Voltammetric determination of uric acid in human serum by the use of a dialysis membrane-covered graphite paste electrode.

Hideaki KINOSHITA and Daisuke YOSHIDA

Kwassui Women's College, Higashiyamate, Nagasaki 850, *Japan*

“透析膜を被覆したグラファイトペースト電極を用いる血清尿酸のボルタメトリック定量”

木　下　英　明・吉　田　大　介

Abstract

A dialysis membrane-covered graphite paste electrode was prepared and tested as a detecting electrode for uric acid in serum. The current magnitude of uric acid on the linear sweep voltammograms was proportional to the concentration of uric acid from 1 μM to 200 μM. Protein, which fouls the surface of a bare lectrode and worsens the property of the electrode, did not affect the anodic current of uric acid at this electrode. Oxidizable compounds in serum except ascorbic acid did not interfere with the current of uric acid measured at 0.5−0.6 V in the acidic solutions by this electrode. The magnitude of the anodic current for a serum solution measured in pH 3−4 could be corrected for the effect of ascorbic acid to obtain the current due to uric acid. Values of uric acid content in serum measured by this method agreed with those by a photometric uricase-peroxidase procedure.

Introduction

Nowadays uric acid in serum is determined mainly by a photometric uricase-peroxidase (or catalase) procedure〔1〕. In this method expensive enzymes are used only once and then thrown away. So immobilization of uricase is developed not only

in a photometric mthod [2, 3] but also in an electrochemical method [7, 8, 9] . An electrochemical method has advantages of rapidity, simplicity, and independence of turbidity and coloration in test solutions. Determination of uric acid by the direct oxidation at a graphite paste electrode [4, 5] , a pyrolytic graphite electrode [6] , and a platinum electrode [7] and by the enzymatic oxidation combined with a peroxidase electrode [8] , with a hydrogen peroxide electrode [7] and with a carbon dioxide electrode [9] have been reported. In the former, protein and ascorbic acid affect the anodic current of uric acid. In the latter, preparations of electrodes are troublesome and the life times of these electrodes are not so long.

We made a dialysis membrane-covered graphite paste electrode. Anodic current magnitude of uric acid at this electrode was linearly proportional to the concentration of uric acid from 1 μM to 200 μM and was not affected by protein in serum, which worsend the property of a bare electrode, and by oxidizable compounds in serum except ascorbic acid, when measured at relatively negative potentials in the acidic solutions. Effect of ascorbic acid on the current magnitude of uric acid could be estimated by measuring the current intensity at 0.35−0.40 V in pH 3.5 as a limiting current of ascorbic acid. In this paper we describe the voltammetric behavior of uric acid at this electrode and an application of the voltammetric method for the determination of uric acid in serum.

Experimental

Preparation of an electrode

Graphite powder (Nippon Kokuen Co.) containing 33% paraffin liquid (Merck Co.) was packed into a carbon paste electrode (BAS Inc.). The surface (0.07 cm^2) was smoothed and the electrode surface was covered with a round−cut (6 mm diameter) dialysis membrane (20 μm thickness, Union Carbide Co.). The whole electrode was covered by a nylon net and parafilm to give it physical strength. An electrode prepared in this way is reffered to below as a membrane electrode.

Reagents and appratus

A reagent kit for the photometric determination of uric acid "Uricolor Ace" containing uricase, peroxidase, 4−aminoantipyrine and standard solution of uric acid was purchased from Toyobo Co. (Osaka). Other reagent grade chemicals were purchased from Nakarai tesque Co. (Kyoto). Samples of patient's serum, which had been

clinically examined at Nagasaki University Hospital, were used. A Yanagimoto Polarographic Analyzer P1100 was used to apply the potential to a two-elctrode system, and currents were measured with a Watanabe X (t)-Y Recorder WX4401.

Electrochemical measurements

McIlvaine's buffer pH 3.5-6.5 were used as base solutions. All measurements were made at 25℃ in an H-type cell containing 2 ml of test solution. The solutions were stirred at 500 rpm with a magnetic stirrer (10 mm diameter) placed in the bottom of the cell except in the experiment shown in Fig. 1. Potentials were measured against an SCE.

Results and Discussion

Voltammetric behavior of uric acid at a dialysis membrane-covered graphite paste electrode.

Dialysis membrane-covered electrodes have been used for the comparison with an uricase membrane-covered platinum electrode in the determination of uric acid at

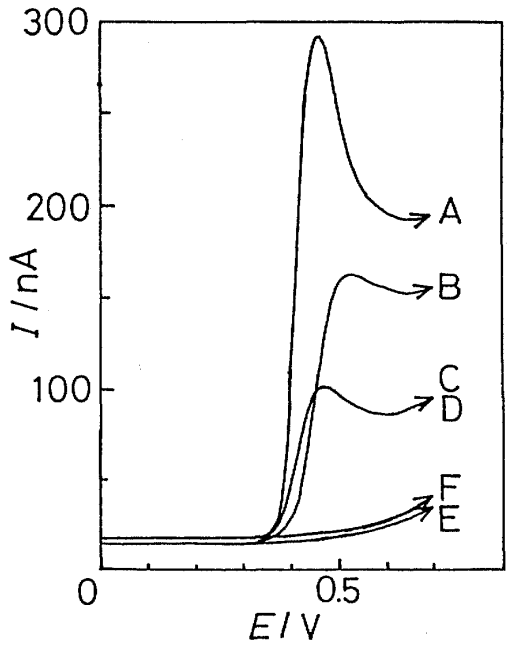

Fig. 1 Linear sweep voltammograms of 5 μg ml^{-1} uric acid at a graphite paste electrode without membrane (A, B) and at a membrane-covered electrode (C, D) in the absence (A, C) and the presence of 50 μg ml^{-1} serum albumin (B, D) at the voltage scan rate, v=5 mV s^{-1}, pH 4, and 25℃. E and F are the voltammograms of McIlvaine's buffer at electrodes without (E) and with membrane (F).

flow system [7] and for the immobilization of enzymes adsorbed onto the surface of graphite paste electrode [10] . Dialysis membrane of these electrodes were not used for the elimination of fouling effect on the electrode surface by adsorptive substances, especially protein. Figure 1 shows linear sweep voltammograms for uric acid obtained with a graphite paste elctrode without membrane (A, B) annd with a membrane electrode (C, D). The current intensity on the voltammograms at the electrode without membrane was strongly influenced by the presence of protein (Fig. 1 −B) and gradually decreased with the exposure time of electrode in the solution containing protein. On the other hand such influence and decrease in current were not observed at the membrane electrode (Fig. 1−D) as expected from the impermiability of the membrane to protein. Many oxidizable compounds such as tochopherol, lipoic acid, ubiquinone, iron (II), and copper (I) exist in the form of conjugation linked to protein in serum [11] . Therefore they may be inactive to the membrane electrode.

Uric acid, ascorbic acid, epinephrine, and glutathione are the compounds usually contained in serum and electroactive. Figure 2 shows the linear sweep voltammograms for these compounds at 1.25 μg ml^{-1} and at the voltage scan rate, v=5 mV s^{-1}, obtained with a membrane electrode in the stirred (500 rpm with a magnetic

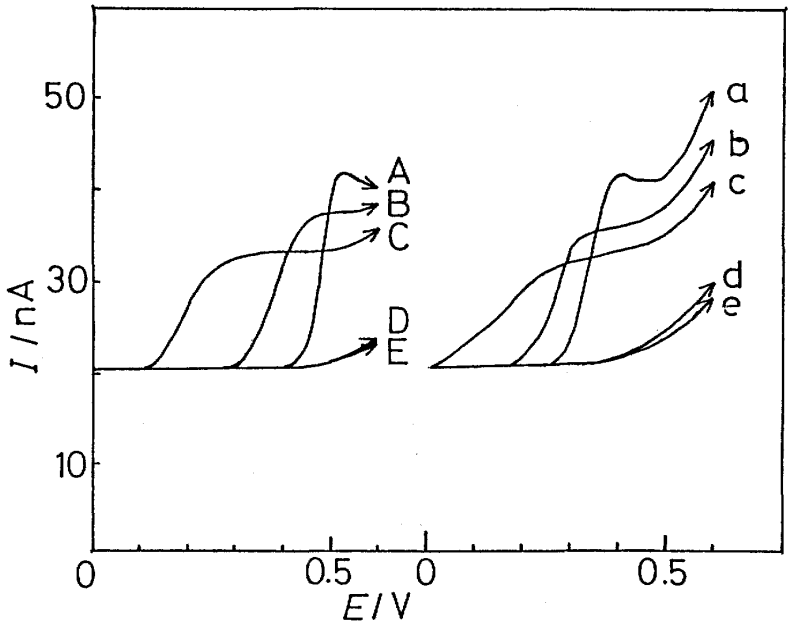

Fig. 2 Linear sweep voltammograms of 1.25 μg ml^{-1} of uric acid (A, a), epinephrine (B, b), ascorbic acid (C, c) and glutathione (D, d) in McIlvaine's buffer pH 3.5 (A−D) and pH 6.5 (a−d) at v=5 mV s^{-1} and 25 ℃. E and e are the voltammograms of buffer at pH 3.5 (E) and pH 6.5 (e). The solutions were stirred at 500 rpm with a magnetic stirrer. (the same as in Fig. 3 and Fig. 4)

stirrer) solution of pH 3.5 and 6.5. Voltammograms obtained in stirred solution superimposed in every scan and were reproducible. So experiments shown below were made in stirred solutions. The oxidation of uric acid began at 0.40 V in pH 3.5 and at 0.25 V in pH 6.5. Then anodic current increased sharply and reached an almost limiting value at 0.5 V in pH 3.5 and 0.4 V in pH 6.5. The net anodic current due to uric acid was obtained by subtracting the current intensity of the base solution from that of uric acid solution. Magnitude of net currents, I, at 0.4−0.5 V in pH 3.5−6.5 were linearly proportional to the concentration of uric acid from 0.2 (1 μM) to 40 μg ml^{-1} (200 μM). Figure 3 shows the example of I–c curve at 0.5 V and pH 3.5. Coefficient of variation for the current intensities of 1.25 μg ml^{-1} uric acid solution at 0.5 V and pH 3.5 was 1.8% (n=7). Anodic current of uric acid at a bare electrode to which protein adsorbed was not linearly proportional to the concentration of uric acid.

The oxidation of glutathione started at 0.5 V (pH 3.5) and 0.4 V (pH 6.5) and the current magnitude of it was very small compared with that of uric acid. So the influence of glutathione on the current of uric acid could be avoided by selecting the measuring potential. (Other−SH compounds such as cysteine and mercaptoethanol

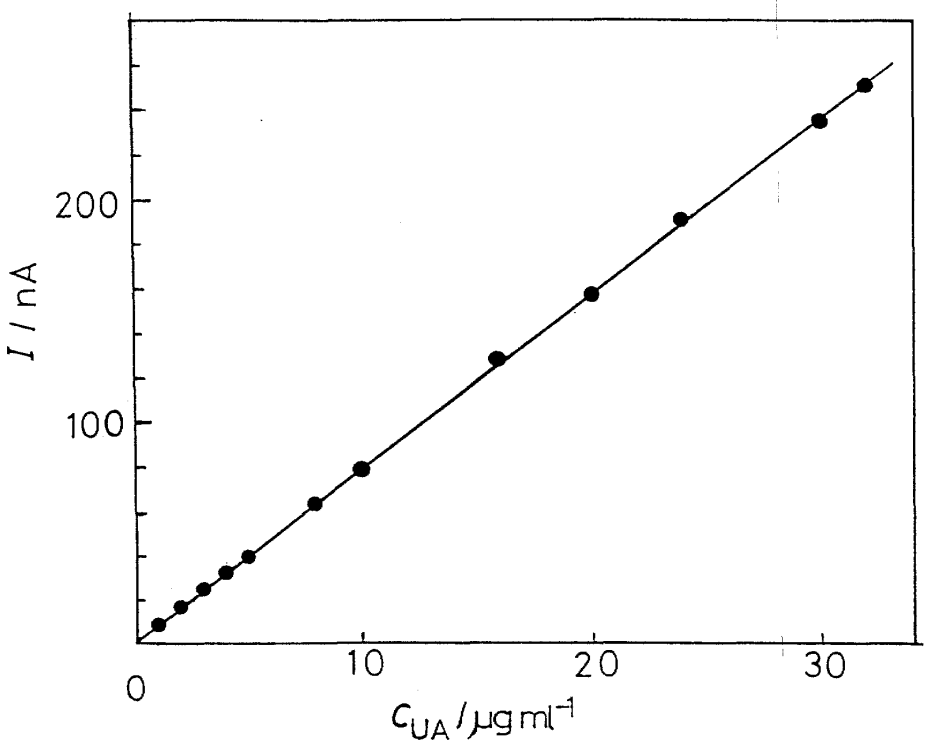

Fig. 3 Dependence of the anodic current on the voltage sweep voltammograms with v=5 mV s^{-1} at 0.5 V on the concentration of uric acid at pH 3.5

gave the similar voltammograms.) The oxidation of ascorbic acid and epinephrine started at more negative potentials than that at which the oxidation of uric acid started. Usually serum contains uric acid 2.6−8.0, ascorbic acid 0.2−1.4 and epinephrine 10^{-7} mg dl^{-1} [11, 12] . So the contribution of epinephrine to the anodic current of uric acid may be neglected but the contribution of ascorbic acid must be estimated for the electrochemical determination of uric acid.

Figure 4 shows the linear sweep voltammograms of 1.25 μg ml^{-1} uric acid solution (B, b), 0.4 μg ml^{-1} ascorbic acid solution (C,c), and solution containing 1.25 μg ml^{-1} uric acid and 0.4 μg ml^{-1} ascorbic acid (D, d) at pH 3.5 (B−D) and 6.5 (b−d). The current magnitude of the solution containing uric acid and ascorbic acid was equal to the sum of currents due to uric acid and ascorbic acid. The same results were obtained with the solutions containing uric acid and ascorbic acid in various ratios as long as the total concentration of these compounds was kept below 1 mg ml^{-1}. In pH 3.5, oxidation of uric acid started at the potential 100 mV more positive than that at which the anodic current of ascorbic acid reached a limiting current. The magnitude of current due to uric acid in serum could be obtained by subtracting the limiting current of ascorbic acid from the current magnitude of serum solution at 0.5−0.6 V.

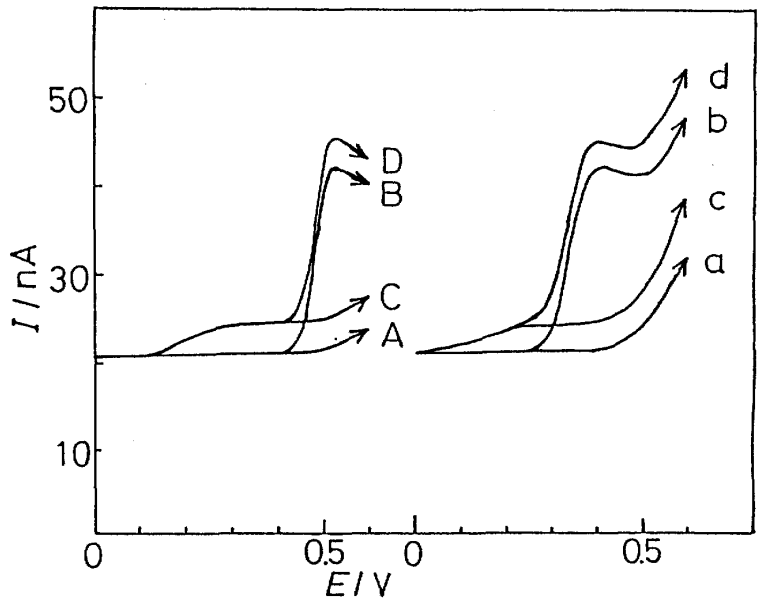

Fig. 4 Linear sweep voltammograms recorded in McIlvaine's buffer pH 3.5 (A−D) and pH (6.5 (a−d) at **v**=5 mV s^{-1} and 25℃ by the use of a membrane electrode.
A, a; buffer solution, B, b; 1.25 μg ml^{-1} uric acid solution, C, c; 0.40 μg ml^{-1} ascorbic acid solution, D, d; buffer solution containing 1.25 ug ml^{-1} uric acid and 0.40 μg ml^{-1} ascorbic acid.

This correction of the current due to ascorbic acid is difficult in the pH range from 4.5 to 6.5.

Presence of glutamine, glutamic acid, asparagine, aspartic acid, alanine, valine, threonine, lysine, glucose, acetylglucosamine, citric acid, albumin (up to 200 μg ml^{-1}), sodium heparine, sodium fluoride, sodium ethylenediamine tetraacetate (up to 100 μg ml^{-1}), and bilirubin (up to 5 μg ml^{-1}) in the test solution did not affect the voltammograms of uric acid solution.

Determination of uric acid in patient's serum.

On the basis of the above results, we measured the anodic current at 0.5 V in the buffer solution of pH 3.5 to determine uric acid in serum. Fifty micro liter of standard uric acid solution (10 mg dl^{-1}) or the same volume of patient's serum was syringed into an H−type cell containing 2 ml of McIlvaine's buffer. Voltammograms recorded in these solution (S, A−D) and buffer (E) are shown in Fig. 5. Net anodic current of uric acid in serum solution (a−d) was obtained by subtracting the limiting cuurent of ascorbic acid measured at 0.35−0.40 V from the current magnitude of serum solution at 0.5 V. The current magnitude of standard uric acid solution diluted 1/40 was 34.3 nA (a). On the basis of this result, uric acid contents of four samples were determined to be 2.2 (7.3 nA), 4.5 (15.3 nA), 8.5 (29.0 nA) and 11.0 mg dl^{-1} (37.8 nA). Coefficients of variation for the results of four samples were 1.3, 1.8, 1.6 and

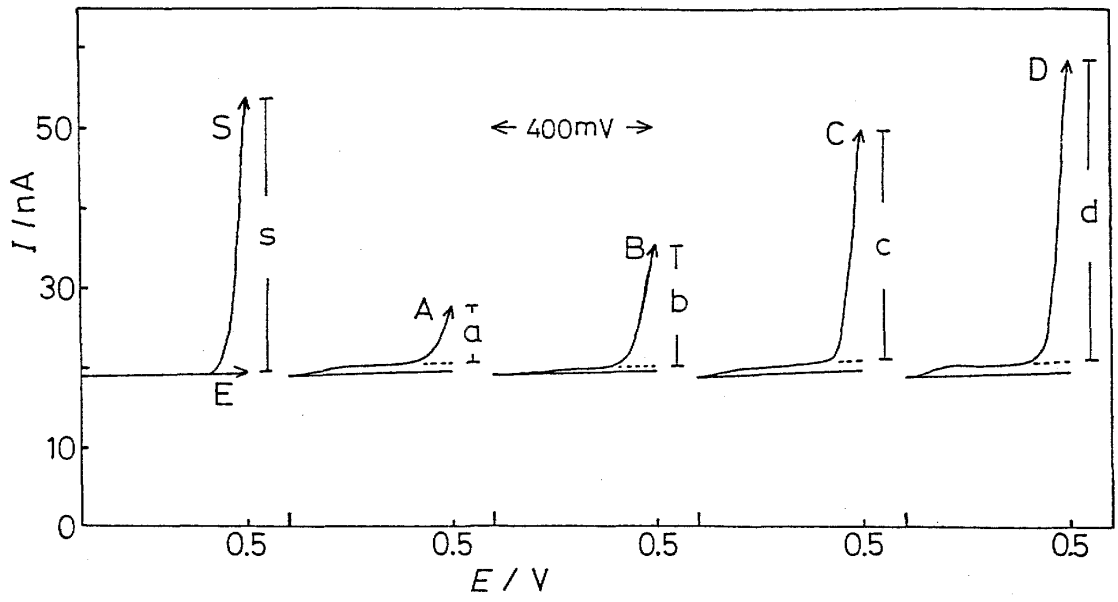

Fig. 5 Linear sweep voltammograms of McIlvaine's buffer pH 3.5 (E), 2.5 μg ml^{-1} uric acid solution (S), and patient's serum solutions (A−D) which were diluted 1/40 with the buffer. a−d are the estimated net current magnitude of uric acid.

1.5%, respectively (n=5). The values determined by the present method, 2.2, 4.5, 8.5 and 11.0 mg dl^{-1}, agreed well with the values determined by a photometric uricase-peroxidase method, 2.0, 4.3, 8.3, and 10.5 mg dl^{-1} respectively. Coefficient of correlation for the uric acid contents of thirty samples between by the electrochemical method and the photometric method was 0.998.

A membrane-covered graphite paste electrode recovered for one min rinsing in buffer solution. The current response to the oxidation of uric acid was not changed after 200 times use in biofluid solutions. The life time of this electrode is semipermanent on storage at 4℃ in buffer.

References

1) Kageyama N. Direct colorimetric determination of uric acid in serum and urine with uricase-catalase system. *Clin Chim Acta* 1971; 31: 421−426.

2) Iob A, Mottola H. A. Uricase immobilized on walls of glass tubes used as reactors in continuous-flow analyses. *Clin Chem* 1981; 27: 195−196.

3) Nakamura N, Murayama K, Kinoshita T. Immobilization of uricase on protamine bound to glass beads and its application to determination of uric acid. *Anal Biochem* 1986; 152: 386−390.

4) Park G, Adams R, White W. R. A rapid accurate electrochemical method for serum uric acid. *Anal Lett* 1972; 5: 887−896.

5) Wang J, Freiha B. A. Preconcentration of uric acid at a carbon paste electrode. *Bioelectrochem Bioenerg* 1984; 12: 225−234.

6) Dryhurst G, De P. K. Direct electrochemical method for the determination of allopurinol and uric acid mixtures. *Anal Chim Acta* 1972; 58: 183−191.

7) Jaenchen M, Gruening G, Bertermann K. An electrochemical determination of uric acid by a flow-through equipment with and without uricase. *Anal Lett* 1985; 18: 1799−1820.

8) Yao T, Sato M, Wasa T. Flow injection analysis for uric acid by the combined use of an immobilized uricase reactor and a peroxidase electrode. Nippon Kagaku Kaishi (Japan) 1985; 189−192.

9) Kawashima T, Arima A, Hatakeyama N, Tomonaga N, Ando M. Uric acid sensors based on the use of a carbon dioxide electrode and activation of uricase membrane. Nippon Kagaku Kaishi (Japan) 1980: 1542−1548.

10) Kinoshita, H, Ikeda T, Senda M, Usui T. α−Amylase assay in humane serum using a mediated amperometric enzyme electrode based on oligosaccharide dehydrogenase. *Bioelectrochem. Bioenerg* 1990; 24; 365−369.

11) Usui T, Sasaki T, Kosaka A. "Rinshokagaku (Clinical Chemistry, in Japanese)", Igakushoin, Tokyo, 1987.

12) Nipponseikagakugakkai, "Seikagakudetabukku 1 (Biochemical Data Book 1 in Japanese)" Tokyo Kagakudojin, Tokyo, 1979.

1991. 1. 31. 受理