Melamine-imprinted polymer grafts through surface photopolymerization initiated by aryl layers from diazonium salts

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ARTICLE INFO

ABSTRACT

In this work, diazonium-based photoinitiators were electrografted on gold electrodes to provide specific, selective and ultrasensitive molecularly imprinted polymer (MIP) grafts for the detection of melamine. This toxic molecule was used as a template, while methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were the functional and crosslinking monomers, respectively. Surface-initiated photopolymerization was conducted because it is a simple and versatile mean for making ultrathin MIP grafts (~25 nm thick). Both cyclic and square wave voltammetry (SWV) were used for investigating rebinding melamine by the MIP grafts. However, the latter permitted to achieve ultrasensitive detection down to 7.7×10^{-10} mol/L in PBS and 3.1×10^{-10} mol/L in milk. Recovery was found to be very good, levelling off at 92%.

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1. Introduction

Melamine (2,4,6-triamino-1,3,5-triazine, MEL) is a toxic organic compound, usually employed to produce melamine—formaldehyde resins which are used as adhesives, and for the preparation of laminates and overlays (Pizzi, 2003, chap. 32). Melamine contains a substantial amount of nitrogen (66.7%) by mass so that, when added to milk powder, it increases the nitrogen content which is taken as a measure of protein concentration. Consequently, this leads to an overvaluation of protein concentration in pet foods and other protein-based food commodities (Li, Qi, & Shi, 2009). This falsification has resulted in the well known "Chinese milk" that provoked severe health problems in China in 2006 such as developing kidney stones with subsequent renal failure and death if infant children were not treated (Pei et al., 2011; Xiu & Klein, 2010; Yan, Zhou, Zhu, & Chen, 2009).

A few previously published articles were reported on the determination of melamine in complex matrices by gas chromatography (Yokley, Mayer, Rezaaiyan, Manuli, & Cheung, 2000), gas chromatography/mass spectrometry (Xu, Ren, et al., 2009; Zhu et al., 2009), high-performance liquid chromatography/tandem mass spectrometry (Desmarchelier, Cuadra, Delatour, & Mottier, 2009; Ehling, Tefera, & Ho, 2007; Ge et al., 2011), high-performance liquid chromatography with UV detection (Ali, Rafiuddin, Ghori, & Khatri, 2008; Chou, Hwang, & Lee, 2003; Lin et al., 2008; Yokley et al., 2000), solid-phase extraction (Zhang, Zhang, Hu, Yang, & Yao, 2011), spectrophotometric absorption measurements (Rima, Abourida, Xu, Cho, & Kyriacos, 2009) and chemiluminescence (Zhang, Wu, Chen, & Song, 2011), etc. However, these analytical methods might not be sensitive enough to detect lower melamine concentrations in complex matrices. Compared to the existing analytical techniques, much importance has been paid to the electrochemical sensors due to their simplicity, sensitivity and high stability. Unfortunately, there are very few reports regarding the utilization of electrochemical methods to determine melamine at solid electrodes. For example, Liao, Chen, Chang, and Zen (2011) have proposed an electrochemical method based on the formation of a polymer film from MEL at the surface of preanodized screen printed carbon electrode. The proposed device allows to reach a limit of detection (LOD) of about 0.8 μmol/L (at signal to noise ratio S/N = 3). However, melamine detection has not been achieved with interferent molecules. In order to bridge the gap between the sensitivity of electrochemical methods and selectivity of the sensor device system one can advantageously

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design molecularly imprinted polymer (MIP) thin layers onto working electrodes for the specific and selective electrochemical detection of MEL. Indeed, numerous research teams have combined MIP sensitive layers and electrochemistry for analytical purposes of a very large of molecules of biomedical, food, environmental importance and so on (Blanco-López, Lobo-Castañón, Miranda-Ordieres, & Tuñón-Blanco, 2004; Suryanarayanan, Wu, & Ho, 2010).

MIPs are synthetic polymeric materials with specific cavities shaped by template molecules trapped within the polymer during the polymerization process (Haupt & Mosbach, 2000). These cavities serve for the specific and selective rebinding of the template molecule after its extraction from the freshly prepared polymer. The retention mechanism involved is based on molecular recognition by capture of molecules adapted in shape and orientation of specific groups with that at the surface of created cavities. MIPs have been prepared to develop a wide range of sensors (Khadro, Sanglar, Bonhomme, Errachid, & Jaffrezic-Renault, 2010; Lattach et al., 2012; Lépinay, Kham, Millot, & Carbonnier, 2012; Malitesta et al., 2012; Uludağ et al., 2007), for enantiomeric separation (Zhang, Wu, Wu, & Zou, 2010), drug delivery (Puoci et al., 2011), detection of toxins (Hadj Ali et al., 2010; Lucci, Derrien, Alix, Pérollier, & Bayoudh, 2010; Yu & Lai, 2010) and explosives (Alizadeh, Zareb, Ganjali, Norouzi, & Tavana, 2010), and destruction of pesticides (Erdem, Say, Ersöz, Denizli, & Türk, 2010), to name but a few. A large set of methods have been developed to produce MIPs, including free radical polymerization (Molinelli, Weiss, & Mizaikoff, 2002), photopolymerization (Lin, Pan, Hwang, & Lee, 2007) or electropolymerisation (Lange, Roznyatouskaya, & Mirsky, 2008; Maouche et al., 2012). Recently, atom transfer radical polymerization (ATRP) has been proposed to prepare ultrathin MIP coatings with controlled thickness and for quick detection of didansyl cystine and lysine by surface plasmon resonance (SPR) (Wei, Li, & Husson, 2005). Provided ATRP initiators are grafted of the substrate via self-assembled monolayers (SAMs) of thiols, it is possible to covalently tether the sensitive MIP layers to the transducer. SAMs have also been combined with radical photopolymerization for grafting MIP sensing layers (Panasyuk-Delaney, Mirsky, & Wolfbeis, 2002). Instead of thiol SAMs, one can also employ silanes with initiator moieties for the in-situ grafting of vinylic (Blanco-López et al., 2004) or sol–gel MIP layers (Xu, Zhou, et al., 2009). In a new strategy of graft polymerization, Gam-Derouich et al. proposed to surface-initiate ATRP (Gam-Derouich, Nguyen, et al., 2010) or radical photopolymerization (Gam-Derouich, Jouini, et al., 2012; Gam-Derouich, Mahouche-Chergui, Truong, Ben Hassen-Chehimi, & Chehimi, 2011) with tethered groups derived from the electrochemical reduction of diazonium salts. These well known compounds have attracted, over the past two decades, a massive number of researchers interested in the surface modification of electrodes and other (nano)materials (Bélanger & Pinson, 2011; Pinson & Podvorica, 2005) because they ensure an efficient, strong and stable substrate-aryl bonding.

In this paper, we take advantage of the suitability of diazonium compounds to modify materials (here electrodes) and their propensity to surface-initiate a range of polymerization methods (Gam-Derouich, Mahouche-Chergui, Ben Romdhane, & Chehimi, 2012, chap. 6; Mahouche-Chergui, Gam-Derouich, Mangeney, & Chehimi, 2011; Salmi, Gam-Derouich, Mahouche-Chergui, Turmine, & Chehimi, 2012) including radical photopolymerization in order to prepare MIP films for the specific and selective electrochemical detection of melamine. Characterization of the chemical properties of the films was performed with polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS) and X-ray photoelectron spectroscopy (XPS). Linear and square wave stripping voltammetry were used to evaluate the binding characteristics of the gold-grafted melamine-imprinted polymer films. Square wave

voltammetry (SWV) was employed in order to reach a very low detection limit as it is known to drastically reduce capacitive current. The selectivity of the electrode-grafted MIP systems was evaluated using a near MEL structural molecule, the 6-methyl-(2,4)-diamino-(1,3,5)triazine (DMT). To the very best of our knowledge, this is the first time that diazonium salts and radical photopolymerization are combined for the development of MIP-based electrochemical sensors against melamine in buffered solutions and in complex milk matrices.

2. Experimental

2.1. Materials

Gold-coated silicon 1×1.5 cm^2 was cut from wafers (Aldrich, nominal Au thickness of 1000 Å). The slides were ultrasonically rinsed with acetone, water and ethanol, dried in a stream of argon, then cleaned in a UV cleaner (Boekel, Inc., Model 135 500) and finally rinsed with acetonitrile (ACN).

Ethylene glycol dimethacrylate (EGDMA: 198.22 g/mol across) was purified by filtering through basic alumina (Al$_2$O$_3$) column, and stored under argon at -4 °C before use. The methacrylic acid (MAA: 86.09 g/mol, Acros) was purified by distillation.

The following chemicals were used as received: benzophenone (Aldrich), triethylamine (TEA, Alfa Aesar), melamine (sigma) and dimethylaniline (Across). Phosphate-buffered saline tablets were purchased from Aldrich. Following the manufacturer protocol we dissolved one tablet in 200 mL of deionized water to yield 0.01 M phosphate buffer, 0.0027 M KCl and 0.137 M NaCl, pH 7.4, at 25 °C. Water was deionized using a Millipore purification system.

2.2. Preparation of photopolymerization macro-initiator

The gold substrates were first cleaned in a UV/ozone cleaner and then modified by electrochemical reduction of BF$_4^-$, $^+$N$_2$–C$_6$H$_4$C(=O)–C$_6$H$_5$ in acetonitrile solution of 0.1 M NBu$_4$BF$_4$. Electrografting was performed by chronoamperometry for 300 s at a potential of -700 mV. This procedure leads to the electrografting of 4-benzoylphenyl groups (BP) onto gold plates (Au–BP).

2.3. Preparation of polymer grafts

The formation of imprinted polymer molecule was carried out in organic solution using mixture of chloroform and methanol, melamine as template molecule, MAA as the functional monomer and EGDMA as the crosslinking agent. The MAA was selected to provide COOH groups assuring electrostatic interaction and hydrogen-bonding with melamine. MIP was prepared using photopolymerization method by dissolving 1 mmol of melamine, 3.2 mmol of MAA, 5 mmol of EGDMA and 1.3 mmol of DMA in 3.5 mL of methanol and 1.5 mL of chloroform. The Au–BP substrate was immersed in this mixture which was purged with argon during 5 min and the photopolymerization was conducted for 800 s. After polymerization, the gold plates (noted Au–MIP) were rinsed three times with methanol and acetone. To remove melamine, the modified gold substrates were extracted with an acid solution (1 mL of acetic acid in 9 mL of water) using a Soxhlet apparatus for 24 h. The Au–MIP plates were washed with distilled water and acetone for three times and dried under vacuum at room temperature.

The non-imprinted polymer-coated gold plates (Au–NIP) were prepared using the same method and conditions but without the addition of the melamine template. Au–NIP served as a control substrate.