Electrochemical oxidation of xanthine at solid electrodes

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The electrochemical oxidation of xanthine at glassy carbon electrode (GCE) and pyrolytic graphite electrode (PGE) has been studied in the pH range of 1.8-10.7 and found to proceed in a 4e, 4H+ reaction via the formation of uric acid at both the electrodes. Cyclic voltammetric behaviour, spectral studies, observed first order rate constant and product identification clearly point out that xanthine initially oxidises in a 2e, 2H+ reaction to give uric acid which on subsequent oxidation gives the final products. The first order decay of UV absorbing intermediate has been monitored and the products have been identified. A plausible mechanism is suggested on the basis of observed experimental behaviour.

Electrochemical oxidation of naturally occurring purines is likely to provide useful insight into the generally complex mechanism of enzymatic and perhaps in vivo redox reactions. Electrochemical and enzymatic oxidation studies of uric acid by Dryhurst and coworkers1-4 clearly illustrate that both the oxidations proceed by identical pathways. In view of such an importance of electrochemical investigations of biologically important purines, it was considered of interest to study the electrooxidation of xanthine at solid electrodes.

Dryhurst5 studied the electrooxidation of xanthine (I) in 1 M acetic acid and reported that electrode process was found to involve adsorption. The product identification was based on rather indirect methods6. Anodic voltammetry of I at a glassy carbon electrode was carried out by Yao et al.7 and a method for the determination of I was suggested. However, no attempt has been made to study the mechanism of electrochemical oxidation of xanthine at solid electrodes. Presently we have studied the electrochemical oxidation of xanthine (I) at glassy carbon electrode (GCE) and pyrolytic graphite electrode (PGE) employing cyclic voltammetry, coulometry and controlled potential electrolysis.

Materials and Methods
Xanthine (I) and uric acid (Sigma Chemical Co., USA) were used as received. Experiments were carried out in phosphate buffers8 of ionic strength 0.5 M.

Equipment used for linear and cyclic sweep voltammetry, coulometry and controlled potential electrolysis were essentially similar to those described earlier9,10. Thin layer chromatography of the electrolysed solutions were carried out using plates coated with silica gel-G and benzene-methanol (4:1) as irrigant. The pyrolytic graphite electrode (area 2.2 mm2) used were prepared by the method reported earlier11. The glassy carbon electrode (PAR, USA) had an area 2.8 mm2. The pre-treatment of glassy carbon electrode before recording the voltammograms was carried out as suggested by Chan et al.12. The IR spectra of the products were recorded on a Beckmann IR-20 spectrophotometer. All potential measurements were made at 25°C against SCE as the reference electrode.

Coulometric studies were carried out in an H-type cell using pyrolytic graphite plate (6 cm x 1 cm) or glassy carbon rod (3 cm x 2.2 mm2) as working electrode, platinum gauze cylinder as counter electrode and SCE as reference electrode. About 4 to 6 mg of the compound were oxidised and the progress of electrooxidation was monitored by recording UV spectra or cyclic voltammograms at different time intervals. The electrolysis was stopped when the oxidation peak completely disappeared. The exhaustively electrolysed solution was removed from the cell, lyopholised and analysed by either TLC or purified by column chromatography on Sephadex G-10 column (2.0 cm x 80 cm) using distilled water as the eluant at a flow rate of 10-12 ml/hr. The fractions (5 ml each) were collected using SICO fraction collector and their absorbances at 210 nm were monitored using Beckmann DU-6
spectrophotometer. The products of electrooxidation were also identified by exhaustively electrolyzing the compound at the desired potential. The resulting solution was lyophilised and the concentrated slurry thus obtained was extracted with ether (2 x 10 ml), the ether extract concentrated and the IR spectrum of the residue recorded.

Solutions of xanthine (0.5 mM) were prepared in appropriate buffers of ionic strength 0.5 M. Purified nitrogen gas was bubbled for 5 to 10 min before recording the voltammograms. UV spectra were recorded using 1.0 cm quartz cells. The UV-absorbing intermediate was generated by electrolyzing the solution for appropriate time and its decay was monitored by recording absorbance at selected wavelengths at different time intervals.

Results and Discussion

Linear sweep voltammetry of xanthine in the pH range of 1.8-10.7 exhibited a single, well-defined oxidation peak (IIa) at a sweep rate of 10 mVs^-1 at both glassy carbon (GCE) and pyrolytic graphite (PGE) electrodes. The peak potential of (IIa) was dependent on pH and shifted towards more negative potential with increase in pH. The dependence of Ep of IIa on pH at GCE and PGE can be described by the relations (1) and (2), respectively.

\[
E_p (pH \text{ 1.8 to 10.7}) = [1.05 - 0.055 \ pH] V \\
E_p (pH \text{ 1.8 to 10.7}) = [1.00 - 0.050 \ pH] V
\]

The peak current values for IIa were found to be practically constant in the entire pH range studied and thus it was concluded that a similar electrode reaction occurs at both the electrodes in the pH range 1.8-10.7. The peak current values for IIa increased with increase in the concentration of xanthine at both the electrodes. The plot of ip versus [xanthine] was linear in the concentration range of 0.01-0.5 mM. The effect of [xanthine] > 0.5 mM could not be studied due to its limited solubility in aqueous buffers. Thus xanthine can be safely estimated in this concentration range using any of the two electrodes. The peak current function (ip/V1/2) was independent of sweep rate. It was thus confirmed that xanthine does not adsorb at GCE or PGE surface at concentration < 0.5 mM.

The cyclic sweep voltammetry of xanthine at GCE at a sweep rate of 150 mVs^-1 exhibited a well-defined oxidation peak IIa, when sweep was initiated in the positive direction. In the reverse sweep three cathodic peaks (IIc, Ic, and IIIc) were observed and in the subsequent sweep towards positive potentials one more anodic peak (Ia) was observed (Fig. 1). Peaks Ic and IIc were observed only at sweep rate > 150 mVs^-1. The ΔEp values for the couples Ic/Ic and IIc/Ic were almost similar (about 50 mV) in the entire pH range indicating the formation of quasi-reversible couple. To check whether peak IIIc is related to peak IIa or is due to independent reduction of xanthine, cyclic voltammograms were also re-
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Fig. 2—Spectral changes observed for 0.05 mM xanthine (A) and uric acid (B) at pH 6.84. [Curves were recorded for (A) at 0(1), 5(2), 7(3), 12(4), 20(5) and 30(6) min of electrolysis. For B at 0(1), 2(2), 5(3), 10(4), 15(5) and 20(6) min of electrolysis.]

Table 1—Observed coulometric n-values for electrooxidation of xanthine and uric acid

<table>
<thead>
<tr>
<th>pH</th>
<th>Electrode</th>
<th>Potential (V vs SCE)</th>
<th>n-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Xanthine</td>
</tr>
<tr>
<td>2.2</td>
<td>PGE</td>
<td>1.1</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>GCE</td>
<td>1.1</td>
<td>4.10</td>
</tr>
<tr>
<td>4.5</td>
<td>GCE</td>
<td>1.0</td>
<td>3.89</td>
</tr>
<tr>
<td>6.8</td>
<td>PGE</td>
<td>0.8</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td>GCE</td>
<td>0.8</td>
<td>3.93</td>
</tr>
<tr>
<td>8.7</td>
<td>GCE</td>
<td>0.8</td>
<td>4.04</td>
</tr>
<tr>
<td>10.0</td>
<td>PGE</td>
<td>0.5</td>
<td>3.89</td>
</tr>
<tr>
<td></td>
<td>GCE</td>
<td>0.5</td>
<td>3.99</td>
</tr>
<tr>
<td>10.7</td>
<td>GCE</td>
<td>0.5</td>
<td>3.88</td>
</tr>
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</table>

For a quasi-reversible couple the ratio of anodic/cathodic should not be very far from unity. However, in the present studies the ratio increased with increase in sweep rate and was found to be 0.2 at a sweep rate of 500 mVs⁻¹. This difference in behaviour is due to the intermediate species gets less time to undergo chemical reaction and thus the ratio increases. The peak potentials of peak I, II and III were also dependent on pH and shifted towards more negative potential with increase in pH. The plots of Eₚ versus pH were linear with slopes of 48, 52 and 56 mV/pH for peaks I, II and III, respectively.

Voltammetric peaks I, II and III were also exhibited by 0.5 mM uric acid in the entire pH range (Fig. 1C). Peak I was pH dependent and exhibited the relation Eₚ (1.8-10.7)= [0.68-0.060 pH] V at a sweep rate of 10 mVs⁻¹. The identical dEₚ/dpH values for peaks I, II and III, of uric acid and of xanthine clearly pointed out that electrochemical oxidation of xanthine proceeded via formation of uric acid. As oxidation of uric acid occurred at less positive potential it readily underwent further oxidation to give the final products of the electrode reaction.

At PGE 0.5 mM xanthine exhibited slightly different behaviour in cyclic voltammetry and reduction peaks I and II were not observed in the entire pH range, probably because of fast consecutive reac-
Table 2—Observed first order rate constants for the decay of UV absorbing intermediate of 0.05 mM xanthine and 0.05 mM uric acid

<table>
<thead>
<tr>
<th>pH</th>
<th>(\lambda) nm</th>
<th>(k) (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xanthine</td>
<td>Uric acid</td>
</tr>
<tr>
<td>2.2</td>
<td>225</td>
<td>0.0009</td>
</tr>
<tr>
<td>2.8</td>
<td>225</td>
<td>0.0018</td>
</tr>
<tr>
<td>4.5</td>
<td>225</td>
<td>0.0018</td>
</tr>
<tr>
<td>6.8</td>
<td>225</td>
<td>0.0033</td>
</tr>
<tr>
<td>8.7</td>
<td>225</td>
<td>0.0017</td>
</tr>
<tr>
<td>320</td>
<td>0.0030</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

A comparison of cyclic voltammograms of xanthine at both the electrodes is presented in Fig. 1.

Controlled potential coulometry of xanthine at a large PGE or GCE corresponding to peak II, potential indicated the involvement of 4.0 ± 0.1 electron in the entire pH range studied. The \(n\)-values observed at different concentrations of xanthine are presented in Table 1. The cyclic voltammogram of an exhaustively electrolysed solution of xanthine did not exhibit any peak indicating thereby that no electroactive species was present in the solution.

**Spectral studies**

The electrochemical oxidation of xanthine at pHs 3.0, 6.84 and 10.0 was monitored by recording UV spectra at different time intervals. As uric acid is the likely product formed from xanthine by 2e, 2\(H^+\) oxidation, UV spectra of uric acid were also recorded under similar conditions. Xanthine (0.05 mM) exhibits two well-defined absorptions at 215 and 270 nm at pH 6.84 (Fig. 2). Upon application of potential corresponding to peak II, the absorbances in the region 217-250 nm and at 270 nm increase for first 5 min and then systematically decrease. The absorbance at longer wavelength (290-350 nm) also increases for first 7 min and then systematically decreases (Fig. 2A). It is thus clear that an intermediate with extensive \(\pi\)-chromophore system is generated, which subsequently decomposes under coulometric conditions to give the products of electrooxidation. Spectral changes of xanthine and of uric acid under identical conditions are presented in Fig. 2B for comparison. In the case of uric acid an increase followed by decrease in absorbance occurs at longer wavelength (310-350 nm) whereas at shorter wavelength (220-250 nm) an increase in absorbance for first 5 min followed by systematic decrease is observed. Thus it is clear that same intermediate species is generated during electrooxidation of xanthine and uric acid. The formation of the same intermediate species in the case of xanthine and uric acid was further established by monitoring the decay of intermediate at selected wavelengths. A typical absorbance versus time curve observed at 320 nm for xanthine is presented in Fig. 3. The absorbance increases for first 5 min on initiation of electrolysis and then decreases when potential is turned off. The absorbance versus time curve clearly indicates a first order kinetics and hence the rate constant value was determined using log (\(A - A_\infty\)) versus time plot. The values of first order rate constant for xanthine and uric acid at different pH values are presented in Table 2. Almost identical values clearly indicate that uric acid is generated during electrooxidation of xanthine and hence the same intermediate species is generated in both the compounds which decays by first order kinetics.

**Product characterization**

The products of electrooxidation of xanthine at pHs 2.2, 6.84, and 10.0 at GCE and PGE were characterised as follows: The exhaustively electrolysed solution of xanthine at pH 2.2 exhibited two spots in TLC with \(R_f\) values of 0.4 and 0.7. The spot with \(R_f = 0.7\) was found to be urea by comparison with an authentic sample. The formation of urea as one of the products indicated the possibility of alloxan as the second product. The product with \(R_f = 0.4\) was identified using a Sephadex column.

The fractions 16-22 were combined (total volume 35 ml) and freeze-dried, IR spectrum of the residue recorded. IR spectrum of the dried material (m.p. 240°) was superimposable over that of an authentic alloxan monohydrate. The formation of alloxan was further confirmed by the mass spectrum of the product which exhibited a molecular ion peak at m/z 160. Thus the products of electrooxidation of xanthine at pH 2.2 were alloxan and urea at both the electrodes. Urea could not be isolated on the Sephadex column as it eluted along with phosphate due to its low molecular weight. Similar observation has also been reported in the case of 6-thiouric acid\(^{14}\). At pH 2.2 uric acid also gave alloxan and urea as the products at GCE and PGE. The formation of alloxan and urea as products in the acidic pH range has also been reported during electrooxidation of uric acid at a PGE\(^{15}\).

At pH 6.84 and 10.0, the electrooxidised solution of xanthine at GCE exhibited only one spot in TLC with \(R_f = 0.32\). The electrolysed solution was lyophilised and extracted with ether. The dried material (m.p. 230°C) gave the molecular ion peak at m/z...
158 in its mass spectrum. This product was identified as allantoin by direct comparison (m.p., IR) with an authentic sample. Thus it is clear that xanthine gives alloxan and urea at pH 2.2 and allantoin as the major product at pH 6.84 and 10.0 at glassy carbon electrode. Electrooxidation of uric acid at pH 6.84 and 10.0 also gave allantoin as the major product, under identical conditions.

Mechanism

Based on the results obtained, electrooxidation of xanthine (I) can be rationalised by a mechanism shown in Scheme 1. Xanthine undergoes initially (2e, 2H⁺) oxidation to give uric acid (II), which readily undergoes further (2e, 2H⁺) oxidation to give corresponding diimine (III). The reverse reaction for peak Iₕ was not observed for xanthine at PGE probably due to fast hydrolysis of diimine (III) at PGE in comparison to that at GCE. The formation of III during electrooxidation of purines at gold electrode has also not been observed earlier. Similarly the non-appearance of peak IIₕ at PGE may be due to direct (4e, 4H⁺) oxidation of xanthine at the electrode surface to give directly III due to close potentials of two 2e steps. Thus conversion of II to I is visible only at GCE. As species III is unstable with half-life of about 30 ms as reported by Owens et al.,¹⁵ it readily undergoes hydrolysis to give the diol (IV). Further, species III is more extensively conjugated than compound I or II, hence it absorbs at longer wavelength and its hydrolysis causes decrease in absorbance. The rate of decay of III was found to be similar to that of uric acid. At pH 2.2, the diol (IV) undergoes cleavage to give alloxan (V) and urea. At pH > 6.0, the pyrimidine ring is cleaved to give allantoin as the major product. The formation of allantoin during electrooxidation of uric acid has been earlier reported in the literature¹⁶. However, formation of 5-hydroxyhydrantoin-5-carboxamide observed¹⁶ during oxidation of uric acid in 0.5 M NaCl medium was not observed under present experimental conditions.

Acknowledgement

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References