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Electrocatalytic Oxidation and Determination of Insulin at Rhodamine B –Multi-Walled Carbon Nanotubes Modified Glassy Carbon Electrode

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Abstract

The modified electrode surface was provided with the electropolymerization of the rhodamine B in the presence of carbon nanotube (CNT). The modified electrode offers dramatic improvements in the stability and sensitivity of voltammetric measurements of insulin compared to the bare and rhodamine B modified glassy carbon electrodes. The enhanced electrocatalytic activity towards insulin is indicated from lowering the potential of the oxidation process (starting around 0.75 vs. Ag/AgCl) and the substantially higher sensitivity over the entire potential range. A linear dynamic range (100–600 nM) was achieved with a detection limit of 5 nM. The accuracy of the modified electrode was indicated by insulin recovery test in the real samples as human plasma and pharmaceutical samples.

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Keywords: Insulin; Multi-Walled Carbon Nanotubes; Rhodamine B; Glassy Carbon Electrode; Electrochemical determination.

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1. Introduction

Insulin is an important polypeptide hormone with extensive effects on both metabolism and several other body functions which regulates the metabolism of glucose, Wang and musameh (2004). The electrochemical oxidation of insulin has been well established as a basis for monitoring cellular processes, Kennedy et al. (1996). Diabetes, as one of the most prevalent and costly diseases in the world, is caused by the insufficient release of insulin or loss of insulin action at target tissues which results in aberrant glucose and lipid metabolism. Therefore, detection of insulin is of critical importance in clinical diagnostic. Direct electrochemical measurements of insulin are of considerable interest in connection to the development of time-resolved in vivo sensors and chromatographic detectors for monitoring insulin secretion and therapeutic insulin formulations. However, such measurements are limited because of the slow kinetics of insulin oxidation at common electrode materials. Various chemically modified electrodes have thus been developed for promoting the oxidation and detection of insulin, Zhang et al. (2005), Pikulski and Gorski (2000), Wang and Musameh (2005). There are high attractions in the development of modified electrodes improve the electrochemical properties and biocompatibility of electrodes in the area of biomaterial sensing, He and Zheng (2012). Rhodamine B (RB) is used in biology as a staining fluorescent dye, sometimes in combination with auramine O, as the auramine-rhodamine stain to demonstrate acid-fast organisms, notably Mycobacterium.

In this study, rhodamine B was electro-polymerized on the glassy carbon electrode in the presence of carbon nanotubes. The effective catalytic oxidation of insulin and highly sensitive and stable voltammetric insulin response at the modified electrode was obtained.

2. Experimental

All chemicals were purchased from Merck and Sigma-Aldrich. The glassy carbon disk working electrode (nominal area of 0.0314 cm² Azar electrode Co. Urmia, Iran) was polished using aqueous slurries of alumina, sonicated in water/ ethanol/water each for 3 min, and then rinsed by double distilled water, thoroughly. Then electropolymerization of RB on the clean surface of the GCE was performed in PBS (pH 3) containing 1 mM RB and 1 mg MWCNTs by cyclic potential sweep in the range of 0.3 to 1.1 V at a scan rate of 100 mVs⁻¹. The potential was continuously cycled until a minimum value of current, which remained constant after further cycling, was observed. This indicated that the electrode surface was completely covered by the polymer. After electropolymerization, the modified electrode was rinsed thoroughly with distilled water and used for electrochemical measurements.

A conventional three electrode cell, consisting of bare or poly-RB/MWCNTs modified GC electrode as working, Ag/AgCl, KCl (saturated, Metrohm) as reference, and a platinum bar (Metrohm) as auxiliary electrode, was used for electrochemical measurements. The measurements were carried out using Potentiostat/Galvanostat Autolab 12 interfaced with a personal computer, and controlled by GPES 4.9 software (Eco Chemie BV, Utrecht, The Netherlands). All potentials mentioned in this paper refer to this reference electrode.

3. Results and Discussion

The nonconducting polymers are those with high resistivity that have been prepared by electropolymerization. The growth of such polymers is self-limited and the film that is formed is much thinner than typical conducting polymer films. Because the thickness of non-conducting polymers is only 10–100 nm, electroactive species diffuse rapidly to the including electrode surface. Also, the nonconducting films are permselective, which might be useful in preventing interfering species from approaching or contaminating the electrode surface. Therefore, fast response time and high selectivity could also be expected for non-conducting polymers modified GCE. The electrochemical behaviour of insulin at the surface of bare GCE, and poly RB/MWCNTs modified GCE has been investigated by CV technique in 0.1 M PBS (pH 3.0). According to Fig. 1, the CV of bare GCE exhibit irreversible behavior with only one broad anodic peak in potential 0.97 V (curve a), while the corresponding peak at the surface of poly RB/MWCNTs GCE is at the 0.82 V (curve b). The comparison of these two curves shows the peak potential of insulin oxidation at the surface of modified electrode shifts by about 0.15 V toward negative values compared with that at the bare electrode.

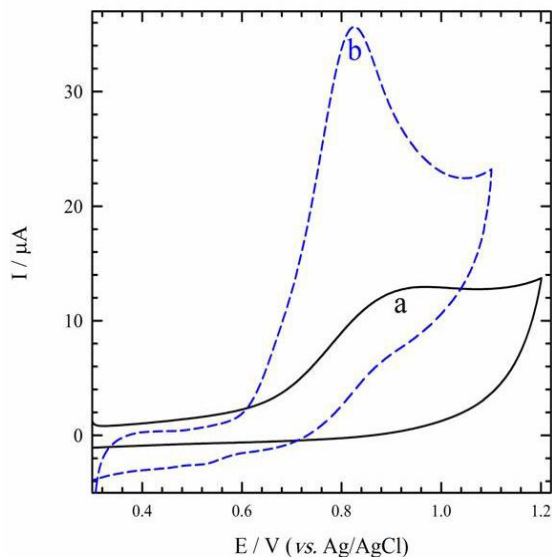


Fig. 1. Cyclic voltammograms of (a) bare GCE; (b) poly RB/MWCNTs modified GCE in buffer solution (pH 3.0) containing 50.0 μM insulin. The scan rate is 100 mVs^{-1} .

Moreover, the related peak currents on the surface of bare GC, show sluggish and small responses, while, substantial increases in the peak currents were observed due to the improvements in the reversibility of the electron transfer processes which suggests an efficient oxidation-reduction reaction of insulin at the modified poly RB/MWCNTs GCE.

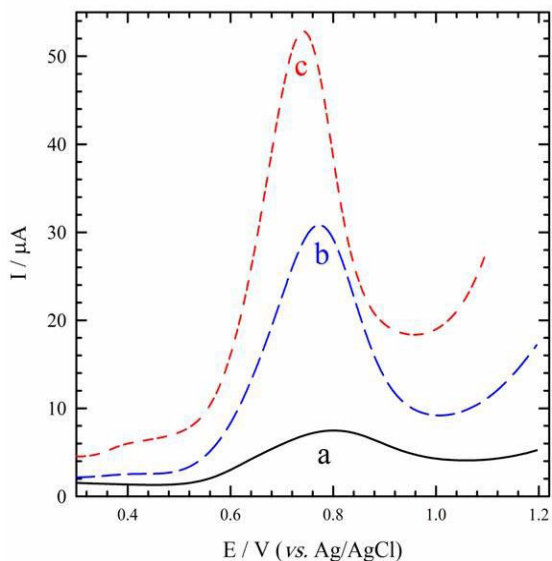


Fig. 2. Differential pulse voltammograms of (a) bare GCE; (b) RB and (c) RB/MWCNTs modified GCE in buffer solution (pH 3.0) containing 50.0 μM insulin. The scan rate is 100 mVs^{-1} .

Since differential pulse voltammetry (DPV) has a much higher current sensitivity, and better resolution than cyclic voltammetry, it was used in study of insulin oxidation at the bare GCE, and poly- RB and poly- RB/MWCNTs modified GCE electrode (Fig. 2). The results are same as CV results. All those above results reveal that poly RB/MWCNTs GCE is more efficient and has exhibited enhanced functional properties comparing to that of bare GCE for determination of insulin. Where, the increase in current and decrease in potential, both are considered as the enhancement of electrocatalysis. Also DPV method was applied for the quantitative insulin analysis. Under the optimum conditions, the DPVs of various concentrations of insulin were recorded. The respected calibration curve of the anodic peak currents for solutions containing different amounts of insulin was plotted and the linear ranges of 100-600 nM were obtained. The detection limits ($S/N = 3$) was 5 nM for insulin at the poly- RB/MWCNTs modified GCE electrode.

4. Conclusions

In this study, it was shown that poly RB/MWCNTs film on the GCE can be considered as a sensitive and selective sensing element in voltammetric determination of insulin. Our results show that the poly RB/MWCNTs modified GCE exhibited excellent electrocatalytic activity towards the oxidation and determination of insulin.

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