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Research paper

Direct electrochemical sensing for oxytetracycline in food using a zinc cation-exchanged montmorillonite

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ABSTRACT

Direct voltammetric determination of oxytetracycline (OTC) at a zinc cation-exchanged montmorillonite modified glassy carbon electrode (Zn-Mt/GCE) was described. The OTC yielded one well-defined oxidation peak at the Zn-Mt/GCE. The experimental parameters, which influence the voltammetric responses of OTC, e.g., the pH value, scan rate and accumulation condition, were optimized. The oxidation peak current changed with the OTC concentration over the range from 0.80 to 40 μM. The detection limit was 0.12 μM for an accumulation time of 4 min. The coefficient of variation, determined at 10 μM OTC, was 3.4% (n = 10). Using this method, OTC in the real food and feedstuff samples was determined.

1. Introduction

Clay minerals are layered silicates, usually aluminosilicates (Schulze, 1989). They have been used for a plethora of applications due to their large adsorption capacity and swelling characteristics in aqueous dispersions (Van Olphen, 1977). Montmorillonite (Mt), a kind of expansible 2:1 smectite clay, is one of the most often studied clay minerals. The nanoporous and nanostructured Mt consists of an octahedral sheet—$M(O, OH)_6$ ($M{=}Al^{III}$, Mg^{II}, Fe^{II}, or Fe^{III}), sandwiched between two SiO_4 tetrahedral sheets. Due to the isomorphic substitutions in the layers, broken bonds, and/or defects in the structure, negative charges in the Mt are resulted in, which are balanced by counter ions adsorbed between the clay mineral layers in the interlayer region (Newmann, 1987). Therefore, cationic-exchange reaction can take place between the cations and other positively charged ions of Mt. Mt also has a high swelling capacity, so intercalation of the metal is efficient (Jha et al., 2013). Furthermore, Mt is not only low cost and abundance (Günister et al., 2007), but also owns high surface area and large aspect ratio (ratio of length to thickness) from 50 to 1000 (Le Corre et al., 2010). These properties make it an excellent filler for next modification. Recent studies have shown that nanohybrids with intercalated an/or exfoliated Mt nanolayers offer significant improvement to the mechanical (Le Corre et al., 2010), thermal (Lewicki et al., 2009) and barrier properties (Labruyère et al., 2009) in comparison with conventional composite materials prepared with fillers dispersed as micrometer-sized structures.

To date, many electrochemical sensors were fabricated based on Mt and its nanohybrids. Wang et al. (Wang et al., 2012) used a modified Mt-modified expanded graphite electrode as an amperometric sensor for *o*-aminophenol determination. The response of *o*-aminophenol was noticeably improved due to the higher accumulation efficiency of modified Mt. Zapp et al. (Zapp et al., 2011) focused on the development and evaluation of a new biosensor for the determination of pesticide methomyl, using Mt as the support. In their work, Mt showed good adsorptive affinity. Bouwe et al. (Bouwe et al., 2011) designed a low-cost electrochemical sensor for lead detection using an organo-clay obtained by the intercalation of 1,10-phenanthroline within Mt. The results showed that Pb(II) could be exchanged into Mt layers, and therefore the accumulation concentration of Pb(II) on electrode surface increased dramatically. El-Desoky et al. (El-Desoky and Ghoneim, 2011) developed a square-wave adsorptive anodic stripping voltammetric method for the determination of silymarin utilizing a Mt–Ca modified carbon paste electrode. The modified electrode was stable, efficient and exhibited excellent selectivity towards the trace determination of silymarin. In addition, our group has reported two electrochemical sensors based on Mt (Gan et al., 2010) and neutral red intercalated Mt (Gan et al., 2011) for the simple and sensitive detection of methyl jasmonate, in which the Mt showed outstanding catalytic ability.

Continuous sub-lethal levels of antibiotics in food have led to the emergence of harmful bacteria resistant to antibiotics (Adrian et al., 2008), caused allergic reactions in sensitized individuals (Dewdney et al., 1991), or affected the intestinal flora (Madden et al., 2005). Therefore, an essential aspect of food safety is the control of drug residuals in food-producing animals. Oxytetracycline (OTC), which belongs to the tetracycline (TC) group, is used in the treatment of vibriosis, enteric redmouth and also furunculosis (Oliveira et al., 2013). The extensive

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use of TCs including OTC in veterinary medicine has led them to be accumulated in food products or to be released into the soil and natural waters via animal metabolism, causing serious threats to human and environmental health (Gräslund and Bengtsson, 2001). A few analytical methods have been developed for OTC determination. However, in most papers dealing with this subject with expensive, complicated and easy denatured chromatography (Chen and Schwack, 2013; Guo et al., 2013), microbiological (Gao et al., 2013) or molecular imprinted techniques (Lian et al., 2013). As far as our knowledge, rare paper reports the direct determination of OTC based on rapid, selective and sensitive electrochemical assay (Nagles et al., 2012).

In this work, Zn nanoparticles were selected to modify Mt through cation-exchange method because Zn possesses high specific energy density, high power density, low cost and low toxicity (Lee et al., 2011). The Zn-Mt was thereafter used to improve the direct electrochemical activity of OTC. OTC exhibited very sensitive oxidation peak on the Zn-Mt modified glassy carbon electrode (GCE). This method has the following advantages: easy to prepare, economical to use, rapid response, easy renewal, low cost and low detection limit.

2. Experimental

2.1. Reagents

OTC and zinc nitrate hexahydrate ($ZnNO_3 \cdot 6H_2O$) were obtained from the Sinopharm Group Chemical Reagent Co. Ltd., China, with analytical grade purity. Nano-sodium Mt (~25 nm) as purchased from the Fenghong Clay Chemicals Co., Ltd. (Zhejiang, China). The content of Mt is 96–98%, the apparent density is 0.25–0.35 g cm^{-3}, and the diameter thickness ratio is 200. OTC was dissolved into 0.1 M HCl to prepare 10.0 mM standard stock solution, which was diluted by 0.1 M HCl to the desired concentration before use. The water used was re-distilled.

2.2. Apparatus

All the electrochemical measurements were carried out with a CHI 660D electrochemical analyzer (Chenhua Instruments, China). A three-electrode system, including Zn-Mt modified GCE working electrode, a platinum wire counter electrode and a saturated calomel reference electrode (SCE), was employed. All potentials were reported versus SCE.

The field emission scanning electron microscopy (SEM) images were taken on Hitachi S-4800 microscopy (Japan). Transmission electron microscopy (TEM) and high-resolution electron microscopy (HR-TEM) images were obtained from a TECNAI G2 20S-TWIN transmission electron microscope (FEI, Holland). X-ray diffraction (XRD) was recorded on a Rigaku D/max-2500, using Ni filtered Cu K_α radiation ($\lambda = 0.154$ nm) as a source (current intensity, 100 mA; voltage, 40 kV) and a Xcelerator detector.

2.3. Preparation of Zn-Mt

The Zn modified Mt was prepared as follows (Jha et al., 2013): 40 mL of 0.2 M-$ZnNO_3 \cdot 6H_2O$ aqueous solution was slowly added into 2.5 g nano-Mt, and stirred for 4 h at room temperature. After filtering, washing with abundant water and drying in a vacuum oven at 373 K for 2 h, the product was grinded to powder and then calcined in a furnace at 573 K for 3 h with a heating rate of 2 °C min^{-1}, which was denoted as Zn-Mt. 20 mg of the prepared Zn-Mt was mixed with 10 mL water and stirred for 48 h to obtain colloidal dispersion.

2.4. Fabrication of Zn-Mt modified GCE

Prior to coating, the GCE was firstly abraded on filter paper, and then polished on the microcloth pads. After that, the electrode was ultrasonicated in ethanol/water (1:1, V/V) and distilled water for about 30 s, respectively. A Zn-Mt coating was achieved by adding 10.0 μL of Zn-Mt colloidal solution on the surface of the pretreated GCE, and finally allowed to dry under infrared lamp.

2.5. Analytical procedure

A 5 mL volume of citric acid–Na_2HPO_4 (pH 2.2) containing a known concentration of OTC was added into the electrochemical cell. After OTC was accumulated under an open-circuit for a certain time while stirring the solution, differential pulse voltammograms (DPV) were recorded from 0.6 to 1.2 V. The oxidation peak current was measured at 1.01 V. All of the measurements were carried out at room temperature.

2.6. Sample pretreatment

The chicken feed, chicken, fish and shrimp samples are purchased from local market. The chicken feed granules were grinded to powder, and the meat samples were sheared to fragment before pretreatment.

2.6.1. Chicken feed

Chicken feed sample spiked with target OTC, was prepared by adding the stock solution of OTC with spiked level of 0.2 mg g^{-1} to the feed sample, and then the sample was shaken for 2 min by a Model XK96-B shaker (Shanghai, China) to mix completely. It was subsequently evaporated to dryness in air and stored at 4 °C in dark. 0.1 g of this spiked chicken feed sample was weighed accurately and mixed with 5.0 mL aliquot of ethyl acetate in a 10 mL centrifuge tube. Then, the sample was treated in an ultrasonic bath for 20 min and centrifuged for 10 min at 4000 rpm. The upper ethyl acetate layer (3.2 mL) was transferred into a 10 mL centrifuge tube, which was evaporated to dryness at room temperature. Finally, the obtained dried deposit was dissolved with 500 μL methanol and diluted to 5.0 mL with water.

2.6.2. Chicken, fish and shrimp

A weighed aliquot of chicken (6 g) was homogenized in 3.0 mL of 0.1 mol L^{-1} EDTA and 2.0 mL of pH 4.0 citric acid–Na2HPO4 buffer with the use of a model HY-4 homogenizer (Shanghai Instrumental Manufacturing, China) at 4000 rpm for 15 min. Such a sample was shaken for 5 min with 3 mL-acetonitrile. It was then, centrifuged at 4000 rpm for 10 min, decanted into a clean polypropylene tube. Furthermore, the fish residue was extracted with another 3.0 mL aliquot of acetonitrile. The two extracts were then combined and evaporated to dryness with nitrogen. Dried extract was reconstituted in 200 μL methanol, which was centrifuged under the same conditions as before and stored at 4 °C in the dark. The fish or shrimp sample was extracted as mentioned above.

3. Results and discussion

3.1. Characterizations

The XRD powder patterns of Mt and Zn-Mt are shown in Fig. 1. At 2θ angle that equals to 6.78, the basal spacing calculated according to the Bragg's Law ($n\lambda = 2d\mathrm{Sin}\theta$) is found to be 10.1 Å for Na-Mt. For Zn-Mt, the 2θ value of the 001 reflection does not shift after ion exchange, indicating that the basal spacing is not affected. The relative low d-spacing is caused by the high-temperature treatment at 300 °C, which leads to none or a little residual water in the interlayer space. The non-basal X-ray reflections, which are a characteristic of Mt (M in Fig. 1) are found at $2\theta = 19.7$ and 34.9°. Other reflections at $2\theta = 20.8$, 26.6 and 27.5° can be attributed to quartz (Q in Fig. 1), which are present as an impurity (Brindley and Brown, 2011).

The morphologies of bare GCE and Zn-Mt film modified GCE are displayed in SEM photographs (Fig. 2a and b). The surface of bare GCE is rather smooth (a). However, the Zn-Mt film which appears in micrograph (b) is completely different. The Zn-Mt film shows uniformly