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Characteristics of a Poly(thionine) Modified Glassy Carbon Electrode and the Detection of Dopamine and Uric acid

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A simple and sensitive electrochemical sensor for the simultaneous detection of dopamine (DA) and uric acid (UA) in the presence of ascorbic acid (AA) was developed, based on the poly(thionine) (PTH) modified glassy carbon electrode (GCE). The PTH/GCE sensor could clearly separate the electro-oxidation signals of DA and UA from that of AA. The accumulated surface charges of the PTH film and pH of the base solutions were optimized for improved simultaneous quantitative detection of DA and UA. The oxidation peak currents of DA and UA were both linear over the range from 5 to 30 μM in the presence of 200 μM AA. The sensor showed sensitivities of 22.87 and 6.02 μA/cm^2/μM with detection limits (S/N=3) of 0.7 and 1.3 μM for DA and UA, respectively. The sensor obtained interference free signals and excellent recoveries from spiked human urine samples for both DA and UA detection.

Keywords: Simultaneous detection, poly(thionine), dopamine, uric acid, differential pulse voltammetry.

1. INTRODUCTION

Dopamine (DA) and Uric acid (UA) are neurochemical and biomedical compounds that have important roles in many human metabolic processes. In particular, DA is one of the most significant catecholamines, which acts as a neurotransmitter in the brain [1, 2]. Unbalanced DA activity causes brain dysfunction and many diseases such as Schizophrenia and Parkinson's [3]. A high level of UA

also causes diseases such as kidney damage, gout and Lesch-Nyan [4]. Therefore, a sensitive and selective analytical method for their simultaneous detection is highly desirable.

Electrochemical detection methods are much more advantageous over other detection approaches such as optical and chromatographic methods with respect to the sensitivity, selectivity and simplicity [5-11]. The electrochemical oxidation potentials of DA and UA are very similar, so most conventional electrodes (e.g. Au and Pt) cannot distinguish their oxidation potentials because of interference by the ascorbic acid (AA), which coexists in biological fluids at high concentrations [12]. Various materials and methods including conducting polymers (CPs) [13-15], nano materials [16-18], covalent modification [19, 20] and self-assembled monolayers [21, 22] have been suggested for modification of the electrode surface to enhance the detection efficiency of the electrode. Electrochemically, a CP film is known to be one of the most effective materials for surface modification since it is easy to prepare and has strong adherence and chemical stability [23]. In recent years, CP films of poly(aniline), poly(pyrrole), poly(3,4-ethylene-dioxythiophene), poly(evans blue), poly(5-amino-1,3,4-thiadiazole-2-thiol), poly(acid chrome blue K), poly(p-xylenolsulfonephthalein), and poly(tiron) [24-30] have been reported as useful electrode surface modifiers for DA detection.

Poly(thionine) (PTH), electrochemically synthesized and deposited from thionine, is another potential CP for the modification of electrode surfaces. The stability and electrochemical properties of PTH films are dependent on the deposition condition. For example, a PTH film prepared by applying a constant positive voltage in $H_2SO_{4(aq)}$ is not stable, whereas a PTH film prepared by applying a constant oxidation potential, followed by potential sweeping in thionine containing a neutral buffer solution, is stable [31]. PTH films, similarly prepared to modify the screen-printed carbon electrode and the conventional electrode, have been used in H_2O_2 biosensor [31], NADH biosensor [32], glucose sensor [33] and for DNA hybridization detection [34]. Previously, we reported the preliminary results of simultaneous detection of DA and UA with use of a PTH modified glassy carbon electrode (GCE) (PTH/GCE) [35]. In this report, we extended our previous study by optimizing the number of cycles (i.e. accumulated surface charge) for PTH formation and the pH of the base solutions for the quantitative detection of both DA and UA. The results demonstrated that the present PTH/GCE sensor at the optimized PTH surface charge and pH showed even higher sensitivity and lower detection limit for both DA and UA in the presence of highly concentrated AA.

2. EXPERIMENTAL PART

All reagents were obtained in analytical grade and used without further purification. Doubly distilled water from a Milli-Q water purifying system (18 MΩ.cm) was used throughout the experiments. Dopamine hydrochloride, L-ascorbic acid, uric acid, thionine, disodium hydrogen phosphate and sodium dihydrogen phosphate were from Sigma-Aldrich. Phosphate buffer solution (PBS) was prepared according to our previously reported procedure [36]. For voltammetric experiments, unless otherwise stated, a 0.1M PBS (pH 6.5) was used. All experiments were carried out at room temperature.

Electrochemical experiments were performed with a CHI 430A electrochemical workstation. A conventional three-electrode system was used, where an anodized glassy carbon electrode (GCE, 3 mm diam.) or PTH modified GCE, a platinum wire, and Ag/AgCl (*aq.* Saturated KCl) were used as the working, counter and reference electrodes, respectively. Electrochemical impedance spectra (EIS) were obtained using 5 mM $K_3[Fe(CN)_6]$ at + 0.30 V (i.e formal potential, (E^o) of $K_3[Fe(CN)_6]$) in the frequency range of 100 KHz to 10 mHz with the ac amplitude of 5 mV (IM6ex, Zahner-Elektrik GmbH & Co. KG). The measured EIS spectra were fitted to the Randles equivalent circuit using Zview software (version 3.1, Scribner Associates Inc., U.S.A.). Atomic force microscopy (AFM) (PSIA, XE100, Korea) was used to investigate the electrode surface topography. A differential pulse voltammogram (DPV) was obtained by scanning the potential from -0.1 to 0.5 V with 100 mV/s pulse amplitude, 2 ms pulse width, and 1000 ms pulse period.

The preparation of PTH film on GCE is identical to that of the previous report [35]. In brief, GCE was anodized by applying a constant potential (+1.8 V) for 400 s in PBS (pH 6.5) and then, electropolymerized with thionine by cycling the potential between –0.4 and +0.1 V in PBS (pH 6.5) containing 0.5 mM thionine. The resulting shiny and deep violet-blue colored film was very stable and hard to be taken off from the surface unless the electrode was heavily polished.

3. RESULTS AND DISCUSSION

3.1. AFM characterization of the anodized GCE and PTH/GCE

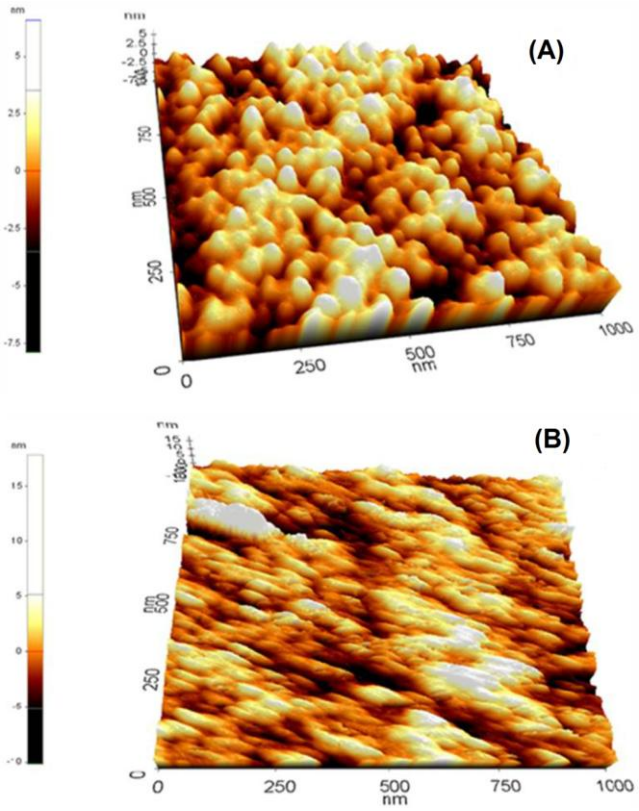

Figure 1. Topographical AFM images of (A) anodized GCE and (B) PTH (20cycles)/GCE.

Figure 1 shows the topographical AFM images of the anodized GCE and the PTH/GCE. The AFM images confirmed that anodization increased the roughness of the GCE [35, 37]. Additionally, anodization increased the amount of oxygen-containing functional groups on the GCE surface [38]. The increased roughness of the anodized GCE facilitated PTH formation. A uniform PTH film covered the anodized GCE surface and reduced its overall roughness, consistent with the previous report [39].

3.2. Cyclic voltammetric and Impedemetric characterization of different PTH/GCE electrodes

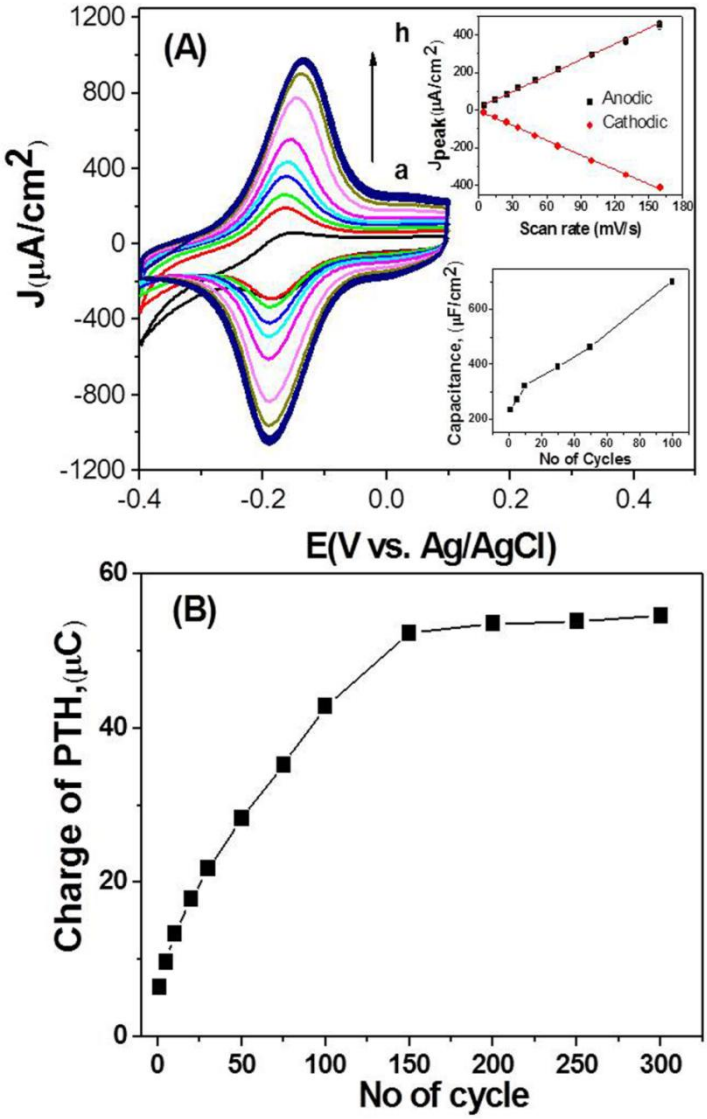

Figure 2. (A) Cyclic voltammograms at an anodized GCE up to 300 potential cycles (a-h) (5, 10, 20, 30, 50, 100, 150, 200-300) for the electropolymerization of thionine. Upper inset shows the plots of PTH (10cycles) peak currents (anodic and cathodic) *vs.* scan rates (5, 15, 25, 35, 50, 70, 100, 130 and 160 mV/s) and lower inset shows the capacitance *vs.* number of cycles. (B) Charge accumulation on the PTH film as a function of number of cycles.

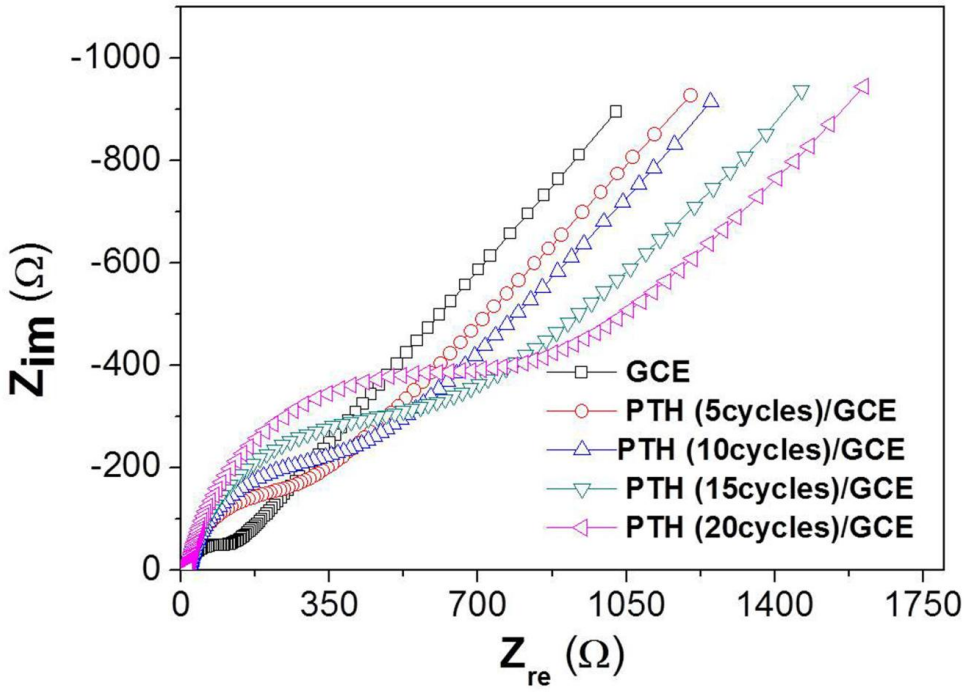

Figure 3. Nyquist plot for GCE and those for different PTH/GCEs with different potential cycles in 5 mM $K_3[Fe(CN)_6]$ containing 1 M KCl.

For the electropolymerization of PTH films, cyclic voltammograms (CVs) with successively increasing numbers of potential cycles are shown in the Figure 2A. The accumulated surface charges for different PTH films were estimated by integrating the voltammograms. The surface charges increased linearly with the number of potential cycles and reached a saturation level after 150 cycles (Fig. 2B). This result was consistent with the continuous increase of the differential capacitance of the PTH/GCE from 233 (1cycles) to 701 μF/cm^2 (100 cycles) (Lower insets, Fig. 2A), calculated from the scan rate dependent charging current. Furthermore, both anodic and cathodic peak currents (i_{pa} and i_{pc}) from the CVs of the PTH/GCE (10 cycles) in PBS increased linearly with scan rate, and the peak currents ratios (i_{pa}/i_{pc}) were almost unity for all scan rates (Upper insets, Fig. 2A). This clearly indicates the formation and growth of the electrochemically active PTH film with highly facile charge transfer functionality [40]. The peak-to-peak separation (ΔE_p) of *ca.* 28 mV (at 10 mVs^{-1}) suggests that two electrons were involved in the PTH redox reaction [29]. The charge transfer resistance (R_{ct}) at the GCE surface increased with increasing number of PTH surface charges or the number of cycles (288.11, 417.75, 517.06 and 881.14 Ω for 5, 10, 20 and 30 cycles, respectively), but was lowest for the anodized GCE (89.26 Ω) (Figure 3). This implies that the electron transfer was less efficient across the thicker PTH/GCE.

3.3. DPV characterization and the optimizations of the number of potential cycles for PTH formation and pH for DA and UA detection

Figure 4(A) shows the DPVs of the mixture solution of AA, DA and UA (500, 20 and 100 μM, respectively) in PBS (pH = 6.5) at both PTH/GCE and anodized GCE. The oxidation waves of AA,

DA and UA at the PTH/GCE were clearly resolved with peak potentials at *ca.* 0.01, 0.18, and 0.31 V, respectively, while an unresolved peak at *ca.* 0.40 V was observed for the mixture at the anodized GCE (Inset, Fig. 4(A)). Although a low oxidation peak appeared at *ca.* 0.26 V, the anodized GCE still could not distinguish the components as easily as the PTH/GCE. The separation of the oxidation peaks at PTH/GCE can be partly attributed to the strong hydrogen-bonding between the analytes and nitrogen atoms of PTH [41].

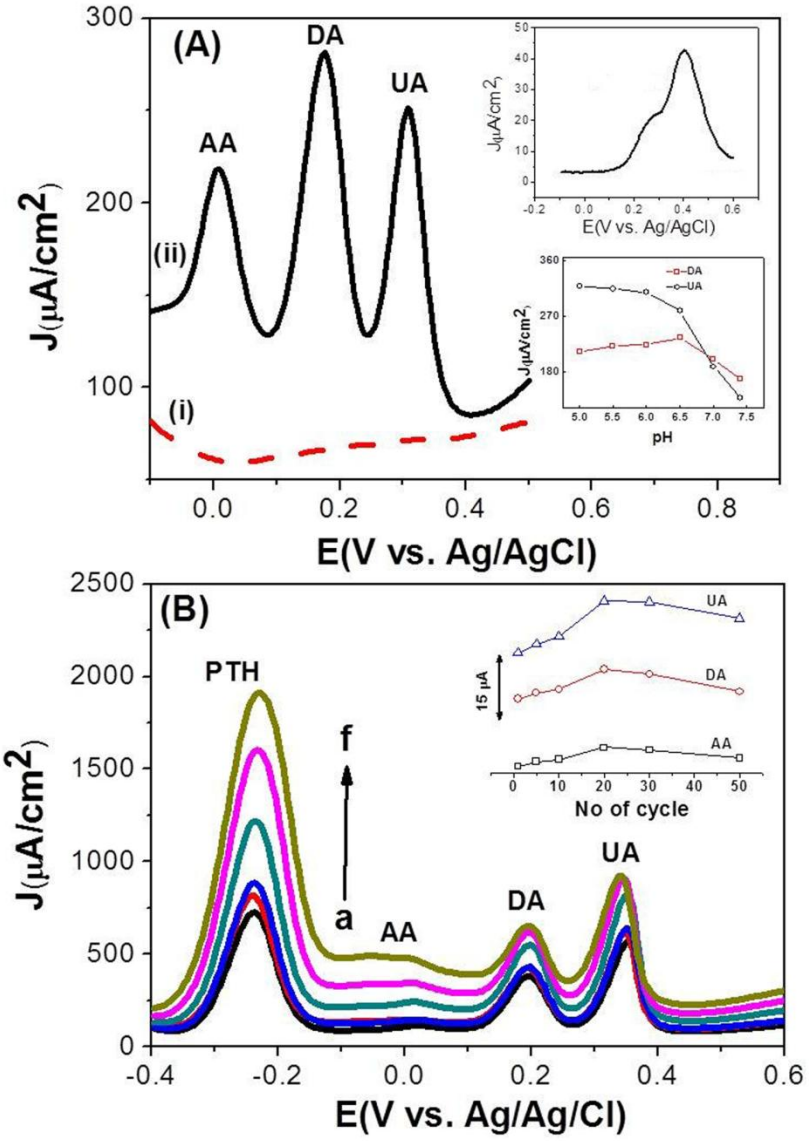

Figure 4. (A) Differential pulse voltammograms (DPVs) for PTH/GCE in PBS (i) and in PBS containing 500 μM AA, 20 μM DA and 100 μM UA (ii). Insets (Upper) show the DPV for the same mixture solution at anodized GCE and (Lower) the pH dependent oxidation peak currents of DA and UA. (B) DPVs for PTH/GCE with different accumulated PTH charges corresponding to potential cycles 1, 5, 10, 20, 30 and 50 (a-f) in PBS containing 200 μM AA, 10 μM DA and 100 μM UA. Inset shows the peak current as a function of the number of cycles.

The peak currents of all analytes were dependent on the number of cycles for PTH formation, and the currents were maximized at the charge corresponding to 20 potential cycles (Fig. 4(B)). DA and UA showed enhanced oxidation behavior above the pH range of 5.0 – 6.5, but their oxidation peak currents decreased with further increase of the pH at the PTH/GCE (20cycles) (Insets, Fig. 4A). Meanwhile, the oxidation peak potentials of DA and UA were shifted negatively with increasing pH, strongly indicating the significant role of protons in the overall electrode processes. Thus, the 20 potential cycled PTH/GCE and the pH 6.5 PBS solution were considered optimal for simultaneous quantitative detection.

3.4. Simultaneous detection of DA and UA

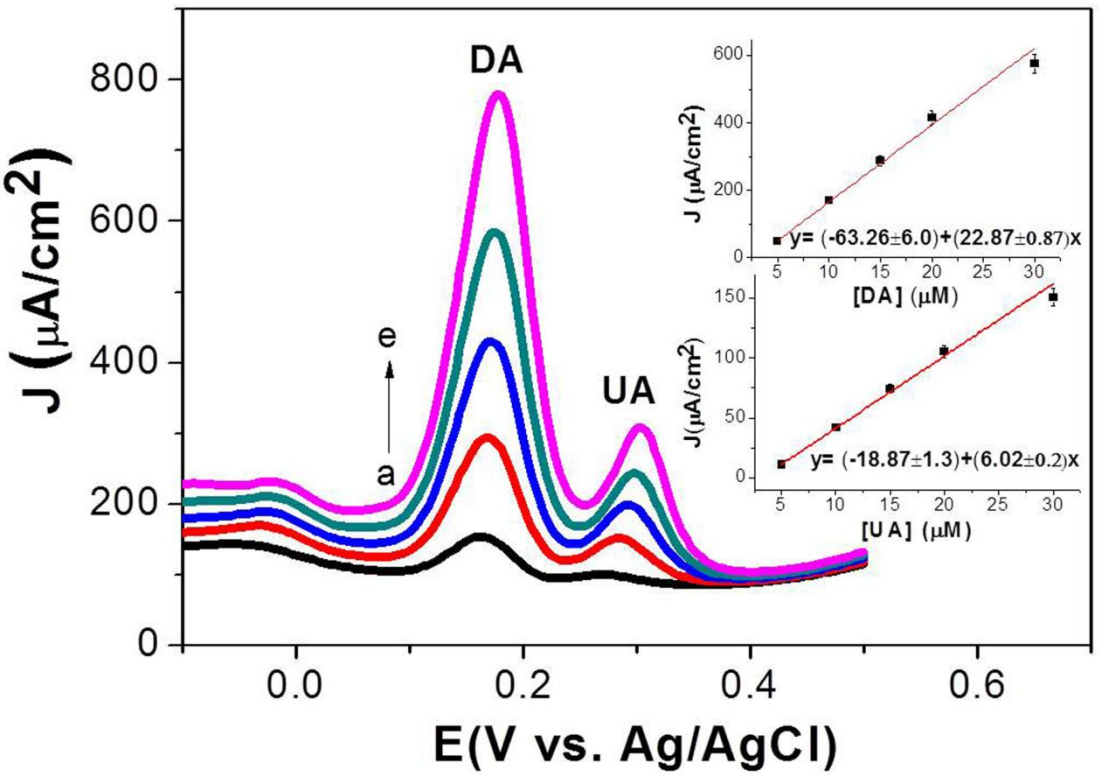

Figure 5. Differential pulse voltammograms (DPVs) of different concentrations of DA and UA mixtures at PTH/GCE in PBS (pH 6.5). DA and UA contents (a-e): 5, 10, 15, 20 and 30 μM. Insets show the calibration plots of DA and UA.

Figure 5 shows the DPV signals of DA and UA in the concentration range from 5 to 30 μM in presence of constant concentration AA (200 μM). The oxidation peak currents of both DA and UA increased linearly with their increasing concentrations (Inset, Fig.5). The sensitivities of DA and UA were 22.87 and 6.02 $\mu A/cm^2/\mu M$, respectively, obtained from their linear regression equations with the regression coefficient of 0.99 for both components. The detection limits (S/N=3) were 0.7 and 1.3 μM for DA and UA, respectively. It is notable that the linear range and the peak currents of DA and UA in the mixture remain almost the same as those of a single component in the same condition. This suggested that the voltammetric responses of DA and UA were independent of each other at PTH/GCE.

3.3. Interference studies and real sample analysis

The PTH/GCE sensor showed an interference free signal for the simultaneous detection of DA (20 μM) and UA (20 μM) in PBS (pH 6.5) in the presence of glucose (1,000 μM), caffeine (1000 μM), ascorbic acid (100 μM), cystine (100 μM), L-cysteine (50 μM) and acetaminophen (50 μM), respectively. Maximum tolerance limits of the foreign substances were considered, inducing a relative signal error of *ca.* ± 5% for DA and UA. The sensor was further tested for its ability to detect DA and UA in human urine samples, to which both analytes of known concentrations were added. The recoveries of both DA and UA were 97.2 – 105.2 % and 97.8 –104.8 %, respectively (Table 1).

Table 1. Recovery results for DA and UA in human urine samples.

Recovery results for DA			
Sample No.	DA added (μM)	DA found (μM)[a]	Recovery (%)
1	10	10.5	105.2
2		9.9	98.7
3		9.7	97.2

Recovery results for UA			
Sample No.	UA added (μM)	UA found (μM)[b]	Recovery (%)
1	10	10.5	104.8
2		10.1	101.4
3		9.8	97.8

[a, b] Average of five measurements.

4. CONCLUSIONS

A highly stable and reproducible PTH film on GCE was obtained by electropolymerization of thionine, following a prior anodization process. This optimal PTH/GCE sensor at the optimal pH showed a wide dynamic range (5-30 μM for both DA and UA), sensitivities of 22.87 and 6.02 μA/cm^2/μM and detection limits of 0.7 and 1.3 μM for DA and UA, respectively, in the presence of

AA (200 μM). The interference free signals and the reliable recoveries from human urine samples suggested the potential practical applicability of this sensor for simultaneous detection of DA and UA.

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