



Original research article

## Preparation of molecularly imprinted polymer based on the magnetic multiwalled carbon nanotubes for selective separation and spectrophotometric determination of melamine in milk samples

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## ABSTRACT

A new molecularly imprinted polymers coated on magnetic multiwalled carbon nanotubes (MIPs/MMWCNTs) was synthesized. Synthesized sorbent were characterized by scanning electron microscopy, X-ray diffraction, FT-IR spectroscopy and via adsorption kinetics and adsorption isotherms. The application of this sorbent was investigated in preconcentration and determination with a simple and selective spectrophotometric determination of melamine (MEL) by charge transfer complexation. The method is based on the adsorption of MEL on MIPs/MMWCNTs and subsequent reaction of desorbed MEL with tetrachloro-*p*-benzoquinone (TCBQ) reagent for final spectrophotometric detection. The effect of experimental parameters affecting separation efficiency and spectrophotometric detection were investigated and optimized. Under optimum conditions, the calibration curve for MEL determination showed a good linearity in the range of 10.0–600.0 ng mL<sup>-1</sup> ( $R^2 = 0.999$ ) and the limit of detection ( $S/N = 3$ ) was estimated to be 3.0 ng mL<sup>-1</sup>. The intra-day and inter-day precision (RSD%) of MEL were in the range of 2.8–3.9%. This method was successfully applied to determination of MEL in milk and milk powder samples. The good spiked recoveries ranging from 94.6 to 102.3% were obtained.

## 1. Introduction

Investigation on food materials toxicity has received much attention in recent years due to their serious health hazard effects (Vasimalai and John, 2013). Melamine (MEL, 1,3,5-triazine-2,4,6-triamine) is a synthetically prepared organic base which has been used for a wide variety of applications, including plastics, adhesives, laminates, paints, permanent-press fabrics, flame retardants, textile finishes, tarnish inhibitors, paper coatings and fertilizer mixtures (Hilts and Pelletier, 2008). Because of its high nitrogen level (66% nitrogen by mass) and low price, MEL has been sometimes added to foodstuff intentionally to show a false elevated protein level. MEL can cause a false enhancement in protein concentration which was estimated from total nitrogen concentration by standard protein analysis tests, such as Kjeldahl and Dumas methods (Thompson et al., 2002). MEL can induce acute kidney failure, urolithiasis, bladder cancer and even death in humans, especially babies and children (Langman et al., 2009; Skinner et al., 2010). Due to the proved toxicity of MEL, the safety limit of MEL ingestion has officially set by the United States Food and Drug Administration (USFDA) as 2.5 ppm for adult food and 1 ppm for infant formula (Ai et al., 2009). The reported methods for quantitative determination of

MEL include enzyme immunoassay (EIA), infrared spectroscopy, gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), vibrational spectroscopy and high performance liquid chromatography (HPLC) with UV detection (Sancho et al., 2005; Filigenzi et al., 2007; Kim et al., 2008; Mauer et al., 2009; Venkatasami et al., 2010; Domingo et al., 2014; Rovina and Siddiquee, 2015). These methods provide high sensitivity and low detection limit, but they suffer from some disadvantages, such as, multi-step operation, extended clean up steps and expensive instrumentation. Also, they need chemical derivatization to overcome interference effects and to increase sensitivity. Therefore, it is of critical important to develop a sensitive, simple, rapid and reliable method for the routine analysis of MEL in milk products. Since the matrices of food samples are often complex, determination of MEL in real samples requires a sample pretreatment step. Solid phase extraction (SPE) has been recognized as a solvent-less and efficient sample preparation procedure for a wide range of samples (Pohl et al., 2012; Hashemi and Taherimaslak, 2014; Wen et al., 2014). The selectivity and efficiency of SPE procedure are mainly dependent on the proper choice of the sorbent material. In the past few years, molecularly imprinted polymers (MIPs) has been considered as a selective adsorbent in SPE, and found wide applications in analysis of

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real samples with complex matrices, as highlighted by several reviews (Lasakova and Jandera, 2009; Ghorani et al., 2015). MIPs are synthetic polymers possessing specific cavities designed for a target molecule and are synthesized by the polymerization of different components. MIPs have attracted increasing attentions due to their advantages involve in predetermined recognition ability, mechanical and chemical stability, relative ease and simple preparation. The use of MIPs for separation and preconcentration of MEL has been previously described in some literatures (He et al., 2009a,b; Liang et al., 2009; Yang et al., 2009; Curcio et al., 2010; Li et al., 2010; Cheng et al., 2013; Hu et al., 2015; Zhu et al., 2015). Recently magnetic- MIPs have been developed for integration of extraction of MEL and separation of adsorbent from sample matrix (He et al., 2014). Also preparation of MEL-MIPs on the surface of MWCNTs has been suggested to improve the binding site accessibility and reduce the binding time (Zhang et al., 2011). The analytical capability of solid supporting for MIPs can be further increased by magnetization of it. Incorporation of MIPs on the magnetic-MWCNTs (MMWCNTs) allows the development of highly selective and fast extraction procedure for MEL and convenient separation of adsorbent from sample. So far, no report has been given for separation of MEL by MIP based MMWCNTs (MIP/MMWCNTs). Compared with the expensive methods, the cost of conventional spectrophotometric method and its simplicity makes it a widely accepted detection method. However the application of this method is mainly limited by its poor selectivity and sensitivity. Therefore, Hyphenation of advanced sample preconcentration and clean up method with ordinary spectrophotometric method can be worthy (Hashemi et al., 2017). The aim of this study, was to develop of a new approach for preparation of MIPs/MMWCNTs based on the functionalization of MMWCNTs by oleic acid (OA) and subsequent polymerization for preparation of a selective MIPs/MMWCNTs composite for separation and enrichment of MEL prior its spectrophotometric determination. Spectrophotometric determination was carried out using charge transfer complex formation between removed MEL and tetrachloro-*p*-benzoquinone (TCBQ), as a  $\pi$ -acceptor reagent. SPE with MIPs/MMWCNTs increases the selectivity and derivatization of extracted MEL improves the sensitivity of the spectrophotometric detection of MEL. The main factors affecting extraction efficiency and spectrophotometric reaction were investigated and optimized. Obtained results for recoveries of spiked real samples revealed the merits of the proposed method for the analysis of MEL in real samples.

## 2. Experimental

### 2.1. Materials and reagents

MWCNTs (mean diameter of 30 nm) were obtained from Iranian Nanomaterials Pioneers Co. (Mashhad, Iran). Methacrylic acid (MAA), 2,2-azobisisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA), melamine (MEL), and tetrachloro-*p*-benzoquinone (TCBQ), were purchased from Sigma-Aldrich (Saint Louis, MO, USA).  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ , dimethyl sulfoxide (DMSO), oleic acid (OA), acetonitrile, acetone, sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol and methanol were obtained from Merck (Darmstadt, Germany).

### 2.2. Apparatus

A UV–vis spectrophotometer model SPECORD 210 (Analytikjena AG, 07795 Jena, Germany) with a 300  $\mu\text{L}$  quartz microcell (Fisher Co., Schwer Germany) was used for spectrophotometric measurements. The synthesized MIPs were characterized by a scanning electron microscopy (SEM), (Philips, SEM 515, Amsterdam, Netherlands), APD 2000 X-ray diffractometer (XRD) (Ital Structures, Riva Del Garda, Italy) and FT-IR spectrometer (Perkin-Elmer, Spectrum version 10.01.00, USA). A Metrohm 713 pH-meter (Herisau, Switzerland) equipped with a

combined glass calomel electrode was used for the pH adjustments at  $25 \pm 1$  °C. A centrifuge Model 16105 (Farayand Co., Tehran, Iran) was used for separation of adsorbents. An ultrasonic water bath Model parsonic 2600 s (Parsnahand Co., Tehran, Iran) was used for dissolution of reagent.

### 2.3. Synthesis and pretreatment of MMWCNTs

The MMWCNTs was synthesized and preconditioned with OA in three steps. At first, the surface of MWCNTs was oxidized by acid treatment (Shen et al., 2007). Briefly, 500 mg of MWCNTs was added to 50 mL of  $\text{H}_2\text{SO}_4/\text{HNO}_3$  (3:1, v/v) solution and the suspension was refluxed at 60 °C for 4 h. The oxidized MWCNTs were filtered and washed with deionized water (200 mL, three times) and dried in a vacuum oven. In the second step, oxidized MWCNTs (0.5 g),  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (2.92 g) and  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (1.07 g) were dissolved in 150 mL deionized water under a nitrogen atmosphere with vigorous stirring at 80 °C. Then, 20 mL of 28% aqueous ammonia solution was added to reaction mixture to yield a black suspension. Finally, the modification of MMCNTs with OA was carried out by adding undiluted OA in five volumes of 0.2 mL at 5 min intervals. The crystal growth was allowed to proceed for 30 min at 80 °C with constant stirring. The suspension was then cooled to room temperature. The suspended particles were flocculated by acetone and are washed five times with 20 mL of an acetone–methanol mixture (1:1, v/v) to remove the excess OA. Then, the prepared suspension (OA/MMWCNTs) was collected by magnetic decantation and dried under vacuum for 24 h.

### 2.4. Synthesis of MEL MIPs/MMWCNTs

0.5 mmol of MEL, 300 mg of OA/MMWCNTs and 4.0 mmol of MAA were added into a 100 mL round flask containing 20 mL of DMSO/methanol (1:3, v/v). The mixture was incubated for 1 h at room temperature for pre-polymerization. Then, 20 mmol of EGDMA was added and the mixture was purged with nitrogen to remove oxygen. Afterwards, 30 mg of AIBN was added for initiation of polymerization. The reaction was allowed to proceed at 60 °C for 24 h. After the polymerization, the polymers were washed with methanol/acetic acid (9:2, v/v) mixture for several times until the template was no longer detectable by spectrophotometry. Finally, the MIPs/MMWCNTs were dried in vacuum at 60 °C for 24 h. For comparison, non-molecularly imprinted polymers-modified magnetic multiwalled carbon nanotubes (NIPs/MMWCNTs) were prepared by the same procedure, only without addition of MEL in the polymerization process. The preparation process of MIPs/MMWCNTs is schematically depicted in Scheme 1.

### 2.5. Preconcentration procedure

A 50 mL of sample solution containing 100 ng mL<sup>-1</sup> of MEL was transferred into a 250 mL beaker. The pH of the solution was adjusted to 7.0. Then 40 mg of MEL – MIPs/MMWCNTs was added to solution and the mixtures were shaken for 20 min. Then, the adsorbents were collected by an external magnetic field and supernatant solution was decanted. Separated adsorbents were eluted with 2 mL methanol/acetic acid (9:2, v/v) mixture. After separation of the adsorbents, the clear solution of eluent, containing eluted MEL, was evaporated to dryness under a gentle nitrogen flow. The residue was reconstituted in 500  $\mu\text{L}$  of TCBQ  $2 \times 10^{-4}$  M in DMSO for charge transfer complexation. After 30 min, the resultant solution was subjected to the final spectrophotometric determination at 500 nm.

### 2.6. Adsorption studies

Adsorption study was performed to examine the adsorption kinetic and adsorption isotherms at 25 °C. The adsorption kinetic of the MIPs/MMWCNTs toward MEL was investigated by following procedure: In a

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