



# Preparation and characterization of magnetic molecularly imprinted polymers for the extraction of hexamethylenetetramine in milk samples

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## ARTICLE INFO

### Keywords:

Hexamethylenetetramine  
Magnetic molecularly imprinted polymers  
Magnetic solid phase extraction  
Milk  
GC-MS/MS

## ABSTRACT

Magnetic molecularly imprinted polymers (M-MIPs) were synthesized as the sorbents for extracting hexamethylenetetramine (HMT) from milk samples. Molecular simulations were used to calculate the interaction energies of the template monomers. The physical properties of M-MIPs were characterized. The adsorption isotherms and kinetics were investigated. Gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) was applied to determine the amount of HMT residue in milk samples. In the optimized method, a linear calibration curve was obtained using a matrix-matched standard in the range of 1.0–50.0  $\mu\text{g L}^{-1}$ . The limit of detection (LOD) and limit of quantification (LOQ) was 0.3  $\mu\text{g kg}^{-1}$  and 1.0  $\mu\text{g kg}^{-1}$ , respectively. The relative standard deviation (RSD) of the intra-day assay ranged from 2.6% to 5.2%, while that of the inter-day assay ranged from 3.6% to 11.5%. The recovery of HMT in milk samples ranged from 88.7% to 111.4%.

## 1. Introduction

Hexamethylenetetramine (HMT,  $\text{C}_6\text{H}_{12}\text{N}_4$ ), also known as methenamine or urotropine, is a common drug used in the treatment of urinary tract infections [1]. Thus, HMT is often applied in farming practices to control livestock disease. The treatment mechanism is attributed to formaldehyde [2], which is derived from the decomposition of HMT. However, such constantly used veterinary drugs may reside in animal-derived food. As such, antibiotic resistance or allergic reactions may be induced by dangerous drug residues in food [3]. Moreover, formaldehyde derived from HMT has carcinogenic effects and acute and chronic toxicity [2]. HMT is not approved for use as a food additive in Australia, China, New Zealand, Russia or the USA [4–6]. In view of the situation, it is indispensable to establish an effective and convenient method to detect HMT residues in animal-derived food.

The preparation process is a crucial step in analysing complex samples. In previous research, HMT in a cheese sample was extracted by solvent, then subjected to centrifugation and membrane filtration [7]. Because the matrix effects for HMT can be observed in mass spectrometric analysis, modified approaches have been used to com-

pensate for matrix effects, such as the combination of isotope dilution mass spectrometry and cation exchange solid phase extraction (SPE) or the QuEChERS method [8,9]. However, the sorbents that are presently used in SPE and QuEChERS are not specific to HMT, and the absolute recovery of HMT is not optimum. Moreover, the isotope-labelled reference material is expensive, making it inaccessible to popularization and promotion. Therefore, it is necessary to find a highly specific and economical sorbent. Molecular imprinting is an effective technique to establish notable recognition properties in templates [10,11] and could be used for the selective binding of target compounds from a matrix. However, the conventional synthetic method for MIPs is bulk polymerization, where the polymer must be ground and sieved [12,13]. This procedure is tedious, and binding sites may be embedded in the polymer matrix [14]. The surface imprinting technique can overcome these problems [15]. Recently, magnetic separation technology combined with the surface imprinting technique has received considerable attention [16,17]. Magnetic molecularly imprinted polymers (M-MIPs) can easily be separated from a matrix using an external magnetic field [18]. Therefore, M-MIPs have been used as selective sorbents to extract target compounds from various matrices [19–21] and have been successfully used in analytical chemistry [22,23]. Recently, molecular

*Abbreviations:* AIBN, 2,2'-Azobisisobutyronitrile; EGDMA, Ethylene glycol dimethacrylate; FT-IR, Fourier transform infrared spectrometer; GC-MS/MS, Gas chromatography coupled with tandem mass spectrometry; HMT, Hexamethylenetetramine; LOD, Limit of detection; LOQ, Limit of quantification; MAA, Methacrylic acid; M-MIPs, Magnetic molecularly imprinted polymers; M-NIPs, Magnetic non-molecularly imprinted polymers; MISPE, Magnetic imprinted solid-phase extraction; PDA, Photo-diode array; RSD, Relative standard deviation; SEM, Scanning electron microscopy; XRD, X-ray diffraction

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<http://dx.doi.org/10.1016/j.talanta.2016.10.080>

Received 9 August 2016; Received in revised form 19 October 2016; Accepted 21 October 2016

Available online 25 October 2016

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simulations have been widely applied to the design and synthesis of MIPs. Simulation methods based on quantum mechanical theories are more economical than experiments [24,25].

In this study, we prepared HMT imprinted polymers on the surface of oleic acid-modified  $\text{Fe}_3\text{O}_4$ . Methacrylic acid (MAA) was the functional monomer, and ethylene glycol dimethacrylate (EGDMA) was the cross-linker. The characterization, selectivity, adsorption isotherms and kinetics of M-MIPs and magnetic non-molecularly imprinted polymers (M-NIPs) were investigated. A magnetic imprinted solid phase extraction (MISPE) method was developed to analyze HMT in milk samples by gas chromatography coupled with tandem mass spectrometry (GC-MS/MS). Method validation parameters, such as linearity, limit of detection (LOD), precision and accuracy, were investigated.

## 2. Materials and methods

### 2.1. Reagents and materials

All reagents were of analytical reagent grade unless otherwise stated. HMT (purity  $\geq 99\%$ ), melamine (purity  $\geq 99\%$ ), L-lysine (purity  $\geq 98\%$ ) and D-(+)-galactose (purity  $\geq 99\%$ ) were purchased from Sigma-Aldrich (St. Louis, USA). HPLC-grade acetonitrile, methanol and ethanol were obtained from Merck KGaA (Darmstadt, Germany). MO, USA). Iron (II) chloride tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ), iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 2,2'-azobisisobutyronitrile (AIBN), oleic acid and polyvinylpyrrolidone (PVP) were obtained from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). Potassium bromide (KBr) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ammonia hydroxide and glacial acetic acid were obtained from Chongqing Chuandong Chemical Co. (Chongqing, China). Ultrapure water with a resistivity of  $18.2 \text{ M}\Omega \text{ cm}^{-1}$  was produced by a Biogen Ultrapure Water Type 1 system (Weston, USA). Organic-phase syringe filters ( $0.22 \mu\text{m}$  nylon) were obtained from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). Standard stock solutions of HMT ( $500 \text{ mg L}^{-1}$ ) were prepared in pure methanol and stored at  $4^\circ\text{C}$  in the dark for no more than a month. Milk samples were randomly collected from the local market in Dali (China). The samples were stored at  $4^\circ\text{C}$  in the dark.

### 2.2. Molecular simulation and calculation of energies

The template-monomer complexes were modelled to demonstrate the interactions between HMT and MAA using the HyperChem 8.0 software (Hypercube, Inc., USA). The structures of HMT, MAA and their complexes were optimized. The energies of the structures were calculated by semiempirical quantum mechanical methods (PM3) [16,26]. The most stable template-monomer complex was selected for calculation of the binding energy ( $\Delta E$ ), which was defined as:

$$\Delta E = E_{\text{complex}} - nE_{\text{template}} - nE_{\text{monomer}} \quad (1)$$

where  $E_{\text{complex}}$  is the energy of the complex;  $E_{\text{template}}$  is the energy of the template;  $E_{\text{monomer}}$  is the energy of the monomer, and  $n$  is the number of the template or monomer.

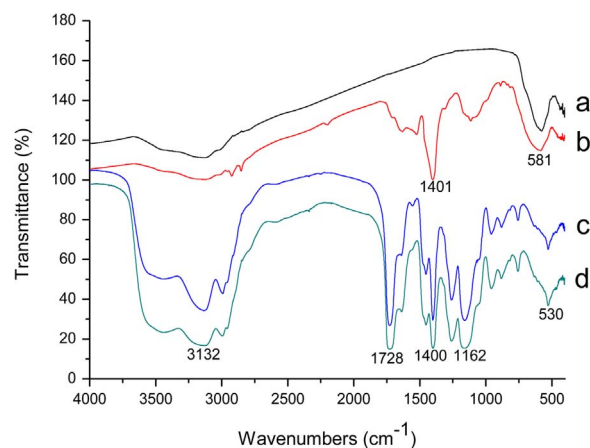
### 2.3. Preparation of M-MIPs

The preparation of  $\text{Fe}_3\text{O}_4$  magnetic particles involved the coprecipitation of  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ . The procedure detailed by He et al. was followed [27]. After coprecipitation, a 1.0 g aliquot of  $\text{Fe}_3\text{O}_4$  magnetic particles was mixed with 1.0 mL of oleic acid, and the mixture was shaken for 10 min by a vortex shaker (Thermo Corp., Waltham, USA) to ensure that the  $\text{Fe}_3\text{O}_4$  magnetic particles were adequately mixed with oleic acid [27]. The mixture was kept in room temperature for 6 h, and the excess oleic acid was removed by washing with ethanol.

**Table 1**

The interaction energies of HMT with MAA molecules for different molar ratio.

HMT/MAA	$E_{\text{complex}}$ (a.u.)	$\Delta E$ ( $\text{kJ mol}^{-1}$ )
1/1	-99.19	-9.28
1/2	-141.60	-33.53
1/3	-184.02	-49.03
1/4	-226.43	-64.79
1/5	-268.83	-29.37
1/6	-311.23	0.23
1/7	-353.63	14.76
1/8	-396.04	22.95



**Fig. 1.** FT-IR spectra of  $\text{Fe}_3\text{O}_4$  (a), oleic acid-modified  $\text{Fe}_3\text{O}_4$  (b), M-MIPs (c) and M-NIPs (d).

For the preparation of M-MIPs, the template (HMT, 1 mmol) was dissolved with 10 mL methanol in a 100 mL borosilicate glass bottle, and then 40 mL of acetonitrile was added. Subsequently, 4 mmol of MAA was added into 20 mmol of EGDMA and the oleic acid-modified  $\text{Fe}_3\text{O}_4$  magnetic particles. This mixture was sonicated for 10 min and shaken overnight at  $40^\circ\text{C}$  to induce prepolymerization. Then, 0.1 g of polyvinylpyrrolidone (PVP) was dissolved in 20 mL of methanol and added to the prepolymerization solution. Next, 0.04 g of AIBN was added, and the mixture was saturated with nitrogen for 10 min. Thereafter, the bottle was placed in a water bath at  $60^\circ\text{C}$  and shaken for 24 h. After polymerization, the magnetic polymers were separated by an external magnetic field and washed by a methanol/acetic acid mixture (8:2, v/v) using a Soxhlet apparatus for 48 h to remove the template molecules. Then, the M-MIPs were washed with methanol for 24 h and dried at  $60^\circ\text{C}$  under vacuum overnight. The magnetic non-molecularly imprinted polymers (M-NIPs) were prepared similarly but without the addition of HMT.

### 2.4. Characterizations

A Spotlight 400 FT-IR spectrometer (Perkin-Elmer, Wellesley, USA) with a  $2 \text{ cm}^{-1}$  resolution and a spectral range of  $400\text{--}4000 \text{ cm}^{-1}$  was used to measure the FT-IR spectra for  $\text{Fe}_3\text{O}_4$  magnetic particles, oleic acid-modified  $\text{Fe}_3\text{O}_4$  magnetic particles, M-MIPs and M-NIPs. The KBr disc technique (sample: KBr=1:100, w/w) was applied in this experiment. The sizes and morphologies of the particle samples were determined using a SWPRATM55 field emission scanning electron microscope (Carl Zeiss, AG, Aalen, Germany) with the analytical method referred to in the literature [28]. The X-ray diffraction (XRD) patterns were analysed on a D/MAX-III B X-ray diffractometer (Rigaku, Akishima, Japan) with  $\text{CuK}\alpha$  radiation at 40 kV and 200 mA, following the analytical procedure referred to in the literature [29]. The magnetic properties were measured using a VersaLab physical property measurement system (Quantum Design, San Diego,

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