Differential pulse voltammetric determination of uric acid at nanogold modified indium tin oxide (ITO) electrode

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The determination of uric acid has been carried out at pH 7.2 (\( \mu = 0.1 \) M) at nanogold modified indium tin oxide electrode by differential pulse voltammetry in absence and presence of ascorbic acid and other common interferents. A well-defined oxidation peak has been observed at around 840 mV vs Ag/AgCl electrode for uric acid at pH 7.2. The plot of peak current versus concentration plot is linear in the range 0.5 - 100 \( \mu M \) with a correlation coefficient of 0.995 and a sensitivity of 0.0004 \( \mu A/\mu M \). The detection limit (3\( \sigma \)) is found to be 0.5 \( \mu M \). Ascorbic acid in concentrations lower than 10 \( \mu M \) does not interfere, whereas, at higher concentrations it interferes. Xanthine, hypoxanthine and adenine do not interfere even at eight fold excess. The method is simple and convenient with the only limitation that the electrode cannot be used more than eight times.

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Uric acid (2,6,8-trihydroxypurine, UA) is the primary end product of purine metabolism in the human system. The normal concentration range of uric acid in blood serum is reported to be in the range 4.1-8.8 mg/100 ml. The excretion of uric acid occurs mainly through urine\(^{1,2}\) and its concentration varies between 250 mg/24h and 750 mg/24h. The presence of higher than normal UA concentration leads to various diseases and physiological disorders. Excess of uric acid may be indicated by leukemia, gout, hyperuricemia, pneumonia and renal failure\(^{3,4}\).

A relationship between serum uric acid and cardiovascular disease has been shown in hypertensive patients\(^{5,6}\). Uric acid is also employed as a clinical marker for renal dysfunction\(^{7}\). Kidneys have also been found to be affected by excess uric acid deposition in kidney tubules\(^{8}\). The excretion of uric acid through urine has been reported to continue at a steady rate even during starvation and during a purine-free diet due to endogenous (tissue) purine metabolism. Ingestion of foods high in nucleoprotein produces a marked increase in uric acid excretion. High concentration of uric acid in humans for a long time has been reported to lead to high risk of diabetes mellitus with abnormal glucose tolerance\(^{9}\). On the other hand, uric acid is an important antioxidant present in human plasma and is involved in many pathological changes and damage recovery\(^{10,11}\). In view of the diseases caused by a higher concentration of uric acid in blood and plasma, the monitoring of uric acid concentration in biological fluids is essential.

Various methods have been employed for uric acid analysis in body fluids, viz., colorimetric, electrochemical, spectrophotometric methods using HPLC and chemiluminescence\(^{12,13}\). However, as electrochemical techniques are more selective, less costly and less time-consuming they have been widely used for the determination of uric acid in body fluids. Carbon nanotubes-modified-electrodes\(^{14}\), electrochemically pre-treated carbon paste electrodes\(^{15}\), gold nanoparticles modified glassy carbon electrodes\(^{16}\) and nano-coated glassy carbon electrode\(^{17}\) have been developed for the selective determination of UA. Different approaches have been attempted to solve the problem of interference during determination of uric acid\(^{18,19}\). Low detection limits are required because of the low concentration of UA in biological systems.

As electrodes modified using nanoparticles show enhanced conductivity, facilitates the electron transfer and improves the analytical sensitivity and selectivity\(^{20,21}\), an attempt has been made herein to determine UA at gold nanoparticles-modified indium tin oxide (ITO) electrode using differential pulse
voltammetric technique at physiological pH 7.2. Gold nanoparticles and carbon nanotubes are the most popular materials to be attached on electrodes. Due to the small size of gold nanoparticles conductive material comes into close vicinity of the active process and provides improved electrocatalytic activity that can be utilized in biosensor devices. In the present paper, we present a relatively simple differential pulse voltammetric method for the sensitive determination of uric acid on gold nanoparticles-modified indium tin oxide (ITO) electrode at physiological pH of 7.2. The electrode has a strong catalytic function towards the oxidation of UA. The voltammetric response of uric acid in the presence of other biologically common interferents has also been studied.

Materials and Methods

Uric acid, ascorbic acid, adenine, xanthine and hypoxanthine were purchased from Sigma, USA. All these compounds were used without further purification. Phosphate buffer solutions (µ = 0.1 M) were prepared according to the method of Christian and Purdy. All other reagents used were of analytical grade. All solutions were prepared in doubly distilled water.

Scanning electron microscopic image was obtained with a field emission SEM instrument (JSM-7400 F, Jeol Japan). The differential pulse voltammetric experiments were performed on BAS (Bioanalytical Systems, West Lafayette, IN, USA) CV-50W voltammetric analyzer. The electrochemical measurements were carried out in a single-compartment three-electrode glass cell with a gold nanoparticles-modified ITO electrode as the working electrode, a platinum wire as counter electrode and Ag/AgCl electrode as reference electrode (model MF-2052 RB-5B). Gold nanoparticles modified ITO electrodes were prepared by the method reported in the literature. All experiments were carried out at an ambient temperature of 25 ± 2 °C.

A stock solution of uric acid (2 mM) was prepared in doubly distilled water. An aliquot (2 ml) of the stock solution was mixed with 2 ml of phosphate buffer solution (µ = 0.1 M, pH = 7.2) and the differential pulse voltammograms were recorded. The parameters used for recording the voltammograms were as follows: Pulse amplitude 5 mV; Sample width: 2 ms; Pulse width: 5 ms; Pulse period: 200 ms; Initial E: 200 mV; Final E: 1000 mV; Scan rate: 20 mV/s; Sensitivity: 100 nA/V. Uric acid has been reported to adsorb at the surface of pyrolytic graphite, glassy carbon and platinum electrodes and therefore the electrode was kept at zero volts vs. Ag/AgCl electrode in buffer solution for 30-40 s after recording each voltammogram.

Results and Discussion

The typical surface image of nanogold modified electrode observed using a field-emission type scanning electron microscopy (FE-SEM) is shown in Fig. 1. It is recognized that gold nanospheres and nanorods are attached over the surface of the ITO crystals, and effectively modified. Differential Pulse Voltammetric (DPV) study of UA was initially carried out on bare ITO electrode at various pH values. In some cases, a broad bump was observed. The $E_p$ of this bump was centered around 1040 mV, which was not very clear specially below pH 7.2. However, when similar studies were carried out on gold nanoparticles-modified ITO electrode, a sharp

![Fig. 1 — Typical FE-SEM image of the gold nanoparticles-modified ITO electrode.](image1)

![Fig. 2 — A typical differential pulse voltammogram of 25 µM uric acid at nanogold-modified ITO electrode at pH 7.2.](image2)
peak was observed. The peak potential of this peak shifted to less positive potential with increase in pH. At pH 7.2, several voltammograms were recorded and the $E_p$ of well-defined peak was found as 840 ± 10 mV vs. Ag/AgCl electrode. The $dE_p/dpH$ of the linear $E_p$ vs. pH plot was 58 mV/pH. The shift of peak potential (ca. 200 mV) to less positive potentials at pH 7.2 with increased peak current indicates that the oxidation of UA is catalyzed at the gold nanoparticles-modified ITO electrode. A typical differential pulse voltammogram of UA at gold nanoparticles-modified electrode is presented in Fig. 2. As we were basically interested in the physiological pH range, we focused our studies in the pH range 6-8.

It was found during voltammetric experiments that peak current increases with increase in concentration of UA solution. The current versus concentration graph was linear in the concentration range 0.5-100 μM as presented in Fig. 3. Thus, uric acid can be safely estimated in this range at gold nanoparticles-modified ITO electrode. The linear dependence of peak current on the concentration of uric acid can be expressed by the relationship:

$$i_p (nA) = 0.412C, \text{ where } C \text{ is in } \mu M/L. \quad \ldots (1)$$

with correlation coefficient 0.995 and sensitivity 0.0004 nA/μM.

The linear behaviour of $i_p$ versus concentration in the range 0.5-100 μM further indicates that in the case of gold nanoparticles-modified ITO electrode, the process is basically diffusion controlled and not adsorption controlled in the concentration range studied at pH 7.2.

Modification of indium tin oxide surface by gold nanoparticles remarkably improves the reactivity of ITO electrode towards uric acid and ascorbic acid oxidations. Thus, by using gold nanoparticles modified ITO electrode, uric acid can be detected in a solution as low as 0.5 μM, with detection limit (3σ) of 0.5 μM.

**Effect of Interferents**

The voltammetric response of UA generally suffers from interference of ascorbic acid as well as xanthine, hypoxanthine and adenine existing in biological samples. Hence, it was considered desirable to monitor the effect of interferents on the response of $E_p$ and $i_p$ of UA. To monitor the effect of these common interferents, differential pulse voltammograms of UA were recorded keeping the concentration of UA constant at 25 μM and varying the concentrations of ascorbic acid, xanthine, hypoxanthine and adenine. The DPV response for the oxidation of UA (25 μM) was then recorded. The peak current response obtained in these cases is compiled in Table 1. In these studies, two electrodes were used which had practically same area and thus exhibited same peak currents for 25 μM uric acid. Ascorbic acid was found to affect the peak current of UA at concentrations greater than 10 μM. A typical differential pulse voltammetric response of 25 μM UA at the gold nanoparticle-modified ITO electrode.

**Table 1** — Effect of interferents on the differential pulse voltammetric response of 25 μM UA at the gold nanoparticle-modified ITO electrodes

<table>
<thead>
<tr>
<th>Interferents</th>
<th>Conc, μM</th>
<th>Peak current, nA</th>
<th>Change in current response, nA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No interferent</td>
<td>25.0</td>
<td>9.92 ± 0.02</td>
<td>—</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>10.0</td>
<td>9.94</td>
<td>+0.00</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td>12.02</td>
<td>+2.10</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>14.35</td>
<td>+4.43</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>24.26</td>
<td>+14.34</td>
</tr>
<tr>
<td>Adenine</td>
<td>25.0</td>
<td>9.92</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>9.94</td>
<td>+0.02</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>9.95</td>
<td>+0.03</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
<td>9.92</td>
<td>0.00</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>25.0</td>
<td>9.92</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>9.94</td>
<td>+0.02</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>9.95</td>
<td>+0.03</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
<td>9.92</td>
<td>0.00</td>
</tr>
<tr>
<td>Xanthine</td>
<td>25.0</td>
<td>9.92</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>50.0</td>
<td>9.96</td>
<td>+0.04</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>9.92</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>200.0</td>
<td>9.86</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

Fig. 3 — Observed dependence of peak current on concentration of uric acid at pH 7.2 at nanogold-modified ITO electrode.
voltamogram of a solution of uric acid with 100 μM ascorbic acid is presented in Fig. 4. It can be seen that the peak current of uric acid is enhanced and no separate peak is obtained for ascorbic acid. Thus, the $E_p$ of uric acid and ascorbic acid appear to be in the same range and hence a combined peak is seen. However, adenine, xanthine and hypoxanthine did not show any interference up to eight-fold excess. In all cases, the oxidation peak potential of UA remained practically constant and the change in current response in the presence of interferents was within ±0.0348 nA relative to their absence. Thus, the response of uric acid at the gold nanoparticles modified ITO electrode is not affected much by the interferents except ascorbic acid.

Uric acid could be easily detected to a much lower concentration with the help of nanoparticles-modified electrode. The present study demonstrates the use of gold nanoparticle-modified electrode for the study of uric acid in solution. A linear relationship between uric acid concentration and current response was obtained in the concentration range of 0.5–100 μM with excellent reproducibility. The gold nanoparticles-modified ITO electrode shows a stable and reproducible response only up to 7–8 runs from 0.0 to 1.0 V, after which the peak current values starts decreasing probably because nanogold particles deposited on ITO surface start detaching from the surface. Hence, it is necessary that the calibration plot and the determination should be made within these runs. Use of a fresh electrode may lead to a new surface area and hence observed peak currents may be slightly different. In other methods, however, UA was determined in the concentration range of 5–53 μM and uric acid was found to be adsorbed on the surface of exfoliated graphite surface. The present method provides a large range of 0.5–100 μM for determination of uric acid. At nanogold-modified ITO electrode, the peak currents became almost constant at concentrations greater than 100 μM. The results of three successive determinations showed a relative standard deviation of <3%. This value, however, increases significantly if the electrode is used more than eight times. Thus, the limitation of the method is that the electrode cannot be used for a long time. Attempts are on to prepare densely attached gold nanoparticles at ITO electrode to solve the problem.

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