conditions being constant, plots of $1/k_{\text{obs}}$ versus $1/[\text{OH}^-]$ ($r = 0.9923, S \leq 0.014$), $1/k_{\text{obs}}$ versus $1/[\text{KET}]$ ($r = 0.9983, S \leq 0.012$) and $1/k_{\text{obs}}$ versus $[\text{H}_3\text{IO}_6^2^-]$ ($r = 0.999, S \leq 0.011$) should be linear and are found to be so (Fig. 4). The slopes and intercepts of such plots lead to values of $K_4$, $K_5$, $K_6$ and $k$ as $(17.8 \pm 0.4) \text{ dm}^3\text{mol}^{-1}$, $(8.3 \pm 0.1) \times 10^{-4} \text{ mol} \cdot \text{dm}^3$, $(26.2 \pm 0.6) \times 10^2 \text{ dm}^3\text{mol}^{-1}$ and $(1.56 \pm 0.02) \times 10^{-2} \text{ s}^{-1}$ respectively. The values of $K_4$ and $K_5$ are in good agreement with earlier literature [20].

These constants were used to calculate the rate constants and compared with the experimental values and found to be in reasonable agreement with each other (Table 1), which fortifies the Scheme 2. The equilibrium constant $K_4$ is far greater than $K_5$. This may be attributed to the greater tendency of DPC to undergo hydrolysis compared to the dissociation of hydrolyzed species in alkaline medium. The negligible effect of ionic strength and dielectric constant of the medium on the rate explains qualitatively the reaction between neutral and negatively charged ions, as seen in Scheme 2.

The thermodynamic quantities for the first, second and third equilibrium steps of Scheme 2 can be evaluated as follows. The $[\text{H}_3\text{IO}_6^2^-]$, [KET] and [OH$^-$] were varied at six different temperatures. The slopes of $1/k_{\text{obs}}$ versus $1/[\text{OH}^-]$, $1/k_{\text{obs}}$ versus $1/[\text{KET}]$ and $1/k_{\text{obs}}$ versus $[\text{H}_3\text{IO}_6^2^-]$ should be linear (Fig. 4). From the slopes and intercepts, the values of $K_4$, $K_5$ and $K_6$ were calculated at different temperatures and these values are given in Table 2. The van’t Hoff’s plots were made for the variation of $K_4$, $K_5$ and $K_6$ with temperature ($\log_{10} K_4$ versus $1/T$ ($r = 0.9915, S \leq 0.004$), $\log_{10} K_5$ versus $1/T$ ($r = 0.989, S \leq 0.007$) and $\log_{10} K_6$ versus $1/T$ ($r = 0.977, S \leq 0.008$)) and the values of enthalpy of reaction $\Delta H$, entropy of reaction $\Delta S$ and Gibbs energy of reaction $\Delta G$, were calculated for the first, second and third equilibrium steps. These values are given in Table 2.

A comparison of the thermodynamic quantities $\Delta H$ of the second step of Scheme 2 ($14.9 \pm 2.0$) from $K_5$ with that of $\Delta H^\#$ ($49.9 \pm 2.0$) of the rate determining step supports the observation that the reaction before the rate determining step of Scheme 2 is fairly fast since it involves a low activation energy [21].

The moderate values of $\Delta H^\#$ and $\Delta S^\#$ were both favorable for electron transfer processes. The negative value of $\Delta S^\#$ suggests that the intermediate complex is more ordered than the reactants [22]. The observed modest enthalpy of activation and as well as a higher rate constant of the slow step indicate that the oxidation presumably occurs via an inner-sphere mechanism. This conclusion is supported by earlier observation [23].

### 4 Conclusion

The simplified kinetic study is consistent and gives a reasonable response to experimental data. Among various species of DPC in alkaline medium, monoperiodatocuprate(III) (MPC) $[\text{Cu(H}_2\text{IO}_6)(\text{H}_2\text{O})_2]$ is considered as the active species for the title reaction. The results indicate that the role of pH in the reaction medium is crucial. On oxidation of ketorolac by the copper(III) periodate complex, at higher pH, it undergoes decarboxylation, giving 5-benzyol-1-(2-hydroxy-ethyl)-1H-pyrole-2-carboxylic acid via the formation of a free radical. Rate constant of slow step and other equilibrium constants involved in the mechanism are evaluated and activation parameters with respect to the slow step of reaction were computed. The overall mechanistic sequence described here is consistent with product studies, mechanistic and kinetic studies.
Fig. 4 Verification of rate law (Eq. 8) for the oxidation of ketorolac by diperiodatocuprate(III). Plots of 
(A) $1/k_{obs}$ versus $1/[\text{KET}]$, 
(B) $1/k_{obs}$ versus $1/[\text{OH}^-]$, 
(C) $1/k_{obs}$ versus $[\text{H}_3\text{IO}_6^{2-}]$, at six different temperatures (conditions as in Table 1)

Appendix

According to Scheme 2,

$$\text{Rate} = -\frac{d[DPC]}{dt} = k[C] = \frac{kK_4K_5K_6[\text{KET}][DPC][\text{OH}^-]}{[\text{H}_3\text{IO}_6^{2-}]} \quad \text{(I)}$$
where the subscripts T and f refer to total and free concentrations.

\[
[DPC]_T = [DPC]_f + [Cu(H_2IO_6^-)(H_3IO_6^-)] + [Cu(H_2IO_6^-)] + [C]
\]

Similarly,

\[
[DPC]_T = [DPC]_f \times \left[1 + K_4[DPC][OH^-] + \frac{K_4K_5K_6[DPC][KET][OH^-]}{[H_3IO_6^-]} \right]
\]

\[
[DPC]_f = \frac{[DPC]_T[H_3IO_6^-]}{[H_3IO_6^-]} + K_4[H_3IO_6^-][OH^-] + K_4K_5[OH^-] + K_4K_5K_6[KET][OH^-]
\]  

(II)

In view of low concentrations of DPC used, the second term in the above equation can be neglected. Therefore,

\[
[KET]_T = [KET]_f + [C]
\]

\[
[KET]_T = [KET]_f + \frac{K_4K_5K_6[DPC][KET][OH^-]}{[H_3IO_6^-]}
\]

Similarly,

\[
[OH^-]_T = [OH^-]_f
\]  

(III)

In view of low concentrations of DPC used, the second term in the above equation can be neglected. Therefore,

\[
[OH^-]_T = [OH^-]_f
\]  

(IV)

Substituting Eqs. II, III and IV in I and omitting the subscripts T and f we get,

\[
\text{Rate} = -\frac{d[DPC]}{dt} = \frac{kK_4K_5K_6[KET][DPC][OH^-]}{[H_3IO_6^-] + K_4[H_3IO_6^-][OH^-] + K_4K_5[OH^-] + K_4K_5K_6[KET][OH^-]}
\]

(V)

References


Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry

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Osmium(VIII) Catalyzed Oxidation of DL-Ornithine Monohydrochloride by a New Oxidant, Diperiodatoargentate(III) in Aqueous Alkaline Medium

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1. INTRODUCTION

Amino acids act not only as the building blocks in protein syntheses but they also play a significant role in metabolism and have been oxidized by a variety of oxidizing agents. The study of the oxidation of amino acids is of interest because of their biological significance and selectivity towards the oxidant to yield the different products. DL-Ornithine monohydrochloride ([±]-2,5-Diaminopentanoic acid monohydrochloride) (OMH) is the most potent amino acid studied for the production of release of growth hormone in the body from the pituitary gland, which in turn helps with fat metabolism. It is further required for a properly functioning immune system and liver, and assists in ammonia detoxification and liver rejuvenation. It is also of use in healing and repairing skin and tissue, and is found in both these body parts. Ornithine has the ability to regenerate the thymus gland, liver, and heart tissue, enhance muscle growth, and increase immune system function.

Diperiodatoargentate(III) (DPA) is a powerful oxidizing agent in alkaline medium with the reduction potential, 1.74 V. It is widely used as a volumetric reagent for the determination of various organic and inorganic species. Jayaprakash Rao et al. have used DPA as an oxidizing agent for the kinetic oxidation of various substrates. They normally found that order with respect to both oxidant and substrate was unity and OH− was found to enhance the rate of reaction. It was also observed that they did not arrive the possible active species of DPA in alkali; on the other hand, they proposed mechanisms by generalizing the DPA as [Ag(HL)L]^{x+1−}. However, Kumar et al. put in an effort to give evidence for the reactive form of DPA in the large scale of alkaline pH. When the Ag(III) species are involved, it would be interesting to know which of the species is the active oxidant. In the present investigation, we have obtained the evidence for the reactive species for the DPA in alkaline medium. The DPA is a metal complex with Ag in 3+ oxidation state like Cu^{3+} in DPC and Fe^{3+} in hemoglobin.

Transition metals are known to catalyze many oxidation-reduction reactions since they involve multiple oxidation states. In recent years, the use of transition metal ions such as osmium, ruthenium, palladium, chromium and iridium either alone, as binary mixtures, or as catalysts in various redox processes has attracted considerable interest. The role of osmium(VIII) as a catalyst in some redox reactions has been reviewed. Although the mechanism of catalysis depends on the nature of the substrate, oxidant and on experimental conditions, it has been shown that metal ions act as catalysts by one of these different paths such as the formation of complexes with reactants or oxidation of the substrate itself or through the formation of free radicals. In an earlier report, it has been observed that Os(VIII) forms a complex substrate, which gets oxidized by the oxidant to form an Os(VII) intermediate followed by the rapid reaction of Os(VII) with one more mole of oxidant to regenerate Os(VIII). In another report, it has been observed that oxidant-substrate complex reacts with Os(VIII) to form Os(VI), which reacts with oxidant in a fast step to regenerate Os(VIII). In some other reports, it is observed that Os(VIII) forms complex with substrate, which gets oxidized by the oxidant with the regeneration of Os(VIII). Hence, understanding the role of Os(VIII)
in redox reactions is important. Osmium(VIII) catalysis in
reactions involves several complexes, different oxidation
states of osmium, etc. We have observed that osmium(VIII)
catalyzes the oxidation of DL-ornithine monohydrochloride by
DPA in alkaline medium in micro amounts.

Literature survey reveals that there is no report on os-
mium(VIII) catalyzed oxidation of DL-ornithine monohy-
drochloride by DPA in alkaline medium. In earlier reports of
DPA oxidation,[18] the order in [OH⁻] was found to be less than
unity and periodate had a retarding effect in most of the reac-
tions, and monoperiodatoargentate(III) (MPA) was considered
be active species. However, in the present study, we have
observed entirely different kinetic observations, and diperioda-
toargentate(III) (DPA) itself is found to be an active form of
oxidant. In order to understand the active species of oxidant and
catalyst, to compute the activity of the catalyst and to propose
the appropriate mechanism, the title reaction is investigated in
detail. An understanding of the mechanism allows the chemistry
to be interpreted, understood and predicted.

2. EXPERIMENTAL

2.1. Materials and Reagents

All reagents used were of analytical reagent grade, and mil-
lipore water was used throughout the work. A solution of DL-
ornithine monohydrochloride (HiMedia Laboratories) was pre-
pared by dissolving an appropriate amount of recrystallised
sample in millipore water. The purity of DL-ornithine mono-
hydrochloride sample was checked by comparing its melting
point, 232°C with literature data [literature melting point =
233°C]. The required concentration of OMH was obtained from
its stock solution. The osmium(VIII) solution was prepared by
dissolving OsO₄ (Johnson Matthey) in 0.50 mol dm⁻³ NaOH. The
concentration was ascertained [19] by determining the un-
reacted [Fe(CN)₆]⁴⁻ with standard Ce(IV) solution in an acidic
medium. A stock standard solution of IO₃⁻ was prepared by
dissolving a known weight of KIO₄ (S.D. fine) in hot water, and
used after keeping for 24 h to complete the equilibrium. Its
concentration was ascertained by iodometrically [20] at neutral
pH maintained using phosphate buffer. The pH of the medium
in the solution was measured by Elico model (LI 120) pH me-
ter. KNO₃ (AR) and KOH (BDH) were used to maintain ionic
strength and alkalinity of the reaction, respectively. Aqueous
solution of AgNO₃ was used to study the product effect, Ag(I).
t-Butyl alcohol (S.D. Fine Chem.) was used to study the dielec-
tric constant of the reaction medium.

2.2. Preparation of DPA

DPA was prepared by oxidizing Ag(I) in presence of KIO₄ as
described elsewhere.[21] The mixture of 28 g of KOH and 23 g of
KIO₄ in 100 cm³ of water along with 8.5 g AgNO₃ was heated
just to boiling and 20 g of K₂S₂O₈ was added in several lots
with stirring and then allowed to cool. It was filtered through a
medium porosity fritted glass filter and 40 g of NaOH was added
slowly to the filtrate, whereupon a voluminous orange precipi-
tate agglomerates. The precipitate is filtered and washed three
to four times with cold water. The pure crystals were dissolved
in 50 cm³ water and warmed to 80°C with constant stirring
thereby some solid was dissolved to give a red solution. The
resulting solution was filtered when it was hot and on cooling at
room temperature, the orange crystals separated out and were
crystallized from water.

The complex was characterized from its UV spectrum, which
exhibited three peaks at 216, 255 and 362 nm. These spectral
features were identical to those reported earlier for DPA.[20] The
magnetic moment study revealed that the complex is diamag-
etic. The compound prepared was analyzed [21] for silver and
periodate by acidifying a solution of the material with HCl, re-
covering and weighing the AgCl for Ag and titrating the iodine
liberated when excess of KI was added to the filtrate for IO₃⁻.
The stock solution of DPA was used for the required [DPA]
solution in the reaction mixture.

2.3. Instruments Used

(i) For kinetic measurements, a Peltier accessory (temperature
control) attached to Varian CARY 50 Bio UV-visible spec-
trophotometer (Varian, Victoria-3170, Australia) was used.
(ii) For product analysis, a QP-2010S Shimadzu gas chromato-
graph mass spectrometer, Nicolet 5700-FT-IR spectrometer
(Thermo, U.S.A), 300 MHz ¹H NMR spectrophotometer
(Bruker, Switzerland) were used.
(iii) For pH measurements ELICO pH meter model LI 120 was
used.

2.4. Kinetic Measurements

The kinetic measurements were performed on a Varian
CARY 50 Bio UV-visible spectrophotometer. The kinetics was
followed under pseudo first-order condition where [OMH] >
[DPA] at 25 ± 0.1°C, unless specified. The reaction was initi-
ated by mixing the DPA to DL-ornithine monohydrochloride so-
lution, which also contained required concentrations of KNO₃,
KOH, catalyst Os(VIII) and KIO₄. The progress of reaction was
followed spectrophotometrically at 360 nm by monitoring de-
crease in absorbance due to DPA with the molar absorbancy
index, ‘ε’ to be 13900 ± 100 dm³ mol⁻¹ cm⁻¹. The spectral
changes during the chemical reaction for the standard condition
at 298 K are shown in Figure 1.

It is evident from the figure that the concentration of DPA
decreases at 360 nm. It was verified that there is a negligible
interference from other species present in the reaction mixture
at this wavelength.

The pseudo first-order rate constants, ‘k’c’, were determined
from the log (absorbance) versus time plots. The plots were
linear up to 85% completion of reaction under the range of
FIG. 1. Spectroscopic changes occurring in the oxidation of DL-ornithine monohydrochloride by alkaline DPA at 25°C, [DPA] = 5.0 × 10⁻⁵, [OMH] = 5.0 × 10⁻⁴, [OH⁻] = 0.08, [IO₄⁻] = 5.0 × 10⁻⁵, [Os] = 5.0 × 10⁻⁶ and I = 0.10 mol dm⁻³ with scanning time of: (1) 1.0, (2) 2.0, (3) 3.0, (4) 4.0, (5) 5.0 and (6) 6.0 min

[OH⁻] used. The orders for various species were determined from the slopes of plots of log kc versus respective concentrations of species except for [DPA] in which non-variation of ‘kc’ was observed as expected to the reaction condition. During the kinetics a constant concentration, viz. 5.0 × 10⁻⁵ mol dm⁻³ of KIO₄ was used throughout the study unless otherwise stated.

Since periodate is present in excess in DPA, the possibility of oxidation of DL-ornithine monohydrochloride by periodate in alkaline medium at 25°C was tested. The progress of the reaction was followed iodometrically. However, it was found that there was no significant reaction under the experimental conditions employed compared to the DPA oxidation of DL-ornithine monohydrochloride. The total periodate concentration was calculated by considering the periodate present in the DPA solution and that was additionally added.

In view of the modest concentration of alkali used in the reaction medium, attention was also directed to the effect of the reaction vessel surface on the kinetics. Use of polythene/acrylic wares and quartz or polyacrylate cells gave the same results, indicating that the surface did not have any significant effect on the reaction rates.

In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates. However, fresh solutions were nevertheless used for carrying out each kinetic only. Regression analysis of experimental data to obtain regression coefficient ‘r’ and the standard deviation ‘S’, of points from the regression line, was performed with the Microsoft office Excel—2003 program.

3. RESULTS

3.1. Stoichiometry and Product Analysis

Different sets of reaction mixtures containing varying ratios of DPA to DL-ornithine monohydrochloride in presence of constant amount of OH⁻, Os(VIII) and KNO₃, were kept for 3 hours in a closed vessel under nitrogen atmosphere. The remaining concentration of DPA was estimated spectrophotometrically at 360 nm. The results indicated 1:2 stoichiometry (OMH:DPA), as given in Scheme 1.

The main oxidation product was identified as 4-aminobutyric acid. The product was extracted with ether and recrystallized from aqueous alcohol. It was characterized by FT-IR, GC-MS and ¹H NMR spectral studies.

The presence of carboxylic acid was confirmed by IR spectroscopy, which showed >C=O stretching of carboxylic acid at 1708 cm⁻¹ indicating the presence of acidic C=O group, O-H stretching of carboxylic acid at 2848 cm⁻¹ indicating the presence of acidic -OH group and N-H stretching at 3427 cm⁻¹ indicating the presence of -NH₂ group in the product. 4-aminobutyric acid was further characterized by ¹H NMR spectrum (CDCl₃) two triplet at 2.31 δ (a) and 2.69 δ (c) and multiplet at 1.84 δ (due to (b) CH₂), 5.44 δ (s, 2H due to –NH₂) and 11.6 δ (s, H due to –COOH), –NH₂ and –OH were vanished on adding D₂O.

Further, the product was subjected to GC-MS spectral analysis. GC-MS data was obtained on a QP-2010S Shimadzu gas chromatograph mass spectrometer. The mass spectrum showed a molecular ion peak at 103 amu confirming 4-aminobutyric acid product (Figure 2).

All other peaks observed in GC-MS can be interpreted in accordance with the observed structure of the 4-aminobutyric acid.

The by-products were identified as ammonia by Nessler’s reagent and the CO₂ was qualitatively detected by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through the tube containing limewater. The formation of free Ag⁺ in solution was detected by adding KCl solution to the reaction mixture, which produced white turbidity due to the formation of AgCl. It was observed that 4-aminobutyric acid does not undergo further oxidation under the present kinetic conditions.
3.2. Reaction Orders

As the diperiodatoargentate(III) oxidation of DL-ornithine monohydrochloride in alkaline medium proceeds with a measurable rate in the absence of Os(VIII), the catalyzed reaction is understood to occur in parallel paths with contributions from both the catalyzed and uncatalyzed paths. Thus, the total rate constant ($k_T$) is equal to the sum of the rate constants of the catalyzed ($k_C$) and uncatalyzed ($k_U$) reactions, so $k_C = k_T - k_U$. Hence, the reaction orders have been determined from the slopes of log $k_C$ versus log (concentration) plots by varying the concentrations of OMH, IO$_4^-$, OH$^-$ and Os(VIII), in turn, while keeping others constant. The uncatalyzed reaction was followed under the condition, [DPA] = $5.0 \times 10^{-5}$, [OMH] = $5.0 \times 10^{-4}$, [OH$^-$] = 0.08, [IO$_4^-$] = $5.0 \times 10^{-5}$, $I = 0.10 / \text{mol dm}^{-3}$. The rate constant of uncatalyzed reaction ($k_U$) was obtained by the plot of log (absorbance) versus time by following the progress of the reaction spectrophotometrically at 360 nm.

3.3. Effect of [diperiodatoargentate(III)]

The DPA concentration was varied in the range of $1.0 \times 10^{-5}$ to $1.0 \times 10^{-4}$ mol dm$^{-3}$ and the linearity of the plots of log (absorbance) versus time up to 85% completion of the reaction ($r \geq 0.9858$, $S \leq 0.016$) indicates a reaction order of unity in [DPA]. This is also confirmed by varying of [DPA], which did not result in any change in the pseudo first-order rate constants, $k_C$ (Table 1).

3.4. Effect of [DL-ornithine monohydrochloride]

The DL-ornithine monohydrochloride concentration was varied in the range of $7.0 \times 10^{-5}$ to $7.0 \times 10^{-4}$ mol dm$^{-3}$ at 25°C while keeping other reactant concentrations and conditions constant. The $k_C$ values increased with the increase in concentration of DL-ornithine monohydrochloride indicating an apparent less than unit order dependence on [OMH] ($r \geq 0.9996$, $S \leq 0.009$) under the concentrations studied (Table 1). This is also confirmed in the plots of $k_C$ versus [OMH]$^{0.79}$ which is linear rather than the direct plot of $k_C$ versus [OMH] (Figure 3).

3.5. Effect of [Alkali]

The effect of alkali on the reaction has been studied in the range of 0.01 to 0.10 mol dm$^{-3}$ at constant concentrations of OMH, DPA, Os(VIII) and a constant ionic strength of 0.10 mol dm$^{-3}$. The rate constants decreased with increasing [alkali] and the order was found to be negative fractional order i.e., $-0.33$ (Table 1) ($r \geq 0.9948$, $S \leq 0.007$).
TABLE 1
Effect of variation of [DPA], [OMH], [OH\(^-\)], [IO\(^-\)\(_4\)] and [Os(VIII)] on the osmium(VIII) catalyzed oxidation of DL-ornithine monohydrochloride by diperiodatoargentate(III) in aqueous alkaline medium at 25°C and I = 0.10 mol dm\(^{-3}\)

<table>
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<th>[DPA] × 10(^5)</th>
<th>[OMH] × 10(^4)</th>
<th>[OH(^-)] × 10(^4)</th>
<th>[IO(^-)(_4)] × 10(^5)</th>
<th>[Os(VIII)] × 10(^6)</th>
<th>k(_T) × 10(^2)</th>
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3.6. Effect of [Periodate]
Periodate concentration was varied from 1.0 × 10\(^{-5}\) to 1.0 × 10\(^{-4}\) at constant [DPA], [OMH], [OH\(^-\)], [Os(VIII)] and ionic strength. It was observed that the added periodate had no effect on the reaction (Table 1).

3.7. Effect of [Os(VIII)]
The [Os(VIII)] concentration was varied from 1.0 × 10\(^{-6}\) to 1.0 × 10\(^{-5}\) mol dm\(^{-3}\) range, at constant concentrations of diperiodatoargentate(III), DL-ornithine monohydrochloride, alkali and ionic strength. The order in [Os(VIII)] was found to be unity from the linearity of the plot of \(k_C\) versus [Os(VIII)] (Table 1) (Figure 4) (\(r \geq 0.9994, S \leq 0.019\)).

3.8. Effect of Ionic Strength (I) and Dielectric Constant of the Medium (D)
The addition of KNO\(_3\) at constant [DPA], [Os(VIII)], [OMH], [OH\(^-\)] and [IO\(^-\)\(_4\)] was found that increasing ionic strength had no significant effect on the rate of the reaction.
OSMIUM(VIII) CATALYZED OXIDATION

\[
[\text{Ag(H}_2\text{IO}_6\text{(H}_2\text{IO}_6\text{)}^2+] + \text{H}_2\text{O} \xrightarrow{K_1} \text{[Ag(H}_2\text{IO}_6\text{)}^2_] + [\text{OH}^-]
\]

\[
\begin{align*}
\text{NH}_2 & \quad \text{COO}^- & \quad + [\text{OsO}_4(\text{OH})_2]^2+ & \text{Complex(C)} \\
& & \xrightarrow{K_2} & \\
\text{NH}_2 & \quad \text{CHO} & \quad + \text{Ag(II)} & \text{fast} + \text{CO}_2 & + 2\text{H}_3\text{IO}_6^{2-} & + [\text{OsO}_4(\text{OH})_2]^2- & + \text{2OH}^- & \text{[Ag(H}_2\text{IO}_6\text{)(H}_3\text{IO}_6\text{)}^2] + \text{H}_2\text{O} \\
\end{align*}
\]

\[
\text{Complex(C)} + [\text{Ag(H}_2\text{IO}_6\text{)}^2_] \xrightarrow{k} \text{slow} \text{NH}_2 + \text{Ag(II)} + \text{Co}_2 & + 2\text{H}_3\text{IO}_6^{2-} & + \text{[OsO}_4(\text{OH})_2]^2- \\
& \text{NH}_2 & \quad + \text{NH}_3 & + \text{Ag(I)} & \text{fast} \\
& \text{NH}_2 & \quad \text{CHO} & \quad + \text{Ag(II)} & \text{fast} + \text{2OH}^- & \text{COOH} & \text{[Ag(H}_2\text{IO}_6\text{)(H}_3\text{IO}_6\text{)}^2] + \text{H}_2\text{O} \\
\]

\text{SCH. 2. Detailed scheme for the Os(VIII) catalyzed oxidation of DL-ornithine monohydrochloride by alkaline diperiodatoargentate(III)}

Varying the t-butyl alcohol and water percentage varied dielectric constant of the medium, ‘D’. The D values were calculated from the equation \( D = D_w V_w + D_B V_B \), where \( D_w \) and \( D_B \) are dielectric constants of pure water and t-butyl alcohol, respectively, and \( V_w \) and \( V_B \) are the volume fractions of components water and t-butyl alcohol, respectively, in the total mixture. There was no effect of dielectric constant on the rate of reaction.

3.9. Effect of Initially Added Products
Initially added products, Ag(I), and 4-aminobutyric acid did not have any significant effect on the rate of reaction.

Thus, from the observed experimental results-
The rate law for Os(VIII) catalyzed reaction is given as:

\[
\text{Rate} = k_C \ [\text{DPA}]^{1.0} \ [\text{OMH}]^{0.79} \ [\text{OH}^-]^{-0.33} \ [\text{Os(VIII)}]^{1.0}
\]

3.10. Test for Free Radicals (Polymerization Study)
The intervention of free radicals was examined as follows, the reaction mixture, to which a known quantity of acrylonitrile scavenger has been added initially, was kept in an inert atmosphere for 1 hour. Upon diluting the reaction mixture with methanol, precipitate resulted, suggesting there is participation of free radicals in the reaction. The blank experiments of either DPA or DL-ornithine alone with acrylonitrile did not induce any polymerization under the same condition as those induced for the reaction mixture. Initially added acrylonitrile decreases the rate of reaction indicating free radical intervention, which is the case in earlier work.\([24]\)

3.11. Effect of Temperature (T)

The influence of temperature on the rate of reaction was studied at 15, 25, 35 and 45°C. The rate constants (k), of the slow step of Scheme 2 were obtained from the slopes and the intercept of the plots of \( [\text{Os(VIII)}]/k_C \text{versus } 1/\text{[OMH]} \) and \( [\text{Os(VIII)}]/k_C \text{versus } \text{[OH}^-] \) at four different temperatures. The values are given in Table 2. The activation parameters for the rate determining step were obtained by the least square method of plot of log \( k \) versus \( 1/T \) and are presented in Table 2.

3.12. Catalytic Activity

It has been pointed out by Moelwyn-Hughes\([25]\) that in presence of the catalyst, the uncatalyzed and catalyzed reactions proceed simultaneously, so that

\[
k_T = k_U + K_C \ [\text{catalyst}]^x
\]

Here \( k_T \) is the observed pseudo first-order rate constant in the presence Os(VIII) catalyst, \( k_U \) the pseudo first-order rate constant for the uncatalyzed reaction, \( K_C \) the catalytic constant and ‘x’ the order of the reaction with respect to [Os(VIII)]. In the
Table 2

Activation parameters and thermodynamic quantities for the osmium(VIII) catalyzed oxidation of DL-ornithine monohydrochloride by diperiodatoargentate(III) in aqueous alkaline medium with respect to the slow step of Scheme 2: (A) Effect of temperature, (B) Activation parameters (Scheme 2), (C) Effect of temperature to calculate $K_1$ and $K_2$ for the Os(VIII) catalyzed oxidation of DL-ornithine monohydrochloride by diperiodatoargentate(III) in alkaline medium, (D) Thermodynamic quantities using $K_1$ and $K_2$

(a) Temperature (K) $k \times 10^{-4}$ (dm$^3$ mol$^{-1}$ s$^{-1}$)

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<th>Temperature (K)</th>
<th>$k \times 10^{-4}$</th>
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(b) Parameters Values

- $E_a$ (kJ mol$^{-1}$) 34.6
- $\Delta H^\#$ (kJ mol$^{-1}$) 32.2
- $\Delta S^\#$ (J K$^{-1}$ mol$^{-1}$) -53.8
- $\Delta G^\#$ (kJ mol$^{-1}$) 48.2
- log $A$ 10.4

(c) Temperature (K) $K_1 \times 10^1$ (mol dm$^{-3}$) $K_2 \times 10^{-3}$ (dm$^3$ mol$^{-1}$)

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<th>Temperature (K)</th>
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(d) Thermodynamic quantities Values from $K_1$ Values from $K_2$

- $\Delta H$ (kJ mol$^{-1}$) -25.6 33.9
- $\Delta S$ (J K$^{-1}$ mol$^{-1}$) -108.4 171.6
- $\Delta G_{298}$ (kJ mol$^{-1}$) 6.6 -17.3

$[\text{DPA}] = 5.0 \times 10^{-5}$, $[\text{OMH}] = 5.0 \times 10^{-4}$, $[\text{OH}^-] = 0.08$, $[\text{IO}_4^-] = 5.0 \times 10^{-5}$, $[\text{Os(VIII)}] = 5.0 \times 10^{-6}$, $I = 0.10$ / mol dm$^{-3}$.

The values of $K_C$ were evaluated at different temperatures and found to vary with temperature. Further, plots of log $K_C$ versus $1/T$ were linear and the values of energy of activation and other activation parameters with reference to catalyst were computed. These results are summarized in Table 3. The value of $K_C$ at 25 °C is $3.7 \times 10^3$.

Table 3

Values of catalytic constant ($K_C$) at different temperatures and activation parameters calculated using $K_C$ values

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<th>Temperature (K)</th>
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- $E_a$ (kJ mol$^{-1}$) 43.2
- $\Delta H^\#$ (kJ mol$^{-1}$) 40.7
- $\Delta S^\#$ (J K$^{-1}$ mol$^{-1}$) -39.9
- $\Delta G^\#$ (kJ mol$^{-1}$) 52.6
- log $A$ 11.1

$[\text{DPA}] = 5.0 \times 10^{-5}$, $[\text{OMH}] = 5.0 \times 10^{-4}$, $[\text{OH}^-] = 0.08$, $[\text{IO}_4^-] = 5.0 \times 10^{-5}$, $[\text{Os(VIII)}] = 5.0 \times 10^{-6}$, $I = 0.10$ / mol dm$^{-3}$.

4. DISCUSSION

In the later period of the 20th century, the kinetics of oxidation of some organic and inorganic substrates have been studied by Ag(III) species, which may be due to its strong versatile
nature of two electrons oxidant. Among the various species of Ag(III), Ag(OH)\(_4\)\(^-\), diperiodatoargentate(III) and ethylenediamine (biguanide), (EBS), silver(III) are of maximum attention to the researchers due to their relative stability.\(^{[26]}\) The stability of Ag(OH)\(_4\)\(^-\) is very sensitive towards traces of dissolved oxygen and other impurities in the reaction medium whereupon it had not drawn much attention. However, the other two forms of Ag(III)\(^{[27]}\) are considerably stable; the DPA is used in highly alkaline medium and EBS is used in highly acidic medium.

The literature survey\(^{[21]}\) reveals that the water soluble diperiodatoargentate(III) (DPA) has a formula [Ag(IO\(_6\))\(_2\)]\(^{7-}\) with dsp\(^2\) configuration of square planar structure, similar to diperiodatocopper(III) complex with two bidentate ligands, periodate to form a planar molecule. When the same molecule is used in alkaline medium, it is unlikely to exist as [Ag(IO\(_6\))\(_2\)]\(^{7-}\) as periodate is known to be in various protonated forms\(^{[28]}\) depending on pH of the solution as given in following multiple equilibria (3)–(5).

\[
\begin{align*}
H_5IO_6 & \rightleftharpoons H_4IO_6^- + H^+ \quad [3] \\
H_4IO_6^- & \rightleftharpoons H_3IO_6^{2-} + H^+ \quad [4] \\
H_3IO_6^{2-} & \rightleftharpoons H_2IO_6^{3-} + H^+ \quad [5]
\end{align*}
\]

Periodic acid exists as H\(_3\)IO\(_6\) in acid medium and as H\(_4\)IO\(_6^-\) near pH 7. Hence, under alkaline conditions as employed in this study, the main species are expected to be H\(_3\)IO\(_6^-\) and H\(_2\)IO\(_6^{3-}\). At higher concentrations, periodate also tends to dimerise.\(^{[4]}\) However, formation of this species is negligible under conditions employed for kinetic study. On contrary, the authors\(^{[7,8]}\) in their recent studies have proposed the DPA species as [Ag(HL)\(_2\)]\(^{3-}\) in which ‘L’ is a periodate with uncertain number of protons and ‘HL’ is a protonated periodate of uncertain number of protons. This can be ruled out by considering the alternative form\(^{[28]}\) of IO\(_7^-\) at pH > 7, which is in the form H\(_3\)IO\(_6^-\) or H\(_2\)IO\(_6^{3-}\). Hence, DPA could be as [Ag(H\(_3\)IO\(_6\))\(_2\)]\(^{-}\) or [Ag(H\(_2\)IO\(_6\))\(_2\)]\(^{3-}\) in alkaline medium. Therefore, under the present condition, diperiodatoargentate(III), may be depicted as [Ag(H\(_3\)IO\(_6\))\(_2\)]\(^{-}\). The similar speciation of periodate in alkali was proposed\(^{[29]}\) for diperiodatocinnickelate(IV).

It is known that DL-ornithine exists in the form of zwitterion in aqueous medium. In highly acidic medium it exists in the protonated form, where as in highly basic medium it is in the deprotonated form,\(^{[30]}\) as [OsO\(_5\)(OH)]\(^{3-}\). At higher concentration of OH\(^-\), [OsO\(_5\)(OH)]\(^{3-}\) is significant. At lower concentrations of OH\(^-\), as employed in the present study, and since the rate of oxidation decreased with increase in [OH\(^-\)], it is reasonable that [OsO\(_4\)(OH)]\(^{2-}\) was operative and its formation is important in the reaction. In earlier reports\(^{[15]}\) it has been observed that in Os(VIII) catalyzed reaction, in view of less than unit order in substrate, unit order in Os(VIII) and oxidant, Os(VIII) is regenerated through formation of Os(VII). In another case,\(^{[16]}\) Os(VIII) is regenerated by Os(VI) intervention in view of unit order each in osmium, substrate and oxidant. In some other reports,\(^{[17]}\) it is observed that Os(VIII) forms a complex with substrate, which is oxidized by the oxidant with regeneration of the catalyst. Hence, the study of behavior of Os(VIII) in catalyzed reaction becomes significant. To explain all the observed orders, Scheme 2 is proposed for osmium(VIII) catalyzed reaction.

In the prior equilibrium step 1, the hydroxyl ion concentration with fractional order in OH\(^-\) concentration, the main oxidant species is likely to be [Ag(H\(_3\)IO\(_6\))\(_2\)]\(^{-}\} and its formation by the above equilibrium is important in the present study. The less than unit order in [OMH] presumably results from the formation of a complex (C) between the Os(VIII) species and DL-ornithine monohydrochloride. This complex (C) reacts with one mole of DPA in a slow step to give the free radical species of OMH, Ag(II) with the regeneration of catalyst, Os(VIII). Further this free radical species of OMH reacts with Ag(II) in a fast step to form 4-aminobutyaldehyde intermediate. This intermediate reacts with one mole of DPA in a further fast step to form the products such as 4-aminobutyric acid, Ag(I) and periodate as given in Scheme 2.

The probable structure of the complex (C) is given as,

![Complex structure](image)

Spectroscopic evidence for the complex formation between Os(VIII) and OMH was obtained from UV–vis spectra of OMH (5.0 \(\times\) 10\(^{-4}\)), Os(VIII) (5.0 \(\times\) 10\(^{-6}\), [OH\(^-\)] = 0.08 mol dm\(^{-3}\)) and a mixture of both. A bathochromic shift of 4 nm from 364 nm to 368 nm in the spectra of Os(VIII) to the mixture of Os(VIII) and OMH was observed. Attempts to separate and isolate the complex were not successful. The Michaelis-Menten plot proved the complex formation between catalyst and substrate, which explains less than unit order in [OMH]. Such a complex between a catalyst and substrate has also been observed in other studies.\(^{[17]}\) The rate law (7) for the Scheme 2

4.1. Mechanism

Osmium(VIII) is known to form different complexes at different OH\(^-\) concentrations, [OsO\(_4\)(OH)]\(^{2-}\) and
could be derived as,
\[
\text{rate} = \frac{-d[DPA]}{dt} = \frac{kK_1K_2[OMH][Os(VIII)][DPA]}{K_1 + K_1K_2[OMH] + [OH^-] + K_2[OMH][OH^-]} \quad [6]
\]
\[
\text{rate} = \frac{[DPA]}{[DPA]} = k_C = k_T - k_U = \frac{kkK_1K_2[OMH][Os(VIII)]}{K_1 + K_1K_2[OMH] + [OH^-] + K_2[OMH][OH^-]} \quad [7]
\]

This explains all the observed kinetic orders of different species. The rate law (7) can be rearranged to be equation (8), which is suitable for verification.

\[
\frac{[Os(VIII)]}{k_C} = \frac{[OH^-]}{kkK_1K_2[OMH]} + \frac{[OH]}{kK_1} + \frac{1}{K_1K_2[OMH]} + \frac{1}{k} \quad [8]
\]

According to Equation (8), other conditions being constant, the plots of \([Os(VIII)]/k_C versus [OH^-]\) and \([Os(VIII)]/k_C versus 1/[OMH]\) should be linear and found to be so (Figure 5). From the intercepts and slopes of such plots, the reaction constants \(K_1\) and \(K_2\) were calculated as \((6.8 \pm 0.1) \times 10^{-2}\) mol dm\(^{-3}\), \((1.07 \pm 0.03) \times 10^3\) dm\(^3\) mol\(^{-1}\), \((2.2 \pm 0.2) \times 10^4\) dm\(^3\) mol\(^{-1}\) s\(^{-1}\), respectively. The value \(K_1\) obtained is in good agreement with previously reported value.\(^{[3]}\) These constants were used to calculate the rate constants and compared with the experimental \(k_C\) values and found to be in reasonable agreement with each other, which fortifies Scheme 2.

The thermodynamic quantities for the different equilibrium steps, in Scheme 2 can be evaluated as follows. The DL-ornithine monohydrochloride and hydroxide ion concentrations (Table 1) were varied at different temperatures. The thermodynamic quantities for the different equilibrium steps, in Scheme 2 can be evaluated as follows. The DL-ornithine monohydrochloride and hydroxide ion concentrations (Table 1) were varied at different temperatures. The plots of \([Os(VIII)]/k_C versus [OH^-]\) and \([Os(VIII)]/k_C versus 1/[OMH]\) (\(r \geq 0.9996, S \leq 0.00133\), \([Os(VIII)]/k_C versus [OH^-]\) (\(r \geq 0.9948, S \leq 0.00142\) should be linear as shown in Figure 5. From the slopes and intercepts, the values of \(K_1\) are calculated at different temperatures. A vant Hoff’s plot was made for the variation of \(K_1\) with temperature [i.e., \(\log K_1 versus 1/T\) \(r \geq 0.9213, S \leq 0.1034\)] and the values of the enthalpy of reaction \(\Delta H\), entropy of reaction \(\Delta S\) and free energy of reaction \(\Delta G\), were calculated. These values are also given in Table 2. A comparison of the \(\Delta H\) value of second step (33.9 k J mol\(^{-1}\)) of Scheme 2 with that of \(\Delta H^0\) (32.2 k J mol\(^{-1}\)) obtained for the slow step of the reaction shows that these values mainly refer to the rate limiting step, supporting the fact that the reaction before rate determining step is fairly slow and involves high activation energy.\(^{[1]}\) In the same manner, \(K_2\) values were calculated at different temperatures and the corresponding values of thermodynamic quantities are given in Table 2.

The negligible effect of ionic strength and dielectric constant in the reaction might be due to the presence of various ions in reaction (Scheme 2). The moderate \(\Delta H^0\) and \(\Delta S^0\) values are favorable for electron transfer reaction. The value of \(\Delta H^0\) was due to energy of solution changes in the transition state. The negative value of \(\Delta S^0\) (\(-53.8\) J K\(^{-1}\) mol\(^{-1}\)) suggests that the intermediate complex (C) is more ordered than the reactants.\(^{[32]}\) The observed modest enthalpy of activation and a higher rate constant for the slow step indicates that the oxidation presumably occurs via an inner-sphere mechanism. This conclusion is supported by earlier observations.\(^{[33]}\) The activation parameters evaluated for the reaction explain the catalytic effect on the reaction. The catalyst Os(VIII) forms the complex (C) with substrate which enhances the reducing property of the substrate than that without catalyst. Further, the catalyst Os(VIII) modifies the reaction path by lowering the energy of activation.
5. CONCLUSIONS

The Os(VIII) catalyzed oxidation of DL-ornithine monohydrochloride by diperiodatooargentate(III) was studied. Oxidation products were identified. Among the various species of Ag(III) in alkaline medium, in earlier reports the monoperiodatoargentate(III) was the active species, whereas diperiodatoargentate(III) itself is considered to be the active species for the title reaction. Active species of Os(VIII) is \([\text{OsO}_4(\text{OH})_2]^2\)−. Activation parameters were evaluated for the catalyzed reaction. Catalytic constants and the activation parameters with reference to catalyst were also computed. The overall sequence described here is consistent with all the experimental evidences including the product, spectral, mechanistic and kinetic studies.

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APPENDIX

According to Scheme 2

\[
\text{rate} = k[C][\text{Ag(H}_3\text{IO}_6)_2]^- = \frac{kK_1K_2[\text{OMH}][\text{Ost(VIII)}][\text{DPA}]}{[\text{OH}^-]}
\]
The total concentration of DPA is given by (where T and F stand for total and free)

\[ [\text{DPA}]_T = [\text{DPA}]_F + [\text{Ag(H}_3\text{IO}_6)_2]^- \]

\[ = [\text{DPA}]_F \left[ \frac{[\text{OH}^-] + K_2}{[\text{OH}^-]} \right] \]

Therefore,

\[ [\text{DPA}]_F = \left[ \frac{[\text{DPA}]_T[\text{OH}^-]}{[\text{OH}^-] + K_1} \right] \]  \hspace{1cm} [2]

Similarly,

\[ [\text{OMH}]_T = [\text{OMH}]_F + [C] \]

\[ = [\text{OMH}]_F + K_2[\text{OMH}]_F[\text{Os(VIII)}] \]

\[ = [\text{OMH}]_F(1 + K_2[\text{Os(VIII)}]) \]

In view of low concentration of Os(VIII) used,

\[ [\text{OMH}]_T = [\text{OMH}]_F \]  \hspace{1cm} [3]

Similarly,

\[ [\text{OH}^-]_T = [\text{OH}^-]_F \]  \hspace{1cm} [4]

\[ [\text{Os(VIII)}]_T = [\text{Os(VIII)}]_F + [C] \]

\[ = [\text{Os(VIII)}]_F + K_2[\text{OMH}][\text{Os(VIII)}]_F \]

\[ [\text{Os(VIII)}]_F \left[ \frac{[\text{Os(VIII)}]_T}{1 + K_2[\text{OMH}]} \right] \]  \hspace{1cm} [5]

Substituting equations (2)–(5) in equation (1) and omitting the subscripts T and F, we get

\[ \frac{\text{rate}}{[\text{DPA}]} = k_C = k_T - k_U \]

\[ = \frac{k K_1 K_2[\text{OMH}][\text{Os(VIII)}]}{K_1 + K_1 K_2[\text{OMH}] + [\text{OH}^-] + K_2[\text{OMH}][\text{OH}^-]} \]
Mechanistic aspects of uncatalyzed and ruthenium(III) catalyzed oxidation of DL-ornithine monohydrochloride by silver(III) periodate complex in aqueous alkaline medium

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ABSTRACT

The oxidation of an amino acid, DL-ornithine monohydrochloride (OMH) by diperiodatoargentate(III) (DPA) was carried out both in the absence and presence of ruthenium(III) catalyst in alkaline medium at 25 °C and a constant ionic strength of 0.10 mol dm⁻³ spectrophotometrically. The reaction was of first order in both catalyzed and uncatalyzed cases, with respect to [DPA] and was less than unit order in [OMH] and negative fraction in [alkali]. The order with respect to [OMH] changes from first order to zero order as the [OMH] increases. The order with respect to Ru(III) was unity. The uncatalyzed reaction in alkaline medium has been shown to proceed via a DPA–OMH complex, which decomposes in a rate determining step to give the products. Where as in catalyzed reaction, it has been shown to proceed via a Ru(III)–OMH complex, which further reacts with two molecules of DPA in a rate determining step to give the products. The reaction constants involved in the different steps of the mechanisms were calculated for both the reactions. The catalytic constant (Kcat,comp.) was also calculated for catalyzed reaction at different temperatures. The activation parameters with respect to slow step of the mechanism and also the thermodynamic quantities were determined.

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1. Introduction

Amino acids act not only as the building blocks in protein synthesis but they also play a significant role in metabolism and have been oxidized by a variety of oxidizing agents [1]. The study of the oxidation of amino acids is of interest because of their biological significance and selectivity towards the oxidant to yield the different products [2]. Ornithine is one of the non-protein amino acids which is derived from the break down of arginine during the citric acid cycle. It is the most potent amino acid studied for stimulating the production of release of growth hormone in the body from the pituitary gland, which in turn helps with fat metabolism. It is further required for a properly functioning immune system and liver and assists in ammonia detoxification and liver rejuvenation. It is also of use in healing and repairing skin and tissue. Ornithine has the ability to regenerate the thymus gland, liver, and heart tissue, enhance muscle growth, and increase immune system function [3].

Diperiodatoargentate(III) (DPA) is a powerful oxidizing agent in alkaline medium with the reduction potential [4], 1.74 V. It is widely used as a volumetric reagent for the determination of various organic and inorganic species [5,6]. Jayaprakash Rao et al. [7,8] have used DPA as an oxidizing agent for the kinetics of oxidation of various substrates. They normally found that order with respect to both oxidant and substrate was unity and OH⁻ was found to enhance the rate of reaction. It was also observed that they did not arrive the possible active species of DPA in alkali and on the other hand they proposed mechanisms by generalizing the DPA as [Ag(HL)L]+[Ru(III)]⁻. However, Kumar et al. [9–11] put an effort to give an evidence for the reactive form of DPA in the large scale of alkaline pH. When the Ag(III) species are involved, it would be interesting to know which of the species is the active oxidant. In the present investigation, we have obtained the evidence for the reactive species for the DPA in alkaline medium. The DPA is a metal complex with Ag in 3+ oxidation state like Cu³⁺ in DPC and Fe⁺ in hemoglobin.

Transition metals are known to catalyze many oxidation–reduction reactions since they involve multiple oxidation states. In recent years, the use of transition metal ions such as osmium, ruthenium, palladium, chromium and iridium either alone or as binary mixtures, as catalysts in various redox processes has attracted considerable interest [12]. The Ru(III) acts as a catalyst in the oxidation of many organic and inorganic substrates [13,14]. The catalyzed mechanism can be quite complicated due to formation of different intermediate complexes, and different oxidation states of ruthenium(III). Although the mechanism of catalysis depends on the nature of the substrate, oxidant and on experimental conditions, it has been shown [15] that metal ions act as catalysts...
by one of these different paths such as the formation of complexes with reactants or oxidation of the substrate itself or through the formation of free radicals. In earlier reports [16], it has been observed that Ru(III) forms a complex with the substrate, which gets oxidized by the oxidant to form Ru(IV)–substrate complex followed by the rapid redox decomposition to regenerate Ru(III). In another report [17], it has been observed that there involves the formation of a Ru(III)–substrate complex with further cleavage in a concerted manner giving rise to a Ru(I) species, which gets rapidly oxidized by the oxidant to regenerate the catalyst. In some other reports [18], it is observed that Ru(III) forms a complex with substrate and is oxidized by the oxidant with the regeneration of the catalyst. Hence, understanding the role of Ru(III) in catalyzed reaction is important. We have observed that, ruthenium(III) catalyzes the oxidation of OMH by DPA in alkaline medium in micro amounts.

The literature survey reveals that there is no report on the uncatalyzed and catalyzed oxidative mechanism of n-ornithine monohydrochloride [(±)-2,5-diaminopentanoic acid monohydrochloride] by diperiodatoargentate(III) in alkaline medium. Such oxidation studies may throw some light on the mechanism of conversions of the compounds in biological systems. In earlier reports of DPA oxidation [18], the order in [OH⁻] was found to be less than unity and periodate had a retarding effect in most of the reactions and monoperiodatoargentate(III) (MPA) was considered to be active species. However, in the present study we have observed entirely different kinetic observations and diperiodatoargentate(III) (DPA) itself is found to be active form of oxidant. In order to understand the active species of oxidant and catalyst, to compute the activity of the catalyst and to propose the appropriate mechanisms, the title reaction is investigated in detail. An understanding of the mechanism allows the chemistry to be interpreted, understood and predicted.

2. Experimental

2.1. Materials and reagents

All reagents used were of analytical reagent grade and millipore water was used throughout the work. A solution of n-ornithine monohydrochloride (HiMedia Laboratories) was prepared by dissolving an appropriate amount of recrystallized sample in millipore water. The purity of n-ornithine monohydrochloride sample was checked by comparing its melting point, 232 °C with the literature data [literature melting point = 233 °C]. The required concentration of OMH was obtained from its stock solution. A standard stock solution of Ru(III) was prepared by dissolving RuCl₃ (S.D. Fine Chem.) in 0.20 mol dm⁻³ HCl. The concentration was determined [19] by EDTA titration.

A stock standard solution of IO₄⁻ was prepared by dissolving a known weight of KIO₄ (S.D. Fine Chem.) in hot water and used after keeping for 24 h to complete the equilibrium. Its concentration was ascertained iodometrically [20] at neutral pH maintained using phosphate buffer. The pH of the medium in the solution was measured by ELICO (LI 120) pH meter. KNO₃ (AR) and KOH (BDH) were used to maintain ionic strength and alkalinity of the reaction, respectively. Aqueous solution of AgNO₃ was used to study the product effect, Ag(I), t-Butyl alcohol (S.D. Fine Chem.) was used to study the dielectric constant of the reaction medium.

2.2. Preparation of DPA

DPA was prepared by oxidizing Ag(I) in the presence of KIO₄ as described elsewhere [21]. The mixture of 28 g of KOH and 23 g of KIO₄ in 100 cm³ of water along with 8.5 g AgNO₃ was heated just to boiling and 20 g of K₂S₂O₈ was added in several lots with stirring and then allowed to cool. It was filtered through a medium porosity fritted glass filter and 40 g of NaOH was added slowly to the filtrate, whereupon a voluminous orange precipitate agglomerates. The precipitate is filtered and washed 3–4 times with cold water. The pure crystals were dissolved in 50 cm³ water and warmed to 80 °C with constant stirring thereby some solid was dissolved to give a red solution. The resulting solution was filtered when it was hot and on cooling at room temperature, the orange crystals separated out and were recrystallized from water.

The complex was characterized from its UV spectrum, which exhibited three peaks at 216, 255 and 362 nm. These spectral features were identical to those reported earlier for DPA [20]. The magnetic moment study revealed that the complex is diamagnetic. The compound prepared was analyzed [21] for silver and periodate by acidifying a solution of the material with HCl, recovering and weighing the AgCl for Ag and titrating the iodine liberated when excess of KI was added to the filtrate for I₂. The stock solution of DPA was used for the required [DPA] solution in the reaction mixture.

2.3. Instruments used

(i) For kinetic measurements, a Peltier Accessory (temperature control) attached to Varian CARY 50 Bio UV–Visible spectrophotometer (Varian, Victoria-3170, Australia) connected to a rapid kinetic accessory (HI-TECH SFA-12, UK) was used.

(ii) For product analysis, a QP-2010S Shimadzu gas chromatograph mass spectrometer, Nicolet 5700-FT-IR spectrometer (Thermo, USA), 300 MHz ¹H NMR spectrophotometer (Bruker, Switzerland) were used.

(iii) For pH measurements ELICO pH meter model LI 120 was used.

2.4. Kinetic measurements

Kinetic measurements were performed on a Varian CARY 50 Bio UV–Visible spectrophotometer. The kinetics was followed under pseudo-first-order conditions where [OMH] > [DPA] in both uncatalyzed and catalyzed reactions at 25.0 ± 0.1 °C, unless otherwise specified. In the absence of catalyst the reaction was initiated by mixing the DPA to OMH solution, which also contained required concentrations of KNO₃, KIO₄ and KOH. The reaction in the presence of Ru(III) catalyst was initiated by mixing the DPA to OMH solution which also contained required concentrations of KNO₃, KOH, KIO₄ and Ru(III) catalyst. Since the initial rate was too fast to be monitored by usual methods in the catalyzed reaction, the kinetic measurements were performed on a Hitachi 150-20 UV–Visible spectrophotometer attached to a rapid kinetic accessory (HI-TECH SFA-12, UK). The progress of the reaction was followed spectrophotometrically at 360 nm by monitoring the decrease in absorbance due to DPA with the molar absorbancy index, ε to be 13900 ± 100 dm² mol⁻¹ cm⁻¹ in both catalyzed and uncatalyzed reaction. It was verified that there is a negligible interference from other species present in the reaction mixture at this wavelength.

The reaction was followed to more than 50% completion of the reaction. Plots of log(absorbance) versus time lead to the first-order rate constants (k_U or k_C). The plots were linear up to 85% completion of reaction. The orders for various species were determined from the slopes of plots of log(k_U or k_C) versus respective concentration of species except for [DPA] in which non-variation of k_U and k_C was observed as expected to the reaction condition. The rate constants were reproducible within ±5%. During the kinetics, a constant concentration viz. 5.0 × 10⁻⁵ mol dm⁻³ of KIO₄ was used throughout the study unless otherwise stated. Thus, the possibility of oxidation of n-ornithine monohydrochloride by perio-
date was tested and found that there was no significant interference due to KIO₄ under both the experimental conditions. The total concentration of OH⁻ and IO₄⁻ was calculated by considering the amount present in the DPA solution and that additionally added. Kinetics runs were also carried out in N₂ atmosphere in order to understand the effect of dissolved oxygen on the rate of reaction. No significant difference in the results was obtained under a N₂ atmosphere and in the presence of air. In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates. The spectral changes during the chemical reaction for the standard condition at 25 °C are shown in Fig. 1 (for uncatalyzed). It is evident from the figure that the concentration of DPA decreases at 360 nm.

In view of the modest concentration of alkali used in the reaction medium, attention was also directed to the effect of the reaction vessel surface on the kinetics. Use of polythene/acrylic wares and quartz or polyacrylate cells gave the same results, indicating that the surface did not have any significant effect on the reaction rates.

Regression analysis of experimental data to obtain regression coefficient ‘r’ and the standard deviation ‘S’, of points from the regression line, was performed with the Microsoft office Excel-2003 program.

3. Results

3.1. Stoichiometry and product analysis

Different sets of reaction mixtures containing varying ratios of DPA to OMH in the presence of constant amount of OH⁻ and KNO₃ in uncatalyzed reaction and a constant amount of Ru(III) in catalyzed reaction were kept for 2 h in a closed vessel under nitrogen atmosphere. The remaining concentration of DPA was estimated spectrophotometrically at 360 nm. The results indicated 1:2 stoichiometry (OMH:DPA) for both the reactions as given in Scheme 1.

The stoichiometric ratio in both the cases suggests the main product as 4-aminobutyric acid. The product was extracted with ether and recrystallized from aqueous alcohol. It was characterized by FT-IR, GC–MS, and ¹H NMR spectral studies.

The presence of carboxylic acid was confirmed by IR spectroscopy [22] which showed >C=O stretching of carboxylic acid at 1708 cm⁻¹ indicating the presence of acidic C=O group, O–H stretching of carboxylic acid at 2848 cm⁻¹ indicating the presence of acidic –OH group and N–H stretching at 3427 cm⁻¹ indicating the presence of –NH₂ group in the product. 4-aminobutyric acid was further characterized by ¹H NMR spectrum (CDCl₃) two triplet at 2.31 δ (a) and 2.69 δ (c) and multiplet at 1.84 δ (due to (b) CH₂).

**Scheme 1.** Stoichiometry of uncatalyzed and ruthenium(III) catalyzed oxidation of DL-ornithine monohydrochloride by alkaline diperiodatoargentate(III).

**Fig. 1.** Spectroscopic changes occurring in the oxidation of α-ornithine monohydrochloride by alkaline DPA at 25 °C, [DPA] = 5.0 × 10⁻⁵, [OMH] = 5.0 × 10⁻⁴, [OH⁻] = 0.08, [IO₄⁻] = 5.0 × 10⁻⁵ and I = 0.10 mol dm⁻³ with scanning time of: (1) 1.0, (2) 2.0, (3) 3.0, (4) 4.0, (5) 5.0 and (6) 6.0 min.

**Fig. 2.** GC–mass spectrum of 4-aminobutyric acid with its molecular ion peak at 103 m/z.
5.44 $\delta$ (s, 2H due to $-\text{NH}_2$) and 11.6 $\delta$ (s, H due to $-\text{COOH}$), $-\text{NH}_2$ and $-\text{OH}$ were vanished on adding D$_2$O. Further, the product was subjected to GC–MS spectral analysis. GC–MS data was obtained on a QP-2010S Shimadzu gas chromatograph mass spectrometer. The mass spectrum showed a molecular ion peak at 103 amu confirming 4-aminobutyric acid product (Fig. 2). All other peaks observed in GC–MS can be interpreted in accordance with the observed structure of the 4-aminobutyric acid.

The by-products were identified as ammonia by Nessler’s reagent [23] and the CO$_2$ was qualitatively detected by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through the tube containing limewater. The formation of free Ag$^+$ in solution was detected by adding KCl solution to the reaction mixture, which produced white turbidity due to the formation of AgCl. It was observed that 4-aminobutyric acid does not undergo further oxidation under the present kinetic conditions.

### Table 1

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<th>[OMH] $\times 10^4$ (mol dm$^{-3}$)</th>
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plots by varying the concentrations of OMH, IO₄⁻, OH⁻ and catalyst Ru(III), in turn while keeping others constant.

3.3. Effect of [diperiodatoargentate(III)]

In the absence and in the presence of Ru(III) catalyst, the DPA concentration was varied in the range of 1.0 × 10⁻⁵–1.0 × 10⁻⁴ mol dm⁻³ and the linearity of the plots of log(absorbance) versus time up to 85% completion of the reaction indicates a reaction order of unity in [DPA] (r ≥ 0.918, S < 0.014). This is also confirmed by varying of [DPA], which did not result in any change in the pseudo-first-order rate constants, k_U (Table 1 uncatalyzed), k_C (Table 2 Ru(III) catalyzed).

3.4. Effect of [α-ornithine monohydrochloride]

In both the cases the α-ornithine monohydrochloride concentration was varied in the range of 7.0 × 10⁻⁵–7.0 × 10⁻⁴ mol dm⁻³ at 25 °C while keeping other reactant concentrations and conditions constant. The k_U and k_C values increased with the increase in concentration of OMH indicating an apparent less than unit order dependence on [OMH] under the conditions of experiment (Table 1 uncatalyzed and Table 2 Ru(III) catalyzed). This was also confirmed by the plots of k_U versus [OMH]⁰.₈₁ and k_C versus [OMH]⁰.₈₂ which were linear rather than the direct plot of k_U versus [OMH] and k_C versus [OMH] (Fig. 3). (r ≥ 0.994, S ≤ 0.003 for uncatalyzed, r ≥ 0.997, S ≤ 0.024 for Ru(III) catalyzed). However, the order with respect to [OMH] changes from first order to zero order as the [OMH] increases.

3.5. Effect of [Ru(III)]

The ruthenium(III) concentration was varied from 1.0 × 10⁻⁶ to 1.0 × 10⁻⁵ mol dm⁻³ range, at constant concentrations of DPA, OMH, alkali and ionic strength. The order in [Ru(III)] was found to be unity from the linearity of the plots of k_C versus [Ru(III)] (r ≥ 0.999, S ≤ 0.023) (Table 2 Ru(III) catalyzed).

3.6. Effect of [alkali] and [periodate]

The effect of alkali in the absence and presence of Ru(III) catalyst reaction was studied in the range of 0.01–0.10 mol dm⁻³ at constant concentrations of OMH, DPA, IO₄⁻ and ionic strength of 0.10 mol dm⁻³. The rate constants decreased with increase in alkali and the order was found to be negative fraction, i.e., –0.32 in uncatalyzed and –0.48 in Ru(III) catalyzed reaction, which is rarely observed in DPA oxidation (r ≥ 0.987, S ≤ 0.004 for uncatalyzed, r ≥ 0.987, S ≤ 0.027 for catalyzed) (Tables 1 and 2).

The periodate concentration was varied in the range of 1.0 × 10⁻⁵–1.0 × 10⁻⁴ at constant concentrations of DPA, OMH, OH⁻ and ionic strength in uncatalyzed and with constant concentration Ru(III) in catalyzed reaction. It was observed that the added periodate did not have any effect on the reaction (Tables 1 and 2), which is also an unusual case in DPA oxidation.

Thus, from the observed experimental results:

The rate law for uncatalyzed reaction is given as:

\[
\text{Rate} = k_U[DPA]^{1.0}[OMH]^{0.81}[OH^-]^{-0.32}
\]

The rate law for Ru(III) catalyzed reaction is given as:

\[
\text{Rate} = k_C[DPA]^{1.0}[OMH]^{0.82}[OH^-]^{-0.48}[Ru(III)]^{1.0}
\]

3.7. Effect of ionic strength (I) and dielectric constant of the medium (D)

The effect of varying [KNO₃] at constant [DPA], [Ru(III)], [OMH], [OH⁻] and IO₄⁻ was found that increasing ionic strength had no significant effect on the rate of the reaction in both the cases of uncatalyzed and catalyzed reactions.

Varying the t-butyl alcohol and water percentage varied dielectric constant of the medium, ‘D’. The D values were calculated from the equation \( D = D_w V_w + D_B V_B \), where \( D_w \) and \( D_B \) are dielectric constants of pure water and t-butyl alcohol, respectively, and \( V_w \) and \( V_B \) are the volume fractions of component water and t-butyl alcohol, respectively, in the total mixture. It was observed that there was no effect of dielectric constant on the rate of uncatalyzed reaction but in case of Ru(III) catalyzed reaction the rate constant \( k_C \) decreased with decrease in dielectric constant of the reaction medium and the plot of log \( k_C \) versus \( 1/D \) was linear with negative slope.

3.8. Effect of initially added products

In both the cases initially added products, Ag(I), and 4-amino-butyric acid did not have any significant effect on the rate of reaction.

3.9. Test for free radicals (polymerization study)

To test the intervention of free radicals for both uncatalyzed and catalyzed reactions, the reaction mixture was mixed with acryloni-
trile monomer and kept for 2 and 1 h, respectively, under nitrogen atmosphere. On dilution with methanol, a white precipitate was formed, indicating the intervention of free radicals in the reactions. The blank experiments of either DPA or α-ornithine alone with acrylonitrile did not induce any polymerization under the same condition as those induced for the reaction mixture. Initially added acrylonitrile decreases the rate of reaction indicating free radical intervention, which is the case in earlier work [24].

3.10. Effect of temperature (T)

The influence of temperature on the rate of reaction was studied for both uncatalyzed and catalyzed reaction at six different temperatures (15, 20, 25, 30, 35 and 40 °C) under varying concentrations of α-ornithine monohydrochloride and alkali keeping other conditions constant. The rate constant was found to increase with increase in temperature. The rate constant (k₁) of the slow step of the uncatalyzed reaction mechanism was obtained from the slopes and intercepts of plots of 1/k₁ versus 1/[OH⁻] at six different temperatures and were used to calculate the activation parameters. The energy of activation corresponding to these constants was evaluated from the Arrhenius plots of log(k₁) versus 1/T (r > 0.988, S ≤ 0.012) and other activation parameters obtained are tabulated in Table 3.

Similarly the rate constant (k₂) of the slow step of catalyzed reaction mechanism was obtained from the slopes and the intercept of the plots of [Ru(III)]/k₂ versus 1/[OMH] and [Ru(III)]/k₂ versus [OH⁻] at six different temperatures. The values are given in Table 4. The energy of activation for the rate determining step was obtained by the least square method of plot of log(k₂) versus 1/T (r > 0.978, S ≤ 0.022) and other activation parameters calculated for the reaction are presented in Table 4.

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3.11. Catalytic activity

It has been pointed out by Moelwyn-Hughes [25] that in the presence of the catalyst, the uncatalyzed and catalyzed reactions proceed simultaneously, so that

\[ k_1 = k_0 + K_{cat, const}[\text{Catalyst}]^x \]  

Here 'k₁' is the observed pseudo-first-order rate constant in the presence Ru(III) catalyst, 'k₀' the pseudo-first-order rate constant for the uncatalyzed reaction, 'K_{cat, const.}' the catalytic constant and 'x' the order of the reaction with respect to [Ru(III)]. In the present investigations, x values for the standard run was found to be unity. Then the value of K_{cat, const.} is calculated using the equation.

### Table 4

Activation parameters and thermodynamic quantities for the oxidation of OMH by diperiodatoargentate(III) in aqueous alkaline medium with respect to the slow step of Scheme 3: (A) effect of temperature, (B) activation parameters (Scheme 2), (C) effect of temperature to calculate K₁ and K₂ for the oxidation of α-ornithine monohydrochloride by diperiodoargentate(III) in alkaline medium, (D) thermodynamic quantities using K₁ and K₂.

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### Table 5

Values of catalytic constant (K_{cat, const.}) at different temperatures and activation parameters calculated using K_{cat, const.} values.

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The values of $K_{\text{Cat, const.}}$ were evaluated at different temperatures and found to vary at different temperatures. Further, plots of log $K_{\text{Cat, const.}}$ versus $1/T$ were linear and the values of energy of activation and other activation parameters with reference to catalyst were computed and the results are summarized in Table 5. The value of $K_{\text{Cat, const.}}$ at 25 °C is $1.7 \times 10^4$.

4. Discussion

In the later period of 20th century the kinetics of oxidation of some organic and inorganic substrates have been studied by Ag(III) species which may be due to its strong versatile nature of two electrons oxidant. Among the various species of Ag(III), Ag(OH)$_2$$^-$, diperiodatocopper(III) and ethylenebis(biguanide), (EBS), silver(III) are of maximum attention to the researchers due to their relative stability [26]. The stability of Ag(OH)$_2$$^-$/Ag(OH)$_3$$^-$ is very sensitive towards traces of dissolved oxygen and other impurities in the reaction medium whereupon it had not drawn much attention. However, the other two forms of Ag(III) [27] are considerably stable; the DPA is used in highly alkaline medium and EBS is used in highly acidic medium.

The literature survey [21] reveals that the water soluble diperiodatocopper(III) (DPA) has a formula [Ag(IO$_6$)$_2$]$^-$ with dsp$^2$ configuration of square planar structure, similar to diperiodatocopper(III) complex with two bidentate ligands, periodate to form a planar molecule. When the same molecule is used in alkaline medium, it is unlikely to exist as [Ag(IO$_6$)$_2$]$^-$ as periodate is known to be in various protonated forms [28] depending on pH of the solution as given in following multiple equilibria Eqs. (3)–(5).

$$H_2IO_6^- = H_2IO_6 + H^+$$ (3)

$$HIO_4^- = HIO_4 + H^+$$ (4)

$$HIO_3^- = HIO_3 + H^+$$ (5)

Periodic acid exists as H$_2$IO$_6$ in acid medium and as H$_3$IO$_6$ near pH 7. Hence, under alkaline conditions as employed in this study, the main species are expected to be H$_3$IO$_6$$^2-$ and H$_2$IO$_6$$^3-$. At higher concentrations, periodate also tends to dimerise [4]. However, formation of this species is negligible under conditions employed for kinetic study. On contrary, the authors [7,8] in their recent studies have proposed the DPA species as [Ag(H$_2$IO$_6$)$_2$]$^{1-}$ in which ‘L’ is a periodate with uncertain number of protons and ‘HL’ is a protonated periodate of uncertain number of protons. This can be ruled out by considering the alternative form [28] of IO$_4$$^-$ at pH > 7 which is in the form H$_3$IO$_6$$^2-$ or H$_2$IO$_6$$^3-$. Hence, DPA could be as [Ag(H$_2$IO$_6$)$_2$]$^-$ or [Ag(H$_3$IO$_6$)$_2$]$^{1-}$ in alkaline medium. Therefore, under the present condition, diperiodatocopper(III), may be depicted as [Ag(H$_2$IO$_6$)$_2$]$^-$ . The similar speciation of periodate in alkali was proposed [29] for diperiodatonicelate(IV).

It is known that α-ornithine exists in the form of zwitterion in aqueous medium. In highly acidic medium it exists in the protonated form, while as in highly basic medium it is in the deprotonated form [30].

4.1. Mechanism for uncatalyzed reaction

The reaction between diperiodatocopper(III) and α-ornithine monohydrochloride in alkaline medium has the stoichiometry 1:2 (OMH:DPA) with a first order dependence on [DPA] and an apparent order of less than unit order in [substrate], a negative fractional order dependence on [alkali]. No effect of added products was observed. Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [DPA], [OMH], [OH$^-$] and [IO$_6$$^3-$] may be well accommodated.

It is interesting to note that in most of the reports [18] on DPA oxidation, OH$^-$ had an increasing effect on the rate of the reaction, periodate retarded the rate of reaction and MPA was considered as active species of DPA. However, in the present kinetic study, different kinetic results have been obtained. In this study, increasing the concentration of OH$^-$ decreases the rate of the reaction and negligible effect of periodate on rate of reaction and DPA itself was the active species of the reaction. The result of decrease in rate of reaction with increase in alkalinity (Table 1) can be explained in terms of prevailing equilibrium of formation of [Ag(H$_2$IO$_6$)$_2$]$_2^-$ from [Ag(H$_3$IO$_6$)$_2$]H$_2$IO$_6$$^-$ hydrolysis as given in the following equation:

$$[\text{Ag}(\text{H}_2\text{IO}_6)_2]/[\text{H}_2\text{IO}_6]^2 + \text{H}_2\text{O} \rightleftharpoons \text{Ag}(\text{H}_2\text{IO}_6)_2^- + [\text{OH}^-]$$ (6)

Such type of equilibrium (6) has been well noticed in the literature [31]. Because of this reaction and fact that $k_U$ values are inverse function of hydroxyl ion concentration with fractional order in OH$^-$ concentration, the main oxidant species is likely to be [Ag(H$_3$IO$_6$)$_2$]$_2^-$ and its formation by the above equilibrium is important in the present study. The less than unit order in [OMH] presumably results from formation of a complex (C$_1$) between the DPA species and α-ornithine monohydrochloride prior to the formation of the products. This complex (C$_1$) decomposes in a slow step to form a free radical derived from OMH. This free radical species further reacts with Ag(II) in a fast step to form 4-aminobutyraldehyde intermediate. This intermediate reacts with one more mole of DPA species in a further fast step to form products such as 4-aminobutyric acid, Ag(I) and periodate as given in Scheme 2.

The direct plot of $k_U$ versus [OMH] was drawn to know the parallel reaction if any along with interaction of oxidant and reducing agent. However the plot of $k_U$ versus [OMH] was not linear. Thus, in Scheme 2, the parallel reaction and involvement of two molecules of OMH in the complex are excluded. The probable structure of the complex (C$_1$) is given as,

$$\text{NH}_2\text{COO}^- \quad \text{NH}_2$$

Spectroscopic evidence for the complex formation between oxidant and substrate was obtained from UV–Vis spectra of α-ornithine monohydrochloride (5.0 × 10$^{-4}$), DPA (5.0 × 10$^{-5}$), [OH$^-$] = 0.08 mol dm$^{-3}$ and mixture of both. A bathochromic shift of 8 nm from 216 to 224 nm in the spectra of DPA to mixture of DPA and OMH was observed (Fig. 4). The Michaelis–Menten plot proved the complex formation between DPA and OMH, which ex-
plains the less than unit order dependence on [OMH]. Such complex between an oxidant and substrate has also been observed in other studies [18]. Scheme 2 leads to the rate law (7):

\[
\text{rate} = \frac{d[DPA]}{dt} = \frac{k_1 K_1 K_2 [OMH][DPA]}{[OH^-] + K_1 + K_1 K_2 [OMH]}
\]

\[
ku = \frac{\text{rate}}{[DPA]} = \frac{k_1 K_1 K_2 [OMH]}{[OH^-] + K_1 + K_1 K_2 [OMH]}
\]

This explains all the observed kinetic orders of different species. The rate law (8) can be rearranged into the following form, which is suitable for verification:

\[
\frac{1}{ku} = \frac{1}{k_1 K_2 [OMH]} + \frac{1}{K_1 K_2 [OMH]} + \frac{1}{k_1}
\]

According to Eq. (9), other conditions being constant, plots of $1/k_1$ versus [OH$^-$] ($r > 0.991, S < 0.012$) and $1/ku$ versus $1/[OMH]$ ($r > 0.999, S < 0.014$) should be linear and are found to be so (Fig. 5). The slopes and intercepts of such plots lead to the values...
of $K_1$, $K_2$ and $k_1$ as $(2.70 \pm 0.05) \times 10^{-2}$ mol dm$^{-3}$, $(3.7 \pm 0.1) \times 10^{3}$ dm$^3$ mol$^{-1}$ and $(6.8 \pm 0.2) \times 10^{-3}$ s$^{-1}$, respectively.

The negligible effect of ionic strength and dielectric constant of medium on the rate might be due to the presence of various ions shown in Scheme 2. A high negative value of $\Delta H^\ominus$ ($-127.5$ kJ mol$^{-1}$) suggests that intermediate complex $(C_1)$ is more ordered than the reactants [33].

The thermodynamic quantities for the first and second equilibrium steps of Scheme 2 can be evaluated as follows. [OMH] and [OH$^-$] (as in Table 1) were varied at six different temperatures. The plots of $1/K_0$ versus [OH$^-$] and $1/K_0$ versus [OMH] should be linear (Fig. 5). From the slopes and intercepts, the values of $K_1$ and $K_2$ were calculated at different temperatures and these values are given in Table 3. The vant Hoff’s plots were made for variation of $K_1$ and $K_2$ with temperature ($\log K_1$ versus $1/T$ ($r > 0.991$, $5 \leq 0.004$) and $\log K_2$ versus $1/T$ ($r > 0.989$, $5 \leq 0.005$)) and the values of enthalpy of reaction $\Delta H^\ominus$, entropy of reaction $\Delta S^\ominus$ and free energy of reaction $\Delta G^\ominus$ were calculated for the first and second equilibrium steps. These values are given in Table 3. A comparison of the $\Delta H$ value of second step [40.6 kJ mol$^{-1}$] of Scheme 2 with that of $\Delta H^\ominus$ (47.2 kJ mol$^{-1}$) obtained for the slow step of the reaction shows that these values mainly refer to the rate limiting step, supporting the fact that the reaction before rate determining step is fairly fast and involves low activation energy [32].

4.2. Mechanism for Ru(III) catalyzed reaction

Ruthenium(III) chloride acts as an efficient catalyst in many redox reactions, particularly in an alkaline medium [24]. It is interesting to identify the probable ruthenium(III) chloride species in alkaline media. In the present study it is quite probable that the [Ru(H$_2$O)$_6$]$_3^+$ species might assume the general form [Ru(III)(OH$_2$)$_5$]$.^+$$. The x value would always be less than six because there are no definite reports of any hexahydroruthenium species. The remainder of the coordination sphere would be filled by water molecules. At higher pH, the electronic spectra studies have confirmed [34] that the ruthenium(III) chloride exists in the hydrated form as [Ru(H$_2$O)$_6$]$.^+$$. Metal ions of the form [Ru(H$_2$O)$_6$]$.^+$ are also known to exist as [Ru(H$_2$O)$_5$OH]$^+$ in an alkaline medium and are most likely mononuclear species. Hence, under the conditions employed, e.g. [OH$^-$] $\gg$ [Ru(III)], ruthenium(III) is mostly present as the hydroxylated species, [Ru(H$_2$O)$_5$OH]$^+$$. Similar species have been reported between Ru(III) and OMH [35]. In earlier reports of Ru(III) catalyzed oxidation [16], it has been observed that, if there is a fractional order dependence with respect to [substrate] and Ru(III) and unit order with respect to [oxidant], Ru(III) forms a complex with the substrate. It gets oxidized by the oxidant to form Ru(IV)/substrate complex followed by the rapid redox decomposition to regenerate Ru(III). In another case [17], if the process shows a zeroth order dependence with respect to [oxidant], first order with respect to [Ru(III)] and a fractional order with respect to [substrate], there involves the formation of a Ru(III)/substrate complex. It undergoes further cleavage in a concerted manner giving rise to a Ru(I) species, which is rapidly oxidized by the oxidant to regenerate the catalyst. In some other reports [18], it is observed that Ru(III) forms a complex with substrate and is oxidized by the oxidant with the regeneration of the catalyst. Hence, the study of behavior of Ru(III) in catalyzed reaction becomes significant.

The equilibrium step 1 and the stoichiometry were same as in the case of uncatalyzed reaction. In the Ru(III) catalyzed reaction, [DPA] was first order dependence, an apparent order of less than unit order in [OMH], a negative fractional order dependence on [alkali] and order with respect to Ru(III) was found to be unity. No effect of added products was observed. Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [DPA], [OMH], [Ru(III)], [OH$^-$] and [IO$_4$$^-$] may be well accommodated.

In the prior equilibrium step 1, the hydroxyl ion concentration with fractional order in OH$^-$ concentration, the main oxidant species is likely to be [Ag(H$_2$O)$_5$OH]$^+$ and its formation by the above equilibrium is important in the present study. The less than unit order in [OMH] presumably results from formation of a complex (C$_2$) between the Ru(III) species and L-ornithine monohydrochloride. This complex (C$_2$) reacts with one mole of DPA in a slow step to give the free radical species of OMH, Ag(II) with the regeneration of catalyst, Ru(III). Further this free radical species of OMH reacts with Ag(II) in a fast step to form 4-aminobutyrildehyde intermediate. This intermediate reacts with one mole of DPA in a further fast step to form the products such as 4-aminobutyrlic acid, Ag(I) and peroxidate as given in Scheme 3. The reduction of Ru(III) to lower oxidation state and then regeneration of Ru(III) by oxidant was not possible under the experimental conditions due to observed orders in different constituents of reaction.

The probable structure of the complex (C$_2$) is given as,

Spectroscopic evidence for the complex formation between Ru(III) and OMH was obtained from UV–Vis spectra of OMH (5.0 $\times$ 10$^{-5}$), Ru(III) (3 $\times$ 10$^{-6}$, [OH$^-$] = 0.08 mol dm$^{-3}$) and a mixture of both. A hypochrome shift of 5 nm from 277 to 272 nm in the spectra of Ru(III) to the mixture of Ru(III) and OMH was observed (Fig. 6). Attempts to separate and isolate the complex were not successful. The Michaelis–Menten plot proved the complex formation between catalyst and substrate, which explains less than unit order in [OMH]. Such a complex between a catalyst and substrate has also been observed in other studies [36]. The rate law (11) for the Scheme 3 could be derived as,

$$\text{rate} = \frac{-d[DPA]}{dt} = \frac{k_1K_2K_4[OMH][Ru(III)][DPA]}{K_1 + K_2K_4[OMH] + [OMH] + K_4[OMH][OH^-]}$$

$$\text{rate} = \frac{[DPA]}{K_5} = \frac{k_1K_2K_4[OMH][Ru(III)]}{K_3 + K_4K_4[OMH][Ru(III)]}$$

This explains all the observed kinetic orders of different species. The rate law (11) can be rearranged to be Eq. (12), which is suitable for verification

$$\frac{[Ru(III)]}{k_5} = \frac{[OMH]}{K_3K_4[OMH]} + \frac{[OMH]}{K_3K_4[OMH]} + \frac{1}{K_2}$$

According to Eq. (12), other conditions being constant, the plots of [Ru(III)]/[k$_5$ versus [OMH]] and [Ru(III)]/[k$_5$ versus 1/[OMH]] should be linear and found to be so (Fig. 7). From the intercepts and slopes of such plots, the reaction constants $K_3$, $K_4$ and $k_2$ were calculated as $(3.4 \pm 0.1) \times 10^{-2}$ mol dm$^{-3}$, $(9.8 \pm 0.3) \times 10^{-3}$ dm$^3$ mol$^{-1}$, $(4.8 \pm 0.2) \times 10^4$ dm$^3$ mol$^{-1}$ s$^{-1}$, respectively. These constants were
used to calculate the rate constants and compared with the experimental $k_C$ values and found to be in reasonable agreement with each other, which fortifies Scheme 3.

The thermodynamic quantities for the different equilibrium steps, in Scheme 3 can be evaluated as follows. The DL-ornithine monohydrochloride and hydroxide ion concentrations (Table 2) were varied at different temperatures. The plots of $[\text{Ru(III)}]/k_C$ versus $1/\text{[OMH]}$ ($r \geq 0.9982$, $S \leq 0.00132$), $[\text{Ru(III)}]/k_C$ versus $[\text{OH}^-]$ ($r \geq 0.9873$, $S \leq 0.00145$) should be linear as shown in Fig. 7. From the slopes and intercepts, the values of $K_3$ are calculated at different temperatures. A vant Hoff’s plot was made for the variation of $K_3$ with temperature [i.e., log $K_3$ versus $1/T$ ($r \geq 0.961$, $S \leq 0.1035$)] and the values of the enthalpy of reaction $\Delta H$, entropy of reaction $\Delta S$ and free energy of reaction $\Delta G$, were calculated. These values are also given in Table 4. A comparison of the $\Delta H$ value of second step (31.5 kJ mol$^{-1}$) of Scheme 3 with that of $\Delta H^\circ$ (49.6 kJ mol$^{-1}$) obtained for the slow step of the reaction shows that these values mainly refer to the rate limiting step, supporting the fact that the reaction before rate determining step is fairly fast and involves low activation energy [18]. In the same manner, $K_4$ values were calculated at different temperatures and the corresponding values of thermodynamic quantities are given in Table 4. The positive value of $\Delta S^\circ$ is normal in case of fast reactions [37] involving distribution of charges as given in Scheme 3.

Fig. 6. Spectroscopic evidence for the complex formation between Ru(III) and OMH. (a) UV–Vis spectra of Ru(III) (327, 272 and 229 nm); (b) UV–Vis spectra of mixture of Ru(III) and OMH (327, 272 and 229 nm); and (c) UV–Vis spectra of OMH.

Fig. 7. Verification of rate law (12) for the Ru(III) catalyzed oxidation of α-ornithine by alkaline diperiodatoargentate(III). Plots of (A) $[\text{Ru(III)}]/k_C$ vs $1/\text{[OMH]}$, (B) $[\text{Ru(III)}]/k_C$ vs $[\text{OH}^-]$, at six different temperatures (conditions as given in Table 2).
The negligible effect of ionic strength might be due to the presence of various ions in both catalyzed and uncatalyzed reactions (Schemes 2 and 3). But in case of Ru(III) catalyzed reaction, the rate constants decreased with decrease in dielectric constant of the medium. The effect of solvent on the reaction rate has been described in detail in the literature [38]. For the limiting case of a zero angle approach between two dipoles or anion–dipole system, Amis [38] has shown that a plot of log $k_1$ versus $1/D$ gives a straight line, with a negative slope for a reaction between a negative ion and a dipole or two dipoles, and with a positive slope for positive ion and dipole interaction. In the present study, the plot observed had a negative slope, which supports the involvement of negative ions as given in Scheme 3. The moderate $\Delta H^\circ$ and $\Delta S^\circ$ values are favorable for electron transfer reaction. The value of $\Delta H^\circ$ was due to energy of solution changes in the transition state. The observed modest enthalpy of activation and a higher rate constant for the slow step indicates that the oxidation presumably occurs via an inner-sphere mechanism. This conclusion is supported by earlier observations [39]. The activation parameters evaluated for the catalyzed and uncatalyzed reactions explain the catalytic effect on the reaction. The catalyst Ru(III) forms the complex (C$_2$) with substrate which enhances the reducing property of the substrate than that without catalyst. Further, the catalyst Ru(III) modifies the reaction path by lowering the energy of activation.

The values of activation parameters for the uncatalyzed and ruthenium(III) catalyzed oxidations of some amino acids by DPA are summarized in Tables 6 and 7. The entropy of the activation for the title reaction falls within the observed range. Variation in the rate within a reaction series may be caused by change in the enthalpy or entropy of activation. Changes in the rate are caused by changes in both $\Delta H^\circ$ and $\Delta S^\circ$, but these quantities vary extensively in a parallel fashion. A plot of $\Delta H^\circ$ versus $\Delta S^\circ$ is linear according to equation

$$\Delta H^\circ = \beta \Delta S^\circ + \text{constant}$$

where $\beta$ is called the isokinetic temperature. It has been asserted that apparently linear correlation of $\Delta H^\circ$ with $\Delta S^\circ$ are sometimes misleading and the evaluation of $\beta$ by means of the above equation lacks statistical validity [40]. Exner [41] advocates an alternative method for the treatment of experimental data. If the rates of several reactions in a series have been measured at two temperatures

![Fig. 8. Plots of (A) log $k_2$ at 303 K vs log $k_1$ at 298 K for uncatalyzed reaction (conditions as given in Table 6); (B) log $k_2$ at 303 K vs log $k_1$ at 298 K for ruthenium(III) catalyzed reaction (conditions as given in Table 7); for (1) L-alanine; (2) L-leucine; (3) L-lysine; and (4) D-ornithine.](image)

### Table 6

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>$k_1 \times 10^3$ (dm$^3$ mol$^{-1}$ s$^{-1}$) at 298 K</th>
<th>$k_2 \times 10^3$ (dm$^3$ mol$^{-1}$ s$^{-1}$) at 303 K</th>
<th>$\Delta H^\circ$ (kJ mol$^{-1}$)</th>
<th>$\Delta S^\circ$ (J K$^{-1}$ mol$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Alanine</td>
<td>3.04</td>
<td>3.66</td>
<td>24</td>
<td>−212</td>
<td>[43]</td>
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<tr>
<td>l-Leucine</td>
<td>4.29</td>
<td>4.98</td>
<td>19.4</td>
<td>−225</td>
<td>[43]</td>
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<tr>
<td>l-Lysine</td>
<td>4.02</td>
<td>5.50</td>
<td>36</td>
<td>−169</td>
<td>[44]</td>
</tr>
<tr>
<td>D-Ornithine</td>
<td>6.83</td>
<td>9.44</td>
<td>47</td>
<td>−127</td>
<td>Present work</td>
</tr>
</tbody>
</table>

### Table 7

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>$k_1 \times 10^{-4}$ (dm$^3$ mol$^{-1}$ s$^{-1}$) at 298 K</th>
<th>$k_2 \times 10^{-4}$ (dm$^3$ mol$^{-1}$ s$^{-1}$) at 303 K</th>
<th>$\Delta H^\circ$ (kJ mol$^{-1}$)</th>
<th>$\Delta S^\circ$ (J K$^{-1}$ mol$^{-1}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Alanine</td>
<td>0.0218</td>
<td>0.0271</td>
<td>12</td>
<td>−128</td>
<td>[45]</td>
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<tr>
<td>l-Leucine</td>
<td>0.100</td>
<td>0.108</td>
<td>9.3</td>
<td>−155</td>
<td>[45]</td>
</tr>
<tr>
<td>l-Lysine</td>
<td>1.9768</td>
<td>2.0767</td>
<td>5.87</td>
<td>−142</td>
<td>[45]</td>
</tr>
<tr>
<td>D-Ornithine</td>
<td>4.8160</td>
<td>6.7523</td>
<td>49.2</td>
<td>11.38</td>
<td>Present work</td>
</tr>
</tbody>
</table>
We have calculated the isokinetic temperatures as 269.4 K by plotting log k₂ at 303 K versus log k₁ at 298 K for uncatalyzed and 150.9 K by plotting log k₂ at 303 K versus log k₁ at 298 K for Ru(III) catalyzed (Fig. 8). The values of β for both uncatalyzed (269.4 K) and Ru(III) catalyzed (150.9 K) are lower than experimental temperature (298 K). This indicates that both the rates are governed by the entropy of activation [42]. The linearity and the slope of the plot obtained may confirm that the kinetics of these reactions follow similar mechanism, as previously suggested.

5. Conclusion

The comparative study of uncatalyzed and ruthenium(III) catalyzed oxidation of α-ornithine monohydrochloride by diperiodatooargentate(III) was studied. Oxidation products were identified. Among the various species of Ag(III) in alkaline medium, DPA itself, i.e., [Ag(H₂IO₆)₂]⁺ is considered as active species for the title reaction. Active species of Ru(III) is found to be [Ru(H₂O₂)OH]²⁺. It becomes apparent that the role of pH in the reaction medium is crucial. Thermodynamic activation parameters of individual steps in the mechanisms were evaluated for uncatalyzed and Ru(III) catalyzed reactions at different temperatures, respectively. The catalytic constants and the activation parameters with reference to catalyst was also computed. The descriptions of the mechanisms are consistent with all the experimental evidences including kinetic, spectral and product studies.

Appendix A

According to Scheme 2

\[
\frac{d[DPA]}{dt} = k_1 K_1 [OMH] [Ag(H₂IO₆)(H₂IO₆)]^2 \quad \text{(A.1)}
\]

where T and F refer to total and free concentrations.

\[
[DPA]_T = [DPA]_F + [Ag(H₂IO₆)]_F + [C₁]
\]

\[
[DPA]_F = \frac{[1 + K_1 + K_2 [OMH]]}{[OMH]} K_1 K_2 [OMH]
\]

Similarly,

\[
[OMH]_T = [OMH]_F + [C₁]
\]

\[
[OMH]_F = \frac{[OMH]}{[OH] + k_1 k_2 [DPA]_F}
\]

In view of low concentrations of DPA used, the second term of above equation is neglected.

Therefore,

\[
[OMH]_T = [OMH]_F
\]

Similarly,

\[
[OH]_F = [OH]_T \quad \text{(A.3)}
\]

Substituting Eqs. (A.2)–(A.4) in Eq. (A.5) and omitting the subscripts T and F we get

\[
\frac{d[DPA]}{dt} = \frac{k_1 K_1 K_2 [OMH][DPA]}{[OH] + k_1 + k_2 [OMH]}
\]

Appendix B

According to Scheme 3

\[
\text{rate} = k_2 [C₁][Ag(H₂IO₆)]_F = k_2 K_1 K_2 [OMH][Ru(III)][DPA] \quad \text{(B.1)}
\]

The total concentration of DPA is given by (where T and F stand for total and free)

\[
[DPA]_T = [DPA]_F + [Ag(H₂IO₆)]_F = [DPA]_F \left[ \frac{[OH] + K_1}{[OH]} \right]
\]

Therefore,

\[
[DPA]_T = \frac{[DPA]_F [OH]}{[OH] + K_1} \quad \text{(B.2)}
\]

Similarly,

\[
[OMH]_T = [OMH]_F + [C₁] = [OMH]_F + K_1 [OMH] [Ru(III)]
\]

In view of low concentration of Ru(III) used,

\[
[OMH]_T = [OMH]_F \quad \text{(B.3)}
\]

Similarly,

\[
[OH]_T = [OH]_F \quad \text{(B.4)}
\]

\[
[Ru(III)]_T = [Ru(III)]_F + [C₁] = [Ru(III)]_F + K_2 [OMH] [Ru(III)]_F
\]

Substituting Eqs. (B.2)–(B.5) in Eq. (B.1) and omitting the subscripts T and F, we get

\[
\text{rate} = k_c = k_1 k_2 k_3 [OMH][Ru(III)] \quad \text{(B.5)}
\]

References

Mechanistic investigations of ruthenium(III) catalyzed oxidation of pentoxifylline by copper(III) periodate complex in aqueous alkaline medium

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Abstract The kinetics of the oxidation of ruthenium(III)-catalyzed oxidation of pentoxifylline (PTX) by diperiodatocuprate(III) (DPC) in aqueous alkaline medium at a constant ionic strength of 0.30 mol dm\(^{-3}\) was studied spectrophotometrically. The reaction between PTX and DPC in alkaline medium in the presence of Ru(III) exhibits 1:2 stoichiometry (PTX:DPC). The reaction was of first order in DPC, less than the unit order in [PTX] and [OH\(^{-}\)] and negative fractional order in [IO\(_4^{-}\)]. The order in [Ru(III)] was unity. Intervention of free radicals was observed in the reaction. The main products were identified by TLC and spectral studies including LC-MS. The oxidation reaction in alkaline medium has been shown to proceed via a Ru(III)-PTX complex, which reacts with monoperiodatocuprate(III) to decompose in a rate determining step followed by a fast step to give the products. The reaction constants involved in different steps of the mechanism were calculated. The activation parameters with respect to the slow step of the mechanism were computed and discussed, and thermodynamic quantities were also determined. The active species of catalyst and oxidant have been identified.

Keywords Kinetics · Mechanism · Diperiodatocuprate(III) · Pentoxifylline · Ruthenium(III) catalysis

Introduction

In recent years, diperiodatocuprate(III) (DPC) has been used as an oxidant in alkaline medium [1]. The oxidation studies of DPC are scanty in view of its limited solubility and stability in aqueous medium. Its use as an analytical reagent is now well recognized [2]. The copper(III) periodate complex exhibits different multiple equilibria involving different copper(III) species in aqueous alkaline medium. It is interesting to know which of the copper(III) species is the active oxidant.

Pentoxifylline (3,7-dihydro-3,7-dimethyl-1-(5-oxohexyl)purine-2,6-dione, PTX), a trisubstituted purine and xanthine derivative, is a hemorheologic agent used for the treatment of peripheral arterial disease [3] and intermittent claudication. PTX improves blood flow through the peripheral circulation by decreasing blood viscosity, inhibiting platelet aggregation, enhancing erythrocyte flexibility, and diminishing fibrinogen concentration [4]. The drug is gaining acceptance for conservative treatment of Peyronie’s disease and neuropathic injuries. It also helps to prevent strokes, manage sickle cell disease, improve blood flow to the brain, improve effects of diabetes, or treat nausea and headaches in high places (high-altitude sickness). It has been shown to reduce mortality in acute alcoholic and non-alcoholic steatohepatitis, presumably through its ability to inhibit TNF-alpha. Its anti-TNF properties indicate its potential for the treatment of ALD, alcoholic liver disease. A study demonstrated the possible use of pentoxifylline administered in conjunction with vitamin E for reducing the extent of fibrotic lesions induced by radiation therapy for breast cancer [5].

Transition metals are known to catalyze many oxidation-reduction reactions since they involve multiple oxidation states. In recent years the use of transition metal ions, such as ruthenium, osmium, palladium, manganese,
chromium, and iridium, either alone or as binary mixtures, as catalysts in various redox processes have attracted considerable interest [6]. Ru(III) acts as a catalyst in the oxidation of many organic and inorganic substrates [7, 8]. The catalyzed mechanism can be quite complicated because of the formation of different intermediate complexes and different oxidation states of ruthenium(III). Although the mechanism of catalysis depends on the nature of the substrate, oxidant, and experimental conditions, it has been shown [9] that metal ions act as catalysts by one of these different paths, such as the formation of complexes with reactant or oxidation of the substrate itself, or through the formation of free radicals.

A literature survey revealed that there are no reports on ruthenium(III)-catalyzed oxidation of PTX by DPC in alkaline medium. We have observed that micro amounts of ruthenium catalyze the oxidation of PTX by DPC in alkaline medium. In earlier reports [10] of DPC oxidation, both periodate and alkali had a retarding effect in most of the reactions. However, in the present study we made entirely different kinetic observations. In view of the medicinal value and potential pharmaceutical importance of PTX, it is important to understand the active species of oxidants and catalysts to compute the activity of the catalyst and to propose the appropriate mechanism. Hence, the title reaction is investigated in detail. An understanding of the mechanism allows the chemistry to be interpreted, understood, and predicted.

### Results and discussion

#### Reaction orders

As the diperiodatocuprate(III) oxidation of pentoxifylline in alkaline medium proceeds with a measurable rate in the absence of ruthenium(III), the catalyzed reaction is understood to occur in parallel paths with contributions from both the catalyzed and uncatalyzed paths. Thus, the total rate constant \( k_T \) is equal to the sum of the rate constants of the catalyzed \( k_C \) and uncatalyzed \( k_U \) reactions, so \( k_C = k_T - k_U \). Hence the reaction orders have been determined from the slopes of \( \text{lg} \) \( k_C \) versus \( \text{lg} \) (concentration) plots by varying the concentrations of PTX, OH\(^-\), IO\(_4^-\), and Ru(III), while in turn keeping the others constant. The uncatalyzed reaction was followed under the conditions [PTX] = 2.0 \( \times \) \( 10^{-3} \); [DPC] = 5.0 \( \times \) \( 10^{-5} \); [OH\(^-\)] = 0.20; \( I = 0.30 \text{ mol dm}^{-3} \). The rate constants of uncatalyzed reaction \( k_U \) and catalyzed reaction \( k_C \) were obtained by the plot of \( \text{lg} \) (absorbance) versus time by following the progress of the reaction spectrophotometrically at 415 nm.

#### Effect of [diperiodatocuprate(III)]

The DPC concentration was varied in the range of 1.0 \( \times \) \( 10^{-5} \)–1.0 \( \times \) \( 10^{-4} \) mol dm\(^{-3}\). The linearity of the plots of \( \text{lg} \) (absorbance) versus time up to 85% completion of the reaction indicates a reaction order of unity in (DPC). This is also confirmed by varying [DPC], which did not result in any change in the pseudo first-order rate constants \( k_C \) (Table 1).

#### Effect of [pentoxifylline]

The pentoxifylline concentration was varied in the range of 6.0 \( \times \) \( 10^{-4} \)–6.0 \( \times \) \( 10^{-3} \) mol dm\(^{-3}\) at constant concentrations of DPC, OH\(^-\), IO\(_4^-\), and at a constant ionic strength of 0.30 mol dm\(^{-3}\) in the presence of Ru(III) catalyst at 298 K. The \( k_C \) values increased with the increase in concentration of pentoxifylline, indicating an apparent less than unit order dependence on [PTX] \((r \geq 0.9978, S \leq 0.027)\); Table 1) under the concentrations of the experiment done. This is also confirmed in the plot of \( k_C \) versus [PTX]\(^0.79\), which is linear rather than the direct plot of \( k_C \) versus [PTX] (Fig. 1).

#### Effect of [alkali]

The effect of increase in concentration of alkali on the reaction was studied in the range of 0.03–0.30 mol dm\(^{-3}\) at constant concentrations of DPC, PTX, IO\(_4^-\), and at a constant ionic strength of 0.30 mol dm\(^{-3}\) in the presence of the Ru(III) catalyst at 298 K. The rate constants increased with increasing [alkali] (Table 1), indicating apparent less than unit order dependence of the rate on alkali concentration, i.e., 0.67 \((r \geq 0.9936, S \leq 0.007)\). This less than unit order is also confirmed by the plot of \( k_C \) versus [OH\(^-\)]\(^{0.67}\), which is linear rather than the direct plot of \( k_C \) versus [OH\(^-\)] (Fig. 2).

#### Effect of [periodate]

The effect of an increase in concentration of periodate on the reaction was studied in the range of 5.0 \( \times \) \( 10^{-6} \)–5.0 \( \times \) \( 10^{-5} \) mol dm\(^{-3}\) at constant concentrations of DPC, PTX, OH\(^-\), and at a constant ionic strength of 0.30 mol dm\(^{-3}\) in the presence of the Ru(III) catalyst at 298 K. It was found that the added periodate had a retarding effect on the rate of reaction. The order with respect to periodate concentration was a negative fractional order, i.e., \(-0.76 \((r \geq 0.9981, S \leq 0.004)\); Table 1).
Table 1 Effect of variation of [DPC], [PTX], [OH\textsuperscript{-}], [IO\textsubscript{4}\textsuperscript{-}], and [Ru(III)] on the ruthenium(III) catalyzed oxidation of pentoxifylline by diperiodatocuprate(III) in aqueous alkaline medium at 298 K and \( I = 0.30 \text{ mol dm}^{-3} \)

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<th>10(^{1}) [OH\textsuperscript{-}] (mol dm(^{-3}))</th>
<th>10(^{5}) [IO\textsubscript{4}\textsuperscript{-}] (mol dm(^{-3}))</th>
<th>10(^{6}) [Ru(III)] (mol dm(^{-3}))</th>
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Fig. 1 Plot of \( k_{C} \) versus [PTX]\(^{0.79}\) and \( k_{C} \) versus [PTX] (conditions as in Table 1)

Fig. 2 Plot of \( k_{C} \) versus [OH\textsuperscript{-}]\(^{0.67}\) and \( k_{C} \) versus [OH\textsuperscript{-}] (conditions as in Table 1)
The influence of temperature on the rate constant, $k$, of the slow step in the mechanism was obtained from the intercept of $[\text{Ru(III)}] \cdot k_C$ versus $1/\text{[PTX]}$ plots at four different temperatures. The values are given in Table 2. The activation parameters for the rate-determining step were obtained by the least square method of plot of $\lg k$ versus $1/T$ and are presented in Table 2.

**Test for free radicals**

The intervention of free radicals was examined as follows: the reaction mixture, to which a known quantity of acrylonitrile monomer had been added initially, was kept in an inert atmosphere for 2 h. On diluting the reaction mixture with methanol, a white precipitate was formed, indicating
the intervention of free radicals in the reaction. The blank experiments of either DPC or PTX alone with acrylonitrile did not induce any polymerization under the same conditions as those induced for the reaction mixture. Initially added acrylonitrile decreased the rate of reaction, indicating free radical intervention, as was the case in earlier work [11].

It was also examined using an electron spin resonance spectrophotometer whose spectrum showed a peak at 3,325.000 G with a g-value of 2.0023 indicating the intervention of free radicals [12].

**Catalytic activity**

It has been pointed out by Moelwyn-Hughes [13] that in the presence of the catalyst, the uncatalyzed and catalyzed reactions proceed simultaneously, so that

\[ k_T = k_U + K_C [\text{Catalyst}]^x \]  
\[ (1) \]

Here \( k_T \) is the observed pseudo first-order rate constant in the presence of Ru(III) catalyst; \( k_U \) is the pseudo first-order rate constant for the uncatalyzed reaction; \( K_C \) is the catalytic constant, and ‘\( x \)’ is the order of the reaction with respect to [Ru(III)]. In the present investigation, the value of \( x \) for the standard run was found to be 1.0. Then the value of \( K_C \) can be calculated using the equation

\[ K_C = \frac{k_T - k_U}{[\text{Catalyst}]^x} = \frac{k_C}{[\text{Catalyst}]^x} \quad \text{(where } k_T - k_U = k_C) \]  
\[ (2) \]

The values of \( K_C \times 10^{-3} \) were obtained as 0.89, 1.74, 3.69, and 7.59 at 288, 298, 308, and 318 K, respectively. Further, plot of \( \log K_C \) versus \( 1/T \) was linear, and the values of energy of activation and other activation parameters with reference to the catalyst were computed as \( E_a = 54.4 \pm 2.1 \text{ kJ mol}^{-1} \), \( \Delta H^\circ = 51.9 \pm 0.4 \text{ kJ mol}^{-1} \), \( \Delta S^\circ = -8.5 \pm 0.8 \text{ JK}^{-1} \text{ mol}^{-1} \), \( \Delta G^\circ = 84.7 \pm 4.2 \text{ kJ mol}^{-1} \), \( \log A = 12.8 \pm 0.3 \).

The water-soluble copper(III) periodate complex is reported [14] to be \([\text{Cu(HIO}_6])_2(\text{OH})_2]^{2-}\). However, in an aqueous alkaline medium and at the high pH range employed in the study, periodate is unlikely to exist as \( \text{HIO}_6^{2-} \) (as present in the complex) as is evident from its involvement in the multiple equilibria [15] (3)–(5), depending on the pH of the solution.

\[ \text{H}_3\text{IO}_6 \Leftrightarrow \text{H}_4\text{IO}_6^- + \text{H}^+ \quad (3) \]
\[ \text{H}_2\text{IO}_5^- \Leftrightarrow \text{H}_3\text{IO}_5^- + \text{H}^+ \quad (4) \]
\[ \text{H}_3\text{IO}_5^- \Leftrightarrow \text{H}_2\text{IO}_6^- + \text{H}^+ \quad (5) \]

Periodic acid exists as \( \text{H}_3\text{IO}_6 \) in acid medium and as \( \text{H}_4\text{IO}_6^- \) near pH 7. Hence, under alkaline conditions as employed in this study, the main species are expected to be \( \text{H}_3\text{IO}_6^- \) and \( \text{H}_2\text{IO}_6^- \). Thus, at the pH employed in this study, the soluble copper(III) periodate complex might be \([\text{Cu(OH}_2]\text{(H}_3\text{IO}_6)]^{2-}\), a conclusion also supported by earlier work [16, 17].

Ruthenium(III) chloride acts as an efficient catalyst in many redox reactions, particularly in an alkaline medium [18]. It is interesting to identify the probable ruthenium(III) chloride species in alkaline media. In the present study it is quite probable that the \([\text{Ru(H}_2\text{O})_5\text{OH}]^{2+}\) species might assume the general form \([\text{Ru(III)(OH)}_3]^{2-x}\). The x value would always be less than six because there are no definite reports of any hexahydroxy ruthenium species. The remainder of the coordination sphere would be filled by water molecules. Hence, under the conditions employed, e.g., \([\text{OH}^-] \gg [\text{Ru(III)}]\), ruthenium(III) is mostly present as the hydroxylated species, \([\text{Ru(H}_2\text{O})_x\text{OH}]^{2+}\). Similar equilibria have been reported between Ru(III) catalyzed oxidation of several other substrates with various oxidants in alkaline medium [19].

The reaction between the Ru(III) catalyzed oxidation of pentoxifylline by diperiodatocuprate(III) complex in alkaline medium has the stoichiometry 1:2 (PTX:DPC) with a first order dependence on [DPC] and [Ru(III)], and an apparent order of less than unity in [substrate], [alkali], and negative fractional order dependence on [periodate]. No effect of added products was observed. Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [catalyst], [oxidant], [reductant], [OH\textsuperscript{−}], and [IO\textsubscript{4}\textsuperscript{−}] may be well accommodated.

In most of the reports [10] on DPC oxidation, both periodate and OH\textsuperscript{−} had a decreasing effect on the rate of the reaction. However in the present kinetic study, different kinetic results have been obtained. In this study OH\textsuperscript{−} increased and periodate retarded the rate of the reaction. The results of an increase in the rate of reaction with increase in alkalinity (Table 1) can be explained in terms of the prevailing equilibrium of formation of \([\text{Cu(H}_2\text{IO}_6\text{(H}_3\text{IO}_6)]^{2-}\) from \([\text{Cu(H}_3\text{IO}_6\text{)}^{2-}\) as given in the following Eq. (6).

\[ [\text{Cu(H}_2\text{IO}_6\text{)}^{2-}] + \text{OH}^- \rightleftharpoons [\text{Cu(H}_2\text{IO}_6\text{(H}_3\text{IO}_6)]^{2-} + \text{H}_2\text{O} \]  
\[ (6) \]

Also the decrease in rate with an increase in \([\text{H}_1\text{IO}_6^{2-}\) (Table 1) suggests that the displacement of a ligand periodate takes place to give free periodate and the equilibrium of copper(III) periodate complex to form monoperiodatocuprate(III) (MPC) species as given in Eq. (7) is established.

\[ [\text{Cu(H}_2\text{IO}_6\text{(H}_3\text{IO}_6)]^{2-} + 2\text{H}_2\text{O} \rightleftharpoons [\text{Cu(H}_2\text{IO}_6\text{)}^{2-} + \text{H}_3\text{IO}_6^- \]  
\[ (7) \]
Such types of equilibria as (6) and (7) have been well noticed in literature [19]. It may be expected that a lower periodate complex such as monoperiodatocuprate(III) (MPC) is more important in the reaction than the DPC. The inverse fractional order in $[\text{H}_3\text{IO}_6^{2-}]$ might also be for this reason. Therefore, MPC might be the main reactive form of the oxidant.

The less than unit order in $[\text{PTX}]$ presumably results from the formation of a complex (C) between the ruthenium(III) species and PTX prior to the formation of the products. This complex (C) reacts with one mole of MPC in a slow step to give the intermediate species of free radical derived from PTX, Cu(II) with the regeneration of catalyst, Ru(III). This free radical species further reacts with one more molecule of MPC in a fast step to form the products such as 3,7-dihydro-1-(4-hydroxybutyl)-3,7-dimethylpurine-2,6-dione, Cu(II), and periodate as given in Scheme 1.

Since Scheme 1 is in accordance with the generally well accepted principle of non-complementary oxidations taking place in sequence of one-electron steps, the reaction between the substrate and oxidant would afford a radical intermediate. A free radical scavenging experiment revealed such a possibility. This type of radical intermediate has also been observed in earlier work [10]. Spectroscopic evidence for the complex formation between Ru(III) and PTX was obtained from UV-Vis spectra of PTX ($2.0 \times 10^{-3}$), Ru(III) ($5.0 \times 10^{-6}$, $[\text{OH}^-] = 0.20 \text{ mol dm}^{-3}$) and a mixture of both. A bathochromic shift of about 4 nm from 271 to 275 nm in the spectra of PTX to the mixture of Ru(III) and PTX was observed (Fig. 4). However, the Michaelis-Menten plot also proved the complex formation between catalyst and reductant, which explains the less than unit order dependence on [PTX]. Such a type of complex between a substrate and a catalyst has been observed in other studies [21].
From Scheme 1, the rate law (8) can be derived as

\[
\text{Rate} = \frac{-d[DPC]}{dt} = kK_1K_2K_3[DPC][PTX][OH^-][Ru(III)]
\]

\[
= \frac{[H_2IO_6^-] + K_1[OH^-] [H_2IO_6^+] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][PTX]}{[H_2IO_6^-] + K_1[OH^-] [H_2IO_6^+] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][PTX]}
\]

(8)

\[
\frac{[\text{DPC}]}{[\text{PC}]} = k_C = k_T - k_U
\]

\[
= \frac{kK_1K_2K_3[PTX][OH^-][Ru(III)]}{[H_2IO_6^-] + K_1[OH^-] [H_2IO_6^+] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][PTX]}
\]

(9)

The rate law (9) can be rearranged into the following form suitable for verification:

\[
\frac{[\text{Ru(III)}]}{k_C} = \frac{[H_2IO_6^-]}{kK_1K_2K_3[OH^-][PTX]} + \frac{[H_2IO_6^+]}{kK_2K_3[PTX]} + \frac{1}{kK_3[PTX]} + \frac{1}{k}
\]

(10)

The plots of [Ru(III)]/kC versus 1/[PTX], [Ru(III)]/kC versus 1/[OH\(^-\)], and [Ru(III)]/kC versus [H_2IO_6\(^2\)-] should be linear and were found to be so (Fig. 5). From the intercepts and slopes of such plots, the reaction constants K_1, K_2, K_3, and k were calculated as (1.3 ± 0.02) dm\(^3\) mol\(^{-1}\) (1.1 ± 0.04) \times 10^{-5} mol dm\(^{-3}\) (7.0 ± 0.2) \times 10^{5} dm\(^3\) mol\(^{-1}\), and (9.04 ± 0.30) \times 10^{5} dm\(^3\) mol\(^{-1}\) s\(^{-1}\), respectively. The values of K_1 and K_2 are in good agreement with previously reported work [20]. The equilibrium constant K_1 is far greater than K_2. This may be attributed to the greater tendency of DPC to undergo hydrolysis compared to the dissociation of hydrolyzed species in alkaline medium. These constants were used to calculate the rate constants over different experimental conditions, and when compared with the experimental k_C.
values, they are found to be in reasonable agreement with each other, which fortifies Scheme 1.

The effect of ionic strength and dielectric constant of the medium on the rate explains quantitatively the reaction between the neutral and positively charged ions, as seen in Scheme 1. The thermodynamic quantities for the different equilibrium steps in Scheme 1 can be evaluated as follows. The [PTX], [OH⁻], and [H₃IO₆²⁻] (Table 1) were varied at four different temperatures. The plots of [Ru(III)]/kC versus 1/[PTX] (r ≥ 0.9999, S ≤ 0.00131), [Ru(III)]/kC versus 1/[OH⁻] (r ≥ 0.9992, S ≤ 0.00097), and [Ru(III)]/kC versus [H₃IO₆²⁻] (r ≥ 0.9993, S ≤ 0.00131) should be found to be so, as shown in Fig. 5. From the slopes and intercepts, the values of K₁, K₂, and K₃ were calculated at four different temperatures. A van't Hoff's plot was made for the variation of K₁, K₂, K₃ with temperature (i.e., lg K₁ versus 1/T, lg K₂ versus 1/T, and lg K₃ versus 1/T). The values of the enthalpy of reaction ΔH, entropy of reaction ΔS, and free energy of reaction ΔG were calculated for the first, second, and third equilibrium steps. These values are also given in Table 2. A comparison of the ΔH value (68 ± 2) from K₁ with that of ΔHᵢ (48.3 ± 2.0) of the rate limiting step supports that the first step of Scheme 1 is fairly slow since it involves a high activation energy [22].

The values of ΔHᵢ and ΔSᵢ were both favorable for electron transfer processes. The favorable enthalpy was due to the release of energy on solution changes in the transition state. The negative value of ΔSᵢ suggests that the intermediate complex is more ordered than the reactants [23]. The observed modest enthalpy of activation and a higher rate constant for the slow step indicate that the oxidation presumably occurs via an inner-sphere mechanism. This conclusion is supported by earlier observations [24, 25]. The activation parameters evaluated for the reaction explain the catalytic effect on the reaction. The catalyst Ru(III) forms the complex (C) with the substrate, which enhances the reducing property of the substrate compared to that without the Ru(III) catalyst. Further, the Ru(III) catalyst modifies the reaction path by lowering the energy of activation.

**Conclusion**

The Ru(III) catalyzed oxidation of pentoxifylline by di-periodatocuprate(III) was studied. Oxidation products were identified. Among various species of Cu(III) in alkaline medium, monoperiodatocuprate(III), Cu(H₂IO₆)(H₂O)₂, is considered to be the active species for the title reaction. The active species of Ru(III) is found to be [Ru(H₂O)₅OH]²⁺.

Activation parameters were evaluated. Catalytic constant and activation parameters with reference to the catalyst were also computed.

**Experimental**

**Materials and reagents**

All chemicals were of reagent grade, and double distilled water was used throughout the work. Stock solution of PTX (Sigma-Aldrich, St. Louis, MO) was prepared by dissolving the appropriate amount of sample in double-distilled water. The required concentration of PTX was used from its aqueous stock solution. A standard stock solution of Ru(III) was prepared by dissolving RuCl₃ (S.D. Fine-chem Ltd., Mumbai, India) in 0.20 mol dm⁻³ HCl. The concentration was determined [26] by EDTA titration.

The copper(III) periodate complex was prepared [27] and standardized by a standard procedure [28]. The UV-Vis spectrum with maximum absorption at 415 nm verified the existence of copper(III) complex. Periodate solution was prepared by weighing the required amount of sample in hot water and used after 24 h to complete the equilibrium. Its concentration was ascertained iodometrically [29] at neutral pH maintained using phosphate buffer. KOH (BDH) and KNO₃ (AR) were employed to maintain the required alkalinity and ionic strength, respectively. The temperature was maintained constant to within ±0.1 °C.

**Instruments used**

For kinetic measurements, a Peltier Accessory (temperature control) attached to a Varian CARY 50 Bio UV-Vis spectrophotometer (Varian, Victoria-3170, Australia) was used. For product analysis, a Nicolet 410-FT-IR spectrometer (Thermo, USA), a 300-MHz ¹H NMR spectrometer (Bruker, Germany), and a LC-MS Agilent 1100 series-API 2000 mass spectrometer using the EI ionization technique were used. For intervention of free radicals, an ESR spectrophotometer (Bruker BioSpin GmbH) was used. For pH measurements an ELICO pH meter model LI 120 was used.

**Kinetic measurements**

The kinetics were followed under pseudo first-order conditions where [PTX] > [DPC] at 25.0 ± 0.1 °C, unless otherwise specified. The reaction was initiated by mixing...
the DPC to PTX solution, which also contained required concentrations of Ru(III), KNO₃, KOH, and KIO₄. The progress of the reaction was followed spectrophotometrically at 415 nm by monitoring the decrease in absorbance due to DPC with the molar absorbancy index ε at 6,235 ± 100 dm³ mol⁻¹ cm⁻¹ (literature ε = 6,230 [27]). It was verified that there is negligible interference from other species present in the reaction mixture at this wavelength. The pseudo-first order rate constants, kᵣ, were determined from the lg(absorbance) versus time plots. The plots were linear up to 85% completion of the reaction under the range of [OH⁻] used.

During the kinetics a constant concentration of 1.0 × 10⁻⁵ mol dm⁻³ of KIO₄ was used throughout the study unless otherwise stated. Since the excess of periodate is present in DPC, the possibility of oxidation of pentoxifylline by periodate in alkaline medium at 298 K was tested. The progress of the reaction was followed iodometrically. However, it was found that there was no significant reaction under the experimental conditions employed compared to the DPC oxidation of PTX. The total concentrations of periodate and OH⁻ were calculated by considering the amount present in the DPC solution and that additionally added. Kinetics runs were also carried out in N₂ atmosphere in order to understand the effect of dissolved oxygen on the rate of reaction. No significant difference in the results was obtained under a N₂ atmosphere and in the presence of air. In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates. The spectral changes during the reaction are shown in Fig. 6. It is evident from the figure that the concentration of DPC decreases at 415 nm.

**Stoichiometry and product analysis**

Different sets of reaction mixtures containing varying ratios of DPC to PTX in the presence of constant amounts of OH⁻, IO₄⁻, KNO₃, and Ru(III) were kept for 6 h in a closed vessel under inert atmosphere. The remaining DPC concentration was estimated by measuring the absorbance at 415 nm spectrophotometrically. The results indicated 1:2 stoichiometry as given in Scheme 2.

The main reaction product was eluted with chloroform and was identified as 3,7-dihydro-1-(4-hydroxybutyl)-3,7-dimethylpurine-2,6-dione. The product was characterized by FT-IR, ¹H NMR, and LC-MS studies.

The IR spectrum showed the absence of aliphatic C=O stretching at 1,719 cm⁻¹, which was shown for the parent molecule, the band at 3,431 cm⁻¹ indicating the presence of an alcoholic OH group. The product was also characterized by an NMR spectrum (CDCl₃) showing a peak at a chemical shift of δ = 1.82 ppm (s, 1H), which disappeared on D₂O exchange. Further, the product was subjected to LC-MS analysis. The mass spectrum (Fig. 7) showed a molecular ion peak (M+1) at m/z = 253.1, confirming the formation of product. Sodium acetate was confirmed by a spot test [30]. It was observed that 3,7-dihydro-1-(4-hydroxybutyl)-3,7-dimethylpurine-2,6-dione did not undergo further oxidation under the present kinetic conditions.

Regression analysis of experimental data to obtain the regression coefficient r and standard deviation S of point.

![Fig. 6 Spectroscopic changes occurring in the oxidation of PTX by alkaline DPC at 298 K, [DPC] = 5.0 × 10⁻⁵, [PTX] = 2.0 × 10⁻³, [OH⁻] = 0.20, [IO₄⁻] = 1.0 × 10⁻⁵, [Ru(III)] = 5.0 × 10⁻⁶, and I = 0.30 mol dm⁻³ with scanning time of (1) 0.5, (2) 1.0, (3) 1.5, (4) 2.0, (5) 2.5, and (6) 3.0 min](image-url)
from the regression line was performed using the Microsoft Excel 2003 program.

Appendix

According to Scheme 1,

\[
\text{Rate} = \frac{-d[DPC]}{dt} = k[C][\text{Cu(H}_2\text{IO}_6)(\text{H}_2\text{O})_2] = \frac{kK_1K_2K_3[DPC][PTX][\text{OH}^-][\text{Ru(III)}]}{[\text{H}_3\text{IO}_6^2^-]} \tag{11}
\]

The total concentration of DPC, \([\text{DPC}]_T\), is given by (subscripts T and f stand for total and free, respectively)

\[
[\text{DPC}]_T = [\text{DPC}]_f + [\text{Cu(H}_2\text{IO}_6)(\text{H}_2\text{O})_2] + [\text{Cu(H}_2\text{IO}_6)(\text{H}_2\text{O})_2^2^-] \tag{12}
\]

\[
[\text{DPC}]_f = [\text{DPC}]_f + K_1[\text{OH}^-][\text{DPC}] + K_2[\text{Cu(H}_2\text{IO}_6)(\text{H}_2\text{IO}_6)]^2^- \tag{13}
\]

Similarly,

\[
[\text{Ru(III)}]_T = [\text{Ru(III)}]_f + [C] = [\text{Ru(III)}]_f + K_3[\text{PTX}]_f[\text{Ru(III)}]_f \tag{14}
\]

\[
[\text{PTX}]_f = \frac{[\text{PTX}]_T}{1 + K_3[\text{Ru(III)}]} \tag{15}
\]

In view of the low concentrations of Ru(III) used, we have

\[
[\text{OH}^-]_T = [\text{OH}^-]_f \tag{16}
\]

Similarly,

\[
[\text{OH}^-]_T = [\text{OH}^-]_f \tag{17}
\]
Substituting Eqs. (12), (13), (14), and (15) in Eq. (11) and omitting the T and f subscripts, we get

\[
\text{Rate} = \frac{-d[DPC]}{dt} = \frac{kK_1K_2K_3[DPC][PTX][OH^-][\text{Ru(III)}]}{[H_3IO_6^{2-}] + K_1[OH^-][H_3IO_6^{2-}] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][PTX] + K_3[H_3IO_6^{2-}][PTX] + K_1K_2[PTX][OH^-][H_3IO_6^{2-}]}
\]

The terms \((K_3[H_3IO_6^{2-}][PTX])\) and \((K_1K_3[PTX][OH^-][H_3IO_6^{2-}])\) of the denominator are neglected in view of their lower values as compared to the periodate used in the study. Therefore,

\[
\text{Rate} = \frac{-d[DPC]}{dt} = \frac{kK_1K_2K_3[DPC][PTX][OH^-][\text{Ru(III)}]}{[H_3IO_6^{2-}] + K_1[OH^-][H_3IO_6^{2-}] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][PTX]}
\]

References

Os(VIII)/Ru(III) Catalysed Oxidation of L-Valine by Ag(III) Periodate Complex in Aqueous Alkaline Medium: A Comparative Kinetic Study

Shweta J. Malode · Nagaraj P. Shetti · Sharanappa T. Nandibewoor

Abstract The kinetics of osmium(VIII) (Os(VIII)) and ruthenium(III) (Ru(III)) catalysed oxidation of L-valine (L-val) by diperiodatoargentate(III) (DPA) in aqueous alkaline medium at 25 °C and a constant ionic strength of 0.006 mol dm⁻³ was studied spectrophotometrically. The stoichiometry is the same in both the catalysed reactions, i.e., [L-val]:[DPA] = 1:1. The reaction is of first order in [Os(VIII)], [Ru(III)], and [DPA] and has less than unit order in [L-val] and negative fractional order in [OH⁻]. Added periodate had no effect on rate of reaction. The products were identified by spot test and characterized by spectral studies. The catalytic constant (K_C) was also calculated for both catalysed reactions at different temperatures. The activation parameters with respect to slow step of the mechanisms were computed and discussed and thermodynamic quantities were also determined. It has been observed that the catalytic efficiency for the present reaction is in the order of Os(VIII) > Ru(III). The probable active species of catalyst and oxidant have been identified.

Keywords Kinetics · Oxidation · L-Valine · Diperiodatoargentate(III) · Osmium(VIII) catalysis · Ruthenium(III) catalysis

1 Introduction

Amino acids act not only as the building blocks in protein syntheses but they also play a significant role in metabolism and have been oxidized by a variety of oxidizing agents [1]. The study of the oxidation of amino acids is of interest because of their biological significance and selectivity towards the oxidant to yield the different products [2–4]. L-Valine is an essential amino acid with hydrocarbon side chains. It is usually found in the interior of proteins. It has an antagonistic property with structurally similar leucine and isoleucine, and imbalance among these three items results in suspension of growth. Particular symptoms of valine deficiency include loss of balance during locomotion, changes in the ventral horn and susceptibility to irritation allergens. Some of valine derivatives have antibiotic action.

Diperiodatoargentate(III) (DPA) is a metal complex with Ag in 3⁺ oxidation state like Cu³⁺ in DPC and Fe³⁺ in hemoglobin. It is used as a powerful oxidizing agent in alkaline medium and also as a volumetric reagent for the determination of various organic and inorganic species [3–5]. Jayaprakash Rao and other researchers [6, 7] have studied DPA as an oxidizing agent for the kinetics of oxidation of various substrates. They normally found that the order with respect to both oxidant and substrate was unity and the presence of OH⁻ ions was found to enhance the rate of reaction. But they did not arrive the possible active species of DPA in alkali and on the other hand they proposed mechanisms by generalizing the DPA as [Ag(HL)L]^(x+1)⁻. However, Anil Kumar [8–10] et al. put an effort to give an evidence for the reactive form of DPA in the alkaline pH. In the present investigation, we have obtained the evidence for the reactive species for the DPA in alkaline medium.

Transition metals are known to catalyse many oxidation–reduction reactions since they involve multiple oxidation states. In recent years the use of transition metal ions such as osmium, ruthenium, palladium, manganese, chromium, iridium either alone or as binary mixtures, as
catalysts in various redox processes has attracted considerable interest [11]. Although the mechanism of catalysis depends on the nature of the substrate, oxidant and experimental conditions, it has been shown [12] that metal ions act as catalysts by one of these different paths such as the formation of complexes with reactants or oxidation of the substrate itself or through the formation of free radicals. Osmium(VIII) (Os(VIII)) and ruthenium(III) (Ru(III)) catalysis in redox reactions involves different degrees of complexity, due to the formation of intermediate complexes and different states of osmium/ruthenium, etc. The uncatalysed reaction of oxidation of L-val by DPA has been studied [13]. We have observed that Os(VIII) and Ru(III) catalyses the oxidation of L-val by DPA in alkaline medium in micro amounts. In order to understand the active species of oxidant and catalyst, to compute the activity of the catalyst and to propose the appropriate mechanism, the title reaction is investigated in detail. An understanding of the mechanism allows the chemistry to be interpreted, understood and predicted.

2 Experimental and Methods

2.1 Materials and Reagents

All chemicals used were of reagent grade and Millipore water was used throughout the work. A solution of L-valine (S. D. Fine Chem.) was prepared by dissolving an appropriate amount of recrystallised sample in Millipore water. The purity of L-valine was checked by comparing its melting point 294 °C with the literature data [Lit. m.p. 296 °C]. The required concentration of L-valine was obtained from its stock solution. The Os(VIII) solution was prepared by dissolving OsO₄ (Johnson Matthey) in 0.50 mol dm⁻³ NaOH. The concentration was ascertained [14] by determining the unreacted [Fe(CN)₆]⁴⁻ with standard Ce(IV) solution in an acidic medium. A standard stock solution of Ru(III) was prepared by dissolving RuCl₃ (S. D. Fine Chem.) in 0.20 mol dm⁻³ HCl. The concentration was determined [15] by EDTA titration. KNO₃ and KOH (BDH) were used to maintain ionic strength and alkalinity of the reaction, respectively. An aqueous solution of AgNO₃ was used to study the product effect, Ag(I). A stock standard solution of IO₄⁻ was prepared by dissolving a known weight of KIO₄ (Riedel-de-Haen) in hot water and used after keeping for 24 h to attain the equilibrium. Its concentration was ascertained iodometrically [16] at neutral pH maintained using phosphate buffer. The pH of the medium in the solution was measured by ELICO (LI 120) pH meter. t-Butyl alcohol (S.D. Fine Chem.) was used to study the dielectric constant of the reaction medium.

2.2 Instruments Used

(i) For kinetic measurements, a Peltier Accessory (temperature control) attached to Varian CARY 50 Bio UV–Visible spectrophotometer (Varian, Victoria-3170, Australia) was used.

(ii) For product analysis, Nicolet 5700-FT-IR spectrometer (Thermo, USA), 300 MHz ¹H NMR spectrophotometer (Bruker, Switzerland) were used.

(iii) For pH measurements ELICO pH meter model LI 120 was used.

2.3 Preparation of DPA

DPA was prepared by oxidizing Ag(I) in presence of KIO₄ as described elsewhere [17]: the mixture of 28 g of KOH and 23 g of KIO₄ in 100 cm³ of water along with 8.5 g AgNO₃ was heated just to boiling and 20 g of K₂S₂O₈ was added in several lots with stirring and then allowed to cool. It was filtered through a medium porosity fritted glass filter and 40 g of NaOH was added slowly to the filtrate, whereupon a voluminous orange precipitate agglomerates. The precipitate is filtered as above and washed three to four times with cold water. The pure crystals were dissolved in 50 cm³ water and heated to 80 °C with constant stirring thereby some solid was dissolved to give a red solution. The resulting solution was filtered when it was hot and on cooling at room temperature, the orange crystals separated out and were recrystallised from water.

The complex was characterized from its UV spectrum, which exhibited three peaks at 216, 255, and 362 nm. These spectral features were identical to those reported earlier for DPA [12]. The magnetic moment study revealed that the complex is diamagnetic. The compound prepared was analysed for silver and periodate by acidifying a solution of the material with HCl [18], recovering and weighing the AgCl for Ag and titrating the iodine liberated when excess of KI was added to the filtrate for IO₄⁻. The stock solution of DPA was used for the required [DPA] solution in the reaction mixture.

2.4 Kinetic Measurements

The kinetic measurements were performed on a Varian CARY 50 Bio UV–Visible spectrophotometer. The kinetics was followed under pseudo-first-order condition where [L-val] ≫ [DPA] at 25 ± 0.1 °C, unless specified. The reaction was initiated by mixing the DPA to L-valine solution which also contained required concentrations of Os(VIII) or Ru(III), KNO₃, KOH and KIO₄. The progress of reaction was followed spectrophotometrically at 360 nm by monitoring decrease in absorbance due to DPA with the
molar absorbancy index, ‘ε’ to be 13900 ± 100 dm$^3$ mol$^{-1}$ cm$^{-1}$. The spectral changes during the chemical reaction for the standard condition at 298 K are shown in Fig. 1. It is evident from the figure that the concentration of DPA decreases at 360 nm. It was verified that there is a negligible interference from other species present in the reaction mixture at this wavelength.

The pseudo-first-order rate constants, ‘$k_C$’, were determined from the log (absorbance) versus time plots. The plots were linear up to 85% completion of reaction under the range of [OH$^-$] used. The orders for various species were determined from the slopes of plots of log $k_C$ versus respective concentration of species except for [DPA] in which non-variation of ‘$k_C$’ was observed as expected to the reaction condition. During the kinetics a constant concentration, viz. 5.0 × 10$^{-5}$ mol dm$^{-3}$ of KIO$_4$ was used throughout the study unless otherwise stated. Since periodate is present in excess in DPA, the possibility of oxidation of l-valine by periodate in alkaline medium at 25 °C was tested. The progress of the reaction was followed iodometrically. However, it was found that there was no significant reaction under the experimental conditions employed compared to the DPA oxidation of l-valine. The total periodate concentration was calculated by considering the periodate present in the DPA solution and that additionally added.

In view of the modest concentration of alkali used in the reaction medium, attention was also directed to the effect of the reaction vessel surface on the kinetics. Use of polythene/acrylic wares and quartz or polyacrylate cells gave the same results, indicating that the surface did not have any significant effect on the reaction rates.

In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates. However, fresh solutions were nevertheless used for carrying out each kinetic only. Regression analysis of experimental data to obtain regression coefficient ‘$r$’ and the standard deviation ‘$S$’, of points from the regression line, was performed with the Microsoft office Excel-2003 program.

3 Results
3.1 Stoichiometry and Product Analysis

Different sets of reaction mixtures containing varying ratios of DPA to l-val in the presence constant amount of OH$^-$, KIO$_4$, KNO$_3$ and Os(VIII)/Ru(III) catalyst were kept for 4 h in a closed vessel under nitrogen atmosphere. The remaining concentration of DPA was estimated by spectrophotometrically at 360 nm. The results indicated 1:1 stoichiometry for both the catalysed reactions as given in Eq. 1.

$$\text{CH}_3\text{CHCH}_2\text{CO}^+ + [\text{Ag(H}_2\text{IO}_6\text{)}_2]^+ + 2\text{OH}^- \rightarrow \text{CH}_3\text{CHCO}^- + \text{Ag(I)} + 2\text{H}_2\text{IO}_6^{2-} + \text{HCO}_3^- + \text{NH}_3$$

The main reaction product was identified as isobutyraldehyde by spot test [19]. The nature of aldehyde was confirmed by its IR spectrum, which showed a carbonyl stretch at 1719 cm$^{-1}$ and a band at 2938 cm$^{-1}$ due to aldehydic C–H stretch and characterized by its $^1$H NMR spectrum (singlet at δ 9.2 ppm due to 1H of –CHO group, multiplet at δ 1.8 ppm due to 1H of –CH, a doublet at δ 1.0 ppm due to 6H of two equivalent –CH$_3$ group), thus confirming the presence of isobutyraldehyde. It was further observed that the aldehyde does not undergo further oxidation under the present kinetic conditions. A test for corresponding acid proved negative.

The by-products were identified as ammonia by Nessler’s reagent [20], the CO$_2$ was qualitatively detected by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through tube containing...
lime water. The formation of free Ag$^+$ in solution was detected by adding KCl solution to the reaction mixture, which produced white turbidity due to the formation of AgCl. It was observed that isobutyraldehyde does not undergo further oxidation under the present kinetic conditions.

3.2 Reaction Orders

As the DPA oxidation of l-valine in alkaline medium proceeds with a measurable rate in the absence of Os(VIII) or Ru(III), the catalysed reaction is understood to occur in parallel paths with contributions from both the catalysed and uncatalysed paths. Thus, the total rate constant ($k_T$) is equal to the sum of the rate constants of the catalysed ($k_C$) and uncatalysed ($k_U$) reactions, so $k_C = k_T - k_U$. Hence, the reaction orders have been determined from the slopes of log $k_C$ versus log (concentration) plots by varying the concentrations of l-val, OH$^-$ and catalysts (Os(VIII) and Ru(III)), in turn while keeping other concentrations and conditions constant. The uncatalysed reaction was followed under the condition [l-valine] = 5.0 x 10^{-3}; [DPA] = 5.0 x 10^{-5}; [OH$^-$] = 0.002; $I = 0.006$ mol dm$^{-3}$. The rate constant of uncatalysed reaction ($k_U$) was obtained by the plot of log (absorbance) versus time by following the progress of the reaction spectrophotometrically at 360 nm.

3.3 Effect of [DPA]

The DPA concentration was varied in the range from 1.0 x 10^{-5} to 1.0 x 10^{-4} mol dm$^{-3}$ at 25 ºC for both the catalysts while keeping other reactant concentrations and conditions constant. The fairly constant pseudo-first-order rate constants, $k_C$, indicate that the order with respect to [DPA] was unity (Tables 1, 2). This was also confirmed by linearity of the plots of log (absorbance) versus time up to 80% completion of the reaction.

3.4 Effect of [l-Valine]

The l-valine concentration was varied in the range from 1.0 x 10^{-5} to 1.0 x 10^{-3} mol dm$^{-3}$ at 25 ºC while keeping other reactant concentrations and conditions constant in the presence of both the catalysts. The $k_C$ values increased with increase in [l-val]. The order with respect to [l-val] was less than unity (Os(VIII) catalysed Table 1 and Ru(III) catalysed Table 2). This was also confirmed by the plots of $k_C$ versus [l-val]$^{0.75}$ (Os(VIII) catalysed) and $k_C$ versus [l-val]$^{0.75}$ (Ru(III) catalysed) which were linear rather than the direct plot of $k_C$ versus [l-val] (Os(VIII) catalysed) and $k_C$ versus [l-val] (Ru(III) catalysed) (Fig. 2) ($r \geq 0.994, S \leq 0.003$ for Os(VIII) catalysed, $r \geq 0.997, S \leq 0.004$ for Ru(III) catalysed). However, the order with respect to [l-val] changes from first order to zero order as the [l-val] increases.

3.5 Effect of [$\text{OH}^-$] and [$\text{IO}_4^-$]

The effect of alkali was studied in the range from 0.2 x 10^{-5} to 6.0 x 10^{-3} mol dm$^{-3}$ at 25 ºC and constant concentrations of DPA, l-val, KNO$_3$, $\text{IO}_4^-$, Os(VIII)/Ru(III) catalysts. The rate constants decreased with increase in [$\text{OH}^-$] and the order was found to be less than unity in both the cases (Table 1 Os(VIII) catalysed) (Table 2 Ru(III) catalysed).

Similarly, the effect of [$\text{IO}_4^-$] was studied in the range from 1.0 x 10^{-5} to 1.0 x 10^{-4} mol dm$^{-3}$ at constant concentrations of DPA, l-valine, $\text{OH}^-$, KNO$_3$ and Os(VIII)/Ru(III) catalysts. It was observed that there was no effect of periodate on the rates of both the reactions (Tables 1, 2).

3.6 Effect of [Os(VIII)] or [Ru(III)]

The Os(VIII) concentration was varied from 1.0 x 10^{-7} to 1.0 x 10^{-6} mol dm$^{-3}$ and [Ru(III)] from 1.0 x 10^{-6} to 1.0 x 10^{-5} mol dm$^{-3}$ range, at constant concentrations of DPA(III), l-valine, $\text{OH}^-$ and ionic strength. The order in [Os(VIII)] or [Ru(III)] was found to be unity from the linearity of the plots of log $k_C$ versus log [Os(VIII)] or log [Ru(III)] (Fig. 3; Tables 1, 2).

3.7 Effect of Initially Added Products

The externally added products, Ag(I) and isobutyraldehyde did not have any significant effect on the rates of both catalysed reactions.

3.8 Effect of Ionic Strength ($I$) and Dielectric Constant of the Medium ($D$)

The addition of KNO$_3$, to increase the ionic strength of the reaction, increased the rate of reaction at constant [DPA], [l-val], [$\text{OH}^-$], [$\text{IO}_4^-$] and [catalyst]. The plot of log $k_C$ versus $\sqrt{I}$ was found to be linear with positive slope (Fig. 4).

Dielectric constant of the medium, ‘$D$’ was varied by varying the t-butyl alcohol and water percentage. The decrease in dielectric constant of the reaction medium decreased the rate and the plot of log $k_C$ versus $1/D$ was linear with negative slope (Fig. 4). The possibility of oxidation of t-butyl alcohol–water media is also checked and found that there was no significant effect under the experimental conditions.

3.9 Polymerization Study

The intervention of free radicals was examined as follows: the reaction mixture, to which a known quantity of acrylonitrile scavenger has been added initially, was kept in an
inert atmosphere for 2 h. Upon diluting the reaction mixture with methanol, precipitate resulted, suggesting there is participation of free radicals in the reaction for both Os(VIII) and Ru(III) catalysis. The blank experiments of either DPA or L-val alone with acrylonitrile did not induce any polymerization under the same condition as those induced for the reaction mixture. Initially added acrylonitrile decreases the rate of reaction indicating free radical intervention, which is the case in earlier work [21].

3.10 Effect of Temperature (T)

The influences of temperature on the rates of reaction were studied at 15, 25, 35 and 45 °C. The rate constants (k₁ and k₂) of the slow step of Schemes 1 and 2 were obtained from the slopes and the intercepts of the plots of [Catalyst]/k_C versus 1/[L-val], and [Catalyst]/k_C versus [OH⁻] at four different temperatures. The values are given in Tables 3 and 4. The activation parameters for the rate determining step were obtained by the least square method of plot of log k₁ and log k₂ versus 1/T and are presented in Tables 3 and 4.

3.11 Catalytic Activity

It has been pointed out by Moelwyn-Hughes [22] that in presence of the catalyst, the uncatalysed and catalysed reactions proceed simultaneously, so that

\[ k_T = k_U + K_C [\text{Catalyst}]^x \]  

Here ‘k_T’ is the total rate constant, ‘k_U’ the pseudo-first-order rate constant for the uncatalysed reaction, ‘K_C’ the catalytic constant and ‘x’ the order of the reaction with respect to [Os(VIII)]/[Ru(III)]. In the present investigations, x values for the standard run were found to be unity for Os(VIII) and Ru(III). Then the value of K_C is calculated using the equation

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<th>[DPA] \times 10^7 (mol dm⁻³)</th>
<th>[l-val] \times 10^4 (mol dm⁻³)</th>
<th>[OH⁻] \times 10^5 (mol dm⁻³)</th>
<th>[IO₄⁻] \times 10^5 (mol dm⁻³)</th>
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\[ \text{Table 1} \] Effect of variation of [DPA], [l-val], [OH⁻], [IO₄⁻] and [Os(VIII)] on the Os(VIII) catalysed oxidation of L-valine by DPA in aqueous alkaline medium at 25 °C and I = 0.006 mol dm⁻³
The values of $K_C$ were evaluated for both the catalysts at different temperatures and found to vary at different temperatures. Further, plots of log $K_C$ versus $1/T$ were linear and the values of energy of activation and other activation parameters with reference to catalysts were computed. These results are summarized in Table 5. The value of $K_C$ for Os(VIII) is 5.9 $\times$ 10$^{4}$ and Ru(III) is 4.5 $\times$ 10$^{3}$ at 298 K. The values of $K_C$ indicate that Os(VIII) is more efficient catalyst compared to Ru(III) in the oxidation of L-valine by DPA in alkaline medium.

4 Discussion

In the later period of twentieth century the kinetics of oxidation of various organic and inorganic substrates have been studied by Ag(III) species which may be due to its strong versatile nature of two electrons oxidant. Among the various species of Ag(III), Ag(OH)$_4^-$, DPA and ethylenebis(biguanide), (EBS), silver(III) are of maximum attention to the researchers due to their relative stability [23]. The stability of Ag(OH)$_4^-$ is very sensitive towards traces of dissolved oxygen and other impurities in the reaction medium whereupon it had not drawn much attention. However, the other two forms of Ag(III) [24] are considerably stable; the DPA is used in highly alkaline medium and EBS is used in highly acidic medium.

The literature survey [17] reveals that the water soluble DPA has a formula $[\text{Ag(IO}_6\text{)}_2]^-$ with dsp$^2$ configuration of square planar structure, similar to diperiodatocopper(III) complex with two bidentate ligands, periodate to form a planar molecule. When the same molecule is used in alkaline medium, it is unlikely to exist as $[\text{Ag(IO}_6\text{)}_2]^-$ as periodate is known to be in various protonated forms [25] depending on pH of the solution as given in following multiple equilibria (4)–(6).
H₅IO₆ ⇌ H₄IO₆⁻ + H⁺  
H₄IO₆⁻ ⇌ H₃IO₆²⁻ + H⁺  
H₃IO₆²⁻ ⇌ H₂IO₆³⁻ + H⁺  

Periodic acid exists as H₅IO₆ in acid medium and as H₃IO₆⁻ near pH 7. Thus, under the present alkaline conditions, the main species are expected to be H₃IO₆²⁻ and H₂IO₆³⁻. At higher concentrations, periodate also tends to dimerise [5]. However, formation of this species is negligible under conditions employed for kinetic study. On contrary, the authors [8] in their recent studies have proposed the DPA species as [Ag(HL)₂]⁻ in which ‘L’ is a periodate with uncertain number of protons and ‘HL’ is a protonated periodate of uncertain number of protons. This can be ruled out by considering the alternative form [25] of IO₄⁻ at pH > 7 which is in the form H₂IO₆³⁻ or H₂IO₆⁻. Hence, DPA could be as [Ag(H₃IO₆)₂]⁻ or [Ag(H₂IO₆)₂]³⁻ in alkaline medium. Therefore, under the present condition, DPA, may be depicted as [Ag(H₃IO₆)₂]⁻. The similar speciation of periodate in alkali was proposed [26] for diperiodatonickelate(IV).

It is known that L-valine exists in zwitterionic form in aqueous medium [27]. In highly acidic medium, it exists in the protonated form, whereas in highly basic medium it is in the fully deprotonated form [27].

4.1 Mechanism of Os(VIII) Catalysis

Os(VIII) is known to form different complexes at different OH⁻ [28] concentrations, [OsO₄(OH)₂]²⁻ and [OsO₄(OH)]³⁻. At higher concentration of OH⁻, [OsO₄(OH)]³⁻ is significant. At lower concentrations of OH⁻, as employed in the present study, and since the rate of oxidation decreased with increase in [OH⁻], it is reasonable that [OsO₄(OH)₂]²⁻ was operative and its formation is important in the reaction. In earlier report [29], it has been observed that in Os(VIII) catalysed reaction, in view
of less than unit order in substrate, unit order in Os(VIII) and oxidant, Os(VIII) is regenerated through formation of Os(VII). In another case [30] Os(VIII) is regenerated by Os(VI) intervention in view of unit order each in osmium, substrate and oxidant. In some other reports [28] it is observed that Os(VIII) forms a complex with substrate, which is oxidised by the oxidant with regeneration of the catalyst. Hence the study of behavior of Os(VIII) in catalysed reaction becomes significant. To explain all the observed orders, Scheme 1 is proposed for Os(VIII) catalysed reaction.

In the prior equilibrium step 1, the hydroxyl ion concentration with fractional order in OH\(^{-}\) concentration, the main oxidant species is likely to be \([\text{Ag(H}_2\text{IO}_6\text{)}_2]\) and its formation by the above equilibrium is important in the present study. The less than unit order in [L-val] presumably results from the formation of a complex (C\(_1\)) between the Os(VIII) species and L-valine. This complex (C\(_1\)) reacts with one mole of DPA in a slow step to give the free radical species of L-val, Ag(II) with the regeneration of catalyst, Os(VIII). Further this free radical species of L-val reacts with Ag(II) in a fast step to form the products isobutyraldehyde, Ag(I) as given in Scheme 1.

Spectroscopic evidence for the complex formation between Os(VIII) and L-val was obtained from UV–Vis spectra of L-val (5.0 \(\times\) \(10^{-4}\)), Os(VIII) (5.0 \(\times\) \(10^{-7}\), \([\text{OH}^{-}\] = 0.002 mol dm\(^{-3}\)) and a mixture of both. A hypsochromic shift of 5 nm from 246 to 241 nm in the spectra of Os(VIII) to the mixture of Os(VIII) and L-val was observed. Attempts to separate and isolate the complex were not successful. The Michaelis–Menten plot proved the complex formation between catalyst and substrate, which explains less than unit order in [L-val]. Such a

![Diagram](image-url)

**Scheme 1** Detailed scheme for the Os(VIII) catalysed oxidation of L-valine by DPA

\[
[\text{Ag(H}_2\text{IO}_6\text{)(H}_3\text{IO}_6\text{)}_2\text{-} + \text{H}_2\text{O} \xrightarrow{K_1} [\text{Ag(H}_3\text{IO}_6\text{)}_2\text{-} + \text{OH}^-}
\]

\[
\begin{align*}
\text{CH}_3\text{CH}-\text{CH}-\text{COO}^- + [\text{OsO}_4\text{(OH)}_2\text{]}^{-2} \xrightarrow{K_2} \text{Complex (C}_1\text{)}
\end{align*}
\]

\[
\text{Complex (C}_1\text{)} + [\text{Ag(H}_3\text{IO}_6\text{)}_2\text{]} \xrightarrow{k_f/\text{slow}} \text{CH}_3\text{CH}-\text{CH}^-\text{COO}^- + \text{HCO}_3^- + \text{Ag(OH)}^+ + 2\text{H}_2\text{IO}_6^{-2} + [\text{OsO}_4\text{(OH)}_2\text{]}^{-2}
\]

\[
\begin{align*}
\text{CH}_3\text{CH}-\text{CH}^-\text{COO}^- + \text{Ag(OH)}^+ \xrightarrow{\text{fast}} \text{CH}_3\text{CH}-\text{CHO} + \text{Ag(I)} + \text{NH}_3
\end{align*}
\]
complex between a catalyst and substrate has also been observed in other studies [28]. The rate law (8) for the Scheme 1 could be derived as

$$\text{Rate} = \frac{-d[DPA]}{dt} = \frac{k_1 K_1 K_2 [\text{L-val}] [\text{Os(VIII)}] [\text{DPA}]}{K_1 + K_1 K_2 [\text{L-val}] + [\text{OH}^-] + K_2 [\text{L-val}] [\text{OH}^-]}$$

Rate

$$\text{[DPA]} = k_C = k_T - k_U$$
$$= \frac{k_1 K_1 K_2 [\text{L-val}] [\text{Os(VIII)}]}{K_1 + K_1 K_2 [\text{L-val}] + [\text{OH}^-] + K_2 [\text{L-val}] [\text{OH}^-]}$$

This explains all the observed kinetic orders of different species. The rate law (8) can be rearranged to be Eq. 9, which is suitable for verification.

$$\frac{[\text{Os(VIII)}]}{k_C} = \frac{[\text{OH}^-]}{k_1 K_1 K_2 [\text{L-val}]} + \frac{[\text{OH}^-]}{k_1 K_1 K_2 [\text{L-val}]} + \frac{1}{k_1 K_1 K_2 [\text{L-val}]} + \frac{1}{k_1}$$

According to Eq. 9, other conditions being constant, the plots of [Os(VIII)]/k_C versus [OH^-] and [Os(VIII)]/k_C versus 1/[L-val] should be linear and found to be so (Fig. 5). From the intercepts and slopes of such plots, the reaction constants \(K_1\) and \(k_1\) were calculated as

\((9.1 \pm 0.1) \times 10^{-4} \text{ mol dm}^{-3}\),
\((1.05 \pm 0.03) \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}\),
\((5.3 \pm 0.2) \times 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}\), respectively. These constants were used to calculate the rate constants and compared with the experimental \(k_C\) values and found to be in reasonable agreement with each other, which fortifies the Scheme 1.

The thermodynamic quantities for the different equilibrium steps, in Scheme 1 can be evaluated as follows. The L-valine and hydroxide ion concentrations (Table 1) were varied at different temperatures. The plots of [Os(VIII)]/k_C versus 1/[L-val] \((r \geq 0.9996, S \leq 0.00133)\), [Os(VIII)]/k_C versus [OH^-] \((r \geq 0.9948, S \leq 0.00142)\) should be linear as shown in Fig. 5. From the slopes and intercepts, the values of \(K_1\) are calculated at different temperatures. A vant Hoff’s plot was made for the variation of \(K_1\) with temperature \([i.e., \log K_1 \text{ versus } 1/T (r \geq 0.9213, S \leq 0.1034)]\) and the values of the enthalpy of reaction \(\Delta H\), entropy of reaction \(\Delta S\) and free energy of reaction \(\Delta G\), were calculated. These values are also given in Table 3. In the same manner, \(K_2\) values were calculated at different temperatures and the corresponding values of thermodynamic quantities are given in Table 3.

The effect of increasing ionic strength on the rate explains qualitatively the reaction between two negatively charged ions, as seen in Schemes 1 and 2. Amis has shown that a plot of \(\log k_C\) versus 1/\(D\) is linear with a negative slope for a reaction between a negative ion and a dipole or two dipoles, and with a positive slope for a positive ion-dipole interaction [32].

However, in the present study, an increase in the content of t-butyl alcohol in the reaction medium leads to the decrease in the reaction rate (Fig. 4a Os(VIII) catalysed), which is in agreement with Amis theory [32].

The moderate values of \(\Delta H^\#\) and \(\Delta S^\#\) were both favorable for electron transfer processes. The favorable enthalpy was due to release of energy on solutions changes in the transition state. The negative value of \(\Delta S^\#\) suggests that the intermediate complex is more ordered than the reactants [33]. The observed modest enthalpy of activation and a higher rate constant for the slow step indicates that the oxidation presumably occurs via an inner-sphere mechanism. This conclusion is supported by earlier observations [34].
4.2 Mechanism of Ru(III) Catalysis

It is interesting to identify the probable Ru(III) chloride species in alkaline media. Electronic spectral studies have confirmed that ruthenium chloride exists in hydrated form as [Ru(H2O)5OH]2+. In the present study, it is quite probable that the [Ru(III)(OH)3]3– species in alkaline media. Electronic spectral studies have confirmed that ruthenium chloride exists in hydrated form as [Ru(H2O)5OH]2+. In the present study, it is quite probable that the [Ru(III)(OH)3]3– species, [Ru(H2O)5OH]2+ is mostly present as the hydroxylated species, [Ru(H2O)5OH]2+ [35].

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The equilibrium step 1 and the stoichiometry were same as in case of Os(VIII) catalysis. In most of the works on DPA oxidation, periodate shows retarding effect and OH⁻ had an increasing effect on the rate of reaction. However, in the present kinetic study, entirely different kinetic observations have been obtained, OH⁻ retarded the rate of the reaction and periodate showed no effect. Hence, the active species of oxidant is found to be [Ag(H2IO6)2]⁻. The less than unit order in [L-valine] indicates the formation of a complex (C2) between the Ru(III) species and L-valine. This complex (C2) reacts with one mole of DPA in a slow step to give the free radical species of L-val, Ag(II) with the regeneration of...
Spectroscopic evidence for the complex formation between Ru(III) and L-val was obtained from UV–Vis spectra of L-val \((5.0 \times 10^{-4})\), Ru(III) \((5 \times 10^{-6})\), \([\text{OH}^-]\) \(= 0.002 \text{ mol dm}^{-3}\) and a mixture of both. A bathochromic shift of 9 nm from 354 to 365 nm in the spectra of Ru(III) to the mixture of Ru(III) and L-val was observed. Attempts to separate and isolate the complex were not successful. The Michaelis–Menten plot proved the complex formation between catalyst and substrate, which explains less than unit order in [L-val]. Such a complex between a catalyst and substrate has also been observed in other studies [36]. The rate law (11) for the Scheme 2 could be derived as

\[
\frac{\text{Rate}}{[\text{DPA}]} = \frac{-d[\text{DPA}]}{dt} = \frac{k_2 K_3 K_4 [\text{L-val}] [\text{Ru(III)}] [\text{DPA}]}{K_3 + K_3 K_4 [\text{L-val}] + [\text{OH}^-] + K_4 [\text{L-val}] [\text{OH}^-]}
\]

(10)

This explains all the observed kinetic orders of different species. The rate law (11) can be rearranged to be Eq. 12, which is suitable for verification.

\[
\frac{[\text{Ru(III)}]}{[\text{K}_C]} = \frac{[\text{OH}^-]}{k_2 K_3 K_4 [\text{L-val}]} + \frac{[\text{OH}^-]}{k_2 K_3} + \frac{1}{K_3 K_4 [\text{L-val}]} + \frac{1}{k_2}
\]

(12)

According to Eq. 12, other conditions being constant, the plots of [Ru(III)]/[K_C] versus [OH^-] and [Ru(III)]/[K_C] versus 1/[L-val] should be linear and found to be so (Fig. 6). From the intercepts and slopes of such plots, the reaction constants \(K_3\), \(K_4\) and \(k_2\) were calculated as \((9.1 \pm 0.4) \times 10^{-4} \text{ mol dm}^{-3}, (9.1 \pm 0.3) \times 10^2 \text{ dm}^3 \text{ mol}^{-1}, (4.4 \pm 0.2) \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}\), respectively. These constants were used to calculate the rate constants and compared with the experimental \(k_C\) values and found to be in reasonable agreement with each other, which fortifies the Scheme 2.

The thermodynamic quantities for the different equilibrium steps, in Scheme 2 can be evaluated as follows. The L-valine and hydroxide ion concentrations (Table 2) were varied at different temperatures. The plots of \([\text{Ru(III)}] / [\text{K}_C]\) versus 1/[L-val] \((r \geq 0.9982, S \leq 0.00132)\), \([\text{Ru(III)}] / [\text{K}_C]\) versus [OH^-] \((r \geq 0.9873, S \leq 0.00145)\) should be linear as shown in Fig. 6. From the slopes and intercepts, the values of \(K_3\) are calculated at different temperatures. A vant Hoff’s plot was made for the variation of \(K_3\) with temperature \([\text{i.e., } \log K_3 \text{ versus } 1/T (r \geq 0.961, S \leq 0.1035)]\) and the values of the enthalpy of reaction
ΔH, entropy of reaction ΔS and free energy of reaction ΔG, were calculated. These values are also given in Table 4. A comparison of the ΔH value of second step (28.7 kJ mol⁻¹) of Scheme 2 with that of ΔH# (39.4 kJ mol⁻¹) obtained for the slow step of the reaction shows that these values mainly refer to the rate limiting step, supporting the fact that the reaction before rate determining step is fairly fast and involves low activation energy [31]. In the same manner, K₄ values were calculated at different temperatures and the corresponding values of thermodynamic quantities are given in Table 4. The negative value of ΔS# suggests that intermediate complex is more ordered than the reactants [33].

The effect of increasing ionic strength on the rate explains qualitatively the reaction between two negatively charged ions, as seen in Scheme 2. Amis has shown that a plot of log k₄ versus I/D is linear with a negative slope for a reaction between a negative ion and a dipole or two dipoles, and with a positive slope for a positive ion–dipole interaction [32]. However, in the present study, an increase in the content of β-butyl alcohol in the reaction medium leads to the decrease in the reaction rate (Fig. 4b for Ru(III) catalysed), which is in agreement with Amis theory [32].

The values of ΔH#, ΔS# and rate constants (k₁ and k₂) indicate that the order of reactivity of the catalysts is Os(VIII) > Ru(III) for the oxidation of l-valine by DPA. The Os(VIII) catalysed reaction, however, is reasonably fast in view of readiness of Os(VIII) to act across the –COO group and the Ru(III) catalysed reaction is slower, probably owing to the less ability of the Ru(III) to act across the –COO group. The activation parameters evaluated for the catalysed and uncatalysed reactions explain the catalytic effect on the reaction. The catalyst Os(VIII) or Ru(III) forms the complex with substrate, which enhances the reducing property of the substrate over that without catalyst (Os(VIII) or Ru(III)). Further, the catalyst Os(VIII) or Ru(III) modifies the reaction path by lowering the energy of activation.

The values of activation parameters for the Os(VIII) and Ru(III) catalysed oxidation of some amino acids by DPA are summarized in Tables 6 (for Os(VIII)) and 7 (for Ru(III)). The entropy of activation for the title reaction falls within the observed range. Variation in the rate within the reaction series may be caused by change in the enthalpy or entropy of activation. Changes in the rate are caused by changes in both ΔH# and ΔS#, but these quantities vary extensively in a parallel fashion. A plot of ΔH# versus ΔS# is linear according to equation

$$\Delta H^# = \beta \Delta S^# + \text{constant}$$

β is called the isokinetic temperature. It has been asserted that apparently linear correlation of ΔH# with ΔS# are sometimes misleading and the evaluation of β by means of the above equation lacks statistical validity [37]. Exner [38] advocates an alternative method for the treatment of experimental data. If the rates of several reactions in a series have been measured at two temperatures and log k’₁ (at T₂) is linearly related to log k₁ (at T₁), i.e., log k’₁ = α + b log k₁, he proposes that β can be evaluated from the equation

$$\beta = T_1 T_2 (b - 1)/(T_2 b - T_1)$$

We have calculated the isokinetic temperature as 157.9 K for Os(VIII) catalysed reaction by plotting log k’₁ at 303 K versus log k₁ at 298 K (r ≥ 0.9893, s ≤ 0.003) as in Fig. 7 and for Ru(III) catalysed reaction, β value as 84.3 K by plotting log k’₂ at 303 K versus log k₂ at 298 K (r ≥ 0.9969, s ≤ 0.005) as in Fig. 8. The values of β for both Os(VIII) (157.9 K) and Ru(III) (84.3 K) catalysed
reactions are lower than experimental temperature (298 K). This indicates that both the rates of reactions are governed by entropy of activation [39]. The linearity and the slope of the plot obtained may confirm that the kinetics of these reactions follow a similar mechanism, as previously suggested.

### 5 Conclusion

The comparative study of Os(VIII) and Ru(III) catalysed oxidation of L-valine by DPA was studied. Oxidation products were identified. Among the various species of Ag(III) in alkaline medium, in earlier reports the monoperiodatoargentate(III) (MPA) was the active species, whereas DPA is considered to be the active species for the title reaction. Active species of Os(VIII) is found to be [OsO₄(OH)₂]⁻ and that for Ru(III) is [Ru(H₂O)₅OH]²⁻. Activation parameters were evaluated for both the catalysed reactions. Catalytic constants and the activation parameters with reference to catalyst were also computed. Catalytic efficiency is Os(VIII) > Ru(III).

Table 6 Activation parameters for Os(VIII) catalyzed oxidation of some amino acids by DPA (for isokinetic temperature)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>$k_1 \times 10^{-4}$ (dm³ mol⁻¹ s⁻¹) at 298 K</th>
<th>$k'_1 \times 10^{-4}$ (dm³ mol⁻¹ s⁻¹) at 303 K</th>
<th>$\Delta H^\circ$ (kJ mol⁻¹)</th>
<th>$\Delta S^\circ$ (J K⁻¹ mol⁻¹)</th>
<th>Reference</th>
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<tr>
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<td>0.47</td>
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<td>0.73</td>
<td>19</td>
<td>−128</td>
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<td>L-Proline</td>
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<td>1.91</td>
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<td>L-Valine</td>
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<td>0.33</td>
<td>32.2</td>
<td>−53.8</td>
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</table>

Table 7 Activation parameters for Ru(III) catalyzed oxidation of some amino acids by DPA (for isokinetic temperature)

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<thead>
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<th>$k'_2 \times 10^{-4}$ (dm³ mol⁻¹ s⁻¹) at 303 K</th>
<th>$\Delta H^\circ$ (kJ mol⁻¹)</th>
<th>$\Delta S^\circ$ (J K⁻¹ mol⁻¹)</th>
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<td>−148</td>
<td>[41]</td>
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<td>7.03</td>
<td>39.4</td>
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<td>Present work</td>
</tr>
</tbody>
</table>

Fig. 7 Plot of log $k'_1$ at 303 K versus log $k_1$ at 298 K for isokinetic temperature in case of Os(VIII) catalysed reaction (Table 6). 1 L-leucine, 2 tyrosine; 3 L-lysine, 4 L-alanine, 5 L-proline, 6 L-valine

Fig. 8 Plot of log $k'_2$ at 303 K versus log $k_2$ at 298 K for isokinetic temperature in case of Ru(III) catalysed reaction (Table 7). 1 L-alanine, 2 L-leucine, 3 L-lysine, 4 tyrosine, 5 L-valine
Appendix A

According to Scheme 1

\[
\text{Rate} = k_1[C_1][\text{Ag(H}_2\text{IO}_6\text{)}]^{-2} = \frac{k_1K_1K_2[l-\text{val}][\text{Os(VIII)}][\text{DPA}]}{[\text{OH}^-]}
\]  

(13)

The total concentration of DPA is given by (where T and F stand for total and free)

\[
[\text{DPA}]_T = [\text{DPA}]_F + [\text{Ag(H}_2\text{IO}_6\text{)}]^{-2}
\]

\[
= [\text{DPA}]_F \left[\frac{[\text{OH}^-] + K_2}{[\text{OH}^-]}\right]
\]

Therefore,

\[
[\text{DPA}]_F = \left[\frac{[\text{DPA}]_T[\text{OH}^-]}{[\text{OH}^-] + K_1}\right]
\]  

(14)

Similarly,

\[
[l-\text{val}]_T = [l-\text{val}]_F + [C_1] = [l-\text{val}]_F + K_2[l-\text{val}]_F[\text{Os(VIII)}]
\]

\[
= [l-\text{val}]_F(1 + K_2[\text{Os(VIII)}])
\]

In view of low concentration of Ru(III) used,

\[
[l-\text{val}]_T = [l-\text{val}]_F
\]  

(15)

Similarly,

\[
[\text{OH}^-]_T = [\text{OH}^-]_F
\]

\[
[\text{Os(VIII)}]_T = [\text{Os(VIII)}]_F + [C_1]
\]

\[
= [\text{Os(VIII)}]_F + K_2[l-\text{val}]_F[\text{Os(VIII)}]_F
\]

\[
= \left[\frac{[\text{Os(VIII)}]_T}{1 + K_2[l-\text{val}]}\right]
\]  

(16)

Substituting Eqs. 14–17 in Eq. 13 and omitting the subscripts T and F, we get

\[
\text{Rate} = \frac{k_1K_1K_2[l-\text{val}][\text{Os(VIII)}]}{K_1 + K_1K_2[l-\text{val}][\text{OH}^-] + K_2[l-\text{val}][\text{OH}^-]}
\]

Appendix B

According to Scheme 2

\[
\text{Rate} = k_2[C_2][\text{Ag(H}_2\text{IO}_6\text{)}]^{-2} = \frac{k_2K_3K_4[l-\text{val}][\text{Ru(III)}][\text{DPA}]}{[\text{OH}^-]}
\]  

(18)

The total concentration of DPA is given by (where T and F stand for total and free)

\[
[\text{DPA}]_T = [\text{DPA}]_F + [\text{Ag(H}_2\text{IO}_6\text{)}]^{-2} = [\text{DPA}]_F \left[\frac{[\text{OH}^-] + K_4}{[\text{OH}^-]}\right]
\]

Therefore,

\[
[\text{DPA}]_F = \left[\frac{[\text{DPA}]_T[\text{OH}^-]}{[\text{OH}^-] + K_3}\right]
\]  

(19)

Similarly,

\[
[l-\text{val}]_T = [l-\text{val}]_F + [C_2] = [l-\text{val}]_F + K_4[l-\text{val}]_F[\text{Ru(III)}]
\]

\[
= [l-\text{val}]_F(1 + K_4[\text{Ru(III)}])
\]

In view of low concentration of Ru(III) used,

\[
[l-\text{val}]_T = [l-\text{val}]_F
\]  

(20)

Similarly,

\[
[\text{OH}^-]_T = [\text{OH}^-]_F
\]

\[
[\text{Ru(III)}]_T = [\text{Ru(III)}]_F + [C_2]
\]

\[
= [\text{Ru(III)}]_F + K_4[l-\text{val}]_F[\text{Ru(III)}]_F
\]

\[
[\text{Ru(III)}]_F = \left[\frac{[\text{Ru(III)}]_T}{1 + K_4[l-\text{val}]}\right]
\]  

(21)

Substituting Eqs. 19–22 in Eq. 18 and omitting the subscripts T and F, we get

\[
\text{Rate} = \frac{k_3K_4[l-\text{val}][\text{Ru(III)}]}{K_3 + K_3K_4[l-\text{val}][\text{OH}^-] + K_4[l-\text{val}][\text{OH}^-]}
\]

References

Mechanistic aspects of oxidation of loop diuretic drug furosemide by Ag(III) periodate complex in alkali media: A kinetic approach

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P.G. Department of Studies in Chemistry. Karnatak University, Dharwad, India

Abstract. The kinetics of oxidation of a loop diuretic drug furosemide (Fur) by diperiodatoargentate(III) (DPA) has been investigated spectrophotometrically in alkaline medium at a constant ionic strength of 0.20 mol dm$^{-3}$. The stoichiometry was 1 : 2 (Fur : DPA). The order of the reaction with respect to [DPA] was unity while the order with respect to [Fur] was less than unity over the concentration range studied. The rate increased with increase in [OH$^{-}$] and decreased with increase in [IO$_4$$^{-}$]$^{-}$. The oxidation products were identified as 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid and Ag(I). The reaction constants involved in the different steps of the reaction mechanism were calculated. Kinetic experiments suggest that [Ag(H$_2$IO$_6$)(H$_2$O)$_2$] is the reactive silver(III) species.

Keywords: Furosemide, diperiodatoargentate(III), oxidation, kinetics

1. Introduction

5-Aminosulphonyl-4-chloro-2-furanylmethyl acid (furosemide) (Fur) is a compound belonging to the high ceiling or loop diuretics group. Its pharmacological activity produces an increase in electrolyte urinary excretion and urine flow. It can increase it by a factor of 30 compared with normal excretion. These drugs are clinically used mainly in the treatment of renal disease, liver cirrhoses, oedema and hypertension [1]. For furosemide analysis in aqueous solutions, one must consider the adequate solubility in alkaline solutions or in the presence of surfactants [2].

Diperiodatoargentate(III) (DPA) is a metal complex with Ag in 3+ oxidation state like Cu$^{3+}$ in DPC and Fe$^{3+}$ in hemoglobin. It is used as a powerful oxidizing agent in alkaline medium and also as a volumetric reagent for the determination of various organic and inorganic species [3–5]. Jayaprakash Rao and other researchers [6, 7] have studied DPA as an oxidizing agent for the kinetics of oxidation of various substrates. They normally found that the order with respect to both oxidant and substrate was unity and the presence of OH$^{-}$ ions was found to enhance the rate of reaction. But they did not arrive the possible active species of DPA in alkalii and on the other hand they proposed mechanisms by generalizing
the DPA as \([\text{Ag(HL)L}]^{3+} \). However, Anil Kumar [8–10] et al. put an effort to give an evidence for the reactive form of DPA in the alkaline pH. In the present investigation, we have obtained the evidence for the reactive species for the DPA in alkaline medium.

Literature survey reveals that, analytical determination [11], photo sensitized oxidation [12] and chemical and enzymatic oxidations of furosemide [13] have been studied. But, there is no report on the uncatalyzed oxidative mechanism of furosemide drug by any oxidant in alkaline medium. Such studies also provide an insight into the interaction of metal ions with the drug in biological systems. In view of complexity of the title reaction and pharmaceutical importance of furosemide, its detailed study becomes important. In earlier reports of DPA oxidation [14], the order in \([\text{OH}^-] \) was found to be negative fractional and periodate had no effect in the reaction and diperoxidoargentate(III) (DPA) was considered to be the active species. However, in the present study, we have observed entirely different kinetic observations. That is monoperiodatoargentate(III) (MPA) is found to be active form of oxidant. In order to understand the active species of oxidant, and to propose the appropriate mechanism, the title reaction is investigated.

2. Experimental

2.1. Materials and reagents

All reagents were of reagent grade and millipore water was used throughout the work. A solution of furosemide (Sigma Aldrich) was prepared by dissolving an appropriate amount of recrystallised sample in 0.004 M alkali, because of its lower solubility in water. The required concentration of furosemide was obtained from its stock solution. The ionic strength was maintained by adding KNO3 solution and the pH was regulated with KOH (BDH) solution. An aqueous solution of AgNO3 was used to study the product effect, Ag(I). A standard stock solution of \(\text{IO}_4^- \) was prepared by dissolving a known weight of KIO4 (S.D. Fine) in hot water and used after keeping for 24 hours to attain the equilibrium. Its concentration was ascertained iodometrically [15] at neutral pH maintained using phosphate buffer. The pH of the medium in the solution was measured by ELICO (LI 120) pH meter.

2.2. Instruments used

(a) For kinetic measurements, a Peltier Accessory (temperature control) attached to Varian CARY 50 Bio UV-visible spectrophotometer (Varian, Victoria-3170, Australia) was used.

(b) For product analysis, Nicolet 5700-FT-IR spectrometer (Thermo, U.S.A), LC-MS by Agilent 1100 series-API 2000 mass spectrometer using the EI ionization technique were used.

(c) For pH measurements ELICO pH meter model LI 120 was used.

2.3. Kinetic measurements

Kinetic measurements were performed on a Varian CARY 50 Bio UV-visible spectrophotometer. The kinetics was followed under pseudo first-order condition where \([\text{Fur}] \gg [\text{DPA}] \) at 27 \(\text{C} \pm 0.1\), unless specified. The reaction was initiated by mixing the DPA to Fur solution, which also contained required concentrations of KNO3, KOH and KIO4. The progress of reaction was followed spectrophotometrically at 360 nm by monitoring the decrease in absorbance due to DPA with the molar absorbancy index, \(\varepsilon \) (base 10) to be 13900 ± 100 dm³ mol⁻¹ cm⁻¹. It was verified that there was a negligible interference from other species present in the reaction mixture at this wavelength.
The reaction was followed to more than 90% completion of the reaction. Plots of log (absorbance) versus time lead to the first-order rate constants ($k_{obs}$). The plots were linear up to 85% completion of reaction (Fig. 1) and rate constants were reproducible within ±5%. During the kinetics, a constant concentration viz. $5.0 \times 10^{-5}$ mol dm$^{-3}$ of KIO$_4$ was used throughout the study unless otherwise stated. Thus, the possibility of oxidation of furosemide by periodate was tested and found that there was no significant interference due to KIO$_4$ under experimental conditions. The total concentrations of periodate and OH$^-$ was calculated by considering the amount present in the DPA solution and that additionally added. Kinetic runs were also carried out in nitrogen atmosphere in order to understand the effect of dissolved oxygen on the rate of reaction. No significant difference in the results was observed under a nitrogen atmosphere and in the presence of air. In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates. The spectral changes during the reaction are shown in Fig. 2. It is evident from Fig. 2 that the concentration of DPA decreases at 360 nm. In view of the modest concentration of alkali used in the reaction medium, attention was also directed to the effect of the reaction vessel surface on the kinetics. Use of polythene/acrylic wares and quartz or polycrylate cells gave the same results, indicating that the surface did not have any significant effect on the reaction rates.

Regression analysis of experimental data to obtain regression coefficient ‘$r$’ and the standard deviation ‘$S$’, of points from the regression line, was performed with the Microsoft office Excel - 2003 program.

3. Results

3.1. Stoichiometry and product analysis

Different sets of reaction mixtures containing varying ratios of DPA to Fur in presence of constant amounts of OH$^-$, KIO$_4$, and KNO$_3$ were kept for 3 hours in a closed vessel under nitrogen atmosphere. The remaining concentration of DPA was assayed by measuring the absorbance at 360 nm. The results indicated a 1:2 stoichiometry as shown in Scheme 1.
Fig. 2. Spectroscopic changes occurring in the oxidation of furosemide by diperiodatoargentate (III) at 27°C, [DPA] = 5.0 × 10⁻⁵, [Fur] = 5.0 × 10⁻⁴, [OH⁻] = 0.10 and I = 0.20 mol dm⁻³ with scanning time interval = 1 min.

Scheme 1. Stoichiometry of the reaction.

The stoichiometric ratio suggests that the main oxidation products were 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid and Ag(I). The presence of carboxylic and ketone groups were identified by spot tests [16]. The nature of the product 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid was also identified by its IR spectrum, which showed sharp absorption peaks at 1625 cm⁻¹, 1776 cm⁻¹ and 1706 cm⁻¹ due to acidic –C=O respectively. The LC-MS spectrum showed a molecular ion peak at m/z at 363.0 amu confirming the product. The formation of free Ag⁺⁺ in solution was detected by adding KCl solution to the reaction mixture, which produced white turbidity due to the formation of AgCl.

3.2. Reaction order

The reaction orders were determined from the slopes of log k_{obs} versus log (concentration) plots by varying the concentrations of Fur, IO₄⁻, OH⁻ in turn while keeping all other concentrations and conditions constant. The reaction was followed under the conditions of [DPA] = 5.0 × 10⁻⁵; [Fur] = 5.0 × 10⁻⁴; [OH⁻] = 0.10; [IO₄⁻] = 5.0 × 10⁻⁵; I = 0.20 mol dm⁻³.

3.3. Effect of [diperiodatoargentate(III)]

The oxidant [DPA] was varied in the range of 1.0 × 10⁻⁵ to 1.0 × 10⁻⁴ mol dm⁻³ at fixed [Fur], [KOH] and [KIO₄]. The fairly constant pseudo first-order rate constant k_{obs} indicate that the order with respect to
Table 1

<table>
<thead>
<tr>
<th>[DPA] (mol dm⁻³)</th>
<th>[Fur] (mol dm⁻³)</th>
<th>[OH⁻] (mol dm⁻³)</th>
<th>[IO₄⁻] (mol dm⁻³)</th>
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<td>5.0</td>
<td>0.10</td>
<td>3.0</td>
<td>5.11</td>
<td>5.36</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>0.10</td>
<td>7.0</td>
<td>4.13</td>
<td>4.43</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>0.10</td>
<td>10.0</td>
<td>3.79</td>
<td>3.92</td>
<td></td>
</tr>
</tbody>
</table>

[DPA] was unity (Table 1). This was also confirmed by linearity of the plots of log (absorbance) versus time ($r \geq 0.999, S \leq 0.04$) up to 85% completion of the reaction (Fig. 1).

3.4. Effect of [furosemide]

The effect of furosemide was studied in the range of $2.0 \times 10^{-4}$ to $2.0 \times 10^{-3}$ mol dm⁻³ at constant concentrations of DPA, OH⁻, IO₄⁻ and a constant ionic strength of 0.20 mol dm⁻³. The $k_{obs}$ values increased with increase in [Fur]. The order with respect to [Fur] was less than unity (Table 1). This was also confirmed by the plot of $k_{obs}$ versus [Fur]⁰.⁵⁶ which is linear rather than the direct plot of $k_{obs}$ versus [Fur] ($r > 0.9948, S \leq 0.007$) (Fig. 3).

3.5. Effect of [alkali]

The effect of alkali was studied in the range of 0.02 to 0.20 mol dm⁻³ at constant concentrations of DPA, Fur, IO₄⁻ and ionic strength. The rate constants increased with increase in [alkali] and the order was found to be less than unity (Table 1) ($r \geq 0.9984, S \leq 0.008$), for the plot of log ($k_{obs}$) versus log [OH⁻].
3.6. Effect of periodate

The effect of periodate was studied in the range of $1.0 \times 10^{-5}$ to $1.0 \times 10^{-4}$ mol dm$^{-3}$ at constant concentrations of DPA, Fur, OH$^-$ and ionic strength. The experimental results indicated that the $k_{obs}$ values decreased with increase in the [IO$^-_4$]. The order with respect to IO$^-_4$ was negative fractional (Table 1) ($r \geq 0.9864$, $S \leq 0.015$), for the plot of log($k_{obs}$) versus log (concentration).

3.7. Effect of initially added products

The externally added products, Ag(I) and 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid did not have any significant effect on the rate of reaction.

On the basis of above experimental findings, the rate laws for the oxidation of furosemide at low concentrations of DPA can be proposed in the form of following equations,

$$\text{Rate} = k_{obs} [\text{DPA}]^{1.0} [\text{Fur}]^{0.56} [\text{OH}^-]^{0.35} [\text{IO}_4^-]^{-0.21}$$

3.8. Effect of ionic strength (I) and dielectric constant of the medium (D)

By the addition of KNO$_3$ at constant [DPA], [Fur], [OH$^-$] and [IO$^-_4$] it was found that increasing the ionic strength had no significant effect on the rate of the reaction.

Varying the t-butyl alcohol and water percentage varied dielectric constant of the medium, ‘D’. The $D$ values were calculated from the equation $D = D_a V_a + D_b V_b$, where $D_a$ and $D_b$ are dielectric constants of pure water and t-butyl alcohol respectively and $V_a$ and $V_b$ are the volume fractions of components water and t-butyl alcohol respectively in the total mixture. There was no effect of dielectric constant on the rate of reaction.
3.9. Effect of temperature (T)

The kinetics was studied at four different temperatures (290, 295, 300 and 305 K) under varying concentrations of furosemide, alkali and periodate, keeping other conditions constant. The rate constant was found to increase with increase in temperature. The rate constant ($k$) of the slow step of Scheme 2 was obtained from the slopes and the intercepts of the plots of $1/k_{obs}$ versus $1/[Fur]$ at four different temperatures. The values are given in Table 2. The activation parameters for the rate determining step were obtained by the least square method of plot of log $k$ versus $1/T$ ($r \geq 0.9925, S \leq 0.011$) and are presented in Table 2.

3.10. Test for free radicals (polymerization study)

To test the intervention of free radicals, the reaction mixture was mixed with acrylonitrile monomer and kept for two hours under a nitrogen atmosphere. On dilution with methanol no precipitate resulted, suggesting there was no participation of free radicals in the reaction.

4. Discussion

The kinetics of oxidation of various organic and inorganic substrates have been studied by Ag(III) species due to its strong versatile nature of two electrons oxidant. Among the various species of Ag(III), Ag(OH)$_4^-$, diperiodatoargentate(III) and ethylenebis (biguanide) silver(III) (EBS) are of maximum attention to the researchers due to their relative stability [17]. The stability of Ag(OH)$_4^-$ is very sensitive towards traces of dissolved oxygen and other impurities in the reaction medium whereupon it had not
Thermodynamic activation parameters for the oxidation of furosemide by DPA in aqueous alkaline medium with respect to the slow step of Scheme 2

(A) Effect of temperature

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>$10^3 \Delta \ln k (s^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>0.67</td>
</tr>
<tr>
<td>295</td>
<td>0.89</td>
</tr>
<tr>
<td>300</td>
<td>1.21</td>
</tr>
<tr>
<td>305</td>
<td>1.63</td>
</tr>
</tbody>
</table>

(B) Activation parameters (Scheme 2)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_a$ (kJ mol$^{-1}$)</td>
<td>$43 \pm 2$</td>
</tr>
<tr>
<td>$\Delta H^\circ$ (kJ mol$^{-1}$)</td>
<td>$41 \pm 2$</td>
</tr>
<tr>
<td>$\Delta S^\circ$ (JK$^{-1}$mol$^{-1}$)</td>
<td>$-146 \pm 6$</td>
</tr>
<tr>
<td>$\Delta G^\circ$ (kJ mol$^{-1}$)</td>
<td>$84 \pm 4$</td>
</tr>
<tr>
<td>$\log A$</td>
<td>$5.6 \pm 0.3$</td>
</tr>
</tbody>
</table>

(C) Effect of temperature to calculate $K_1$, $K_2$ and $K_3$ for the oxidation of furosemide by diperiodatoargentate(III) in alkaline medium

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>$10^3 K_1$ (dm$^3$ mol$^{-1}$)</th>
<th>$10^3 K_2$ (mol dm$^{-3}$)</th>
<th>$10^{-3} K_3$ (dm$^3$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>290</td>
<td>$3.1 \pm 0.1$</td>
<td>$4.1 \pm 0.1$</td>
<td>$1.6 \pm 0.07$</td>
</tr>
<tr>
<td>295</td>
<td>$4.6 \pm 0.3$</td>
<td>$2.6 \pm 0.1$</td>
<td>$1.9 \pm 0.08$</td>
</tr>
<tr>
<td>300</td>
<td>$7.5 \pm 0.15$</td>
<td>$1.1 \pm 0.05$</td>
<td>$2.2 \pm 0.06$</td>
</tr>
<tr>
<td>305</td>
<td>$8.7 \pm 0.2$</td>
<td>$0.4 \pm 0.02$</td>
<td>$4.0 \pm 0.10$</td>
</tr>
</tbody>
</table>

(D) Thermodynamic quantities using $K_1$, $K_2$ and $K_3$

<table>
<thead>
<tr>
<th>Thermodynamic quantities</th>
<th>Values from $K_1$</th>
<th>Values from $K_2$</th>
<th>Values from $K_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H$ (kJ mol$^{-1}$)</td>
<td>$55 \pm 3$</td>
<td>$-121 \pm 3$</td>
<td>$44 \pm 2$</td>
</tr>
<tr>
<td>$\Delta S$ (JK$^{-1}$mol$^{-1}$)</td>
<td>$2 \pm 0.1$</td>
<td>$16.0 \pm 0.7$</td>
<td>$-19.0 \pm 0.8$</td>
</tr>
<tr>
<td>$\Delta G$ (kJ mol$^{-1}$)</td>
<td>$214 \pm 8$</td>
<td>$214 \pm 8$</td>
<td>$214 \pm 8$</td>
</tr>
</tbody>
</table>

[DPA]$=5.0 \times 10^{-5}$, [FUR]$=5.0 \times 10^{-4}$, [OH$^-$]$=0.10$, [IO$^-_4$]$=5.0 \times 10^{-5}$, $I=0.20$mol dm$^{-3}$.

drawn much attention. However, the other two forms of Ag(III) [8–10, 18] are considerably stable; the DPA is used in highly alkaline medium and EBS is used in highly acidic medium.

The water soluble diperiodatoargentate(III) (DPA) has a formula [Ag(IO$_6$)$_2$]$^{7-}$ with dsp$^2$ configuration of square planar structure and two bidentate periodate ligands [19]. When the same molecule is used in alkaline medium, it is implausible to exist as [Ag(IO$_6$)$_2$]$^{7-}$ as periodate is known to be in various protonated forms [20] depending on the pH of the solution as given in following multiple equilibria (1)–(3).

\[
\begin{align*}
\text{H}_2\text{IO}_6 & \rightleftharpoons \text{H}_3\text{IO}_5^- + \text{H}^+ \\
\text{H}_2\text{IO}_5^- & \rightleftharpoons \text{H}_3\text{IO}_4^- + \text{H}^+ \\
\text{H}_2\text{IO}_6^2^- & \rightleftharpoons \text{H}_3\text{IO}_5^- + \text{H}^+
\end{align*}
\]
Periodic acid exists as H₃IO₆ in acid medium and as H₄IO⁶⁻ at pH 7. Thus, under the present alkaline conditions, the main species are expected to be H₃IO₂⁶⁻ and H₂IO₃⁶⁻. At higher concentrations, periodate also tends to dimerise [3]. However, formation of this species is negligible under conditions employed for kinetic study. On the contrary, the authors [6, 7] in their recent studies have proposed the DPA species as [Ag(HL)₂]⁻ in which ‘L’ is a periodate with uncertain number of protons and ‘HL’ is a protonated periodate of uncertain number of protons. This can be ruled out by considering the alternative form [20] of IO₄⁻ at pH > 7 which is in the form H₃IO₂⁶⁻ or H₂IO₃⁶⁻. Hence, DPA could be as [Ag(H₁IO₆)₂]⁻ or [Ag(H₂IO₆)₂]⁻ in alkaline medium. Therefore, under the present condition, diperiodatoargentate(III), may be depicted as [Ag(H₃IO₆)₂]⁻. The similar speciation of periodate in alkali was proposed [21] for diperiodatonicelate(IV). It is well known that, in alkali media, furosemide exists fully as anionic form.

The reaction between diperiodatoargentate(III) and furosemide in alkaline medium presents 1 : 2 stoichiometry (Fur : DPA) with a first order dependence on [DPA] and an apparent order of less than unit order in both [Fur] and [alkali] and a negative fractional order dependence on periodate. No effect of added products was observed. Based on the experimental results, a mechanism is proposed for which all the observed orders in each constituent such as [oxidant], [reductant], [OH⁻] and [IO₄⁻] may be well accommodated.

It is interesting to note that, in earlier reports [14] of DPA oxidation, OH⁻ had retarding effect on the rate of the reaction, no effect of periodate on the rate of reaction, DPA itself was considered as active species of diperiodatoargentate(III) complex and free radical intervention was observed. However in the present kinetic study, different kinetic results have been obtained. In view of the observed experimental results, monoperiodatoargentate(III) (MPA) is considered to be the active species. The fractional order dependence of kₚₑₓ on [OH⁻] suggests that OH⁻ takes part in the pre-equilibrium step 1 with DPA to give a deprotonated diperiodatoargentate(III). The plot of 1/kₚₑₓ versus [IO₄⁻] is linear with a positive intercept indicating a dissociative equilibrium in which the DPA loses a periodate ligand from its coordination sphere, forming a reactive monoperiodatoargentate(III) complex (MPA) in the second step, which is evidenced by decrease in the rate with increase in [IO₄⁻]. It may be expected that lower Ag(III) periodate species such as MPA will be more important in the reaction than DPA. The fractional order with respect to Fur primarily results from the complex formation between MPA and Fur prior to the slow step. The increase in rate of reaction with increase in alkalinity (Table 1) can be explained in terms of prevailing equilibrium of formation of [Ag(H₂IO₆)(H₃IO₆)]²⁻ from [Ag(H₃IO₆)₂]⁻ as given in the following equation.

\[
[Ag(H₃IO₆)₂]⁻ + OH⁻ \overset{K₁}{\rightleftharpoons} [Ag(H₂IO₆)(H₃IO₆)]²⁻ + H₂O
\]  

(4)

Also decrease in rate with increase in [H₃IO₆²⁻] (Table 1) suggests that equilibrium of silver(III) periodate complex to form monoperiodatoargentate(III) (MPA) species as given in Eqn. (5) is established.

\[
[Ag(H₂IO₆)(H₃IO₆)]²⁻ + 2H₂O \overset{K₂}{\rightleftharpoons} [Ag(H₁IO₆)(H₂IO₂)]⁻ + H₃IO₂⁶⁻
\]  

(5)

Such types of equilibria (4) and (5) have been well noticed in literature [22]. It may be expected that a lower periodate complex such as monoperiodatoargentate(III) (MPA) is more important in the reaction than the DPA. The inverse fractional order in [H₁IO₆²⁻] might also be due to this reason. Therefore, MPA might be the main reactive form of the oxidant.

Less than unit order in [Fur] presumably results in the reaction involving formation of a complex (C) between the MPA species and furosemide prior to the formation of the products. This complex (C) decomposes in a slow step to form 4-chloro-2-(2,5-dioxo-pentamino)-5-sulfamoyl-benzoic acid as an
intermediate species. This intermediate species further reacts with another molecule of MPA in a fast step to form the products such as 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-3-sulfamoyl-benzoic acid, Ag(I) and periodate as given in Scheme 2.

The direct plot of $k_{obs}$ versus [Fur] was drawn to know the parallel reaction if any, along with the interaction of oxidant and reductant. However, the plot of $k_{obs}$ versus [Fur] was not linear. Thus, in Scheme 2, the parallel reaction and involvement of two molecules of Fur in the complex are excluded. The probable structures of DPA, MPA and complex (C) are given in Scheme 3. Spectroscopic evidence for the complex formation between oxidant and substrate was obtained from UV-vis spectra of (DPA $(5.0 \times 10^{-5} \text{ mol dm}^{-3})$, (furosemide $(5.0 \times 10^{-4} \text{ mol dm}^{-3})$, [OH$^{-}$] $(0.10 \text{ mol dm}^{-3})$) and mixture of both. A hypsochromic shift of about 4 nm from 273 nm to 268 nm in the spectra of DPA to the mixture of DPA and Fur was observed (Fig. 4). However, the Michaelis-Menten plot proved the complex formation between DPA and Fur, which explains the less than unit order dependence in [Fur]. Such complex between oxidant and substrate has also been observed in other studies [23].

Scheme 2 leads to the rate law (7)

$$
\frac{\text{rate}}{[\text{DPA}]} = \frac{kK_1K_2K_3[\text{Fur}][\text{OH}^-][\text{DPA}]}{[\text{H}_2\text{IO}_3^-] + K_1K_2[\text{OH}^-] + K_1[\text{OH}^-][\text{H}_2\text{IO}_3^-] + K_1K_2K_3[\text{OH}^-][\text{Fur}]}
$$

(6)

$$
\frac{k_{obs}}{[\text{DPA}]} = \frac{kK_1K_2K_3[\text{Fur}][\text{OH}^-]}{[\text{H}_2\text{IO}_3^-] + K_1K_2[\text{OH}^-] + K_1[\text{OH}^-][\text{H}_2\text{IO}_3^-] + K_1K_2K_3[\text{OH}^-][\text{Fur}]}
$$

(7)

Scheme 3. Probable structures of DPA, MPA and complex (C).

Fig. 4. Spectroscopic evidence for the complex formation between DPA and furosemide. (a) UV-vis spectra of DPA complex (360 nm); (b) UV-vis spectra of mixture of DPA and Fur (268 nm); (c) UV-vis spectra of Fur (273 nm).
This explains all the observed kinetic orders of different species. The rate law (7) can be rearranged into the following form, which is suitable for verification.

\[
\frac{1}{k_{\text{obs}}} = \frac{[\text{H}_3\text{IO}_3^-]}{kK_1K_2K_3} [\text{OH}^-] [\text{Fur}] + \frac{1}{kK_3} [\text{Fur}] + \frac{1}{k} \text{[Fur]} + \frac{1}{k} \text{[H}_2\text{IO}_3^-] \frac{1}{k} \text{[Fur]}
\]

According to equation (8), other conditions being constant, plots of \(1/k_{\text{obs}}\) versus \(1/[\text{Fur}]\) (\(r \geq 0.99772, S \leq 0.014\)), \(1/k_{\text{obs}}\) versus \(1/[\text{OH}^-]\) (\(r \geq 0.98627, S \leq 0.011\)) and \(1/k_{\text{obs}}\) versus \([\text{H}_2\text{IO}_3^-]\) (\(r \geq 0.9859, S \leq 0.021\)) should be linear and are found to be so (Fig. 5). The slopes and intercepts of such plots lead to the values of \(K_1\), \(K_2\), \(K_3\) and \(k\) as \((0.75 \pm 0.15) \text{ dm}^3 \text{ mol}^{-1}\), \((1.1 \pm 0.05) \times 10^{-3} \text{ mol dm}^{-3}\), \((2.2 \pm 0.06) \times 10^{3} \text{ dm}^3 \text{ mol}^{-1}\) and \((1.21 \pm 0.10) \times 10^{-2} \text{ s}^{-1}\) respectively. The values of \(K_1\) and \(K_2\) are in good agreement with earlier literature [22]. The negligible effect of ionic strength and dielectric constant of medium on the rate explains qualitatively the reaction between neutral and negatively charged ions, as seen in Scheme 2.

The thermodynamic quantities for the first, second and third equilibrium steps of Scheme 2 can be evaluated as follows; \([\text{Fur}]\), \([\text{IO}_4^-]\) and \([\text{OH}^-]\) (as in Table 1) were varied at four different temperatures. The plots of \(1/k_{\text{obs}}\) versus \(1/[\text{Fur}]\), \(1/k_{\text{obs}}\) versus \(1/[\text{OH}^-]\) and \(1/k_{\text{obs}}\) versus \([\text{IO}_4^-]\) were linear at different temperatures. From the slopes and intercepts, the values of \(K_1\), \(K_2\) and \(K_3\) were calculated at different temperatures and these values are given in Table 2. The vant Hoff’s plots were made for variation of \(K_1\), \(K_2\) and \(K_3\) with temperature (log \(K_1\) versus \(1/T\) (\(r \geq 0.9759, S \leq 0.0088\)), log \(K_2\) versus \(1/T\) (\(r \geq 0.9816, S \leq 0.010\)) and log \(K_3\) versus \(1/T\) (\(r \geq 0.9896, S \leq 0.009\))) and the values of enthalpy of reaction \(\Delta H\), entropy of reaction \(\Delta S\) and free energy of reaction \(\Delta G\) were calculated for the first, second and third equilibrium steps. These values are given in Table 2.

A comparison of the thermodynamic quantities of first step of Scheme 2 with those obtained for the slow step of the reaction shows that these values mainly refer to the rate limiting step, supporting the fact that the reaction before rate determining step is fairly fast and involves low activation energy [24]. A high negative value of \(\Delta S^\#\) (\(-146 \text{ JK}^{-1} \text{ mol}^{-1}\)) suggests that intermediate complex is more ordered than...
the reactants [25]. The moderate $\Delta H^\circ$ and $\Delta S^\circ$ values are favourable for electron transfer reaction. The value of $\Delta H^\circ$ was due to energy of solution changes in the transition state. The observed modest enthalpy of activation and a higher rate constant for the slow step indicates that the oxidation presumably occurs via inner-sphere mechanism. This conclusion is supported by earlier observations [26].

5. Conclusion

Among the various species of Ag(III) in alkaline medium, monoperiodatoargentate(III) (MPA) i.e., $[Ag(H_2IO_6)(H_2O)_2]$ is considered as active species for the title reaction. It becomes apparent that in carrying out this reaction, the role of reaction medium is crucial. Rate constant of slow step and other equilibrium constants involved in the mechanism were evaluated and activation parameters with respect to slow step of the reaction were computed. The overall sequence described is consistent with product, mechanistic and kinetic studies.

Appendix

Appendix According to Scheme 2

\[
\text{rate} = -\frac{d[DPA]}{dt} = \frac{k K_1 K_2 [Fur][Ag(H_2IO_6)]^+ [OH^-]}{[H_2IO_3^-]} \quad (1)
\]

Total DPA can be written as (where $T$ and $f$ refer to total and free concentrations).

\[
[DPA]_T = [DPA]_f + [Ag(H_2IO_6)(H_2O)_2]_f + [Ag(H_2IO_6)(H_2O)_2]^{2-} + [C]
\]

\[
[DPA]_T = [DPA]_f \left[ 1 + \frac{K_1 K_2 [OH^-]}{[H_2IO_3^-]} + K_3 [OH^-] + \frac{K_1 K_2 K_3 [Fur][OH^-]}{[H_2IO_3^-]} \right] \quad (2)
\]

Similarly,

\[
[Fur]_T = [Fur]_f + (C)
\]

\[
[Fur]_T = [Fur]_f \left[ \frac{[H_2IO_3^-]}{[H_2IO_3^-] + K_1 K_2 [DPA][OH^-]} \right] \quad (3)
\]

In view of low concentrations of DPA used, the second term of above eqn. is neglected. Therefore,

\[
[Fur]_T = [Fur]_f \quad (3)
\]

Similarly,

\[
[OH^-]_T = [OH^-]_f \quad (4)
\]

Substituting Eqns. (2), (3) and (4) in (1) and omitting the subscripts $T$ and $F$ we get

\[
\text{rate} = -\frac{d[DPA]}{dt} = \frac{k K_1 K_2 [Fur][OH^-][DPA]}{[H_2IO_3^-] + K_3 [OH^-] + K_3 [OH^-][H_2IO_3^-] + K_1 K_2 K_3 [OH^-][Fur]} \quad (5)
\]
References

Mechanistic aspects of Os(VIII) catalysed oxidation of loop diuretic drug furosemide by Ag(III) periodate complex in aqueous alkaline medium

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Abstract. The kinetics of oxidation of a loop diuretic drug furosemide (Fur) by diperiodatoargentate(III) (DPA) has been investigated in the presence of osmium(VIII) (Os(VIII)) used as homogeneous catalyst in alkaline medium at a constant ionic strength of 0.20 mol dm\(^{-3}\) spectrophotometrically attached with HI-TECH SFA-12 stopped flow accessory. The stoichiometry was 1:2 (Fur:DPA). The order of the reaction with respect to [DPA] was unity while the order with respect to [Fur] was less than unity over the concentration range studied. The rate increased with an increase in [OH\(^–\)] and decreased with an increase in [IO\(^4–\)]. The order with respect to [Os(VIII)] was unity. The oxidation products were identified as 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid and Ag(I). A suitable mechanism was proposed. The reaction constants involved in the different steps of the reaction mechanism were calculated. Kinetic experiments suggest that [Ag(H\(_2\)IO\(_6\))(H\(_2\)O)\(_2\)] is the reactive silver(III) species and [OsO\(_4\)(OH)\(_2\)]\(^–\) is the reactive Os(VIII) species.

Keywords. Kinetics; oxidation; diperiodatoargentate(III); furosemide; Os(VIII) catalysis; mechanism.

1. Introduction

5-Aminosulphonyl-4-chloro-2-furanyl methyl acid (furosemide) (Fur) is a compound belonging to the high ceiling or loop diuretics group. Its pharmacological activity produces an increase in electrolyte urinary excretion and urine flow. It can increase it by a factor of 30 compared with normal excretion. These drugs are clinically used mainly in the treatment of renal disease, liver cirrhoses, oedema and hypertension.\(^1\)

Diperiodatoargentate(III) (DPA) is used as a powerful oxidizing agent in alkaline medium and also as a volumetric reagent for the determination of various organic and inorganic species.\(^2\)–\(^4\) Jayaprakash Rao and other researchers\(^5\)–\(^6\) have studied DPA as an oxidizing agent for the kinetics of oxidation of various substrates. They found that the order with respect to both oxidant and substrate was unity and the presence of OH\(^–\) ions was found to enhance the rate of reaction. But they did not arrive at the possible active species of DPA in alkali and on the other hand, they proposed mechanisms by generalizing the DPA as [Ag(HL)L]\(^{(x+1)–}\). However, Anil Kumar and others\(^7\)–\(^9\) put an effort to give an evidence for the reactive form of DPA in the alkaline pH. In the present investigation, we have obtained the evidence for the reactive species for the DPA in alkaline medium.

The role of osmium(VIII) as a catalyst in some redox reactions has been reviewed.\(^10\),\(^11\) Although the mechanism of catalysis depends on the nature of the substrate, oxidant and on experimental conditions, it has been shown\(^12\) that metal ions act as catalysts by one of these different paths such as the formation of complexes with reactants or oxidation of the substrate itself or through the formation of free radicals. In earlier report,\(^13\) it has been investigated that Os(VIII) forms a complex with substrate, which is reduced to Os(VI) then osmium(VII) species, followed by the rapid reaction of Os(VII) with one mole of oxidant to regenerate Os(VIII). In another report,\(^14\) it has been observed that oxidant-substrate complex reacts with Os(VIII) to form Os(VI) species, which again reacts with oxidant in a fast step to regenerate Os(VIII). In some other reports,\(^15\) it is observed that Os(VIII) forms a complex with substrate which is oxidized by the oxidant with the regeneration of Os(VIII). Hence understanding the role of Os(VIII) in the catalysed reaction is important. Catalysis by osmium(VIII) in redox reactions involves different degrees of complexity, due to the formation of different intermediate complexes and different oxidation states of osmium. We
have observed that, osmium(VIII) catalyses the oxidation of furosemide by diperiodatoargentate(III) in micro amounts in alkaline medium.

There is no report on Os(VIII) catalysed oxidative mechanism of furosemide drug by any oxidant in alkaline medium. Such studies also provide an insight into the interaction of metal ions with the drug in biological systems. Using stopped-flow accessory with UV-visible spectroscopy is an effective method to detect the spectra of intermediate species and for understanding the reaction mechanism. In view of complexity of the title reaction and pharmaceutical importance of furosemide, its detailed study becomes important. In earlier reports, Os(VIII) catalysed DPA oxidation, the order in [OH$^-\$] was found to be negative fractional and periodate had no effect in the reaction and diperiodatoargentate(III) (DPA) was considered to be the active species. However, in the present study, we have observed entirely different kinetic observations. That is monoperiodatoargentate(III) (MPA) is found to be active form of oxidant. In order to understand the active species of oxidant, and catalyst and to propose the appropriate mechanism, the title reaction is investigated using a sophisticated accessory stopped flow technique.

2. Experimental

2.1 Materials and reagents

All reagents were of reagent grade and millipore water was used throughout the work. A solution of furosemide (Sigma Aldrich) was prepared by dissolving an appropriate amount of recrystallised sample in 0.004M alkali, because of its less solubility in water. The required concentration of furosemide was obtained from its stock solution. The osmium(VIII) solution was prepared by dissolving OsO$_4$ (Johnson Matthey) in 0.50 mol dm$^{-3}$ NaOH. The concentration of Os(VIII) was ascertained by adding excess [Fe(CN)$_6$]$^{4-}$ and titrating the unreacted [Fe(CN)$_6$]$^{4-}$ with standard Ce(IV) solution in an acidic medium. The ionic strength was maintained by adding KNO$_3$ solution and the pH value was regulated with KOH (BDH) solution. An aqueous solution of AgNO$_3$ was used to study the product effect, Ag(I). A standard stock solution of IO$_4$$^-\$ was prepared by dissolving a known weight of KIO$_4$ (S.D. Fine) in hot water and used after keeping for 24 h to attain the equilibrium. Its concentration was ascertained iodometrically at neutral pH maintained using phosphate buffer. The pH of the medium in the solution was measured by ELICO (LI 120) pH meter.

2.2 Preparation of DPA

DPA was prepared by oxidizing Ag(I) in the presence of KIO$_4$ as described elsewhere; the mixture of 28 g of KOH and 23 g of KIO$_4$ in 100 cm$^3$ of water along with 8.5 g AgNO$_3$ was heated just to boiling and 20 g of K$_2$S$_2$O$_8$ was added in several lots with stirring and then allowed to cool. It was filtered through a medium porosity fritted glass filter and 40 g of NaOH was added slowly to the filtrate, whereupon a voluminous orange precipitate agglomerates. The precipitate was filtered as above and washed three to four times with cold water. The pure crystals were dissolved in 50 cm$^3$ water and heated to 80$^\circ$C with constant stirring thereby some solid was dissolved to give a red solution. The resulting solution was filtered when it was hot and on cooling at room temperature, the orange crystals separated out and were recrystallized from water.

The complex was characterized from its UV spectrum, which exhibited three peaks at 216, 255 and 360 nm. These spectral features were identical to those reported earlier for DPA. The magnetic moment study revealed that the complex was diamagnetic. The compound prepared was analysed for silver and periodate by acidifying a solution of the material with HCl, recovering and weighing the AgCl for Ag and titrating the iodine liberated when excess KI was added to the filtrate for IO$_4$$^-\$. The aqueous solution of DPA was used for the required [DPA] solution in the reaction mixture.

2.3 Instruments used

(i) For kinetic measurements, a Peltier Accessory (temperature control) attached to Varian CARY 50 Bio UV-visible spectrophotometer (Varian, Victoria-3170, Australia) connected to a rapid kinetic accessory (HI-TECH SFA-12, U.K) was used.

(ii) For product analysis, Nicolet 5700-FT-IR spectrometer (Thermo, U.S.A), LC-MS by Agilent 1100 series-API 2000 mass spectrometer using the EI ionization technique were used.

(iii) For pH measurements, ELICO pH meter model LI 120 was used.

2.4 Kinetic measurements

Since the initial reaction (Os(VIII) catalysed reaction) was too fast to be monitored by usual methods, kinetic measurements were performed on a Varian CARY 50 Bio UV-visible spectrophotometer connected to a rapid
Mechanistic aspects of Os(VIII) catalysed oxidation

kinetic accessory (HI-TECH SFA-12). The kinetics was followed under pseudo first-order condition where \([\text{Fur}] >> [\text{DPA}]\) at 27°C ± 0.1, unless specified. The reaction was initiated by mixing the DPA to Fur solution which also contained required concentrations of KNO₃, KOH, KIO₄ and Os(VIII) catalyst. The progress of reaction was followed spectrophotometrically at 360 nm by monitoring the decrease in absorbance due to DPA with the molar extinction coefficient, \(\varepsilon\) to be 13900 ± 100 dm³ mol⁻¹ cm⁻¹. It was verified that there was a negligible interference from other species present in the reaction mixture at this wavelength. The reaction was followed to more than 90% completion of the reaction. Plots of log (absorbance) versus time lead to the first-order rate constants of the catalysed (\(k_c\)) reactions. The plots were linear up to 85% completion of reaction.

The orders for various species were determined from the slopes of plots of log \(k_c\) versus log of respective concentrations of species except for [DPA] in which non-variation of ‘\(k_c\)’ was observed as expected to the reaction condition. The rate constants were reproducible within ±5%. During the kinetics, a constant concentration viz. \(5.0 \times 10^{-5}\) mol dm⁻³ of KIO₄ was used throughout the study unless otherwise stated. Thus, the possibility of oxidation of furosemide by periodate was tested and found that there was no significant interference due to KIO₄ under the experimental condition. The total concentrations of periodate and OH⁻ was calculated by considering the amount present in the DPA solution and that additionally added. Kinetic runs were also carried out in nitrogen atmosphere in order to understand the effect of dissolved oxygen on the rate of reaction. No significant difference in the results was observed under a nitrogen atmosphere and in the presence of air. In view of the ubiquitous contamination of carbonate in the basic medium, the effect of carbonate was also studied. Added carbonate had no effect on the reaction rates. The spectral changes during the reaction are shown in figure 1. It is evident from the figure 1 that the concentration of DPA decreases at 360 nm.

In view of the modest concentration of alkali used in the reaction medium, attention was also directed to the effect of the reaction vessel surface on the kinetics. Use of polythene/acyrlic wares and quartz or polyacrylate cells gave the same results, indicating that the surface did not have any significant effect on the reaction rates. Regression analysis of experimental data to obtain regression coefficient ‘\(r\)’ and the standard deviation ‘\(S\)’, of points from the regression line, was performed with the Microsoft Office Excel - 2003 program.

\[\text{Figure 1. Spectroscopic changes occurring in the oxidation of furosemide by diperiodatoargentate (III) at 27°C, [DPA] = 5.0 \times 10^{-5}, [\text{Fur}] = 5.0 \times 10^{-4}, [\text{OH}^-] = 0.10 \text{and I} = 0.20 \text{mol dm}^{-3} \text{with scanning time interval} = 1 \text{min.}\]

3. Results

3.1 Stoichiometry and product analysis

Different sets of reaction mixtures containing varying ratios of DPA to Fur in the presence of constant amount of OH⁻, KIO₄, and KNO₃ and Os(VIII) were kept for 3h in a closed vessel under nitrogen atmosphere. The remaining concentration of DPA was assayed by measuring the absorbance at 360 nm. The results indicated 1:2 stoichiometry as given in scheme 1. The stoichiometric ratio suggests that the main products were 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid and Ag(I). Presence of carboxylic and ketone group was identified by spot test.20

The nature of the product 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid was also identified by its IR spectrum, which showed sharp absorption peaks at 1625 cm⁻¹, 1776 cm⁻¹ and 1706 cm⁻¹ due to acidic – C = O respectively. The LC-MS spectrum showed a molecular ion peak at m/z at 363.0 amu confirming the product. The formation of free Ag⁺ in solution was detected by adding KCl solution to the reaction mixture, which produced white turbidity due to the formation of AgCl.

3.2 Reaction order

As the diperiodatoargentate(III) oxidation of furosemide in alkaline medium proceeds with a measurable
rate in the absence of Os(VIII), the catalysed reaction is understood to occur in parallel paths with contributions from both the catalysed and uncatalysed paths. Thus, the total rate constant \( k_t \) is equal to the sum of the rate constants of the catalysed \( k_c \) and uncatalysed \( k_u \) reactions, so \( k_c = k_t - k_u \). Hence, the reaction orders have been determined from the slopes of \( \log k_c \) versus log (concentration) plots by varying the concentrations of Fur, \( \text{IO}_4^- \), OH\(^-\) and catalyst Os(VIII), in turn, while keeping others constant. The rate constant, \( k_u \) was evaluated in the absence of catalyst, by the plot of \( \log(\text{absorbance}) \) versus time by following the progress of the reaction spectrophotometrically at 360 nm.

### 3.3 Effect of [diperiodatoargentate(III)]

The oxidant [DPA] was varied in the range of \( 1.0 \times 10^{-5} \) to \( 1.0 \times 10^{-4} \text{ mol dm}^{-3} \) at fixed [Fur], [KOH] and [KIO\(_4\)]. The fairly constant pseudo first-order rate constants, \( k_c \), indicate that the order with respect to [DPA] was unity (table 1). This was also confirmed by

#### Table 1. Effect of [DPA], [Fur], [OH\(^-\)], [IO\(_4\)] and Os(VIII) on the osmium(VIII) catalysed oxidation of furosemide by DPA in alkaline medium at 27°C, \( I = 0.20 \text{ mol dm}^{-3} \).

<table>
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<tr>
<th>( 10^5 \text{ [DPA]} ) (mol dm(^{-3}))</th>
<th>( 10^4 \text{ [Fur]} ) (mol dm(^{-3}))</th>
<th>( 10^5 \text{ [OH}^-) (mol dm(^{-3}))</th>
<th>( 10^5 \text{ [IO}_4^-) (mol dm(^{-3}))</th>
<th>( 10^6 \text{ [Os(VIII)]} ) (mol dm(^{-3}))</th>
<th>( 10^2 k_t ) (s(^{-1}))</th>
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<td>5.0</td>
<td>16.26</td>
<td>4.61</td>
<td>15.79</td>
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</table>

**Found** | **Calculated**
linearity of the plots of log (absorbance) versus time \((r \geq 0.999, S \leq 0.04)\) up to 85% completion of the reaction.

### 3.4 Effect of [furosemide]

The effect of furosemide was studied in the range of \(2.0 \times 10^{-4}\) to \(2.0 \times 10^{-3}\) mol dm\(^{-3}\) at constant concentrations of DPA, OH\(^-\), IO\(_4^-\), Os(VIII) and a constant ionic strength of 0.20 mol dm\(^{-3}\). The order with respect to [Fur] was less than unity (table 1). This was also confirmed by the plot of \(k_C\) versus [Fur] \((r \geq 0.9982, S \leq 0.014)\) (figure 2).

### 3.5 Effect of [alkali]

The effect of alkali was studied in the range of 0.02 to 0.20 mol dm\(^{-3}\) at constant concentrations of DPA, Fur, IO\(_4^-\), Os(VIII) and at a constant ionic strength. The order was found to be less than unity (table 1) \((r \geq 0.9985, S \leq 0.017)\) for the plot of log \((k_C)\) versus log [OH\(^-\)].

### 3.6 Effect of [periodate]

The effect of periodate was studied in the range of \(1.0 \times 10^{-3}\) to \(1.0 \times 10^{-2}\) mol dm\(^{-3}\) at constant concentrations of DPA, Fur, OH\(^-\), Os(VIII) and a constant ionic strength. The order with respect to IO\(_4^-\) was negative fractional (table 1) \((r \geq 0.9830, S \leq 0.021)\) for the plot of log \((k_C)\) versus log [IO\(_4^-\)].

### 3.7 Effect of [Os(VIII)]

The [Os(VIII)] was varied from \(5.0 \times 10^{-7}\) to \(5.0 \times 10^{-6}\) mol dm\(^{-3}\) range, at constant concentrations of DPA, Fur, alkali and ionic strength. The order in [Os(VIII)] was found to be unity from the linearity of the plot of \(k_C\) versus [Os(VIII)] \((r \geq 0.9986, S \leq 0.011)\) (table 1).

### 3.8 Effect of initially added products

The externally added products, Ag(I) and 2-(4-carboxy-2-oxo-but-3-enylamino)-4-chloro-5-sulfamoyl-benzoic acid did not have any significant effect on the rate of reaction.

On the basis of above experimental findings, the rate laws for the oxidation of furosemide at low concentrations of DPA in the presence of Os(VIII) as homogeneous catalyst can be proposed in the form of following equations,

\[
\text{Rate} = k_C [\text{DPA}]^{1.0} [\text{Fur}]^{0.50} [\text{OH}^-]^{0.36} \\
\times [\text{IO}_4^-]^{-0.20} [\text{Os} (\text{VIII})]^{0.99}.
\]

### 3.9 Effect of ionic strength (I) and dielectric constant of the medium (D)

By the addition of KNO\(_3\) at constant [DPA], [Fur], [OH\(^-\)], [IO\(_4^-\)] and [Os(VIII)] it was found that increasing the ionic strength of the reaction medium increases the rate of reaction; the plot of log \(k_C\) versus \(\sqrt{I}\) was found to be linear with positive slope. Varying the \(t\)-butyl alcohol and water percentage varied dielectric constant of the medium, ‘\(D\)’. The \(D\) values were calculated from the equation \(D = D_w V_w + D_B V_B\), where \(D_w\) and \(D_B\) are dielectric constants of pure water and \(t\)-butyl alcohol, respectively and \(V_w\) and \(V_B\) are the volume fractions of components water and \(t\)-butyl alcohol, respectively in the total mixture. The oxidation of \(t\)-butyl alcohol–water mixture by DPA in the presence and absence of Os(VIII) was checked and found that there was no significant effect under the experimental conditions. The decrease in dielectric constant of the reaction medium increased the rate and the plot of log \(k_C\) versus \(1/D\) was linear with positive slope.

### 3.10 Effect of temperature (T)

The kinetics was studied at four different temperatures (290, 295, 300 and 305 K) under varying concen-
trations of furosemide, alkali, periodate and catalyst, keeping other conditions constant. The rate constant was found to increase with increase in temperature. The rate constant ($k$) of the slow step of scheme 2 was obtained from the slopes and the intercepts of the plots of $[\text{Os(VIII)}]/k_C$ versus $1/[\text{Fur}]$ at four different temperatures. The values are given in table 2(A). The activation parameters for the rate determining step were obtained by the least square method of plot of log $k$ versus $1/T$ ($r \geq 0.9951, S \leq 0.019$) and are presented in table 2(B).

### 3.11 Test for free radicals (polymerization study)

To test the intervention of free radicals, the reaction mixture was mixed with acrylonitrile monomer and kept for one hour, under nitrogen atmosphere. On

### Table 2. Thermodynamic activation parameters for the osmium(VIII) catalysed oxidation of furosemide by DPA in aqueous alkaline medium with respect to the slow step of scheme 2.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>$10^{-4} k$ (dm$^3$ mol$^{-1}$ s$^{-1}$)</th>
<th>(B) Activation parameters (scheme 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parameters</td>
</tr>
<tr>
<td>290</td>
<td>5.75 ± 0.03</td>
<td>$\Delta H^\circ$ (kJ mol$^{-1}$)</td>
</tr>
<tr>
<td>295</td>
<td>6.46 ± 0.09</td>
<td>$\Delta S^\circ$ (JK$^{-1}$ mol$^{-1}$)</td>
</tr>
<tr>
<td>300</td>
<td>7.06 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>305</td>
<td>7.66 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>
dilution with methanol no precipitate resulted, suggesting there was no participation of free radicals in the reaction.

3.12 Catalytic activity

It has been pointed out by Moelwyn–Hughes that in the presence of catalyst, the uncatalysed and catalysed reactions proceed simultaneously, so that

$$k_T = k_U + K_C \text{Catalyst}^x,$$

(1)

where ‘$k_T$’ is the total rate constant, ‘$k_U$’ the pseudo first-order rate constant for the uncatalysed reaction, ‘$K_C$’ the catalytic constant and ‘$x$’ the order of the reaction with respect to Os(VIII). In the present investigations, $x$ values for the standard run was found to be unity. The values of $K_C$ were evaluated at different temperatures and found to vary at different temperatures. Further, plot of log $K_C$ versus $1/T$ was linear and the values of energy of activation and other activation parameters with reference to catalyst were computed. The values of $K_C \times 10^{-4}$ were obtained as 1.70, 2.27, 2.69, and 3.21 at 290, 295, 300, and 305 K, respectively. Further, plot of log $K_C$ versus $1/T$ was linear, and the values of energy of activation and other activation parameters with reference to the catalyst were computed as $E_a = 38.0 \pm 0.5$ kJ mol$^{-1}$, $\Delta H^\circ = 35.0 \pm 0.7$ kJ mol$^{-1}$, $\Delta S^\circ = -43.0 \pm 0.8$ JK$^{-1}$ mol$^{-1}$, $\Delta G^\circ = 48.0 \pm 1.1$ kJ mol$^{-1}$, $\log A = 10.9 \pm 0.3$.

4. Discussion

The kinetics of oxidation of various organic and inorganic substrates have been studied by Ag(III) species due to its strong versatile nature of two electrons oxidant. Among the various species of Ag(III), Ag(OH)$_3^-$, diperiodatoargentate(III) and ethylenbis (biguanide) silver(III) (EBS) are of maximum attention to the researchers due to their relative stability. The stability of Ag(OH)$_3^-$ is very sensitive towards traces of dissolved oxygen and other impurities in the reaction medium whereupon it had not drawn much attention. However, the other two forms of Ag(III) are considerably stable; the DPA is used in highly alkaline medium and EBS is used in highly acidic medium.

The water soluble diperiodatoargentate(III) (DPA) has a formula $[\text{Ag(IO}_6\text{)}_2]^{2-}$ with dsp$^2$ configuration of square planar structure and two bidentate periodate ligands. The same molecule is used in alkaline medium, it is impossible to exist as $[\text{Ag(IO}_6\text{)}_2]^{2-}$ as periodate is known to be in various protonated forms depending on the pH of the solution as given in following multiple equilibria (2)–(4).

$$\text{H}_5\text{IO}_6 \iff \text{H}_4\text{IO}_6^- + \text{H}^+, \quad (2)$$

$$\text{H}_4\text{IO}_6^- \iff \text{H}_3\text{IO}_6^{2-} + \text{H}^+, \quad (3)$$

$$\text{H}_3\text{IO}_6^{2-} \iff \text{H}_2\text{IO}_3^{-} + \text{H}^+. \quad (4)$$

Periodic acid exists as $\text{H}_3\text{IO}_6$ in acid medium and as $\text{H}_4\text{IO}_6^-$ at pH 7. Thus, under the present alkaline conditions, the main species are expected to be $\text{H}_3\text{IO}_6^-$ and $\text{H}_2\text{IO}_3^-$. At higher concentrations, periodate also tends to dimerise. However, formation of this species is negligible under conditions employed for kinetic study. On the contrary, the authors in their recent studies have proposed the DPA species as $[\text{Ag(HL)}_2]^2-$ in which ‘L’ is a periodate with uncertain number of protons and ‘HL’ is a protonated periodate of uncertain number of protons. This can be ruled out by considering the alternative form of IO$_4^-$ at pH > 7 which is in the form $\text{H}_2\text{IO}_6^2-$ or $\text{H}_3\text{IO}_6^-$. Hence, DPA could be as $[\text{Ag(H}_2\text{IO}_6^-\text{)}_2]^-$ or $[\text{Ag(H}_2\text{IO}_6^2^-\text{)}_2]^2-$ in alkaline medium. Therefore, under the present condition, diperiodatoargentate(III), may be depicted as $[\text{Ag(H}_2\text{IO}_6^2^-\text{)}_2]^2-$. The similar speciation of periodate in alkali was proposed for diperiodatonicelicate(IV). It is well-known that, in alkaline media, furosemide exists fully as anionic form.

Osmium(VIII) is known to form different complexes at different OH$^-$ concentrations as $[\text{OsO}_4\text{(OH)}_2]^2-$ and $[\text{OsO}_4\text{(OH)}]^3-$. At higher concentration of OH$^-$, $[\text{OsO}_4\text{(OH)}]^3-$ is significant. At lower concentrations of OH$^-$, as employed in the present study, and since the rate of oxidation increased with increase in [OH$^-$], it is reasonable that $[\text{OsO}_4\text{(OH)}]^2-$ was operative and its formation is important in the reaction. Mechanistically most studies propose electron transfer between the substrate and catalyst in the rate determining steps leading to the products, followed by the rapid oxidation of osmium(VI/VII) to osmium(VIII) by the main oxidants, resulting in zeroth order kinetics with respect to the main oxidant. In another case, Os(VIII) is regenerated by Os(VI) intervention in view of unit order in each osmium, substrate and oxidant. In some other reports, it is observed that Os(VIII) forms complex with substrate, in view of apparent less than unit order in substrate concentrations which is oxidized by the oxidant with the regeneration of the catalyst. Hence the study of the behaviour of Os(VIII) becomes significant.

In the Os(VIII) catalysed reaction, [DPA] was first order dependence, an apparent order of less than unit order in both [Fur] and [alkali], a negative fractional
order dependence on [periodate] and unit order with respect to Os(VIII) concentrations. No effect of added products was observed. Based on the experimental results, a mechanism is proposed as in scheme 2 for which all the observed orders in each constituent such as [oxidant], [reductant], [catalyst], [OH$^-$] and [IO$_4^-$] may be well-accommodated.

In the prior equilibrium step 1, the [OH$^-$] deprotonates the DPA to give a deprotonated diperiodatoargentate(III); in the second step displacement of ligand, periodate takes place to give free periodate which is evidenced by decrease in the rate with increase in [periodate] (table 1). It may be expected that lower Ag(III) periodate species such as MPA is more important in the reaction than the DPA. The inverse fractional order in [H$_3$IO$_6^-$] might also be due to this reason. In the pre rate determining stage, the Os(VIII) species combines with one mole of MPA in a rate determining step to give the intermediate species derived from furosemide and regeneration of catalyst, Os(VIII). This intermediate species derived from furosemide further reacts with another molecule of MPA species in further fast step to give the products as given in scheme 2.

Spectroscopic evidence for the complex formation between Os(VIII) and Fur was obtained from UV-vis spectra of (Fur (5.0 × 10$^{-4}$ mol dm$^{-3}$)), (Os(VIII) (1.0 × 10$^{-6}$ mol dm$^{-3}$), OH$^-$ (0.1 mol dm$^{-3}$)) and a mixture of both. A bathochromic shift of about 7 nm from 273 nm to 280 nm in the spectra of Os(VIII) to mixture of Os(VIII) and Fur was observed. The Michaelis–Menten plot proved the complex formation between catalyst and reductant, which explains less than unit order in [Fur]. Such complex between a catalyst and substrate has also been observed in other studies.$^{26}$ The nature of the complex (C) is a chelate complex. An attempt to isolate the complex was not successful. According to scheme 2, the rate law could be derived as,

$$\text{rate}_T = \text{rate}_U + \text{rate}_C = \frac{-d[DPA]}{dt} = \frac{kK_1K_2K_3[Fur][OH^-][\text{Os (VIII)}][DPA]}{K_1K_2[OH^-] + [H_2IO_6^-] + K_1[OH^-][H_2IO_6^-] + K_1K_2K_3[Fur][OH^-]},$$  \hspace{1cm} (5)

$$\frac{\text{rate}}{[DPA]} = \frac{kC = k_T - k_U}{k_kK_1K_2K_3[Fur][OH^-][\text{Os (VIII)}]} = \frac{kK_1K_2K_3[Fur][OH^-][\text{Os (VIII)}]}{K_1K_2[OH^-] + [H_2IO_6^-] + K_1[OH^-][H_2IO_6^-] + K_1K_2K_3[Fur][OH^-]},$$  \hspace{1cm} (6)

This explains all the observed kinetic orders of different species. The rate law (6) can be rearranged to be equation (7), which is suitable for verification.

$$\frac{[\text{Os (VIII)}]}{k_C} = \frac{1}{kK_3[Fur]} + \frac{[H_2IO_6^-]}{kK_1K_2K_3[OH^-][Fur]} + \frac{[H_2IO_6^-]}{kK_2K_3[Fur]} + \frac{1}{k}. \hspace{1cm} (7)$$

According to equation (7), the plots of [Os(VIII)]/$k_C$ versus 1/[Fur], [Os(VIII)]/$k_C$ versus 1/[OH$^-$] and [Os(VIII)]/$k_C$ versus [IO$_4^-$] should be linear and are found to be so. From the intercepts and slopes of such plots, the reaction constants $K_1$, $K_2$, $K_3$ and $k$ were calculated as (0.55 ± 0.06) dm$^3$ mol$^{-1}$, (1.3 ± 0.05) × 10$^{-2}$ mol dm$^{-3}$, (3.0 ± 0.1) × 10$^3$ dm$^3$ mol$^{-1}$ (7.1 ± 0.4) × 10$^4$ dm$^3$ mol$^{-1}$ s$^{-1}$, respectively. The value $K_1$ and $K_2$ obtained are in good agreement with previously reported value.$^{26}$ These constants were used to calculate the rate constants and compared with the experimental $k_C$ values and found to be in reasonable agreement with each other, which fortifies the scheme 2.

The thermodynamic quantities for the different equilibrium steps, in scheme 2 can be evaluated as follows. The furosemide, periodate and hydroxide ion concentrations (table 1) were varied at different temperatures. The plots of [Os(VIII)]/$k_C$ versus 1/[Fur] (r ≥ 0.9899, S ≤ 0.0012), [Os(VIII)]/$k_C$ versus 1/[OH$^-$] (r ≥ 0.9799, S ≤ 0.0015) and [Os(VIII)]/$k_C$ versus [IO$_4^-$] (r ≥ 0.9847, S ≤ 0.0019) were linear at different temperatures. From the slopes and intercepts, the values of $K_1$, $K_2$, $K_3$ were calculated at different temperatures. A vant Hoff's plot was made for the variation of $K_1$,
with temperature (i.e., log $K_1$ versus $1/T$, log $K_2$ versus $1/T$ and log $K_3$ versus $1/T$) and the values of the enthalpy of reaction $\Delta H$, entropy of reaction $\Delta S$ and free energy of reaction $\Delta G$, were calculated. These values are also given in table S1. A comparison of the latter values with those obtained for the slow step of the reaction shows that these values mainly refer to the rate limiting step, supporting the fact that the reaction before the rate determining step is fairly slow and involves high activation energy.\textsuperscript{27}

The increase in the rate in increase in the ionic strength is in favour of a reaction between oppositely charged species of reactant, as presented in scheme 2 of the proposed mechanism. The effect of solvent on the reaction rate is described in detail in the literature.\textsuperscript{28} For the limiting case of a zero angle approach between two dipoles or anion-dipole system, Amis\textsuperscript{,29} has shown that a plot of log $k_\ell$ versus $1/D$ gives a straight line, with a negative slope for a reaction between a negative ion and a dipole or two dipoles and with a positive slope for positive ion and dipole interaction. In the present study, the observed plot had a positive slope, which is in the right direction as shown in scheme 2. The moderate $\Delta H^\#$ and $\Delta S^\#$ values are favourable for electron transfer reaction. The value of $\Delta H^\#$ was due to energy of solution changes in the transition state. The negative value of $\Delta S^\#$ value suggests that the intermediate complex is more ordered than the reactants.\textsuperscript{28} The observed modest enthalpy of activation and a higher rate constant for the slow step indicates that the oxidation presumably occurs via inner-sphere mechanism. This conclusion is supported by earlier observations.\textsuperscript{30} The activation parameters evaluated for the reaction explains the catalytic effect on the reaction. The catalyst Os(VIII) forms the complex (C) with substrate which enhances the reducing property of the substrate than that without catalyst. Further, the catalyst Os(VIII) modifies the reaction path by lowering the energy of activation.

5. Conclusions

The Os(VIII) catalysed oxidation of furosemide by diperiodatoargentate(III) was studied. Oxidation products were identified. Among the various species of Ag(III) in alkaline medium, monoperiodatoargentate(III) (MPA) i.e., [Ag(H$_2$IO$_6$) (H$_2$O)$_2$] is considered as active species for the title reaction. Active species of Os(VIII) is found to be [OsO$_4$(OH)$_2$]$^{2-}$. It becomes apparent that in carrying out this reaction, the role of reaction medium is crucial. Activation parameters were evaluated. Catalytic constants and the activation parameters with reference to catalyst were also computed. The overall sequence described is consistent with all the experimental evidences including the product, spectral, mechanistic and kinetic studies.

Supporting information

Table S1 is given as electronic supplementary data (see www.ias.ac.in/chemsci).

Appendix A

According to scheme 2,

$$\text{Rate} = k \left[ \text{C} \right] \left[ \text{Ag} \left( \text{H}_2\text{IO}_6 \right) \left( \text{H}_2\text{O} \right)_2 \right] = \frac{kK_1K_2K_3 \left[ \text{Fur} \right] \left[ \text{Os} \left( \text{VIII} \right) \right] \left[ \text{DPA} \right] \left[ \text{OH}^- \right]}{\left[ \text{H}_2\text{IO}_6^- \right]} \quad \text{(A)}$$

The total concentration of DPA is given by (where T and f stands for total and free)

$$[\text{DPA}]_T = [\text{DPA}]_f + \left[ \text{Ag} \left( \text{H}_2\text{IO}_6 \right) \left( \text{H}_2\text{O} \right)_2 \right]^{2-} + \left[ \text{Ag} \left( \text{H}_2\text{IO}_6 \right) \left( \text{H}_2\text{O} \right)_2 \right]$$

$$= [\text{DPA}]_f \left[ \frac{K_1K_2 \left[ \text{OH}^- \right] + \left[ \text{H}_2\text{IO}_6^- \right] + K_1 \left[ \text{OH}^- \right] \left[ \text{H}_2\text{IO}_6^- \right]}{\left[ \text{H}_2\text{IO}_6^- \right]} \right] \quad \text{(B)}$$

Therefore,

$$[\text{DPA}]_f = \frac{[\text{DPA}]_T \left[ \text{H}_2\text{IO}_6^- \right]}{K_1K_2 \left[ \text{OH}^- \right] + \left[ \text{H}_2\text{IO}_6^- \right] + K_1 \left[ \text{OH}^- \right] \left[ \text{H}_2\text{IO}_6^- \right]} \quad \text{(B)}$$

Similarly,

$$[\text{Fur}]_T = [\text{Fur}]_f + (\text{C})$$

$$= [\text{Fur}]_f + K_3 \left[ \text{Fur} \right]_f \left[ \text{Os} \left( \text{VIII} \right) \right] = [\text{Fur}]_f \left[ 1 + K_3 \left[ \text{Os} \left( \text{VIII} \right) \right] \right].$$
In view of low concentrations of Os(VIII) used, 
\[ [\text{Fur}]_T = [\text{Fur}]_f. \]  
\[ \text{(C)} \]

Similarly, 
\[ [\text{OH}^-]_T = [\text{OH}^-]_f \text{ and } [\text{H}_2\text{IO}_3^-]_T = [\text{H}_2\text{IO}_3^-]_f, \]  
\[ \text{(D)} \]

Substituting (B), (C), (D) and (E) in (A) and omitting the subscripts T and f, we get 
\[
\text{rate} = -\frac{d[DPA]}{dt} = \frac{k K_1 K_2 K_3 [\text{Fur}] [\text{OH}^-] [\text{Os(VIII)}] [DPA]}{K_1 K_2 [\text{OH}^-] + [\text{H}_2\text{IO}_3^-]^+ + K_1 [\text{OH}^-][\text{H}_2\text{IO}_3^-]^+ + K_1 K_2 K_3 [\text{Fur}] [\text{OH}^-]}.
\]

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Voltammetric oxidation and determination of loop diuretic furosemide at a multi-walled carbon nanotubes paste electrode
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Electrochemical oxidation of loop diuretic furosemide (4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid) was studied in 5.0 pH with 0.04 M Britton–Robinson buffer as supporting electrolyte at 25 ± 0.1 C at a multi-walled carbon nanotubes-paraffin oil paste electrode (CNTPE) using cyclic and differential-pulse voltammetric (DPV) techniques. The electrochemical process was observed to be adsorption controlled, irreversible and involving two-electron oxidation. Effects of anodic peak potential (Epa), anodic peak current (Ipa) and heterogeneous rate constant (kH) have been discussed. Under optimal conditions, the peak current was proportional to furosemide concentration in the range of 8.0 × 10−6 to 2.0 × 10−4 M with a detection limit of 2.9 × 10−7 M by differential pulse voltammetry. A differential pulse voltammetric method with good precision and accuracy was developed for the determination of furosemide in pharmaceutical formulations and urine as a real sample.

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1. Introduction

Furosemide (FUR) is a diuretic which is an antranilic acid derivative. Chemically, it is 4-chloro-N-furfuryl-5-sulfamoylbenzoic acid [1]. It is used in the treatment of edema associated with renal impairment [2], nephrotic syndrome, hypertension, heart failure [3], hepatic cirrhosis [4], adjunct in cerebral/pulmonary edema where rapid diuresis is required and also used in the management of severe hypercalcemia in combination with adequate rehydration. The high degree of efficacy is largely due to this unique site of action. Furosemide with a prompt action is fairly rapidly absorbed after oral administration and shows a strong diuretic effect of short duration [5]. Its bioavailability ranges from 60% to 70% and its plasma half-life is about 1–2 h [6], with structural formula shown in Scheme 1.

A number of methods for the individual determination of these diuretics in both pharmaceutical preparations and biological fluids have been reported. Thus, furosemide is normally determined by liquid chromatography with spectrophotometric [7], spectrophuorimetric detection [8], chemiluminescent [9] and micellar electrokinetic chromatographic methods [10]. Most often, the procedures involve some extraction, solvent-usaive intensive, expensive devices and are thus time consuming.

In recent years, the electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time, as compared with other techniques. Electrochemical have proven to be useful for development of very sensitive and selective methods for the determination of organic molecules including drugs. In addition application of electro analytical techniques include the determination of electrode mechanisms. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmaceutical activity [11,12]. This drug was also be quantified with electrochemical methods using glassy carbon electrode [13], gold electrode [14], hanging mercury drop electrode and graphite electrode [15].

A comparison between the analytical parameters obtained by various electrochemical procedures is presented in Table 1. A limit of detection close to 1.5 × 10−7 mol dm−3 is found at glassy carbon electrodes, but with the necessity of renewal of the electrode surface after each measurement, in order to achieve reproducible responses [13]; limits of detection of 1.7 × 10−7 mol dm−3 (using flow injection analysis) and 5.5 × 10−7 mol dm−3 using HPLC with electrochemical detection were found in [16] at glassy carbon electrodes. In the latter case, the oxidation peak potential had a relatively high value, close to +1.25 V vs. Ag/AgCl (1 M KCl); electrode surface pre-treatment being needed before each measurement. For electrochemical determinations, the main problem which causes most difficulties is adsorption of the analyte or its reaction products on the electrode surface. Apart from solid carbon electrodes, such as glassy carbon, paste electrodes have also been investigated for use in oxidative electroanalytical procedures.
2. Experimental

2.1. Reagents

A stock standard solution of furosemide (Sigma Aldrich), 1 mM was prepared in HPLC grade methanol (S.D. Fine) and stored in the dark under refrigeration (4 °C) to avoid possible decomposition. More dilute solutions (10⁻⁶ to 10⁻⁴ M) were prepared by diluting the stock solution. Multi-walled carbon nanotubes were from Sigma–Aldrich (>90%, O.D.: 10–15 nm, I.D.: 2–6 nm, length: 0.1–10 μm). Britton–Robinson (BR) buffer (0.04 mol/l) was prepared and used as supporting electrolyte. Buffer solutions were adjusted by adding the necessary amounts of KOH or HCl in order to obtain the appropriate pH value. Furosemide tablets i.e., Lasix (40 mg/tablet) were purchased from Aventis Pharma. Limited, India. Rest of the reagents was of analytical-reagent grade, and millipore water was used through out the experiment.

2.2. Apparatus

Electrochemical measurements were carried on a CHI-1110A electrochemical analyzer (CH Instruments Ltd. Co., USA, version 4.01) coupled with a conventional three-electrode cell. A three-electrode system consisting of a Ag/AgCl as reference electrode, a Pt wire as counter electrode and a self-made carbon nanotubes paste electrode as a working electrode were used. All the potentials in this paper are given against the Ag/AgCl (3 M KCl). The pH measurements were made by Elico LI120 pH meter (Elico Ltd., India) and Nicolet Impact 410 FTIR, Varian Cary 50 Bio UV-visible Spectrophotometer were used to identify the product.

2.3. Preparation of electrode

The carbon nanotubes paste was prepared by mixing multi-walled carbon nanotube powder and paraflin oil in an agate mortar in a ratio of 60.0% nanotubes powder to 40.0% paraflin oil (w/w) and this mixture was then homogenized. The ratio was used due to its success in previous applications [30]. A portion of the resulting paste was packed firmly into a cavity of a polytetraflouro ethylene tube (PTFE). The surface of the electrode was smoothed against weighing paper and rinsed with water. Unless otherwise stated, the paste was carefully removed prior to pressing a new portion into the electrode after every measurement. The resulting electrode was noted as CNTEP.

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM K₃Fe(CN)₆ in 0.1 M KCl by recording the current voltage curve at different scan rates. For a reversible process, the following Randles–Scheil formula can be used [31].

\[ I_{pa} = (2.69 \times 10^5) n^{1/2} A D_R^{1/2} \nu^{1/2} C_0 \]  

where \( I_{pa} \) refers to the anodic peak current, \( n \) is the number of electrons transferred, \( A \) is the surface area of the electrode, \( D_R \) is diffusion coefficient, \( \nu \) is the scan rate and \( C_0 \) is the concentration of K₃Fe(CN)₆. For 1.0 mM K₃Fe(CN)₆ in 0.1 M KCl electrolyte, \( n = 1, D_R = 7.6 \times 10^{-6} \text{cm}^2 \text{s}^{-1} \), then from the slope of the plot of \( I_{pa} \) versus \( \nu^{1/2} \), relation, the electro active area was calculated.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Electroanalytical procedure for determination of furosemide in the literature.</th>
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<tr>
<td>Detection</td>
<td>Media</td>
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<tr>
<td>Amperometric detection at carbon fiber microelectrodes (+1.25 V vs. Ag/AgCl) coupled to HPLC and FIA</td>
<td>Acetonitrile–water (25:75) with 5 mmol/l NaH₂PO₄ (HPLC), and 5 mmol/l NaH₂PO₄ pH 6.5 (FIA)</td>
</tr>
<tr>
<td>Graphite–polyurethane composite electrode (+1.0 V vs. SCE)</td>
<td>1.0 mmol/l NaOH</td>
</tr>
<tr>
<td>Multi-walled carbon nanotubes-paraffin oil paste electrode</td>
<td>Methanol–water (10:90)</td>
</tr>
</tbody>
</table>
experiment the slope was $7.58 \times 10^{-6} \mu A (V s^{-1})^{-1/2}$ and the area of electrode was calculated to be 0.102 cm$^2$.

2.4. Analytical procedure

The CNTPE was first activated in BR buffer (pH 5.0) by cyclic voltammetric sweeps between 0.0 and 2.0 V until a stable cyclic voltammogram was obtained. Then electrodes were transferred into another cell of BR buffer (pH 5.0) containing proper amount of FUR. The potential scan was initiated and cyclic voltammograms were recorded between $+0.60$ and $+1.60$, with a scan rate of 50 mV s$^{-1}$. All measurements were carried out at room temperature of $25.0 \pm 0.1$ °C.

2.5. Sample preparation

A quantity of 10 tablets were weighed and ground to a homogeneous fine powder in a mortar. A portion equivalent to a stock solution of a concentration of about 0.01 M was accurately weighed and transferred into a 100 ml calibrated flask and completed to the volume with methanol. The contents of the flask were sonicated for 10 min to affect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with the BR buffer solutions. Each solution was transferred to the voltammetric cell and analyzed by standard addition method. The differential-pulse voltammograms were recorded between 0.60 and 1.60 V. The oxidation peak current of FUR was measured. The parameters for differential-pulse voltammetry (DPV) were pulse width of 0.06 s, pulse increment of 4 mV, pulse period of 0.2 s, pulse amplitude of 50 mV and scan rate of 20 mV s$^{-1}$. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage form, recovery experiments were carried out. The concentration of FUR was calculated using standard addition method.

3. Results and discussion

3.1. Cyclic voltammetric behavior of FUR

The voltammetric behavior of furosemide at the CNTPE was examined. Fig. 1 shows the cyclic voltammograms of $1.0 \times 10^{-3}$ M furosemide in BR buffer of pH 5.0 in the potential range of 0.60–1.60 V, with a scan rate of 50 mV s$^{-1}$. Furosemide exhibited two anodic peaks, one at 1.09 V with anodic current of 6.28 μA and another at 1.26 V with anodic current of 10.2 μA.

No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. Nevertheless, it was found that the oxidation peak current of FUR showed a remarkable decrease during the successive cyclic voltammetric sweeps (Fig. 2). After the second sweep, the peak current decreased greatly and finally remained unchanged. This phenomenon may be due to the fact that the adsorption of FUR or its oxidative product occurs at the electrode surface. Therefore, the voltammograms corresponding to the second cycle and peak B were generally recorded, since peak B was intense than A.

3.2. Influence of pH

Within the range of pH 3.0–11.2, the peak potential shifted to less positive values for both the peaks, together with a decrease in peak currents with increasing the pH of the buffer solution (Fig. 3A). However, by increasing the pH, the potential of the peak B is shifted to less positive values till pH 7.0, then becomes almost pH independent (Fig. 3B). Basically, two linear regions are obtained, one between pH 3.0 and 7.0 with a slope of 50 mV/pH and another between pH 7.0 and 11.2 with a slope of 27 mV/pH. The maximum peak current value was obtained at acidic pH value i.e., the intensity of peak B was increased to a high value at pH 5.0, and then the peak intensity decreases continuously. The highest peak intensity for the peak A was obtained around pH 7.0. Above pH 9.0, the peak A was no longer present. This effect of pH on the electrochemical properties of soluble ions in solution can be attributed to the acid–base equilibrium constants of this diuretic due to the iminium, carboxylic and sulphonamidic groups [13]. As compared to peak B, peak A was less intense and moreover it can be observed within pH 9.0.

3.3. Influence of scan rate

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of FUR at different scan rates from 10 to 200 mV s$^{-1}$ (Fig. 4A) was studied. It was noticed that the first oxidation peak became broader and almost disappeared at higher scan rates. There is a good linear relationship between peak current and scan rate. The equations are $I_{pa1} = 98.51\nu + 1.4702; r = 0.9870$ and $I_{pa2} = 129.59\nu + 2.5953; r = 0.9865$, for peaks A and B, respectively. In addition, there was
a linear relation between \( \log I_p \) and \( \log v \), corresponding to the following equation: 
\[
\log I_p = \log I_0 + \frac{2.303RT}{anF} \log \left( \frac{RTn}{anF} \right) + 0.0898 \log v
\]
for peaks A and B, respectively (Fig. 4B). The slopes of 0.7846 and 0.8028 are close to the theoretically expected value of 1.0 for an adsorption controlled process [32]. This indicates that the electrode process was controlled by adsorption rather than diffusion.

The peak potential shifted to more positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate can be expressed as 
\[
E_{pa1} = 1.2108 + 0.0898 \log v; \quad r = 0.9902 \quad \text{and} \quad E_{pa2} = 1.372 + 0.0985 \log v; \quad r = 0.9951,
\]
for peaks A and B, respectively (Fig. 4C). This behavior was consistent with the electrochemical nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step [33].

As for an irreversible electrode process, according to Laviron [34], \( E_p \) is defined by the following equation:
\[
E_p = E^{0} + \frac{2.303RT}{anF} \log \left( \frac{RTn}{anF} \right) + \alpha \frac{2.303RT}{anF} \log v \tag{2}
\]
where \( \alpha \) (alpha) is the transfer coefficient, \( E^{0} \) the standard heterogeneous rate constant of the reaction, \( n \) the number of electrons transferred, and \( \log v \) (nu) the scan rate and \( E^{0} \) is the formal redox potential. Other symbols have their usual meanings. Thus the value of \( \alpha \) can be easily calculated from the slope of \( E_{pa1} \) versus \( \log v \) and \( E_{pa2} \) versus \( \log v \). In this system, for first plot slope is 0.0898, therefore, the \( \alpha \) calculated to be 0.66 and for the second plot slope is 0.0985, therefore \( \alpha \) calculated to be 0.60, taking \( T = 298 \), \( R = 8.314 \) and \( F = 96,480 \). According to Bard and Faulkner [35], \( \alpha \) can be given as
\[
\alpha (\text{mV}) = \frac{47.7}{E_p - E_{p/2}}
\tag{3}
\]

Fig. 3. (A) Influence of pH on the shape of anodic peak: pH 3.0 (a), 4.0 (b), 5.0 (c), 6.0 (d), 7.0 (e), 8.0 (f) and 9.0 (g). Other conditions are as in Fig. 1. (B) Influence of pH on the peak potential of FUR for peaks A and B. Other conditions are as in Fig. 1.

Fig. 4. (A) Cyclic voltammograms of \( 1.0 \times 10^{-3} \) M FUR on CNTPE with different scan rates. (a)–(g) were 10, 30, 50, 80, 100, 180 and 200 mV s\(^{-1}\), respectively. Other conditions are as in Fig. 1. (B) Dependence of the logarithm of peak current on logarithm of scan rate. (C) Relationship between peak potential and logarithm of scan rate.
where $E_{pa2}$ is the potential where the current is at half the peak value. So, from this we got the value of $\alpha$ to be 0.56. Further, the number of electron ($n$) transferred in the electro oxidation of FUR, was calculated to be 1.1~1 for first peak and 1.07~1 for second peak. Totally the number of electron ($n$) transferred in the electro oxidation of FUR was found to be two. The value of $k^0$ can be determined from the intercept of the above plot if the value of $E^0$ is known. The value of $E^0$ in Eq. (2) can be obtained from the intercept of $E_{pa1}$ versus $v$ curve by extrapolating to the vertical axis at $v = 0$ [36]. For peak A, the intercept for $E_{pa1}$ versus log $v$ plot was 1.2108 and $E^0$ was obtained to be 1.057, the $k^0$ was calculated to be $0.941 \times 10^3$ s$^{-1}$. Similarly for the peak B, $k^0$ was $1.321 \times 10^3$ s$^{-1}$.

3.4. Mechanism and identification of product of electrolysis

The chemical oxidation of FUR by dimethylidioxirane in acetone, which involves a Mannich-like reaction for the formation of different products, is proposed by earlier workers [37].

But based on the voltammetric experiment, the number of electrons transferred ($n$) was calculated and found to be two. The IR spectrum of the product shows a sharp intense band at 1705 cm$^{-1}$ due to $\mathrm{C=O}$ stretching frequency of carboxylic group, a broad band at 3401 cm$^{-1}$ due to acidic OH; two sharp band at 3055 and 3021 cm$^{-1}$ due to NH$_2$ stretching, a band at 1625 cm$^{-1}$ due the presence of $\mathrm{C=\text{N}}$ which was absent in the IR spectrum of furosemide. The UV spectra of 1.0mM FUR in 0.04 ml/l BR buffer solution at pH 5.0, before and after electrolysis are shown in Fig. 5. Three absorption peaks are found at 226, 271 and 316 nm (curve a), but after depleting electrolysis the relative absorption peak, a slight blue shift to 229, 277 and 329 nm occurs (curve b). The electro-oxidation might have led to excitation of $\pi\rightarrow\pi^*$ transitions due to the formation of $\mathrm{C=\text{N}}$ bond. Further, LC–ESI-MS analysis of product showed a molecular ion peak m/z at 329, confirming the product.

Based on the spectral characterization, the electro-chemical oxidation product of FUR in BR buffer was identified as 2-chloro-4-[furan-2-yl(methylene)-amino]-benzenesulfonamide. Hence, the proposed mechanism is shown in Scheme 2. The voltammetric studies show that oxidative pathways of electrochemical and chemical process are different.

3.5. Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the differential-pulse voltammetric mode, because the peaks are sharper and better defined at lower concentration of FUR than those obtained by cyclic voltammetry, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative analysis of FUR. The BR buffer solution of pH 5.0 was selected as the supporting electrolyte for the quantification as FUR gave maximum peak current at pH 5.0. Differential-pulse voltammograms obtained with increasing amounts of FUR showed that the peak current increased linearly with increasing concentration, as shown in Fig. 6. It was found that the plot of $I_{pa1}$ and $I_{pa2}$ versus concentrations showed

![Scheme 2](image-url)
linearity over the drug concentration range of $8.0 \times 10^{-6}$ to $2.0 \times 10^{-4}$ M (from DPV) suggesting further that the electron de process was adsorption controlled [38]. By selecting the anodic peak ($I_{pa2}$) of FUR, the DPV procedure was developed. The linear equation was $I_{pa2} (\mu A) = 4.21 + 0.236C (r = 0.9970, C$ is in mM). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of FUR or its oxidation product on the electrode surface [39]. Related statistical data of the calibration curves were obtained from the five different calibration curves. Limit of detection (LOD) and quantification (LOQ) were calculated based on the peak current ($I_{pa2}$) using the following equations shown below [40]:

$$\text{LOD} = 3s/m; \quad \text{LOQ} = 10s/m$$

where $s$ is the standard deviation of the peak currents of the blank (five replicates), and $m$ is the slope of the calibration curve. The LOD and LOQ values were calculated to be $2.92 \times 10^{-7}$ and $9.73 \times 10^{-7}$ M, respectively. Low values of the both LOD and LOQ values confirmed the sensitivity of the proposed method as compared to reported electrochemical method performed by graphite–polyurethane composite electrode. The LOD and LOQ values calculated by the present method are better compared to the reported work [41]. Analyzing five replicates, for the process of the validation within-day variations and for intraday assay were studied.

### 3.6. Reproducibility of the multi-walled carbon nanotubes paste electrode

The regeneration and reproducibility of the electrode was investigated. It was found that after determination the surface of the CNTP electrode could be regenerated by successively cycling between 0.6 and 1.6 V in 5.0 pH with 0.04 M Britton–Robinson buffer for 5 cycles, respectively. As an example, a 1.0 mM FUR solution was measured successively for 10 times with the same electrode regenerated through such procedure after every determination, the relative standard deviation (RSD) of the peak current was 3.54%. As to the reproducibility between days, it was similar to that of within a day if the temperature was kept almost unchanged. Owing to the adsorption of FUR or its oxidative products on to the electrode surface, the current response of the modified electrode would decrease after successive use. In this case, the electrode should be prepared again.

### 3.7. Interference

FUR was formulated in single as well as multi-component tablets. The oxidation peaks of interferents should not appear where the peak corresponds to FUR appears. So in order to investigate the effect of co-formulated substances such as glucose, starch, dextrose, sucrose etc. on the voltammetric response of FUR, this study was carried out. Differential-pulse voltammetric experiments were carried out for $1.0 \times 10^{-4}$ M FUR in the presence of 0.1 mM of each of the interferents. The results are listed in Table 2.

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Concentration (mmol dm$^{-1}$)</th>
<th>Signal change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>1.0</td>
<td>– 11.1</td>
</tr>
<tr>
<td>Dextrose</td>
<td>1.0</td>
<td>– 1.47</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0</td>
<td>– 10.3</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>1.0</td>
<td>– 6.43</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.0</td>
<td>+1.76</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>1.0</td>
<td>– 10.9</td>
</tr>
<tr>
<td>Starch</td>
<td>1.0</td>
<td>– 10.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.0</td>
<td>– 3.21</td>
</tr>
</tbody>
</table>

### 3.8. Tablet analysis

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, a commercial medicinal sample containing FUR i.e., Lasix (40 mg/tablet) was used. The tablets were grounded to powder, dissolved in methanol and then further diluted so that FUR concentration falls in the range of calibration plot. The contents of the flasks were sonicated for 10 min to affect complete dissolution. Differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential-pulse voltammograms for plotting calibration plot. The results are in good agreement with the content marked in the label. The detected content was 39.3 mg/tablet with 98.25% recovery.

The recovery test of FUR ranging from $3.0 \times 10^{-5}$ to $2.0 \times 10^{-4}$ M was performed using differential-pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulations of FUR. The recoveries in different samples were found to lie in the range from 98.5% to 100.8%, with R.S.D. of 1.0%.

### 3.9. Detection of FUR in urine samples

The applicability of the proposed method for the determination of FUR in biological fluid of human urine was attempted. Furosemide is a potent diuretic which, if given in excessive amounts, can lead to a profound diuresis with water and electrolyte depletion. It is detectable in urine 36–72 h following injection [2–5]. Therefore, it analyzes directly in biological fluids. Drug-free human and urine samples, obtained from healthy volunteers, filtrated through a filter paper and stored frozen until the assay. The developed differential-pulse voltammetric method for the FUR determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of FUR. The urine samples were diluted 100 times with the BR buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of FUR into the detect system of urine sample. The calibration graph was used for the determination of spiked FUR in urine samples. The detection results of four urine samples obtained are listed in Table 3. The recovery determined was in the range from 98.72% to 99.43% and the R.S.D. was 0.38%. Good recoveries of FUR were achieved from these matrices, denoting that application of the proposed method to the analysis of FUR in biological fluid could be easily assessed.

### 4. Conclusions

In this work, multi-walled carbon nanotubes paste electrode has been successfully used for the oxidation of FUR in BR buffer solution (pH = 5.0). Based on the study, influence of several...
Acknowledgement

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Appendix A. Supplementary data


References

Voltammetric behavior of theophylline and its determination at multi-wall carbon nanotube paste electrode

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ABSTRACT

The voltammetric behavior of theophylline was investigated using cyclic and differential-pulse voltammetric techniques. The cyclic voltammetric results indicate that multi-wall carbon nanotube paste electrode can remarkably enhance electrocatalytic activity toward the oxidation of theophylline in pH 3.0 phosphate buffer solution than the carbon paste electrode. The oxidation of theophylline was observed to be a two-electron process, irreversible with diffusion character. Effects of anodic peak potential (Epk), anodic peak current (Ip) and heterogeneous rate constant (kθ) have been discussed. Under optimal conditions, the anodic peak current was proportional to theophylline concentration in the range of 2.0 × 10⁻⁶ to 1.5 × 10⁻⁴ M with a detection limit of 1.97 × 10⁻⁸ M using differential pulse voltammetry. The proposed method was employed to determine theophylline in pharmaceutical formulations and urine as a real sample.

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1. Introduction

Theophylline (1,3-dimethyl-7H-purine-2,6-dione, TP), as shown in Scheme 1 is a methylxanthine drug used in therapy for respiratory diseases. TP is a bronchodilator drug used in treating asthma and other airway diseases. It opens airways by relaxing the smooth muscles in the walls of the airways. TP can also be helpful in patients with emphysema and chronic bronchitis when their symptoms are partially related to reversible airway narrowing. TP also strengthens right heart function and diaphragm movement. Like other methylated xanthine derivatives, TP is both a competitive nonselective phosphodiesterase inhibitor [1] and nonselective adenosine receptor antagonist [2]. The rate of metabolism of TP varies considerably from one individual to another. As a consequence of the variation of pharmacokinetics between patients, it is necessary to monitor concentration of drugs in individual patients to ensure the maximum clinical response and to avoid undesirable side effects.

A number of methods have been used to determine TP, including spectrophotometry [3], high-performance liquid chromatography [4], capillary electrophoresis [5], thin layer chromatography [6] and immunoassay [7]. Most often, the procedures involve some extraction, intensive solvent-usage, and expensive devices and are thus time consuming.

The electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time, as compared with other techniques. Electrochemical techniques have proven to be useful for development of very sensitive and selective methods for the determination of organic molecules including drugs. In addition application of electro analytical techniques include the determination of electrode mechanisms. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmaceutical activity [8,9].

For electrochemical determinations, the main problem which causes most difficulties is adsorption of the analyte or its reaction products on the electrode surface. Apart from solid carbon electrodes, such as glassy carbon, paste electrodes have also been investigated for use in oxidative electroanalytical procedures due to their unique characteristics such as versatility of chemical modification, renewability of the electrode surface, and compatibility with various electron mediators [10].

Carbon nanotubes (CNTs) continue to receive remarkable attention in electrochemistry [11]. Since their synthesis by Iijima [12] in 1991 using transmission electron microscopy, CNTs have been the subject of numerous investigations in chemical, physical and material areas due to their novel structural, mechanical, electronic and chemical properties [13]. The subtle electronic properties suggest that CNTs have the ability to promote charge transfer reactions when used as an electrode [14]. The modification of electrode substrates with multi-walled carbon nanotubes (MWCNTs) for use in analytical sensing has been documented to result in low detection limits, high sensitivities, reduction of over potentials and
resistance to surface fouling. MWCNTs have been introduced as electrocatalysts [15,16] and CNTs-modified electrodes have been reported to give super performance in the study of a number of biological species [17]. Generally there are two ways to fabricate CNTs based electrodes. One way is to cast CNTs suspension on the surface of solid electrodes such as Pt, Au and glassy carbon electrodes to make CNTs film modified electrodes [18,19]. Another method is to mix CNTs with bonds such as nujol [20], bromiform [21] or mineral oil [22], and then pack the mixture into a pipe to prepare paste electrodes. Naturally, the characteristics of CNT's paste electrodes depend on the type of CNTs and the bonds used. Especially, the bonds show greater influence on the accumulation efficiency of electroactive species and blank current due to their different hydrophobicity. Every bond has its characteristics and suits for some purposes. Paraffin oil is commercially available and paraffin oil based paste electrode exhibits some characteristics; hence it is frequently used as bond [23]. Although CNTs modified electrodes are commonly used, carbon nanotubes paste electrodes are rarely used in electro oxidation. TP was quantified with electrochemical methods [24] using carbon paste electrode, nano-sized cobalt phthalocyanine particles modified electrode, xanthine oxidase electrode, boron-doped diamond electrode, Nafion/lead ruthenium oxide pyrochlore chemically modified glassy carbon electrode, multi-wall carbon nanotube modified glassy carbon electrode, disposable modified carbon paste electrode and platinum nanoparticles decorated multiwalled carbon nanotubes–ionic liquid composite film coated electrodes, cytremethyl ammonium bromide modified carbon paste electrode [24].

In this work, the voltammetric behavior of TP has been studied at a multi-wall carbon nanotube paste electrode (MWCNT-PE), and electrocatalytic activity of MWCNTs for the oxidation of TP has been explored by comparing its voltammetric responses at MWCNT-PE with that of carbon paste electrode (CPE). This modified electrode was used for the analysis of TP in pharmaceutical and urine samples. The resulted biosensor exhibits high sensitivity, rapid response, good reproducibility, low detection limit, renewal of the surface and freedom from other potentially interfering species.

2. Experimental

2.1. Reagents

Pure TP in powdered form was obtained from Spectrum, India and used as received. A stock solution of TP (1.0 × 10^{-2} M) was prepared in doubly distilled water. Multi-walled carbon nanotubes were from Sigma–Aldrich (>90%, O.D.: 10–15 nm, I.D.: 2–6 nm, length: 0.1–10 μ m). Phosphate buffer solutions (ionic strength = 0.2 M) were prepared according to the literature method [25]. Rest of the reagents was of analytical–reagent grade, and doubly distilled water was used through out the experiment.

2.2. Apparatus

Electrochemical measurements were carried out on a CHI-630D electrochemical analyzer (CH Instruments Inc., U.S.A.) coupled with a conventional three-electrode cell. A three-electrode system consisting of a Ag/AgCl as reference electrode, a Pt wire as counter electrode and a self-made multi-wall carbon nanotube paste electrode as a working electrode were used. All the potentials in this paper are given against the Ag/AgCl (3 M KCl). The pH measurements were made by Elico LI120 pH meter (Elico Ltd., India).

2.3. Preparation of electrode

The MWCNT-PE was prepared by mixing multi-wall carbon nanotube powder and paraffin oil in an agate mortar in a ratio of 60.0% nanotubes powder to 40.0% paraffin oil (w/w) and this mixture was then homogenized. The ratio was used due to its success in previous applications [26]. A portion of the resulting paste was packed firmly into a cavity of a polytetrafluoro ethylene tube (PTFE). The surface of the electrode was smoothed against weighing paper and rinsed with water. Unless otherwise stated, the paste was carefully removed prior to pressing a new portion into the electrode after every measurement. The resulting electrode was noted as MWCNT-PE.

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM K3Fe(CN)6 in 0.1 M KCl by recording the current voltage curve at different scan rates. For a reversible process, the following Randles–Sevcik formula can be used [27].

$$I_p = (2.69 \times 10^5)n^{1/2}A_D^{1/2}u^{1/2}C_0$$

(1)

where $I_p$ refers to the anodic peak current, $n$ is the number of electrons transferred, $A$ is the surface area of the electrode, $D_k$ is diffusion coefficient, $\nu$ is the scan rate and $C_0$ is the concentration of $K_3Fe(CN)_6$. For 1.0 mM K3Fe(CN)6 in 0.1 M KCl electrolyte, $n = 1$, $D_k = 7.6 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$, then from the slope of the plot of $I_p$ versus $\nu^{1/2}$, relation, the electro active area was calculated. In our experiment the slope was 7.59 × 10^{-6} μA (Vs^{-1})^{1/2} and the area of electrode was calculated to be 0.102 cm².

2.4. Analytical procedure

The MWCNT-PE was first activated in phosphate buffer (pH 3.0) by cyclic voltammetric sweeps between 0.0 and 2.0 V until a stable cyclic voltammogram was obtained. Then electrodes were transferred into another cell of phosphate buffer (pH 3.0) containing proper amount of TP. The potential scan was initiated and cyclic voltammograms were recorded between +0.80 and +1.40, with a scan rate of 50 mV s⁻¹. All measurements were carried out at room temperature of 25.0 ± 0.1 °C.

2.5. Sample preparation

A quantity of 10 tablets were weighed and ground to a homogeneous fine powder in a mortar. A portion equivalent to a stock solution of a concentration of about 1.0 × 10⁻³ M was accurately weighed and transferred into a 100 ml calibrated flask and completed to the volume with double distilled water. The contents of the flask were sonicated for 10 min to affect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with the phosphate buffer solutions. Each solution was transferred to the voltammetric cell and analyzed by standard addition method. The differential-pulse voltammograms (DPV) were recorded between 0.80 and 1.40 V after open-circuit accumulation for 10 s with stirring. The oxidation peak current of TP was measured. The parameters for DPV were pulse width of 0.06 s, pulse increment of 4 mV, pulse period of 0.2 s, pulse amplitude of 50 mV. To study the accuracy of the proposed method and to check the interferences from excipients used in the dosage form, recovery experiments were carried out. The concentration of TP was calculated using standard addition method.
3. Results and discussion

3.1. Voltammetric behavior of TP

The voltammetric responses of $5.0 \times 10^{-4}$ M TP at CPE and MWCNT-PE in pH 3.0 phosphate buffer in the potential range of 0.80–1.40 V, with a scan rate of 50 mV s$^{-1}$ were compared and the results are shown in Fig. 1. At CPE, a poorly defined oxidation peak was observed. The peak current was smaller. The TP exhibits an anodic peak at about 1.24 V (curve b) at the CPE but in case of MWCNT-PE the oxidation peak current increases greatly (curve a). This indicates that MWCNT-PE can make the electron transfer of TP more easily. The larger effective surface area of MWCNTs and the stronger adsorption of TP at MWCNT-PE were responsible for the electrocatalytic oxidation of TP at the MWCNT-PE.

No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. Nevertheless, it was found that the oxidation peak current of TP showed a remarkable decrease during the successive cyclic voltammetric sweeps (Fig. 2). After the second sweep, the peak current decreased greatly and finally reached a constant level. This phenomenon may be due to the fact that the adsorption of TP or its oxidative product occurs at the electrode surface. Therefore, the voltammograms corresponding to the first cycle was generally recorded.

3.2. Influence of accumulation time

It was important to fix the accumulation potential and time when adsorption studies were undertaken. Both conditions could affect the amount of adsorption of TP at the electrode. Bearing this in mind, the effect of accumulation potential and time on peak current response was studied by CV. Open circuit accumulation is widely used in electro analytical chemistry to accumulate analyte and improve the determining sensitivity. The influence of accumulation time ranging from 0 to 200 s on the oxidation of TP at MWCNT-PE is shown in Fig. 15. The peak current increased gradually as accumulation time increased from 0 to 10 s. However, with further increasing, the accumulation time beyond 10 s the peak current tends to decrease and then almost stable. Therefore, the optimal accumulation time of 10 s was employed in further experiments.

3.3. Influence of pH

The influence of solution pH on the response of $5.0 \times 10^{-4}$ M TP was examined over the pH range 3.0–11.2 in phosphate buffer solution by CV at a scan rate of 50 mV s$^{-1}$. With increase in the solution pH (Fig. 3A), the potential of the peak is shifted to less values till pH 8.0, then becomes almost pH independent (Fig. 3B). This indicates that hydrogen ions were concerned with the electrode reaction. The linear relationship between $E_p$ and pH can be expressed as follows:

$$E_p = 1.3866 - 0.0482pH$$

($E_p$ in V, correlation coefficient $(r)=0.996$).

A slope of 0.0482 per pH unit suggested that the number of electron transfer is equal with that of hydrogen ions taking part in the electrode reaction [24]. The solution pH influenced the peak current considerably. The peak current decreased with the increase of solution pH. So, the buffer solution with pH 3.0 was chosen for determination of TP due to highest peak current ($I_p$).

3.4. Influence of scan rate

The effect of scan rate $(\nu)$ on the peak current and peak potential of TP was evaluated (Fig. 4A). The influence of the square root of the scan rate on the peak current showed a linear relationship between 10 and 200 mV s$^{-1}$, which is of typical diffusion controlled process [28] and the equation can be expressed as follows:

$$I_p = 21.99\nu^{1/2} - 0.0444; \quad r = 0.9979$$

In addition, there was a linear relation between log $I_p$ and log $\nu$ (Fig. 4B), corresponding to the following equation:

$$\log I_p = 0.5106 \log \nu + 1.3504; \quad r = 0.9985$$

The slope of 0.5106 is close to the theoretically expected value of 0.5 for a purely diffusion controlled process [23]. This indicates that the electrode process was controlled by diffusion rather than adsorption. The peak potential shifted to positive values with increasing the scan rates. The linear relation between peak potential and logarithm of scan rate can be expressed as:

$$E_p = 1.313 + 0.0534 \log \nu; \quad r = 0.9901$$
This behavior was consistent with the electrochemical nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step [29]. As for an irreversible electrode process, according to Laviron [30], $E_p$ is defined by the following equation

$$E_p = E^\circ + \left( \frac{2.303RT}{nF} \right) \log \left( \frac{\alpha nF}{RTk^o} \right) + \left( \frac{2.303RT}{anF} \right) \log \nu$$

(2)

where $\alpha$ (alpha) is the transfer coefficient, $k^o$ the standard heterogeneous rate constant of the reaction, $n$ the number of electrons transferred, $\nu$ (nu) the scan rate and $E^\circ$ is the formal redox potential. Other symbols have their usual meanings. Thus the value of $an$ can be easily calculated from the slope of $E_p$ versus log $\nu$. In this system, the slope is 0.0534, the $an$ calculated to be 1.11, taking $T = 298K$, $R = 8.314KJ^{-1}mol^{-1}$ and $F = 96480C$ mol$^{-1}$. According to Bard and Faulkner [31], $\alpha$ can be given as

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \text{mV}$$

(3)

where $E_{p/2}$ is the potential where the current is at half the peak value. So, from this we got the value of $\alpha$ to be 0.56. Further, the number of electron ($n$) transferred in the electro oxidation of TP, was calculated to be $1.98 \pm 2$. The value of $k^o$ can be determined from the intercept of the above plot if the value of $E^\circ$ is known. The value of $E^\circ$ in Eq. (2) can be obtained from the intercept of $E_p$ versus $\nu$ curve by extrapolating to the vertical axis at $\nu = 0$ [32]. In our system the intercept for $E_p$ versus log $\nu$ plot was 1.313 and $E^\circ$ was obtained to be 1.218, the $k^o$ was calculated to be $2.574 \times 10^4$ s$^{-1}$.

3.5. Mechanism and identification of product of electrolysis

UV spectra of $4.0 \times 10^{-5}$ M TP in 0.2 M phosphate buffer solution in pH 3.0 before and after electrolysis are shown in Fig. 2S. Two absorption peaks were found at 203 and 272 nm before electrolysis (curve a). The electrolysis was carried out for 6 h. After depleting electrolysis, the relative weak absorption peak at 272 nm vanishes, the strong absorption peak at short wavelength remains, but a slight blue shift to 201 nm occurs (curve b). We speculate that electro
oxidation might have lead to destruction of the π bond conjugate system in TP. This was also supported by the earlier report [33]. The number of electrons and hydrogen ions transferred was 2. With all the previous experimental results, a possible electrode reaction mechanism for TP might be expressed as shown in Scheme 2.

### 3.6. Calibration curve

In order to develop a voltammetric method for determining the drug, we selected the differential-pulse voltammetric mode, because the peaks are sharper and better defined at lower concentration of TP than those obtained by CV, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative analysis of TP. The phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification as TP gave maximum peak current at pH 3.0. Differential-pulse voltammograms obtained with increasing amounts of TP showed that the peak current increased linearly with increasing concentration, as shown in Fig. 5. It was found that the plot of \( I_p \) versus concentration showed linearity except the range of \( 1.5 \times 10^{-4} \) to \( 3.0 \times 10^{-4} \) M (from DPV). The linear equation was \( I_p (\mu A) = 0.4392 + 10.708c (\mu M) \) (from Fig. 5). Deviation from linearity was observed for more concentrated solutions, due to the adsorption of TP or its oxidation product on the electrode surface [34]. Related statistical data of the calibration curves were obtained from the five different calibration curves. Limit of detection (LOD) and quantification (LOQ) were calculated [35] based on the peak current using the following equations shown below.

\[
\text{LOD} = \frac{s}{m} \quad \text{and} \quad \text{LOQ} = \frac{3s}{m}
\]

where \( s \) is the standard deviation of the peak currents of the blank (five replicates), and \( m \) is the slope of the calibration curve.

### Table 1

<table>
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<tr>
<th>Electrodes</th>
<th>Detection limits</th>
<th>Refs.</th>
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<td>[24]</td>
</tr>
<tr>
<td>Xanthine oxidase electrode</td>
<td>( 2.0 \times 10^{-7} )</td>
<td>[36]</td>
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<tr>
<td>Amperometric enzyme oxidase electrode</td>
<td>( 2.0 \times 10^{-6} )</td>
<td>[37]</td>
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<tr>
<td>Nefion/lead-ruthenium oxide</td>
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</tbody>
</table>

The LOD and LOQ values were calculated to be \( 1.97 \times 10^{-8} \) and \( 6.57 \times 10^{-9} \) M, respectively. The LOD and LOQ values calculated by the present method are better compared to the reported work [24,36–39]. The detection limits reported at different electrodes are tabulated in Table 1. Analyzing five replicates, for the process of the validation within-day variations and for intraday assay were studied.

### 3.7. Reproducibility of the multi-wall carbon nanotube paste electrode

The regeneration and reproducibility of the electrode was investigated. It was found that after determination the surface of the MWCNT-PE could be regenerated by successively cycling between 0.8 and 1.4 V in 3.0 pH with 0.2 M phosphate buffer for 5 cycles, respectively. As an example, a 1.0 mM TP solution was measured successively for 10 times with the same electrode regenerated through such procedure after every determination, the relative standard deviation (R.S.D.) of the peak current was 3.42%. As to the reproducibility between days, it was similar to that of within a day if the temperature was kept almost unchanged. Owing to the adsorption of TP or its oxidative products on to the electrode surface, the current response of the modified electrode would decrease after successive use. In this case, the electrode should be prepared again.

### 3.8. Interference

TP was formulated in single as well as multi-component tablets. The oxidation peaks of interferents should not appear where the peak corresponds to TP appears. So in order to investigate the effect of co-formulated substances such as glucose, starch, dextrose and sucrose on the voltammetric response of TP, this study was carried out. Differential-pulse voltammetric experiments were carried out for \( 1.0 \times 10^{-5} \) M TP in the presence of 1.0 mM of each of the interferents. It was observed that 100 folds of citric acid, dextrose, glucose, gum acacia, lactic acid, oxalic acid, starch and sucrose did not interfere with the voltammetric signal of TP.

### 3.9. Tablet analysis

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, a commercial medicinal sample containing TP i.e., Theo-Asthilin (100 mg per tablet) was used. The tablets were grounded to powder, dissolved in double distilled water and then further diluted so that TP concentration falls in the range of calibration plot. The contents of the flask were sonicated for 10 min to affect complete dissolution. Differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential-pulse voltammograms for plotting calibration plot. The results are in good agreement with the reference values.

**Fig. 5.** Differential-pulse voltammograms of MWCNT-PE in TP solution at different concentrations: blank (1), 2.0 (2), 10.0 (3), 20.0 (4), 40.0 (5), 60.0 (6), 80.0 (7), 100.0 (8) and 150.0 (9) \mu M. Inset: plot of the peak current against the concentration of TP.
agreement with the content marked in the label. The detected content was 99.3% mg per tablet with 99.3% recovery.

The recovery test of TP ranging from $5.0 \times 10^{-6}$ to $1.0 \times 10^{-4}$ M was performed using differential-pulse voltammetry. Recovery studies were carried out after the addition of known amounts of the drug to various pre-analyzed formulations of TP. The results are listed in Table 2. The recoveries in different samples were found to lie in the range from 96.5% to 100.2%, with R.S.D. of 1.3%.

3.10. Detection of TP in urine samples

The applicability of the proposed method for the determination of TP in biological fluid of human urine was attempted. Drug-free human and urine samples, obtained from healthy volunteers, filtered through a filter paper and stored frozen until the assay. The developed differential-pulse voltammetric method for the TP determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of TP. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of TP into the detect system of urine sample. The calibration graph was used for the determination of spiking TP in urine samples. The detection results of four urine samples obtained are listed in Table 3. The recovery determined was in the range from 98.3% to 99.8% and the R.S.D. was 0.71%. Good recoveries of TP were achieved from these matrices, denoting that application of the proposed method to the analysis of TP in biological fluid could be easily assessed.

4. Conclusions

The voltammetric behavior and oxidation mechanism of TP were investigated at a MWCNT-PE by CV in phosphate buffer solution (pH = 3.0). Based on the study, influence of several physicochemical parameters like potential scan rate, pH and concentration were investigated. The oxidation of TP was found to be an irreversible two-electron and two-proton process with diffusion character. MWNTs showed electrocatalytic action for the oxidation of TP, characterizing by the enhancement of the peak current and the reduction of the peak potential, which was probably due to the larger effective surface area of MWNTs and the stronger adsorption of TP at MWCNT-PE. This method has been successfully used to determine TP in the pharmaceutical sample. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. In addition, the results obtained in the analysis of TP in spiked urine samples demonstrated the applicability of the method for real sample analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2012.04.010.

References