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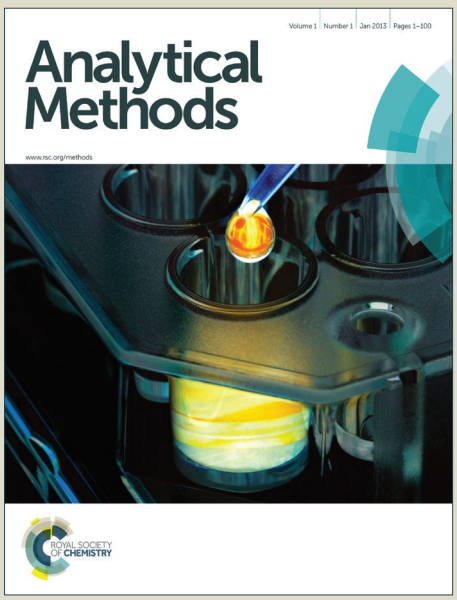

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PAPER

Electrochemical sensor based on silver nanowires modified electrode for determination of cholesterol

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In this manuscript, the fabrication of an amperometric cholesterol biosensor based on silver nanowires (AgNWs) and cholesterol oxidase (ChOx)-graphene oxide (GO)-chitosan (CS) film is reported. The electrochemical behaviour of the ChOx/Ag/GO/CS/ITO biosensor was studied using cyclic voltammetry (CV), which revealed that the developed biosensor possessed high sensitivity(13.628μA $mM^{-1}cm^{-2}$) and low detection limit (0.427$mgdL^{-1}$). The apparent Michaelis-Menten constant, K_M^{app} of this biosensor was very low (2.813mM), originating from the effective immobilization process and the nanopwires structure of the substrate. The biosensor expressed a wide linear range up to 400$mgdL^{-1}$ in a physiological condition (pH 7.0), making it very promising for the clinical determination of cholesterol.

1. Introduction

Cholesterol is essential for human life since its abnormality in the blood may cause serious cardiovascular diseases, such as heart diseases, coronary artery disease, arteriosclerosis, hypertension, cerebral thrombosis and so on[1]. The Adult Treatment Panels suggests the normal blood cholesterol level should be lower than 5.2mM (200$mgdL^{-1}$) in total, with over 6.2mM (240$mgdL^{-1}$) as a high level[2]. Therefore, precise cholesterol has been regarded significant in clinical diagnosis.

Cholesterol oxidase (ChOx) is usually modified on the biosensor to monitor cholesterol. Cholesterol is oxidized by molecular oxygen to produce 4-cholestene-3-one and hydrogen peroxide(H_2O_2) due to ChOx[3]. The H_2O_2 determination is deemed an indirect cholesterol quantification method. Many researchers have looked for different peroxidases to sense the formed H_2O_2. However, the applications of multiple enzyme-based biosensors were hindered due to the high cost and rigorous store environment.

Recently, metallic nanorods/nanowires have become extensively applied in electrochemical biosensors. Compared with equivalent larger-scale materials, one dimensional metallic nanowire perhaps possesses excellent unique physical and chemical properties due to their controllable size and high density in corner or edge surface sites. Featured by high conductivity, direct and real-time electrical signal transduction, remarkable stability and large surface to volume ratio which is essential in fast reaction kinetics, it is believed that one-dimensional (1-D) silver nanowires (AgNWs) can exhibit enhanced eletrocatalytic performances[4]. With superior electrical properties, AgNWs enjoy great popularity and can act as effective carriers for loading various signal molecule storages for different types of electrochemical sensing[5]. It is known that AgNWs own the abilities to catalyze the reduction of H_2O_2 and keep bioactivities of immobilized enzymes, so it could act as a platform for cholesterol sensing applications[6].

Instead of peroxidases, AgNWs based cholesterol biosensor reduced H_2O_2 at low applied potential, it allows a low consumption of enzyme (reagent saving) and high sensitivity for the detection.

The popularity of grapheme can be attributed to its honeycomb lattice molecular structure which possesses outstanding physical and chemical properties like electrical conductivity, large surface area, rapid electrontransfer and excellent capability of absorbing a variety of aromatic biomolecules through a π-π stacking interaction and/or electrostatic interaction[7]. In order to enhance sensitivity of cholesterol sensors, the AgNWs were modified with the reduced graphene oxide (GO)[8]. Due to the unique structure with numbers of -NH_2 and -OH functional groups, chitosan (CS) was considered as a good material to immobilize AgNWs on electrode surfaces. Moreover, GO-CS nanocomposite membrane could improve the catalytic activity of AgNWs[9].

This study shows the application of GO-CS nanocomposite in fabricating the electrochemical cholesterol biosensor for sensitive detection by using AgNWs as illustrated in Scheme 1. The GO-CS-AgNWs composite membrane could enhance the surface area for the adsorption of enzymes and electronic transfer rate[10]. Due to the excellent biocompatibility, hypotoxicity, and biodegradability of CS, the fabricated cholesterol biosensor was free of any special materials toxic to the environment and human[11]. Our electrochemical measurements show that the nanostructured electrode exhibited high sensitivity, a wide linear range and low detection limit for electrochemical detection of cholesterol.

2. Experimental

2.1 Materials

Ethylene glycol, poly(vinyl pyrrolidone), silver nitrate, graphene oxide, chitosan, isopropanol, cholesterol, TritonX-100 (t-octylphenoxypolyethoxyethanol), cholesterol oxidase, sodium phosphate dibasic, sodium phosphate monobasic, potassium hexacyanoferrate, potassium ferricyanide, potassium chloride

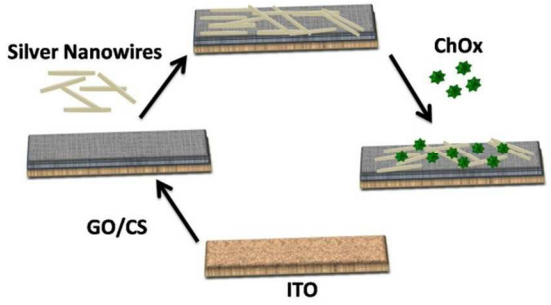
were purchased from Sigma-Aldrich. The doubly distilled water was used to prepare all experiments. A mixture of 5mL Triton X-100 and isopropanol was used to prepare stock solution of cholesterol. All other chemicals were of analytical grade and used without further purification.

2.2 Preparation of AgNWs

The aspect ratio-controlled AgNWs were synthesized according to a reported procedure[12]. 10mL silver nitrate(0.10g $AgNO_3$ dissolved in 10mL Ethylene glycol (EG)) was added in a stirred mixture solution of 10mL EG and 0.40g poly(vinyl pyrrolidone) (PVP, K30). 1mL $AgNO_3$ solution treated to the Ag crystal seeds at the first 3-5 min. then the successive 9mL $AgNO_3$ solution was added using a syringe. After the solution turned into a grey emulsion, continued boiling it for half an hour. Finally, the emulsion was cooled down to room temperature. Almost all AgNWs deposited at the bottom of flask for one day.

2.3 Preparation of the cholesterol biosensor

A sheet of ITO glass was cleaned by the ultrasonic cleaning machine and dried in a nitrogen gas flow. Firstly, we dropped an amount of GO-CS solution onto ITO and dried it at room temperature. Secondly, after immersing the modified electrode in the AgNWs solution for 5min, and drying it in nitrogen, we got the Ag/GO/CS/ITO modified electrode. Finally, we dropped a certain amount of ChOx onto Ag/GO/CS/ITO electrode surface and dried to form ChOx/Ag/GO/CS/ITO. Without use, all prepared electrodes were stored at 4℃. The fabrication process of ChOx/Ag/GO/CS/ITO electrode is shown in Scheme 1.



Scheme 1 Formation process of ChOx/Ag/GO/CS/ITO electrode.

2.4 Apparatus

The scanning electron microscopy(SEM) images were carried out by Hitachi S-4800 (Japan). The UV-vis absorption spectra was obtained with a PerkinEImer spectrometer (Lambda 35 650s). Cyclic voltammetric (CV), amperometric and impedance experiments were performed with a AUT84875 electrochemical workstation (Metrohm AUTOLAB). All experiments were carried out using a conventional three-electrode system with ChOx/Ag/GO/CS/ITO electrode as the working electrode, a platinum coil as a counter electrode, and an Ag/AgCl (saturated KCl) electrode as the reference electrode.

3. Results and discussion

3.1 Characterizations of modified electrode

Scanning electron microscopy(SEM) was used to investigate the micro structure and morphology of prepared nanomaterials(Fig. 1). The SEM image of the high purity AgNWs appears as straight lines, the diameters of the randomly formed line structures vary from tens to hundreds of nanometers, which can promote electron transfer. Different from silver nanoparticles, two typical absorption peaks of AgNWs were observed at wavelengths of 350 nm and 390 nm(Fig 2). This confirmed the high purity of the as-synthesized AgNWs.

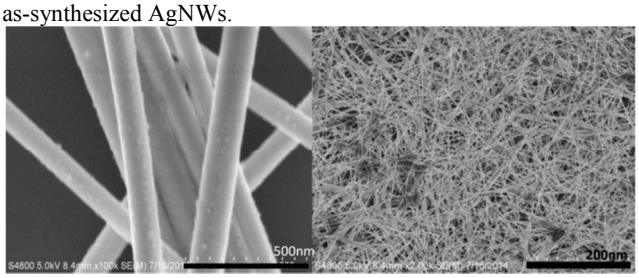


Fig. 1 The AgNWs interface byScanning Electronic Microscopy.

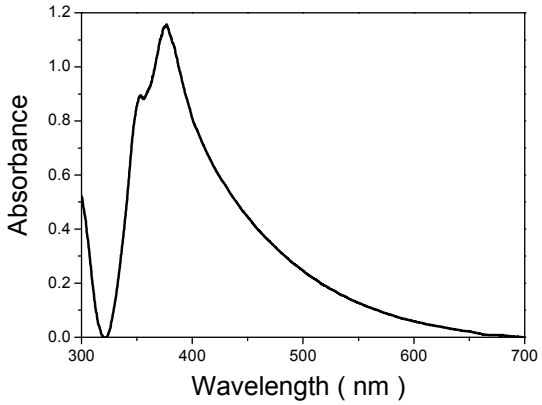


Fig. 2 UV-vis absorption spectra of AgNWs in 0.1 M sodium phosphate buffer(pH 7.0).

3.2 Electrocatalytic response to cholesterol

The reduced grapheme oxide is applied to fabricate high-efficiency AgNWs-GO nanocomposites based cholesterol sensors so as to improve the performances of AgNWs. AgNWs-GO hybrids perform better in sensing than the AgNWs without GO due to the excellent synergetic properties of novel metal nanowires and GO, see in Fig. 3(curve a and b).

A comparative investigation of the sensing based on either metallic nanowires or nanoparticles has been conducted. The nanowires based sensors with better stability has excellent analyte delivery and transduction[13].

Our work gets a similar result that the electrochemical behavior of AgNWs modified ITO electrode is better than the nanoparticles based sensor in cholesterol solution Fig. 3(curve c).

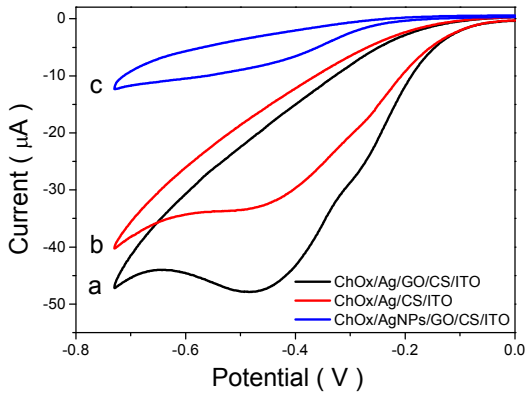


Fig.3 Caption Cyclic voltammetrics response of (a) ChOx/Ag/GO/CS/ITO (b) ChOx/Ag/CS/ITO and (c) ChOx/AgNPs/GO/CS/ITO electrode in present cholesterol solution(pH7.0)with scan rate at 50mV/s.

The cyclic voltammetric behavior of the modified electrode detected in cholesterol solution(pH 7.0) is 200 $mgdL^{-1}$ and 10mM PBS shown in Fig. 4. The ChOx/Ag/GO/CS/ITO biosensor can be used to detect the cholesterol with electrons released through the redox reaction. It can be seen from Fig. 4 that both ChOx/Ag/GO/CS/ITO electrode in PBS (curve a) and Ag/GO/CS/ITO electrode in cholesterol solution(curve b) exhibit very low current, and no redox peaks could be observed in the CV spectrum, respectively. In cholesterol solution, when ChOx was immobilized onto the Ag/GO/CS/ITO electrode surface, the electrode gave a strong response to cholesterol at quite negative potential due to the insulating character of the enzymes (curve c). This result validated the successful attachment of ChOx on the electrode. The biosensor exhibited an excellent response to cholesterol reveals that catalytic activity of ChOx toward cholesterol and electrocatalytic activity of AgNWs in the reduction of H_2O_2.

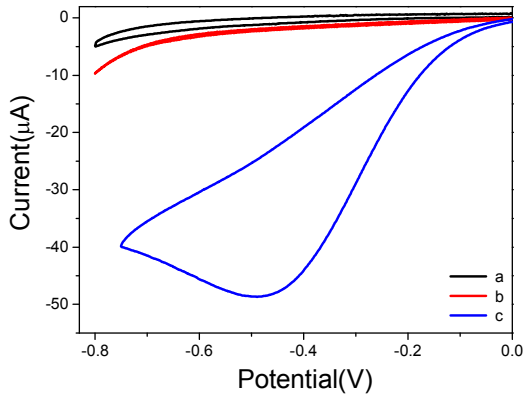


Fig. 4 Cyclic voltammetrics response of ChOx/Ag/GO/CS/ITO electrode in (a) PBS (pH7.0), (b) Ag/GO/CS/ITO electrode and (c) ChOx/Ag/GO/CS/ITO electrode in present cholesterol solution(pH7.0) with scan rate at 50mV/s.

The immobilization of ChOx/Ag/GO/CS/ITO electrode was confirmed from electrochemical impedance spectroscopy (EIS) analysis. The electron transfer kinetics is limited by the interfacial changes of biosensor. The impedance properties of bare ITO, GO-CS, AgNWs and ChOx are presented in Fig.5. As can be seen in Fig. 5b, the Nyquist semicircle is a little larger than bare ITO (Fig.5a). This is due to chitosan hindered the electron transfer between GO and bare ITO. When AgNWs were modified on the top of GO-CS layer, the Nyquist semicircle became smaller than GO/CS/ITO, which indicated that AgNWs promoted the electron transfer (Fig. 5c). ChOx modified Ag/GO/CS/ITO shows a larger semicircle reveals that ChOx is poor electrical conductors.

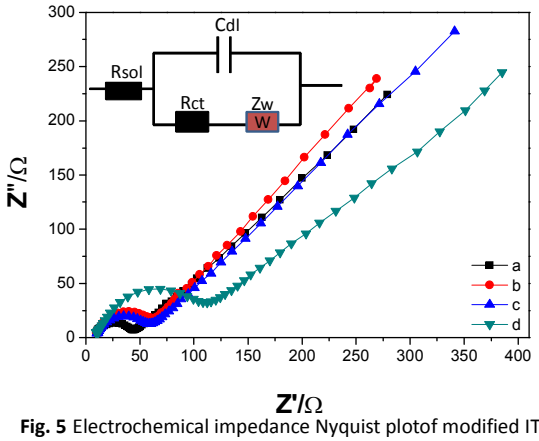


Fig. 5 Electrochemical impedance Nyquist plotof modified ITO electrodes: (a): nake ITO, (b): GO/CS/ITO, (c):Ag/GO/CS/ITO, (d): ChOx/Ag/GO/CS/ITO.

3.3 Electrochemical characterization of ChOx/Ag/GO/CS/ITO

In order to monitor cholesterol, ChOx catalyzed cholesterol molecules to H_2O_2. And then, AgNWs accelerated the reduction of H_2O_2. In Fig. 6, the low concentration of cholesterol solution produced trace amounts of H_2O_2, the current response was found at ca. -0.45V. In 5mM potassium ferricyanide solution (pH 7.0) condition, the surface-confide redox reactions can be typically exhibited by cyclic voltammetry. With the increase of scan rates(25-500mV s^{-1}), the peak currents(anodic and cathodic) and potentials are increased. It indicates that the pair of redox waves originates from the surface confined molecules. With the increase of the scan rate, the oxidation peak shifts to more positive potentials, while the reduction peak shifts to more negative potentials. This is in agreement with Laviron theory[14].

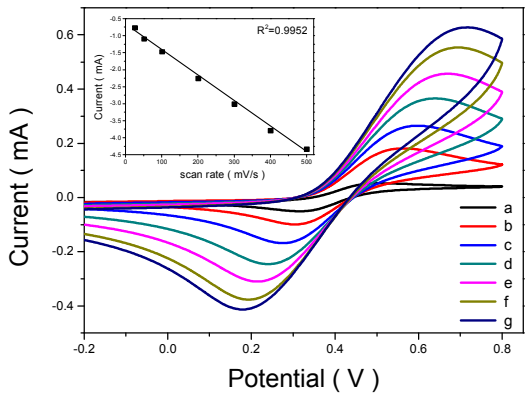


Fig. 6 Cyclic voltammamograms of 200mgdL^{-1} cholesterol solution on the ChOx/Ag/GO/CS/ITO electrode at different scan rates (curves a-g25, 50, 100, 200, 300, 400, 500mVs^{-1}) containing 5mM potassium ferricyanide solution (pH 7.0).

As seen in Fig. 7, when the reduction peak current arises dramatically with the increase of the concentration of cholesterol solution, the cyclic voltammetric responses of the ChOx/Ag/GO/CS/ITO biosensor to different concentrations of cholesterol solutions shows a linear dynamic range from 0.5mgdL^{-1} upto 400mgdL^{-1} with a correlation coefficient of 0.9946, the detection limit of 0.427mgdL^{-1} (S/N=3), and the sensitivity of 13.628μA mM^{-1}cm^{-2}. A smaller K_M^{app} value means a higher catalytic efficiency of enzyme onto the ITO electrode, which results in higher affinity of ChOx toward cholesterol. Based on Michaelis-Menten mechanism, we can obtain K_M^{app} as 2.813mM by using a Lineweaver-Burk plot[15]:

$$1/I = 1/I_{\max} + K_M^{app}/I_{\max}[S]$$

where I is the steady-state current, I_{max} is the maximum current obtained at the cholesterol saturated level , and [S] is the concentration of cholesterol.

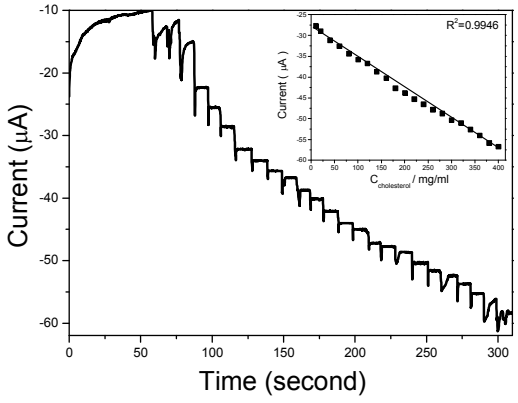


Fig.7 Amperometric responses of ChOx/Ag/GO/CS/ITO to successive addition of cholesterol at -0.45V in phosphate buffer (pH 7.0). Inset shows the plot of chronoamperometric current of vs. concentration of cholesterol.

Due to the outstanding electrical properties, we believe the detection limit of nanowires sensors is lower with a wider linear detection range compared with that of nanoparticles sensors. The performance of fabricated cholesterol sensor compared with other ChOx based sensors is shown in Table 1. It confirms that the functionalized ChOx/Ag/GO/CS modified ITO electrode exhibited good sensing performance.

Table 1. Comparison of ChOx/Ag/GO/CS/ITO biosensor with other ChOx based biosensors.

4. Conclusions

In summary, we have successfully fabricated a novel cholesterol sensor using silver nanowires-graphene oxide- chitosan. Instead of peroxidases, AgNWs own the catalytic activity of H_2O_2 reduction which occurs at low potential. The AgNWs-based cholesterol sensor not only reduces consumption of enzyme, but also enhances sensitivity and selectivity in the detection. The production of cholesterol reaction, hydrogen peroxide, is considered to be an indirect cholesterol quantification measure. Current experimental results demonstrate that the fabricated sensor possesses an excellent electrocatalytic activity, a wide linear range and low limit of detection. Therefore, AgNWs are expected to be a potential material for any enzyme-based sensor with the merit of forming H_2O_2.

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Notes and references

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Table 1. Comparison of ChOx/Ag/GO/CS/ITO biosensor with other ChOx based biosensors.

Electrode	Linear range	Detection limit	References
ChEt-ChOx/MWCNT/SiO_2-CS/ITO	10-500 mg/dL	0.634mg/dL	16
ChOx/PAni-Au-CS/ITO	50-500 mg/dL	37.89mg/dL	17
ChOx/$NiFe_2O_4$/CuO/FeO-CS/ITO	50-5000 mg/dL	31.3 mg/dL	18
ChOx/Nano-CdS/ITO	2-500 mg/dL	1.87 mg/dL	19
ChOx/α-Fe_2O_3/Ag	0.1-8.0mM	0.018mM	20
ChOx/Ag/GO/CS/ITO	0.5- 400mg/dL	0.427 mg/dL	this work

MWCNT: multi-walled carbon nanotubes, PAni : polyaniline, ZNT: zinc oxide nanotube, ChEt : cholesterol esterase.

1mM*38.67=1mg/dL