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Mechanistic study on the oxidation of L-phenylalanine by copper(III) in aqueous alkaline medium: a kinetic approach

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Abstract Oxidation of the amino acid L-phenylalanine by diperiodatocuprate(III) in alkaline medium at constant ionic strength of 0.25 mol dm^{-3} was studied spectrophotometrically at different temperatures (298–313 K). The reaction between diperiodatocuprate(III) and L-phenylalanine in alkaline medium exhibits 1:2 stoichiometry. Intervention of free radicals was observed in the reaction. Based on the observed orders and experimental evidence, a mechanism involving monoperiodatocuprate(III) as the reactive oxidant species has been proposed, proceeding through the formation of a complex and reaction of the intermediate of L-phenylalanine with monoperiodatocuprate(III) to give the products. The products were identified by spot test, infrared (IR), and gas chromatography-mass spectrometry (GC-MS). The reaction constants involved in the different steps of the mechanism were calculated. The activation parameters with respect to the slow step of the mechanism were computed and are discussed. The thermodynamic quantities were determined for different equilibrium steps. The isokinetic temperature was also calculated and found to be 331 K.

Keywords Kinetics · Mechanism · L-Phenylalanine · Oxidation · Diperiodatocuprate(III)

Introduction

Amino acids not only act as building blocks in protein synthesis but also play a significant role in metabolism.

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They have been oxidized by a variety of oxidizing agents [1]. The study of oxidation of amino acids is of interest because of their biological significance and selectivity towards the oxidant to yield different products [2–5].

L-Phenylalanine (L-PA) is an essential amino acid. It forms active sites of enzymes and helps in maintaining their proper conformation by keeping them in proper ionic states. So, oxidation of L-PA may help in understanding some aspects of enzyme kinetics (Appendix). L-Phenylalanine supplementation helps in the suppression of pain and aids weight loss through the suppression of appetite. It is converted to tyrosine, which is a precursor to dopamine, having a definite function in sexual desire.

In recent years, the study of the highest oxidation states 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) [6], diperiodatoargentate(III) [7–9], and diperiodatonickelate(IV) [10] are good oxidants in a medium with appropriate pH value. Periodate and tellurate complexes of copper in its trivalent state have been extensively used in the analysis of several organic compounds [11]. The kinetics of self-decomposition of these complexes was studied in some detail [12]. Copper(III) is shown to be an intermediate in the copper(II)-catalyzed oxidation of amino acids by peroxydisulfate [13]. The oxidation reaction usually involves the copper(II)–copper(I) couple, and such aspects are detailed in different reviews [14, 15]. The use of diperiodatocuprate(III) (DPC) as an oxidant in alkaline medium is new and restricted to a few cases due to 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 [16, 17]. Copper complexes have occupied a major place in oxidation

chemistry due to their abundance and relevance in biological chemistry [18, 19]. Copper(III) is involved in many biological electron-transfer reactions [20]. It has also been used [21] 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.

A literature survey reveals that there are no reports on oxidation of L-phenylalanine (L-PA) by diperiodatocuprate(III). The present study deals with the title reaction to investigate the redox chemistry of DPC in alkaline media and to arrive at a suitable mechanism on the basis of kinetic and spectral results and to calculate the thermodynamic quantities of various steps.

Results and discussion

Reaction orders

Reaction orders were determined from the slope of lg k_{obs} versus lg(concentration) plots by varying the concentrations of L-phenylalanine, alkali, and periodate in turn while keeping all other concentrations and conditions constant.

Effect of [diperiodatocuprate(III)]

The oxidant DPC concentration was varied in the range of 1.0×10^{-5} to 1.0×10^{-4} mol dm^{-3}, and the fairly constant k_{obs} values indicate that order with respect to [DPC] was unity (Table 1). This was also confirmed by linearity of the plots of lg(absorbance) versus time ($r \geq 0.986$, $S \leq 0.014$) up to 80% completion of the reaction, as shown in Fig. 1.

Effect of [L-phenylalanine]

The effect of L-phenylalanine on the rate of reaction was studied at constant concentrations of alkali, DPC, and periodate at constant ionic strength of 0.25 mol dm^{-3}. The substrate L-PA was varied in the range of 3.0×10^{-4} to 3.0×10^{-3} mol dm^{-3}. The k_{obs} values increased with increase in concentration of L-phenylalanine (Table 1). The order was found to be less than unity ($r \geq 0.995$, $S \leq 0.009$).

Effect of [alkali]

The effect of alkali on the reaction has been studied in the range of 0.02–0.20 mol dm^{-3} at constant concentrations of L-phenylalanine, DPC, and periodate at constant ionic strength of 0.25 mol dm^{-3} at 25 °C. The rate constants

Table 1 Effect of [DPC], [L-PA], [OH$^-$], and [IO$_4^-$] on oxidation of L-phenylalanine by DPC in alkaline medium at 25 °C

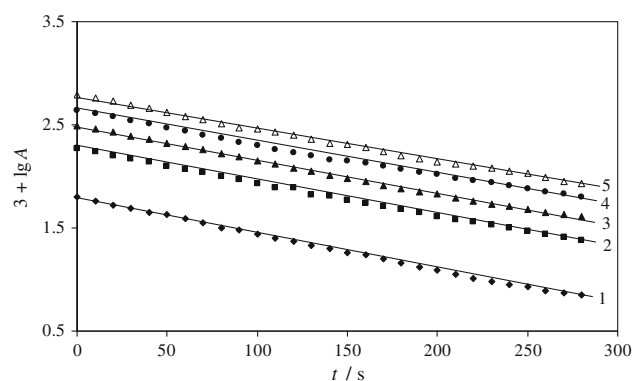| 10^5 [DPC] (mol dm^{-3})	10^4 [L-PA] (mol dm^{-3})	[OH$^-$] (mol dm^{-3})	10^4 [IO$_4^-$] (mol dm^{-3})	10^3 k_{obs} (s^{-1})	10^3 k_{calc} (s^{-1})
1.0	5.0	0.15	1.0	7.06	7.14
3.0	5.0	0.15	1.0	7.09	7.14
5.0	5.0	0.15	1.0	7.08	7.14
8.0	5.0	0.15	1.0	7.12	7.14
10.0	5.0	0.15	1.0	7.11	7.14
5.0	3.0	0.15	1.0	6.58	6.28
5.0	5.0	0.15	1.0	7.08	7.14
5.0	10.0	0.15	1.0	7.63	8.01
5.0	20.0	0.15	1.0	8.36	8.58
5.0	30.0	0.15	1.0	9.80	9.37
5.0	5.0	0.02	1.0	4.57	4.52
5.0	5.0	0.05	1.0	5.88	6.06
5.0	5.0	0.08	1.0	6.38	6.63
5.0	5.0	0.15	1.0	7.08	7.14
5.0	5.0	0.20	1.0	7.84	7.51
5.0	5.0	0.15	0.5	7.75	7.62
5.0	5.0	0.15	0.8	7.47	7.33
5.0	5.0	0.15	1.0	7.08	7.14
5.0	5.0	0.15	3.0	5.81	5.71
5.0	5.0	0.15	5.0	4.94	4.76

Fig. 1 First-order plots for oxidation of L-phenylalanine by diperiodatocuprate(III) in aqueous alkaline medium at 25 °C. [Diperiodatocuprate(III)] $\times 10^5$ (mol dm^{-3}): *1* 1.0; *2* 3.0; *3* 5.0; *4* 8.0; *5* 10.0

increase with increase in alkali concentration, and order was found to be less than unity (Table 1) ($r \geq 0.988$, $S \leq 0.007$).

Effect of [periodate]

The effect of increasing concentration of periodate was studied by varying the periodate concentration from 5.0×10^{-5} to 5.0×10^{-4} 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 negative less than unity ($r \geq 0.984$, $S \leq 0.004$) (Table 1).

Effect of ionic strength and dielectric constant of the medium

It was found that ionic strength and dielectric constant of the medium had no significant effect on the rate of the reaction.

Effect of initially added products

The externally added products, phenylacetaldehyde and copper(II) (CuSO₄), did not have any significant effect on the rate of the reaction.

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 4 h in an inert atmosphere. On diluting the reaction with methanol, a white precipitate was formed, indicating the intervention of free radicals in the reaction [22]. The blank experiments of either DPC or L-phenylalanine 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 [23, 24].

Effect of temperature (T)

The kinetics were studied at four different temperatures (298, 303, 308, and 313 K) under varying concentrations of L-phenylalanine, alkali, and periodate, keeping other conditions constant. The rate constants were found to increase with increase in temperature. The rate constants (k) of the slow step of Scheme 1 were obtained from the slopes and intercepts of $1/k_{obs}$ versus $1/[\text{L-PA}]$, $1/[\text{OH}^-]$, and $[\text{H}_3\text{IO}_6^{2-}]$ plots at four 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 lg k versus $1/T$ ($r \geq 0.976$, $S \leq 0.009$). Other activation parameters obtained are tabulated in Table 2.

The water-soluble copper(III) periodate complex is reported [25] to be $[\text{Cu}(\text{HIO}_6)_2(\text{OH})_2]^{7-}$. However, in an aqueous alkaline medium and at the high pH range employed in the study, periodate is unlikely to exist as HIO_6^{4-} (as present in the complex), as is evident from its involvement in the multiple equilibria (1–3) [26], depending on the pH of the solution.

$$\text{H}_5\text{IO}_6 \rightleftharpoons \text{H}_5\text{IO}_6^- + \text{H}^+ \tag{1}$$

$$\text{H}_4\text{IO}_6^- \rightleftharpoons \text{H}_3\text{IO}_6^{2-} + \text{H}^+ \tag{2}$$

$$\text{H}_3\text{IO}_6^{2-} \rightleftharpoons \text{H}_2\text{IO}_6^{3-} + \text{H}^+ \tag{3}$$

Periodic acid exists as H_5IO_6 in acid medium and as H_4IO_6^- near pH 7. Hence, under alkaline conditions as employed in this study, the main species are expected to be $\text{H}_3\text{IO}_6^{2-}$ and $\text{H}_2\text{IO}_6^{3-}$. Thus, at the pH employed in this study, the soluble copper(III) periodate complex might be
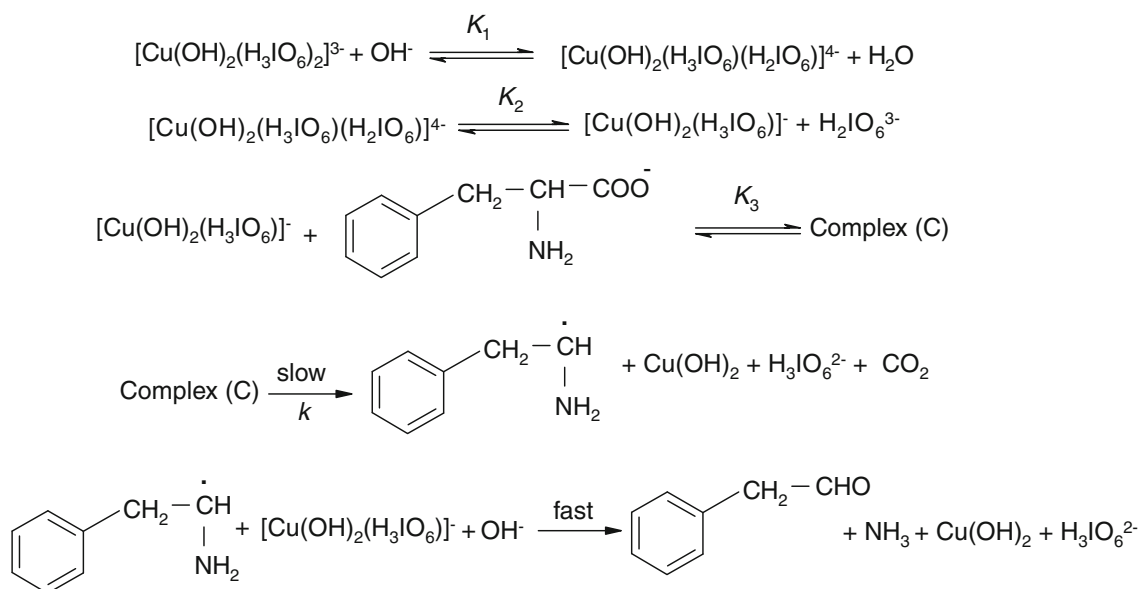
Scheme 1

Table 2 Thermodynamic activation parameters for oxidation of L-phenylalanine by DPC in aqueous alkaline medium with respect to the slow step of Scheme 1

T (K)	$10^2\,k$ (s^{-1})		
(a) Effect of temperature (K)			
298	0.92		
303	2.34		
308	3.09		
313	4.04		

Parameters	Values		
(b) Activation parameters (Scheme 1)			
E_a (kJ mol^{-1})	73 ± 3		
$\Delta H^{\ddagger}$ (kJ mol^{-1})	71 ± 4		
$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	-46 ± 2		
$\Delta G^{\ddagger}$ (kJ mol^{-1})	85 ± 2		
lg A	10 ± 0.6		

T (K)	K_1 (dm^3 mol^{-1})	$10^4\,K_2$ (mol dm^{-3})	$10^{-4}\,K_3$ (dm^3 mol^{-1})
(c) Effect of temperature to calculate K_1, K_2, and K_3 for oxidation of L-phenylalanine by DPC in alkaline medium			
298	2.66	2.73	1.59
303	2.72	4.29	0.75
308	2.79	6.17	0.24
313	2.88	7.44	0.19

Thermodynamic quantities	Values from K_1	Values from K_2	Values from K_3
(d) Thermodynamic quantities using K_1, K_2, and K_3			
ΔH (kJ mol^{-1})	4.2 ± 0.2	52 ± 2	-115 ± 4
ΔS (J K^{-1} mol^{-1})	22 ± 0.4	108 ± 5	-308 ± 6
ΔG_{298} (kJ mol^{-1})	-2.5 ± 0.1	20 ± 0.2	-24 ± 0.2

$[Cu(OH)_2(H_3IO_6)_2]^{3-}$, a conclusion also supported by earlier work [4, 5].

The reaction between the diperiodatocuprate(III) complex and L-phenylalanine in alkaline medium has stoichiometry of 1:2 (L-PA:DPC) with a first-order dependence on [DPC], apparent less than unit order in [substrate] and [alkali], and a negative fractional order in [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 [IO$_4^-$] may be well accommodated. It is known that L-phenylalanine exists in the form of a zwitterion [27] in aqueous medium. In highly acidic medium, it exists in the protonated form, whereas in highly basic medium it is in the fully deprotonated form [27]. The results of increase in reaction rate with increase

in alkalinity (Table 1) and also decrease in rate with increase in [H$_3$IO$_6^{2-}$] (Table 1) suggest an equilibrium of the copper(III) periodate complex to form a monoperiodatocuprate(III) (MPC) species. Such types of results have been well noticed in literature [28]. It may be expected that a lower periodate complex such as monoperiodatocuptrate(III) is more important in the reaction than the DPC. The inverse fractional order in [H$_3$IO$_6^{2-}$] might also be due to this reason. Therefore, MPC might be the main reactive form of the oxidant.

The less than unit order in [L-PA] presumably results from formation of a complex (C) between the MPC species and L-phenylalanine prior to the formation of the products. This complex (C) decomposes in a slow step to form a free radical derived from L-phenylalanine. This free radical species further reacts with another molecule of MPC in a fast step to form products as given Scheme 1.

Since Scheme 1 is in accordance with the generally well-accepted principle of noncomplementary 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 (see below). This type of radical intermediate has also been observed in earlier work [29]. The probable structure of the complex is given in Scheme 2.

Spectroscopic evidence for the complex formation between oxidant and substrate was obtained from UV–Vis spectra of L-phenylalanine (5.0×10^{-4}), DPC (5.0×10^{-5}), [OH$^-$] = 0.15 mol dm^{-3} and mixture of both. A bathochromic shift of about 5 nm from 259 to 264 nm in the spectra of DPC was observed. The Michaelis–Menten plot also proved the complex formation between DPC and L-phenylalanine, which explains the less than unit order dependence on [L-PA]. Such a type of complex between a substrate and an oxidant has been observed in other studies [29].

Scheme 1 leads to the rate law (5).

$$\text{Rate} = \frac{-d[\text{DPC}]}{dt}$$
$$= \frac{kK_1K_2K_3[\text{L-PA}][\text{OH}^-]\left[Cu(OH)_2(H_3IO_6)_2\right]^{3-}}{[\text{H}_2\text{IO}_6^{3-}]},$$
(4)

$$\frac{\text{Rate}}{[\text{DPC}]} = k_{obs}$$
$$= \frac{kK_1K_2K_3[\text{L-PA}][\text{OH}^-]}{[\text{H}_2\text{IO}_6^{3-}] + K_1K_2K_3[\text{OH}^-][\text{L-PA}] + K_1K_2[\text{OH}^-] + K_1[\text{H}_2\text{IO}_6^{3-}][\text{OH}^-]}.$$
(5)

This explains all the observed kinetic orders of different species. The rate law (5) can be rearranged into the following form, which is suitable for verification:
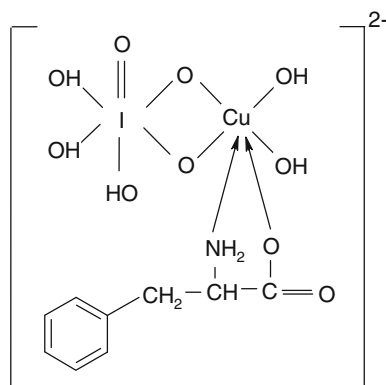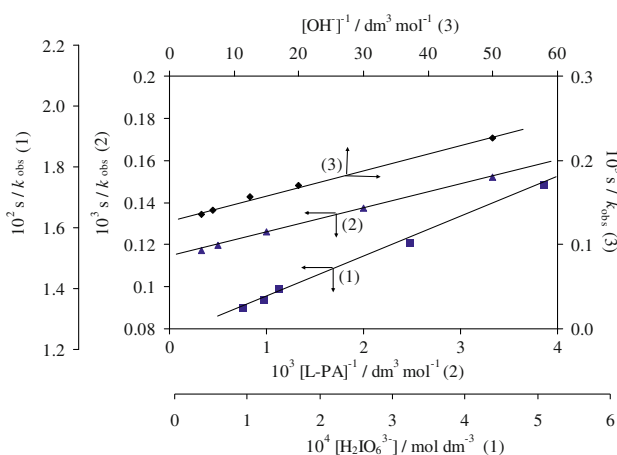
Scheme 2

Fig. 2 Verification of rate law (6) for oxidation of L-phenylalanine by diperiodatocuprate(III) at 25 °C

$$\frac{1}{k_{obs}} = \frac{\left[H_2IO_6^{3-}\right]}{kK_1K_2K_3[OH^-][L\text{-}PA]} + \frac{\left[H_2IO_6^{3-}\right]}{kK_2K_3[L\text{-}PA]} + \frac{1}{kK_3[L\text{-}PA]} + \frac{1}{k}. \tag{6}$$

According to Eq. 6, other conditions being constant, plots of $1/k_{obs}$ versus $1/[OH^-]$ ($r \geq 0.995$, $S \leq 0.014$), $1/k_{obs}$ versus $1/[L\text{-}PA]$ ($r \geq 0.999$, $S \leq 0.012$), and $1/k_{obs}$ versus $[H_3IO_6^{2-}]$ ($r \geq 0.999$, $S \leq 0.011$) should be linear, and are indeed found to be so (Fig. 2). The slopes and intercepts of such plots lead to the values of K_1, K_2, K_3, and k as (2.6 ± 0.1) dm^3 mol^{-1}, $(2.7 \pm 0.1) \times 10^{-4}$ mol dm^{-3}, $(1.6 \pm 0.05) \times 10^4$ dm^3 mol^{-1}, and $(0.92 \pm 0.02) \times 10^{-2}$ s^{-1}, respectively. The value of K_1 is in good agreement with earlier literature [28]. These constants were used to calculate the rate constants for comparison with experimental values, which were found to be in reasonable agreement (Table 1), which fortifies the Scheme 1. The equilibrium constant K_1 is far larger than K_2. This may be attributed to the greater tendency of DPC to undergo hydrolysis compared with the dissociation of hydrolyzed

Table 3 The rate constant of the slow steps at different temperatures of some acids (for isokinetic temperature)

Amino acids	k_1 at 298 K (dm^3 mol^{-1} s^{-1})	k_2 at 303 K (dm^3 mol^{-1} s^{-1})	Reference
L-Lysine	0.021	0.041	Hiremath et al. [44]
L-Cystine	0.025	0.056	Hosamani et al. [28]
L-Phenylalanine	0.0092	0.023	Present work

species in alkaline medium. The effect of ionic strength and dielectric constant of the medium on the rate qualitatively explains the reaction between two negatively charged ions, as seen in Scheme 1.

The thermodynamic quantities for the first, second, and third equilibrium steps of Scheme 1 can be evaluated as follows. $[H_3IO_6^{2-}]$, $[L\text{-}PA]$, and $[OH^-]$ (as in Table 1) were varied at four different temperatures. The plots of $1/k_{obs}$ versus $1/[OH^-]$, $1/k_{obs}$ versus $1/[L\text{-}PA]$, and $1/k_{obs}$ versus $[H_3IO_6^{2-}]$ should be linear (Fig. 2). From the slopes and intercepts, the values of K_1, K_2, and K_3 were calculated at different temperatures and are given in Table 2. van't Hoff plots were made for variation of K_1, K_2, and K_3 with temperature [lg K_1 versus $1/T$ ($r \geq 0.985$, $S \leq 0.004$), lg K_2 versus $1/T$ ($r \geq 0.973$, $S \leq 0.007$), and lg K_3 versus $1/T$ ($r \geq 0.992$, $S \leq 0.008$)], and the values of enthalpy of reaction ΔH, entropy of reaction ΔS, and free energy of reaction ΔG were calculated for the first, second, and third equilibrium step. These values are given in Table 2. Comparison of the thermodynamic quantities of the first step of Scheme 1 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 [30, 31].

The moderate values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are both favorable for electron-transfer processes. The negative value of $\Delta S^{\ddagger}$ suggests that the intermediate complex is more ordered than the reactants [32]. The observed modest enthalpy of activation and the higher rate constant of the slow step indicate that the oxidation presumably occurs via an inner-sphere mechanism. This conclusion is supported by earlier observations [21, 33, 34].

The activation parameters for oxidation of some acids by DPC are summarized in Table 3. 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 rate are caused by changes in both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, but these quantities vary extensively in a parallel fashion. A plot of $\Delta H^{\ddagger}$ versus $\Delta S^{\ddagger}$ is linear according to the following equation:

$$\Delta H^{\ddagger} = \beta \, \Delta S^{\ddagger} + \text{constant}.$$

β is called the isokinetic temperature; it has been asserted that apparently linear correlations of $\Delta H^{\ddagger}$ with $\Delta S^{\ddagger}$ are sometimes misleading and the evaluation of β by means of the above equation lacks statistical validity [35]. Exner [36] 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 lg k_2 (at T_2) is linearly related to lg k_1 (at T_1), i.e., lg $k_2 = a + b$lg k_1, 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 to be 331 K by plotting lg k_2 at 303 K versus lg k_1 at 298 K ($r \geq 0.997$, $S \leq 0.006$) (Fig. 3). The value of β (331 K) is higher than the experimental temperature (298 K). This indicates that the rate is governed by the enthalpy of activation [37]. The linearity and the slope of the plot obtained may confirm that the kinetics of these reactions follows a similar mechanism, as previously suggested.

Conclusions

Among various species of DPC in alkaline medium, monoperiodatocuptrate(III) $[Cu(OH)_2(H_3IO_6)]^-$ is considered as active species for the title reaction. The results indicated that the role of pH in the reaction medium is crucial. The rate constant of the slow step and other equilibrium constants involved in the mechanism were evaluated, and activation parameters with respect to the slow step of the reaction were computed. The overall mechanistic sequence described herein is consistent with product studies, and mechanistic and kinetic studies.
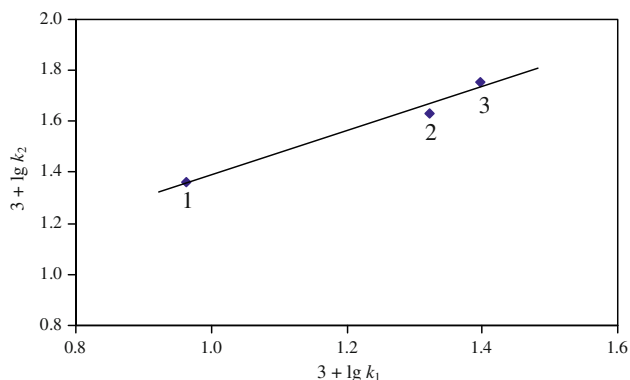

Fig. 3 Plot of lg k_2 at 303 K versus lg k_1 at 298 K for isokinetic temperature (Table 3). *1* L-phenylalanine; *2* L-lysine; *3* L-cystine

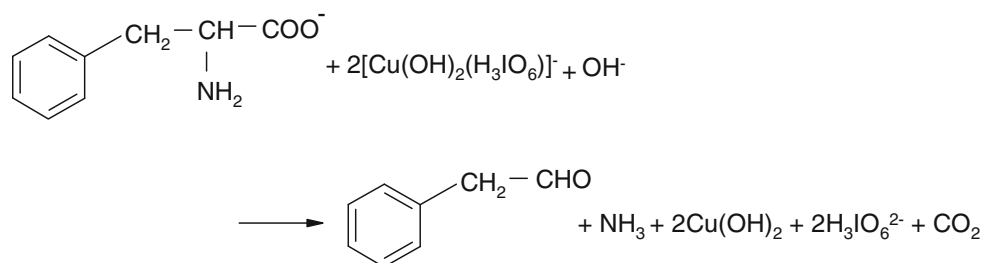## Experimental

Materials

All chemicals used were of reagent grade, and double-distilled water was used throughout the work. The copper(III) periodate complex was prepared [38, 39] and standardized by standard procedures [40]. The UV–Vis spectrum with maximum absorption at 415 nm verified the existence of a copper(III) complex. A solution of L-phenylalanine (HiMedia Laboratories) was prepared by dissolving an appropriate amount of recrystallized sample in double-distilled water. The purity of L-PA was checked by its m.p. of 273 °C [Lit. m.p. 275 °C]. The IR spectrum agreed with literature. The required concentration of L-PA was used from its stock solution, and copper sulfate (BDH) was prepared by dissolving known amounts of the samples in distilled water. Periodate solution was prepared and standardized iodometrically [41]. Required alkalinity and ionic strength were maintained by KOH (BDH) and KNO_3 (Analar), respectively, in the reaction solutions.

Kinetic measurements

Since the initial rate of reaction is fast, kinetic measurements were performed using a Varian CARY 50 Bio UV–Vis spectrophotometer. The kinetics was followed under pseudo-first-order condition, where [L-PA] > [DPC] at 298 ± 0.1 K, unless specified. The reaction was initiated by mixing the DPC into L-PA solution which also contained the required concentration of KNO_3, KOH, and KIO_4. Reaction progress was followed spectrophotometrically at 415 nm by monitoring the decrease in absorbance due to DPC with the molar absorbancy index ε to be $6{,}235 \pm 100$ dm^3 mol^{-1} cm^{-1}. 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 k_{obs} were determined from lg(absorbance) versus time plots. The plots were linear up to 80% completion of reaction under the range of [OH$^-$] used. The orders for various species were determined from the slopes of plots of lg k_{obs} versus respective concentration of species, except for [DPC] in which no variation of k_{obs} was observed as expected for the reaction condition. During the kinetics, a constant concentration of 1.0×10^{-4} mol dm^{-3} of KIO_4 was used throughout the study unless otherwise stated. Since periodate is present in excess in DPC, the possibility of oxidation of L-phenylalanine 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 with the DPC oxidation of

Scheme 3

$$\text{CH}_2\!-\!\text{CH}\!-\!\text{COO}^- \qquad + 2[\text{Cu(OH)}_2(\text{H}_3\text{IO}_6)]^- + \text{OH}^-$$
$$\underset{\text{NH}_2}{|}$$

$$\longrightarrow \qquad \text{CH}_2\!-\!\text{CHO} \qquad + \text{NH}_3 + 2\text{Cu(OH)}_2 + 2\text{H}_3\text{IO}_6^{2-} + \text{CO}_2$$

L-phenylalanine. The total concentration of periodate and OH^- was calculated by considering the amount present in the DPC solution and that additionally added. Kinetics runs were also carried out in N_2 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 N_2 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.

Stoichiometry and product analysis

Different sets of reaction mixtures containing varying ratios of DPC to L-phenylalanine in presence of constant amounts of OH^-, KNO_3, and KIO_4 were kept for 5 h in a closed vessel under nitrogen atmosphere. The remaining concentration of DPC was estimated spectrophotometrically at 415 nm. The results indicate 1:2 stoichiometry as given in Scheme 3.

The main oxidation product was identified as aldehyde (phenylacetaldehyde) by a spot test [42]. Phenylacetalde-

peak at $m/z = 119$ (m-1) amu, confirming the presence of phenylacetaldehyde (Fig. 4). All other peaks observed in GC-MS can be interpreted in accordance with the observed structure of phenylacetaldehyde.

The by-products were identified as ammonia by Nessler's reagent [43], and the CO_2 was qualitatively detected by bubbling nitrogen gas through the acidified reaction mixture and passing the liberated gas through a tube containing limewater. The presence of $Cu(OH)_2$ was also tested.

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

Appendix

According to Scheme 1

$$\text{Rate} = \frac{-d[\text{DPC}]}{dt} = k[C]$$
$$= \frac{kK_1K_2K_3[\text{L-PA}][\text{OH}^-]\left[\text{Cu(OH)}_2(\text{H}_3\text{IO}_6)_2\right]^{3-}}{\left[\text{H}_2\text{IO}_6^{3-}\right]}, \quad (7)$$

$$[\text{DPC}]_\text{T} = [\text{DPC}]_\text{f} + \left[\text{Cu(OH)}_2(\text{H}_3\text{IO}_6)(\text{H}_3\text{IO}_6)\right]^{4-} + \left[\text{Cu(OH)}_2(\text{H}_3\text{IO}_6)\right]^- + \text{Complex (C)}$$
$$= [\text{DPC}]_\text{f}\left[\frac{\left[\text{H}_2\text{IO}_6^{3-}\right] + K_1\left[\text{H}_2\text{IO}_6^{3-}\right][\text{OH}^-] + K_1K_2[\text{OH}^-] + K_1K_2K_3[\text{OH}^-][\text{L-PA}]}{\left[\text{H}_2\text{IO}_6^{3-}\right]}\right], \quad (8)$$

hyde was confirmed by its IR spectrum, which showed C=O stretching at 1,720 cm^{-1}, indicating the presence of aldehydic C=O, and C–H stretching at 2,845 cm^{-1}. Furthermore, phenylacetaldehyde was subjected to GC-mass spectral analysis. GC-mass data were obtained using a 17A Shimadzu gas chromatography with a QP-5050A Shimadzu mass spectrometer using the electrospray ionization (EI) technique. The mass spectrum showed a molecular ion

where $[\text{DPC}]_\text{T}$ and $[\text{DPC}]_\text{f}$ refer to total and free DPC concentrations, respectively. The free [DPC] is given by

$$[\text{DPC}]_\text{f}$$
$$= \frac{[\text{DPC}]_\text{T}\left[\text{H}_2\text{IO}_6^{3-}\right]}{\left[\text{H}_2\text{IO}_6^{3-}\right] + K_1\left[\text{H}_2\text{IO}_6^{3-}\right][\text{OH}^-] + K_1K_2[\text{OH}^-] + K_1K_2K_3[\text{OH}^-][\text{L-PA}]}.$$
$$(9)$$

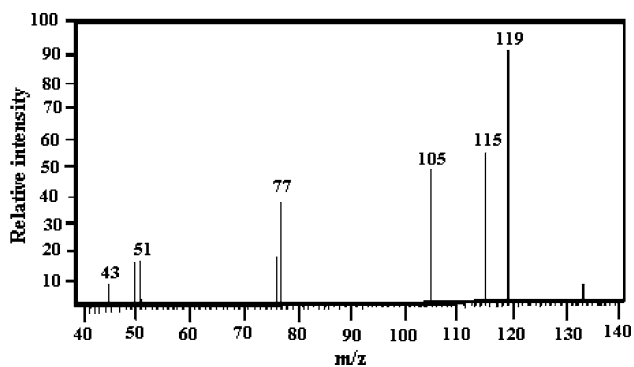**Fig. 4** GC-mass spectrum of phenylacetaldehyde with its molecular ion peak at $m/z = 119$

Similarly, total $[OH^-]$ can be calculated as

$$[OH^-]_T = [OH^-]_f + \left[Cu(OH)_2(H_3IO_6)(H_2IO_6)\right]^{4-} + \left[Cu(OH)_2(H_3IO_6)\right]^- + [C], \quad (10)$$

$$= [OH^-]_f + K_1[OH^-]\left[Cu(OH)_2(H_3IO_6)_2\right]^{3-}$$
$$+ \frac{K_1K_2\left[Cu(OH)_2(H_3IO_6)_2\right]^{3-}[OH^-]}{[H_2IO_6^{3-}]}$$
$$+ \frac{K_1K_2K_3\left[Cu(OH)_2(H_3IO_6)_2\right]^{3-}[OH^-][\text{L-PA}]}{[H_2IO_6^{3-}]}. \quad (11)$$

In view of the low concentrations of DPC used, the second, third, and fourth term in the above equation are neglected; therefore,

$$[OH]_T = [OH]_f, \quad (12)$$

similarly

$$[\text{L-PA}]_T = [\text{L-PA}]_f. \quad (13)$$

Substituting Eqs. 9, 10, and 12 into Eq. 7, we get

Rate
$$= \frac{kK_1K_2K_3[\text{L-PA}][OH^-][DPC]}{[H_2IO_6^{3-}] + K_1[H_2IO_6^{3-}][OH^-] + K_1K_2[OH^-] + K_1K_2K_3[OH^-][\text{L-PA}]}.$$

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