Selective Detection of Uric Acid in the Presence of Ascorbic Acid and Acetaminophen at a Glassy Carbon Paste Electrode in an Alkaline Solution

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Selective determination of uric acid (UA) was conducted in the presence of ascorbic acid (AA) and acetaminophen (APAP) as interference species at an unmodified glassy carbon paste electrode (GCPE). Using the GCPE prepared in our lab, we succeeded in detecting UA in the presence of AA in acidic and alkaline media. In addition, the oxidation peak potential of APAP is very close to that of UA in neutral media, but in alkaline media, a peak separation of ca. 140 mV was obtained between UA and APAP on the differential pulse voltammogram (DPV).

UA is the primary end product of purine metabolism, and is a fluid of great importance in human diagnosis. The typical concentration of UA in blood is in the range of 120–450 μM.[1,2] Abnormal concentrations of UA can indicate the presence of one of numerous diseases and/or physiological disorders. An elevated concentration of UA is observed in patients suffering from diseases such as gout and hyperuricaemia.[3,4] Because of its clinical relevance, it is crucial to develop simple and rapid methods for UA determination in routine analysis. Various methods have been used to accomplish this, such as enzyme-based systems, fluorescence, chemiluminescence, capirally electrophoresis, and liquid chromatography. The advantages of an electrochemical technique for the determination of UA are high sensitivity, low cost, and rapid measurement time. One major problem for the electrochemical detection of UA is interfering species such as ascorbic acid (AA) or APAP, which are oxidized at a similar potential to that of UA. Although many electrodes have been developed to exclude AA interference,[5–10] few electrodes have been reported to exclude the influence of neutral or positively charged molecules such as APAP.

Carbon paste electrodes are widely used for electrochemical applications because of their advantages over other electrodes, including ease of construction and renewal. One drawback of this electrode is its slow electron transfer, which prevents selective analysis. Cai et al. reported that AA and UA were separated well on an electrochemically pretreated carbon paste electrode.[1] This paper describes a strategy to eliminate the influence of AA and APAP. The peak separation between UA and APAP increases with solution pH, and these peaks can be separated well in an alkaline solution. An untreated GCPE exhibits a much better peak separation between AA and UA than a traditional carbon paste electrode, and this device was used to selectively determine UA in the presence of AA and APAP.

UA and APAP were purchased from Sigma and AA was purchased from Wako. Glassy carbon powder (1–12 μm) and mineral oil were purchased from Aldrich. Solutions of UA, APAP, and AA were prepared using ultrapure water, and were used directly for detection in an open-circuit. Ultrapure water (18.2 MΩ cm^{-1}) from a Millipore-MilliQ system was used to prepare all solutions.

DPVs were performed with a BAS 100 analyzer. A platinum wire and an Ag/AgCl (3 M NaCl, BAS) were used as the counter and reference electrodes, respectively. All experiments were conducted at room temperature (22 ± 2 °C). The working electrode was a GCPE prepared by hand mixing 90 wt % GC powder with 10 wt % mineral oil. The mixture was introduced to a Teflon tube (1-mm diameter) with a stainless wire used for the electrical contact. The electrode surface was renewed by cutting an electrode using a cutter knife. The peak current was determined on the DPV of the second scan.

Two distinct oxidation peaks corresponding to those of UA and AA were observed in DPVs of mixed solutions of UA and AA in neutral and alkaline media. The peak separation of UA and AA was 160 mV in 0.01 M NaOH solution (data not shown). Figure 1 shows the DPVs of UA and APAP at a GCPE at pH 7.0 (top) and pH 12 (bottom). At pH 7.0, the peak potentials of UA and ACAP were 0.29 and 0.34 V, respectively, so the peak separation between UA and APAP was only ca. 50 mV. Peak potentials of UA and APAP shifted positively in an acidic solution and negatively in an alkaline solution, because these species are oxidized with the loss of protons, as is well known. Peak potentials of UA and APAP at pH 3.1 were 0.59 and 0.60 V, respectively. Peak separation was smaller than that in a neutral solution. In a 0.01 M NaOH solution, peak potentials of UA and APAP were −0.02 and 0.12 V, respectively. Peak separation was 140 mV, larger than that in the pH 3.1 or pH 7.0 solutions.

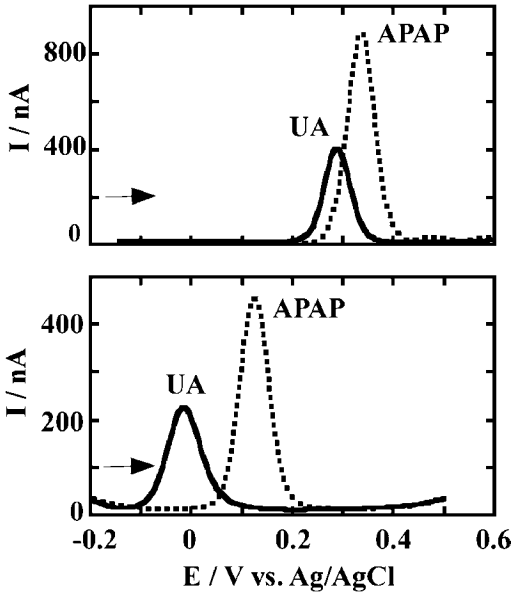

Figure 1. DPVs of 50 μM UA and APAP at a GCPE in a phosphate solution at pH 7.0 (top) and a 0.01 M NaOH.

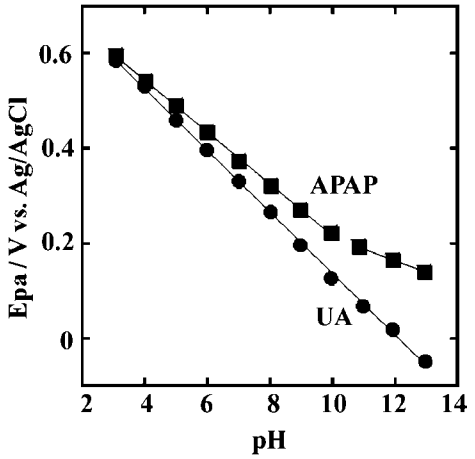

Figure 2. Effect of pH on the anodic peak potential of UA and APAP on CVs. Scan rate: 20 mV s^{-1}.

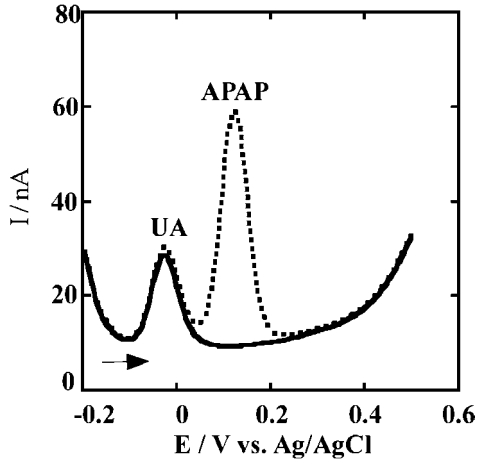

Figure 3. DPVs of 5 μM UA only (solid line) and 5 μM UA and 5 μM APAP (dotted line) in a 0.01 M NaOH solution.

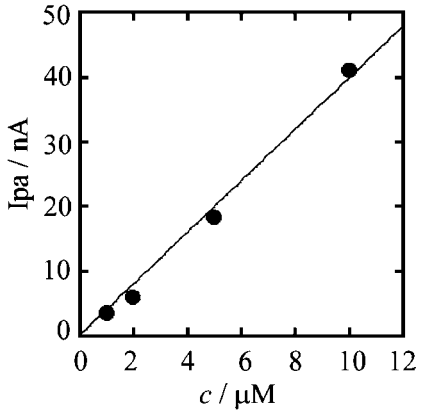

Figure 4. Calibration plot of UA on a GCPE in a 0.01 M NaOH solution.

Figure 2 shows the effect of solution pH on the peak potentials of UA and APAP. For UA, peak potential had a linear decrease from pH 3 to 13 with a slope of 65 mV/pH, suggesting that an identical number of electrons and protons are present. Since UA oxidation is a two electron process, the number of protons involved is predicted to be two;

$$\text{UA (red)} - 2H^+ - 2e \rightarrow \text{UA (ox)} \text{ (pH 3–13)} \quad (1)$$

For APAP, the slope was 55 mV/pH from pH 3 to 10. The APAP oxidation is predicted to be a two electron process, so the number of electrons and protons involved should be two. On the other hand, the slope was 25 mV/pH from pH 11 to 13, suggesting 1 proton was involved per 2 electrons in this region;

$$\text{APAP (red)} - H^+ - 2e \rightarrow \text{APAP (ox)} \text{ (pH 11–13)} \quad (2)$$

Peak separation between UA and APAP increased with pH, and the peak separation reached 170 mV in a 0.1 M NaOH solution.

The ability for the selective determination of UA in the presence of APAP was investigated. A DPV of 5 μM UA only, and 5 μM UA in the presence of 5 μM APAP gave almost identical peaks in a 0.01 M NaOH solution, as shown in Figure 3. This indicates that UA can be selectively identified in the presence of APAP. A 0.01 M NaOH solution was used for further investigation. AA gave an oxidation peak at −0.18 V in a 0.01 M NaOH solution, which is more negative by ca. 160 mV than that of UA. The peak current of UA did not change even in the presence of 10-fold higher AA. Although the oxidation peak potentials are very close to each other on a traditional carbon paste electrode,[11] a much better separation was obtained between AA and UA on an untreated GCPE. We hypothesize that the preservation state of the electrodes after construction may be one reason for such a better electron transfer rate constant. Further experiments on this phenomenon will be done in the future.

The determination of UA was conducted in a 0.01 M NaOH solution for further study. Peak currents increased as with the UA concentration. Over the concentration range from 1 to 10 μM, a linear calibration plot was obtained for UA as shown in Figure 4.

The effect of preconcentration time was also investigated. Adsorption phenomena have been observed on some electrodes, and the peak current depends on the preconcentration time. When 30 s, 1, 2, and 3 min were used as preconcentration times on a GCPE, there was no clear change in the peak current of UA, indicating that preconcentration time has little impact.

In summary, UA was successfully determined on an untreated GCPE in the presence of AA and APAP in an alkaline solution.

References

1 X. Cai, K. Kalcher, C. Neuhold, B. Ogorevc, *Talanta* **1994**, *41*, 407.
2 J.-M. Zen, J.-S. Tang, *Anal. Chem.* **1995**, *67*, 1892.
3 V. S. E. Dutt, H. A. Mottola, *Anal. Chem.* **1974**, *46*, 1777.
4 J.-M. Zen, *Analyst* **1998**, *123*, 1345.
5 L. Xiao, J. Chen, C.-S. Cha, *J. Electroanal. Chem.* **2000**, *495*, 27.
6 C. R. Raj, T. Ohsaka, *J. Electroanal. Chem.* **2003**, *540*, 69.
7 J.-S. Ye, Y. Wen, W. D. Zhang, L. M. Gan, G. Q. Xu, F.-S. Sheu, *Electroanalysis* **2003**, *15*, 1693.
8 S. B. Khoo, F. Chen, *Anal. Chem.* **2002**, *74*, 5734.
9 K. Shi, K.-K. Shiu, *Electroanalysis* **2001**, *13*, 1319.
10 P. Ramesh, S. Sampath, *Electroanalysis* **2004**, *16*, 866.
11 A. Safavi, N. Maleki, O. Moradlou, F. Tajabadi, *Anal. Biochem.* **2006**, *359*, 224.

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