UNIVERSITI PUTRA MALAYSIA

PREPARATION AND CHARACTERIZATION OF MOLECULARLY IMPRINTED POLYMER FOR MELAMINE

SITI KHADIJAH BT AB. RAHMAN

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PREPARATION AND CHARACTERIZATION OF MOLECULARLY IMPRINTED POLYMER FOR MELAMINE

By

SITI KHADIJAH BT AB. RAHMAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

PREPARATION AND CHARACTERIZATION OF MOLECULARLY IMPRINTED POLMER FOR MELAMINE

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SITI KHADIJAH BT AB. RAHMAN

July 2013

Chair : Professor Dr. Nor Azah Yusof, PhD

Faculty : Science

Molecularly imprinted polymers (MIPs) are synthetic polymers having a predetermined selectivity for a given analyte, or group of structurally related compounds, that make them ideal materials to be used in separation processes. In this research, MIP was synthesized and used for selective removal of melamine compound. The MIP was prepared using precipitation polymerization method where melamine as template, 9-vinylcarbazole or methacrylic acid as functional monomer, ethylene glycol dimethacrylate (EGDMA) as a crosslinker and benzoyl peroxide (BPO) as initiator in the same phase. The total weight of yield of MIP increased, by increasing the ratio of initiator, monomer and cross-linker and the suitable temperature was chosen for this polymerization process at 60 ^{o}C. The best ratio of monomer to crosslinker is 1:8 for methacrylic acid and 1:5 for 9-vinylcarbazole. The synthesized Mel-MIP-9VC and Mel-MIP-MAA were characterized using fourier transform infrared spectroscopy (FTIR), thermal gravimetric analysis (TGA), field scanning electron microscopy (FESEM) and brunauer, emmet teller (BET) for determining the polymerization and imprinting process were occurred successfully.

Adsorption process for the determination of melamine using molecular imprinted solid phase extraction (MISPE) method and adsorption capacities of melamine by Mel-MIP-9VC and Mel-MIP-MAA were analysed by using ultra performance liquid chromatography (UPLC). The optimum adsorption capacity is achieved at pH 5.0 for both MIP (Mel-MIP-9VC and Mel-MIP-MAA). The adsorption isotherm data is well defined by the Freundlich model for both MIP with R^2 value of Mel-MIP-9VC and Mel-MIP-MAA are 0.980 and 0.896 respectively. The adsorption of melamine can be modeled by pseudo-second order reaction. The percentage removal of melamine increases with increasing dosage of MIP. The selectivity study shows the adsorption capacity of melamine by MIP is higher than NIP. The k' value for Mel-MIP-9VC and Mel-MIP-MAA of melamine/cyanuric acid is 3.1651 and 1.6070 respectively. The adsorption capacity of melamine in milk sample by Mel-MIP-MAA and Mel-MIP-9VC is higher than NIP-MAA and NIP-9VC.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENYEDIAAN DAN PENCIRIAN POLMER CETAKAN MOLEKUL BAGI MELAMIN

Oleh

SITI KHADIJAH BT AB. RAHMAN

Julai 2013

Pengerusi : Profesor Dr. Nor Azah Yusof, PhD

Fakulti : Sains

Polimer cetakan molekul (MIP) adalah polimer sintetik yang mempunyai pemilihan terhadap analyte tertentu, atau kumpulan struktur bagi sebatian yang berkaitan. Justeru, ia menjadikan MIP sebagai bahan yang sesuai untuk digunakan dalam proses pemisahan. Dalam kajian ini, MIP disintesis dan digunakan untuk menyingkirkan sebatian melamin. MIP telah disintesis melalui kaedah pempolimeran pemendakan dengan menggunakan melamin sebagai acuan, 9-vinilkarbazol dan asid metakrilik sebagai monomer berfungsi, etelina glikol dimetakrilik (EGDMA) sebagai penggabung dan benzoil peroksida (BPO) sebagai pemula. Jumlah hasil MIP adalah menaik, dengan peningkatan nisbah monomer, pemula, dan penggabung. Suhu yang sesuai telah dipilih untuk proses pempolimeran ini pada 60^{o}C. Nisbah terbaik monomer kepada penggabung adalah 1:8 bagi asid metakrilik dan 1:5 bagi 9-vinilkarbazol. Mel-MIP-9VC dan Mel-MIP-MAA yang telah disistensis, dicirikan pula menggunakan spektroskopi fourier inframerah transformasi (FTIR), analisis gravimetrik terma (TGA), medan pengimbas mikroskopi elektron (FESEM) dan brunauer, emmet teller (BET) bagi menentukan proses pempolimeran dan pencetakan telah berjaya. Proses penjerapan bagi penentuan melamin menggunakan

kaedah molekul cetakan pengekstrakan fasa pepejal (MISPE). Kapasiti penjerapan melamin oleh Mel-MIP-9VC dan Mel-MIP dianalisis dengan menggunakan prestasi ultra kromatografi cecair (UPLC) pada gelombang 235 nm. Kapasiti penjerapan optimum telah dicapai pada pH 5.0 bagi kedua-dua MIP (Mel-MIP-9VC dan Mel-MIP-MAA). Data penjerapan isoterma bagi melamin boleh dihuraikan melalui model Freundlich untuk kedua-dua MIP. Nilai R^2 bagi Mel-MIP-9VC dan Mel MIP-MAA masing-masing adalah 0.980 dan 0.896. Penjerapan melamin adalah boleh dimodelkan sebagai tindak balas tertib kedua. Peratusan penyingkiran melamin adalah meningkat dengan peningkatan dos MIP. Kajian selektiviti menunjukkan kapasiti penjerapan bagi melamin oleh MIP lebih tinggi berbanding NIP. Nilai k' untuk Mel-MIP-9VC dan Mel-MIP MAA melamin/cyanurik asid masing-masing adalah 3.1651 dan 1.6070. Kapasiti penjerapan bagi melamin dalam sampel susu oleh Mel-MIP MAA-dan Mel-MIP-9VC lebih tinggi berbanding NIP-MAA dan NIP-9VC.

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I certify that a Thesis Examination Committee has met on **1 July 2013** to conduct the final examination of **Siti Khadijah Bt Ab. Rahman** on her thesis entitled "**Preparation and Characterization of Molecularly Imprinted Polymer for Melamine**" in accordance with the Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

Abdul Halim Abdullah, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mansor Hj Ahmad, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Tan Yen Ping, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mohd Noor Ahmad, PhD
Professor
School of Materials Engineering
Universiti Malaysia Perlis
(External examiner)

_____

NORITAH OMAR, PhD
Assoc. Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 2 August 2013

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree master of science. The members of the Supervisory Committee were as follows:

Nor Azah Yusof, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Nor Azowa Ibrahim, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohd Zobir Hussein, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been dully acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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SITI KHADIJAH BT. AB RAHMAN

Date: 1 July 2013

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LIST OF ABBREVIATIONS

FTIR	Fourier transform infrared
BPO	Benzoyl peroxide
TGA	Thermal gravimetric analysis
DTG	Differential thermogravimetric analysis
MIP	Molecularly imprinted polymer
NIP	Non imprinted polymer
Mel-MIP-9VC	Melamine-Molecularly imprinted polymer-9-vinylcarbazole
NIP-9VC	Non imprinted polymer- 9-vinylcarbazole
Mel-MIP-MAA	Melamine-Molecularly imprinted polymer-Methacrylic acid
MAA	Methacrylic acid
9VC	9-vinylcarbazole
NIP-MAA	Non imprinted polymer- Methacrylic acid
FESEM	Field emission scanning electron microscope
UPLC	Ultra performance liquid chromatography
EGDMA	Ethylene glycol dimethacrylate
TEM	Transmission electron microscope
BET	Brunauer emmett teller
NPN	Non-protein nitrogen
TDI	Tolerable daily intake
MISPE	Molecularly imprinted solid phase extraction
NISPE	Non-imprinted solid phase extraction

SPE	Solid phase extraction
LOD	Limit of detection
LOQ	Limit of quantitation
BJH	Barret-joyner-halenda

CHAPTER 1

INTRODUCTION

1.1 Melamine and Toxicity of Melamine

Melamine, chemically known as 2,4,6-triamino-1,3,5-triazine, is produced in large amounts (1.2 million tons in 2007) primarily for use in the synthesis of melamine formaldehyde resins for the fabrication of laminates, plastics coatings, commercial filters, glues or adhesives, as well as for dishware and kitchenware (Lutter *et al.,* 2010). Melamine contamination has been reported in products such as milk, infant formula, frozen yogurt, pet food, biscuits, candy, and coffee drinks. It was previously considered a non-protein nitrogen (NPN) supplement for cattle feed, however, this use has been discontinued (Gopalakrishnan *et al.,* 2010).

Recent cases of melamine-tainted infant formula in China was responsible for severe adverse health effects on babies (WHO/FAO, 2008), raised the need for appropriate controls on milk supply chain and finished products. WHO/FAO experts stated that a limit of 1 mg/kg in infant formula would provide a sufficient margin of safety for dietary exposure relative to the tolerable daily intake (TDI). It was recently shown that the presence of melamine in cow's milk may be due to melamine-contaminated ingredients used for animal feeds (Cruywagen *et al.,* 2009).

In recent years, melamine has been found to be used illicitly as a food additive for a false increase of protein level and due to its high nitrogen content (66.7%). Recent reports demonstrated that this man-made non-nutritive additive was involved in an

outbreak of renal damage in infants who ingested regularly the melamine-contained milk products. The melamine-induced acute nephrotoxicity has resulted in several deaths and more than 10,000 of hospitalizations and invoked public anxiety on the food safety in China.

Pharmacological study in animals indicated that melamine alone was of low toxicity. This compound can be readily cleared by the kidney and become undetectable in animal tissues in 24 h after administration. However, the coexistence of melamine and its hydrogen-bonding partners uric acid and cyanuric acid, the latter is a by-product in melamine synthesis, may trigger renal damage by formation of insoluble complexes through hydrogen bonds between these molecules (Puschner *et al.*, 2007). The results demonstrated that mixing melamine and cyanuric acid resulted in the formation of insoluble particles and that the insoluble melamine-cyanurate complex induced membrane damages of human erythrocytes. The membrane damages included hemolysis, alterations in cell shape and membrane fragility, and inhibition of enzymatic activity. By contrast, either melamine or cyanuric acid alone had no effect on erythrocyte membranes (Wang *et al.*, 2010)

1.2 Molecularly Imprinted Polymer

Molecularly imprinted polymers (MIPs) are tailor made synthetic materials with selective molecular recognition capability (Tao *et al.*, 2007). Molecular imprinting is a technique for creating artificial receptor sites in a polymer. MIP is produced by forming a polymer around a molecule that is used as the template. Upon removal of the template, molecular cavities remain which are specific in shape and size to the

target molecule (Lisa and Evangelyn, 2005). The resulting MIP are being used in a wide range of applications, such as chiral separation, chemical sensing, competitive drug assays, chemical catalysis, solid phase extraction and drug delivery system(Pouci *et al.,* 2009).

MIP has unique advantages over natural biological receptors in terms of physical and chemical stability (stable against mechanical stress, elevated temperatures and high pressures, resistant against treatment with acid, base or metal ions and stable in a wide range of solvents), ease of preparation, low cost and application in harsh environmental conditions (Zeng-peng and Chun-jing, 2009). The long term endurance of the polymer is also very high: storage for several years at ambient temperature leads to no apparent reduction in performance. Further, the polymers can be used repeatedly, in excess of 100 times during periods of years without loss of 'memory effect' (memory of active sites of the template) (Svenson *et al*., 2001).

The principles of molecular imprinting process can be classified into covalent imprinting approach, non-covalent imprinting and semi-covalent imprinting, based on the type of interactions involved between the functional and template during the polymerization process occurred. Non covalent approach has been used more extensively due to following reasons: (1) Non covalent protocol, avoiding the tedious synthesis of prepolymerization complex. (2) Removal of the template is generally much easier. (3) A greater variety of functionality can be introduced into the MIP binding site using non covalent methods (Hongyuan and Kyung, 2006)

Synthesis of MIP is a relatively straight forward and inexpensive procedure. The MIP is prepared by mixing the functional monomer, template, cross-linker, and initiator in a proper solvent (Hongyuan and Kyung, 2006). Functional monomer as a component that is responsible for binding with template, where the template is the target analyte. Other components like cross-linker, the polymerization initiator, and the porogen influence the structures and performance of the polymer. When free-radical polymerization is employed, polymerization can be initiated either thermally or photochemically, thus starting the cleavage of the initiator to generate free radicals. This radical formation then initiates the polymerization of the functional monomers and the crosslinker, which leads to the formation of a crosslinked polymer network. Note that polymerization is carried out in the presence of the template molecules, and therefore the template molecules are 'trapped' inside the polymer network at the end of this process (Ramstron and Yan, 2005).

Before the MIP can be used for rebinding studies, extraction of the original template molecules from the polymer matrix can be done. In covalent approach, an appropriate reagent is needed to break the bonds formed between the template and the functional element. For non-covalent approach, which is easier to remove template, extraction can be done by washing in aqueous solution of an acid, a base or methanol, thus facilitating the removal of the template molecule from the polymer network. After of the template molecule is extracted out, the resulting imprinted polymer will possess a permanent memory of the imprint species. This will enable the resultant polymer selectively to rebind the imprint molecule from a mixture of closely related compound.

In the last 5 years, many methods for the preparation of MIPs have been proposed. Most reported MIPs are prepared by bulk polymerization. But it has low capacity and poor site accessibility for the template and the process of crushing and sieving can break the imprinted sites. Thus, in order to overcome these drawbacks, new polymerization strategies have been proposed, such as dispersion polymerization, multi-step polymerization, surface polymerization, in situ polymerization and precipitation polymerization. Precipitation polymerization has recently emerged as a desirable and scalable approach for the production of high quality, high uniform, spherical imprinted particles. Unlike the suspension polymerization to generate spherical polymer particles, the precipitation technique does not require the use of surfactant or steric stabilisers (Tao *et al.,* 2007)

1.3 Statement of the Problem

Melamine was used as a source of non-protein nitrogen (or NPN) in cattle feed from 1958 to 1978 in China. While melamine alone is of low toxicity, it could form insoluble crystals in combination with cyanuric acid, leading to the formation of kidney stones, which may cause kidney failure and ultimately death. Investigation revealed that the infant formula made from adulterated raw milk was the cause of the urinary tract stone epidemic. During the adulteration, melamine was illegally added into raw milk to falsely increase the protein content of milk after dilution with water (Yong-Ning *et al.,* 2009).

The most frequently used methods for the determination of melamine are gas chromatography–mass chromatography (GC-MS) and liquid chromatography-

tandem mass chromatography (LC-MS-MS) (Limin *et al.*, 2009), which always involves in traditional sample pretreatment procedures, such as solid phase extraction and solid phase micro-extraction (SPME). The main problem associated with traditional sorbents of SPE/SPMSE is the low selectivity or low adsorption capacity (Shoufang *et al.*, 2011).

A new type of high efficiency adsorbents MIPs due to high sample load capacity, high selectivity, low cost and easy preparation, have been widely applied for preconcentration and high efficient separation of trace analytes in diverse matrices, such as natural, agricultural, food products and environmental samples (Xingliang *et al.*, 2009). Due to their favorable molecular recognition capability and stability, potential application of MIPs has been investigated in a broad scientific area, such as ligand binding assays, SPE, sensors and catalysis. Among these applications one of the most widely applied is the molecularly-imprinted solid phase extraction (MISPE), which has been used for the extraction of a broad range of analytes (Mu *et al.*, 2010).

In this study MIP for melamine was synthesized based on radical polymerization (precipitatation polymerization) using methacrylic acid and 9-vinyl carbazole as the monomer. The synthesized MIPs were characterized using fourier-transform infrared spectroscopy, field emission scanning electron microscopy, thermal gravimetric analysis and brunauer emmett for confirming the polymerization and imprinting process were occurred successfully. Combining molecular imprinting and solid phase extraction (MI-SPE) used as sorbents for selective removal of melamine were

investigated. The parameters studied include pH, dosage, isotherms, kinetic, selectivity study and removal of melamine in milk sample.

1.4 Objectives of this research

Objectives of this study are:

1. To synthesize molecularly imprinted polymer for melamine by precipitation polymerization.
2. To characterize the synthesized MIP using fourier-transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), thermal gravimetric analysis (TGA) and brunauer emmett teller (BET).
3. To study the adsorption capacity of melamine using the synthesized MIP based on pH, dosage, kinetic, isotherm and selectivity studies.

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