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A square wave voltammetric method for the detection of microorganism populations using a MWNT-modified glassy carbon electrode

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ABSTRACT

A novel method for determination of trace amounts of microorganism populations in solution was developed by using a multiwalled carbon nanotube (MWNT) modified glassy carbon electrode and square wave voltammetry (SWV). The simultaneous combination of MWNT and SWV allowed the electrochemical signal of electro-active materials in *Escherichia coli* O157:H7 (*E. coli*) to be dramatically amplified. Compared with a bare glassy carbon electrode, the MWNT-modified glassy carbon electrode showed catalytic properties in the oxidation of electro-active materials on cell surfaces. Moreover, SWV was proved to be more sensitive than cyclic voltammetry (CV) for investigation of the electrochemical behavior of cells. In this paper, a linear relationship was obtained between the SWV peak current and the cell concentration in the range 2×10^2–2×10^8 cell mL^{-1} with a detection limit of 2×10^2 cell mL^{-1}. The effect of antibiotic drug Gentamycin Sulfate injection (GSI) on the growth of *E. coli* was also investigated.

1. Introduction

Detection of living microbial cells, in particular the pathogenic bacterium *Escherichia coli* (*E. coli*) O157:H7 [1], is important in clinical, environmental, and bioindustrial fields. Methods based on colony formation and plate count have been used for the detection of viable cells in microbiology. However, these procedures require visual counting and a growth period of more than 48 h, resulting in errors due to bacterial contamination or to visual factors [2]. To circumvent these problems, other non-culturing methods have been developed for the determination of cell populations without a plate count. These include voltammetry [2], surface plasmon resonance [3], piezoelectric quartz crystal techniques [4], polymerase chain reaction (PCR)-based analysis [5], flow cytometry methods [6], fluorescence by *in situ* hybridization (FISH) [7] or conjugated fluorene-based glycopolymers [8], wireless sensors [1], and electrochemical immunosensing [9,10] methods. Many of these have failed to gain wide acceptance due to the relatively high degree of user expertise required and the high cost of labeling reagents. The electrochemical approach is promising for its speed and broad applicability in the determination of cells, but assays of food and environmental samples have been limited by lack of sensitivity [2].

Modification of the working electrode surface can enhance the sensitivity and selectivity once a judicious choice of the modifier has been achieved. Since their discovery in 1991 [11], carbon nanotubes (CNTs) have shown unique topologically controlled electronic properties for use in electrochemical investigations, for instance, in direct electrochemistry of proteins [12], construction of electrochemical sensors and biosensors [13,14], electrocatalysis [15], and electrode materials in batteries [16]. However, to the best of our knowledge, the electrochemical behavior of living microbial cells on CNT-modified electrodes has scarcely been reported, and the capability of these electrodes to enhance electrochemical response signals is still unexplored.

Voltammetric techniques offer the possibility of determining analyte concentrations directly in samples without any pretreatment or separation. It is also possible to analyze colored materials and samples with dispersed solid particles and to determine several analytes simultaneously [17]. Because of the low faradaic currents and high sensitivity, square wave voltammetry (SWV) has proven to be extremely sensitive for the detection of organic molecules, while the conventional voltammetric techniques have detection limits of 10^{-5} mol L^{-1}, the use of SWV enabled detection limits three orders of magnitude lower [18].

This paper describes the rapid, direct and sensitive measurement of microbial populations using a multiwalled carbon nanotube (MWNT)-modified glassy carbon electrode with square wave voltammetry. Using this novel technique, we were able to

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directly quantify *E. coli* O157:H7 concentrations in the range of 2×10^2–2×10^8 cell mL^{-1} and to monitor the effect of gentamycin sulfate injection (GSI) on proliferation of the bacteria. The electrochemical results were compared with morphological observations.

2. Experimental

2.1. Chemicals

Multiwalled carbon nanotubes (10–20 nm in diameter) were purchased from Shenzhen Nanotech Port Co., Ltd (Shenzhen, China). Prior to use, the MWNTs were purified by refluxing in concentrated nitric acid for 8 h, followed by filtering, rinsing with ultrapure water and drying. Gentamycin sulfate injection (GSI) was purchased from Nanyang Pukang Pharmaceutical Co., Ltd (Henan province, China).

Phosphate-buffered saline (PBS) solution consisting of 136.7 mM NaCl, 2.7 mM KCl, 9.7 mM Na_2HPO_4 and 1.5 mM KH_2PO_4 was used as the diluent for dispensing microbial suspensions or samples. A stock solution of GSI (2×10^{-3} g mL^{-1}) was prepared with PBS and sterilized by filtration using a 0.22 μm filter (Whatman). Ultrapure water (18.2 MΩ cm resistivity) produced from a Millipore Milli-Q system was used throughout. All other chemicals were of reagent grade and used as received.

2.2. Apparatus

Cyclic voltammogram (CV) and SWV experiments were performed with a CH Instrument model 660A-electrochemical analyzer (Shanghai Chenhua Apparatus Company, China) and a conventional three-electrode system: a MWNT-modified glassy carbon electrode (GC, disk shape with diameter of 3 mm) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum electrode as the auxiliary electrode. All measurements were carried out in a 10 mL detection cell. All potentials measured in this report were with respect to SCE.

2.3. Materials

E. coli O157:H7 was generously provided by the Department of Life Science of Hunan Normal University (China). Phosphate-buffered saline (pH 7.4, 25 °C) after sterilization was used as the diluent for dispensing microbial suspensions or samples. The basic culture medium of the microorganism had the composition: yeast extract 0.5 g, NaCl 1.0 g, tryptone 1.0 g, agar (for solid medium) 1.5 g, water 100 mL.

2.4. Modification of electrode

The glassy carbon electrode surfaces were hand-polished with Al_2O_3 (0.7 μm and 0.05 μm), and washed with ultrapure water before use. The MWNT film was constructed *via* casting 5 μL 0.75 mg mL^{-1} MWNT suspension in ultrapure water onto the surface of glassy carbon electrode. After being carefully rinsed with water, the MWNT-modified electrode was immersed in PBS and electrochemically treated by cyclic voltammetry in the potential range from 0.0 to 1.0 V *vs.* SCE until a stable voltammogram was obtained. Then the modified electrode was transferred to the cell suspension in PBS and electrochemical measurements were carried out.

2.5. Detection of E. coli cells

A certain amount of cells, such as 2×10^9 cells, were suspended in 10 mL phosphate buffer (pH 7.4) to get a concentration of 2×10^8 cell mL^{-1}. Then, CVs and SWVs were obtained at ambient temperature, 25 ± 2 °C. The cell number was determined by colony counting method based on the colony formation on culture dishes after 36 h of incubation at 37 °C.

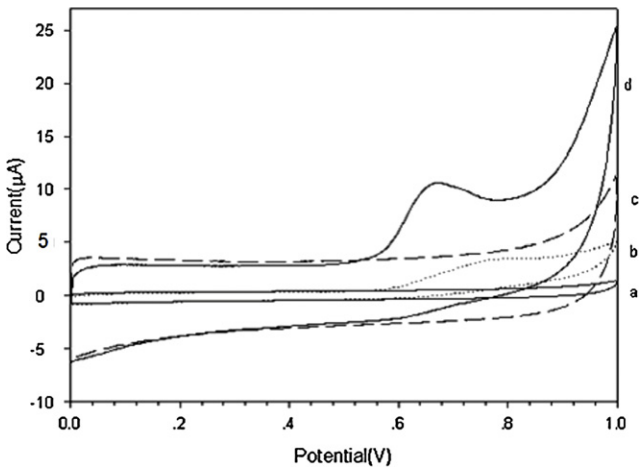

Fig. 1. Cyclic voltammograms at bare (a, b) and MWNT-modified (c, d) glassy carbon electrodes in PBS in the absence (a, c) and presence (b, d) of *E. coli* cells. *E. coli* cell concentration, 2×10^8 cell mL^{-1}; scan rate, 50 mV s^{-1}.

3. Results and discussion

3.1. Voltammetric behavior of E. coli cells

Fig. 1 shows the typical cyclic voltammograms of *E. coli* cells at bare and MWNT-modified glassy carbon electrodes. No redox peak can be observed at either electrode in the potential range of 0.0 and 1.0 V in PBS without cells (curves a and c). The background current of the MWNT-modified electrode was apparently larger than that of the bare glassy carbon electrode, due primarily to the enhanced effective surface area of the MWNT-modified electrode. In the presence of freshly collected *E. coli* cells, an oxidative peak at about +0.672 V appeared (curve d) at the MWNT-modified electrode, while at the bare glassy carbon electrode, the anodic current increased slightly starting at about 0.6 V, but no obvious oxidative peak was observed (curve b). This observation indicates that MWNTs catalyzed the electrochemical oxidation of electroactive species in the *E. coli* cell suspension. The MWNT-modified electrode CV peak current was about twice as high as that on the bare electrode and the peak position moved to lower potential. The results in Fig. 1 indicate that the MWNT-modified glassy carbon electrode provides better peak shape, larger peak current, and lower peak position, all leading to improved electrochemical analysis. No corresponding reduction peak waves were observed in the reverse scans, indicating a clearly irreversible voltammetric character of the cell suspension. Many previous studies have proposed that the voltammetric response of cells was related to enzymatic action [2,19,20], but the intrinsic reason for cell voltammetric behavior has not been sufficiently investigated.

In order to study the electrochemical reaction mechanism, the MWNT-modified glassy carbon electrode was used for a continuous scan in the cell suspension. As shown in Fig. 2 that the anodic wave of the second scan moved to more positive potentials, and the peak current decreased compared with that of the first scan.

To determine if the sharp decline in the peak current was related to cell death, we prepared two colonies (*E. coli* concentration was 2×10^8 cell mL^{-1}) in the same medium, and stirred them under the same conditions. Electrochemical scanning was continued for one sample, while the other sample served as a control, and the turbidities (measured by the absorbance at 600 nm) of