



Selective Determination of Ranitidine in the Presence of Metronidazole at an Activated Glassy Carbon Electrode

Research Article

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Abstract : In this report, a facile and very sensitive electrochemical method has been developed by the anodization of glassy carbon electrode for the determination of ranitidine (RT) selectively in presence of metronidazole (MT). Characterization of surface morphology after the activation of GCE (AGCE) was studied by field emission scanning electron microscopy (FE-SEM), and the surface charge transfer property of AGCE was characterized by cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS) analysis. An excellent catalytic activity of AGCE was observed to reduce RT and MT. The AGCE can separate the reduction peak current of RT and MT in both cyclic voltammogram (CV) and differential pulse voltammogram (DPV). The large value of peak potential separation (ca. 0.5 V) implies the sensitivity of AGCE for the determination of RT in the presence of MT. Moreover, the cathodic current of RT was in a linear relationship within a concentration range 1 to 100 μM in the presence of 100 μM RT. The detection limit ($S/N = 3$) of RT was calculated 0.65 μM from the calibration curve obtained on the basis of reduction peak current in DPV analysis.

Keywords: Selective determination • Activated glassy carbon electrode • Ranitidine • Metronidazole • Voltammetry

1. Introduction

Ranitidine (RT), an active compound in the field pharmaceutical formulation, averts gastric acid secretion that makes it widely exploited for treating duodenal and gastric ulcers. It thoroughly resists the function of histamine on the H₂-receptors of partial cells (Xiaobo and Guangri, 2014). Excessive consumption of ranitidine hydrochloride may cause severe hepatitis, hepatotoxicity, and anarchy in visual accommodation as side effects (William R *et al.* 2011). RT together with metronidazole (MT) has been effectively employed with antibiotics for treating gastric *Helicobacter pylori* infections (M. Mahbubur *et al.* 2012). Drugs education and clean-up them from biological extract are usually the most labored step in bioanalysis due to the need to selectively dispel

interferents. Therefore, the development of highly sensitive and effective analytical method to selectively detect RT in the presence of MT is strongly required and vice versa.

Various traditional methods such as; spectrophotometry (Ekram and Christine, 2020), ion selective electrode (Eman *et al.*, 2011], fluorometric method (D Yin, 2011), chromatography (LOTI D K, 2014), and near infrared reflectance spectrometry (E V Stepanova, 2009] have been exploited to selectively determine RT in the presence of MT. The chromatographic method has complicated instrumental arrangements and generally consumes too much time for a single experiment because additional steps are required for separation and

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pretreatment of analytes before signal detection (Haiyun Z, 2015). On other hand, an additional reagent is required for the signal generation in optical methods. Nevertheless, they are very sensitive but they are not convenient enough for selective determination of such compounds. Furthermore, the mutual interaction of reagents can be one of the reasons for lowering sensitivity for chromatographic or optical signal detection. Thus, a developed and highly sensitive analytical method without any preliminary step for sample preparation is very promising for selective detection of RT in the presence of MT.

Electrochemical techniques are advantageous over optical and chromatographic methods for the selective determination of such mutual interfering compounds, as these methods are not only facile and sensitive but also much reliable, takes very short times to detect analytes and useful for miniaturization of analysis. Not but what considerable attention on the improvement of electrochemical methods have been reported for the determination RT selectively in the presence of MT (Xiaobo and Guangri, 2014) but among them many methods yet facing difficulties because of the overlapping of signals of RT and MT. To resolve the overlapping signal a chemically modified electrode (CME) is a potential substitute (Abdollah S *et al*, 2007). Here introducing a modifier on the surface of the bare electrode significantly differ the extent of interaction from one analyte to other. Although the response of CME is highly selective and sensitive for the selective detection of RT but relatively intricate schemes are required to immobilize the modifiers and also for the optimization steps. In general, higher value of double layer current founds for CME, is not adjuvant for high sensitivity either. To minimize or overcome these problems, development of highly sensitive sensors that do not require any intricate scheme, is crucial for chemical modification.

It is well acquainted, catalytic function of carbon-based electrodes such as GCE can be improved without difficulty by performing various pretreatment or activation processes (T.S. Sunil *et al*, 2018. Hoshyar *et al*, 2017. Jayant I G *et al*, 2017. Tiago A S *et al*, 2016. , Qin X *et al* 2007). Among the different processes, the electrochemical activation process is the easiest process which is proceeded by the anodization of electrode and many works already have been reported where GCE was anodically activated to improve the catalytic activity (Hailemariam K *et al*, 2013. Enrico C *et al*, 2016. Chelladurai K *et al*, 2015].

Here, we are presenting a very facile and sensitive electrochemical method for detecting RT in the presence of MT with an activated GCE. To the best of our

knowing, activated GCE has been successfully used for the first time to detect RT in presence of MT. Therefore, this must be the easiest method ever reported for detecting RT selectively.

2. Experimental

2.1 Chemicals

Ranitidine (RT), metronidazole (MT), Potassium ferricyanide ($K_3[Fe(CN)_6]$), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), were obtained from Sigma-Aldrich (USA) which were in analytic grade and used without any changes. Water Milli-Q purifying system ($18\text{ M}\Omega/\text{cm}$) was employed to extract doubly distilled water which was used throughout the experiment to make solutions and wash the electrode and cell. Neutral solution of phosphate buffer (PBS) was prepared by following the reported procedure (A. J. Saleh *et al*, 2011). Nitrogen gas (N_2) was passed through all the solutions of RT and MT to remove the dissolved oxygen from the solution which may interfere during the electrochemical response. All electrochemical experiments in this report were proceeded at room temperature.

2.2 Apparatus

CHI 660E (CH Instruments, USA) was used as an electrochemical working station to perform electrochemical experiment. A three-electrode system was used to carry out the experiments, where a platinum wire was used as a counter electrode, GCE or electrochemically AGCE and Ag/AgCl (3 M KCl) as a working electrode and reference electrode respectively. The FE-SEM analysis was perform applying JEOL JSM-7600 F, USA. EIS experiment was proceeded within the frequency range 1 Hz-100 kHz. In all the experiments 0.1 M PBS (pH= 7) was used as a supporting electrolyte. Using potential window -0.4 to -1.3V, differential pulse voltammogram was run with 100mV /s pulse amplitude for 1000 ms pulse period.

2.3 Preparation of the activated GCE

To have mirror like finish GGE was polished before anodization, using liquid suspension of 0.05mm alumina slurry on a polishing cloth. After polishing, the electrode was cleaned with the fast flow of DI water. Then the polished GCE was dipped into PBS (pH = 7.0) and applied a constant potential +1.8for 400s. After activation, the color of the surface turned into purple due to the formation of oxide layer on the surface [A. J. Saleh *et al*, 2010]. Here, the activated GCE was washed with 0.1M PBS (pH =7.0) and gently flow of nitrogen gas was used to dry the surface.

3. Results and Discussions

3.1 Surface characterization by FE-SEM analysis

Generally surface morphology of GCE changes after electrochemical anodization [Enrico C et al, 2016]. Here, characterization of surface morphology of GCE and activated GCE was done by FE-SEM analysis. Fig. 1 displays equal magnification FE-SEM images for AGCE (B) and GCE (A)

A smooth and flat surface without any remarkable features was observed for GCE. The FE-SEM image after activation (Fig. 1B) shows densely granular fracture, uniformly distributed over the surface of GCE. Here the formation of the oxide layer after electrochemical anodization produced an uneven surface of GCE.

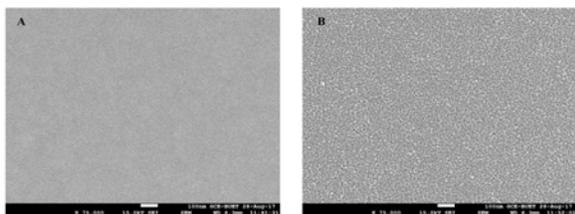


Figure 1: FE-SEM images of the surface of GCE(A) and activated GCE (B)

3.2 CV and EIS characterization of activated GCE

The electrochemical property of GCE after anodic treatment was studied in 5mM $K_3[Fe(CN)_6]$ (in 1mM KCl) by CV and EIS analysis. The results found in CV and EIS analysis are shown in Fig. 2A and 2B, respectively. There was a significant change in the electrochemical property after electrochemical activation of GCE. Fig. 2A shows decreased reduction and oxidation peak current at 0.229V and 0.308V for activated GCE which is consistent with the increasing charge transfer resistance (R_{ct}) obtained from Nyquist plot diameter of the semicircle (Fig. 2B). The R_{ct} value at higher frequency was calculated 80 Ω and 200 Ω for bare

GCE and activated GCE, respectively. Here 2-3 times increase in R_{ct} for activated GCE can be ascribed due to the oxide layer formed on the surface of GCE. The oxide layer developed a negative charge on the surface of the electrode and averted the electron transfer of negatively charged $[Fe(CN)_6]^{3-}$ at interface of activated GCE and aqueous system.

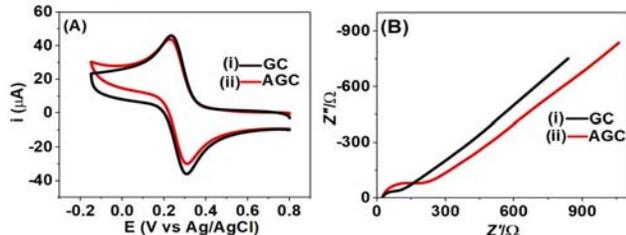


Figure 2: (A) CVs at 50 mV/s and (B) EIS plot for GCE (i) and activated-GCE (ii) in 5mM $K_3[Fe(CN)_6]$

3.3 Electrochemical response of RT and MT at an activated GCE

CV was performed to study the response of RT and MT at an AGCE and a bare GCE in 0.1 M PBS (pH 7.0). Fig.3A and 3B shows results obtained from CVs for 0.5 mM of RT and MT in 0.1 M PBS (pH=7.0) at AGCEs and bare GCE. The reduction peak for RT and MT was founded at ca. -0.8 and -0.7 V for the bare GCE. While for the AGCE, two significantly distinctive reduction peaks with increased peak current at ca. -1.10 and -0.55 V were observed for RT and MT respectively. These dramatic increases of reduction peak currents for both RT and MT at the activated GCE is ascribed to a strong electrocatalytic activity of the oxide layers, which is formed after activation and contains lots of carbonyl and carboxylic functional groups [Xiao-B et al, 2017]. Here, the oxygen containing groups helped to accelerate the electron transfer by forming hydrogen bond (O—H—O) with the nitro group of RT and MT.

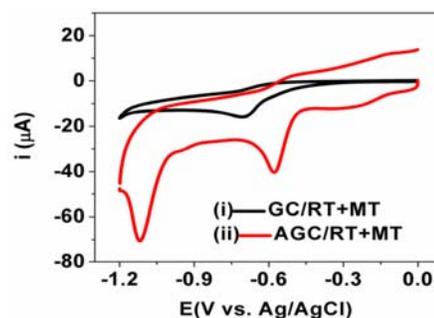


Figure 3: CVs results for (A) 0.5 mM RT in 0.1M PBS (pH 7.0) at bare GCE (i) and AGCE (ii), (B) 0.5 mM MT in 0.1M PBS (pH 7.0) at GCE (i) and AGCE (ii) with scan rate: 50 mV/s.

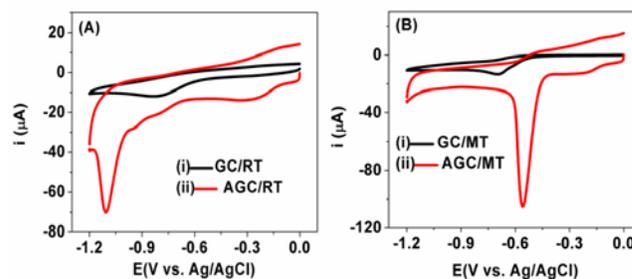


Figure 4: CVs obtained in PBS (pH 7.0) containing a mixed solution of 0.5 mM of RT and MT at bare GCE (i) and AGCE (ii).

Figure. 4 displays the CVs response for the mixture of 0.5 mM of RT and MT in 0.1M PBS (pH 7.0) at the AGCE and bare GCE. The reduction peak of RT and MT were clearly resolved at the AGCE with peak potentials of -1.12 and -0.58 V, respectively. In contrast, the bare GCE showed unresolved and broad peak current at ca. -0.71 V. Overall, we noted that due to the disparity in the

degree of potential shift for RT and MT, the peak current for these two components were entirely resolved. In this case, the peak position of RT shifted towards lower value than the MT at the AGCE.

3.4 Selective determination of RT by using DPV

The results discussed in the above evidently imply that both RT and MT can be detected selectively at an AGCE by performing CV technique. We further observed the catalytic activity of AGCE for quantitative and selective determination of the RT at the AGCE. Fig.5 shows the DPVs responses observed for different concentrations of RT in the presence of constant amount (50 μM) MT. It was observed that the reduction peak current of RT at -1.09 V, increased linearly as the concentration of RT increased while the peak current of MT, at -0.59 V, essentially unvaried. The inset of Fig. 5 shows the calibration plot which was constructed from the DPV responses for RT. A linear relationship was observed within a concentration range from 1 to 100 μM with a regression coefficient of 0.99 for RT was obtained. The reproducibility of electrode performance expressed in terms of relative standard deviation (RSD) of the same electrode for 10 consecutive successive CVs response was 0.40% for 0.1 μM RT.

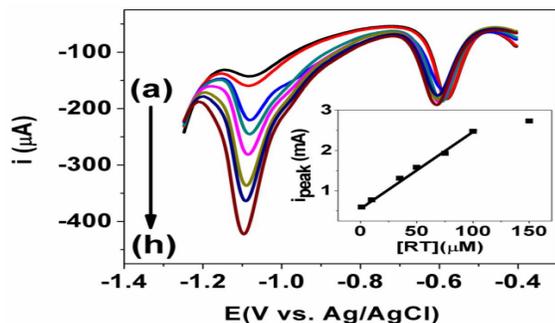


Figure 5: DPVs at AGCE in a mixture containing different concentrations (a – h: 1, 10, 35, 50, 75, 100, 150, 200 mM) of RT and 100 mM MT. Inset shows the calibration plot of RT

In addition, this proposed method was highly reproducible with RSD value 2.65% found by preparing four electrodes in the same condition. After 30 consecutive CV measurements, the reduction peak current of RT was decreased to 96.5% of the elemental response in the same condition. This implied excellent performance durability of AGCE. Moreover, the storage stability of the AGCE was also explored. The electrode was dipped in PBS (pH7.0) and kept in the refrigerator for three weeks at 4 °C temperature, the sensor showed more than 95% of its initial current response. Study of stability analysis implied that AGCE showed excellent storage stability for quantitative and selective detection of RT in the presence of MT.

4. Conclusions

For the selective and quantitative determination of RT a very facile and sensitive electrochemical method is constructed using an activated GCE. This developed method is very simple and reliable than other modification methods of electrode and does not need any extra chemicals. The AGCE could resolve the reduction peak current of RT and MT in both DPV and CV with a peak separation of ca.0.52V and 0.54 V, respectively. The large peak separation value implies that RT can be detected selectively in the presence of MT. Therefore, activated GCE showed excellent catalytic property towards the reduction of RT and MT. The peak current of RT showed an increasing linear response over a concentration range 1 - 100 μM and the detection limit was calculated to be 0.65 μM for RT. More trifles of this work together with optimization, sensing mechanism real sample analysis etc. are in progress.

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References

- Abdollah S., Mohadeseh I., Rahman H., Manochehr R. 2007. Simultaneous Determination of Ranitidine and Metronidazole at Glassy Carbon Electrode Modified with Single Wall Carbon Nanotubes, *Electroanalysis*, 19: 1668–1676.
- Ahmmad, A.J.S., Tamanna I., Md. Mahedi H.M., N. Islam M., Rejwana K., Noyon O., Poly R.P, Subrata S., Dong M.K. 2011. Highly sensitive and simultaneous determination of hydroquinone and catechol at poly(thionine) modified glassy carbon electrode, *Electrochimica Acta*, 56: 5266–5271, 2011.
- Ahmmad, A.J.S., Subrata S., Md Aminur R., Jae J.L. 2010. Simultaneous determination of hydroquinone and catechol at an activated glassy carbon electrode, *Electroanalysis*, 22(6): 694–700.
- Chelladurai K., Rajkumar D., Shen M.C., Danial A., Selvakumar P., Veerappan M., V.S. Vasantha. 2015. Fabrication of Nickel Tetrasulfonated Phthalocyanine Functionalized Multiwalled Carbon Nanotubes on Activated Glassy Carbon Electrode for the Detection of Dopamine, *Electroanalysis*, 27: 485 – 493.
- D Yin X.C., Yue Q.Q. , Li M.D., Chang F.L., Min G. 2011. Determination of ranitidine , nizatidine, and cimetidine by a sensitive fluorescent probe, *Analyst*, 136: 4168–4173.

- Ekram H.M., Christine M.E. 2020. Eco-friendly-assessed TLC-densitometry and absorptivity coefficient based spectrophotometric methods for resolution and simultaneous analysis of two gastrointestinal acting drugs with superimposed spectra, *Microchem. J.*, 158: June, 105161.
- Eman Y.Z.F, A. Ahmed M.K.M., Gehad G.M., Ebtesam E.A. 2011. Construction and Performance Characterization of Ion Selective Electrodes for Potentiometric Determination of Ranitidine Hydrochloride in Pharmaceutical Preparations and Biological Fluids, *Int. J. Electrochem. Sci*, 6: 3508–3524.
- Enrico C., Silvia B., Agnese G., Mery M., Claudia B., Enrico P., Davide V., Ornella A. 2016. Electrocatalysis in the oxidation of acetaminophen with an electrochemically activated glassy carbon electrode, *Electrochim. Acta*, 192: 139–147.
- E.V. Stepanova, A.P. Arzamastsev, A.V. Titova. 2009. Analysis of Ranitidine Hydrochloride by Near Infrared Spectroscopy, *Pharmaceutical Chemistry Journal*, 43(7): 425–427.
- Hailemariam K., Alemnew G., Shimelis A. 2013. Voltammetric Determination Of Nicotine In Cigarette Tobacco at Electrochemically Activated Glassy Carbon Electrode, *Bull. Chem. Soc. Ethiop.*, 27(3): 321-328,
- Haiyun Z., Zhixian L., Zuanguang C., Haihang W., Zhenping L., Zihao S., Qing Z. 2015. Simultaneous detection of metronidazole and chloramphenicol by differential pulse stripping voltammetry using a silver nanoparticles/sulfonate functionalized graphene modified glassy carbon electrode, *Electrochim. Acta*, 171: 105–113.
- Hoshyar S.A, Abdullah A.A., Pınar T.P., Yavuz Y., Zühre S. 2017. Simultaneous voltammetric determination of vanillin and caffeine in food products using an anodically pretreated boron-doped diamond electrode: Its comparison with HPLC-DAD, *Talanta*, 170: 384-391.
- Jayant I.G, Girish S.H, Sharanappa T.N. 2017. Pretreated Graphite Pencil Electrode Based Voltammetric Sensing of Albendazole, *Analytical Chemistry Letters*, 7(3): 389 – 401.
- Loti D.K, Roderick B.W. 2014. HPLC Method for Simultaneous Analysis of Ranitidine and Metronidazole in Dosage Forms, *Analyst*, 26(2): 426–430.
- Md. Mahbubur R., Xiao B.L, Young D.J., Soo J.L., Jae J.L. 2012. Simultaneous Determination of Ranitidine and Metronidazole at Poly (thionine) Modified Anodized Glassy Carbon Electrode, *Journal of Electrochemical Science and Technology*, 3(2) 90–94.
- Qin X., Ren X.G., Chen Y.W., Xiao Y.H. 2007. Application of activated glassy carbon electrode for the detection of nuciferine in lotus leaves, *Talanta*, 73: 262-268.
- Tiago A.S., Gabriel F.P., Orlando F.F., Katlin I.B.E., Giancarlo R.S.B. 2016. Electroanalytical sensing of indigo carmine dye in water samples using a cathodically pretreated boron-doped diamond electrode, *Journal of Electroanalytical Chemistry*, 769: 28–34.
- T.S. Sunil K.N., B.E. Kumara S. 2018. Pre-treated glassy carbon electrode sensor for catechol: A voltammetric study, *J. Electroanal. Chem.*, 826: 23-28.
- William R., de Araújo, Thiago R.L.C.P. 2011. Amperometric detection of ranitidine using glassy carbon modified with ruthenium oxide hexacyanoferrate adapted in a flow injection system, *Electroanalysis*, 23(11): 2549–25542012.
- Xiao B.L., Md. Mahbubur R., Chuang Y.G., Guang R.X., Jae J.L. 2017. A Poly(trypan blue)-Modified Anodized Glassy Carbon Electrode for the Sensitive Detection of Dopamine in the Presence of Uric Acid and Ascorbic Acid, *J. Electrochem. Soc.*, 164(2): B34–B39.
- Xiao B.L., Guangri X. 2012. Simultaneous determination of ranitidine and metronidazole in pharmaceutical formulations at poly (chromatropone 2B) modified activated glassy carbon electrodes, *J. Food Drug Anal.*, 22(3): 345–349.