Journal of Electrochemical Science and Technology
Vol. 3, No. 2, 2012, 90-94
http://dx.doi.org/10.5229/JECST.2012.3.2.90

THE KOREAN ELECTROCHEMICAL SOCIETY **Journal of Electrochemical Science and Technology**

Simultaneous Determination of Ranitidine and Metronidazole at Poly(thionine) Modified Anodized Glassy Carbon Electrode

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ABSTRACT :

A simple and sensitive electrochemical sensor for simultaneous and quantitative detection of ranitidine (RT) and metronidazole (MT) was developed, based on a poly(thionine)-modified anodized glassy carbon electrode (PTH/GCE). The modified electrode showed the excellent electrocatalytic activity towards the reduction of both RT and MT in 0.1 M phosphate buffer solution (PBS, pH 7.0). The peak-to-peak separations (ΔE_p) for the simultaneous detection of RT and MT between the two reduction waves in CV and DPV were increased significantly from *ca.* 100 mV at anodized GCE, to *ca.* 550 mV at the PTH/GCE. The reduction peak currents of RT and MT were linear over the range from 35 to 500 μM in the presence of 200 and 150 μM of RT and MT, respectively. The sensor showed the sensitivity of 0.58 and 0.78 $\mu A/cm^2/\mu M$ with the detection limits (S/N = 3) of 1.5 and 0.96 μM, respectively for RT and MT.

Keywords : Simultaneous detection, Poly(thionine), Ranitidine, Metronidazole, Differential pulse voltammetry.

Received June 9, 2012 : Accepted June 25, 2012

1. Introduction

In pharmaceutical drug formulation, ranitidine (RT) is the most frequently used drug to reduce gastric acid secretion under daytime and nocturnal basal conditions.[1)] It is used for the short term treatment of active duodenal ulcer, active and benign gastric ulcer, and for the treatment of photogenic gastrointestinal hypersecretory condition such as Zollinger-Ellison Syndrome.[2)] Metronidazole (MT), the most important derivative of nitromidazole, is a type of cytostatic drugs. It is well known to be effective against trichomonas, Vincent's organisms, anaerobic bacteria, giardias, and amoebiasis with the effective concentration in human serum ranging from 2 to 8 μg/mL.[3)] The combination of these two drugs has been used successfully with antibiotics for the treatment of gastric *Helicobacter pylori* infections.[4)] They have the low binding efficiency (20%) to plasma proteins and their effective plasma concentrations are 100 ng/mL and 6 mg/mL, respectively.[1,2)]

Quantitative detection of RT and MT is difficult and crucial for the bioanalysis in biological fluids and drug formulation due to the need to selectively remove the interferents such as proteins. Various methods such as; spectrophotometry,[5)] atomic absorption spectrometry,[6)] ion selective electrodes,[7)] fluorimetric method,[8)] near infrared reflectance spectrometry,[9)] and chromatography[2)] have been used for the determination of RT and MT. However, lack of selectivity has restricted the application of these methods for pharmacokinetic studies in pharmaceutical and biological samples. Moreover, some of these methods are poorly

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sensitive and require expensive apparatus. Most of them involve several manipulation steps in their course of getting the final result of the analysis. Therefore, it is greatly desirable to develop a simple instrumental method with high sensitivity and selectivity for the simultaneous detection of RT and MT. In this regards, electrochemical methods have showed several advantages including their easiness to operate, high sensitivity and selectivity to the specific analytes, and fast and label-free detection etc.[10)]

RT and MT contain electrochemically active reducible nitro groups that corroborate the development of electrochemical sensor for their simultaneous detection.[1)] Electrochemical detection of these two drugs at carbon fiber microdisk electrode has been reported.[11)] However, the use of bare electrodes for the detection of RT and MT has the limitations of low sensitivity, poor reproducibility, slow electron transfer kinetics, and low stability over a wide range of solution compositions. In addition, high overpotential required usually caused an interference effect.[12)] Meanwhile, the modification of a bare glassy carbon electrode (GCE) with conducting polymers (CPs),[12)] carbon nanotube (CNT),[1)] and metal oxide[13)] offered potential benefits in the context of selectivity, sensitivity, and fast electron transfer kinetics.

In the present study, we developed a poly(thionine) modified anodized glassy carbon electrode (PTH/GCE) for the simultaneous determination of RT and MT. Various experimental conditions (e.g. pH and scan rates) effecting the quantitative detection of RT and MT were investigated.

2. Experimental

2.1. Chemicals

All reagents were obtained as analytical grade and used without further purification. Doubly distilled water obtained from a Milli-Q water purifying system (18 MΩ/cm) was used throughout the experiments. Ranitidine (RT), metronidazole (MT), thionine, disodium hydrogen phosphate (Na_2HPO_4), and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, MS, USA). Phosphate buffer solutions (PBS) of different pH were prepared by our previously reported protocol.[12)] All sample solutions of RT and MT were purged with oxygen free nitrogen (N_2) to get rid of interference effects of dissolved oxygen. All experiments were performed at room temperature.

2.2. Apparatus

Electrochemical experiments were performed with a CHI430A electrochemical workstation (CH Instruments, USA). A conventional three-electrode system was used where anodized glassy carbon electrode (GCE, 3 mm diameter) or PTH/GCE, a platinum wire, and a Ag/AgCl (3MKCl) electrode were used as working, counter, and reference electrodes, respectively. All potentials in this study referred to this reference electrode. Differential pulse voltammograms (DPV) were obtained by scanning the potential at different potential window with the following pulse amplitude;100 mV/s, pulse width; 2 ms, and pulse period; 1000 ms. All electrochemical experiments were performed in 0.1 M PBS (pH 7.0), unless specified otherwise.

2.3. Preparation of the PTH-modified anodized GCE

GCE was first subjected to electrochemical anodization in PBS (pH 7.0) by applying a constant potential +1.8 V for 5 min to enhance its catalytic activity.[10)] Prior to the anodization, GCE was polished with 0.05 mm alumina/water slurry on a polishing cloth (Buekler, Germany) to a mirror-like finish, followed by sonication and rinsed with water. The anodized GCE was then subjected to electropolymerization of thionine by potential cycling from 0.1 to –0.4 V at 50 mVs^{-1} in 0.1 M of PBS (pH 7.0) containing 0.5 mM of thionine (Fig. 1). The potential cycling was applied for 20 cycles to prepare the optimized PTH

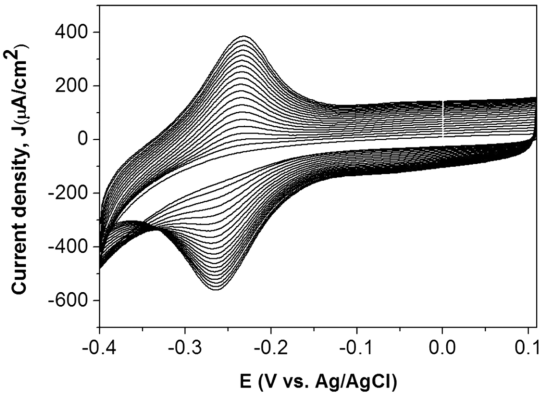

Fig. 1. Cyclic voltammograms for the electropolymerization of thionine at anodized glassy carbon electrode.

amount[14] and the resulting PTH/GCE was rinsed with and stored in PBS (pH 7.0) prior to electrochemical measurements.

3. Results and Discussions

3.1. Electrochemical behavior of RT and MT at PTH-modified anodized GCE

Fig. 2 shows the cyclic voltammetric (CV) responses of individual solution of RT and MT, and co-solution of RT and MT at anodized GCE and PTH/GCE in PBS (pH 7.0). From Fig. 2(A), it was observed that RT was electrochemically active at anodized GCE and showed a reduction peak at *ca.* −0.8 V, while a significantly distinguishable and increased reduction peak current density (J_{peak}) with the negative shifting of reduction peak at *ca.* −1.15 V was observed at PTH/GCE. MT also showed a significantly distinguishable reduction peak and increased J_{peak} at the PTH/GCE compared to the anodized GCE with the reduction peak at *ca.* −0.58 V (Fig. 2(B)). These dramatic increases of the reduction J_{peak} for both RT and MT at the PTH/GCE is attributed to the strong catalytic activity of PTH. In order to investigate the simultaneous determination of RT and MT, CV of the co-solution of RT and MT in PBS (pH 7.0) was measured (Fig. 2(C)). RT and MT showed two clearly distinguishable reduction peak without altering the reduction peak potential at PTH/GCE with a significant increase of reduction J_{peak}, while the reduction of these two drugs at anodized GCE did not showed neither a well- separated peak nor an increased J_{peak}. It is noteworthy that the oxidation and reduction peaks, observed at *ca.* −0.24 and *ca.* −0.28 V, respectively, were arisen from the PTH itself.

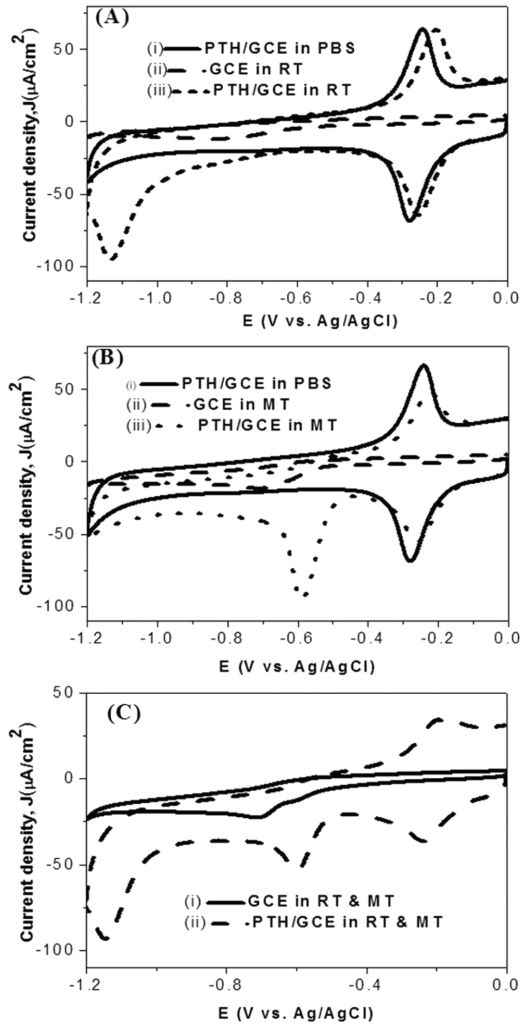

Fig. 2. Cyclic voltammograms of (A) PTH/GCE in PBS (i); 0.5 mM RT at anodized GCE (ii) and PTH/GCE (iii), (B) PTH/GCE in PBS (i); 0.5 mM MT at anodized GCE (ii) and PTH/GCE (iii), and (C) 0.5 mM co-solution of RT and MT at anodized GCE (i) and PTH/GCE (ii). Scan rate 50 mV/s

3.2. Effect of pH

In order to optimize the electrocatalytic response of the PTH/GCE towards RT and MT, the effect of pH on their catalytic reduction were investigated by CV in the pH range of 4.0-9.0 and the corresponding J_{peak} and peak potential were summarized against pH (Fig. 3). With the increase of the pH of the co-solutions of RT and MT, both the reduction peaks were shifted negatively, and maximized the reduction J_{peak} at pH 7.0. Considering the physiological pH, interference which occurs at more negative potential and the nitrogen containing functional group of PTH which may become deprotonated and possesses negative charges at higher pH,[12,15] and the highest reduction J_{peak}, pH 7.0 was chosen for the simultaneous detection of RT and MT.

3.3. Effect of scan rate

Fig. 4 shows the typical CVs of the electroreduction of RT and MT at different scan rate. The reduction peak potentials for both RT and MT were observed to shift negatively with the increase of the scan rate. The J_{peak}s for both RT and MT were directly proportional

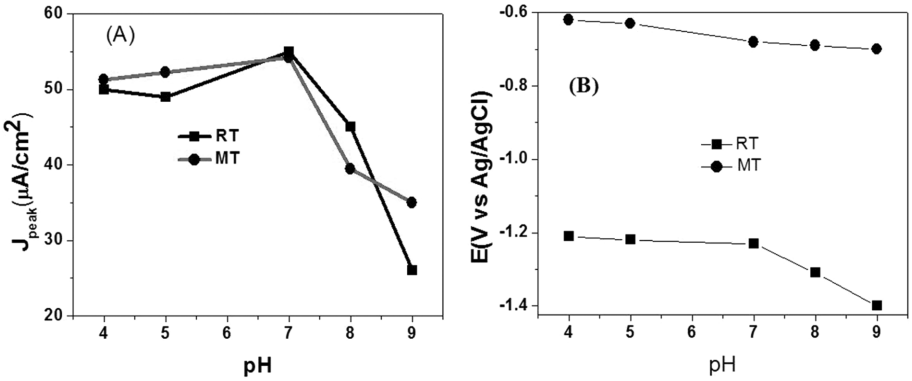

Fig. 3. Effect of pH on the cathodic peak current density (J_{peak}) (A) and peak potentials (B) of RT and MT (0.5 mM of each).

to the square root of the scan rate over the range of 10-120 mV/s with the regression coefficient of 0.96 and 0.99 for RT and MT, respectively. This observation strongly suggests that the reduction of RT and MT were governed by diffusion control.[16)]

3.4. Simultaneous determination of RT and MT using differential pulse voltammetry

Fig. 5 shows the DPVs recorded for various concentrations of RT and MT in the presence of constant concentration of counterpart (200 μM of RT for MT exp.

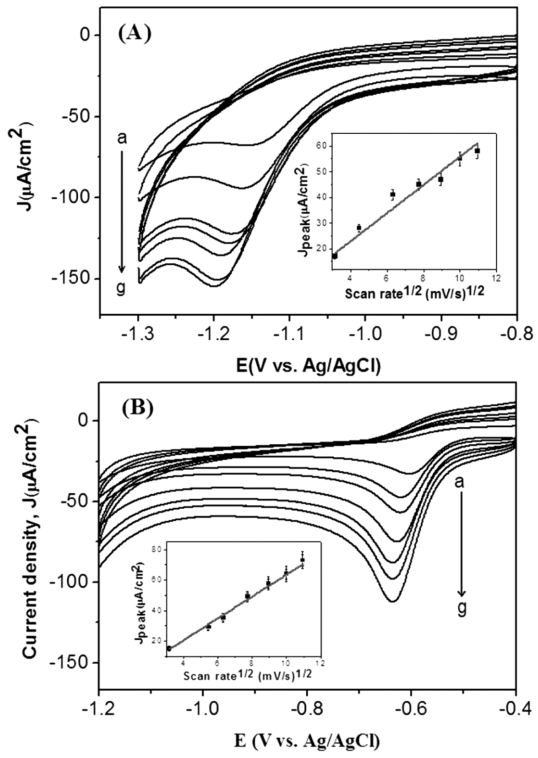

Fig. 4. Cyclic voltammograms of 0.5 mM RT (A), and 0.5 mM MT (B) at PTH/GCE in pH 7.0 at different scan rates (a-g: 10, 30, 40, 60, 80, 100, 120 mV/s). Inset of each graph show the plot of peak current density (J_{peak}) *vs.* square root of scan rates.

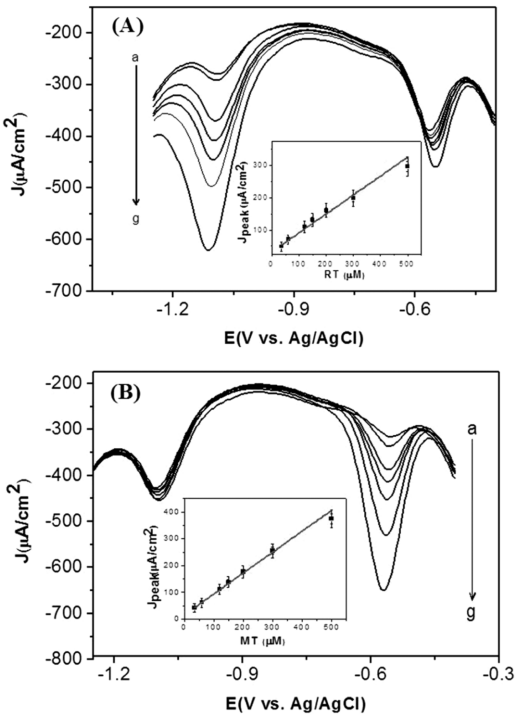

Fig. 5. Differential pulse voltammograms for PTH/GCE in solution of different concentrations (a-g: 35, 60, 120, 150, 200, 300, and 500 μM) of RT containing 150 μM MT (A) and different concentrations (a-g: 35, 60, 120, 150, 200, 300, and 500 μM) of MT containing 200 μM RT (B). Insets show the calibration plots of RT and MT.

and 150 μM of MT for RT exp.). The cathodic current of RT, peaked at *ca.* −1.10 V, increased linearly as the concentration of RT increased, while the peak current of constant concentration of MT at *ca.* −0.55 V, remained essentially unchanged. Similar patterns were observed for MT with the cathodic peak at *ca.* −0.55 V in the presence of RT, which shows no mutual interference. The insets of both panels in Fig. 5 show the calibration plots constructed from the DPV responses. From these results we can infer regression equations as J_{peak} (μA/cm^2) = (31.72±4.27) + (0.58±0.03) [RT] (μM), and J_{peak} (μA/cm^2) = (14.90±2.20) + (0.78± 0.024) [MT] (μM) with the regression coefficient of 0.97 and 0.99, respectively. This corresponded the sensitivity values of 0.58 and 0.78 μA/cm^2/μM with the detection limit (S/N = 3) of 1.5 and 0.96 μM, respectively for RT and MT.

4. Conclusions

A simple and sensitive electrochemical sensor for the simultaneous and quantitative detection of RT and MT was developed using a PTH/GCE. The PTH/GCE showed two well defined reduction waves for RT and MT in both CV and DPV with a peak potential separation of *ca.* 0.55 V, which was largely enough for the simultaneous detection of them. Excellent catalytic activity for the electrochemical reduction of RT and MT were achieved at the PTH/GCE with the sensitivity of 0.58 and 0.78 μA/cm^2/μM, and detection limits of 1.5 and 0.96 μM, respectively for RT and MT.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2012-0001174, 2012047703, 20120007133) and by the Ministry of Education, Science Technology (MEST) and National Research Foundation of Korea (NRF) through the Human Resource Training Project for Regional Innovation. It was also supported by the Converging Research Center Program through the Ministry of Education, Science and Technology (2012K001287) and by Seoul R&BD Program (WR090671).

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