Electrochemical and peroxidase catalysed oxidation of 1,7-dimethyluric acid and effect of methyl groups on the oxidation mechanism

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The electrochemical oxidation of 1,7-dimethyluric acid has been studied over a wide pH range of 2.2–10.3 at solid electrodes. Based on the results obtained from linear and cyclic sweep voltammetry, coulometry, spectroscopic studies and various analytical studies, a reaction mechanism has been proposed. The enzymatic oxidation of 1,7-dimethyluric acid has also been found to follow an identical pathway. The products of oxidation have been separated and characterized by using mp, ^{1}H NMR and mass spectra. *N*-Methylation of the pyrimidine ring of purine causes protonation of nitrogen and affects the oxidation mechanism by not permitting the ring contraction of the diol intermediate.

Introduction

Electrochemical investigations of purines have proven useful in providing information about the biological redox reactions of these compounds. The intimate involvement of purines and pyrimidines (constituents of nucleic acids) in biological processess, particularly neuromodulation, has been recognised in recent years.[1,2] The use of 3′-azido-3′-deoxythymidine (azidothymidine) for the treatment of AIDS[3,4] prompted scientists to study further this important class of compounds. In a recent report[5] from this laboratory we described electrochemical and peroxidase-catalysed oxidation of 9-methyluric acid and it was observed that both the oxidations proceed by identical mechanisms.

In the present paper electrochemical and peroxidase-catalysed oxidation of 1,7-dimethyluric acid is presented at three solid electrodes, *viz.* pyrolytic graphite (PGE), glassy carbon (GCE) and platinum (Pt). A comparison of the observed behaviour has also been made with uric acid[6] and 1,3,7-trimethyluric acid[7] to evaluate the effects of *N*-methylation on the oxidation behaviour.

Experimental

1,7-Dimethyluric acid was obtained from Adams Chemical Co., Round Lake, Illinois and used as received. Type VIII peroxidase (R_z *ca.* 3.4) and catalase were the products of Sigma Chemical Co., USA. The stock solution of 1,7-dimethyluric acid (0.5 mmol dm^{-3}) was prepared in double distilled water. For voltammetric experiments 5.0 cm^3 of the stock solution was mixed with 5.0 cm^3 of the buffer of the desired pH (μ = 0.5 mol dm^{-3}) so that the ionic strength of the solutions was 0.25 mol dm^{-3}. For spectral studies during electrooxidation an effective concentration of 0.1 mmol dm^{-3} 1,7-dimethyluric acid was used at all three electrodes. The equipment used and procedure for electrochemical and spectral studies were essentially the same as have been described earlier.[8,9] All potentials are referenced to the saturated calomel electrode (SCE) at an ambient temperature of 20 ± 2 °C. TLC was carried out using Silica Gel-G as adsorbent and methanol–benzene (1:9) as the eluent. Silylation of the products obtained was carried out by using acetonitrile and *N*,*N*-bis(trimethylsilyl)triflouroacetamide (BSTFA) by the procedure reported earlier.[8,9]

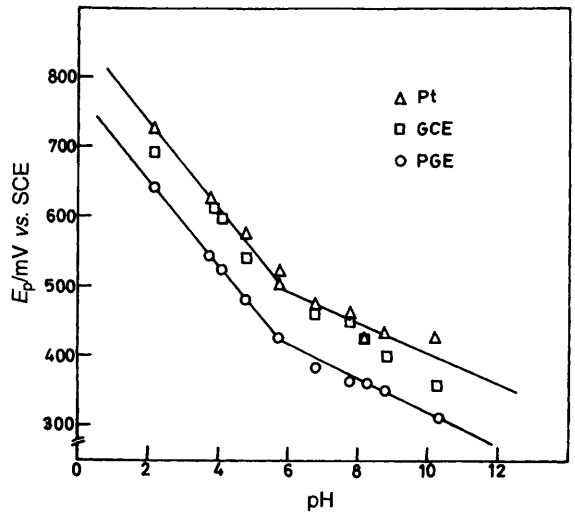


Fig. 1 Plot of peak potential (E_p) *vs.* pH for the voltammetric oxidation of peak Ia for 0.25 mmol dm^{-3} of 1,7-dimethyluric acid at different solid electrodes

Results and discussions

Linear and cyclic sweep voltammetry

Linear sweep voltammetry of 1,7-dimethyluric acid at a sweep rate of 10 mV s^{-1} in the pH range 2.2–10.3 exhibited a single, well defined, pH-dependent oxidation peak Ia at all the three electrodes PGE, GCE and platinum. The peak at the platinum electrode was found to be broader in comparison with that observed at PGE and GCE. The peak potential of peak Ia was dependent on pH and shifted to a less positive potential with an increase in pH. The peak potentials of peak Ia were more or less the same at GCE and Pt whereas at PGE the peak potential values were 80–100 mV less positive. The E_p *versus* pH plot at all the three electrodes exhibited a break at *ca.* pH 5.8 (Fig. 1) and corresponded to the pK_a of 1,7-dimethyluric acid. This pK_a value is similar to the values reported for several *N*-methylated uric acids in the literature.[10,11] The dependence of E_p on the pH (i) at Pt and GCE can be expressed by eqns. (1) and (2) and (ii) at PGE by eqns. (3) and (4).

$$E_p \text{ (pH 2.2–5.8)} = [860 - 62.5 \times \text{pH}] \text{ mV } \textit{vs.} \text{ SCE} \quad (1)$$

$$E_p\,(\text{pH } 5.8\text{–}10.3) = [625 - 22.2 \times \text{pH}]\ \text{mV } \textit{vs.}\ \text{SCE} \quad (2)$$

$$E_p\,(\text{pH } 2.2\text{–}5.8) = [775 - 60.9 \times \text{pH}]\ \text{mV } \textit{vs.}\ \text{SCE} \quad (3)$$

$$E_p\,(\text{pH } 5.8\text{–}10.3) = [560 - 25 \times \text{pH}]\ \text{mV } \textit{vs.}\ \text{SCE} \quad (4)$$

The E_p–pH dependence clearly indicates that the conjugate base is the species oxidised which predominates in the bulk of the solution. 1,7-Dimethyluric acid possesses two ionizable protons at positions 3 and 9. Hence, an anion can be formed by the loss of a proton either from the N-3 or from the N-9 position. The ionisation constants for the imidazole ring have been found to change on fusion with a benzene or pyrimidine ring. Thus, when an imidazole ring is combined with a benzene or pyrimidine ring, its acid strength becomes stronger and its base strength weaker. As the base strength of pyrimidine is very weak [12] and as it is a more electron-attracting nucleus than benzene, it cannot be disputed that the anion is formed by the loss of a proton from the imidazole ring. Thus it seems reasonable to infer that the loss of a proton occurs from position 9.

Cyclic voltammetry of 1,7-dimethyluric acid at a sweep rate of 100 mV s^{-1} gives one well defined oxidation peak Ia at all three electrodes. The nature of the peaks at different electrodes were the same as observed in linear sweep voltammetry. However, entirely different behaviour was observed for cathodic peaks at each electrode. At PGE two reduction peaks Ic and IIc were observed in the reverse sweep. Peak Ic was noticed in the entire pH range whereas peak IIc was clearly observed only up to pH 6.8. At GCE only peak Ic was observed in the reverse sweep in the entire pH range studied whereas no cathodic peak was observed at the platinum electrode in the entire pH range. A comparison of some typical cyclic voltammograms at different electrodes is presented in Fig. 2. Peak Ic observed at PGE and GCE formed a quasi-reversible couple with peak Ia in the entire pH range. The peak potentials of peak Ic and IIc were dependent on pH and shifted to a more negative potential with an increase in pH. The ratio of peaks Ia/Ic at PGE was *ca.* 18 in the pH range 2.2–7.8, whereas at pH > 7.8, the ratio decreased to *ca.* 10. Similarly at GCE the ratio decreased from *ca.* 7 to *ca.* 4 with an increase in pH (Table 1). This behaviour clearly indicates that the species responsible for peak Ic is more predominant in alkaline medium than in acidic medium. Thus, the product generated in the peak Ia reaction is unstable and undergoes acid-catalysed follow-up chemical reactions.

The effect of concentration on peak Ia was studied in the concentration range 0.01–1.0 mmol dm^{-3} at all the three electrodes. The plot of i_p *versus* concentration was linear at platinum and GCE in the entire concentration range indicating that no complications arise due to adsorption. At PGE the i_p increased with increase in concentration. The plot of i_p *versus* concentration was linear up to 0.25 mmol dm^{-3} and at concentrations >0.25 mmol dm^{-3}, the peak current became more or less constant. This behaviour indicates the adsorption of 1,7-dimethyluric acid at the surface of PGE. The adsorption at PGE was further confirmed [13–15] by the increase in peak current function ($i_p v^{-\frac{1}{2}}$) with increase in log v (Fig. 3). The ratio of peaks Ic/IIc at PGE was found to remain practically constant (*ca.* 1.0) with increase in concentration.

The peak potential of peak Ia was found to be dependent on sweep rate and shifted to a more positive potential with increase in sweep rate in the range 5 × 10^{-3} to 10 V s^{-1} at all the three electrodes. The peak potential (E_p) was found to shift by 15 mV per ten-fold increase in sweep rate in the range 5–200 mV s^{-1}, whereas at higher sweep rates the shift decreased to 8–10 mV s^{-1}. The nature of the plots of $[(\Delta E_{p/2})/\Delta \log v]$ *vs.* log v were S-shaped at all the three electrodes suggesting thereby the nature of the electrode reaction as EC in which charge transfer is followed by irreversible chemical reactions. [16,17]

Controlled potential electrolysis of 1,7-dimethyluric acid in the pH range 2.2–10.3 was carried out at all the three electrodes at a potential 100 mV more positive than peak Ia. The number of electrons involved in the electrooxidation n was calculated at different pH and concentrations by graphical integration of the current–time curve. [18] The decrease in peak current with time was more or less exponential. However, log i_p = f(t) was a straight line for the first 20 min of oxidation after which a large deviation was noticed due to competitive chemical reactions. The n value obtained under different conditions at all the three electrodes was found to be 1.9 ± 0.2 (Table 2).

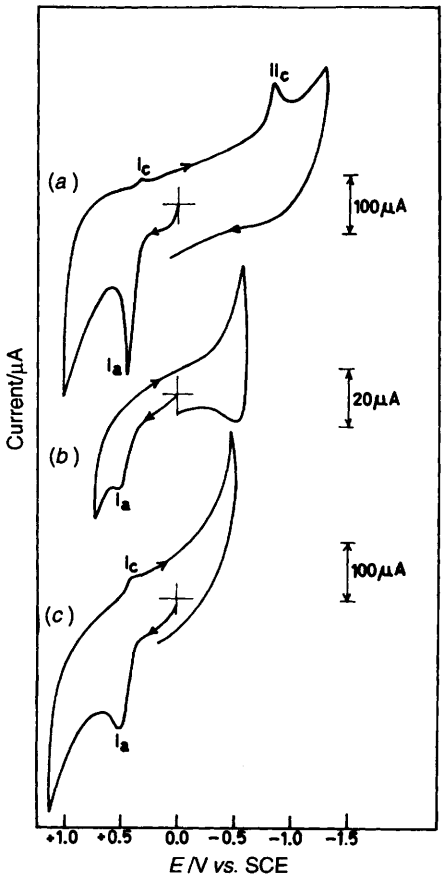


Fig. 2 Cyclic voltammograms of 0.25 mmol dm^{-3} of 1,7-dimethyluric acid in phosphate buffer at pH = 5.7 at (*a*) PGE, (*b*) Pt and (*c*) GCE. Sweep rate = 100 mV s^{-1}. Geometric area of PGE *ca.* 9 mm^2; Pt *ca.* 3 mm^2; GCE *ca.* 12 mm^2.

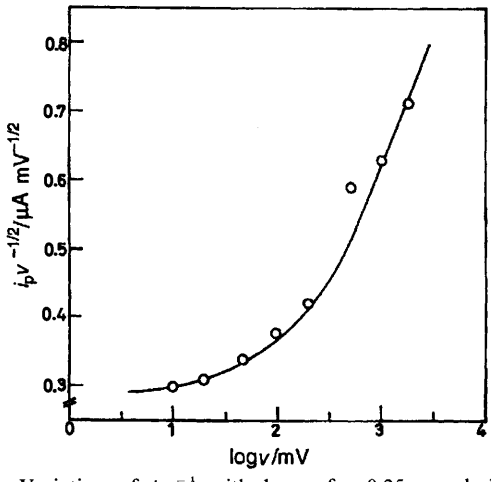


Fig. 3 Variation of $i_p v^{-\frac{1}{2}}$ with log v for 0.25 mmol dm^{-3} 1,7-dimethyluric acid at PGE, pH = 6.8

Spectral studies

The UV spectra of 1,7-dimethyluric acid were recorded in the pH range 2.2–10.3 to determine the pK_a value. 1,7-Dimethyluric

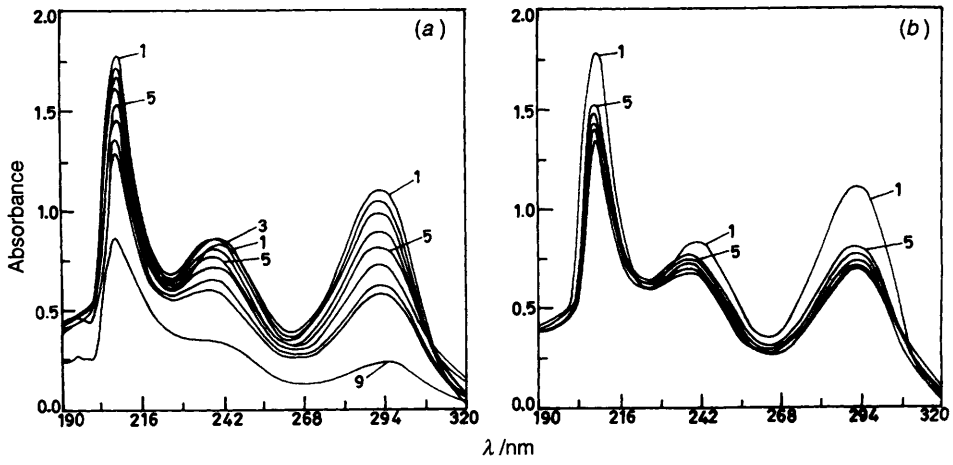


Fig. 4 Spectral changes observed for 0.1 mmol dm^{-3} 1,7-dimethyluric acid electrolysing at 0.57 V in phosphate buffer at pH 6.8 at GCE. (*a*) Curves were recorded (1) before electrolysis; (2) 5; (3) 10; (4) 20; (5) 30; (6) 40; (7) 55; (8) 70 and (9) 180 min after electrolysis. (*b*) Spectra observed when the applied potential coresponding to curve **5** in (*a*) was turned off. Curves were recorded (5) 0; (6) 5; (7) 10; (8) 20; (9) 35; (10) 50; (11) 80 min after turning off the potential.

Table 1 Observed values of peak potentials (E_p) for different peaks with change in pH and the ratio of peak currents Ia/Ic at different solid electrodes[a]

	Pyrolytic graphite electrode				Platinum	Glassy carbon electrode		
pH	E_p(Ia)/mV	E_p(Ic)/mV	E_p(IIc)/mV	Ia/Ic	E_p(Ia)/mV	E_p(Ia)/mV	E_p(Ic)/mV	Ia/Ic
2.2	640	-575	700	18.5	725	692	587	5.0
3.8	540	462	775	17.2	625	612	500	7.5
4.1	525	450	780	17.2	587	587	490	6.9
4.8	480	400	812	17.9	575	540	450	7.3
5.8	425	330	850	18.2	500	520	400	7.0
6.8	382	310	895	17.5	475	462	475	7.0
7.8	362	287	—	17.7	462	450	362	6.2
8.3	360	275	—	12.8	425	425	350	4.3
8.8	350	262	—	9.7	430	400	350	4.2
10.3	312	237	—	10.2	425	362	300	4.3

[a] Average of at least three replicate determinations.

Table 2 Coulometric *n* values observed for the electrooxidation of 1,7-dimethyluric acid at different solid electrodes

pH	Electrode	*E*/V	Conc./ mmol dm^{-3}	n^a
2.2	PGE	0.7	0.1	1.86
	GCE	0.8	0.1	1.90
	Pt	0.8	0.1	1.88
4.8	PGE	0.6	0.2	2.04
	GCE	0.7	0.2	1.96
	Pt	0.7	0.1	1.90
6.8	PGE	0.5	0.2	1.88
	GCE	0.5	0.2	1.96
	Pt	0.5	0.2	1.90
8.3	PGE	0.5	0.1	1.86
	GCE	0.5	0.2	1.70
	Pt	0.5	0.1	1.92

[a] Average of at least two replicate determinations.

acid exhibited three well defined λ_{max} at 209, 239 and 293 nm in the entire pH range. The plot of absorbance at λ_{max} *versus* pH gave an inflection at pH 5.8 corresponding to the pK_a of 1,7-dimethyluric acid. This pK_a value was identical to that obtained from the E_p *versus* pH plot (Fig. 1).

The spectral changes during electrooxidation were monitored at pH 3.8, 4.8, 6.8 and 8.6 in phosphate buffers at all the three electrodes. Fig. 4 depicts the spectral behaviour of 1,7-dimethyluric acid at pH 6.8 during electrooxidation at the glassy carbon electrode. 1,7-Dimethyluric acid exhibited three absorption bands at 293, 239 and 209 nm [Fig. 4(*a*), curve 1]. Upon application of a potential of 100 mV more positive than peak Ia, the bands at 293 and 209 nm decrease systematically whereas the band at 239 nm first increases, shifts to a shorter wavelength and then decreases systematically. The exhaustively electrolysed solution exhibited a single absorption band at 209 nm. If after scanning curve 5 in Fig. 4(*a*) (when 50% of the 1,7-dimethyluric acid has been electrooxidized) electrolysis is terminated by switching off the potential, a systematic decrease in absorbance in the region 190–300 nm is observed [Fig. 4(*b*)]. Thus, a UV-absorbing intermediate species is generated on electrochemical oxidation of 1,7-dimethyluric acid which decays after turning off the potential in a competitive chemical reaction. Similar behaviour was observed at PGE and platinum.

The kinetics of the decay of the UV-absorbing intermediate were monitored at different pH and at selected wavelengths. At all the three electrodes used the plots of absorbance *versus* time showed an exponential nature. The plots of log $(A - A_\infty)$ *versus* time were linear indicating that the decomposition reaction of the UV-absorbing intermediate followed first-order kinetics (Fig. 5). The values of the rate constant observed at different pH and at different electrodes are presented in Table 3. The rate constants obtained at all the three electrodes were in the range (1.5–3.0) × 10^{-3} s^{-1} and thus indicate the probability of a similar electrode reaction at all the three electrodes.

The nature of the species generated in peak IIc reaction at PGE was also studied spectrophotometrically. For this purpose after 30 min of electrooxidation (Fig. 6, curve 2), the electrode is potentiostatted at a potential more negative than that of reduction peak IIc. The results observed are presented in Fig. 6.

Table 3 Comparison of first-order rate constants[a] observed for the decay of UV-absorbing intermediate generated during oxidation of 1,7-dimethyluric acid at solid electrodes

		$k^a/10^{-3}$ s^{-1}			
pH	λ/nm	GCE	PGE	Pt	Enzymic
4.8	293	2.2	2.5	2.9	2.9
	239	2.0	2.0	3.0	2.7
6.8	293	1.8	1.5	1.9	2.3
	239	1.5	1.5	2.0	2.2
8.6	293	3.0	3.2	2.8	—
	239	3.0	2.9	2.9	—

[a] Average of at least three replicate determinations.

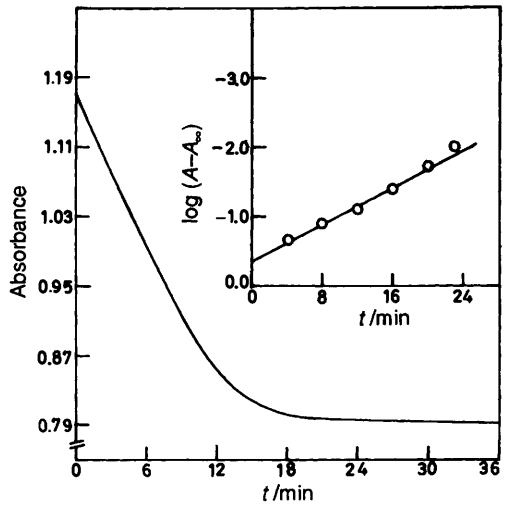


Fig. 5 Absorbance *vs.* time and log $(A - A_\infty)$ *vs.* time curves observed at 293 nm for the first-order decay of the UV-absorbing intermediate generated during electrooxidation of 1,7-dimethyluric acid at PGE, pH 4.8

In this case the absorbance at λ_{max} increased systematically and a spectrum similar to that of 1,7-dimethyluric acid was obtained. Thus, it is reasonable to conclude that reduction of the UV-absorbing intermediate species generated upon electrooxidation of 1,7-dimethyluric acid at a potential corresponding to peak IIc leads to the regeneration of the starting material 1,7-dimethyluric acid. The non-appearance of peak IIc at pH > 6.8 in cyclic voltammetry is probably due to the rapid disappearance of the product of peak Ia in competitive chemical reactions.

Product characterization

Products of electrooxidation of 1,7-dimethyluric acid corresponding to peak Ia were identified at pH 3.0 and 7.0 at all the three electrodes. The exhaustively electrolysed solution was lyophilized and the freeze-dried material obtained was passed through a glass column packed with Sephadex G-10. The absorbance of the fractions was monitored at 210 nm and the absorbance *versus* volume plotted.

At pH 3.0 the dried material obtained exhibited two spots in TLC having R_f values 0.1 and 0.25 thereby indicating the formation of two products. Further, the plot of absorbance *versus* volume exhibited three peaks P_1, P_2 and P_3. The first peak between volume 80–130 cm^3 (P_1) was found to contain phosphate and hence discarded. The volume (130–150 cm^3) under the second peak (P_2) was lyophilized. The dried material obtained exhibited a single spot in TLC with a mp 92 °C. The mass spectrum of this material gave a clear molecular ion peak at m/z = 74 suggesting the product is *N*-methylurea. However, no attempt was made to explain the fragmentation pattern. The formation of *N*-methylurea as one of the products indicated

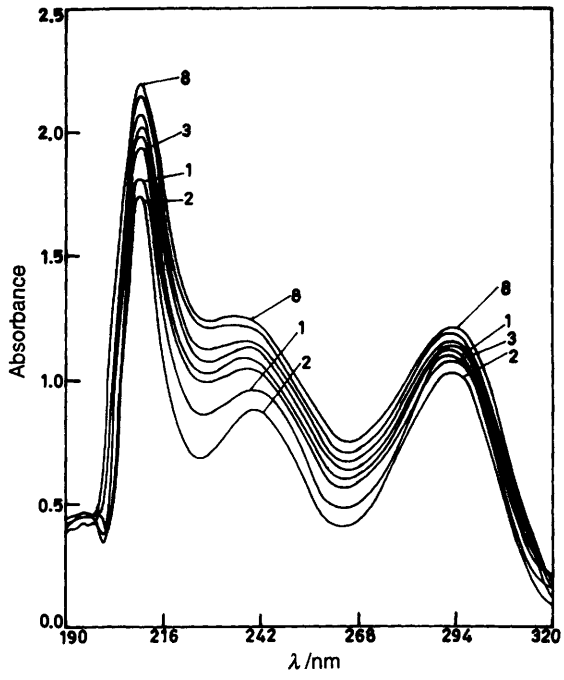
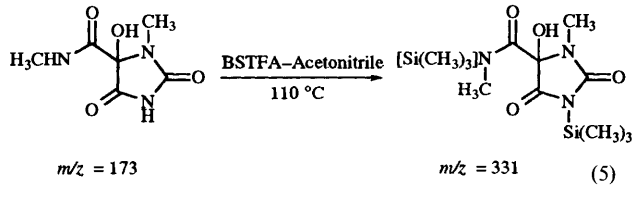


Fig. 6 Spectra observed for the reduction of the UV-absorbing intermediate generated upon electrooxidation of 0.1 mmol dm^{-3} 1,7-dimethyluric acid at PGE, pH = 6.8. Curve (1) is 1,7-dimethyluric acid before electrolysis. Curve (2) is the spectrum 30 min of electrolysis at 0.49 V. Curve (3) is the spectrum 10; (4) 20; (5) 35; (6) 50; (7) 70 and (8) 180 min after switching the potential to − 1.0 V *vs.* SCE.

that the rupture of the imidazole ring takes place at pH 3.0 and the other product should be 1-methylalloxan.

The volume 200–260 cm^3 under peak P_3 was lypophilized and the colourless material obtained had an R_f value of 0.25. The mass spectrum of the product exhibited a molecular ion peak at m/z = 174.0 (100%) and corresponds to hydrated 1-methylalloxan. Some other high mass peaks observed in the fragmentation were at 138 (1.2%), 137 (10.7%), 136 (5.5%), 129 (5.0%) and 128 (79.6%). No attempt was however made to explain the fragmentation pattern. The product was further confirmed as 1-methylalloxan by the ^{1}H NMR spectrum. The signals at δ 2.86 (s, N–H), 8.3 (3 H, s, C–H) and 8.42 (2 H, s) were observed and hence it was concluded that the electrooxidation products of 1,7-dimethyluric acid at pH 3.0 are 1-methylalloxan and *N*-methylurea.

At pH 7.0, two peaks were obtained in gel permeation chromatography. The first peak (80–130 cm^3) was due to phosphate and hence discarded. The volume under the other peak was lyophilized. The colourless dried material obtained exhibited one spot in TLC, thereby indicating the formation of only one product at pH 7.0. This product did not exhibit a sharp mp (decomp. 180 °C) and the attempts to determine its mass were unsuccessful probably due to its non-volatile nature. Therefore, the product was heated with BSTFA and acetonitrile and its trimethylsilyl derivative was prepared [reaction (5)].



(5)

The derivatised sample gave only one major peak in GC–MS at *ca.* 24 min having a molar mass of 331 and was due to 1-methyl-5-hydroxyhydantoin-5-(*N*-methylcarboxamide) which possesses three silylable sites.

It is expected that 1-methyl-5-hydroxyhydantoin-5-(*N*-meth-

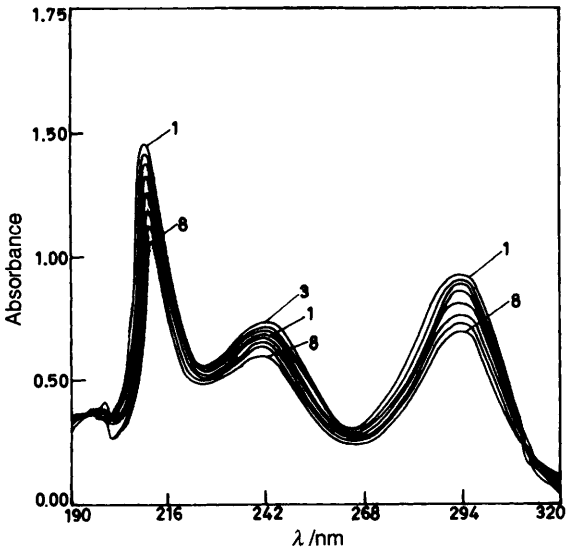


Fig. 7 Spectra observed upon enzymatic oxidation of 1,7-dimethyluric acid in phosphate buffer pH = 6.8. Curves were recorded at (1) 0; (2) 5; (3) 15; (4) 25; (6) 40; (7) 60 and (8) 120 min after adding 0.6 mmol dm^{-3} H_2O_2.

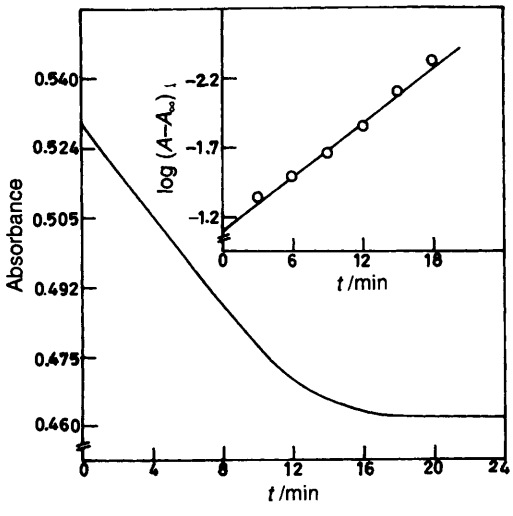


Fig. 8 Absorbance *vs.* time and log $(A - A_\infty)$ *vs.* time plots observed at 239 nm for the UV-absorbing intermediate generated during enzymatic oxidation of 0.1 mmol dm^{-3} 1,7-dimethyluric acid at pH = 6.8

ylcarboxamide) should undergo silylation at three positions, however, only two positions were silylated under the conditions employed for silylation. It appears that the –OH present between C=O and N–CH_3 does not undergo silylation owing to the steric hindrance which prevents introduction of a bulky silyl group between the two groups. Such behaviour has also been observed in the silylation of 5-hydroxyhydantoin-5-carboxamide by Goyal *et al.*[19]

Hence, it was concluded that the oxidation of 1,7-dimethyluric acid at pH 3.0 causes the rupture of the imidazole ring resulting in the formation of 1-methylalloxan and *N*-methylurea, whereas at pH 7.0 the rupture of the pyrimidine ring occurs which leads to the formation of 1-methyl-5-hydroxyhydantoin-5-(*N*-methylcarboxamide).

Enzymatic oxidation

Enzymatic oxidation of 1,7-dimethyluric acid in the presence of Type VIII peroxidase and H_2O_2 gave results which were essentially identical to those obtained during electrochemical oxidation as shown in Fig. 7. Thus, a 0.1 mmol dm^{-3} solution of 1,7-dimethyluric acid at pH 6.8 gives three peaks at 293, 240 and 210 nm. Upon initiation of the oxidation reaction by addition of H_2O_2 (0.6 mmol dm^{-3}) the UV absorption bands at 293 and

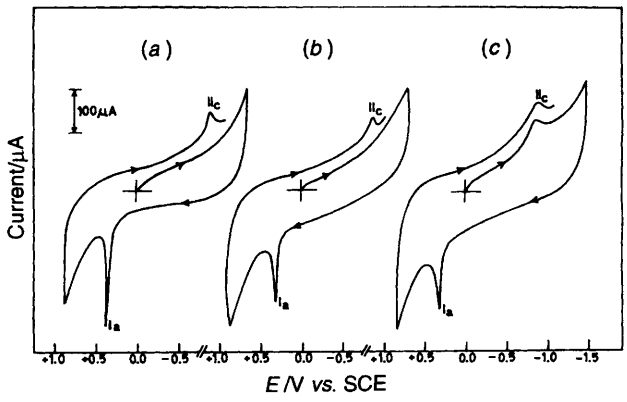


Fig. 9 Cyclic voltammograms observed during enzymatic oxidation of 1,7-dimethyluric acid at pH = 6.8 (*a*) 1,7-dimethyluric acid (0.1 mmol dm^{-3}); (*b*) after adding horse radish peroxidase (0.002 mmol dm^{-3}); (*c*) 2 min after adding H_2O_2 (0.6 mmol dm^{-3})

210 nm decreased systematically whereas the absorbance at 240 nm first increased and then decreased. If the oxidation reaction is terminated by adding catalase, the decrease in absorbance in the region 200–300 nm continues. Thus, the spectral changes during peroxidase-catalysed oxidation were essentially the same as observed during electrochemical oxidation.

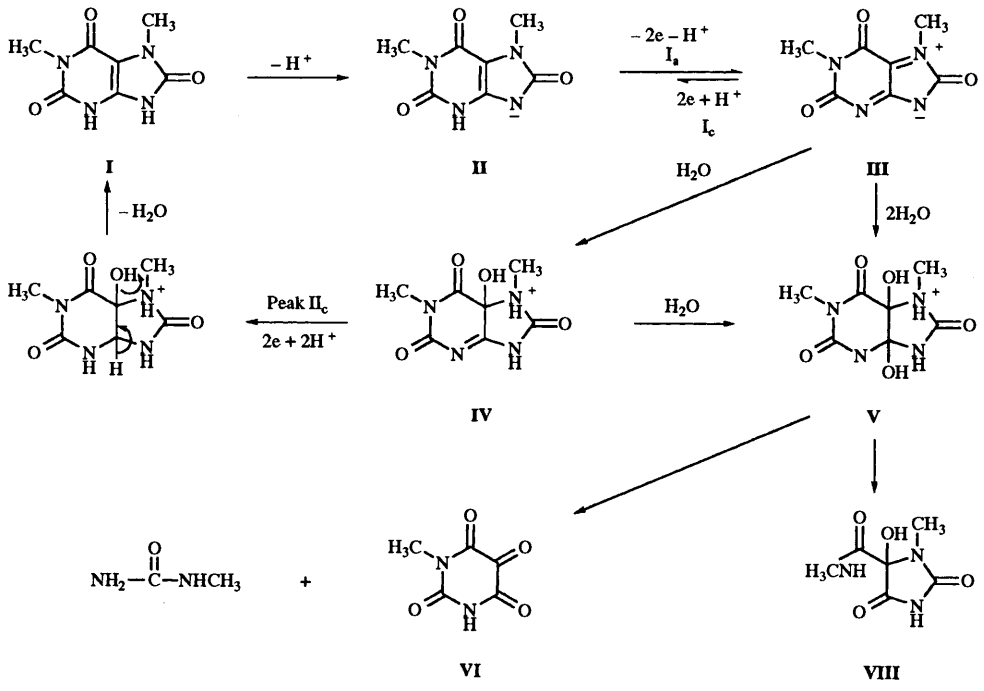
The kinetics of the decomposition of the UV-absorbing intermediate generated was studied by following the decay of absorbance at predetermined wavelengths after terminating the reaction by the addition of catalase when 50% of 1,7-dimethyluric acid has been oxidized. The change in absorbance *versus* time was exponential and the plots of log $(A - A_\infty)$ *versus* time were linear (Fig. 8). The first-order rate constants observed during enzymatic oxidation were similar to that observed in electrochemical oxidation (Table 3).

Cyclic voltammetric studies were also carried out during enzymatic oxidation of 0.2 mmol dm^{-3} solution of 1,7-dimethyluric acid at pH 6.8. Fig. 9(*a*) depicts the voltammogram of 0.2 mmol dm^{-3} 1,7-dimethyluric acid and peak IIc is not observed if the initial direction of sweep is negative. Fig. 9(*b*) is obtained after the addition of 0.002 mmol dm^{-3} peroxidase and the voltammogram is basically similar to Fig. 9(*a*). Fig. 9(*c*) is the voltammogram of the solution after 2 min initiation of oxidation by the addition of H_2O_2 (0.6 mmol dm^{-3}). It is observed that peak IIc is observed in the first sweep towards negative potentials. The peak potential of this peak was at exactly the same potential as observed for reduction peak IIc in cyclic voltammetry of 1,7-dimethyluric acid. Thus, under electrochemical and peroxidase-catalysed oxidation a similar intermediate species is generated which is reduced at peak IIc potentials.

The appearance of peak IIc in cyclic voltammograms during enzymatic oxidation at the same potential and the similarity in the UV spectral changes and identical *k* values with that observed during electrochemical oxidation suggests that the enzymatic oxidation of 1,7-dimethyluric acid follows a similar mechanism as that occurring at the surface of the solid electrodes used.

Redox mechanism

The experimental results obtained indicate that 1,7-dimethyluric acid oxidized in a $2e^-$, $1H^+$ quasi-reversible step gives an unstable diimine which in subsequent steps gives different end-products depending on pH. The dependence of E_p on pH suggests that the conjugate base is the species oxidized which predominates in the bulk of the solution. Below pK_a the species present is a cation with two positive charges whereas at pH > pK_a the species present is an anion with two positive charges. The dE_p/dpH values observed at all the three electrodes used were the same. However, the less positive values



Scheme 1 Proposed tentative electrode reactions for the electrochemical oxidation of 1,7-dimethyluric acid

of E_p at PGE may be due to the difference in the nature of the carbon surface.

Peak Ic is assigned to the quasi-reversible reduction of the diimine **III** back to 1,7-dimethyluric acid **II** (Scheme 1). The diimine formed by $2e^-$, $2H^+$ oxidation of uric acid has also been found unstable with a half-life of 10–20 ms by Owens *et al.*[20] The diimine **III** obtained from 1,7-dimethyluric acid is thus also expected to be unstable and can be attacked by a molecule of water to give an imine–alcohol intermediate **IV**. The formation of the imine–alcohol from the diimine is the chemical follow-up step and the studies of the kinetics of the decay of the UV-absorbing intermediate generated during oxidation clearly indicate that the diimine disappeared in a pseudo-first-order reaction. The imine–alcohol **IV** is also attacked by another molecule of water to give diol **V**. This diol, on decomposition in a series of reactions at pH 3.0, gives 1-methylalloxan and *N*-methylurea as products.

At pH 7.0 decomposition of the diol occurs at the pyrimidine ring and leads to the formation of 1-methyl-5-hydroxyhydantoin-5-(*N*-methylcarboxamide). The formation of alloxan at pH 3.0 and the hydroxyhydantoin derivative at pH 7.0 is not unusual. It is well reported in the literature[21–23] that in acidic medium the imidazole ring of purine undergoes rupture whereas, in neutral and alkaline medium the pyrimidine ring is unstable and opens to give different products.

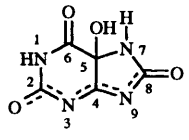
The appearance of peak IIc in enzymatic oxidation and similar rate constants for the decay of the UV-absorbing intermediate generated during electrochemical and enzymatic oxidation indicate that both oxidation reactions proceed through the same path.

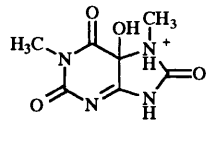
A comparison of the electrochemical behaviour of 1,7-dimethyluric acid at PGE, Pt and GCE indicate that peak IIc is not observed at Pt and GCE: this is probably caused by the low hydrogen overvoltage at these electrodes. Peak Ic was noticed only at PGE and GCE, but not at platinum. One of the possible reasons for such behaviour is non-adsorption of 1,7-dimethyluric acid at the surface of the platinum electrode. As at PGE, where adsorption apparently occurs, the potential given is of the adsorbed species.

The above results obtained from cyclic voltammetric, spectral and enzymatic studies of 1,7-dimethyluric acid at PGE, GCE and platinum electrodes were also compared with uric acid[6] and 1,3,7-trimethyluric acid.[7] Like uric acid and 1,3,7-trimethyluric acid a well defined oxidation peak Ia was obtained in the present studies, the peak potential of which was dependent on pH and shifted towards a less positive potential with increase in pH. The pK_a value obtained for 1,7-dimethyluric acid (*ca.* 5.8) was also comparable to that of uric acid (*ca.* 5.75) and 1,3,7-trimethyluric acid (*ca.* 6.0).

In the case of 1,3,7-trimethyluric acid the E_p of peak Ia was independent of pH at pH > pK_a whereas in 1,7-dimethyluric acid E_p was dependent on pH in the entire pH range studied. The electroactive species in all the cases is the conjugate base. However, *N*-methylation of uric acid causes protonation of the N atom with $pK_a > 11.0$.[24] A comparison of concentration studies on peak Ia in 1,7-dimethyluric acid and 1,3,7-trimethyluric acid indicated that the 1,3,7-derivative is strongly adsorbed at PGE as well as at GCE, whereas the dimethyl derivative adsorbed only at the surface of PGE and adsorption was not observed at GCE. Thus, the introduction of a third methyl group seems to further enhance the complications due to adsorption. However, the overall nature of the electrode reaction in all the three compounds was found as EC in which charge transfer is followed by irreversible chemical reactions.

The spectral studies during electrooxidation also provided interesting information; a UV-absorbing intermediate at longer wavelengths in neutral and alkaline media is observed during electrooxidation of uric acid,[25] whereas in the case of 1,7-dimethyl- and 1,3,7-trimethyl-uric acids the intermediate at longer wavelengths was never observed. One of the possible explanations for such behaviour is that the diimine formed in the case of uric acid is attacked by a molecule of water to give an imine–alcohol intermediate which has several possible resonating structures. This imine–alcohol has an extended conjugated π-chromophore which can extend over seven atoms O–C-2–N-3–C-4–N-9–C-8–O and is more extended than that of the anion





of uric acid, thus exhibiting UV absorption bands at longer wavelengths than uric acid. In the case of 1,7- and 1,3,7-

methylated derivatives, the presence of electron donating methyl group at N-1, N-3 and/or N-7 positions causes protonation of the ring nitrogen atom at these positions. The protonation also produces a positive charge on these N atoms and hence does not allow extended conjugation in the π-chromophore system. Thus, the imine–alcohol formed in methylated uric acids does not absorb at a longer wavelength than the parent molecule.

The present studies indicate that the presence of the methyl groups at nitrogen of the pyrimidine or imidazole ring of uric acid affects the mechanism of the reaction at the electrode. It is well known[6] that electrooxidation of uric acid gives urea and alloxan as ultimate products at pH 3 and 5-hydroxy-hydanatoin-5-carboxamide and allantoin at pH 7. In the case of methylated uric acid having a methyl group in the pyrimidine ring, allantoin was never obtained as one of the products at pH 7. This difference in behaviour can be explained on the basis of the fact that the presence of the electron-donating methyl group in the pyrimidine ring causes protonation of the nitrogen which in turn produces positive charge in the pyrimidine ring. This protonation with $pK_a > 11.0$ does not permit ring contraction to yield 1-carbohydroxy-2,4,6,8-tetraaza-3,7-dioxobicyclo[3,3,0]oct-4-ene which is a primary requirement for the formation of allantoin. On the other hand the presence of a CH_3 group in the imidazole ring does not affect the contraction of the ring and hence allantoin is obtained in such cases as has been observed in 9-methyluric acid[5] and 7-methyluric acid.[26]

Thus, it can be concluded that *N*-methylation of the pyrimidine ring affects the ring contraction of the diol resulting in products different from those obtained in the case of uric acid.

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