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Analytical Methods

Extraction of melamine from milk using a magnetic molecularly imprinted polymer

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ABSTRACT

A novel magnetic molecularly imprinted polymer (MMIP) for the preconcentration of melamine, a non-protein nitrogen food additive from complex matrices was synthesized and characterized using FT-IR, XRD, SEM and VSM techniques. Surface imprinting was done on vinyltrimethoxysilane coated Fe_3O_4 (Fe_3O_4-VTMS) using 2-acrylamido-2-methylpropane sulfonic acid (AMPS), N,N′-methylenebisacrylamide (MBA) and potassium persulfate (KPS) as functional monomer, crosslinker and initiator respectively. Saturation magnetization value obtained for MMIP was 1.72 emu g^{-1}. Binding studies showed that MMIP exhibits good recognition to melamine compared to magnetic non imprinted polymer (MNIP). The optimum pH for the binding of melamine was found to be 4.5. Binding process was very fast and pseudo-second-order model fitted well with the kinetic data. Binding isotherm followed Langmuir isotherm model of monolayer adsorption with a maximum melamine binding efficiency of 62.25 mg g^{-1}. The HPLC-UV analysis results revealed the applicability of MMIP in solid phase extraction and determination of melamine from milk samples.

1. Introduction

Many of our food products are adulterated with toxic substances nowadays. Melamine, a nitrogen rich chemical is often used in the production of plastics, kitchen ware, adhesives etc. But recently melamine contamination has been reported in food products such as infant formulas and milk products. Melamine is added to the food stuffs as a non-protein nitrogen additive in order to falsify the apparent nitrogen content. Melamine can induce serious health problems in children as well as in adults. Ingestion of melamine contaminated food causes formation of kidney stones, kidney failure and bladder cancer in infants (Rovina & Siddiquee, 2015; Sun et al., 2010). U.S Food and Drug Administration has recommended the maximum residue levels of melamine in infant formulas to be 1.0 mg kg^{-1} and 2.5 mg kg^{-1} for milk and milk products (Li, Xu, & Sun, 2015). So monitoring of melamine content in food stuffs is necessary to prevent its illegal addition. Determination of melamine in the complex food matrices requires effective preconcentration prior to instrumental analysis (Zhang, Zhang, Hu, Yang, & Yao, 2011).

Molecular imprinting technique is a familiar method for the selective extraction of specific molecules referred to as templates. Molecularly imprinted polymers (MIPs) possess predetermined sites for the effective binding of templates. The preparation of MIPs involves the polymerization of suitable functional monomer and crosslinker in the presence of template molecule using an initiator. Finally the template is washed out using a suitable solvent to form MIPs. The cavities left behind in such a way are highly selective towards a particular template and can be used for the solid phase extraction of the template molecule from the complex matrix. MIPs possess several advantages such as easy fabrication, high stability, low cost, high selectivity etc. So they find applications in the fields of solid phase extraction, drug delivery, antibody mimics, enantiomeric separation, chromatographic separation and in chemical sensing (Chen, Xu, & Li, 2011; Maier & Lindner, 2007; Schirhagl, 2014). Molecular imprinting at the surface of magnetic nanoparticles has further advantage that they can be easily manipulated by an external magnetic field so that tedious centrifugation steps can be avoided. Surface imprinting also provide fast adsorption kinetics. Thus surface imprinting at magnetic nanoparticles illustrates an easy and rapid way for the extraction of template from complex matrices (Chen & Li, 2013; Su et al., 2015). Magnetic molecularly imprinted polymers (MMIP) are widely used in analytical chemistry for preconcentration process (Chen & Li, 2012). Only a few works have been reported on surface molecular imprinting technique combined with HPLC-UV analysis for the preconcentration

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and determination of melamine (Cheng, Liu, & Wang, 2013; Zhu, Xu, Wei, Yang, & Hu, 2015).

In the present study a highly selective MMIP for the preconcentration of melamine was developed by surface imprinting at superparamagnetic iron oxide nanoparticles. 2-acrylamido-2-methylpropane sulfonic acid (AMPS) and N,N′-methylenebisacrylamide (MBA) were used as functional monomer and crosslinker respectively. AMPS possess easily ionizable sulfonic acid group so that it can readily interact with protonated melamine. AMPS and MBA improve water compatibility of the imprinted polymer so that it can interact well with the template present in the aqueous matrix of food samples (Duan et al., 2014). Adsorption characteristics of the MMIP were checked at varying concentration of melamine, pH and time. Melamine spiked milk samples were chosen as the complex matrix to check the potential of the prepared MMIP in real samples. Thus a less time-consuming preconcentration procedure with fast adsorption kinetics and selective adsorption capacity was developed for the analytical determination of melamine from adulterated milk samples.

2. Materials and methods

2.1. Materials

Melamine (99%), 2-acrylamido-2-methylpropane sulfonic acid sodium salt (AMPS, 50 wt% solution in water) and vinyltrimethoxysilane (VTMS, 98%) were purchased from Aldrich, USA. Iron(III) trichloridehexahydrate [$FeCl_3{\cdot}6H_2O$ (98.0%)], and Iron(II) sulphate heptahydrate [$FeSO_4{\cdot}7H_2O$ (99.0%)] were obtained from Fisher, USA. N,N′-methylenebisacrylamide (MBA) procured from SRL, Mumbai. Potassium persulfate (KPS), ammonia (25%), methanol (HPLC grade), trifluoroacetic acid and acetic acid were purchased from Merck, Mumbai. Resorcinol was procured from Sisco-Chem Industries, Mumbai. Double distilled water was used throughout the study.

2.2. Preparation of vinyl functionalized magnetic particles

2.2.1. Synthesis of Fe_3O_4 nanoparticles

Scheme 1 represents the synthesis procedure adopted for MMIP preparation. Iron oxide nanoparticles were synthesized as reported elsewhere (Chen, Xie, & Shi, 2013). Typically 0.5 M Iron(II) sulphate heptahydrate and 1.0 M Iron(III) chloride hexahydrate were mixed well with a magnetic stirrer at 1000 rpm and heated to 80 °C in nitrogen atmosphere. The solution was made alkaline by adding 25% aqueous ammonia solution. A black precipitate was formed at the alkaline pH. The reaction mixture was stirred further for 1 h at the same temperature. The black precipitate was collected with the help of an external magnet, washed with distilled water to remove excess reagents and dried in an oven at 50 °C.

2.2.2. Synthesis of vinyl functionalized magnetite nanoparticles

The vinyl functionality was introduced into the surface of iron oxide nanoparticles using vinyltrimethoxysilane. The obtained iron oxide nanoparticles were dispersed in 500 mL of ethanol and sonicated for 30 min. Then a mixture of 3 mL, 25% ammonia and 3 mL of VTMS were added to the above dispersion and stirred in a magnetic stirrer at 900 rpm for 24 h at room temperature. VTMS coated iron oxide particles were collected using an external magnet, washed with ethanol and dried in an air oven (Liang, 2014).

2.2.3. Synthesis of MMIP and MNIP

For the synthesis of MMIP, the ratio of template: functional monomer: crosslinker was 1:4:24. Melamine (1 mmol) was dissolved in minimum amount of methanol-water mixture (1:1 V/V). AMPS (4 mmol) and 20 mL of acetonitrile were added to this solution and stirred at 900 rpm for 1 h to make a prepolymerisation mixture. Then 0.8 g KPS was added and heated to 60 °C and then cooled. A solution of MBA (24 mmol) in acetonitrile along with 0.5 g of Fe_3O_4-VTMS was added to the above solution. The solution was then again heated to 70 °C and stirred at 1000 rpm under nitrogen atmosphere. The melamine imprinted polymer was formed. The reaction mixture was stirred further for 3 h to complete the polymerization. The polymers were collected, washed with acetonitrile and finally with water to remove excess reagents. In order to remove the template, the polymer was washed with methanol-acetic acid solution (8:2 V/V) until no peak corresponding to melamine was observed at 235 nm in UV spectra. The magnetic non-imprinted polymer (MNIP) was also prepared in the same way without the addition of template. The polymers were dried in an air oven at 50 °C and stored in glass vials.

2.3. Instruments and methods of characterization

The Fourier Transform Infrared (FTIR) spectra of the samples were recorded with FTIR spectrometer (Agilant Technologies, Cary 630) in the wavelength range 4000–650 cm^{-1}. The X-ray diffraction patterns of the samples were recorded with Bruker AXS D8 Advance X-ray diffractometer using CuK_∞ radiation at a wavelength of 1.5406 Å. SEM images were taken from the JEOL JSM 6390 LA Scanning Electron Microscope. Magnetic properties of MMIP were determined using Vibrating sample magnetometer [Quantum Design Physical Property Measurement System (PPMS, USA)]. The concentration of melamine in solution was determined spectrophotometrically on a Jasco UV–visible (model-530, India) spectrophotometer at a wavelength of 235 nm. All the pH measurements were carried out on a Systronic Microprocessor pH meter (Model μ-362, India). HPLC system (Model: PU-2080) equipped with a Jasco 2075/2070 UV detector was used for chromatographic studies. Measurements were done at 235 nm. Mobile phase was 0.1% trifluoroacetic acid in distilled water (Milli-Q water) and methanol (90:10) with a flow rate of 1 mL min^{-1}. The chromatographic separation was done through an inertsil WP300-C18 column (4.6 mm × 250 mm) and injection volume was 20 μL. The chromatographic calculations were done using Borwin software.

2.4. Rebinding experiments

Adsorption experiments were carried out in a thermostatic waterbath shaker at 30 °C with a shaking speed of 200 rpm using 100 mL Erlenmeyer flasks. To determine the optimum pH for the rebinding of melamine to MMIP, two sets of experiments with concentrations 25 and 50 mg/L were done. 25 mL of melamine standard solution was added to 0.05 g of MMIP at different pH values ranging from 2.0 to 9.0. The solutions were shaken for 3 h. Then the melamine captured MMIP was separated with the help of an external magnet. The supernatant was analysed using UV–visible spectrophotometer at 235 nm. The percentage of adsorption is given by the formula

$$\text{Adsorption}\,\% = \frac{(C_0 - C)}{C_0} \times 100 \qquad (1)$$

where C_0 and C are the initial and final concentrations of melamine in mg L^{-1} before and after the addition of adsorbent respectively. The optimum pH obtained was used for further studies. The kinetic studies were conducted using four different initial concentrations (10, 25, 50, 100 mg L^{-1}). About 0.1 g of MMIP was suspended in 50 mL of melamine solution at different concentrations and shaken in a water bath at room temperature for 3 h. Solutions were withdrawn at different time intervals; supernatant was collected and