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Procedia Engineering 47 (2012) 1235 – 1238

Procedia Engineering

www.elsevier.com/locate/procedia

Proc. Eurosensors XXVI, September 9-12, 2012, Kraków, Poland

Voltammetric Sensor for Direct Insulin Detection

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Abstract

This work covers an area of planar electrochemical sensors for fast and reliable detection of species in environment under field conditions or in vitro/vivo biodetection. In this work, there were fabricated several planar carbon working electrodes using standard thick film technology which were modified with multiwalled carbon nanotubes (MWCNTs) to promote the electrochemical oxidation of insulin. These standalone planar working electrodes were successfully used for direct detection of insulin using cyclic voltammetry in electrochemical cell against conventional Ag/AgCl reference electrode and platinum auxiliary electrode.

Keywords: Electrochemical sensor; working electrode; insulin; electrochemical detection , voltammetry, MWCNTs

1. Introduction

Insulin is a very important polypeptide hormone which controls glucose level in blood and it is used for the treatment of insulin-dependent type I diabetes. The standard analytical methods for insulin detection include bioassays, immunoassays, and chromatography. These methods are time-consuming and slow and frequently require derivatizations of insulin with isotopes or fluorogenic labels to increase the sensitivity and selectivity [1, 2]. Direct and rapid electrochemical insulin detection is therefore very interesting. Several articles about in vitro electrochemical insulin detection using modified common electrodes as a sensing element have been already reported [2, 3], but no work describing an electrochemical three-electrode sensor especially made for insulin detection have been reported. The aim of our work is to

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doi:10.1016/j.proeng.2012.09.376

prepare a disposable electrochemical sensor for direct insulin determination in aqueous solutions which could be also used as a base for intelligent sensors and biosensors [4, 5]. This paper reports on the fabrication of planar multiwalled carbon nanotubes (MWCNTs) thin layer modified carbon working electrodes (WE) using standard thick film technology (TFT) suitable for direct voltammetric detection of insulin. Characterization of fabricated microelectrodes and preliminary results of insulin voltammetric detection using these electrodes are presented here.

2. Materials and Methods

2.1. Electrode substrate fabrication

Working electrode substrate was designed as a working electrode of three-electrode planar sensor system [6] deposited on alumina substrate using standard TFT. Because the output current response depends on the size of the electrode active area, the electrode diameter was designed as large as possible. The diameter of the WE was chosen to be 3 mm. Working TFT microelectrodes were fabricated from polymer thick-film pastes on the alumina substrate. The DuPont paste 7102 (DuPont, USA) was used for contact, ESL 243-S (ESL Electroscience, UK) for covering layer and DuPont BQ 221 for WE fabrication.

2.2. Electrode substrate modification

2 mg of MWCNTs (Sigma-Aldrich, OD = 40-70 nm, ID = 5-40 nm, lenght = 0.5-2 μm, purity 95 %) was dissolved in 1 mL of N,N-dimethylformamide (DMF, Penta), and sonicated for 1 h. Then, 2 μL of the resulting MWCNTs/DMF dispersion were drop-casted on the planar carbon WE surface. The modified WE was allowed to dry at room temperature for at least 2 h.

2.3. Voltammetric measurement

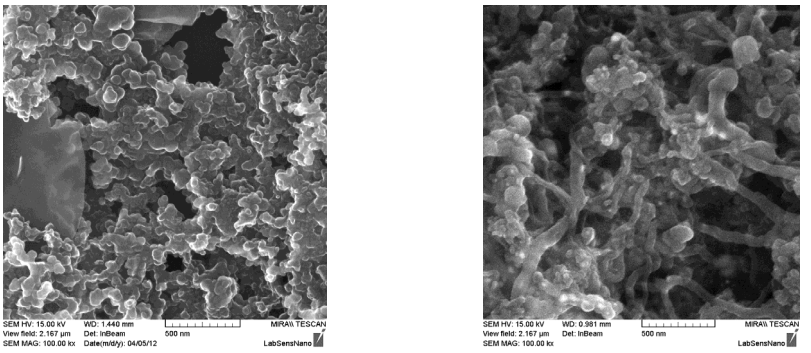
Electrochemical detection was carried out in a three-electrode voltammetric cell using 0.05 $mol.L^{-1}$ phosphate buffer solution (pH 7.4) as a supporting electrolyte against standard Ag/AgCl reference electrode (RE) and platinum auxiliary electrode (AE). The cyclic voltammetric (CV) analysis was done using PalmSens potentiostat in potential range from 0 to 1 V with scan rate of 50 $mV.s^{-1}$. Stock solution of insulin with concentration of 0.50 $mmol.L^{-1}$ was prepared by dissolving powdered insulin from porcine pancreas (Sigma-Aldrich, ≥ 27 USP units/mg) in 0.02 M HCl (Penta) containing 0.02 % (v/v) Tween-80 (Merck). This stock solution was further diluted with 0.05 M phosphate buffer (pH 7.4) solution to make working solutions with desired concentrations.

3. Results and discussion

New planar working microelectrodes were fabricated using standard polymer TFT (see Fig 1) and their surface was modified with MWCNTs. Both types of electrodes were examined optically using SEM. Bare surface of BQ221 carbon electrode is shown in the Fig 2a and the electrode surface modified with MWCNTs is shown in the Fig 2b. From the Fig 2b is clear that the modification was successful and we obtained high-porous electrode surface full of free MWCNTs.

Fig. 1. carbon TFT electrode underlay made of special polymer BQ221 paste (DuPont)

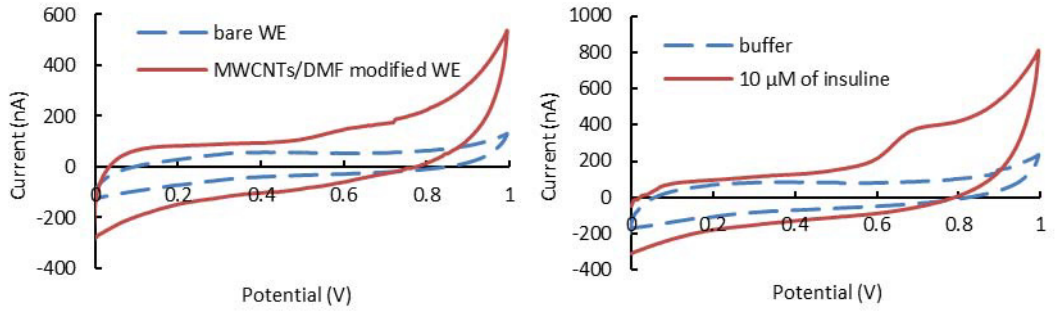
The electrochemical characterization of all electrodes was done using CV in 0.05 mol.L^{-1} phosphate buffer solution (pH 7.4). Obtained voltammograms for bare carbon TFT underlay and MWCNTs/DMF modified electrode are shown in the Fig 3a. From the Fig 3a is clear that the current response of modified electrode is just a little bit higher than the signal from bare electrode.



(a) (b)

Fig. 2. SEM microimages of fabricated electrodes, magnification 100 kx, (a) carbon TFT underlay BQ221 (DuPont); (b) MWNTs/DMF modified electrode

The basic detection of insulin on the MWCNTs/DMF modified electrode was done on sample with higher insulin concentration in order to examine the detection abilities of the electrode. Example of current response to 10 µmol.L^{-1} addition of insulin into the buffer solution is shown in the Fig 3b. The CV response shown in the Fig 3b confirmed that the MWCNTs/DMF modified electrode is able to detect insulin.



(a) (b)

Fig. 3. (a) CV at the bare carbon TFT underlay (DuPont BQ221) (dashed line) and MWCNTs/DMF modified electrode (continuous line) in 0.05 mol.L^{-1} phosphate buffer solution (pH 7.4) using a scan rate of 50 mV.s^{-1}; (b) CV response of MWCNTs/DMF modified electrode to buffer solution (dashed line) and to 10 µmol.L^{-1} insulin solution (continuous line) using a scane rate of 50 mV.s^{-1}

Subsequently the detection limit and calibration curve of modified electrode have been investigated. We were able to determine insulin from the concentration of 250 nmol.L^{-1}. The obtained calibration curve is shown in the Fig 4. We obtained linear calibration curve in the range of insulin concentration from 250 nmol.L^{-1} to 1.6 µmol.L^{-1}.

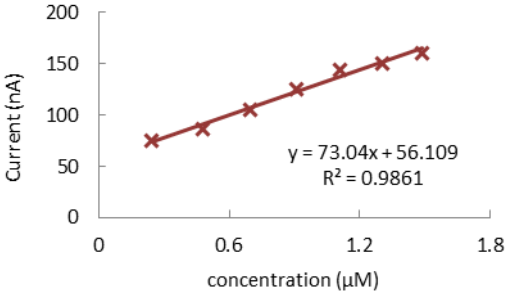


Fig 4 Calibration curve of planar thick film MWNTs/DMF modified electrode obtained for insulin concentrations from 250 nmol.L^{-1} to 1.6 μmol.L^{-1} using a scan rate of 50 mV.s^{-1}.

4. Conclusion

The planar carbon working microelectrodes modified with MWCNTs/DMF to promote the electrochemical oxidation of insulin were fabricated. Our experiments using cyclic voltammetry confirmed the possibility of electrochemical insulin detection on our working electrode. We were able to determine insulin from the concentration of 250 nmol.L^{-1}. We obtained linear calibration curve in the range of insulin concentration from 250 nmol.L^{-1} to 1.6 μmol.L^{-1}. Considering these first results could be concluded that there is high possibility to fabricate special three-electrode sensor for fast insulin detection.

Acknowledgements

Financial support from NANIMEL GACR 102/08/1546, and CEITEC CZ.1.05/1.1.00/02.0068 is highly acknowledged.

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