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Thermodynamic quantities for different steps involved in the mechanism of the oxidation of 6-mercaptopurine by diperiodatocuprate(III) in aqueous alkaline medium

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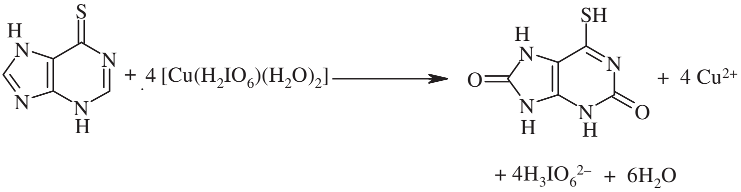
The kinetics of oxidation of 6-mercaptopurine (6-MP) by diperiodatocuprate(III) (DPC) in aqueous alkaline medium at a constant ionic strength of 0.01 mol/dm^3 was studied spectrophotometrically. The reaction between DPC and 6-MP in an alkaline medium exhibits 1:4 stoichiometry (6-MP:DPC). The reaction is of first order in [DPC] and has less than unit order in both [6-MP] and [alkali]. However, the order of [6-MP] and [alkali] changes from first order to zero order as their concentration increases. Intervention of free radicals was observed in the reaction. An increase in periodate concentration decreases the rate. The oxidation reaction in an alkaline medium has been shown to proceed via a monoperiodatocuprate(III)–6-MP complex, which decomposes slowly in a rate-determining step followed by other fast steps to give the products. The main oxidative products were identified by spot test, IR, GC-MS and HPLC studies. The reaction constants involved in the different steps of the mechanism are calculated. The activation parameters with respect to a slow step of the mechanism are computed and discussed, and thermodynamic quantities are also determined.



Keywords: 6-mercaptopurine; diperiodatocuprate(III); oxidation; kinetics; mechanism

1. Introduction

6-Mercaptopurine (6-MP) is a thiopurine drug in which carbon 6 of purine has been substituted with a sulfur-containing group. This drug with methotrexate is the drug of choice in the treatment of childhood acute lymphoblastic leukemia, especially in those cases where prolonged duration of remission is required (*1*). 6-MP together with its prodrug azathioprine is used

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as immunomodulatory agent in the treatment of inflammatory bowel disease (*2*). It has been suggested that in the cell, 6-MP is first converted to thioinosinic acid, by hypoxanthine–guanine phosphoribosyl-transferase and then metabolized to thioguanine ribonucleotide and deoxyribonucleotide; incorporation of these compounds into RNA and DNA results in the anti-tumour effect of the drug (*3*). The first route is thought to involve the initial oxidation of 6-MP to 8-oxo-6-mercaptopurine followed by the conversion to 6-thiouric acid (*4*). However, Zimm *et al.* (*5*) have identified 6-thioxanthine in the urine samples of some patients who received 6-MP by intravenous infusion, which may indicate that 6-MP is converted to 6-thiouric acid via 6-thioxanthine.

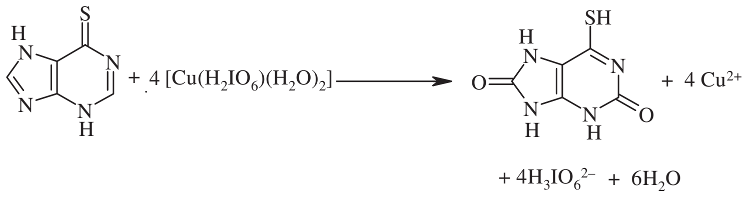
In recent years, the study of the highest oxidation state of transition metals has intrigued many researchers. Transition metals in a higher oxidation state can be stabilized by chelation with suitable polydentate ligands. Metal chelates such as diperiodatocuprate(III) (DPC) (*6*), diperiodatoargentate(III) (*7*), and diperiodatonickelate(IV) (*8*) are good oxidants in a medium with an appropriate pH value. Copper(III) is shown to be an intermediate in the copper(II)-catalyzed oxidation of amino acids by peroxydisulfate (*9*). The oxidation reaction usually involves the copper(II)–copper(I) couple, and such aspects are detailed in different reviews (*10*). Copper complexes have occupied a major place in oxidation chemistry because of their abundance and relevance in biological chemistry (*11*). Copper(III) is involved in many biological electron-transfer reactions (*12*). When the copper(III) periodate complex is oxidant and multiple equilibria between different copper(III) species (*13*) are involved, it would be interesting to know which of the species is the active oxidant.

To the best of our knowledge, oxidation of 6-MP by DPC has not been reported yet. In view of the medicinal value and the potential pharmaceutical importance of 6-MP and lack of the literature on the oxidation mechanism of this drug by DPC, there was a need for understanding the oxidation mechanism of this bioactive compound. 6-MP contains different functional groups and which group gets oxidized by the oxidant is of vital importance. Hence, we have undertaken a careful study of the title reaction.

2. Results

2.1. *Stoichiometry and product analysis*

Different sets of reaction mixtures containing different concentrations of DPC to 6-MP in the presence of constant amounts of OH^- and KNO_3 were kept for 4 h in a closed vessel at 25°C under an inert atmosphere (to avoid any potential reaction between oxygen and 6-MP). The remaining concentration of DPC was estimated spectrophotometrically. The results indicated a 1:4 stoichiometry as given in Equation 1.



(1)

The reaction product was extracted with ether and recrystallized from water and subjected to HPLC and spectral studies. Only one product was obtained as evidenced by a single peak on the HPLC spectrum and a single spot on thin layer chromatography, which was identified as 6-thiouric acid by spot test (*14*) and was characterized by FT-IR and GC-MS spectral studies.

6-Thiouric acid was confirmed by its IR spectrum, which showed a band at 1654 cm^{-1} due to >C=O stretching, 3427 cm^{-1} for −NH stretching and 2925 cm^{-1} for −CH stretching of acid. GC-MS data were obtained on a QP-2010S Shimadzu gas chromatograph mass spectrometer. The mass spectral data showed a molecular ion peak at 185 m/z, confirming the presence of 6-thiouric acid. All other peaks observed in GC-MS can be interpreted in accordance with the observed structure of 6-thiouric acid. Another product Cu(II) was identified by UV–VIS spectra. The reaction products do not undergo further oxidation under the present kinetic conditions. Similar product was noticed (*15*) in *in vitro* study of 6-MP oxidation catalyzed by aldehyde oxidase and xanthine oxidase.

2.2. *Reaction orders*

The reaction orders were determined from the slope of log k_{obs} versus log (concentration) plots by varying the concentrations of 6-MP, alkali, and periodate in turn, while keeping all other concentrations and conditions constant, except for DPC concentration.

2.3. *Effect of [DPC]*

The oxidant, DPC, concentration was varied in the range $1.0 \times 10^{-5} - 1.0 \times 10^{-4}$ mol/dm^3, and lack of variation in the k_{obs} values indicate that order with respect to [DPC] was unity (Table 1). This was also confirmed by linearity of the plots of log [absorbance] versus time ($r \geq 0.9986$, $S \leq 0.013$) up to 90% completion of the reaction (Figure 1).

2.4. *Effect of [6-MP]*

The effect of [6-MP] on the rate of reaction was studied at constant concentrations of alkali, DPC, and periodate at a constant ionic strength of 0.01 mol/dm^3. The substrate, 6-MP, was varied in

Table 1. Effect of [DPC], [6-MP], [IO_4^-], and [OH^-] on the oxidation of 6-MP by DPC in alkaline medium at 25°C, $I = 0.01$ mol/dm^3.

[DPC] × 10^5 (mol/dm^3)	[6-MP] × 10^4 (mol/dm^3)	[IO_4^-] × 10^4 (mol/dm^3)	[OH^-] × 10^3 (mol/dm^3)	$k_{obs} \times 10^2$ (s^{-1})	$k_{cal} \times 10^2$ (s^{-1})
1.0	5.0	5.0	4.0	1.12	1.10
3.0	5.0	5.0	4.0	1.13	1.10
5.0	5.0	5.0	4.0	1.07	1.10
8.0	5.0	5.0	4.0	1.19	1.10
10.0	5.0	5.0	4.0	1.09	1.10
5.0	1.0	5.0	4.0	0.31	0.31
5.0	3.0	5.0	4.0	0.78	0.77
5.0	5.0	5.0	4.0	1.07	1.10
5.0	8.0	5.0	4.0	1.49	1.46
5.0	10.0	5.0	4.0	1.65	1.62
5.0	5.0	1.0	4.0	1.86	1.87
5.0	5.0	3.0	4.0	1.39	1.39
5.0	5.0	5.0	4.0	1.07	1.10
5.0	5.0	8.0	4.0	0.82	0.85
5.0	5.0	10.0	4.0	0.71	0.73
5.0	5.0	5.0	1.0	0.46	0.46
5.0	5.0	5.0	2.0	0.76	0.75
5.0	5.0	5.0	4.0	1.07	1.10
5.0	5.0	5.0	8.0	1.41	1.44
5.0	5.0	5.0	10.0	1.63	1.54

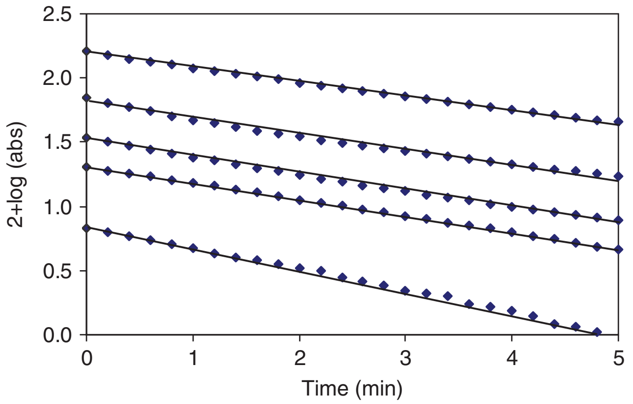


Figure 1. First-order plots for the oxidation of 6-MP by DPC in aqueous alkaline medium at 298 K (10^5 DPC mol/dm^3): (1) 1.0; (2) 3.0; (3) 5.0; (4) 8.0; (5) 10.0.

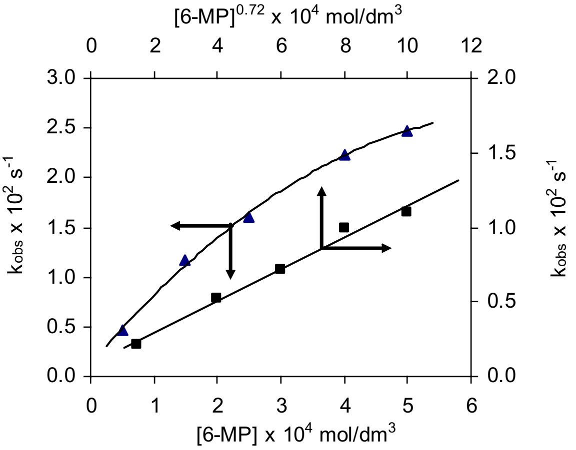


Figure 2. Plots of k_{obs} versus [6-MP]$^{0.72}$ and k_{obs} versus [6-MP] (from Table 1).

the range $1.0 \times 10^{-4} - 1.0 \times 10^{-3}$ mol/dm^3. The k_{obs} values increased with an increase in the concentration of 6-MP. The order with respect to [6-MP] was found to be less than unity (Table 1) ($r \geq 0.9995$, $S \leq 0.007$) under the experimental concentrations. This less than unit order in the 6-MP was also confirmed by the linear plot of k_{obs} versus [6-MP]$^{0.72}$. The plots of k_{obs} versus [6-MP] were nonlinear (Figure 2). However, at lower concentrations of 6-MP, the reaction was first order in [6-MP] and at a high concentration of 6-MP, the reaction was independent of [6-MP]. The order of [6-MP] changes from first order to zero order as [6-MP] increases.

2.5. *Effect of [Alkali]*

The effect of the increase in the concentration of alkali on the reaction was studied at constant concentrations of 6-MP, DPC, and periodate at a constant ionic strength of 0.01 mol/dm^3 at 25°C. The rate constants increased with the increase in the alkali concentration (Table 1), indicating apparent less than unit order dependence of rate on the alkali concentration ($r \geq 0.9994$, $S \leq 0.006$). Similar to that in the case of 6-MP, the order of alkali changes from first order to zero order as [OH$^-$] increases.

2.6. *Effect of [Periodate]*

The effect of the increasing concentration of periodate was studied by varying the periodate concentration from 1.0×10^{-4} to 1.0×10^{-3} mol/dm^3 keeping all other reactants' 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 being negative less than unity (Table 1).

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

The addition of KNO_3 at constant [DPC], [6-MP], [OH^-], and [IO_4^-] was found that with the increasing ionic strength had no significant on the rate of the reaction.

Dielectric constant of the medium, D, was varied by varying 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 the 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 the reaction.

Thus, from the observed experimental results, the rate law for the reaction is given as follows:

$$\text{Rate} = k_{\text{obs}}[\text{6-MP}]^{0.72}[\text{DPC}]^{1.0}[\text{OH}^-]^{0.52}[\text{IO}_4^-]^{-0.41}$$

2.8. *Effect of added products*

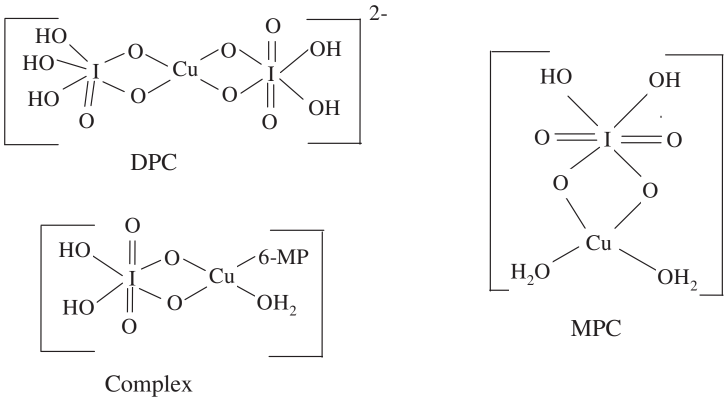
The externally added products, 6-thiouric acid and copper(II) ($CuSO_4$) in the range 1.0×10^{-4} – 1.0×10^{-3} mol/dm^3 did not have any significant effect on the rate of the reaction.

2.9. *Polymerization study (test for free radicals)*

DPC is a single equivalent oxidant. Hence, intervention of a free radical, generated from the organic compound, was expected. In view of this, for the reaction, the possibility of the formation of free radicals was detected as follows. The reaction mixture, to which a known quantity of acrylonitrile (scavenger) 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 6-MP alone with acrylonitrile did not induce any polymerization under the same condition as those induced for a reaction mixture. Initially, added acrylonitrile decreases the rate of reaction, indicating free radical intervention, which is the case in earlier work (*16*).

2.10. *Effect of temperature*

The kinetics was studied at five different temperatures under varying concentrations of 6-MP, alkali, and periodate, keeping other conditions constant. The rate constants were found to increase with the increase in temperature. The rate constants of the slow step (k) of Scheme 1 were obtained from the slopes and intercepts of $1/k_{\text{obs}}$ versus 1/[6-MP], $1/k_{\text{obs}}$ versus [$H_3IO_6^{2-}$], and $1/k_{\text{obs}}$ versus $1/[OH^-]$ plots at five different temperatures and were used to calculate the activation parameters. The activation parameters corresponding to these constants were evaluated from the Eyring plot of $(R \ln k/T \times h/k_B)$ ($r \geq 0.9977$, $S \leq 0.011$) ($h =$ Planck′s constant, $k_B =$ Boltzmann constant) versus $1/T$ and are tabulated in Tables 2 and 3.



Scheme 1. Detailed mechanistic pathways for the oxidation of 6-MP by alkaline DPC.

Table 2. Effect of temperature on the rate constant of the slow step of Scheme 1, k, for the oxidation of 6-MP by DPC in aqueous alkaline medium.

Temperature (K)	$k \times 10^2\ s^{-1}$
288	1.19
293	1.91
298	3.03
303	4.45
308	6.69

Table 3. Thermodynamic activation parameter for the oxidation of 6-MP by DPC in aqueous alkaline medium with respect to slow step of Scheme 1.

Parameters	Values
E_a (kJ/mol)	63 ± 4
$\Delta H^{\#}$ (kJ/mol)	61 ± 4
$\Delta S^{\#}$ (J/K mol)	-70 ± 6
$\Delta G^{\#}_{298\,K}$ (kJ/mol)	81 ± 1
$\log A$	9.6 ± 1.0

3. Discussion

The water-soluble copper(III) periodate complex is reported (*17*) to be $[Cu(HIO_6)_2]^{5-}$. However, in an aqueous alkaline medium and at a high pH range as 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 (*18*) (Equations (2)–(4)) depending on the pH of the solution.

$$H_5IO_6 \rightleftharpoons H_4IO_6^- + H^+ \quad K_1 = 5.1 \times 10^{-4} \tag{2}$$

$$H_4IO_6^- \rightleftharpoons H_3IO_6^{2-} + H^+ \quad K_2 = 4.9 \times 10^{-9} \tag{3}$$

$$H_3IO_6^{2-} \rightleftharpoons H_2IO_6^{3-} + H^+ \quad K_3 = 2.5 \times 10^{-12} \tag{4}$$

Periodic acid exists in an acid medium as H_5IO_6 and as $H_4IO_6^-$ around pH 7. Thus, under the conditions employed in the alkaline medium, the main species are expected to be $H_3IO_6^{2-}$ and

$H_2IO_6^{3-}$. At higher concentrations, periodate also tends to dimerize (*19*). However, the formation of this species is negligible under conditions employed for kinetic study. Hence, at the pH employed in this study, the soluble copper(III) periodate complex exists as DPC, $[Cu(H_2IO_6)(H_3IO_6)]^{2-}$, a conclusion also supported by earlier work (*6*).

The reaction between the DPC complex and 6-MP in the alkaline medium has the stoichiometry 1:4 (6-MP:DPC) with a first-order dependence on [DPC] and an apparent order of less than unity in [substrate], [alkali] and negative-fractional-order dependence on the periodate. 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. Lister (*20*) proposed three forms of copper(III) periodate in the alkaline medium as DPC, monoperiodatocuprate(III) (MPC) and tetrahydroxocuprate(III). The tetrahydroxocuprate(III) is ruled out, as its equilibrium constant is 8.0×10^{-11} at 40°C. Hence, in the present study, DPC and MPC are considered to be active forms of the copper(III) periodate complex. The result of the increase in the rate of the reaction with an increase in alkalinity (Table 1) can be explained in terms of prevailing equilibrium of the formation of $[Cu(H_3IO_6)(H_2IO_6)]^{2-}$ from $[Cu(H_3IO_6)_2)]^-$ as given in Equation (5).

$$[Cu(H_3IO_6)_2]^- + OH^- \overset{K_4}{\rightleftharpoons} [Cu(H_2IO_6)(H_3IO_6)]^{2-} + H_2O \qquad (5)$$

Also the decrease in the rate with the increase in periodate concentration suggests that the displacement of a ligand periodate takes place to give a free periodate and MPC species from $[Cu(H_2IO_6)(H_3IO_6)]^{2-}$ as given in Equation (6).

$$[Cu(H_2IO_6)(H_3IO_6)]^{2-} + 2H_2O \overset{K_5}{\rightleftharpoons} [Cu(H_2IO_6)(H_2O)_2] + H_3IO_6^{2-} \qquad (6)$$

Such types of equilibria have been well noticed in the literature (*21*). It may be expected that a lower periodate complex such as MPC is more important in the reaction than the DPC in view of the observed inverse fractional order in periodate. With the known equilibrium constants (*21*), the individual concentrations of $[DPC]_f$, DPC, and MPC were calculated. It was found that MPC was in higher concentration and nearly paralleled the rate variation with different $[OH^-]$. Furthermore, the spectra of Cu(III) periodate complex was dependent on $[OH^-]$ and the absorption becomes almost constant, indicating the predominance of one species, presumably $[Cu(H_2IO_6)(H_2O)_2]$. Because of this and the fact that rate is a function of $[OH^-]$ (less than unit order), the main oxidation species is likely to be $[Cu(H_2IO_6)(H_2O)_2]$ and its formation equilibrium (6) is of importance in the reaction.

The less than unit order in [6-MP] presumably results from the formation of a complex (C) between the MPC species and 6-MP prior to the formation of the products. K_6 is the composite equilibrium constant comprising the equilibrium to bind active species MPC to 6-MP species to form a complex (C). This complex (C) undergoes decomposition in a slow step to give the free radical species of 6-MP, periodate, and Cu(II). This free radical species of 6-MP reacts with one mole of MPC species in a fast step to form 6-thioxanthine, Cu(II), and periodate. This 6-thioxanthine further reacts with one more mole of MPC in a fast step to form a free radical species of 6-thioxanthine, Cu(II), and periodate. Further, this free radical reacts with one more mole of MPC to form the final products, *i.e.* 6-thiouric acid, Cu(II), and periodate. So, the detailed mechanistic pathways for the oxidation of 6-MP by DPC is presented 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 the 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 (*22*). Spectroscopic evidence for the complex formation between the oxidant and the substrate was obtained from the UV–VIS spectra of 6-MP (5.0×10^{-4} mol/dm^3), DPC

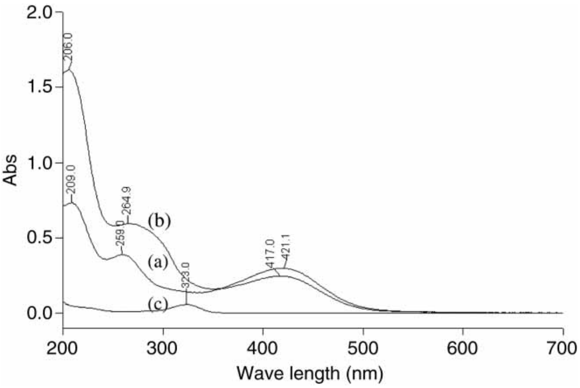


Figure 3. Spectroscopic evidence for the complex formation between DPC and 6-mercaptopurine: (a) UV–VIS spectra of DPC complex (259 nm), (b) UV–VIS spectra of mixture of DPC and 6-MP (265 nm), and (c) UV–VIS spectra of 6-MP.

(5.0×10^{-5} mol/dm^3), [OH$^-$] (4.0×10^{-3} mol/dm^3), and a mixture of both. A bathochromic shift of about 6 nm from 259 to 265 nm in the spectra of DPC was observed (Figure 3). The Michaelis–Menten plot also proved the complex formation between DPC and 6-MP, which explains the less than unit order dependence on [6-MP] (Figure 4). Such a complex between a substrate and an oxidant has been observed in other studies (*23*). The diamagnetic (dsp^2) square planar structure of Cu(III) periodate in the form of DPC and MPC and the paramagnetic (sp^3) complex of Cu(III) and 6-MP can be formulated as shown below. Similar structures have been reported in other studies (*24*).

Scheme 1 leads to the rate law (7)

$$\begin{aligned}\text{Rate} &= \frac{-\mathrm{d}[\text{DPC}]}{\mathrm{d}t}\\ &= \frac{kK_4K_5K_6[\text{DPC}][\text{6-MP}][\text{OH}^-]}{[\text{H}_3\text{IO}_6^{2-}] + K_4[\text{H}_3\text{IO}_6^{2-}][\text{OH}^-] + K_4K_5[\text{OH}^-] + K_4K_5K_6[\text{OH}^-][\text{6-MP}]},\end{aligned} \tag{7}$$

which explains all the observed kinetic orders of different species.

$$\begin{aligned}k_{\text{obs}} &= \frac{\text{Rate}}{[\text{DPC}]}\\ &= \frac{kK_4K_5K_6[\text{6-MP}][\text{OH}^-]}{[\text{H}_3\text{IO}_6^{2-}] + K_4[\text{H}_3\text{IO}_6^{2-}][\text{OH}^-] + K_4K_5[\text{OH}^-] + K_4K_5K_6[\text{OH}^-][\text{6-MP}]}.\end{aligned} \tag{8}$$

The rate law (8) can be rearranged into the following form, which is suitable for verification:

$$\frac{1}{k_{\text{obs}}} = \frac{[\text{H}_3\text{IO}_6^{2-}]}{kK_4K_5K_6[\text{6-MP}][\text{OH}^-]} + \frac{[\text{H}_3\text{IO}_6^{2-}]}{kK_5K_6[\text{6-MP}]} + \frac{1}{kK_6[\text{6-MP}]} + \frac{1}{k}. \tag{9}$$

According to Equation (9), other conditions being constant, plots of $1/k_{\text{obs}}$ versus 1/[OH$^-$] ($r \geq 0.9983$, $S \leq 0.029$), $1/k_{\text{obs}}$ versus 1/[6-MP] ($r \geq 0.9995$, $S \leq 0.016$), and $1/k_{\text{obs}}$ versus [H$_3$IO$_6^{2-}$] ($r \geq 0.9994$, $S \leq 0.011$) should be linear and are found to be so (Figure 4). The slopes and intercepts of such plots lead to the values of K_4, K_5, K_6, and k as 24.6 dm^3/mol, 1.33×10^{-3} dm^3/mol, 5.93×10^3 dm^3/mol, and 3.03×10^{-2} s^{-1}, respectively. The magnitudes of K_4 and K_5 are in good agreement with those obtained by the earlier work (*23*), which supports the proposed mechanism. Using these K_4, K_5, K_6, and k values, the rate constants under different experimental conditions were calculated by Equation (8) and compared with experimental data. There is a good agreement between them (Table 1), which fortifies Scheme 1.

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

The thermodynamic quantities for the first, second, and third equilibrium steps of Scheme 1 can be evaluated as follows. The $[H_3IO_6^{2-}]$, [6-MP], and $[OH^-]$ (as in Table 1) were varied at five different temperatures. The plots of $1/k_{obs}$ versus $1/[OH^-]$, $1/k_{obs}$ versus 1/[6-MP], and $1/k_{obs}$ versus $[H_3IO_6^{2-}]$ should be linear. From the slopes and intercepts, the values of K_4 were calculated at different temperatures and these values are given in Tables 4 and 5. A van't Hoff's plot was made for the variation of K_4 with temperature ($\log K_4$ versus $1/T$ ($r \geq 0.9811$, $S \leq 0.006$)) and the values of the enthalpy of reaction ΔH, entropy of reaction ΔS, and free energy of reaction ΔG were calculated for the first equilibrium step (Tables 4 and 5). A comparison of the ΔH value

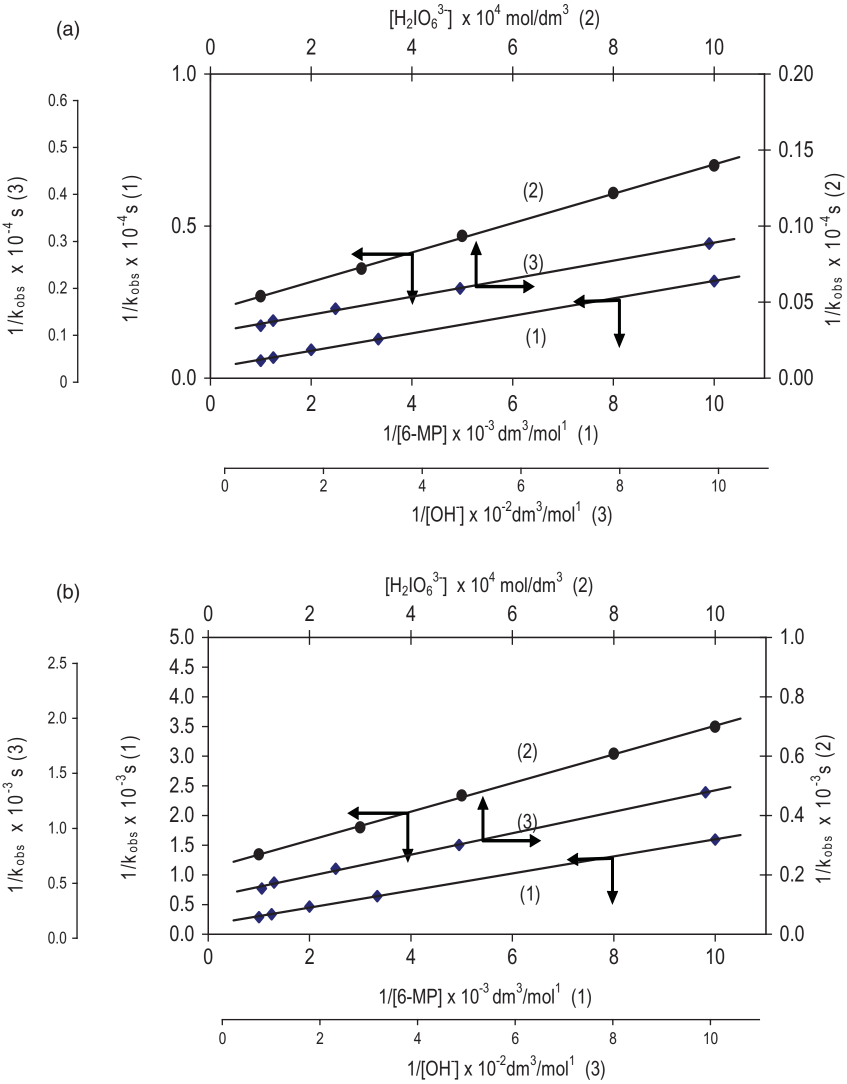


Figure 4. Verification of rate law (8) in the form of Equation (9) for the oxidation of 6-MP by DPC. (a) At 15°C, (b) at 20°C, (c) at 25°C, (d) at 30°C, and (e) at 35°C.

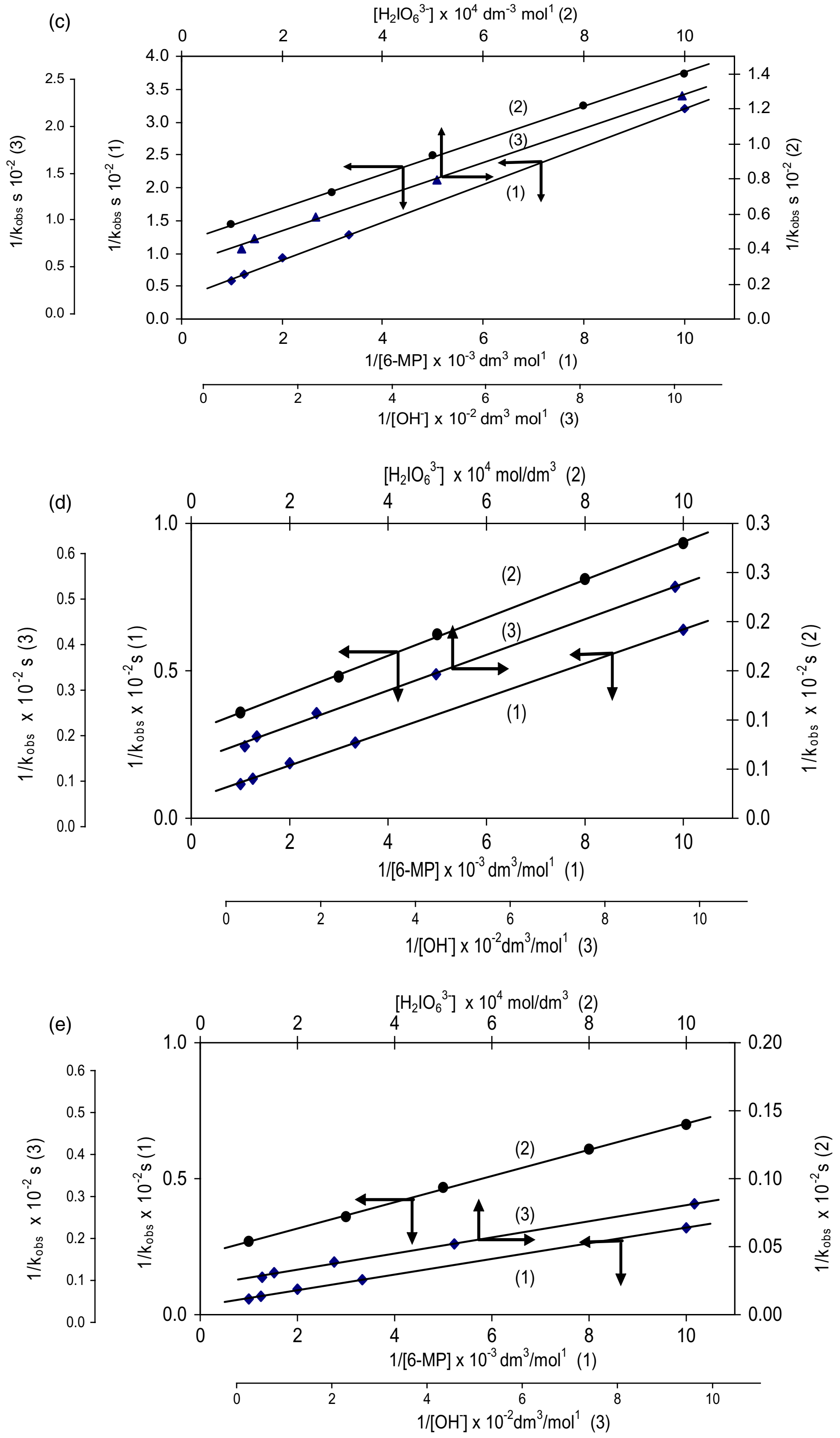


Figure 4. Continued.

Table 4. Effect of temperature on equilibrium constants of Scheme 1 for the oxidation of 6-MP by DPC in aqueous alkaline medium.

Temperature (K)	K_4 (dm^3/mol)	$K_5 \times 10^3$ (mol/dm^3)	$K_6 \times 10^{-3}$ (dm^3/mol)
288	10.3	3.67	1.48
293	17.2	2.27	2.51
298	24.6	1.33	5.93
303	32.5	0.92	6.59
308	41.4	0.51	8.76

Table 5. Thermodynamic quantities with respect to K_4, K_5, and K_6.

Thermodynamic quantities	Values from K_4	Values from K_5	Values from K_6
ΔH (kJ/mol)	51 ± 3	-71 ± 3	67 ± 3
ΔS (J/K mol)	196 ± 10	-293 ± 15	294 ± 15
$\Delta G_{298\,\mathrm{K}}$ (kJ/mol)	-8.0 ± 0.4	16 ± 1	-21 ± 2

(51 ± 3 kJ/mol) from K_4 with that of $\Delta H^{\#}$ (61 ± 4 kJ/mol) of the rate-limiting step supports the fact that the first step of Scheme 1 is fairly fast since it involves low activation energy compared with that obtained for the slow step of the reaction (*25*). In the same manner, K_5 and K_6 values were calculated at different temperatures and their corresponding values of the thermodynamic quantities are given in Tables 4 and 5.

The values of $\Delta H^{\#}$ and $\Delta S^{\#}$ were both favorable for electron-transfer processes. The negative value of $\Delta S^{\#}$ indicates that the complex (C) is more ordered than the reactants (*26*). The value of $\Delta S^{\#}$ within the range for radical reaction has been ascribed (*27*) to the nature of electron pairing and unpairing processes and to the loss of degrees of freedom formerly available to the reactants upon the formation of a rigid transition state. The observed modest enthalpy of activation and a relatively low value of the entropy of activation 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 an earlier observation (*28*).

4. Conclusions

Among the various species of DPC in an aqueous alkaline medium, $[\mathrm{Cu(H_2IO_6)(H_2O)_2}]$ is considered as an active species for the title reaction. The results indicated that in carrying out this reaction, 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 are evaluated. The activation parameters with respect to the slow step of the mechanism were computed. The overall mechanistic sequence described here is consistent with all the experimental evidences including the product, spectral, mechanistic, and kinetic studies.

5. Experimental

5.1. *Materials and reagents*

All chemicals used were of reagent grade, and doubly distilled water was used throughout the work. A solution of 6-MP (Sigma Aldrich) was prepared by dissolving an appropriate amount of 6-MP in doubly distilled water. The required concentration of 6-MP was prepared from its stock solution. The copper(III) periodate complex was prepared by the standard procedure (*29*). The existence of copper(III) complex was verified by its UV–VIS spectrum, which showed an absorption band with the maximum absorption at 415 nm. The aqueous solution of copper(III) was standardized by iodometric titration and gravimetrically by the thiocyanate (*30*) method. The copper(II) solution was prepared by dissolving the known amount of copper sulfate (BDH) in distilled water. Periodate solution was prepared by weighing the required amount of sample in hot water and used after keeping it for 24 h. Its concentration was ascertained iodometrically (*31*) at neutral pH by phosphate buffer. Aqueous solutions of KOH and KNO_3 (BDH, AR) were employed to maintain the required alkalinity and ionic strength, respectively, in reaction solutions.

5.2. *Instruments used*

(i) For kinetic measurements, a Peltier Accessory (temperature control) attached Varian CARY 50 Bio UV–VIS spectrophotometer (Varian, Victoria-3170, Australia) was used.
(ii) For product analysis, a QP-2010S Shimadzu gas chromatograph mass spectrometer and a Nicolet 5700-FT-IR spectrometer (Thermo, USA) and for pH measurements, an Elico pH meter model LI120 were used.

5.3. *Kinetic measurements*

The kinetics was followed under pseudo-first-order condition, where [6-MP] $>$ [DPC] at $25 \pm 0.1°\mathrm{C}$, unless specified. The reaction was initiated by mixing the DPC with 6-MP solution, which also contained the required concentration of KNO_3, KOH, and KIO_4. The progress of the reaction was followed spectrophotometrically by measuring the decrease in the absorbance of DPC in the reaction mixture as a function of time, in a 1 cm quartz cell in a thermostated compartment of Hitachi 150-20 UV–VIS spectrophotometer, at its $\lambda_{\max} = 415$ nm. Previously, it was verified that there is negligible interference from other species present in the reaction mixture at this wavelength. That the absorbance by DPC obeys Beer's law at its $\lambda_{\max} = 415$ nm was verified earlier, and the molar absorbency index, ε was found to be $6230 \pm 100\ \mathrm{dm^3/mol\,cm}$.

The pseudo-first-order rate constants, k_{obs}, were determined from the log (absorbance) versus time plots and were the average of duplicate runs (Table 1). The plots were linear up to 90% completion of reaction under the range of $[\mathrm{OH^-}]$ used and the rate constants were reproducible to within ±5%. The orders for various species were determined from the slopes of plots of $\log k_{\mathrm{obs}}$ versus log respective concentration of species except for [DPC] in which no variation of k_{obs} was observed as expected to the reaction condition. During the kinetics, a constant concentration viz. $5.0 \times 10^{-4}\ \mathrm{mol/dm^3}$ of KIO_4 was used throughout the study unless otherwise stated. Since periodate is present in excess in DPC, the possibility of the oxidation of 6-MP 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 with the DPC oxidation of 6-MP. The total concentrations of periodate and $\mathrm{OH^-}$ was calculated by considering the amount present in the DPC solution and that additionally added. Kinetic 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. The spectral changes during the reaction are shown in Figure 5. It is evident from the figure that the concentration of DPC decreases by observing the absorbance at 415 nm.

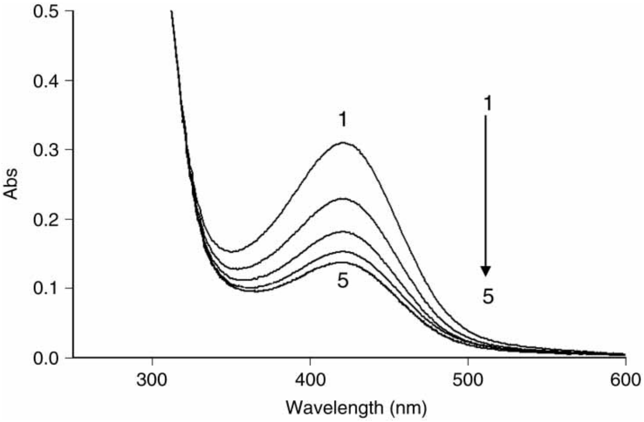


Figure 5. UV–VIS spectra changes during the oxidation of 6-mercaptopurine by alkaline DPC at 298 K, [DPC] $= 5.0 \times 10^{-5}$, [6-MP] $= 5.0 \times 10^{-4}$, $[\mathrm{OH}^-] = 4 \times 10^{-3}$, and $I = 0.01$ mol/dm^3 with scanning time of (1) 0.5 min, (2) 1.0 min, (3) 1.5 min, (4) 2.0 min, and (5) 2.5 min.

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 2003 Excel program.

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Appendix

Derivation of rate law for the reaction

According to Scheme 1:

$$\text{Rate} = -\frac{\text{d[DPC]}}{\text{d}t} = k[\text{Complex}]$$

$$\text{Rate} = kK_6[\text{6-MP}][\text{Cu}]$$

$$\text{Rate} = \frac{kK_5K_6[\text{6-MP}][\text{Cu}^{2-}]}{[\text{H}_3\text{IO}_6^{2-}]}$$

$$\text{Rate} = \frac{kK_4K_5K_6[\text{6-MP}][\text{DPC}][\text{OH}^-]}{[\text{H}_3\text{IO}_6^{2-}]} \qquad \text{(A1)}$$

The total concentration of DPC, $[\text{DPC}]_\text{T}$, is given by

$$[\text{DPC}]_\text{T} = [\text{DPC}]_\text{f} + [\text{Cu}^{2-}] + [\text{Cu}] + \text{Complex}$$

where T and f refer to total and free concentrations.

$$[\text{DPC}]_\text{f} = \frac{[\text{DPC}]_\text{T}[\text{H}_3\text{IO}_6^{2-}]}{[\text{H}_3\text{IO}_6^{2-}] + K_4[\text{OH}^-][\text{H}_3\text{IO}_6^{2-}] + K_4K_5[\text{OH}^-] + K_4K_5K_6[\text{OH}^-][\text{6-MP}]} \qquad \text{(A2)}$$

Similarly, the concentration of 6-MP is

$$[\text{6-MP}]_\text{T} = [\text{6-MP}]_\text{f} + [\text{Complex}]$$

$$[\text{6-MP}]_\text{f} = \frac{[\text{6-MP}]_\text{T}[\text{H}_3\text{IO}_6^{2-}]}{[\text{H}_3\text{IO}_6^{2-}] + K_4K_5K_6[\text{OH}^-][\text{Cu}^-]}$$

In view of the low concentrations of DPC used, the term in $K_4K_5K_6[\text{OH}^-][\text{Cu}^-]$ compared with $[\text{H}_3\text{IO}_6^{2-}]$ can be neglected. Hence,

$$[\text{6-MP}]_\text{f} = [\text{6-MP}]_\text{T} \qquad \text{(A3)}$$

Similarly,

$$[\text{OH}^-]_\text{f} = [\text{OH}^-]_\text{T} \qquad \text{(A4)}$$

Substituting Equations (A2)–(A4) into Equation (A1), we get

$$\text{Rate} = \frac{-\text{d[DPC]}}{\text{d}t} = \frac{kK_4K_5K_6[\text{DPC}][\text{6-MP}][\text{OH}^-]}{[\text{H}_3\text{IO}_6^{2-}] + K_4[\text{H}_3\text{IO}_6^{2-}][\text{OH}^-] + K_4K_5[\text{OH}^-] + K_4K_5K_6[\text{OH}^-][\text{6-MP}]}$$