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Electrochemical determination of chloramphenicol in milk and honey using vertically ordered silica mesochannels and surfactant micelles as the extraction and anti-fouling element

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ABSTRACT

Chloramphenicol (CAP), an antibiotic, is closely related to the food safety, ecological environment and human health. It is therefore of great importance to develop simple and rapid methods for CAP determination. We report in this work the utilization of indium tin oxide (ITO) electrodes modified with a binary assembly of vertical silica mesochannels (VSMs) and cylindrical surfactant micelles (CSMs) for the electrochemical detection of CAP. Each hard VSM of 2–3 nm in diameter supports and confines a soft CSM of cetyltrimethylammonium bromide (CTAB), which has a hydrocarbon core capable of extracting and concentrating lipophilic organic analytes from sample solutions. So the CSM@VSM modified electrode exhibited an analytical performance for CAP apparently superior to bare and VSM modified electrodes. Under optimized conditions, two linear dynamic concentration ranges were obtained for CAP determination using differential pulse voltammetry, namely 0.1 to 3.6 ppm and 3.6 to 15.0 ppm, as well as a low limit of detection at 40 ppb. Moreover, thanks to the ultrasmall size of silica channels and the lipophilic microenvironment of micelle cores, the CSM@VSM film displayed an excellent hydrophobic selectivity and anti-fouling ability by preventing unwanted substances from accessing to and contaminating the underlying electrode surface. Therefore, reliable results were obtained for direct electrochemical determination of CAP in real samples, such as milk and honey, without pre-treatment.

1. Introduction

Antibiotic residues and additives in animal-derived foods have received considerable public attention, despite their irreplaceable role in medicine and agriculture as prophylactic and therapeutic agents to resist animal sickness [1]. Chloramphenicol (CAP), a widely used antibiotic inhibiting the activity of both Gram-positive and Gram-negative bacteria, was first obtained from the culture of a soil bacterium, namely Streptomyces venezuelae, and chemically synthesized in 1948 [2,3]. It is the primary choice for the treatment of vancomycin-resistant *Enterococcus*, tetracycline-resistant *Vibrio cholerae*, and for the patients suffering severe penicillin or cephalosporin allergy. Although it is clinically important, quite a few side effects have appeared. So far, many countries, including USA, Canada and China, have already banned the use of CAP in the food producing animals. The Food and Drug administration in USA has set the "minimum required performance limit" (MRPL) of CAP as 0.3 μg kg^{-1} in 2002 for the detection of its residues in food products [4,5]. Therefore, it is exceptionally important to develop a fast, highly sensitive and simple method for the monitoring and detection of CAP *in vitro* and in samples of different sources from clinical, environmental and pharmaceutical fields.

A large number of analytical methods and techniques have been developed and utilized for the determination of CAP, such as thin-layer chromatography [6], gas chromatography (GC) [7,8], immunoaffinity chromatography [9], gel-based non-instrumental immunoassay [10], piezoelectric immunosensor [5], homogenous light-induced chemiluminescence immunoassay [11], liquid chromatography [12–14], GC–mass spectrometry [15–17], capillary zone electrophoresis with amperometric detection [2], surface plasmon resonance biosensor [18] and photoinduced chemiluminescence [19]. Although these traditional separation-based methods are reliable and sensitive, their practical use is still limited by time-consuming sample preparation, complex operation, consumption of great amount of reagents/solvents and reliance on expensive apparatus [20,21]. To resolve these issues, the utilization of electrochemical sensors represents a good option, due to their fast response, simplicity, low cost and high sensitivity [22]. Moreover, various materials, such as single-wall carbon nanotube–gold nanoparticle–ionic liquid composite film [23], nitrogen-doped graphene nanosheets decorated with gold nanoparticles [24], surfactants [25], molecularly imprinted polymer [26], can be used to improve the analytical performance of electrochemical sensors.

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In this work, we report a facile electrochemical approach for the detection of CAP by modifying the electrode surface with mesoporous silica films, which not only have the characteristics of conventional mesoporous silica but also allow the rapid and selective access of analytes to the underling substrate via vertically aligned channels. The film can be simply fabricated on electrode surface by the electrochemically assisted self-assembly or Stöber-solution growth approaches using cetyltrimethylammonium bromide (CTAB) as the surfactant template [27–29]. As-prepared films are composed of regularly aligned vertical silica mesochannels (VSMs) and cylindrical surfactant micelles (CSMs), with the latter ones physically confined in the former. VSMs after removing surfactant micelles are hydrophilic due to a high density of silanol groups on the walls, which have been successfully applied in the fields of electrochemical sensing, nanomaterial synthesis and molecular separation [30–35]. If retaining CSMs, the hydrocarbon tails of CTAB molecules set up an array of highly ordered hydrophobic nanodomains [36], which can significantly improve the affinity for lipophilic analytes [37]. As we shall show in this work, the CSM@VSM film is able to rapidly extract and concentrate trace CAP from aqueous solutions and subsequently allow their mass transport to the underlying electrode surface, whereby the amount of CAP can be quantitatively determined. In addition, due to the ultrasmall channel size (ca. 2.3 nm in diameter) and lipophilic micelle nanodomains, the film is only permeable to small and hydrophobic organic analytes, with an apparent advantage of inhibiting the surface-fouling and contamination by large substances or unwanted species in solutions. So the CSM@VSM film modified electrode can be utilized for electrochemical determination of CAP in real samples, such as milk and honey, and complicated sample pre-treatment is not needed.

2. Experimental section

2.1. Reagents and solutions

A stock solution of CAP (Aladdin, 98%) was prepared in ethanol and stored at 4 °C. The working solutions for voltammetric investigations were prepared by diluting the stock solution with water. CTAB (≥98%) and hydroxymethylferrocene (FcMeOH, 97%) were obtained from Alfa Aesar. Tetraethoxysilane (TEOS, ≥99.0%) and hexaammineruthenium(III) chloride ($Ru(NH_3)_6Cl_3$, 98%) were purchased from Sigma and used as received. Concentrated ammonia aqueous solution (25 wt%) and potassium ferricyanide ($K_3[Fe(CN)_6]$) were ordered from Aladdin. All other chemicals were analytical grade and used without further purification. Phosphate buffer solution (PBS, pH 7.0, 0.1 mol/L) was prepared by mixing two stock solutions of 0.1 mol/L NaH_2PO_4 and 0.1 mol/L Na_2HPO_4 by a certain volume ratio. All aqueous solutions were prepared with ultrapure water (18.2 MΩ cm). Milk and honey samples were obtained from Zhejiang University campus supermarket. Indium tin oxide (ITO) coated glasses (surface resistivity < 17 Ω/square, thickness 100 ± 20 nm) were firstly treated by 1 M NaOH, followed by sonication in acetone, ethanol and deionized water successively, prior to use.

2.2. Preparation of CSM@VSM/ITO and VSM/ITO electrodes

The Stöber-solution growth approach as previously reported was used for the preparation of the CSM@VSM/ITO electrodes [29]. Typically, CTAB (0.16 g) was dissolved in a water and ethanol mixture (water/ethanol: 70 mL/30 mL), to which the concentrated ammonia aqueous solution (10 μL, 25 wt%) and TEOS (80 μL) were added under stirring. Subsequently, the ITO electrodes were immersed in the above solution for 24 h under quiescent condition at 60 °C to grow the CSM@VSM film on the surface. By immersing the CSM@VSM/ITO electrode in an ethanol solution containing 0.1 M HCl under moderate stirring for 5 min, CSMs were dissolved and thus obtained electrode was designated as the VSM/ITO electrode. In both cases, the electrode surface was rinsed by water to remove loosely adsorbed chemicals and further aged at 100 °C overnight, prior to use.

2.3. Electrochemical measurements

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed on a CHI 440A electrochemical workstation (CH Instrument, Shanghai). A conventional three-electrode system was adopted. The working electrode was a bare or modified ITO electrode, the auxiliary and reference electrodes were platinum wire and Ag/AgCl electrode (saturated with KCl), respectively. The solutions were thoroughly deoxygenated by bubbling argon for 15 min and the argon atmosphere was maintained over the solution during the experiment. The parameters for DPV are as follows: increment potential 0.01 V, amplitude 0.05 V, pulse width 0.05 s and pulse period 0.5 s. The effective area of the working electrode immersed in the electrolyte is 1 cm × 1 cm.

2.4. Electron microscopy measurements

Scanning electron microscopy (SEM) images were obtained on a SU8010 field-emission scanning electron microscope (Hitachi, Japan) at an accelerating voltage of 5.0 kV. Transmission electron microscopy (TEM) images were captured on a HT7700 electron microscope (Hitachi, Japan) operated at 100 kV. To prepare TEM specimens, the fragments of CSM@VSM film were scraped from the ITO electrode surface and dispersed in ethanol. Then a few drops of dispersion were dropped on the copper grids.

3. Results and discussions

3.1. Characterization of the CSM@VSM film

The morphology and thickness of the CSM@VSM film were characterized by TEM and SEM measurements. As shown in Fig. 1a, the TEM image exhibits well-defined mesopores (appeared as the bright spots) homogeneously and regularly distributed over a large domain without cracking. The diameter of mesopores was about 2–3 nm. In a solution, the surfactant cations (CTA^+) can adsorb and form spherical CTAB micelle assemblies adsorbed on a negatively charged substrate (ITO). When TEOS molecules are added, they are slowly hydrolyzed to form negatively charged oligomeric silica species which deposited at the junction between the spherical micelles and the substrate *via* electrostatic interaction. Furthermore, the presence of ammonia facilitates the transformation of the spherical CTAB–silica structure to cylindrical micelles at the solution/substrate interface, leading to a continuous, large-domain film growth of vertical silica mesochannels. As displayed in the SEM image (Fig. 1b), three layers can be clearly identified from the cross-sectional view of the film, corresponding to the CSM@VSM film, ITO layer (~100 nm) and glass substrate, respectively. The thickness of the CSM@VSM film was ca. 86 nm. As previously reported, the obtained film is a binary assembly composed of highly ordered and regularly aligned VSMs and CSMs, with the latter ones physically confined in the former.

Furthermore, the molecular permeation property of the CSM@VSM film was investigated by cyclic voltammetry (CV). Three redox probes, namely neutral FcMeOH, negatively charged $Fe(CN)_6^{3-}$ and positively charged $Ru(NH_3)_6^{3+}$ were employed for this purpose. Fig. 2 compares the CVs obtained for three different electrodes, namely a bare ITO, CSM@VSM/ITO and VSM/ITO as exemplified in the insets. At a bare ITO electrode, well-defined current waves were observed for all three redox probes. However, at the CSM@VSM/ITO electrode, an obvious current response was only displayed for FcMeOH but not $Ru(NH_3)_6^{3+}$ or $Fe(CN)_6^{3-}$. It indicates that neutral FcMeOH can permeate the hydrophobic CTAB micelles and further transfer to the underlying ITO surface, and that the CSMs block the diffusion of charged redox probes

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