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Molecularly imprinted electrochemical sensor for the highly selective and sensitive determination of melamine

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ABSTRACT

A selective and sensitive molecularly imprinted electrochemical sensor was developed for the determination of melamine. Carbon nanotube–ionic liquid composite was used as a carrier to enhance the number of imprinted cavities and then improve the selectivity and sensitivity of the sensor. The proposed sensor showed a linear relationship with the concentration of melamine in the range of 0.4 to 9.2 μM, with a detection limit of 0.11 μM. The sensor was successfully applied to the determination of melamine in milk products with good recovery.

1. Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) is a kind of triazine analogue which has three amino groups. Because it contains a substantial amount of nitrogen (66% by mass), some unethical manufacturers added it to the milk products to obtain high protein contents. The US Food and Drug Administration (FDA), the European community, and other countries and regions have established criteria of maximum residue limits for melamine in various food products. Standard limits of 1 ppm for melamine in infant formula and 2.5 ppm in other milk products have been legislated by many countries [1]. Ingestion of melamine at levels above the safety limit can cause urinary system diseases, especially in babies and children [2]. Therefore, a sensitive and reliable method is essential for the determination of melamine in milk products for children.

Many methods have been developed for the determination of melamine, such as liquid chromatography [3,4], capillary electrophoresis [5–7], chromatography– mass spectrometry [8–10], enzyme-linked immuno-assay [11] and electrochemical techniques [12–15]. Among these methods, electrochemical techniques have the characteristics of simplicity, low cost, accuracy and easy to on-site detection. Moreover, electrochemical techniques have provided a sensitive and selective approach for the detection of numerous biological and environmental compounds [16–24]. Thus, in recent years, electrochemical techniques have attracted more and more attentions in analytical chemistry due to their wide applications.

For the formation of specific recognition sites to improve the selectivity of electrochemical method, molecularly imprinting technique has been proposed and developed fastly in recent years. Firstly, the affinity matrix is prepared by the polymerization of functional monomers and crosslinkers in the presence of template molecules. Then, the template molecules are removed from the polymer. Thus, the molecularly imprinted polymer (MIP) is obtained with the specific cavities complementarily in shape, size and functional groups to the template molecule, which could selectively recognize template molecule. Molecularly imprinted electrochemical sensors have received considerable attention for the determination of melamine because of their high selectivity and stability [25–27]. Recently, functional nanomaterial is often used to improve the conductivity and catalytic ability of the sensors and then greatly enhance the intensity of the electrochemical signal [28]. Because of the large surface area, functional nanomaterial could also be used as a carrier in the preparation of molecularly imprinted polymers to increase the number of imprinted cavities.

Carbon nanotubes (CNTs) have been widely used in electrochemical field due to their unique properties such as large specific surface area, good mechanical stability and high electronic conductivity [29–31]. Ionic liquid (IL) is a salt in the liquid state. IL is largely made of ions and short-lived ion pairs. Room temperature ionic liquids consist of bulky and asymmetric organic cations such as 1-alkyl-3-methylimidazolium, 1-alkylpyridinium, N-methyl-N-alkylpyrrolidinium and ammonium ions. It has unique properties such as good ionic conductivity, high viscosity, high chemical and thermal stabilities [32]. Thus, it has been widely used as powerful solvents and electrolytes in electrochemistry. The capability of IL combining with CNT to form conductive composite makes it very attractive for the synthesis of functional nanomaterial. Therefore, CNT–IL composite could be used as a kind of robust and advanced carrier material for the preparation of molecularly imprinted polymers.

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Pyrrole is an electroactive functional monomer, which is often employed to fabricate MIP sensor for a variety of molecules recognition and detection [33,34]. The binding sites of polypyrrole molecules could effectively bind to the melamine through hydrogen bonds. In this study, a molecularly imprinted electrochemical sensor was prepared for the determination of melamine. Pyrrole was used as monomer, CNT–IL composite was used as a carrier to enhance the number of imprinted cavities and then improve the selectivity and sensitivity of the sensor. The preparation process and the electrochemical response of the sensor to melamine were shown in Fig. 1. The proposed electrochemical sensor showed a high selectivity toward melamine as well as a broad linear range and a low detection limit under the optimized conditions. Satisfactory results were also obtained for the determination of melamine in real samples.

2. Experimental

2.1. Reagents and instrumentation

Multi-walled carbon nanotubes (MWCNTs) were obtained from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China) with a typical diameter of 10–20 nm. The purity was more than 98%. The ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF_4) was purchased from Lanzhou Centre for Green Chemistry and Catalysis, LICP, CAS (Lanzhou, China). All chemicals were of analytical grade and used as received without further purification. Melamine, glycine, and histidine were obtained from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Pyrrole, lactose, and uric acid were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All other chemical reagents were obtained from Nanjing Chemical Reagent Company (Nanjing China). All the solutions were prepared with double distilled water.

All electrochemical experiments were carried out on a CS350 Electrochemical Workstation (Wuhan Corrtest Instruments CO., LTD, Wuhan, China). A conventional three-electrode cell configuration was employed for the electrochemical measurements. A modified glassy carbon electrode (disc diameter of 3 mm) was used as the working electrode, with saturated calomel electrode (SCE, saturated KCl) and platinum wire for the reference and the counter electrode, respectively. All the potentials are versus saturated calomel electrode (saturated KCl). Transmission electron microscopy (TEM) images were obtained by using JEM-200CX (JEOL Ltd., Japan).

2.2. Preparing CNT–IL/MIP composite and fabrication of modified electrode

Prior to use, the glassy carbon electrode (GCE) was carefully polished with a leather containing 0.05 μm Al_2O_3 slurry and then ordinal ultrasonically cleaned in ethanol and distilled water.

Firstly, 5 mg of the MWCNT was dispersed in 10 ml of [Bmim]BF_4 by ultrasonic agitation for about 1 h. Then, the polymerization reaction was initiated with the addition of 0.3 mL H_2O_2 to the mixture containing 10 mL of CNT–IL composite, 0.1 mL of pyrrole, 0.02 g $FeCl_2$, 0.1 g melamine and 40 mL water. After 3 h reaction with stirring, the reactants were washed with water to remove the unreacted substances. Subsequently, 10 μL of the composite dispersion was dropped on the surface of GCE and dried in air.

The extraction of template molecules from polymer matrix is often using organic reagents or buffer solution as eluent. However, it is time consuming and the template cannot be removed entirely. In this work, a simple method, cyclic voltammetry was used to extract melamine molecules from the imprinted polymer matrix. The embedded melamine molecules were extracted by scanning the modified electrode between − 0.3 and 0.8 V in 0.3 mol L^{-1} KOH and 0.1 mol L^{-1} KCl for several cycles until no redox peak for melamine was observed, which demonstrated the complete removal of melamine. Thus, the CNT–IL/MIP/GCE (where MIP is molecularly imprinted polymers) was obtained.

Fabrication of CNT–IL/NIP/GCE (where NIP is non-molecularly imprinted polymers) was the same as the process mentioned above but without the addition of melamine. In addition, 10 μL of the CNT–IL composite dispersion was dropped on the clean GCE surface and then dried in air to prepare CNT–IL/GCE. The modified electrodes were stored in air at room temperature until used.

2.3. Experimental procedures

A standard three-electrode cell connected to the CS350 Electrochemical Workstation was used for electrochemical measurements. The CNT–IL/MIP/GCE was immersed in the solution containing 0.2 mol L^{-1} H_2SO_4, 0.1 mol L^{-1} KCl and certain amount of melamine for 120 s, then the cyclic voltammograms were recorded from 0.2 to 0.9 V at a scan rate of 100 mV/s, the square wave voltammograms were recorded from 0.2 to 0.9 V with a step increment of 4 mV, amplitude of 25 mV and frequency of 15 Hz.

3. Results and discussion

3.1. Morphology characterization of CNT–IL/MIP composite

To investigate the morphology of the fabricated CNT–IL/MIP composite, TEM images were carried out and results were shown in Fig. 2. The image of pure CNT shows a typically smooth surface in Fig. 2A. By contrast, the CNT–IL composite looks like gel-like substance (Fig. 2B). However, many particles were observed on the surface of CNT–IL composite after polymerization, which attributed to the formation of polypyrroles (Fig. 2C).

3.2. Electrochemical behavior of melamine at modified electrodes

Fig. 3A shows the cyclic voltammograms (CVs) of 20 μM melamine at CNT–IL/MIP/GCE (a), CNT–IL/GCE (b) and CNT–IL/NIP/GCE (c) in the electrolyte solution containing 0.2 mol L^{-1} H_2SO_4 and 0.1 mol L^{-1} KCl recorded after 120 s preconcentration. As shown in curve a, a pair of well-defined redox peaks are observed, which is attributed to the redox reaction of melamine at CNT–IL/MIP/GCE. It is also found that melamine shows similar electrochemical response at CNT–IL/NIP/GCE or CNT–IL/GCE, however, the peak current is much lower than that of CNT–IL/MIP/GCE. The oxidation peak current of CNT–IL/MIP/GCE is about 3 times that of CNT–IL/NIP/GCE and 2.2 times that of CNT–IL/

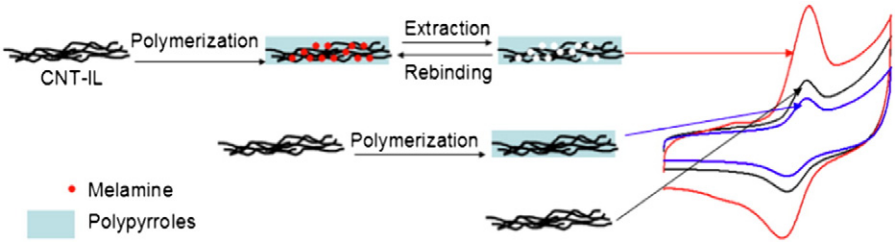

Fig. 1. The preparation process of CNT–IL/MIP and CNT–IL/NIP, and the comparison of electrochemical response of melamine at different modified electrodes.