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Molecular Imprinted Polymer for Atrazine Detection Sensor: Preliminary Study

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A molecular imprinted polymer was synthesized by non-covalent method, which enables the recognition of a small pesticide target molecule, atrazine. The highest recovery was obtained from MIP compared with non-imprinted polymer (NIP) by using a commercial C8 sorbent. The study discusses the washing solvent selection with optimum volume that can removed the interference with maximum binding. Specific atrazine binding was nearly 3 times greater than non-specific binding. Optimum formulation of MIP that have mol ratio of monomer, cross-linker and template has been investigated.

1. Introduction

During recent years, the different chemical substances and pharmaceuticals, so-called emerging pollutants, have been identified in municipal wastewater effluents, surface water and in extreme cases, even in drinking water. Currently the environmental monitoring of water is of particular importance because of a sharp increase in amounts of a number of potential dangerous substances that may contaminate surface water (Maricelaet al., 2013). More than 70,000 different chemical compounds were used throughout the world by the end of the last century. Therefore, the development of selective and express methods of monitoring of emerging contaminants in water is highly desirable. Presently, on worldwide basis, intoxications attributed to pesticides have been estimated to be as high as 3 million cases of acute and severe poisoning annually, with many unreported cases with around 220,000 deaths. In Malaysia, little attention has been given to the presence of pesticides in the source of drinking water and its adverse effects on human health. The effect of pesticides on the environment is very complex as undesirable transfers occur continually among different environmental sections. Pesticides that are sprayed in the air may eventually end up in soils or water. This situation creates urgency to come up with a solution to detect the contaminant in the water stream.

Molecular Imprinted (MI) technology offers considerable potential as a cost-effective alternative to the use of biomolecule-based recognition in a variety of sensor application. Molecular Imprinted Polymers (MIP) enables the creation of specific recognition sites in synthetic polymers by a process that involves co-polymerization of functional monomers and cross-linkers around template molecules. Molecular imprinting of synthetic polymers is a process where functional and cross-linking monomers are co-polymerized in the present of the target analyte (the imprint molecule), which acts as a molecular template (Figure 1). The MIP is obtained by the polymerization of functional monomers and crosslinker in a suitable porogen (solvent) in the presence of a template molecule. The template is either covalently or non-covalently bound to the polymer network during polymerization reaction (Poole, 2003). The advantages and the disadvantages of these two have been summarized in Table 1. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. Subsequent removal of the imprint molecule reveals binding sites that are complimentary in size and shape to the analyte. In that way, a molecular memory is introduced into the polymer, which is now capable of rebinding the analyte with very high specificity (Kryscio and Peppas, 2012). Summary of the advantages and disadvantages of molecular imprinted polymer (MIP) are described in Table 2.

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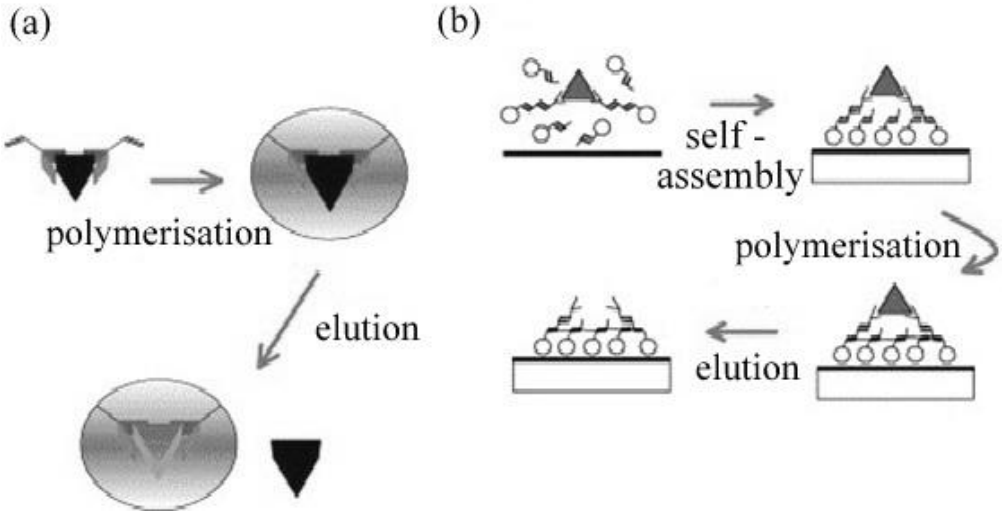

Figure 1: Scheme of: (a) three-dimensional and (b) two-dimensional imprinting polymerisation (Piletsky, Turner et al., 2006)

MI technology offers considerable potential as a cost-effective alternative of biomolecule-based recognition in a variety of sensor application. Molecular Imprinting Polymers (MIPs) is a creation of specific recognition sites in synthetic polymers by a process that involves co-polymerization of functional monomers and cross-linkers around template molecules. The molecules are removed from the polymer, rendering complementary binding sites capable of subsequent template molecule recognition. Although deposition of MIPs onto the surface of nanostructures may improve sensitivity for recognition of a range of organic compounds, electronic nanosensors capable of recognizing proteins continue to be a challenge to implement, in part, because: 1) the MIP film may attenuate signals generated in response to template binding (due to the thickness); 2) the detection mechanisms do not readily allow effective signal conversion of template molecule binding; and 3) the sensor platforms do not support highly sensitive detection. The use of stable molecular imprinted polymers having a specific receptor structure “plastic antibody” seems to be a possible alternative to develop the artificial sensor system (Holthoff and Bright, 2007).

Table 1: The advantages and disadvantages of covalent and non-covalent binding of MIP (Komiyama, Takeuchi et al. 2004)

	Covalent	Non-covalent
Advantages	- More homogeneous recognition sites as possibility to control the stoichiometry of the imprint materials. - Very stable and selective	- Monomer/template complex can be formed by self-assembly just by mixing the interacting molecules - Template is removed under mild conditions with high recognition sites
Disadvantages	- Limited number of functional groups to interact with target molecules and exhibit slow binding kinetics - Repetitive use in cleaving and rebinding templates can be difficult because of the restricted interactions - Different from natural recognition at molecular level that occurs in biological system	- Template-functional monomer interaction are less strong - The use of excess functional monomer to move the equilibrium versus the formation of the template-monomer complexes lead to random distribution of monomer in the polymeric matrix and generate non-homogeneous and/or selective recognition sites.

Table 2: Advantages and disadvantages of MIP (Vasapollo, Sole et al. 2011)

Advantages	Disadvantages
• High affinity and selectivity for the target molecule (template). • MIPs have higher physical strength, robustness, • Resistance to elevated pressure and temperature and inertness against various chemicals (organic solvents, acids, bases, and metal ions) compared to biological media such as proteins and nucleic acids. • Production costs are low • Lifetimes can be as long as several years at room temperature	• Slow mass transfer kinetics, • Binding site heterogeneity, and • Low population of high-affinity binding sites

2. Materials and Methods

2.1 Chemical products

All chemicals were of analytical grade. The ethylene glycol dimethylacrylate (crosslinker), methacrylic acid (monomer), 1,1'-Azobis(cyclohexanecarbonitrile) (radical initiator) and atrazine were purchased from Sigma-Aldrich. Toluene (porogen) and methanol were purchased from Merck (USA). All chemical are used without further purification. A commercial HPLC column was obtained from Agilent.

2.2 Apparatus

Chromatographic studies were performed by Agilent Technologies 1200 Series HPLC system, equipped with a pump (quaternary) and a photodiode array detector (DAD). HPLC conditions were first optimized and the following optimum conditions were obtained and used for all analyses. Atrazine was analyzed by HPLC-DAD at 222 nm and the mobile phase was a mixture of methanol and water (80:20, v/v) at a flow rate 0.8 $mLmin^{-1}$. The analysis was performed on an Eclipse XDB-C8 column (5 µm particle size, 150 mm × 4.6 mm I.D., Agilent). The injection volume was 10 µL. The retention time of atrazine was at 2.765 min. The relative standard deviation of the retention time was less than 1 %.

2.3 Solutions preparation

The protocol for MIP synthesis was modified from Kueseng et.al. (2009). The atrazine-imprinted polymer was prepared by dissolving template (atrazine) in the porogen (toluene, 0.7 mL) in a 7 mL glass tube. Functional monomer (MAA), crosslinker (EGDMA) and initiator (ABCN) were then added to the mixture and purged with nitrogen for 5 min. The tube was sealed and placed in a water bath at 60 °C for 20 h. As a control, non-imprinted polymer (NIP) was simultaneously prepared in the same manner but without the addition of the template. The bulk polymer obtained was smashed, ground and dried in a desiccator for 24 h.

2.4 Molecular imprinted solid phase extraction

The atrazine was extracted by washing the sample with methanol. 5 mL of methanol was added to 0.0250 g of sample (MIP/NIP). The mixture then was homogenized by using vortex. A centrifuge was used to separate the sample from the solution. The steps were repeated for 6 times.

2.5 MIP binding

The binding study of atrazine on MIP and NIP was performed by the dynamic method or non-equilibrium binding. In this method, 5 mL of an aqueous solution containing 60 ppm of atrazine were applied to the washed sample. The atrazine concentrations in the elution step, representing the amount of analyte not bound to the polymer was determined by HPLC-DAD as described above.

3. Results and Discussions

3.1 Type of washing solvent

A washing solvent was applied to MIP with the aim to maximize the specific interactions and disrupt the non-specific interactions between the analytes and the polymer matrix and also the interfering compounds, while it should not disrupt the interaction between MIP and the analyte (Pichon and Chapuis-Hugon, 2008). Two types of solvent, i.e. methanol and water were investigated as washing solvent. NIP was also tested in the same procedure as a control. The recovery in the washing fraction was evaluated. It should

provide high recovery for NIP but low recovery for MIP because atrazine strongly adsorbed on the MIP, so it should not be released in the washing step.

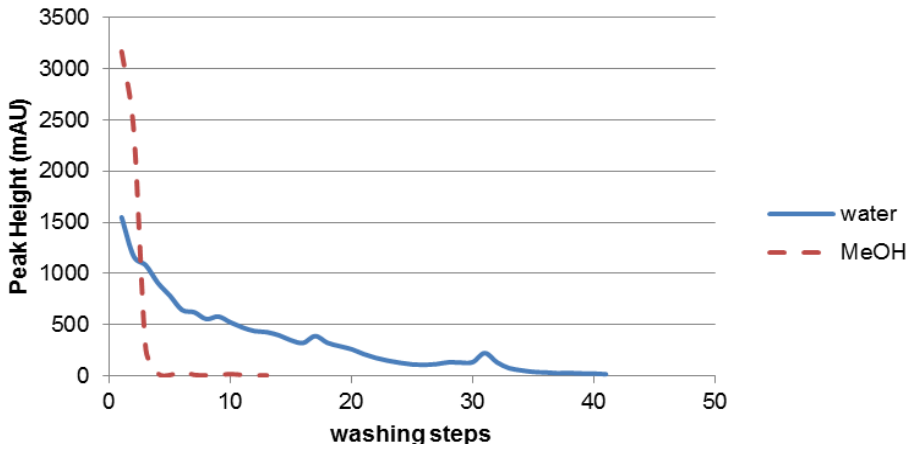

Figure 2: Washing steps and peak height for MIP

As shown in Figure 2, the analyte was easily washed out by using methanol as washing solvent. While for NIP all the interferences were also can be easily removed by using methanol rather than water.

3.2 Volume of washing solvent

The volume of the washing solvent should be enough to remove the non-specific interaction from MIP, while the analyte would remain bound to MIP (Kueseng, Noir et al., 2009). The volume of washing solvent was investigated at 10 and 5.0 mL. The volume of 5 mL was enough to effectively remove nonspecific binding and provided highest selectivity. This was selected as washing volume to remove interference.

3.3 Eluting steps

The minimum steps of eluting solvent required to complete the elution of analyte was investigated. The use of fewer steps can reduce the experimental time and solvent consumption. Figure 3 shows by using methanol the steps can be reduce from 40 to 6 steps only. This can be explained by the different in polarity index between both solvent.

3.4 MIP binding

The binding of the imprinted polymer with 5 mL aqueous solution containing 60 ppm of atrazine aqueous solution was verified by the dynamic binding experiment.

Table 3: MIP binding of analyte with different monomer, cross linker and : template mol ratio

Sample	Ratio (M:Cr:T)	Analyte binding (mAU.s)
MIP5	5:3:1	1002.12
NIP5	5:3:1	480.29

As shown in the Table 3, the optimum formulation between the monomer, cross-linker and template mol ratio is 5:3:1. Specific atrazine binding was nearly 3 times greater than non-specific binding. The higher amount bound of MIP than NIP that confirmed the presence of the imprinted cavities in their structure. Therefore it could bind through specific binding site and non-specific binding site while NIP can bind only through a non-specific binding site. The selective binding sites are mainly formed by hydrogen bonding or electrostatic forces during self-assembly of the template and monomer. In this work, atrazine bound with MAA by hydrogen bond and also formed the strong hydrogen bond complexes with amides through cooperative, multipoint attachment (Muldoon and Stanker, 1997).

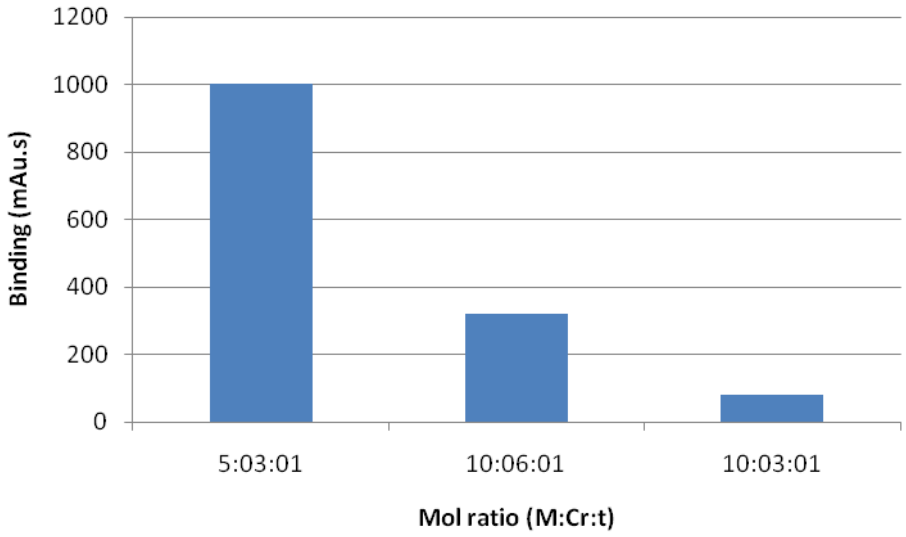

Figure 3: MIP binding of analyte with different monomer, cross linker and: template mol ratio

The analyte binding shown in Figure 3 for MIP7 and MIP8 are lower than MIP5. This can be explained as the imprinted polymers are showing several type of binding effect. This includes the effect of shape, functionality and size (Bruckner-Lea and Division, 2004). The low degrees of cross-linking and high template concentration reduced the formation of individual cavities (Yungerman and Srebnik, 2006). The template aggregate in the pre-polymerization mixture and reduced the deformation of recognition site. Polymers with cross-linker molar ratios in excess 80 % with respect to the monomer are usually used to obtain materials with adequate mechanical stability and good recognition performance.

4. Conclusions

As a conclusion, the study shows that the MIP washed using methanol, with the volume of 5mL, is 6 times less steps than using water. The binding capacity of analyte towards MIP and NIP has found to be optimum at mole ratio of 5:3:1 with monomer, cross-linker and template.

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