Oxidation of 3,9-Dimethylxanthine† at Stationary Pyrolytic Graphite Electrode

Rajendra N. Goyal, Amit K. Srivastava and Vandana Bansal
Department of Chemistry, University of Roorkee, Roorkee, India

Electrochemical oxidation of 3,9-dimethylxanthine has been studied in the pH range 3.0–10.8 at stationary pyrolytic graphite electrode. A single 4e, pH dependent peak was observed. The charge transfer was followed by chemical reaction and various products were isolated. The aim of the study was to determine the effect of methyl groups on the oxidation mechanism. It was found that the electron releasing methyl groups at positions 3 and 9 completely alter the mechanism. A tentative EC mechanism for the oxidation is also suggested.

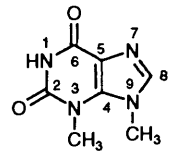
Oxidation reactions play an important role in the degradation of biologically important purines,[1] however, the pathway of such oxidation reactions is still largely unknown. Electrochemical techniques have the potential to provide useful and often uniquely invaluable insights about the redox reactions of many biologically significant molecules.[2,3] In recent years electrochemical investigations coupled with other methods, such as spectroscopy, have been used to probe the mechanisms of biological redox reactions.[4,5]

Dialkylxanthines have remarkable potency as adenosine antagonists.[6] Diethylxanthine has proved an excellent ligand for A_1-adenosine receptors in bovine forebrain membranes.[7] As adenosine antagonists cause facilitation of bronchodilation, renal vasodilation and stimulation of the central nervous system,[8,9] it was considered desirable to study the electrochemical behaviour of a dialkylxanthine, *viz.*, 3,9-dimethylxanthine, at pyrolytic graphite electrode. The presence of alkyl groups at positions 3 and 9 limits the number of possible tautomeric forms of unsubstituted xanthine. A complete interpretation of the redox behaviour of 3,9-dimethylxanthine (**1**) is presented in this paper.

Experimental

3,9-Dimethylxanthine (Research Biochemicals Inc., Natick, MA, USA) and 3,9-dimethyluric acid (Adams Chemical Co., Round Lake, IL, USA) were used as received. *N,N*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and silylation grade acetonitrile were obtained from Supelco, USA. The pH of the solutions was maintained using phosphate buffers[10] of ionic strength 0.5 mol dm^{-3}. The linear and cyclic sweep voltammetric studies were carried out using Micronics cyclic voltammeter. Pyrolytic graphite electrode (area 0.04 cm^2) was used as working electrode and platinum wire and SCE were used as auxillary and reference electrodes respectively. Controlled potential electrolysis was carried out using a home-made potentiostat, designed from the details given in the literature.[11] The spectral changes during electrooxidation were monitored using Beckman DU-6 spectrophotometer. The detailed description of the equipment used for electrochemical studies has been described elsewhere.[12] Stationary pyrolytic graphite electrode (PGE) was prepared by the method reported earlier.[13]

Voltammetric studies were carried out by mixing stock solution (5 cm^3) of the compound **1** (1 mmol dm^{-3}) with buffer (5 cm^3) of appropriate pH. The solution was deaerated by passing through it a purified stream of nitrogen gas for 8–10 min before recording the voltammograms. The coulometric experiments were carried out in a conventional three compartment



1

cell using platinum gauze as counter, SCE as reference and pyrolytic graphite plate (6 × 1 cm^2) as working electrodes. Normally 1 mmol dm^{-3} solution of the 3,9-dimethylxanthine was electrooxidised in a buffer of appropriate pH and the progress of the electrolysis was monitored by recording cyclic voltammograms at different time intervals. When the oxidation peak had completely disappeared, the electrolysis was stopped and the exhaustively electrolysed solution was removed from the cell and lyophilised. The colourless, dried material obtained was dissolved in distilled water (1–2 cm^3) and passed through a column packed with Sephadex G-10 (Sigma, bead size 40–120 μ) using doubly distilled water as eluent. Fractions (5 cm^3 each) were collected and their absorbance was monitored at 210 nm. The absorbance was then plotted against volume. The first peak (P_1) between 150–200 cm^3 obtained in the liquid chromatogram was found to contain phosphate and hence was discarded. The volume under the other two peaks (P_2 and P_3) was collected separately and lyophilised. The dried material thus obtained was analysed by various analytical techniques.

The potential measurements for change in E_p with sweep rate, which require more accurate determination to establish the nature of the electrode reaction, were carried out by decreasing the potential axis to 50 mV cm^{-1}. The deviation of E_p for such measurements was less than ± 2 mV.

The UV spectra in 0.5 mol dm^{-3} phosphate buffers of different pH were recorded using Beckman DU-6 spectrophotometer to determine the pK_a of 3,9-dimethylxanthine and uric acid. The UV–VIS spectral studies were carried out using 1.0 cm quartz cell and the UV absorbing intermediate was generated by electrolysing the solution till the absorbance at absorption maximum reduced to 50%. The decay of the intermediate generated during electrolysis was monitored by change in absorbance with time at selected wave lengths.

For silylation about 50–100 μg of the product was treated with BSTFA–acetonitrile (100 mm^3 each) in a sealed vial (Pierce Chemical Co. USA) at 110 °C for 10–12 min in an oil bath. The vial was then cooled to room temperature and 5 mm^3 of the sample was then injected into the GC-MS.

Results and Discussion

Linear sweep voltammetry of the 0.5 mmol dm^{-3} solution of compound 1 exhibited one sharp, well defined anodic peak (i)

† 3,9-Dimethylxanthine = 3,9-dimethyl-9*H*-purine-2,6(1*H*,3*H*)-dione

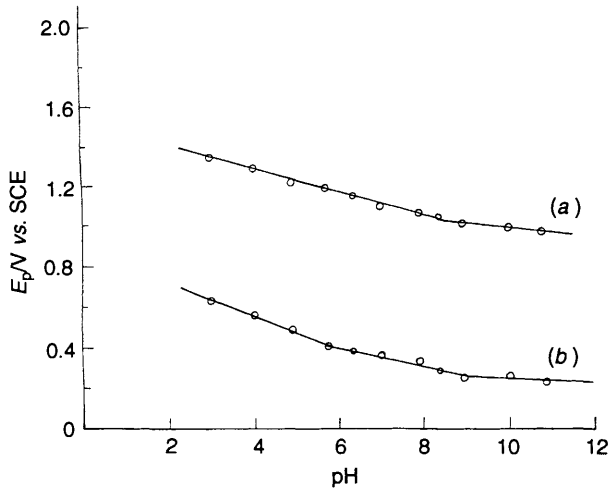


Fig. 1 Dependence of E_p on pH for the voltammetric oxidation peaks [(*a*), (i); (*b*), (ii)] of 0.5 mmol dm^{-3} 3,9-dimethylxanthine in phosphate buffers of different pH, sweep rate 10 mV s^{-1}

[Fig. 1(*a*)] in the pH-range 3.0–10.8 at a sweep rate of 10 mV s^{-1}. The peak potential of the peak (i) was dependent on pH and the change in slope of the E_p–pH plot (Fig. 1) indicates presence of an acid–base equilibrium. The dependence of E_p on pH can be represented by eqns. (1) and (2).

$$E_p(\text{pH } 2.2 - 8.5) = [1.53 - 0.062\ \text{pH}]\ \text{V } vs.\ \text{SCE} \quad (1)$$

$$E_p(\text{pH } 8.5 - 10.8) = [1.24 - 0.031\ \text{pH}]\ \text{V } vs.\ \text{SCE} \quad (2)$$

pH-Dependence of E_p at pH 8.5–10.8 indicates that electroactive species oxidised in a two-electron step is not the species which predominates in the bulk of the solution, but a species bearing one less proton. Hence the scheme is given by eqns. (3)–(5), assuming formation of uric acid as potential determining.

$$XH^{2+} \rightleftharpoons XH^{+} + H^{+} \quad (3)$$

$$XH^{+} \rightleftharpoons X + H^{+} \quad (4)$$

$$X \rightleftharpoons p + 2e \quad (5)$$

The break at pH 8.6 in E_p–pH plot corresponds to the pK_a of 3,9-dimethylxanthine, as determined by spectrophotometric methods ($pK_a \sim 8.8$) and is similar to reported for xanthine derivatives.[14] The pK_a 8.6 represents the dissociation of amidic NH group in position 1. This attribution is plausible, since the pK_a of 5,5-disubstituted 1-methylbarbituric acid is 7.8. But 3,9-dimethylxanthine can be protonated on N-3 or N-9 with $pK_a > 10$ and hence the species predominating at pH < 10 will be a cation with two positive and one negative charge.

In cyclic sweep voltammetry at a sweep rate of 100 mV s^{-1}, one well defined, pH dependent oxidation peak (i) was observed when the sweep was initiated in the positive direction. When the direction of the sweep was reversed, no reduction peak was noticed at sweep rate $\leqslant$100 mV s^{-1}. However, at higher sweep rates peak (iii) was noticed. In the subsequent sweep towards positive potentials, one more anodic peak (ii) was observed at less positive potentials. Some typical voltammograms observed are presented in Fig. 2. The peak potential of peak (ii) was also dependent on pH and shifted to less positive potential with increase in pH. The plot of E_p versus pH for peak (ii) exhibits breaks at around pH 5.4 and 9.0, corresponding to pK_a of 3,9-dimethyluric acid. The pK_a values determined spectrophotometrically were also found as 5.2 and 9.0 and were similar to many other uric acid derivatives.[14] The ratio of peak (i) : (ii) was

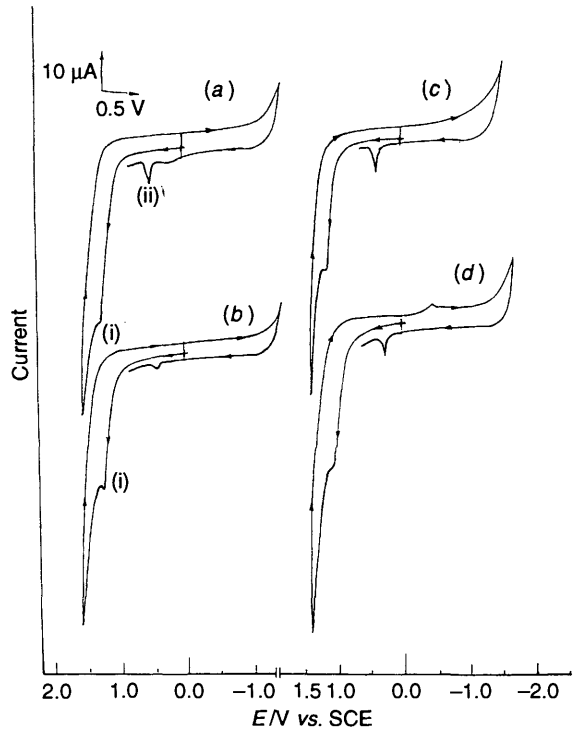


Fig. 2 Typical cyclic voltammograms of 0.5 mmol dm^{-3} 3,9-dimethylxanthine at pH (*a*) 3.2, (*b*) 4.9, (*c*) 6.9, (*d*) 8.8, sweep rate 100 mV s^{-1}

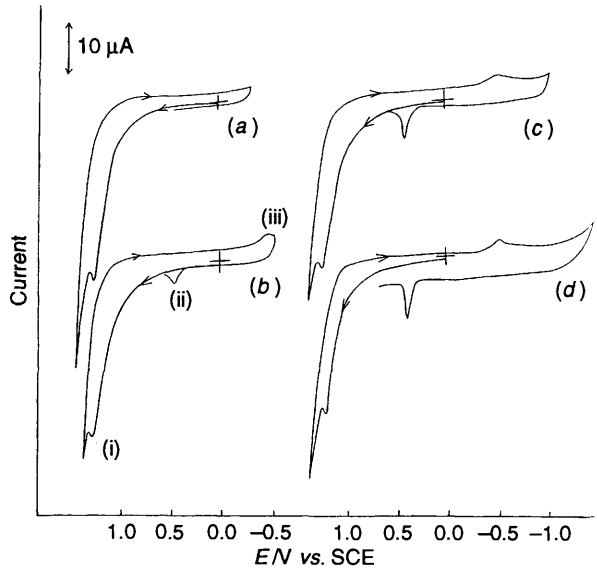


Fig. 3 Effect of potential of reversal observed on peak (ii) for 0.5 mmol dm^{-3} 3,9-dimethylxanthine at pH 7.0

independent of pH in the entire pH-range studied and was close to 4.5. The ratio of peaks (i) : (ii) clearly suggests that the species responsible for peak (ii) (3,9-dimethyluric acid) is formed not by oxidation of **1** but by reduction of the 4e product at negative potentials. This was confirmed by changing the direction of negative-heading sweep at different potentials. Fig. 3 depicts cyclic voltammograms of 3,9-dimethylxanthine at pH 7.0. It is observed that if direction of sweep is changed before peak (iii) potentials, peak (ii) is not observed [Fig. 3(*a*)]. However, peak (ii) systematically increased when the sweep is extended to more negative potentials [Figs. 3(*b*) to (*d*)]. The ratio of peaks (i) : (ii) was also found to be dependent on sweep rate, and decreased from 4.5 to 3.4 when sweep rate was changed from 50 mV s^{-1} to 500 mV s^{-1}. This behaviour clearly suggests that species

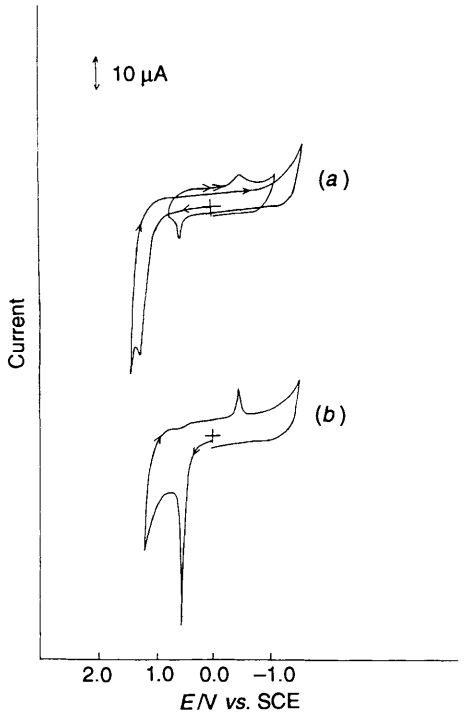


Fig. 4 Comparison of cyclic voltammograms of (*a*) 0.5 mmol dm^{-3} 3,9-dimethylxanthine and (*b*) 3,9-dimethyluric acid at pH 3.2, sweep rate 100 mV s^{-1}

responsible for peak (iii) is unstable and undergoes competitive chemical reactions. It was also interesting to observe that if the direction of the second sweep is reversed after peak (ii), a sharp cathodic peak at peak (iii) potentials was observed (Fig. 4). This behaviour further supported the formation of 3,9-dimethyluric acid from the reduction of 4e product generated at the surface of PGE. The effect of potential reversal in positive extremes was also investigated by changing the direction of sweep at different potentials after recording peak (i). The ratio of peaks (i):(ii) was found to decrease slightly with increase in potential reversal and decreased from 4.5 to 4.0.

As the electrochemical oxidation of xanthine has been found to proceed *via* uric acid,[15] it was interesting to check whether the electrooxidation of 3,9-dimethylxanthine proceeded through 3,9-dimethyluric acid. Therefore, cyclic voltammograms of 3,9-dimethyluric acid in the pH range 2.2–10.8 were recorded. A well defined anodic peak was noticed and it was observed that the $dE_p/d(pH)$ value for oxidation peak was identical to that observed for peak (ii) of 3,9-dimethylxanthine. A comparison of cyclic voltammograms of 3,9-dimethylxanthine and 3,9-dimethyluric acid at pH 3.2 is presented in Fig. 4. Hence, it was concluded that oxidation of 3,9-dimethylxanthine proceeds through the formation of 3,9-dimethyluric acid.

The peak currents (i_p) for peaks (i) [Fig. 5(*a*)] and (ii) [Fig. 5(*b*)] increased linearly up to 1.6 mmol dm^{-3} and between 1.6 and 2.2 mmol dm^{-3} became practically constant. This behaviour suggested adsorption of 3,9-dimethylxanthine at the electrode surface, which was further confirmed by the linear dependence of peak current on sweep rate (V) (Fig. 6) as suggested by Wopschall and Shain.[16] The peak current for peak (ii) was also found to increase with increasing concentration of 3,9-dimethylxanthine. The ratio of peaks (i):(ii) was more or less constant throughout a ten-fold increase in concentration of 3,9-dimethylxanthine. The peak current increased with sweep rate increasing from 5 to 500 mV s^{-1} and the ratio (i):(ii) was constant (~4.2), in the entire sweep range studied. The peak potential of peak (i) shifted to more positive potential with increase in sweep rate. The shift in E_p was 8–10 mV per ten-fold

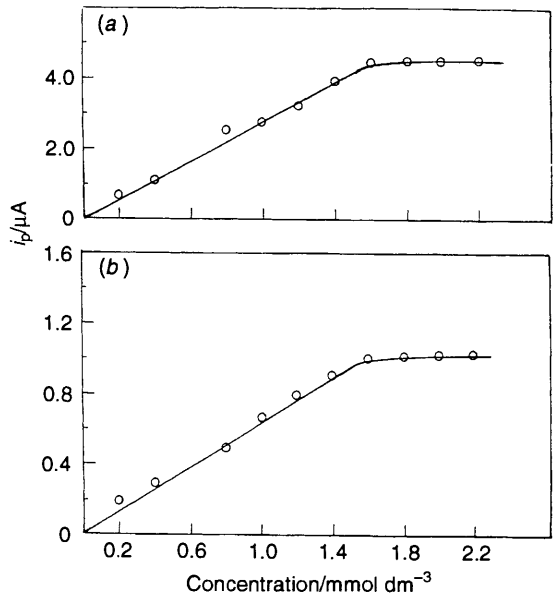


Fig. 5 Observed dependence of peak current (i_p) on concentration for the oxidation peaks [(*a*), (i); (*b*), (ii)] at pH 7.0

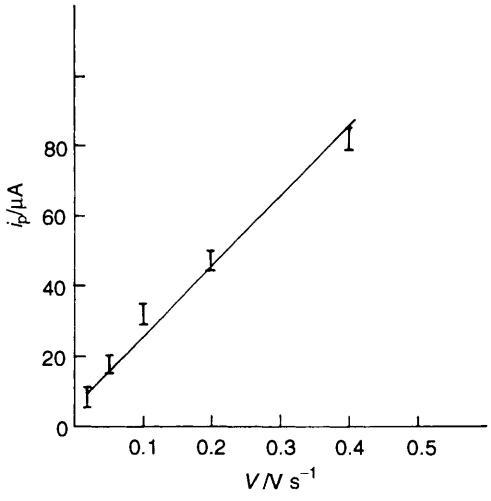
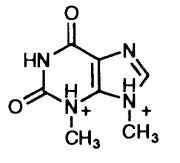


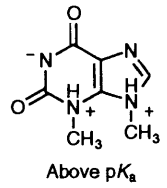
Fig. 6 Variation of peak current with sweep rate for the voltammetric oxidation peak (i) of 0.5 mmol dm^{-3} 3,9-dimethylxanthine at pH 7.0

increase in the sweep rate in the range 5 to 50 mV s^{-1} and decreased to 5 mV at higher sweep rates (50–500 mV s^{-1}). The plot of $\Delta E_{p/2}/\Delta \log V$ *vs.* $\log V$ was found to be S-shaped which suggests that the nature of electrode reaction is EC in which charge transfer is followed by irreversible chemical reaction.[17,18]

Spectral Studies.—The UV spectra of 3,9-dimethylxanthine were recorded in the entire pH-range of 3.0–10.8 to determine the pK_a value. In the entire pH-range 3,9-dimethylxanthine exhibited three well-defined bands having λ_{max} 207, 238 and 271 nm. The absorbances at λ_{max} were plotted against pH and the resulting dissociation curve gave an inflection point at around



Below pK_a



Above pK_a

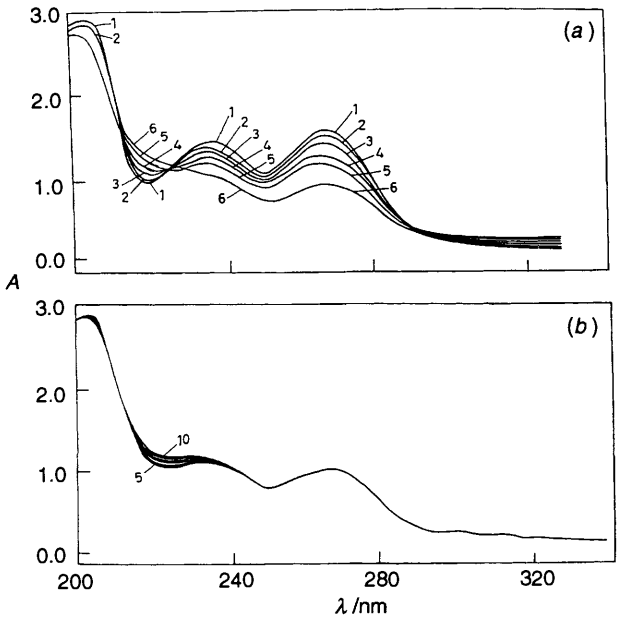


Fig. 7 (*a*) Spectral changes observed during electrooxidation of 0.1 mmol dm^{-3} 3,9-dimethylxanthine at pH 7.0, *V* 1.20 V *vs.* SCE. Spectra 1, 2, 3, 4, 5, 6 taken at *t* 0, 10, 25, 40, 55, 90 min. (*b*) Spectral changes observed on turning off the potential after recording spectrum 5 in Fig. 7(*a*). Spectra 5, 6, 7, 8, 9, 10 taken at *t* 55, 59, 63, 67, 71, 75 min.

pH 8.6, which was in good agreement with the pK_a value obtained from the E_p *versus* pH plot, and is similar to the values reported in the literature for xanthine derivatives.[14,19] Thus, it was concluded that below pK_a 3,9-dimethylxanthine exists as a cation, and at pH > pK_a as a cation with two positive and one negative charge.

The progress of electrolysis was also monitored by recording spectral changes at different time intervals. At pH 7.0 the UV spectrum of the compound **1** exhibited three bands with λ_{max} 207, 238 and 271 nm [curve 1, Fig. 7(*a*)]. Upon application of potential 100 mV more positive to that of peak (i), the absorbance at 238 and 271 nm systematically decreased. An increase in absorbance was observed in the regions 210–225 and 290–330 nm during the course of electrolysis. Curve 6 was recorded after 90 min of electrolysis and exhibited a broad maximum at around 230–240 nm. The isosbestic point at around 230 nm was observed definitely up to 25 min, deviations clearly after 55 min also indicating a slow consecutive reaction.

In a separate experiment, solution of 3,9-dimethylxanthine was electrooxidised for 40–50 min and then the applied potential was turned off. The changes observed in the UV spectrum after turning off the potential are shown in Fig. 7(*b*), and clearly indicate that absorbance in the region 212–232 nm systematically increases. Hence, it was concluded that UV-absorbing intermediate is generated during oxidation of 3,9-dimethylxanthine which absorbs in the wavelength region 212–232 nm. The kinetics of decomposition of the UV-absorbing intermediate were monitored at 220 nm. For this purpose solution of 3,9-dimethylxanthine was electrooxidised and when the absorbance at λ_{max} reached to 50% (usually 40–50 min) the potential was turned off. The change in absorbance with time was monitored at selected wavelength and an exponential decay was observed. The value of the rate constant was calculated by plotting $\log(A - A_\infty)$ *versus* time. The values of *k* obtained from the linear plot of $\log(A - A_\infty)$ *versus* time are summarised in Table 2. It is clear that the rate constant is pH-independent between pH 3 and 8. It indicates that hydrolysis is neither acid nor base catalysed. Most probable seems addition of water, followed by a faster irreversible process, *e.g.* ring opening.

Table 1 Coulometric *n* values observed for the electrooxidation of 3,9-dimethylxanthine and 3,9-dimethyluric acid at PGE

pH	*V*/V	Conc./mmol dm^{-3}	$n^{a,b}$	$n^{a,c}$
3.0	1.4	0.25	4.13	1.96
		0.50	4.06	1.90
4.2	1.4	0.50	4.10	2.08
5.0	1.3	0.50	4.08	2.10
7.0	1.2	0.50	3.89	1.96
8.0	1.2	0.25	3.90	2.14
		0.50	3.90	1.88
9.2	1.1	0.50	4.10	2.06

[a] Average of at least three replicate determinations. [b] 3,9-Dimethylxanthine. [c] 3,9-Dimethyluric acid.

Table 2 Comparison of observed first order rate constants at 224 nm for the decomposition of UV-absorbing intermediates at different pH

			$k^a/10^3$ s^{-1}	
pH	Conc./mmol dm^{-3}	*V*/V	3,9-Dimethyl-xanthine	3,9-Dimethyluric acid
3.0	0.2	1.4	2.87	2.84
	0.5	1.4	3.01	3.14
5.0	0.5	1.3	3.07	2.95
7.2	0.05	1.2	2.95	2.90
	0.1	1.2	2.87	2.79
	0.2	1.2	2.98	2.88
	0.5	1.2	2.88	2.70
	1.0	1.2	3.10	2.96
8.0	0.2	1.2	2.76	2.81

[a] Average of at least three replicate determinations.

The number of electrons *n*, involved in the electrochemical oxidation of the 3,9-dimethylxanthine were determined by the controlled potential electrolysis at large PGE (area 6 cm^2) electrodes. A solution of 0.5 mmol dm^{-3} 3,9-dimethylxanthine was electrooxidised at potential 100 mV more positive than the peak potential of peak (i). At pH 7.0 3,9-dimethylxanthine exhibited a well defined oxidation peak (i) before electrolysis. With the progress of electrolysis peak currents of peaks (i) and (ii) decreased systematically. No new oxidation or reduction peaks were observed during the course of electrolysis. Both the peaks disappeared at the end of electrolysis. The exhaustively electrolysed solution also did not exhibit any peak, even when the solution was allowed to stand at room temperature for several more hours. The plots of (i) and (ii) as a function of time were exponential, whereas the plots of $\log i_p$ as a function of time were linear for the peaks (i) and (ii) only for the first 20–30 min of oxidation. The slope of $\log \mathbf{1} = f(t)$ plots for both the oxidation peaks was more or less same (0.5) for the first 20–30 min, after which time a large deviation (smaller conversion) from the straight line was observed. The deviation clearly suggested that the oxidation follows a simple path only for the first 20–30 min of electrolysis, and after 30 min follow up chemical reactions play an important role, as suggested by Nicholson and Shain[20] and others.[21] The *n*-values determined were found close to 4.0 ± 0.3 and are summarised in Table 1.

As cyclic voltammetric studies indicate that peak (ii) is due to the formation of 3,9-dimethyluric acid, spectral changes were also monitored for 3,9-dimethyluric acid. At pH 7.0 3,9-dimethyluric acid exhibited well defined bands at λ_{max} 210, 233 and 287 nm [Fig. 8(*a*)]. Upon electrolysis a systematic decrease in absorbance at all the three λ_{max} was observed. If potential is turned off after recording curve 4, a systematic increase in the region 220–250 nm was noticed [Fig. 8(*b*)]. The first-order rate constants for the decay of UV-absorbing intermediate for 3,9-dimethyluric acid were also determined at different pH and are presented in Table 2.

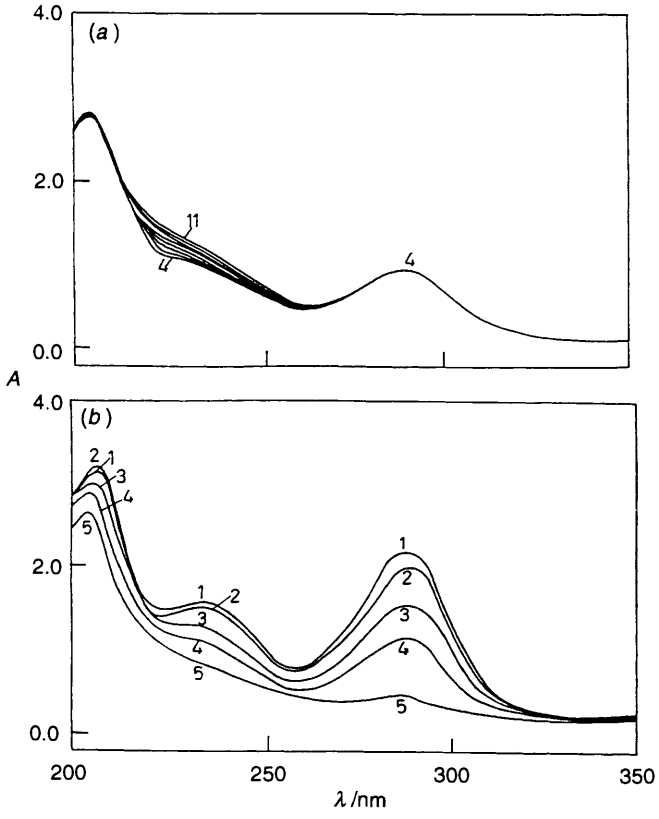


Fig. 8 (*a*) Spectral changes observed during electrooxidation of 0.1 mmol dm^{-3} 3,9-dimethyluric acid at pH 7.0, *V* 0.5 V *vs.* SCE. Spectra 1, 2, 3, 4, 5 taken at *t* 0, 10, 30, 45, 90 min. (*b*) Spectral changes observed on turning off the potential after recording curve 4 in Fig. 8(*a*). Spectra 4, 5, 6, 7, 8, 9, 10, 11 taken at *t* 45, 49, 53, 57, 61, 65, 69, 73 min.

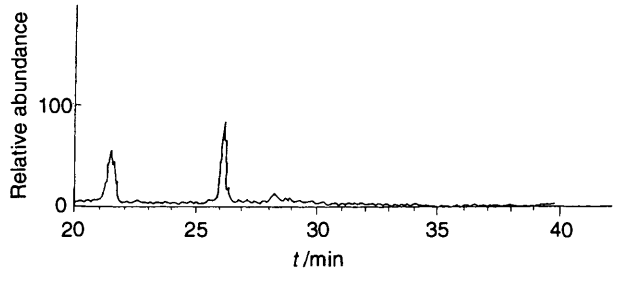
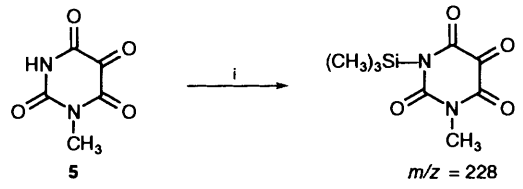


Fig. 9 Total ion current chromatogram obtained upon GC-Mass analysis of the material obtained under chromatographic peak P_3 collected at pH 5.5

The spectral changes observed at pH 3.2 for 3,9-dimethylxanthine were essentially similar to that observed at pH 7.0. The identical nature of the UV spectral changes for 3,9-dimethylxanthine and 3,9-dimethyluric acid, and practically similar values for the decomposition of the UV-absorbing intermediate, indicate that electrooxidation of 3,9-dimethylxanthine proceeds through the formation of 3,9-dimethyluric acid in the entire pH-range.

Analysis of Oxidation Products.—The products of electrooxidation followed by consecutive slow reaction of 3,9-dimethylxanthine were analysed at pH 3.0, 5.5 and 8.0. The exhaustively electrolysed solution was lyophilised and products were separated by gel permeation chromatography (see Experimental). In gel permeation chromatography at pH 5.5, three peaks P_1, P_2 and P_3 were observed. Peak P_1, obtained between 150–200 cm^3 was found to contain phosphate and hence was discarded. The volume under peak P_2 (220–260 cm^3) on lyophilisation give coloured material, which exhibited a single spot in TLC (R_f = 0.32), m.p. 150 °C. The IR spectrum of the product gave sharp bands at 3615, 3512, 2827, 2732, 2489, 2386, 2315, 1740, 1450, 1120 and 805 cm^{-1} indicating the product to be alloxane derivative. The mass spectrum of the material at an electron beam voltage of 70 eV gave a clear molecular ion peak at *m/z* = 174 and suggested the product as 1-methylalloxane monohydrate. The high mass peaks observed in the mass spectrum were at 137 (12.1%), 136 (6.2), 129 (6.3), 128 (89.2), 109 (21) and 100 (51.6). However, no attempt was made to explain the fragmentation pattern.

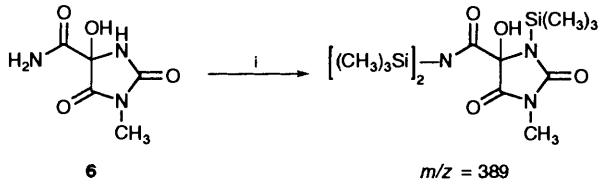
Peak P_3 was never well separated from peak P_2 (260–290 cm^3). The freeze dried material under peak P_3 always gave two spots in TLC (R_f = 0.32 and 0.6). Therefore, it was considered necessary to use gas chromatography coupled with mass spectrometry to analyse the second product. The silylated material exhibited two major peaks in GC-Mass (Fig. 9). The first peak at R_t ~ 2 min had a molecular ion peak at *m/z* 228 (76.4%) and corresponds to 1-methylalloxane **5** having one trimethylsilyl group (Scheme 1).



5

Scheme 1 *Reagents and conditions:* i, BSTFA, acetonitrile, 110 °C

The second peak at R_t ~ 26.6 min exhibited a small peak at *m/z* = 389 (6.4%) and a large peak at *m/z* = 374 (28.5%) indicating the loss of a methyl group. Hence, the molar mass of the silylated species was 389 and corresponds to 5-hydroxy-3-methylhydantoin-5-carboxamide **6** (Scheme 2). Some other high mass peaks observed in the fragmentation pattern were at 389 (6.4%), 375 (1.8), 374 (28.5), 299 (2.0), 273 (2.9), 272 (8.5), 262 (2.3), 257 (1.1), 256 (4.1), 186 (4.0), 185 (31.0) and 184 (5.7). The base peak was at 73.1 (100%).



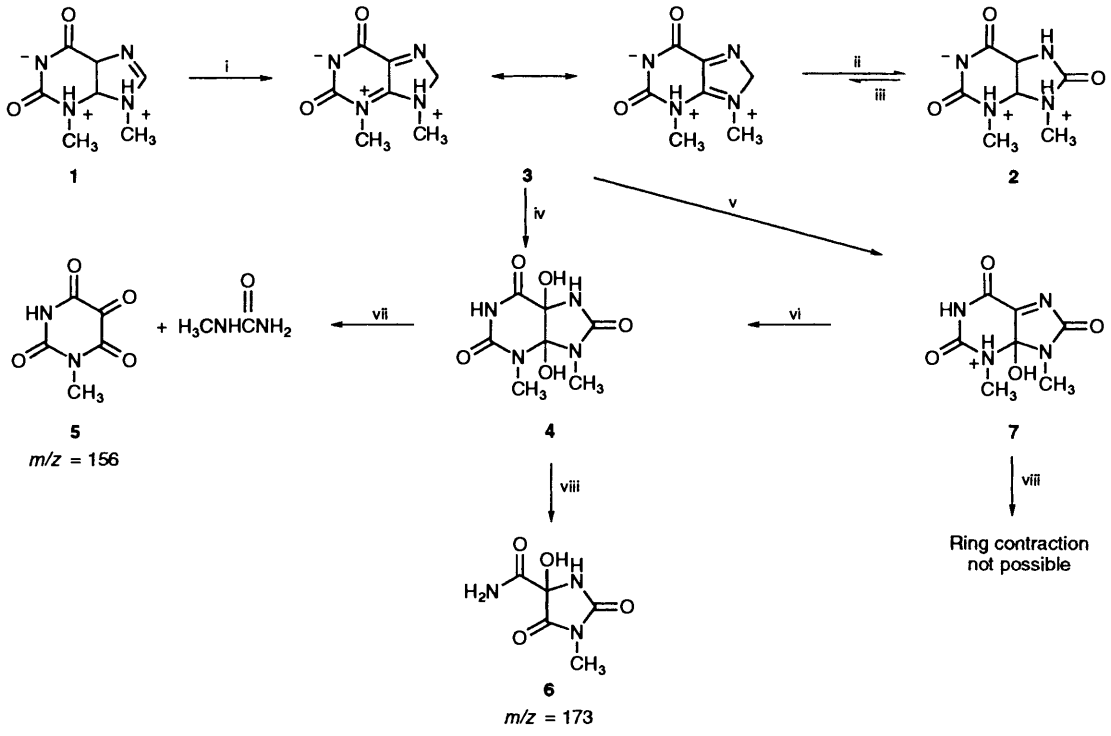
6

Scheme 2 *Reagents and conditions:* i, BSTFA, acetonitrile, 110 °C

As species **6** possesses four sites for silylation, it was expected that reaction should take place at all the four available sites. However, the OH group situated between C=O and NH does not undergo silylation due to bulky nature of trimethylsilyl group. A similar observation has also been reported by other workers during oxidation of uric acid.[22,23]

The formation of 1-methylalloxane and 5-hydroxy-3-methylhydantoin-5-carboxamide suggests that monomethyl urea and methylamine should also be formed as the products, but these compounds were not observed. One of the possible reasons is that owing to their low molecular weights, these compounds elute together with phosphate. This assumption was confirmed by passing monomethyl urea and methylamine through the Sephadex G-10 column. It was observed that both the compounds eluted between 150 to 200 cm^3 and would therefore be eluted with phosphate.

The gel permeation chromatogram at pH 3.0 exhibited only peaks P_1 and P_2 whereas at pH 8.0 only peaks P_1 and P_3 were observed. Hence, it was concluded that the electrooxidation of 3,9-dimethylxanthine gives 1-methylalloxane at pH 3.0 and 5-hydroxy-3-methylhydantoin-5-carboxamide at pH 8.0 as the



Scheme 3 Tentative mechanism proposed for the electrooxidation of 3,9-dimethylxanthine

products. At pH >3.0 and <8.0, both the products were obtained.

The products of electrooxidation of 3,9-dimethyluric acid were also analysed at pH 5.0 and 1-methylalloxane and 5-hydroxy-3-methylhydantoin-5-carboxamide were obtained as the products. Thus, it is concluded that the products of oxidation of 3,9-dimethyluric acid and 3,9-dimethylxanthine are same.

Oxidation Mechanism.—Linear and cyclic sweep voltammetry, coulometry and product identification of 3,9-dimethylxanthine suggested that peak (i) is a 4e reaction. The product of the 4e process formed was found to be unstable due to competitive chemical reactions hence its reduction at peak (iii) potentials was observed only at sweep rates >100 mV s^{-1}. However, the appearance of peak (ii) in the second positive sweep in cyclic voltammetry indicated that the reduction of the product (of a 4e process), formed at negative potentials, gave 3,9-dimethyluric acid, which readily oxidised at less positive potentials. The formation of 3,9-dimethyluric acid was also found to be dependent on the extent of negative sweep as shown in Fig. 3. The 4e oxidation of compound **1** can also occur in two, 2e steps. Thus, compound **1** on 2e, $2H^+$ oxidation gives 3,9-dimethyluric acid **2** which is more easily oxidisable than **1** and gives diimine species **3** at less positive potential. The formation of 3,9-dimethyluric acid has also been confirmed by the similar dE_p/dpH values for the oxidation peak (ii). The UV-spectral changes and the practical identical rates of the decay of the UV-absorbing intermediates also confirmed the formation of 3,9-dimethyluric acid. As the diimine species **3** is doubly positively charged, it is readily attacked by water to give diol **4** which on undergoing series of reactions give 1-methylalloxane **5** and 5-hydroxy-3-methylhydantoin-5-carboxamide **6** as the products (Scheme 3).

The oxidation behaviour of 3,9-dimethylxanthine was found very different than that of xanthine, where alloxane was found as the major product in acidic medium and allantoin was obtained in neutral and alkaline media.[15,24] However, in the present studies methylated allantoin was not obtained, rather 5-hydroxy-3-methylhydantoin-5-carboxamide was obtained as the major product. The difference in behaviour may be due to the availability of a different electroactive tautomeric form in 3,9-dimethylxanthine. The introduction of methyl groups in position 3 and 9 limits the number of possible tautomeric forms in addition to steric effects of methyl group.

The formation of allantoin during oxidation of xanthine occurs from the imine alcohol which on ring contraction gives 1-carboxy-3,7-dioxo-2,4,6,8-tetraazabicyclo[3.3.0]octadec-4-ene. A series of follow up reactions then gives allantoin.[23,25] Attack of one molecule of water on the positively charged diimine **3** would take place on $C{=}N^+$ bond yielding an imine alcohol **7**. As ring contraction and formation of carbohydroxy derivative is not possible from species **7**, methylated allantoin was not obtained as the product of electrooxidation. Thus, the rupture of pyrimidine ring occurs at position 4 giving 5-hydroxy-3-methylhydantoin-5-carboxamide as the major product of oxidation.

It is therefore believed that the products of oxidation of 3,9-dimethylxanthine are different than that of xanthine and uric acid. The presence of methyl groups at positions 3 and 9 does not permit the ring contraction previously observed in xanthine and hence 5-hydroxy-3-methylhydantoin-5-carboxamide is obtained as the product in place of allantoin. As presence of methyl groups also restricts the number of possible tautomeric forms of xanthine, it is also likely that different electroactive forms are available in the oxidation of xanthine and 3,9-dimethylxanthine.

Acknowledgements

Financial assistance for this work was provided by the Department of Science and Technology, New Delhi through grant No. SP/S1/G21/91.

References

1 J. Davidson, *The Biochemistry of Nucleic Acid*, Academic Press, New York, 1976.
2 K. J. Volk, R. A. Yost and A. B. Toth, *Anal. Chem.*, 1992, **64**, 21A.

3 K. Rajeshwar, R. O. Lezna and N. R. Tecconi, *Anal. Chem.*, 1992, **64**, 429A.
4 A. Anne and J. Moiroux, *J. Org. Chem.*, 1988, **53**, 2816.
5 F. Zhang, R. N. Goyal, C. L. Blank and G. Dryhurst, *J. Med. Chem.*, 1992, **35**, 82.
6 K. A. Jacobson, L. Kiriasis, S. Barone, B. J. Bradbury, U. Kammula, J. M. Compagne, S. Secunda, J. W. Daly, J. L. Neumeyer and W. Pfleiderer, *J. Med. Chem.*, 1989, **32**, 1873.
7 C. Londos and J. Wolff, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 5482.
8 E. M. Vander Wenden, A. P. Ijzerman and W. Soudiju, *J. Med. Chem.*, 1992, **35**, 629.
9 M. G. Collis, G. S. Baxter and J. R. Keddie, *J. Pharm. Pharmacol.*, 1986, **38**, 850.
10 G. D. Christian and W. C. Purdy, *J. Electroanal. Chem.*, 1962, **3**, 363.
11 J. L. Owens, H. A. Marsh and G. Dryhurst, *J. Electroanal. Chem.*, 1978, **91**, 231.
12 R. N. Goyal, A. Kumar and A. Mittal, *J. Chem. Soc., Perkin Trans. 2*, 1991, 1369.
13 R. N. Goyal, S. K. Srivastava and R. Agrawal, *Bull. Soc. Chim. Fr.*, 1985, 606.
14 F. Bergman and S. Dikstein, *J. Am. Chem. Soc.*, 1955, **77**, 691.
15 R. N. Goyal, *Indian J. Chem., Sect. A*, 1989, **28**, 467.
16 R. H. Wopschall and I. Shain, *Anal. Chem.*, 1967, **39**, 1514.
17 G. Cauquis and V. D. Parker, *Organic Electrochemistry*, ed. M. M. Baizer, Marcel Dekker, New York, 1973, 134.
18 P. H. Reiger, *Electrochemistry*, Prentice Hall, New Jersey, 1987, 343.
19 *IUPAC Dissociation Constants of Organic Acids and Bases*, Butterworths, London, 1961, 535.
20 R. S. Nicholson and I. Shain, *Anal. Chem.*, 1964, **36**, 706.
21 E. C. Brown and R. F. Large, in *Techniques of Chemistry*, ed. A. Weissberger and R. W. Rossiter, Wiley, New York, 1974, 423.
22 R. N. Goyal and M. S. Verma, *J. Chem. Soc., Perkin Trans. 2*, 1993, 1241.
23 R. N. Goyal, A. Brajter-Toth and G. Dryhurst, *J. Electroanal. Chem.*, 1982, **131**, 181.
24 G. Dryhurst, *J. Electrochem. Soc.*, 1972, **119**, 1659.
25 G. P. A. Bongaerts and G. D. Vogels, *Biochim. Biophys. Acta*, 1979, **567**, 295.

Paper 3/06260K
Received 19*th October* 1993
Accepted 26*th January* 1994