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Simultaneous detection of metronidazole and chloramphenicol by differential pulse stripping voltammetry using a silver nanoparticles/sulfonate functionalized graphene modified glassy carbon electrode

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ABSTRACT

A novel silver nanoparticles/sulfonated functionalized graphene modified glassy carbon electrode (AgNPs/SF-GR/GCE) was fabricated to determine chloramphenicol and metronidazole simultaneously. Taking advantage of sulfonic group, AgNPs were successfully electrodeposited on functionalized GR immobilized on the surface of a GCE. Scanning electron microscopy and energy spectrum analysis results confirmed that AgNPs were deposited on the functionalized GR film. Compared to the bare GCE or the pristine SF-GR modified electrode, AgNPs/SF-GR/GCE exhibited excellent electroreduction towards chloramphenicol and metronidazole. In addition, the two antibacterial drugs were separated completely in 0.10 M citric acid-sodium citrate buffer (pH 4.0) by differential pulse stripping voltammetry under optimum conditions. The cathodic current was linearly related with 0.02~20.0 μM chloramphenicol and 0.10~20.0 μM metronidazole, with the detection limits of 0.01 μM and 0.05 μM respectively. Furthermore, AgNPs/SF-GR/GCE was applied to the simultaneous determination of chloramphenicol and metronidazole in an aquatic product.

1. Introduction

Food safety has become a global concern in recent years, which is being threatened by, the abuse of food additives, especially unpermitted antibiotics. Chloramphenicol (CAP), i.e. [d(-)-threo-2-dichloro-acetamido-1-pnitro-phenyl-1,3-propanediol](Scheme 1), is an effective antibiotic against gram-positive and gram-negative bacteria, which has been widely used since the 1950s to treat animals mainly because of its high antibacterial property and low cost. It inhibits the synthesis of bacterial proteins by binding a site on the 50S subunit of ribosome. Nevertheless, CAP may cause severe toxic effects such as bone marrow depression, aplastic anemia, cardiovascular collapse and gray baby syndrome [1]. Considering this toxicity in humans, CAP has been completely banned in food-producing animals within the European Union (EU) [2]. A minimum required performance limit for CAP detection was set by the EU at 0.3 $\mu g\,kg^{-1}$ in all foods of animal origin [3]. Consequently, it is of great significance to propose a sensitive method to detect CAP.

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol, MTZ) (Scheme 1) has been employed in both human and veterinary medicines to treat diseases caused by anaerobic bacteria (*Bacteroides*,*Fusobacterium*, *Campylobacter*, *Clostridium*) and protozoa (*Trichomonas*, *Treponema*, *Histomonas*) [4]. Unfortunately, this drug is carcinogenic, mutagenic and toxic [5], so the EU and Ministry of Agriculture of the People's Republic of China have prohibited adding MTZ to animal-derived foods. Recently, CAP and MTZ are still added intentionally into foods or feeds, especially in aquatic products, to prevent bacterial and *Trichomonas* infections, causing serious harm to human.

A variety of analytical methods have been developed to determine CAP in food samples, of which chromatographic methods are mainly used, including high performance liquid chromatography (HPLC) [6,7], gas chromatography-mass spectrometry (GC-MS) [8], liquid chromatography-mass spectrometry (LC-MS) [9] and LC-MS/MS [10]. In addition, electrochemical immunoassay [11] and electrochemical detection [12,13] have also been applied to determine CAP. Similarly, GC [14], HPLC [15,16],

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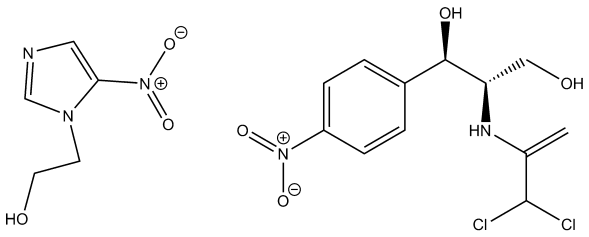

Scheme 1. Chemical structure of MTZ and CAP.

electrophoresis [17] and electroanalytical technique [18–20] have been used to detect MTZ. However, most of these chromatographic techniques require time-consuming pre-processing steps, expensive instruments and skillful operators. Besides, these large instruments are bulky and need considerable warm-up time before sample determination. In contrast, electrochemical analysis, which offers unique advantages such as simple operation, use of low-dose organic solvents, high sensitivity and ultra-low detection limits, has thus been widely used to detect CAP or MTZ. Borowiec et al. determined CAP by using a glassy carbon electrode (GCE) that was modified with synthetic nitrogen-doped graphene (GR) nanosheets decorated with gold nanoparticles (NPs), with the detection limit of 0.59 μM [21]. By using a sensitive voltammetric method, Yang et al. determined CAP based on molybdenum disulfide nanosheets and self-doped polyaniline composite modified GCEs [22]. Gholivand et al. constructed a novel highly selective and sensitive voltammetric sensor based on a molecularly imprinted polymer-carbon paste electrode to detect MTZ with differential cathodic stripping voltammetry [23]. With GR-room temperature ionic liquid (IL) of 1-butyl-3-methylimidazolium hexafluorophosphate composite modified GCE, Peng et al. determined MTZ at a low limit of 0.047 μM [24]. However, CAP, especially when coexisting with MTZ in foodstuff, has never been determined by using electrochemical methods erenow.

As is known to all, NPs of metals, especially those of noble metals (e.g. Au, Ag and Pt), usually exhibit high electrocatalytic activities towards test compounds. Particularly, AgNPs have remarkable conductivity and biocompatibility. Moreover, AgNPs modified electrodes are conducive to the cathodic reactions of compounds. Lorella et al. electrodeposited AgNPs on pure graphite sheets immobilized on GCE and studied the electrochemical behaviors of nitrite, nitrate and idoate respectively [25]. Li et al. electrodeposited AgNPs on GCE modified IL functionalized multiwall carbon nanotube composites, and the fabricated electrode showed high catalytic activity towards H_2O_2 reduction [26]. By preparing an AgNPs electrode, Liu et al. also proved that AgNPs had extraordinary electrocatalytic activity towards the dechlorination of chloroacetic acids [27]. Furthermore, AgNPs can stabilize sensors and accelerate the electron transfers of electrochemical reactions [28,29].

GR, a two-dimensional sheet of sp^2-bonded carbon atoms arranged in a honeycomb lattice, has been widely applied in nanoelectronics [30], biomedicines [31] and sensors [32]. Due to extraordinary electronic transport properties, as well as high electrical conductivity and surface-to-volume ratio [33], GR is able to improve the electrocatalytic performance on the surface of modified electrode. Meanwhile, the unique two-dimensional plane structure allows it to support NPs of metal and metal-oxide catalysts [34]. However, being hydrophobic, GR is prone to irreversible agglomeration or even restacking into graphite through Van der Waals bonding and strong π-π conjugation. To circumvent these problems, surfactants, β-cyclodextrin and IL have been mixed with GR to improve its dispersibility, or some specific groups have been introduced via chemical or physical functionalization. Samulski et al. synthesized a water-soluble GR [35] by introducing $-SO_3^-$ units to increase stability without adding surfactants or polymer, with which a sensor was constructed to detect dopamine [36]. Besides, AgNPs are readily deposited on the surface of GR in the presence of $-SO_3^-$ units because of the interaction between Ag^+ and SO_3^-, managing to control the reaction of electrodeposition.

Thereby motivated, the electrochemical activities of CAP and MTZ were herein evaluated with an AgNPs/sulfonated functionalized GR modified GCE (AgNPs/SF-GR/GCE) by differential pulse stripping voltammetry (DPSV). The cathodic currents of CAP and MTZ were significantly increased on the modified electrode. Notably, the modified electrode displayed much stronger electrochemical responses towards CAP and MTZ in food samples than other working electrodes (except for electrochemical immunosensors) did, with satisfactory recoveries. This facile, time-saving, fast-responding analytical method had high sensitivity and stability, as well as low detection limits.

2. Experiment

2.1. Reagents and chemicals

All chemicals were analytical-grade unless otherwise required. Citric acid, trisodium citrate dihydrate, potassium nitrate, potassium permanganate, potassium hexacyanoferrate (III), sodium nitrite, sodium carbonate and hydrogen peroxide (30%) were obtained from Damao Chemical Reagent Co., Ltd. (Tianjing, China) or Guangzhou Chemical Reagent Co., Ltd. (Guangzhou, China). Silver nitrate was received from Guanghua Chemical Factory (Shantou, China). Graphite, CAP and MTZ were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Nafion (5 wt% solution in a mixture of lower aliphatic alcohols and water) was from Sigma-Aldrich (Steinheim, Germany). Citric acid-trisodium citrate dihydrate buffers with pH ranging from 3.0 to 5.5 were prepared by mixing stock solutions of 0.1 M citric acid and 0.1 M trisodium citrate dihydrate, potassium nitrate at different proportions. CAP and MTZ standard solutions (1.0×10^{-3} M) were prepared in deionized water daily.

2.2. Instrumental conditions

All electrochemical measurements were performed on a CHI 660 E electrochemical workstation (CH Instrument, Shanghai, China). A conventional three-electrode system was used for all electrochemical experiments. Bare or modified GCE (Φ = 3 mm) acted as the working electrode, and a double junction saturated calomel electrode (SCE/KNO_3) and a platinum electrode served as the reference and auxiliary electrodes (Gaoss Union, Wuhan, China) respectively. All the electrochemical measurements were operated in 0.1 M N_2-saturated citric acid-trisodium citrate dihydrate buffers (pH 4.0). The morphologies of samples were viewed by scanning electron microscope (SEM) and energy dispersive spectrometer (SHIMADZU SSX-550, Japan).

2.3. Synthesis of graphene oxide (GO) and SF-GR

GO was synthesized from graphite powders according to the modified Hummers method [37]. SF-GR sheets were prepared from GO by the following three steps [35,36]. Briefly, first, 150 mg dried GO was dispersed in 150 mL of deionized water by sonication for 1 h. After centrifugation at 4000 rpm and removal of sediments, a clear and brown GO dispersion formed. Then, 1.2 g sodium borohydride in 30 mL of deionized water was added into the dispersion of GO after its pH value was adjusted to 9∼10 with 5%