Determination of Uric Acid in the Presence of Ascorbic Acid Using Poly(3,4-ethylenedioxythiophene)-Modified Electrodes

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Abstract
A poly(3,4-ethylenedioxythiophene) (PEDOT) modified glassy carbon electrode (GCE) was used to determine uric acid in the presence of ascorbic acid at physiological pH facilitating a peak potential separation of ascorbic acid and uric acid oxidation (ca. 365 mV), which is the largest value reported so far in the literature. Also, an analytical protocol involving differential pulse voltammetry has been developed using a microchip electrode for the determination of uric acid in the concentration range of 1 to 20 \( \mu \text{M} \) in presence of excess of ascorbic acid.

Keywords: Conducting polymer, Poly(3,4-ethylenedioxythiophene), Uric acid, Ascorbic acid, Voltammetry

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1. Introduction
Uric acid (UA) is a protein metabolism waste present in large quantities in some foods. It causes problems because humans do not possess the enzyme to digest it to a soluble form. When uric acid precipitates it can cause kidney stones or gout. Gout is a problem where uric acid crystals deposit in the joints, causing a painful inflammatory response [1]. Hence, detection of uric acid concentration in biological samples in the presence of other constituents is an important task in analytical research. Different approaches such as colorimetric, enzymatic and electrochemical techniques are reported in the literature to determine UA concentration. Though colorimetric techniques are more popular, the interferences from compounds such as ascorbic acid (AA) and other reducing agents complicate the determination of UA concentration accurately [2]. The enzymatic method for the determination of UA is promising due to its high selectivity but this method is inherently more expensive and could not achieve the required detection limits [3]. Alternatively, the determination of UA by electrochemical methods have received much interest because they are more selective, less expensive and less time consuming than those based on colorimetric and enzymatic methods [4–6].

Determination of UA by electrochemical methods received much interest in the context of more selectivity, sensitivity besides being economic and less time consuming. One of the major problems in the determination of UA is the co-existence of ascorbic acid (AA) in the biological fluids as interference at high concentrations, which also undergoes oxidation at a closer potential [7]. Hence, the detection of UA in presence of excess of AA is a challenging task in electroanalytical research. Several approaches based on electrochemical routes have been reported [8–18], such as electrodes modified with poly(4-vinylpyridine) [8], polypyrrole [9], poly(N,N'-dimethylaniline) [10], polyglycine [11], osmium complex Nafion bilayer [12], clay-Nafion [13], methylene blue/sol-gel [14] and electrodes modified with self-assembled monolayers of thiols [15].

In the realm of modified electrodes, electrodeposited conducting polymer matrices show advantages such as: (a) thin, uniform and adherent polymer films can be obtained; (b) polymer films can be deposited electrochemically on a small surface area with a high degree of geometrical conformity; and (c) deposition can be effected on selected areas, especially in the case of microsensors. Among the numerous polymeric materials developed and studied over the past few decades, polyanilines, polypyrroles and polythiophenes constitute an important class [19]. Among these, polythiophenes have received a significant amount of attention as electrode modifiers for a variety of applications in organic light-emitting devices, sensors, polymer batteries, electrochromic windows, etc. [20–21]. In the course of investigations, we found PEDOT to be resistant to fouling by the AA oxidation products that provided us the impetus to pursue studies on other analytes such as uric acid that is important in the analytical estimation. We recently reported the use of PEDOT modified electrodes for the selective determination of dopamine (DA) in the presence of excess AA [22–23]. In this paper, we report our studies on the selective determination of UA in the presence of AA on a PEDOT modified glassy carbon electrode (GCE) using cyclic voltammetric and differential pulse voltammetric techniques. Further, electroanalysis is demonstrated for uric
acid detection using microfabricated sensor chip containing individual micrometer-scale sensing elements patterned on an alumina substrate.

2. Experimental

2.1. Materials

The monomer 3,4-ethylenedioxythiophene (EDOT, Baytron M) was a gift sample provided by Bayer-AG (Germany). Uric acid (E-Merck), ascorbic acid (E-Merck), tetrabutylammonium perchlorate (TBAPC) (Aldrich), potassium dihydrogen phosphate (E-Merck), sodium hydroxide (E-Merck) were used as received. The aqueous solutions were prepared using Milli-Q water (18.3 Ω) (Millipore).

For voltammetric studies, a 3-mm diameter glassy carbon working electrode (BAS Inc.), a platinum wire coil auxiliary electrode and an Ag | AgCl (3 M NaCl) reference electrode were used and the potential values mentioned in this text are against this reference electrode, unless otherwise mentioned. Phosphate (0.1 M) buffer solutions of pH: 7.4 were employed as aqueous electrolytes.

2.2. Instrumentation

Voltammetric experiments were carried out using a BAS 100B potentiostat/galvanostat (Bioanalytical Systems Inc.) at ambient temperature (25 ± 1 °C). To record differential pulse voltammograms (DPV), the following input parameters were used: scan rate: 30 mV s⁻¹, sample-width: 17 ms, pulse-amplitude: 50 mV, pulse-width (modulation time): 50 ms, pulse-period (interval): 200 ms and quiet-time: 2 s.

PEDOT films (coated on ITO glass substrates supplied by Donnelly Corp., USA) were characterized by PicoSPM Atomic Force Microscopy (Molecular Imaging, USA) operated in contact mode. A gold coated SiN₃ cantilever (Force Constant 0.12 N/m) was used as the force sensor and operated in contact mode. A gold coated SiN₃ cantilever and an Ag | AgCl (3 M NaCl) reference electrode were used and the potential values mentioned in this text are against this reference electrode, unless otherwise mentioned. Phosphate (0.1 M) buffer solutions of pH: 7.4 were employed as aqueous electrolytes.

2.3. Methods

2.3.1. Preparation of PEDOT-Modified GCE

The GCE surface was polished first on a fine emery paper, then with 1.0 and 0.06 μm alumina powder, and finally sonicated in Milli-Q water for 5 minutes. Before electropolymerization, the polished electrode was pretreated by cycling it between −0.9 and +1.5 mV vs. Ag wire at 10 mV s⁻¹ in acetonitrile containing TBAPC for 10 min. Then PEDOT was electropolymerized onto the electrode from a solution of 10 mM EDOT + 0.1 M TBAPC in acetonitrile by potential cycling between −0.9 to 1.5 V vs Ag wire pseudoreference electrode. PEDOT film was allowed to grow on GC surface over five successive scans, as seen from the increasing anodic and cathodic peak current densities. The electropolymerization of EDOT was highly reproducible and the cyclic voltammograms obtained through the electropolymerization agreed closely with that reported earlier [24 – 25]. This modified electrode is hereafter referred to as GC|PEDOT.

2.3.2. Fabrication of Microchip Electrode

The microchip electrode was fabricated on an alumina substrate (8 × 5.0 × 0.6 mm). Before film deposition, the alumina substrate was cleaned using consecutive solvent rinses in acetone, methanol, 2-propanol, and water, blown dry in a stream of N₂, and finally exposed to UV/ozone for 5 minutes. Initially, the microchip electrode pattern was designed having line width and spacing of 250 micron using “L-edit software”. The mask was prepared using screen stencil having 325 Stainless Steel mesh. Pt thick film paste was printed on the alumina substrate. Printed alumina substrate was dried at 150 °C for 10 minutes and fired at 850 °C peak firing temperature. The fired thickness of Pt conductor is about 12 micron. The pattern is shown in Scheme 1. Leads were attached by soldering and protected using Beeswax (Aldrich), leaving the sensing areas open.

3. Results and Discussion

3.1. Characterization of PEDOT Films

The surface characteristics of the PEDOT film coated on an ITO glass substrate were examined by AFM. The film was grown by cycling the electrode potential between −0.9 and 1.5 V vs. Ag wire pseudoreference (5 cycles) in acetonitrile solution containing TBAPC. Figure 1a, b shows the typical topography and its three-dimensional version for the same area of the film. The topographic image gives a clear description of the surface that shows a non-uniform globular structure, which is densely packed. From the horizontal cross section analysis, the minimum and maximum globule size was estimated to be in the range of 50 – 500 nm. The thickness of the film was measured to be approximately 100 ± 20 nm. The surface smoothness of the film was found to have ca. 10 nm variations in the z direction, which shows the deposition of a smooth film.

3.2. Cyclic Voltammetry

Figure 2 shows the cyclic voltammograms (CVs) of UA (1 mM) on GC and GC|PEDOT electrodes. On a bare GC, UA is oxidized at ca. 350 mV yielding an oxidation peak current of 27 ± 3 μA. During the reverse scan, no cathodic peak was observed. The absence of a current peak in the reverse scan shows that the oxidation process is irreversible. On GC|PEDOT, UA undergoes oxidation at 330 mV, yielding an oxidation peak current of 96 ± 3 μA. When compared to bare GC, a small cathodic shift in peak potential for UA oxidation on GC|PEDOT is observed. Moreover, the oxidation peak current increases 3.5 times, which indicates the catalytic oxidation of UA on the modified electrode. On scan reversal, a cathodic peak is observed at 265 mV and $\Delta E_p$ is found to be 70 ± 2 mV, characteristic of a quasireversible process. The potential shifts in the negative direction and the UA oxidation peak current increases compared to that of the bare GC electrode. This shows that the GC|PEDOT electrode catalyses the UA oxidation.

The electrochemical oxidation of UA proceeds in a 2 e\textsuperscript{-}, 2H\textsuperscript{+} process to lead to an unstable diimine species which is then attacked by water molecules in a step wise fashion to be converted into an imine-alcohol and then uric acid-4,5-diol. The uric acid 4,5-diol compound produced is unstable and decomposes to various products depending on the solution pH [26]. Further, the products formed like xanthine or its derivatives can undergo oxidation and the small peak observed at 520 mV can be assigned to the oxidation of the xanthine-related compounds.

The CVs for the oxidation of AA (1 mM) on GC and GC|PEDOT electrodes are shown in Figure 3. On bare GC, AA is oxidized at 220 mV and a peak current of 15 ± 2 μA is noticed. The oxidation peak is found to be broad and no peak in the reverse scan is observed, indicating that the oxidation of AA at bare electrode is irreversible. During successive cycles, the reproducibility of the electrode response is affected possibly due to the fouling effect caused by the adsorption of the oxidation product of AA on the electrode surface.

On GC|PEDOT electrode, AA oxidation peak occurs at around –35 mV, indicating a cathodic shift of 250 mV when compared to the bare electrode. Further, the oxidation peak is sharp on the modified electrode and no peak in the reverse direction is observed. A comparison of peak currents reveals

Fig. 1. AFM images obtained for an as-grown PEDOT film in a 0.1 M TBAPC acetonitrile solution and their cross section analysis. The scan area was 2 × 2 μm.

Fig. 2. Cyclic voltammetric behavior of a) GC|PEDOT electrode in PBS of pH 7.4, b) bare GC in PBS of pH 7.4 containing 1 mM UA, and c) GC|PEDOT in PBS of pH 7.4 containing 1 mM UA. Scan rate: 50 mV s\textsuperscript{-1}. 

that oxidation current at GC$\mid$PEDOT is 2.4 times higher, showing the catalytic oxidation of AA at the modified electrode. The observed large negative shift (ca. 250 mV) of the peak potential and the enhanced oxidation current are attributed to electrostatic interactions between the surface groups and the reactant species in the solution. The cationic sites on the PEDOT film leads to accumulation of ascorbate anions at the interface. This is corroborated by an earlier finding that metal like electrodes with fixed cationic sites can enhance the oxidation rates of anion when compared to bare electrode, by means of electrostatic attraction [27].

The peak potentials for the oxidation of UA and AA at GC$\mid$PEDOT are observed to shift anodically with the increase in scan rate. The plot of oxidation peak current versus the square root of scan rate in the range from 10 to 100 mV s$^{-1}$, yields a straight line passing through origin, which suggests that the oxidation of AA and UA at GC$\mid$PEDOT is diffusion controlled (Fig. 4).

Having examined individually the oxidation behaviour of AA and UA on GC$\mid$PEDOT, details of the interdependence of AA and UA oxidation at the modified electrode is in order. Figure 5 shows CVs for the oxidation of UA (1 mM) and AA (1 mM) on GC and GC$\mid$PEDOT electrodes from a solution containing both. On bare GC, the two species undergo oxidation and their peaks are found to coalesce to appear as a single with a preceding shoulder. On the other hand, on GC$\mid$PEDOT electrode two distinct oxidation peaks are observed at around –30 mV and 335 mV corresponding to the oxidation of AA and UA respectively. In other words, GC$\mid$PEDOT electrode affords a significant peak separation of about ca. 365 mV for the oxidation of UA and AA. The peak separation observed in the case of GC$\mid$PEDOT modified electrode (ca. 365 mV), is the highest for detection of UA on different modified electrodes reported in the literature (Table I).

Thus the large peak separation allows one to measure quantitatively and selectively UA present in the sample. The distinct peak separation achieved above is mainly attributed to the cationic (electrostatic) and hydrophobic characteristics of PEDOT film.

UA has a pK$_a$ value of 5.75 and at physiological pH (pH 7.4), UA exists in its anionic form (urate) and UA is more hydrophobic than AA because the solubility of UA in water is lower than that of AA [10]. Further, it is reasoned

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**Fig. 3.** Cyclic voltammetric behavior of AA (1.0 mM) at a) bare GC and b) GC$\mid$PEDOT electrodes in PBS of pH 7.4. Scan rate: 50 mV s$^{-1}$.

**Fig. 4.** Plot of oxidation peak current vs. square root of scan rate a) 1 mM UA and b) 1mM AA.

**Fig. 5.** Cyclic voltammetric behavior of UA (1 mM) + AA (1 mM) at a) bare GC b) GC$\mid$PEDOT electrodes in PBS of pH 7.4. Scan rate: 50 mVs$^{-1}$.

3.3. Electroanalysis Using Microchip Electrode

The separation of UA and AA in neutral buffer solution was demonstrated for uric acid determination using microfabricated sensor chip containing individual micrometer scale sensing elements on alumina surface. In these studies, we have tried to incorporate polymer materials onto a microelectrode chip that acts as an amperometric sensor. Since the electropolymerization solution is non-aqueous in nature, the device does not suffer from instability as in the case of acidic environment.

Scheme 1 (Sec. 2.3.2) shows a microchip electrode, where platinum was deposited on selected individual micrometer scale sensing elements within the sensor chip. About 1500 μm × 240 μm × 12 μm 3-individually addressable platinum electrode legs were arranged on the alumina substrate to form a 3-electrode configuration.

Electropolymerization provides a means for depositing controlled amounts of sensing materials on desired locations by manipulating the potential and exposed area of the location. This makes possible the patterning of the conducting polymer, a technique that could be attractive, particularly for practical device applications.

To demonstrate the versatility of the electrodeposition technique, we deposited PEDOT from its monomer solution onto the 3-element amperometric sensor configuration (Scheme 1). The packaged device was encapsulated with insulating Beeswax (Aldrich) to electrically isolate the wire bonds prior to use. In this configuration, one element will serve as the sensing element and the other two as counter and reference electrodes, respectively. The reference electrode platform was coated with Ag from a silver-plating solution and anodized in chloride solution to obtain Ag | AgCl. Using this three-electrode configuration, electrodeposition was carried out. A thin film of PEDOT can be easily identified by its blue color. The film has excellent coverage on the plate area. What is significant is that no deposition was observed on the other two microelectrodes, i.e., counter and reference electrodes, suggesting that the electrodeposition is shown as a practical method for patterning PEDOT on miniaturized devices.

The sensitivity and selectivity of the microchip electrode with an electrodeposited PEDOT film was evaluated. Fig. 6 exhibits the DPV of UA and AA oxidation at microchip electrode modified with PEDOT. Here, the uric acid concentration was varied from 1 to 20 μM whereas the ascorbic acid concentration was kept constant. The UA peak current increased linearly with the increase in UA concentration with the correlation coefficient of 0.9883 (N = 10) and sensitivity of 0.48 μA μM⁻¹ which is the minimum detectable concentration using these electrodes (see inset Fig. 6).

The stability of the electrode was checked by immersing it in phosphate buffer solution for 3–4 days and then measuring its response for both AA and UA. The oxidation potentials and peak currents for AA and UA were found to be the same as those obtained at the freshly prepared polymer film-coated electrodes and thus the electrode showed a stable response. The films also appear to be free from fouling by the analytes and/or the oxidized products, as their penetration into the thin film modifier is likely to be minimal (nearly 2%). However, repeated cycling of the electrode in a pH 7.4 buffer is found to completely remove the incorporated analytes. Further, a series of 20 repetitive voltammetric determinations of sample solutions containing 1 μM, UA and 1 mM, AA is found to evaluate the stability of the modified electrode. The variation of peak current was found to be <5%, indicating that the modified electrode is not subject to surface fouling by the oxidation products, which are originally known to foul glassy carbon surfaces. The advantages accruing from the catalytic function of the film improved the selectivity and sensitivity of the voltammetric determination of UA in presence of AA.
concentration of UA.

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6. References


4. Conclusions

In this work, we have presented a convenient approach for forming PEDOT film and demonstrated that the PEDOT polymeric film favors peak separation of UA and AA voltammetric signals. Unlike the bare glassy carbon electrode, the PEDOT coated GC electrode showed a separation of ca. 365 mV in the peak potentials of UA and AA present in the solution. This peak separation can be explained based on a combination of effects such as electrostatic interactions responsible for the negative shift of peak potential of oxidation of AA and weak hydrophobic interactions of UA with the polymer matrix. In effect, the PEDOT modified GC electrode could detect UA at 1 μM even in the presence of AA in concentrations as high as 1 mM, i.e., 1 : 1000 ratio, which reflects the difference in their concentration under physiological conditions. Further, this application was demonstrated using microchip electrode configuration containing individual micrometer scale sensing elements on an alumina surface. The modified electrodes also showed excellent sensitivity, selectivity and anti-fouling property.