MOLECULARLY IMPRINTED POLYMER FOR THE MOLECULAR RECOGNITION OF ENDOCRINE DISRUPTOR BISPHENOL-A

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Abstract: Bisphenol-A (BPA) is a component of a coating material in can packaging and is known to leach out to the canned food. It is a known endocrine disruptor, carcinogen and mutagen hence detection and control of BPA should be a concern to protect the consumers. This study aims to develop a molecularly imprinted polymer (MIP) for the molecular recognition of BPA. This paper presents the synthesis and characterization of BPA- imprinted polymer as potential sorbent for bisphenol compounds. Rational design of MIP using computational technique defined the favorable binding energies of the interacting template-monomer systems. As such, the new BPA-imprinted polymer was synthesized by precipitation polymerization using mecthacrylic acid (MAA), bisphenol-A (BPA), trimethylolpropane trimethacrylate (TRIM), and 2,2'azobis(isobutyronitrile) (AIBN) as functional monomer, template, cross-linker, and initiator, respectively. Successful synthesis was confirmed by infrared spectroscopy, fluorescence microscopy and elemental analysis. Scanning electron micrograph (SEM) showed that the MIP's are relatively uniform microparticles with high degree of porosity. Binding studies using the Freundlich isotherm showed the heterogeneity of the MIP binding sites. Scatchard analysis showed the MIP as having two binding recognition types: the stronger affinity binding type exhibits dissociation constant (Kd) 20 times smaller than that of the weaker type with binding capacity of 68.597 mg BPA/g MIP. Binding of the BPA in MIP showed high imprinting factor suggesting an efficient sorbent material for BPA.

Keywords: *Molecularly Imprinted Polymer, Bisphenol-A, Molecular recognition*

1. INTRODUCTION

The presence of packaging related contaminants in canned foods has been a major concern worldwide. This is because canned packagings are inner coated with epoxyphenolic resin. The most common of this resin is based on Bisphenol-A (BPA) and its close derivatives (Goodson, 2002). These have been shown to cause defects in the endocrine system and considered to be mutagenic and carcinogenic to animals and humans. Studies have shown that these can leach to the food hence these contaminants have been the subject of concern of some regulatory agencies and researchers because of their adverse health effects (Vichez, 2001, Cottier, 1997). Regulatory agencies such as the European Union (EU) have imposed a specific migration limit (SML) on these compounds in the food. Hence a selective and sensitive analytical method to detect trace amount of these compounds in food products is highly sought. In this work, a new molecularly imprinted polymer specific for the molecular recognition of BPA will be synthesized and the binding sites and recognition properties of this imprinted polymer will be investigated via rebinding experiments.

2. METHODOLOGY

A computational approach using MMFF94 was conducted to design the MIP considering the monomer-template interactions, monomer-template ratio, and rebinding solvents. The new BPA-imprinted polymer was synthesized via precipitation polymerization using methacrylic acid (MAA) as functional monomer with trimethylolpropane methacrylate (TRIM) as crosslinker, and BPA as template. The morphological structure of the polymer was studied by SEM, and the polymer assembly was then characterized using FM, EA, and IR spectroscopy studies. Molecular recognition analysis by rebinding experiments focused on the binding capacity and specificity/selectivity of the imprinted polymer for the target molecules. Characterization of the binding sites was conducted using Scatchard analysis and Freundlich adsorption isotherm model.

3. RESULTS AND DISCUSSION

The computational approach for designing molecularly imprinted polymers was developed prior to the experimental study in order to determine the ideal template, optimal monomer-template ratio and the rebinding solvent systems. Results show that the binding energies of the complex increase significantly up to 4:1 monomer-template molar ratio (Figure 1), which is expected to exhibit good imprinting characteristics. Among other solvents tested, the rebinding solvent using toluene was shown to enhance the interaction between BPA target molecule and the MIP.

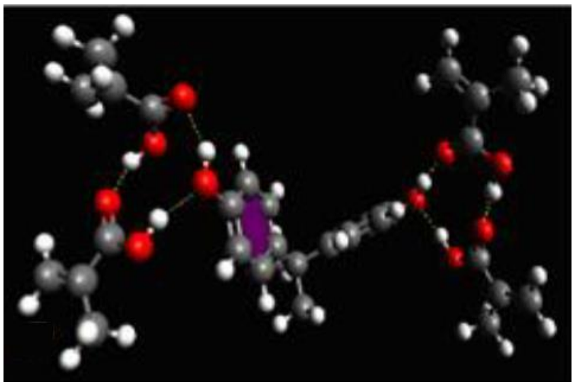

Figure 1. Geometry of stable complex of 4:1 MAA:BPA molar ratio

The synthesis of the MIP using the results obtained in the computational part gave a white fine powder. Similar process was likewise done to prepare a Non-imprinted polymer (NIP) for comparison.

The assembly of the BPA template into the polymer matrix was studied by infrared spectroscopy by comparing the absorption peaks of the imprinted and non-imprinted polymers. A wide OH stretching of free BPA at 3340 cm_{-1} became weak for the BPA in MIP. Moreover, a very notable observation shows that the intensity of C=O stretching vibration at 1728 cm^{-1} of MIP is weaker compared to the NIP suggesting that the polymer had been synthesized successfully with good assembly of BPA in the MAA of the polymer matrix via H-bonding.

Elemental analysis showed a significant increase in the percentage of C, H and O atoms in the BPA-MIP compared with the NIP revealing additional organic matter present in the MIP, suggesting that the BPA template had been assembled and loaded during polymerization. Moreover, the presence of the BPA template in the MIP matrix was mapped by fluorescence microscopy where the BPA-MIP showed fluorescence owing to its UV-active property at 278 nm while the MIP where BPA has been removed showed no fluorescence.

Scanning electron micrograph (Figure 2) showed highly uniform microparticles that are porous in nature with sizes ranging from are ~160 nm to ~200 nm. The uniform shape and size morphology suggests that the synthesized imprinted polymers is suitable as sorbent for column separation. The homogeneous and porous microstructures of the polymers are very helpful for fast adsorption of the target molecules (Guo, 2011). Moreover, the porosity of the MIP which consequently imparts greater permeability would allow the mobile phase to flow through with low flow resistance making it ideal as sorbent in chromatographic separation.

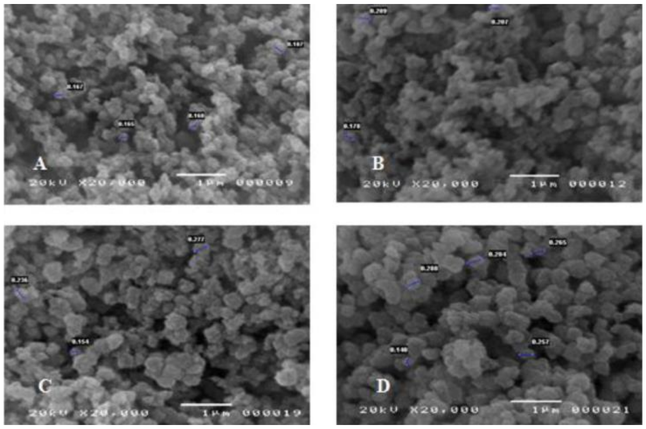

Figure 2. SEM image of the polymers synthesized through 4:1 MAA-BPA molar ratio**,** (A) BPA-MIP, (B) BPA-NIP, (C) MIP, (D) NIP

The characterization data using various techniques (SEM, FM, IR, elemental analysis) all indicate that the BPA imprinted polymer had been successfully prepared. The extraction of the BPA template then afforded the molecularly imprinted polymer (MIP).

The MIP was then studied for its capacity to rebind the BPA. A series of MIP or NIP with increasing concentration of BPA was equilibrated for 24 hours and the supernatant liquid was analysed for free BPA using GC-MS. The data were analysed and the plots of binding isotherm (Figure 3) show that the curve of the MIP isotherm has a saturation point at about 600 ppm. These plots suggest that the imprinted polymer has higher affinity for BPA than the non-imprinted polymer (NIP) as evidenced by the amount of BPA bound in the MIP which is considerably higher than the corresponding NIP.

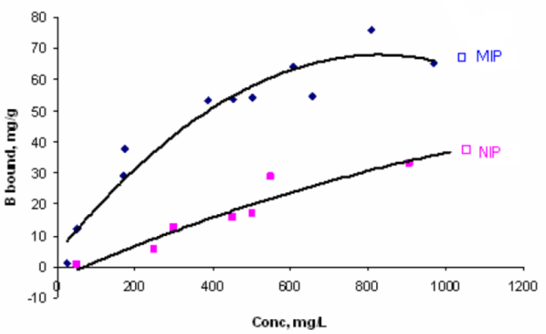

Figure 3. Binding isotherm of BPA-imprinted and non-imprinted polymer at 24-hour equilibration time

CONCLUSION

This work described the development of BPA-imprinted polymer-based extraction sorbent for selective binding and recognition of Bisphenol-A compounds. Computational techniques using MMFF94 were used to design the MIP considering the monomer-template interactions, monomer-template ratio, and rebinding solvents. The BPA-imprinted and non-imprinted polymers were synthesized via precipitation polymerization. Characterization of the MIP using SEM, infrared spectroscopy, fluorescence microscopy and elemental analysis revealed molecular assembly of the template in the polymer matrix. The new BPA-imprinted polymers were found to have porous structure with relatively uniform microparticles. Studies on the binding characteristics of the MIP indicate a saturation profile of ~600 ppm with high binding affinity and selectivity towards BPA under 50%ACN/50%toluene rebinding solvent system. The size and shape uniformity of the MIP and favorable binding characteristics suggests that the new MIP microparticles are suitable as sorbent for column separation that could be used as sample preparation technique in analyzing BPA, which may provide an alternative method for sample clean-up and pre-concentration for detection of BPA in food samples.

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