Portable cholesterol detection with polyaniline-carbon nanotube film based interdigitated electrodes

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Abstract

Polyaniline-carboxylic multiwalled carbon nanotubes composite film (PANi-MWCNT) has been polymerized on the surface of interdigitated platinum electrode (fabricated by MEMS technology) which was compatibly connected to Autolab interface via universal serial bus (USB). An amperometric biosensor based on covalent immobilization of cholesterol oxidase (ChOx) on PANi–MWCNT film with potassium ferricyanide (FeCN) as the redox mediator was developed. The mediator helps to shuttle the electrons between the immobilized ChOx and the PANi-MWCNT electrode, therefore operating at a low potential of $-0.3 \, \text{V}$ compared to the saturated calomel electrode (SCE). This potential precludes the interfering compounds from oxidation. The bio-electrode exhibits good linearity from 0.02 to 1.2 mM cholesterol concentration with a correlation coefficient of 0.9985.

Keywords: cholesterol, polyaniline (PANi), interdigitated electrodes (IDE), carbon nanotubes (CNT)

Classification numbers: 2.04, 5.10, 5.11, 5.14, 6.09, 6.12

1. Introduction

A biosensor is an analytical device that converts the concentration of the analyte into a signal (e.g. electrical signal) by integrating a biological sensing element into a transducer [1]. A very important advancement in producing biosensor membranes is the use of electrically conducting membranes that contain the enzyme, cofactor and mediator. The conducting polymers have recently been applied to biosensors since they can be used both as immobilization matrix and as redox system for the transport of electrical charge. With the discovery of carbon nanotubes (CNT) by Iijima [2], the combination of CNT with polyaniline, one of the most intensively investigated conducting polymer with excellent stability, good electronic property and strong biomolecular interaction [3, 4] was proved to improve significantly mechanical, electrical properties and reversible control of conductivity both by charge-transfer doping and protonation [5–9].

Cholesterol level is an important parameter in clinical diagnosis [10, 11]. In general, cholesterol is measured by spectrophotometry [12] or chromatography [13], however, the procedure is complicated and expensive. Thus, the amperometric biosensor is a preferential choice thanks to its biochemical analysis because of its good selectivity, rapid response and low cost [14]. The most common biosensors based on redox enzymes bound to electrode transducers generally rely upon the electrochemical oxidation of hydrogen peroxide ($\text{H}_2\text{O}_2$). The major drawback for this type of oxidase-based amperometric biosensors is that high operating
potential of ~600–700 mV must be applied at the enzyme–electrode interface which leads to high background currents and consequently to insufficient selectivity of the electrodes to common interference species such as ascorbic acid, uric acid and acetaminophen [15, 16]. Another strategy suggests the application of artificial electron transfer mediators such as organic dyes, ferrocyanide [17] and ferrocene derivatives [18] to replace oxygen, commonly serving as a natural electron acceptor for enzyme reactions. These mediators are capable of shuttling the electrons between the redox centers of enzymes and the electrode surface and therefore, to considerably lowering operating working potential.

The primary objective of this work is to design a sensitive interface for electrochemically multiplexed analyses. Several advantageous features of this platform will be developed. Firstly, interdigitated electrode (IDE) is attractive for its possibility of eliminating the main drawbacks of the electrochemical sensors such as the phenomenon of ‘electrode fouling’, the ‘memory effect’ from one sample to another as well as the possibility to be produced inexpensively on a large scale. Secondly, designed hybrid organic–inorganic electrode interface is expected to express a synergic effect to the overall system and thus improve sensing characteristics. Actually, some metal oxide nanoparticles such as iron oxide (Fe$_3$O$_4$), zinc oxide (ZnO) and especially carbon nanotube (CNT) and graphene having a large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency and strong adsorption ability were proved to be useful for improving sensor stability and sensitivity. In this study, multifunctional PANi-MWCNT composite film was polymerized electrochemically. Then, ChOx was grafted as probe to detect cholesterol, using potassium ferricyanide (FeCN) as an electron mediator. It is expected that the presence of cholesterol at IDE/analyte solution interface strongly influences the switching rate of the conducting polymer, so that on-site detection could be realized in a direct and portable format of IDE with an enhanced selectivity and rapidity.

2. Experimental

2.1. Materials

The enzyme cholesterol oxidase (EC1.1.3.6, from Brevibacterium) with specific activity of ≥50 units mg$^{-1}$ was purchased from Sigma-Aldrich, USA. Phosphate buffer potassium salt (PBS) (20 mM KH$_2$PO$_4$ + 20 mM K$_2$HPO$_4$ + 0.1 M KCl) was used as supporting electrolyte. A stock solution of cholesterol (10 mM) was prepared by dissolving 0.1933 g of cholesterol in a 50 ml flask containing 5 ml of Triton X-100 in a bath at 60° C and then diluting with 0.02 M PBS (pH 7.0).

Carboxylic multiwall carbon nanotube (MWCNT) was purchased from Shenzhen Nanotech Port Co. Ltd, China (purity CNTs >98%, out diameter: 10–20 nm, length: 5–15 μm, carboxyl ratio: 2.31 wt%). Monomer aniline (Merck, 99.5%) was distilled under vacuum prior to polymerization. Glutaraldehyde, potassium ferricyanide (FeCN) and other reagents were analytical grade.

2.2. Apparatus

Amperometric and cyclic voltammetric experiments were performed with the Autolab PGSTAT30 (Eco Chemie, Netherlands). The three-electrode system consists of a working electrode IDE/PANI-MWCNT–ChOx, a reference SCE reference electrode and a platinum wire counter electrode.

Field emission scanning electron microscopy (FE-SEM) images of the biocomposite film surfaces were obtained on S-4800 field emission scanning electron microanalyzer (Hitachi, Japan).

For the infrared spectroscopy (IR) experiment, small amounts of PANi films, peeled off from IDE, were pressed into KBr (Aldrich, 99%) pellets and the spectra were recorded on the Nicolet NEXUS 670 equipment in the region of 4000–400 cm$^{-1}$, with resolution of 4 cm$^{-1}$.

2.3. Sensor fabrication

Conductometric transducers, consisting of two identical pairs of platinum thin film electrodes, were designed to match compatibly with the universal serial bus (USB) that makes possible the direct connection to AUTOLAB software. The electrodes were designed by Institute of Materials Science (IMS, VAST, Vietnam) and fabricated at Hanoi University of Science and Technology (HUST, Vietnam). This pair of Pt (400 nm thick) interdigitated electrodes (IDE) was made by the lift-off process on the substrate of silicon wafer. A 50 nm thick intermediate Cr layer was used to improve the adhesion of Pt to the substrate. The IDE was fabricated with a finger width 70 μm and gap size of 30 μm (figure 1).

2.4. Electropolymerization of PANi-MWCNT film

The PANi-MWCNT film was electropolymerized on the IDE using cyclic voltammetry within the potential range from
−0.2 to +1.0 V (versus SCE) with sweep rate of 50 mV s⁻¹, for 20 cycles in a fresh solution containing 0.1 M ANi in 0.5 M H₂SO₄ and 0.8 wt% MWCNT (weight percent with respect to ANi monomer) [19]. The redox mediator was attached to the PANi–MWCNT film by dropping 1 ml of 0.5 M FeCN on the IDE surface.

2.5. Enzyme immobilization

ChOx was immobilized in PANi–MWCNT film by cross-linking method [20]. The ChOx was deposited manually in composite film, and it was treated by saturated vapor of glutaraldehyde. Finally, PANi–MWCNT–ChOx IDE electrode was stored at 4°C, in 20 mM phosphate buffer, containing 0.5 M FeCN.

3. Results and discussion

3.1. Composite characterization by FE-SEM and IR

To confirm the structural features of the PANi–MWCNT nanocomposites, FE-SEM was applied to study its surface morphology. It was revealed that PANi–MWCNT composite consists of porous networks formed by MWCNT and PANi (figure 2). Being uniform and porous, this structure is suitable for biocomponent immobilization.

In this study, we do not take upon ourselves to study thoroughly the interaction between PANi and MWNT but emphasize our efforts on designing a sensitive interface of PANi and MWNT for cholesterol detection. In this regard, Fourier transform infrared (FTIR) spectra of PANi–MWCNT were recorded (solid line, figure 3). It presents benzenoid (B) and quinoid (Q) ring stretching bands (C=) appeared at 1460 and 1612 cm⁻¹ [21]. The peaks at 1110 and 3415 cm⁻¹ can be attributed to B–N⁺=Q stretching and –N–H stretching vibrations respectively of PANi in the composite film. The peak at 1702 cm⁻¹ is unambiguously attributed to –COO⁻ stretching vibration, clearly confirming the presence of carboxyl group (–COOH) at the surface. This feature is very important for effective immobilization not only of cholesterol but also other biomolecules on IDE surface.

3.2. Electrochemical study by cyclic voltammetry

Figure 4 presents cyclic voltammograms for the PANi-MWCNT IDE in the absence/presence of 0.2 mM cholesterol (dotted/solid lines respectively) in 20 mM PBS buffer at pH 7.0 at scan rate of 50 mV s⁻¹.

In the presence of cholesterol a significant peak at about −0.3 V was registered, indicating the electrochemical response of IDE to cholesterol. Effectively, the enzymatic reaction corresponding to this response and transforming flavin adenine dinucleotide in quinine form (FAD) into that in hydroquinone form (FADH₂) can be written as:

\[
\text{Cholesterol} + \text{ChOx(FAD)} \rightarrow \text{Cholestenone} + \text{ChOx(FADH₂) + Fe(CN)₆}^{3⁻} \\
\rightarrow \text{ChOx(FAD)} + \text{Fe(CN)}₆^{4⁻} \rightarrow \text{Fe(CN)}₆^{3⁻} + e. 
\]
of pure PANI. Effectively, PANi-MWCNT displayed a linear response to cholesterol in the concentration range from 0.02 to 0.12 mM with a correlation coefficient (R) of 0.9985. The linear equation was $I (\mu A) = 0.0017 + 4.301C$ (mM) and the sensitivity of the PANi-MWCNT biosensor was $464 \mu A \text{mM}^{-1} \text{cm}^{-2}$.

3.4. Interference tests

The most common electrochemical interfering species such as ascorbic acid and uric acid were evaluated. Thanks to less applied potential, addition of 0.06 mM ascorbic acid and 0.3 mM uric acid to 2.0 mM cholesterol did not induce any observable interference in the biosensor response (figure 6).

4. Conclusion

The developed biosensor showed a linear response to cholesterol concentration with sensitivity as high as $464 \mu A \text{mM}^{-1} \text{cm}^{-2}$. As for the transduction scheme, it is proposed that the presence of cholesterol was detected via change in signal transduction related to the redox group of FeCN as the redox mediator. One powerfully advantageous aspect of IDE is the ability to array multiple copies of the same probe (to control technical variability) as well as to array multiple probes against the same target on each electrode of IDE (to control biological variability). With the functional conducting PANi-MWCNT platform, using immobilized ChOx as affinity capture reagent, the proof of concept of reagentless electrochemical arrays was proposed. This concept can be extended to other enzyme-based biosensors such as glucose, urea, lactate, choline etc.

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