Imprinted sol–gel electrochemical sensor for melamine direct recognition and detection

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ABSTRACT

A sol–gel based electrochemical sensor was developed for melamine (Mel) direct and selective detection in real samples. Thin film of molecularly imprinted sol–gel polymers with specific binding sites for Mel was fabricated on glassy carbon electrode surface by electropolymerization method. Oxidation peak of Mel was chosen as a detected signal for the properties study of the sensor. The molecularly imprinted polymers film modified method, electropolymerization scan rate, scan cycles, potential range, amount of Mel and extraction conditions for sensor preparation were optimized. Under the optimized conditions, the sensor displayed an excellent recognition capacity towards Mel compared with other structural similar molecules. With acceptable regeneration and stability the sensor could detect Mel in the range of 6.3×10^{-7}–1.1×10^{-4} mol L^{-1} with a detected limit of 6.8×10^{-8} mol L^{-1} ($S/N = 3$). The result of real samples detection indicated that the proposed low cost electrochemical sensor might be potential to determine Mel in real samples to ensure food safety.

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1. Introduction

Melamine (Mel, 1,3,5-triazine-2,4,6-triamine), a trimer of cyanamide, is usually used to manufacture melamine–formaldehyde resin [1,2]. In recent years, it has been illegally added to dairy products to show a false increase in protein concentration because it contains a substantial amount of nitrogen (66%) by mass. Investigation of Mel at levels above the safety limit (2.5 ppm in the United States and European Union; 1.0 ppm for infant formula milk powder in China) may cause kidney failure and even death [3–5]. Therefore, determination of Mel has been of great concern in food safety. So far, many analytical techniques have been employed to investigate Mel, such as gas chromatography [6], liquid chromatography [7], enzyme colorimetric assay [8], high performance liquid chromatography/mass spectrometry [9], and surface enhanced Raman spectroscopy [10]. However, complicated preconcentration process, time-consuming steps and high-cost instruments limit the application of these traditional methods although they are mostly accurate and sensitive. Therefore, there is still an increasing demand for fast, simple, convenient, and sensitive methods to solve these problems.

Electrochemical methods have been applied for the determination of Mel due to their simplicity, sensitivity, and stability [11–14]. Unfortunately, it is difficult for the direct detection of Mel because Mel is highly stable with poor electroactivity in the commonly used experiment conditions. Thus, most reported electrochemical methods for Mel were indirect. Zhu et al. developed an electrochemical sensor for analysis of Mel based on its conversion to an electroactive complex by coordination of copper salt to Mel with a low detection concentration of 0.25 ppb [15]. An electrochemical sensor was proposed by Hu et al. for the detection of subnanomolar Mel based on electrochemical accumulation coupled with enzyme colorimetric assay, which has been successfully applied for the detection of Mel in infant milk powders and fish feed samples [16]. Oligonucleotides film modified gold electrode was prepared for Mel detection by using ferricyanide as an electrochemical probe to investigate the interactions between oligonucleotides and Mel, which was reported by Zhao et al. [17].

Although electrochemical sensors showed high sensitive, electrochemical detection of Mel from interfering molecules has not been yet achieved widely. Molecular imprinting technique, the design and construction of biomimetic receptor system with predetermined recognition for target molecule, has been proposed [18,19] and developed rapidly in these years [20–22]. With the significant advantages in easy preparation, low cost, predictable specific recognition and high stability, the synthesized molecularly imprinted polymers (MIPs) has also been combined with electrochemical sensor to improve the selectivity of the sensor. Qin

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et al. [23] prepared a MIPs membrane based ion-selective electrode for the selective detection Mel in the presence of high concentration of ions co-existing in milk samples. An electropolymerized MIPs of para-aminobenzoic acid on glassy carbon electrode surface has been fabricated by Deng et al. [24]. With hexacyanoferrate as an electroactive probe, the MIPs sensor was successfully applied to the selective determination of Mel in milk products. Hexacyanoferrate was also used for the indirect determination of Mel by Jin et al. on a molecularly imprinted nano-porous film based electrochemical sensor [25]. A MIPs impedimetric sensor constructed for selective detecting Mel was studied by Lu et al. [26]. The properties of the sensor were investigated by impedance spectroscopy, cyclic voltammetry and differential pulse voltammetry by using hexacyanoferrate as a probe. The sensor also showed high sensitivity and wide linear range.

Sol–gel chemistry has sparked many researchers' interest from the electrochemical community as a versatile way for the preparation of modified electrodes. Compared with other methods, sol–gel method has unique advantages including mild reaction conditions, higher selectivity to the template, lower nonspecific binding and faster kinetics of adsorption [27,28]. Thus, sol–gel method has been combined with molecularly imprinted technique to prepare electrochemical sensor. Kong et al. fabricated a sensitive and selective electrochemical sensor for detection of the antidepressant imipramine [29].The sensor was fabricated onto the indium tin oxide electrode via stepwise modification of Au-NPs by self-assembly and a thin film of molecularly imprinted polymers via sol–gel technology. Zhang group prepared a human serum albumin imprinted film on the surface of piezoelectric quartz crystal Au-electrode modified with thioglycolic acid by combining sol–gel and self-assembly technology [30] . This self-assembly sol–gel imprinting technique was proved to be an alternative method for the preparation of biomacromolecule-imprinted thin film.

Compared to the reported indirect electrochemical methods, direct detection of Mel should be much more simple and convenient. Diazonium-based photoinitiators were electrografted on gold electrodes to provide specific, selective and ultrasensitive molecularly imprinted polymer grafts for the detection of Mel by Khlifi et al. [31]. In this study, an electrochemical sensor was prepared by the combination of molecularly imprinted technique and sol–gel technique. The sensitive and selective detection of Mel was directly implemented by the determination of Mel in an acidic solution. The prepared electrochemical sensor exhibited a high recognition capability toward Mel, as well as a broad linear range and a low detection limit under the optimized conditions. Satisfactory results were also obtained for the Mel determination in real samples.

2. Experimental

2.1. Chemicals and instruments

2.1.1. Chemicals

Melamine (Mel, 99%), cyanuric acid (CA, 98%), trithiocyanuric acid (TA, 95%), glycine (Gly, 99.5%), ascorbic acid (AA, 99%), L-histidine (L-His, 99%) were ordered from Aladdin (Aladdin, China). Methyltrimethoxysilane (MTMOS, >98%), phenyltrimethoxysilane (PTMOS, >97%) were provided by Sigma (Sigma, USA). All other reagents were of at least analytical-reagent grade, and double-distilled deionized water was used for all solutions.

2.1.2. Apparatus

Electrochemical experiments, such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed on a CHI 660C workstation (Chenhua Instruments Co., Shanghai, China)

with a conventional three-electrode system. A bare or modified glassy carbon electrode (GCE) served as the working electrode, and a saturated calomel electrode and a platinum wire electrode were used as the reference and counter electrodes, respectively. Field emission scanning electron microscope (FE-SEM) images were obtained on an S-4800 field emission scanning electron microanalyser (Hitachi, Japan).

2.2. Fabrication of electrochemical sensor

An organic–inorganic hybrid sol was prepared by the following procedure. 6.0 mL TEOS (27.0 mmol) was added into 6.0 mL ethanol, subsequently with the slowly addition of 0.74 mL PTMOS (3.96 mmol) and 0.60 mL MTMOS (4.10 mmol). Then, 2.0 mL H_2O and 2.0 mL HCl (0.1 mol L^{-1}) were dropwise added into above mixed solution under the gentle stirring. The sol was kept under stirring for 2 h to get a transparent stock solution. 12 mg Mel was added into above stock solution under sonication for 10 min, obtaining imprinted sol.

Prior to modification, bare GCE was polished with 0.3 and 0.05 μm alumina slurry on a micro-cloth pad and sonicated sequentially in water and ethanol. Then the electrode was activated in a H_2SO_4 (0.5 mol L^{-1}) by CV for several cycles until peak current kept the same. CV method was employed to fabricate imprinted sensor from 0 V to +1.9 V for 30 cycles at a scan rate of 50 mV s^{-1} by immersing GCE into above imprinted sol. Then the embedded Mel molecules were extracted by scanning in 1 mol L^{-1} H_2SO_4 for several cycles until no obvious redox peak of Mel could be observed, giving MIPs modified GCE (MIPs/GCE). The procedure for the preparation of the MIPs/GCE is depicted in Scheme 1.

As a control, non-molecularly imprinted polymers (NIPs) modified GCE (NIPs/GCE) was also prepared and treated in exactly the same manner, except for the omission of Mel in sol.

2.3. Electrochemical property measurements

Electrochemical measurements were performed in an electrolyte of 1 mol L^{-1} H_2SO_4 and 0.1 mol L^{-1} KCl by DPV method. CT, TA, AA, Gly and L-His were selected as the interferents to evaluate the recognition capacity of the prepared sensor.

3. Results and discussion

3.1. Electrochemical behaviors of Mel

Although most of electrochemical methods reported for Mel determination were indirect, direct detection of Mel was investigated in this study to find an easier approach for Mel detected application. Electrochemical behavior of Mel on GCE surface was investigated in an acidic electrolyte, a mixed solution of 1 mol L^{-1} H_2SO_4 and 0.1 mol L^{-1} KCl. As shown in Fig. 1a, a pair of reversible redox peaks (0.65 V for reduction current and 0.72 V for oxidized current) could be seen, which was assumed to be the formation of a radical cation through electrochemical oxidation [32–34]. Then it could be detected by its reversible redox peaks instead of the probe.

In order to get selective recognition property of the MIPs, the template molecules should keep its structure during the polymerized process. Since the electropolymerization of MIPs on GCE surface was also carried out in acidic solution (1.0 mL of 0.1 mol L^{-1} HCl was added into prepolymerized solution before the electropolymerization), the stability of Mel molecules during the electropolymerized process was studied, as shown in Fig. 2. Electropolymerized curves of MIPs (Fig. 2Aa) and NIPs (Fig. 2Ab) did not show any apparent difference. And the CV curve of the first