Electrochemical and enzymic oxidation of 9-methyluric acid at solid electrodes

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The electrochemical oxidation of 9-methyluric acid has been studied in the pH range 2.0–11.3 at pyrolytic graphite, platinum and glassy carbon electrodes. The oxidation was found to occur in a single $2e^-$, H^+ pH-dependent reaction at all the three electrodes. Adsorption complications were observed at the pyrolytic graphite and glassy carbon electrodes whereas no such behaviour was observed at platinum. The UV-spectral changes and the kinetics of the decay of the UV-absorbing intermediate generated during the reaction were found to be essentially the same at all the three electrodes. The peroxidase catalysed oxidation of 9-methyluric acid was also found to be similar to the electrochemical oxidation. The products of oxidation were the same at all the electrodes suggesting thereby that adsorptive action at the surface of PGE and GCE did not affect the EC mechanism.

Introduction

Electrochemical studies have been found useful in recent years to provide invaluable information about biologically relevant redox behaviour of purine drugs and their metabolites.[1,2]

Purines have been found to implicate the mechanism of action of nearly every major class of psychotherapeutic agent.[3] As our ultimate goal is to study the redox behaviour of purine nucleosides and nucleotides which possess a ribose or phosphorylated ribose moiety as N(9)-substituent, it was considered necessary to study relatively simple purines having substituents at the N-9 position. In the present work 9-methyluric acid is selected in which an electron donating methyl group is attached to the N-9 position. The methyl group, besides producing an electron releasing effect in uric acid, also restricts the number of resonating structures.

The electrochemical oxidation of purines has attracted considerable attention in the past.[4,5] However, most of these studies were carried out at a pyrolytic graphite electrode. It is observed in these studies that the electrode reaction involves excessive adsorption at the surface of PGE. To avoid adsorption which leads to adsorption activation,[6,7] glassy carbon and platinum electrodes were also used in the present studies and a comparison of the electrochemical behaviour of 9-methyluric acid at different solid electrodes is presented in this paper. The results of horseradish peroxidase type VIII catalysed enzymic oxidation is also presented.

Experimental

9-methyluric acid was used as received. Horseradish peroxidase type VIII ($R_z \approx 3.2$) and catalase from bovine liver were obtained from Sigma Chemical Co. and silylation grade acetonitrile and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were obtained from Pierce Chemical Co., USA. The phosphate buffers used in this investigation had an ionic strength of 0.5 mol dm^{-3} and were prepared by the method reported in the literature.[8] All potentials are referred to the SCE.

The equipment used for voltammetry, controlled potential electrolysis and coulometry has been described elsewhere.[9,10] The glassy carbon electrode was prepared by the literature method[11] and had an area of *ca.* 12 mm^2. The platinum electrode used in the studies had an area of *ca.* 3 mm^2. The surface area of the PGE used for comparison purposes had an area of *ca.* 9 mm^2.

Platinum and pyrolytic electrodes were polished on a 600 grit metallographic polishing disc after each voltammogram. This polishing procedure resulted in a significantly new surface in each run. The surface of the glassy carbon electrode was renewed each time by first washing with 95% ethanol, then with chloroform and tissue paper dipped in chloroform was touched onto it as suggested by Chan *et al.*[12] For determining peak current values an average of at least three runs were taken for each electrode.

Controlled potential electrolyses of 9-methyluric acid at a potential 100 mV more positive than peak Ia potentials were carried out in a three compartment cell. A glassy carbon rod (*l* 6, *d* 0.5 cm), platinum foil (2 × 2 cm) or pyrolytic graphite plate (6 × 1 cm^2) was used as the working electrode, cylindrical platinum gauze as the auxiliary and SCE as the reference electrode, respectively. Spectral studies during electrolysis were carried out with a Beckmann DU-6 spectrophotometer. The kinetic studies were carried out on a Shimadzu 160-A spectrophotometer. The values of n, the number of electrons involved in the electrooxidation, were determined by graphical integration of the current–time curve as reported by Lingane.[13]

Procedure

A stock solution of 9-methyluric acid (1 mmol dm^{-3}) was prepared in doubly distilled water. For recording voltammograms 4.0 cm^3 of the stock solution was mixed with 4.0 cm^3 of phosphate buffer (ionic strength = 1.0 mol dm^{-3}) of appropriate pH, so that the overall ionic strength of the prepared solution was 0.5 mol dm^{-3}. Before recording the curves nitrogen gas was passed through the solution for 12–15 min. The electro-oxidation products of 9-methyluric acid were separated by using gel-permeation chromatography. For this purpose the exhaustively electrolysed solution of 9-methyluric acid at pH 3.0 and 7.2 was lyophilized. The colourless freeze-dried material was dissolved in 2 cm^3 of water and passed through a glass column packed with Sephadex G-10 resin (Sigma, bead size 40–120 μmol dm^{-3}) using doubly distilled water as the eluent. Fractions of 5 cm^3 each were collected and their absorbance at 210 nm was determined. The first peak in gel-permeation chromatography (140–180 cm^3) was always found to contain phosphate and hence was discarded. The other peaks observed were collected separately and lyophilized to obtain the pure material. The silylation of the lyophilized material was carried out by taking a small amount of material in a 3.0 cm^3 vial. 50 mm^3 (1 mm^3 = 1 μl) each of acetonitrile and BSTFA were

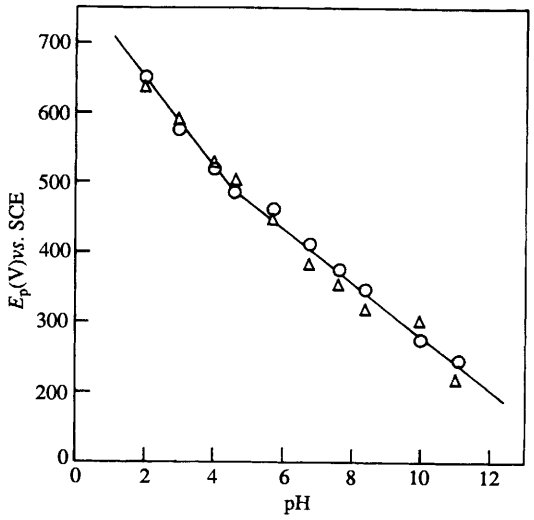


Fig. 1 Dependence of peak potential (E_p) for peak Ia on pH for 0.5 mmol dm^{-3} 9-methyluric acid at different solid electrodes: (○) PGE; (△) GCE

Table 1 Peak current values observed for peaks Ia and Ic at PGE for 9-methyluric acid at different pH values

pH	Ia[a]/μA	Ic[a]/μA	Ia/Ic
1.98	29.5	3.5	8.4
3.05	19	3.0	6.3
4.06	30	6.1	4.9
4.61	28	6.1	4.6
5.68	30	6.5	4.6
6.75	26	6.0	4.3
7.62	25	5.2	4.8
8.40	23	5.5	4.2
9.97	29	6.2	4.7

[a] Average of at least three replicate determinations.

added and the tightly closed vial was heated in an oil bath for 10–12 min. The vial was then cooled and 5 mm^3 of the sample was injected into the GC–MS.

The enzymic oxidation of 9-methyluric acid was carried out by mixing 2 cm^3 of uric acid (0.2 mmol dm^{-3}) and 0.5 cm^3 (0.002 mmol dm^{-3}) of horseradish peroxidase (M_w 20 000). The oxidation was catalysed by adding 0.5 cm^3 H_2O_2 (0.6 mmol dm^{-3}). All solutions were prepared in phosphate buffers of the desired pH, having an ionic strength of 0.5 mol dm^{-3}. The cyclic voltammograms were then recorded. For monitoring the decay of the UV absorbing intermediate generated during enzymatic oxidation, 9-methyluric acid was oxidised for 3–4 min and then the reaction was terminated by addition of 0.1 cm^3 catalase (1 mg cm^{-3}) solution. The change in absorbance at selected wavelengths were monitored at different times.

Results and discussion

Linear and cyclic sweep voltammetry

Linear sweep voltammetry of 9-methyluric acid at a sweep rate of 50 mV s^{-1}, pH 2.0–11.3, exhibited a single well defined oxidation peak (Ia) at all the three electrodes. The peak at the platinum electrode was much broader in comparison to GCE and PGE and the shape of the peak Ia was spiky at PGE. The peak potential of the oxidation peak Ia was dependent on pH and shifted towards less positive potential with increase in pH. It was interesting to observe that peak potentials at the glassy carbon electrode and at the PGE were more or less the same in the entire pH range (Fig. 1) whereas at the platinum electrode

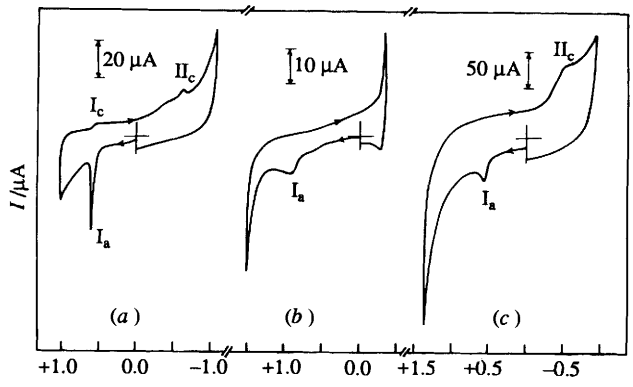
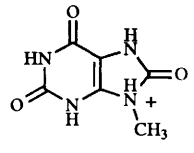
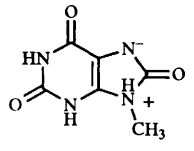


Fig. 2 Typical cyclic voltammogram of 0.5 mmol dm^{-3} 9-methyluric acid in phosphate buffer of pH = 3.6 (μ = 0.5 mol dm^{-3}) at different solid electrodes. Sweep rate = 50 mV s^{-1}. (*a*) PGE; (*b*) Pt; (*c*) GCE.

the peak potentials were at least 25–50 mV more positive than at the GCE or PGE. The E_p *vs.* pH plots at all the three electrodes exhibited a break at *ca.* pH 4.5 (Fig. 1), indicating the pK_a of 9-methyluric acid, and representing the dissociation of the amidic NH group at position 7. 9-Methyluric acid will also be protonated at N-9 due to the presence of an electron-donating CH_3 group, with pK_a > 10. Hence, the species predominating at pH < 10 will be a cation with one negative charge. 9-Methyluric acid possesses three ionizable protons at positions 1, 3 and 7. The base strength of pyrimidine has been reported as very weak and as it is a more electron attracting nucleus than benzene it seems reasonable to conclude that the anion is formed by the loss of a proton from the imidazole ring.[14]



below pH 4.6



pH 4.6–10.0

The E_p–pH relationship can be expressed by eqns. (1) and (2):

$$E_p(\text{pH } 2.0 - 4.5) = (1775 - 62.5\,\text{pH})\ \text{mV } \textit{vs.}\ \text{SCE} \quad (1)$$

$$E_p(\text{pH } 4.6 - 11.3) = (660 - 38.0\,\text{pH})\ \text{mV } \textit{vs.}\ \text{SCE} \quad (2)$$

The E_p–pH dependence clearly indicates that the conjugate base is the species oxidized over the entire pH range studied. The slope of the E_p–pH curve at pH > 4.6 ($\approx$38 mV) suggested that the number of protons involved in oxidation is one.

In cyclic voltammetry at a sweep rate of 200 mV s^{-1}, a well defined oxidation peak (Ia) is observed at all the three electrodes. However, in the reverse sweep different behaviour was noticed. At the PGE two reduction peaks (Ic and IIc) were observed in the entire pH range studied, whereas peaks Ic and IIc were observed at the GCE only at pH > 5.0. No reduction peaks were noticed at the platinum electrode in the reverse sweep. A comparison of cyclic voltammograms at all the three electrodes is presented in Fig. 2. Peak Ic formed a quasi-reversible couple with peak Ia as established by peak potential separation of anodic and cathodic peaks at PGE and GCE. The peak potential of peaks Ic and IIc were also dependent on pH and shifted towards more negative potential with the increase in pH. The ratio of peaks Ia/Ic was found to be 8.4 at pH < 3.0. The ratio then decreased, with increase in pH and became more or less constant (4.3–4.9) at pH > 4.0 (Table 1). This behaviour indicated that the species responsible for peak Ic was less

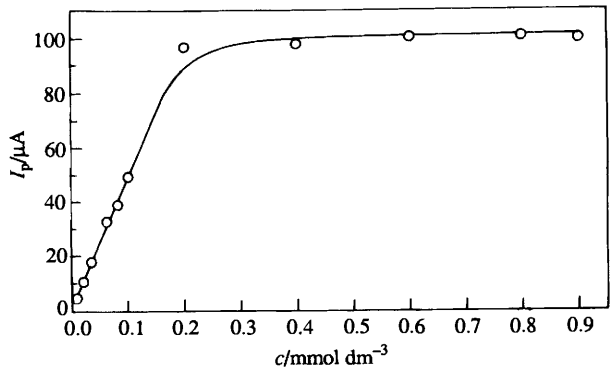


Fig. 3 Observed dependence of peak current (I_p) for peak Ia on concentration for 9-methyluric acid at PGE; pH = 7.2

Table 2 Observed dependence of peaks Ia and Ic of 0.5 mmol dm^{-3} 9-methyluric acid on sweep rate at pH 7.0 on GCE

Sweep rate/V s^{-1}	Ic[a]/μA	Ia[a]/μA	Ic/Ia
0.01	5	332	0.015
0.02	10	370	0.025
0.05	55	362	0.150
0.1	105	515	0.200
0.2	190	730	0.260
0.5	440	1240	0.350
1.0	900	2075	0.430

[a] Average of at least three replicate determinations.

available at the surface of the PGE at pH < 3.0, whereas, at higher pH it was more available. Thus, it was concluded that the species reduced in peak Ic undergoes acid catalysed chemical reaction. An identical behaviour was noticed at the glassy carbon electrode whereas peak Ic was never observed at the platinum electrode.

The peak current of peak Ia increased with an increase in concentration of 9-methyluric acid at all the three electrodes. The plot of I_p *vs.* concentration was approximately linear up to *ca.* 0.2 mmol dm^{-3} at the PGE and the GCE and then attained a more or less constant value (Fig. 3) and at platinum the I_p increased linearly in the entire concentration range used. This behaviour indicated that 9-methyluric acid adsorbed[15] at the surface of the PGE and the GCE whereas no adsorption occurred at the platinum electrode. The ratio of peak current for peaks IIc and Ic was found to be practically constant (4.0) with an increase in concentration of 9-methyluric acid at the glassy carbon as well as pyrolytic graphite electrodes.

It was interesting to observe that the peak current of peak Ic increased with an increase in sweep rate in the range 10 mV s^{-1} to 1.0 V s^{-1} at the GCE and the PGE. The ratio of peak Ic/Ia increased with increase in sweep rate and reached 0.43 at 1.0 V s^{-1}. This behaviour indicated that the species involved in the reduction of peak Ic is unstable and hence is more available at a higher sweep rate (Table 2).

The potential of peak Ia was also found to shift to a more positive potential with an increase in sweep rate in the range 5–1000 mV s^{-1} at the three electrodes. The plots of $[(\Delta E_{p/2})/\Delta \log v]$ *vs.* log v were S-shaped at all the three electrodes and the value reached 15 mV at 1 V s^{-1}. Hence the nature of the electrode reaction is established as EC at all the three electrodes in which charge transfer is followed by an irreversible chemical reaction.[16] The value of n, the number of electrons involved in the oxidation, was determined at all the three electrodes by controlled potential electrolysis. The change in peak current for peak Ia was monitored with time and the plot of I_p *vs.* t was exponential. However the log I_p *vs.* t plot was linear only for the

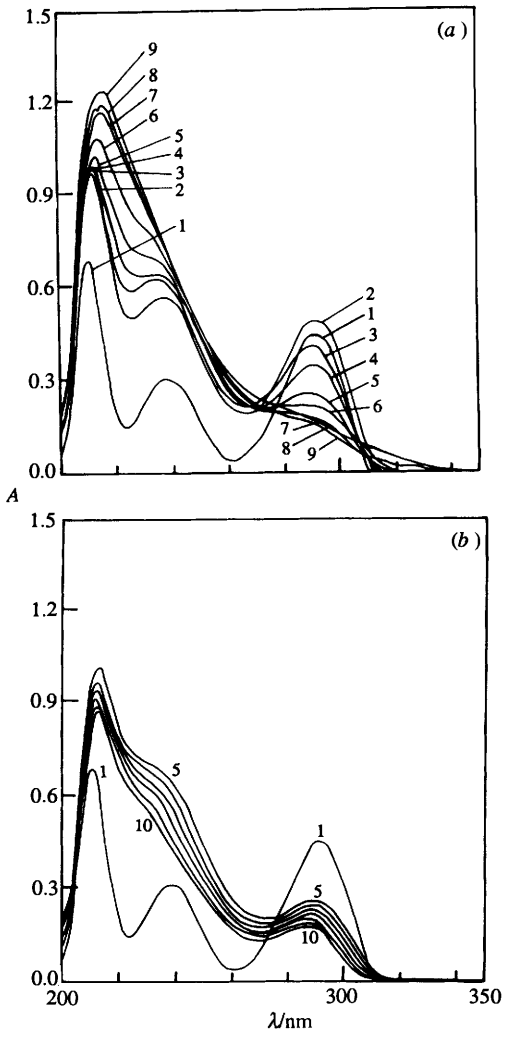


Fig. 4 (*a*) Spectral changes observed during electrooxidation of 0.1 mmol dm^{-3} 9-methyluric acid at the platinum electrode at pH = 7.0; potential 0.8 V *vs.* SCE. Curves were recorded at (1) 0; (2) 5; (3) 10; (4) 15; (5) 25; (6) 35; (7) 45; (8) 55; (9) 65 min of electrolysis. (*b*) Spectral changes observed after turning off the potential corresponding to curve 5 in Fig. 4(*a*). Curves were recorded at (5) 25; (6) 30; (7) 40; (8) 50; (9) 60; (10) 75 min.

first 15–20 min of electrolysis and thereafter a large deviation was observed. The deviation from a straight line clearly indicated that after 15–20 min a subsequent chemical reaction plays an important role, as suggested by various workers.[16,17] The n values determined at all the three electrodes were close to 2.0 ± 0.2.

Spectral studies

UV-Spectra of 0.1 mmol dm^{-3} 9-methyluric acid were recorded at different pH values to determine the pK_a. In the entire pH range 9-methyluric acid exhibited three well defined λ_{max} values at 214, 238 and 292 nm. The absorbance at 292 nm was plotted against pH and the resulting dissociation curve gave an inflection at pH 4.5 indicating the pK_a of the 9-methyluric acid as reported in the literature.[18] The E_p *vs.* pH plot also indicated a break at around pH 4.5, further confirming the pK_a of the species.

The spectral changes during electrooxidation were monitored at pH 3.0, 5.6 and 7.0 at all the three electrodes. A UV spectrum of 9-methyluric acid at pH 7.0 just before electrooxidation is presented by curve 1 in Fig. 4. Upon application of potential corresponding to peak Ia at the platinum electrode, the

Table 3 Rate constants observed for decomposition of UV-absorbing intermediate of 9-methyluric acid at different pH values on PGE

pH	λ/nm	$k^a/10^{-3}$ s^{-1}
3.0	288	4.5
	234	4.9
4.5	288	2.7
	235	2.8
7.0	292	1.8
	262	1.2
	238	1.0
8.8	293	1.6
	209	1.9

[a] Average of at least three replicate determinations.

Table 4 Observed first-order rate constants for the decay of a UV-absorbing intermediate generated during oxidation of 9-methyluric acid

		$k^a/10^{-3}$ s^{-1}			
pH	λ/nm	GCE	PGE	Pt	Enzymic
5.6	238	1.2	1.1	1.8	1.7
	262	1.1	1.5	1.5	1.0
7.0	238	1.3	1.0	1.0	1.2
	262	1.0	1.2	1.9	1.4

[a] Average of at least three replicate determinations.

absorbance at λ_{max} 292 nm first increased and then systematically decreased (curves 2 to 9). The absorbance in the region 210–270 nm systematically increased and the exhaustively electrolysed solution exhibited a λ_{max} value at 218 nm. The absence of a clear isosbestic point at 270 nm further indicated the involvement of subsequent chemical reactions. It was interesting to observe that if the potential at any stage of electrolysis was turned off, the absorbance systematically decreased in the region 200–300 nm. Fig. 4(*b*) presents the change observed after turning off the potential after recording curve 5 in Fig. 4(*a*). The decay in absorbance was monitored with time and the resulting curve was exponential in nature (Fig. 5). The value of first order rate constants determined with the help of log $(A - A_\infty)$ *vs.* *t* plots are presented in Table 3. It was interesting to observe that the value of *k* at pH 3.0 was about four times larger than observed at pH > 5.6. Also, the value of *k* at pH 4.5 was almost twice that at pH > 5.6. The higher value of *k* at pH ≤ 4.5 indicates that the species responsible for peak Ic undergoes a subsequent fast chemical reaction in acidic medium. The spectral changes and the decay of a UV-absorbing intermediate were monitored at different electrodes and at different pH values. The spectral changes at all the three electrodes were essentially similar and the rate constants at different pH values are summarized in Table 4. The values of *k* are found to be more or less the same at all the three electrodes, indicating that the electrode reaction at all the three electrodes is the same.

Product characterization

The products of electrooxidation of 9-methyluric acid were characterized at pH 3.0 and 7.0 at all the three electrodes used. The exhaustively electrolysed solution at pH 3.0 at the glassy carbon electrode was lyophilized and the freeze dried material obtained on passing through a Sephadex column (see Experimental) exhibited two peaks. Peak P_1 (140–180 cm^3) was found to contain phosphate and was discarded. The other peak (200–270 cm^3) was collected and lyophilized. The colourless dried material exhibited a single spot in TLC ($R_f \approx 0.42$) with mp 240 °C. The IR spectrum of this material exhibited strong

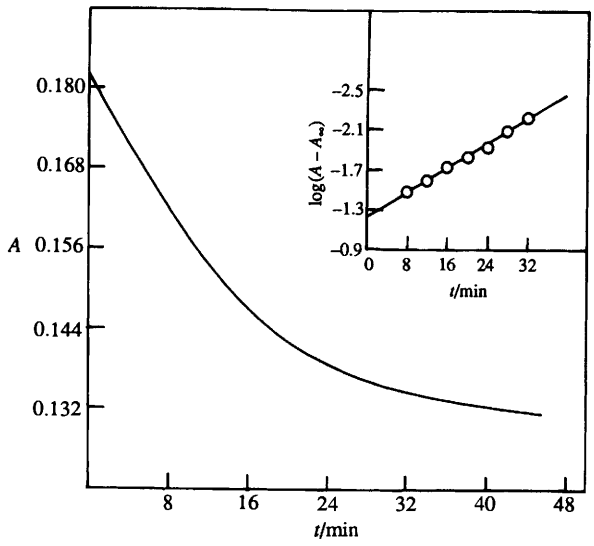


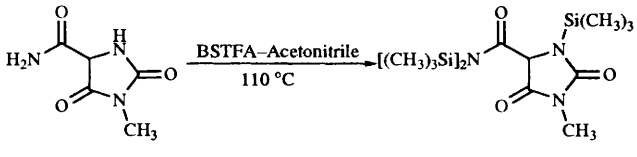
Fig. 5 *A vs. t* and log $(A - A_\infty)$ *vs. t* plots observed at 262 nm for the decay of UV absorbing intermediate generated during electrooxidation of 9-methyluric acid at the platinum electrode; pH = 7.0

bands at 1720, 1640, 1410, 1260, 1170, 1020, 808 and 780 cm^{-1} and was similar to that observed for authentic alloxan. The formation of alloxan as one of the products suggested the other product as methylurea which could not be separated as it always eluted with phosphate in gel-permeation chromatography.

The exhaustively electrolysed solution of 9-methyluric acid at pH 7.0 exhibited two spots in TLC with R_f 0.32 and 0.48. The dried material on passing through Sephadex always exhibited only one peak in gel-permeation chromatography indicating that both the compounds eluted under the same peak. To identify the compound, the volume under the peak was collected and lyophilized. The dried material obtained under the peak again exhibited two spots in TLC. At this stage it was considered necessary to use GC–MS to analyse the two compounds.

The colourless dried material obtained was then treated with BSTFA and acetonitrile at 110 °C to convert the compounds into their volatile trimethylsilyl derivatives. The derivatized sample gave two prominent GC–MS peaks at retention times of 24.8 and 26.2 min. The first peak had *m/z* = 389 with other high mass peaks at 374.2 (M − 15, 1.8%), 299 (2), 274 (1.6), 273 (2.9), 272 (8.5), 262 (2.3), 257 (1.1), 256 (4.1), 218 (1.5), 186 (4.0) and 185 (31).

The molar mass of 389 suggested the material to be 3-methyl-5-hydroxyhydantoin-5-carboxamide with three silylable sites.



m/z = 389

The second GC–MS peak at retention time 26.2 min gave a molar mass 460 (62.8%) with a significant mass peak at 445 (19.0%, M − 15). Thus, the molar mass of 460 of the silylated material suggested the product to be methylated allantoin. As allantoin has been obtained as the major oxidation product of uric acid [19] it is not unusual to get methylated allantoin from 9-methyluric acid. The other high mass peaks observed in the fragmentation pattern were at 461 (25%), 462 (12.5), 447 (2.4),

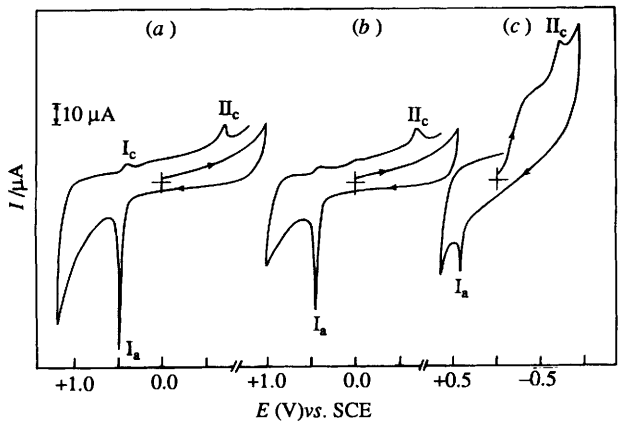


Fig. 6 Cyclic voltammograms observed during enzymic oxidation of 9-methyluric acid at pH 5.6: (*a*) 0.2 mmol dm^{-3} 9-methyluric acid; (*b*) after adding 0.002 mmol dm^{-3} horseradish peroxidase; (*c*) after adding 0.6 mmol dm^{-3} H_2O_2 into (*b*)

446 (3.6), 370 (4.2), 347 (8.9), 346 (16.9), 345 (55.3), 332 (6.0), 331 (8.5), 330 (28.0) and 316 (68.1).

Thus, methyl allantoin (*m*/*z* 172) on silylation at four positions gave a molar mass of 460.

Hence, it was concluded that oxidation of 9-methyluric acid at pH 3.0 gave alloxan and *N*-methylurea as the major products whereas methylated allantoin and 3-methyl-5-hydroxyhydantoin-5-carboxamide are formed at pH 7.0. The same products were obtained at the PGE and platinum electrodes.

Enzymatic oxidation

The enzymic oxidation of 9-methyluric acid catalysed by horseradish peroxidase was also studied at pH 5.6 and 7.0. As peak IIc observed in the cyclic voltammogram of 9-methyluric acid was found to be due to the reduction of species generated in the peak Ic reaction, it was considered worthwhile to detect the peak IIc during enzymic oxidation. Fig. 6(*a*) represents the cyclic voltammogram of 9-methyluric acid at pH 5.6 with the initial negative sweep direction. Peak IIc was not observed in the initial negative sweep. Fig. 6(*b*) represents the cyclic voltammogram obtained after adding peroxidase. Peak IIc was again observed only in the second negative going sweep. Basically, however, the cyclic voltammogram was the same as that observed without adding enzyme. After adding H_2O_2 (0.6 mmol dm^{-3}), peak IIc started appearing in the first negative sweep [Fig. 6(*c*)] at the same potential as was observed during electrochemical oxidation. The shape of the cyclic voltammogram distorted after adding H_2O_2 as an oxygen peak was also noticed at a less positive potential due to addition of H_2O_2. This behaviour clearly indicated that a species similar to electrochemical oxidation is generated during enzymic oxidation which reduced in peak IIc at identical potentials. The spectral changes during enzymic oxidation was also monitored at different pH values and compared with changes during electrochemical oxidation. Thus, at pH 5.6, a 0.1 mmol dm^{-3} solution of 9-methyluric acid exhibited three λ_{max} values at 214, 235 and 292 nm [curve 1, Fig. 7(*a*)]. This UV spectrum did not change on adding peroxidase type VIII except that the region 200–220 nm was not clearly observed due to the absorption of peroxidase. On adding H_2O_2 the absorbance in the region 220–320 nm systematically decreased [curves 2–7, Fig. 7(*a*)]. If the oxidation was terminated by adding catalase after recording curve 3, the absorbance in the region 230–290 nm still decreased. Thus, the UV-changes during and after enzymic oxidation were similar to those observed during electrochemical oxidation [Fig. 7(*b*)] at pH 5.6 and it was

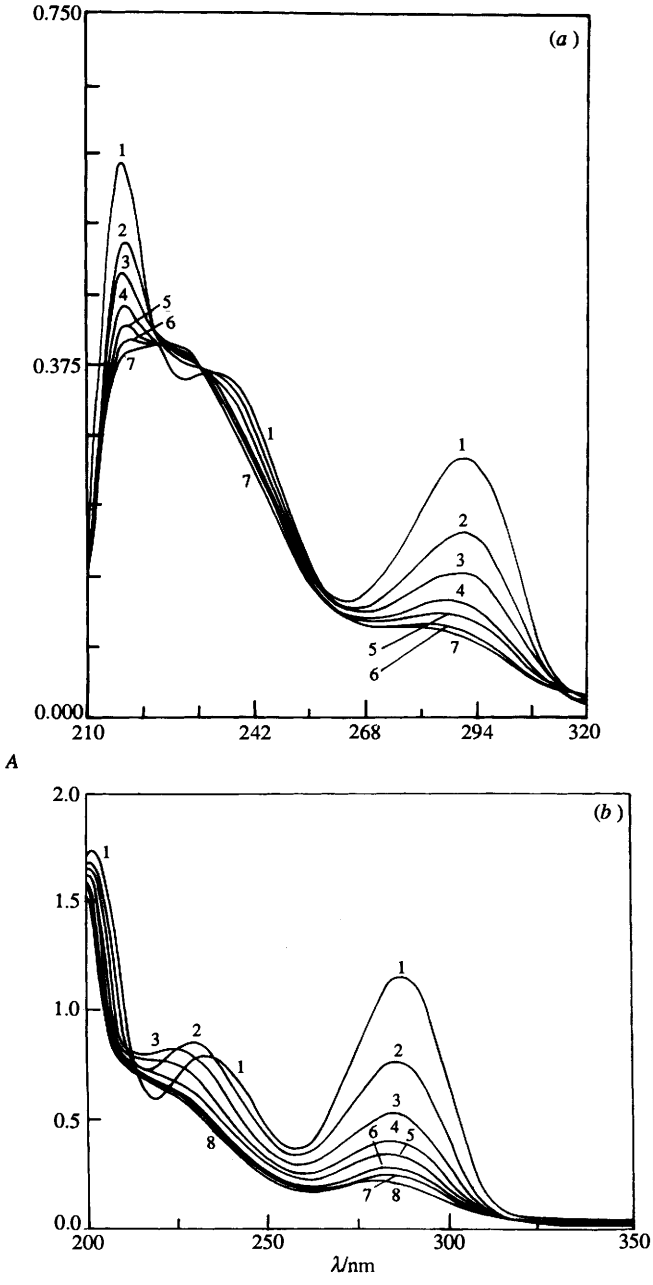
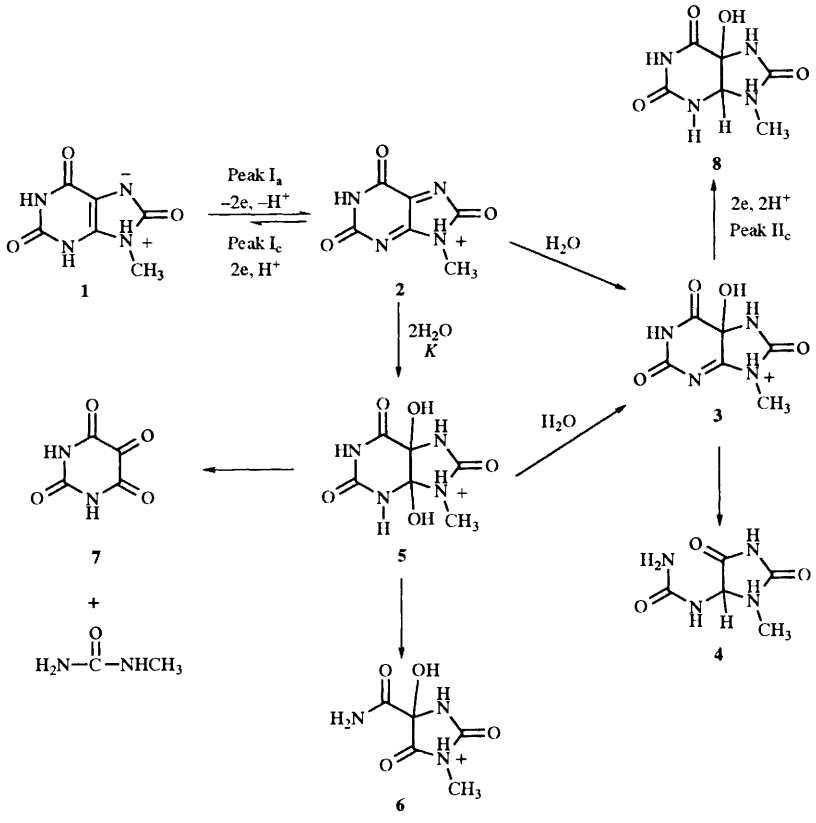


Fig. 7 Comparison of electrochemical and enzymic oxidation of 9-methyluric acid at pH 5.6. (*a*) Observed spectral changes during enzymic oxidation of 0.2 mmol dm^{-3} 9-methyluric acid at pH 5.6. Curves were recorded at an interval of (1) 0; (2) 3; (3) 3; (4) 4; (5) 5; (6) 5; (7) 5 min of electrolysis. (*b*) Observed spectral changes during electrooxidation of 0.2 mmol dm^{-3} 9-methyluric acid at PGE, pH = 5.6. Curves were recorded at an interval of (1) 0; (2) 5; (3) 5; (4) 10; (5) 10; (6) 15; (7) 15; (8) 15 min of electrolysis.

concluded that a UV-absorbing intermediate is also generated during enzymic oxidation of 9-methyluric acid.

The kinetics of decay of the UV-absorbing intermediate generated was also studied. For this purpose, the enzymic oxidation was quenched by adding catalase when the absorbance at 292 nm reached *ca.* 50%. The change in absorbance with time was monitored and the value of *k* was determined using linear log $(A - A_\infty)$ *vs.* *t* plots. The value of the rate constants observed are presented in Table 4 and were essentially similar to that observed during electrochemical oxidation.

Thus, the similar UV spectral changes, rate constants (*k*) and appearance of peak IIc in the cyclic voltammetry during



Scheme 1 Tentative mechanism proposed for the electrooxidation of 9-methyluric acid

enzymic oxidation clearly indicate that the electrochemical oxidation of 9-methyluric acid parallels the enzymic oxidation.

Redox mechanism

The experimental results presented above indicate that oxidation of 9-methyluric acid occurs in a single $2e^-$, H^+ process. The dependence of E_p on pH suggests that the anion with positive charge is the electroactive species which gives an unstable diimine (**2**). The unstable nature of the diimine was also observed in the cyclic voltammeteric studies in which Ic was observed only at higher sweep rates. The ratio of peaks Ia/Ic was also dependent on pH and the higher value in acidic medium suggested that species responsible for peak Ic was less available and hence a small peak Ic was noticed. These sweep rate studies further confirmed the unstable nature of peak Ic because the ratio of Ic/Ia increased systematically with increase in sweep rate.

Thus, when the time allowed between monitoring peak Ia and Ic was less (at higher sweep rates), the ratio of peaks Ic/Ia was higher and with decrease in sweep rate the ratio decreased. As the nature of the electrode reaction was established as EC, the subsequent chemical reaction was acid catalysed and the attack of water on diimine (**2**) occurred fast in an acidic medium. The possibility of the attack of phosphate on diimine as has been suggested in the oxidation of several purines,[20,21] was ruled out on the basis of recent studies reported on the effect of phosphate on the oxidation of uric acid[22] in which the Debye–Huckle relationship was applicable.

The attack of two molecules of water on diimine (**2**) to give diol (**5**) may occur in two steps (Scheme 1) *via* the formation of imine alcohol (**3**). The kinetics of the decay of the UV-absorbing intermediate generated during oxidation indicated that the decay of species (**2**) occurred in the first order reaction. The higher values of k at pH 3.0 and 4.6 further indicated that the decay is fast in an acid medium. The imidazole ring of diol (**5**) then readily undergoes rupture at pH 3.0 giving alloxan and methylurea as the product. The rupture of the imidazole ring of purines has also been observed during chemical oxidation of a variety of purines in an acidic medium.[14,23] At pH 7.0, the pyrimidine ring was found to break to give 1-methylallantoin (**4**) and 3-methyl-5-hydroxyhydantoin-5-carboxamide (**6**) as the major products. The unstable nature of the pyrimidine ring in neutral and alkaline media has also been reported by various workers.[14,23] The appearance of peak IIc at the PGE and GCE in the cyclic voltammograms is believed to be due to the reduction of imine alcohol species (**3**) which on $2e^-$, $2H^+$ reduction will give a dihydro species (**8**). The ratio of peaks Ic/IIc was found to be more or less constant (0.30) in the entire pH range studied and thus hydration of imine alcohol (**2**) occurs at a much faster rate than the disappearance of species (**3**). Peak IIc was never observed at the platinum electrode due to the limited negative range available for reduction at this electrode.[24]

It was interesting to observe that peak Ic was never observed at the glassy carbon electrode at pH < 5.0 even at the sweep rate of 1 V s^{-1}. This behaviour is due to the nature of the electrode. Purines have been found to adsorb strongly at the surface of PGE and hence spiky peaks are noticed.[25] The strong adsorption of 9-methyluric acid at PGE caused the appearance of peak Ic, whereas, the limited adsorption characteristic of GCE due to a mirror-like surface did not permit peak Ic to appear. Peaks Ic and IIc did not appear at platinum due to non-adsorption of 9-methyluric acid at the surface of this electrode.

The cyclic voltammetric studies of the enzymic oxidation of 9-methyluric acid clearly indicated that peak IIc appeared in the negative sweep at the same potential at which it was observed during electrochemical oxidation.

Hence, it is concluded that the imine alcohol species (**3**) is formed during the enzymic oxidation which reduced in peak IIc in the initial negative going sweep. The identical spectral

changes during electrochemical oxidation at different electrodes and enzymic oxidation clearly suggested that the electrochemical and enzymic oxidation proceed by an identical mechanism. The similar observed rate constants for the decay of UV-absorbing intermediate (**2**) for electrochemical and enzymic oxidations further supported the view that both the oxidations are essentially similar.

The results provide some additional insight into the electrochemical and enzymic oxidation of 9-methyluric acid. It is apparent that adsorption occurred at PGE and GCE which caused peak Ic and IIc to appear in the cyclic voltammograms. As the products of EC oxidation of 9-methyluric acid were the same at all the electrodes it seems reasonable to conclude that adsorptive action at the surface of PGE and GCE did not cause activation and thereby a changing mechanism of oxidation as has been observed in the case of polar aromatic compounds.[26]

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