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Voltammetric analysis of anti-arthritis drug, ascorbic acid, tyrosine, and uric acid using a graphene decorated-functionalized conductive polymer electrode

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ABSTRACT

Simultaneous voltammetric analysis of anti-arthritis drug (piroxicam, PX) and its major interferences, L-ascorbic acid (AA), tyrosine (Tyr), and uric acid (UA) in a urine sample was carried out using a graphene decorated-functionalized conductive polymer electrode. Graphene oxide (GO) was firstly interacted with an aminopyrimidyl group on the conductive polyterthiophen backbone, then it was electrochemically converted to reduced GO (RGO). The modified surface was characterized employing FE-SEM, XPS, impedance spectroscopy, cyclic voltammetry, and differential pulse voltammetry. The voltammograms of the analytes displayed well-shaped individual oxidation peaks with high catalytic currents along with a large potential separation between AA and UA (+0.29 V), UA and PX (+0.17 V), and PX and Tyr (+0.15 V). The dynamic ranges of AA, UA, PX, and Tyr were between 0.07 - 0.90, 0.03 - 0.52, 0.05 - 0.82, and 0.05 - 0.60 mM with detection limits of 14.50 ± 0.03, 1.86 ± 0.06, 5.29 ± 0.02, and 5.97 ± 0.07 μM, respectively. A noticeable voltammetric signal was obtained for PX in the sample containing interfering species with 500, 300, and 400-fold higher concentrations. The reliability of sensor was evaluated with human urine samples.

1. Introduction

Piroxicam (PX) has been used in several pharmacological and therapeutic applications, because it provides a rapid response for the treatment of many diseases [1]. PX was launched in European markets in 1980 [2] as an anti-arthritic and anti-inflammatory drug, however, it had side effects such as skin rashes, palpitations, dizziness, headache, edema, and tinnitus, and habitual use caused ulcers and bleeding ulcers [1,3]. Hence, the monitoring of the PX concentration in urine, plasma, and serum is important to confirm these side effects. However, AA, UA, and Tyr in clinical samples might be major interfering compounds in selective analysis of PX, because they exist in higher concentrations in body fluids than other biomolecules [4–6]. Thus, the analysis of PX along with major species of Tyr, UA, and AA is beneficial for patients suffering from diseases treated by PX, because their concentration level in the clinical sample may help the clinical diagnosis of disease [7]. Nonetheless, no method was reported for the simultaneous analysis of PX along with the above interfering species. Therefore, we developed a method of the simultaneous analysis of these species.

Diverse methods for the individual analysis of PX, Tyr, UA, and AA in pharmaceutical and clinical samples have previously been reported, including chromatography, spectrophotometry, fluorimetry, and capillary electrophoresis [8–11]. However, these methods have relatively high costs and are time-consuming processes. Meanwhile, electrochemical methods offer several advantages compared to other methods, like high sensitivity, selectivity, less analysis time, low cost, and good reproducibility. Although various electrochemical modified electrodes have been used as a sensor for the detection of PX [12,13], Tyr, UA, and AA [14–16], none of these sensors has been used for the simultaneous analysis of PX with its main interferences (AA, UA, and Tyr). Thus, we tried to prepare a sensitive positively charged sensor probe material composited with an aminopyrimidyl group functionalized-conductive polymer and reduced graphene oxide (RGO) for the selective detection of these negatively charged species.

Conductive polymers (CPs) have broad applications in various fields [17]. We have effectively utilized various types of CPs for different electrocatalytic applications, such as polymer dyes for

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solar cell and sensor substrates [18–22]. However, demand for more enhanced performance of CPs has brought functionalization of itself and the preparation of the composite materials of them. As one of the composite components, graphene has been receiving much attention for sensor applications recently, due to their excellent conductivity because of π-π stacking and synergetic effects with other materials [23,24]. We recently observed that the highly conductive and positively charged surface can be prepared using aminopyrimidyl group bearing CP through the composition of RGO.

The present study was focused on the application of the RGO-CP composite for the simultaneous analysis of PX with AA, UA, and Tyr in human body fluids (urine). Various experimental parameters affecting the analysis using the composite probe were optimized and characterized using voltammety and surface analysis techniques.

2. Experimental

2.1. Reagent and apparatus

The synthesis of 3′-(2-Aminopyrimidyl)-2,2′:5′,2”-terthiophene (APT) was carried out by a previously published method [20]. Tetrabutylammonium perchlorate (TBAP), acetonitrile, L-ascorbic acid (≥99%), uric acid (+99%), piroxicam (≥98%), potassium hexacyanoferrate(II) trihydrate (98.5-102.0%), potassium ferricyanide(III) powder (99.99+%), sodium perchlorate monohydrate (98%), and graphite powders were obtained from Sigma-Aldrich Co. (U.S.A.). L-Tyrosine (+99%) was from Junsei Chemical Co. Ltd. (Tokyo, Japan). All other chemicals for the synthesis of GO, including sulfuric acid, sodium nitrate, hydrogePHn peroxide, potassium permanganate, and hydrochloric acid were purchased from Dae-jung Chem. (S. Korea). The buffer solution was prepared using Na_2HPO_4 and NaH_2PO_4 mixtures (PBS).

2.2. Instruments

Differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were performed using a potentiostat/galvanostat (Kosentech, model PT-2; S. Korea). The electrochemical cell contained with modified sensor probe (dia. 3.0 mm), Pt wire, and Ag/AgCl (saturated KCl) electrodes, which was internally calibrated against the ferrocyanide/ferricyanide redox couple. The impedance spectra were obtained using a EG&G Princeton Applied Research PARSTAT 2263 at an open circuit voltage from 100.0 kHz down to 100.0 mHz and a sampling rate of five points per decade (AC amplitude: 10.0 mV). Images of field emission scanning electron microscope (FE-SEM) were obtained employing a Carl Zeiss SEM (SUPRA 40VP, Germany). The XPS experiments were performed using a VG Scientific ESCALAB 250 XPS spectrometer with a monochromatic Al-Kα source with charge compensation at KBSI (Busan). All spectra were obtained after Ar ion gas etching for 50 sec, and C1s peak (284.6 eV) is used as the internal standard for the calibration.

2.3. Preparation of probe materials

The GC electrode was cleaned by hand-polishing for 3 min on a wet soft polishing cloth with alumina powder (0.5 and 0.03 mm, successively).The electrode substrate was cleaned with water and then sonicated in absolute ethanol followed by water for 2 min before modification. The synthesized APT monomer was confirmed by FT-IR, ^{13}C-NMR, ^{1}H-NMR, and mass spectrometry. The monomer was used to prepare a polymer layer of APT (p-APT) on the electrode in 0.1 M TBAP containing dichloromethane through electrochemical polymerization with five potential cycles from 0.0 to +1.5 V.

The GO was prepared from the graphite powder according to a modified Hummer's method, which has been described in the detail [25], previously. Graphite (5.0 g) and sodium nitrate (2.5 g) were mixed in 120.0 mL of sulfuric acid (95%) and vigorously stirred for 30 min in an ice bath (≤0 °C). Potassium permanganate (15.0 g) was reacted with the mixture with stirring overnight at temperature <20 °C. After that, 150.0 mL of double-distilled water was slowly added, which formed light brownish paste, and the suspension was stirred at 98 °C for one day. 30% hydrogen peroxide was then added to the mixture, and the product was washed with 5% HCl and water, then the GO was filtered and dried under vacuum. Subsequently, the p-APT layer initially was formed on the GC surface from a APT monomer solution, where the polymerization procedure was similar to a previously reported method [26]. 5.0 μL of 1.0 mg/mL GO dispersed in water was drop cast on the p-APT-modified surface and maintained at 40 °C for 20 min. The p-APT/GO surface was reduced to p-APT/RGO by applying a reduction potential to -1.5 from 0.0 V with five potential cycles in 0.1 M PBS (pH7.2).

2.4. Analytical procedure and the analysis in urine

The analysis of PX, Tyr, UA, and AA was carried out a p-APT/RGO sensing probe employing voltammetry. For the CV measurements, 50.0 mV/s of the scan rate was used. DPV measurements were performed in the following conditions; pulse amplitude: 50.0 mV, pulse period: 100.0 ms, pulse width: 50.0 ms, and sampling width: 10.0 ms were used. The voltammograms were then recorded under the above mentioned optimized experimental conditions. N_2 atmosphere is used for all electrochemical measurements at 27 °C. All electrochemical experiments were performed in 0.1 M PBS that has been degassed by purging with nitrogen gas for 20 min before measurements. The urine samples from normal persons were filtered through a membrane (pore size: 0.5 μm, Millipore).

3. Results and discussion

3.1. Characterization of the sensor probe

Scheme 1 presents a schematic representation for modification of p-APT/RGO on a GC electrode. After polymerization, the negatively charged GO was uniformly trapped on the p-APT layer through a charge interaction and π-π interaction. Furthermore, the GO adsorbed on p-APT was electrochemically reduced to the neutral state by applying the reduction potential. A peak was appeared at approximately -1.15 V in the first scan which represented the formation of the reduced form of GO (RGO), and further cycles diminished the reduction peak towards baseline, suggesting that the complete reduction of GO to RGO was achieved on the p-APT layer. This p-APT/RGO surface was confirmed to be positively charged from the enhance CV peak currents of the negatively charged fericyanide ion, and it was used for further electrochemical characterizations and sensor applications. The analytical parameters for PX analysis using the p-APT/RGO-modified surface were optimized in terms of GO concentration (2, 3, 5, 7, and 10 μL), the number of potential cycles for APT electropolymerization (1, 3, 5, 7, and 10 cycles), and drop cast temperature of 25, 30, 35, 40, 45, and 50 °C. The maximum anodic peak current for PX was obtained with p-APT after five potential cycles from the monomer solution, 5 μL of GO, and a drop cast temperature of 40 °C. Thus, all subsequent experiments were performed at the same condition.

To confirm the modification of probe surface, each modified layer was characterized using XPS and FE-SEM analysis. Fig. 1(A) shows FE-SEM images of (a) p-APT, (b) p-APT/GO, and (c) p-APT/RGO surfaces. P-APT clearly exhibits granular-shaped agglomerates after electropolymerization (Fig. 1(A(a))). The surface of drop-coated GO on the p-APT layer does not alter the physical structure or morphology of the GO nanosheets, and similar