Anodic Voltammetry and Its Analytical Application to the Detection and Simultaneous Determination of Hypoxanthine, Xanthine, and Uric Acid

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The voltammetric oxidation of hypoxanthine, xanthine, and uric acid was investigated by means of linear-sweep voltammetry, cyclic voltammetry, and controlled-potential coulometry. Hypoxanthine, xanthine, and uric acid each gave a single oxidation peak at a different potential, with a stationary glassy carbon electrode, over the wide pH range of 0—13. Hypoxanthine was initially oxidized in a two-electron step to xanthine, which was then further oxidized in a two-electron step to uric acid, as well as the enzymatic oxidation. All three substances were strongly adsorbed on the surface of the glassy carbon electrode, so that the concentration *vs.* anodic peak current curves were not linear. The adsorption on the electrode was very dependent on the pH, and especially at pH values around neutrality the anodic peaks were ill-defined. However, in such acid solutions as H_3PO_4 and H_2SO_4, the voltammetric oxidation was a diffusion-controlled process and the differences between the peak potentials were most pronounced (*ca.* 400 mV). Consequently, it was possible to determine simultaneously three hydroxypurine bases by using 1 M H_2SO_4 or 1 M H_3PO_4 as a supporting electrolyte, without prior treatment or separation procedures.

The electrochemical oxidation and reduction of biologically important compounds have been investigated with much interest. It is important to understand fully their redox behavior in considering the methabolism and analytical determination of biologically important compounds. Several works[1-3] have suggested a possible parallelism between the modes of electrochemical behavior and the biological transformation, *e.g.*, the enzymatic oxidation.

The electrochemical reduction of purine bases, which are important components of nucleic acids, has been investigated in both aqueous[4-6] and nonaqueous solutions[7,8] at the dropping mercury electrode (DME). However, in aqueous solutions none of the purine bases except adenine were easily reduced at the DME.

A few studies of the electrochemical oxidation of purine bases have been done at the pyrolytic graphite electrode (PGE)[9-11] and the glassy carbon electrode (GCE).[12] Both adenine and guanine gave a anodic peak at different potentials over a wide pH range. Our own previous paper[12] described a method whereby adenine, guanine, and their nucleosides could be determined simultaneously by linear-sweep voltammetry using a stationary planar GCE.

In the present work, the oxidation behavior of three hydroxypurine bases (hypoxanthine, xanthine, and uric acid) was investigated by means of linear-sweep voltammetry, cyclic voltammetry, and controlled-potential coulometry. The mutual relation of the primary oxidation process of each of these hydroxypurine bases was investigated. Also, in order to develop a satisfactory analytical method of the three hydroxypurine bases it was found necessary to investigate in some detail the adsorption behavior on the GCE. This paper will describe the oxidation behavior and a method for the simultaneous determination of hypoxanthine, xanthine, and uric acid.

Experimental

Chemicals. The hypoxanthine, xanthine, and uric acid were all obtained from the Wako Pure Chemical Co. The other chemicals were of a reagent grade and were used without further purification.

Apparatus. The anodic voltammograms were recorded using a Yanagimoto P-8 type Polarograph. Cyclic voltammograms were obtained with a versatile solid-state instrument constructed in this laboratory following the design of Goolsby and Sawyer,[13] with the triangular voltage sweeps supplied by a NF circuit design block Co. Model FG-104T function generator. The current-voltage curves were recorded on a IWATSU Model DS-5016 dual-beam oscilloscope equipped with a camera. Controlled-potential coulometry was done with a NICHIA NP-1 type potentiostat, and a glassy carbon beaker (40 ml in volume, Tokai Electrode Co.) was used as the working electrode.

A three-electrode voltammetric cell maintained at (25±0.1) °C was used for all the experiments. A saturated calomel electrode (SCE) was employed as a reference electrode, while a platinum wire was employed as a counter electrode. A working electrode was constructed from a 3-mm glassy carbon rod (Grade GC-20, Tokai Electrode Co.). The rod was cut into a length of 10 mm and was sealed in one end of a glass tube (3 mm in diameter) with epoxy cement. The end of the glass tube-carbon rod assembly was polished with 400-grade emery paper until it was quite smooth. Then, by polishing with 1500-grade emery paper and then with a paste of CeO_2 on asphalt pitch, the surface of the electrode was brought to a mirror finish.

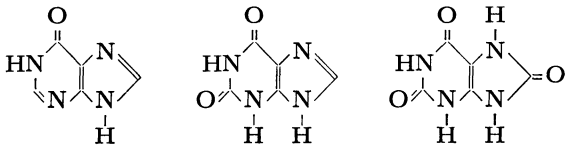
Voltammetric Procedure. In order to obtain reproducible results, a standard pretreatment procedure was applied before recording each voltammogram. The GCE was polished for 30 s with 1500-grade emery paper and then with a paste of CeO_2 on asphalt pitch until the surface of the electrode was brought to a mirror finish. The surface of the electrode was then washed with a fine spray of distilled water. Any water remaining on the electrode surface and the shaft of the electrode was removed by touching the surface very gently with a piece of soft absorbent paper. The test solutions were not deaerated. The voltage scan was commenced after quiescency

for about 20 s at the starting potential (usually 0.0 V *vs.* SCE).

At least three replicate voltammograms were recorded for each test solution. A current-voltage curve of the background solution was recorded in the same way, while the peak current was obtained by arithmetrically subtracting the observed background current at the peak potential from that of the test solution.

Results and Discussion

Linear-Sweep Voltammetry. *Effect of pH*: Hypoxanthine, xanthine, uric acid each gave an oxidation peak at a stationary GCE over the wide pH range of 0—13. The anodic voltammograms recorded at various pH values are shown in Fig. 1 for uric acid and in Fig. 2



Hypoxanthine Xanthine Uric acid

for xanthine. The oxidation peaks of uric acid and xanthine tended to become ill-defined with the increase in pH, and their peak currents decreased gradually. Similar behavior was also observed for hypoxanthine.

The half-peak potentials ($E_{p/2}$) of the oxidation peaks of the three hydroxypurine bases (uric acid, xanthine, and hypoxanthine), as is shown in Fig. 3, shifted linearly towards negative potentials with the increase in pH; $E_{p/2}$ (*vs.* SCE)/V=0.69—0.050 pH for uric acid, $E_{p/2}$ (*vs.* SCE)/V=1.08—0.059 pH for xanthine, and $E_{p/2}$(*vs.* SCE)/V=1.50—0.068 pH for hypoxanthine. The analytical significance of these experimental results lies in the fact that the voltammetric oxidation peak-potential for each of the three hydroxypurine bases is quite different at each of the pH values studied.

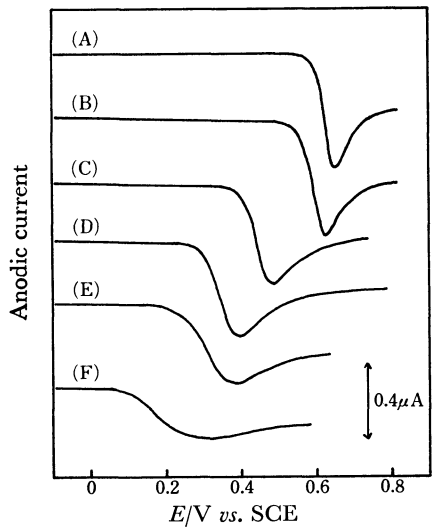


Fig. 1. Anodic voltammograms of 0.1 mM uric acid recorded at different pH values.
Supporting electrolytes: (A) 1M H_3PO_4; Britton-Robinson buffer (B) pH 2.03, (C) pH 4.39, (D) pH 6.36, (E) pH 8.20, (F) pH 10.04.
GCE geometric area: 7.1 mm², scan rate: 3.3 mV/s.

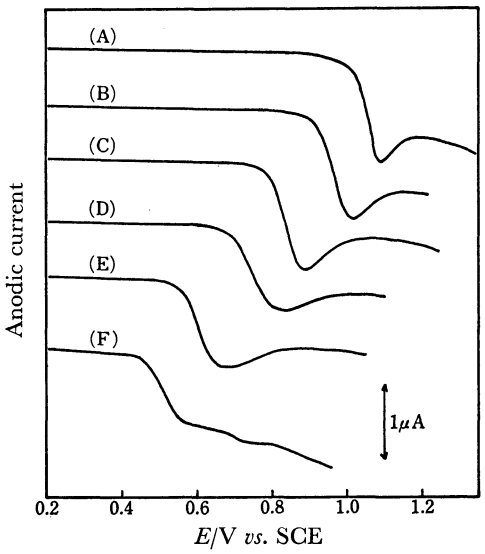


Fig. 2. Anodic voltammograms of 0.1 mM xanthine recorded at different pH values.
Supporting electrolytes: (A) 1M H_3PO_4; Britton-Robinson buffer (B) pH 2.08, (C) pH 4.05, (D) pH 6.07, (E) pH 8.02, (F) pH 9.92.
GCE geometric area: 7.1 mm², scan rate: 3.3 mV/s.

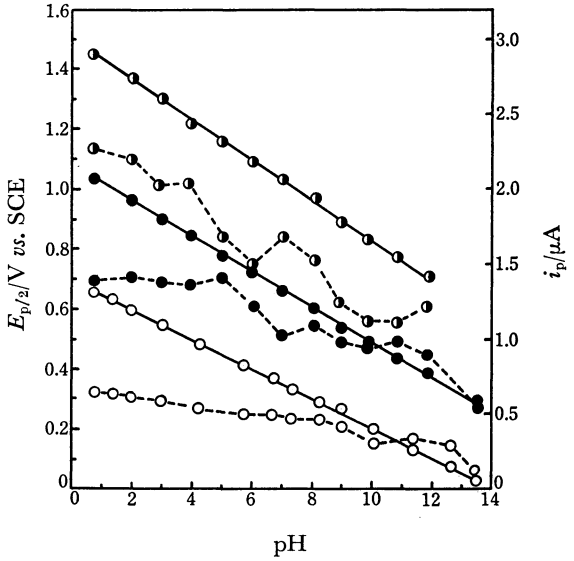


Fig. 3. The effect of pH on the peak current and the half-peak potential of the voltammetric oxidation peaks of 0.1 mM uric acid (○), xanthine (●), and hypoxanthine (◑).
GCE geometric area: 7.1 mm², scan rate: 3.3 mV/s.
----: i_p, —— : $E_{p/2}$.

The peak current of the oxidation peaks gradually decreased with the increase in pH, as is shown in Fig. 3, particularly in the case of hypoxanthine. This experiment was undertaken at a relatively low concentration (0.1 mM) of the hydroxypurine bases. When the depolarizer concentration was fairly high (1.0 mM), the pH values were found to have a pronounced effect on the peak current of the hydroxypurine bases. That is, the peak current decreased extremely at the pH values

around neutrality, as is shown in Fig. 4 for hypoxanthine. However, the peak current was relatively constant in the Britton-Robinson buffer at pH 2.1 and in acid solutions (1 M H_2SO_4, $HClO_4$, and H_3PO_4) as supporting electrolytes. Also, in electrolytes containing chloride ions such as HCl, the oxidation peak of hypoxanthine was masked by the facile oxidation of the chloride ion in the background electrolyte. Similar relationships between the peak current and the pH values were also obtained for the oxidation peaks of xanthine and uric acid.

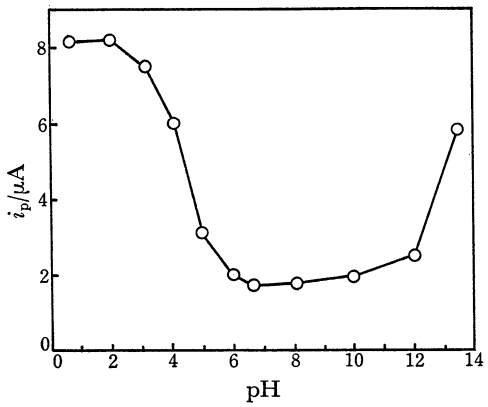


Fig. 4. The effect of pH on the peak current of 1.0 mM hypoxanthine.
GCE geometric area: 7.1 mm², scan rate: 3.3 mV/s.

Effect of the Scan Rate: In order to study the adsorption of the hydroxypurine bases on the stationary GCE, the effect of the voltage scan rate on the peak current of each hydroxypurine base was examined.

For a linear diffusion-controlled peak, the peak current function, $i_p/ACV^{1/2}$, should be a constant[14] in spite of the variation in the scan rate, where A is the area of the electrode surface; C, the bulk concentration of depolarizer; and V, the scan rate. In 1 M H_2SO_4 or H_3PO_4, the peak current function for each of the three hydroxypurine bases was almost independent of the scan rate. In the electrolyte solution with pH values around neutrality, however, the peak current functions increased with the increase in the scan rate, especially in the case of hypoxanthine. This behavior is typical of an electrode process where the reactant is adsorbed on the electrode,[15] but in 1 M H_2SO_4 or H_3PO_4 the voltammetric oxidation is a diffusion-controlled process.

Effect of the Concentration: In the 0.5 M phosphate buffer (pH 6.6), the relationship between the peak current and the concentration were nearly linear for both xanthine and uric acid, but non-linear for hypoxanthine, as is shown in Fig. 5. The peak potential was also dependent on the concentration and slightly shifted to more positive values with the increase in the concentration. Over the concentration range of 0.02—0.2 mM, this shift was about 14 mV for uric acid, 29 mV for xanthine, and 55 mV for hypoxanthine. In the Britton-Robinson buffer with pH values of 8—10, hypoxanthine showed a post-peak at a more positive potential than the main peak at concentrations above *ca.* 1 mM. This post-peak appeared at rather variable potentials.

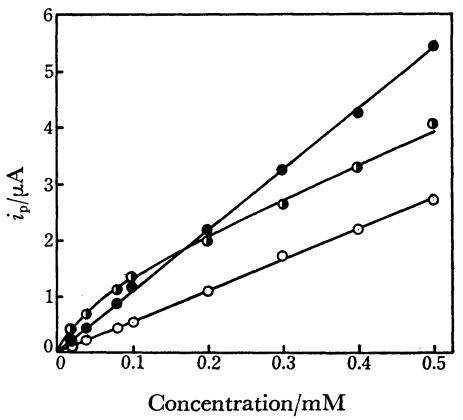


Fig. 5. The relationships between the peak current and the concentration in 0.5 M phosphate buffer (pH 6.6).
○: Uric acid, ●: xanthine, ◑: hypoxanthine.

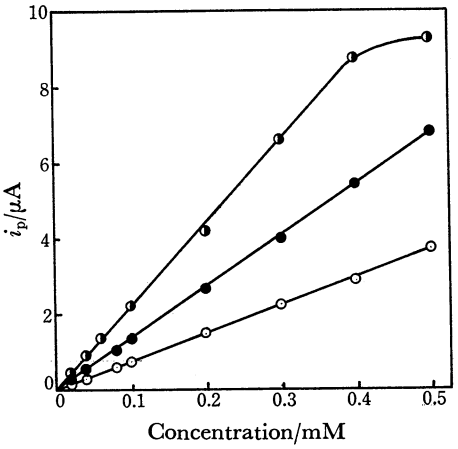


Fig. 6. The relationships between the peak current and the concentration in 1 M H_3PO_4.
○: Uric acid, ●: xanthine, ◑: hypoxanthine.

However, in such acid solutions as 1 M H_3PO_4 and H_2SO_4, each of these hydroxypurine bases showed a good linear relationship between the peak current and the concentrations ranging from 0.02 to 0.4 mM, as is shown in Fig. 6. Also, the peak potential was independent of the concentration.

These effects of the concentration and the pH on the peak current imply that the adsorption of the depolarizer on the GCE is very dependent on the pH, but in 1 M H_3PO_4 or H_2SO_4 solutions the voltammetric oxidation is a diffusion-controlled process.

Mutual Relation of Primary Oxidation Processes. Cyclic voltammograms at fast scan rates were recorded for each of the three hydroxypurine bases. The results are shown in Fig. 7. Scanning from 0.0 V at a clean electrode toward a positive potential, only a single anodic peak was observed for hypoxanthine. The principle point of interest in this voltammogram is that two new and small anodic peaks (Peaks I and II) were observed on the second sweep toward the positive potential, Peak I at 0.65 V and Peak II at 0.99 V (*vs.* SCE) in the Britton-Robinson buffer at pH 2.1. However, the peak potentials of these new peaks were

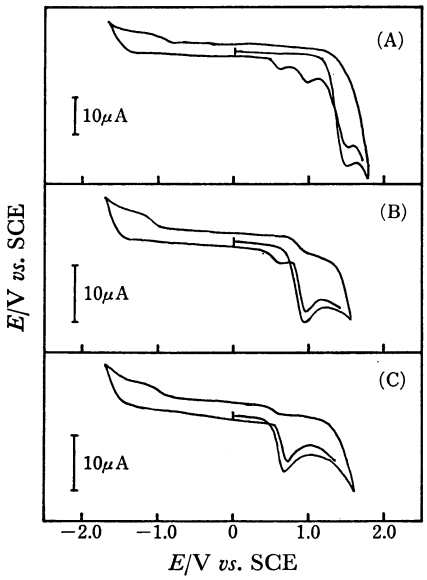


Fig. 7. Cyclic voltammograms of hypoxanthine (A), xanthine (B), and uric acid (C) in the Britton-Robinson buffer at pH 2.1.
Scan rate; (A): 10.3 V/s, (B): 6.4 V/s, (C): 6.6 V/s.

TABLE 1. COULOMETRIC n VALUES FOR THE CONTROLLED-POTENTIAL ELECTROLYSIS OF OXYPURINE BASES IN 1 M H_3PO_4

Oxypurine bases	Applied potential (V *vs.* SCE)	n[a)]
Uric acid	0.80	2.21
Xanthine	1.20	4.16
Hypoxanthine	1.60	5.88

a) Average value of triplicate determinations.

dependent on the pH of electrolyte solutions. At various pH values, the peak potentials for these two anodic peaks, I and II, were the same as those of the anodic peaks for the oxidation of uric acid and xanthine respectively. Similarly, xanthine showed a new anodic peak on the second sweep toward the positive potential. This peak appeared at almost the same peak potential as the anodic peak of uric acid.

Controlled-potential coulometry for each of three hydroxypurine bases was carried out in 1 M H_3PO_4. The electrolysis was continued until all of the depolarizers were completely oxidized. Typical coulometric data are presented in Table 1; the n value for uric acid was about two, for xanthine it was four, and for hypoxanthine it was six.

In summary, the cyclic voltammetric and coulometric data suggest that hypoxanthine is initially oxidized in a two-electron step to produce xanthine, which is then further oxidized in a two-electron step to produce uric acid. It is also known[16] that uric acid is oxidized in a two-electron process to produce 4,5-dihydroxy-4,5-dihydrouric acid, which is unstable and which fragments predominantly to allantoin, but a very small fraction is further oxidized to parabanic acid. Hypoxanthine is also enzymatically oxidized to uric acid *via* xanthine by the action of the xanthine oxidase. Thus, it is interesting that the electrochemical oxidation is very similar to the enzymatic oxidation.

Analytical Application. The anodic voltammograms for the mixtures of uric acid, xanthine, and hypoxanthine were recorded in three electrolyte solutions (Fig. 8). In 1 M H_2SO_4 three successive well-defined oxidation peaks were observed, but in the 0.5 M phosphate buffer and 1 M NaOH solutions the three peaks were ill-defined, as a result that the increase in the pH of the electrolyte solutions made the voltammetric peaks sensitive to the adsorption of depolarizers on the GCE. Accordingly, for the detection and quantitative determination of the three hydroxypurine bases, the use of a 1 M H_2SO_4 or H_3PO_4 solution as the supporting electrolyte was suitable. In order to test the quantitative method developed here, the voltammograms for some mixtures of three hydroxypurine bases were recorded in 1 M H_2SO_4 at a stationary GCE. The concentration of each hydroxypurine base was determined by the calibration-curve method from the respective heights of the resulting voltammetric oxida-

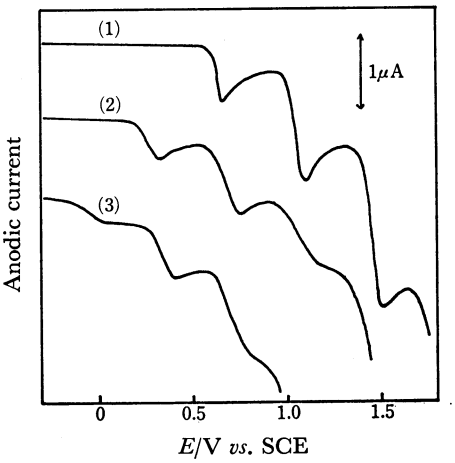


Fig. 8. Anodic voltammograms for the mixtures of uric acid, xanthine, and hypoxanthine.
Concentration of each of uric acid, xanthine, and hypoxanthine: 0.1 mM. Supporting electrolyte; (1): 1 M H_2SO_4, (2): 0.5 M phosphate buffer (pH 6.6), (3): 1 M NaOH.

TABLE 2. VOLTAMMETRIC RESULTS FOR THE DETERMINATION OF EACH COMPONENT IN MIXTURES OF URIC ACID, XANTHINE, AND HYPOXANTHINE

Taken (mM)			Found (mM)		
A	B	C	A	B	C
0.05	0.05	0.05	0.04	0.05	0.05
0.10	0.10	0.10	0.10	0.10	0.09
0.10	0.10	0.30	0.10	0.09	0.32
0.20	0.20	0.20	0.19	0.21	0.20
0.40	0.40	0.20	0.41	0.38	0.18
0.20	0.20	0.30	0.20	0.20	0.29

A, Uric acid; B, xanthine; C, hypoxanthine. Supporting electrolyte, 1 M H_2SO_4; GCE geometric area, 7.1 mm^2; scan rate, 3.1 mV/s.

tion peaks. Some typical analytical results are shown in Table 2. Clearly this method is readily capable of determining simultaneously each component in mixtures of the three hydroxypurine bases.

The principal advantage of the proposed method is the rapidity of analysis. The speed of analysis is aided by the fact that no prior treatment or separation procedure is needed. Furthermore, as dissolved oxygen does not interfere with the anodic voltammetry, no deaeration is required.

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