

Screening of pesticide from contaminated water using Molecular Imprinted Polymer-Solid phase extraction method

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Abstract

The quality of natural water is deteriorating continuously due to the accumulation of undesirable constituents into it. The main sources of the contamination are industrialization, domestic activities, agricultural activities and other environmental changes. These activities and changes, if improperly controlled, can destroy the quality of our environment. Molecularly imprinted polymers (MIPs) with recent advancements have created synthetic materials that can mimic the function of chemical and biological receptors but with less stability constraints. These polymers can provide high sensitivity and selectivity while maintaining excellent thermal and mechanical stability. In the present study molecularly imprinted membrane has

been fabricated for the specific recognition of pesticide like Deltamethrin which is being used widely to control insects in crop management system. The composite membranes have been prepared by using methacrylic acid (MAA) as functional monomer, selected by electrostatic interactions based computational simulation and ethylene glycol dimethacrylate (EGDMA) as cross linker. The garbing of deltamethrin MIP on membrane matrix was confirmed by the SPE, UV-VIS spectrophotometer and FTIR.

Keywords: Sensor | molecularly imprinted polymer (MIP) | SPE

Introduction

In order to increase the food production, uses of pesticides in agriculture become a general thing. But excessive and uncontrolled uses of pesticides contaminate water. These pesticides like Deltamethrin have very severe health effects. Deltamethrin may come in contact with human body through inhalation, ingestion and the dermal routes of eye and skin. Each of

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these pathways can possibly lead to acute health effects. Allergic reactions have included the following effects: anaphylaxis, bronchospasm, eosinophilia, fever, hypersensitivity, pneumonia, pallor, polyposis, sweating, sudden swelling of the face, eyelids, lips and mucous membranes and tachycardia.

In the last decade, one of the most promising technical applications based on the use of MIPs has been molecularly imprinted solid-phase extraction (MISPE). The technique for the first time was used for making MIP by Sellergren *et al.* for imprinting small target molecules. With the same concept, Nematollahzadeh *et al.* developed the techniques based on MIP. Most of the studies performed have focused on extracting compounds from biological samples. Bio fluids have been the samples analysed in most of the biological studies with only a few papers reporting the extraction of analyte from tissue samples. In the last few years, MISPE has also been applied to extracting compounds from other matrices, such as environmental Samples (water and soils), food, plants and tobacco, although the number of studies of some of these sample types is limited. There are many recent reports of molecularly imprinted polymer which are used to develop detection systems for pharmaceuticals and environmental contaminants. Nematollahzadeh *et al.* developed a general technique, so-called polymerization packed bed, to obtain a hierarchically structured high capacity protein imprinted porous polymer beads by using silica porous particles for protein recognition and capture. MIPs show a promising future in the developing knowledge and application in food

sciences.

In the present paper a plan is designed by using MISPE to extract Deltamethrin from Deltamethrin contaminated water. SPE in particular is widely applied in this area due to its simplicity, important savings in time and cost, and, last but not least, because it is an environmentally friendly technique compared to classical solvent extraction.

Materials and Method

Preparation of Template

A template of Deltamethrin (1.006 gm) was taken in a 15 ml reaction vial and solvent (Acetonitrile, 5 ml), cross linker (EGDMA, 4.8ml), functional monomer (Methacrylic acid, 0.5ml) and initiator [4, 4'- azo-bis (4-cyanovaleric acid) - 0.05 gm] were added to it. All the components were thoroughly mixed. Blank polymer without Deltamethrin was also prepared. After that reaction vials were kept at 70-85⁰c in oven for overnight for complete polymerization. The polymer was transferred from the vial to mortar, pistol, grinded and filtered with methanol. The polymers of 40-125 µm were collected through methanol solvent extraction. Remaining residue filtrate was also collected for future use. An amount of 400 mg of MIP were packed in cartridges with frits with the help of frit settling rod.

Solid Phase Extraction

A rapidly growing application area for MIPs is what has become known as molecularly imprinted polymer solid-phase extraction (MISPE). The use of MIPs as the stationary phase in solid phase extraction makes it

possible to perform specific enrichments to facilitate analysis of substances available only at trace levels in samples. Obvious areas of interest for this technique are the analysis of drugs and environmental pollutions. Solid-phase extraction is by far the technique in which MIPs have found most of their applications, mainly due to its speed, robustness, and simplicity. While traditional SPE stationary phases offer generic selectivity related to the hydrophobic/hydrophilic and/or ionic character of the targeted compounds, MIPs introduce inherent selectivity for a specific analyte or family thereof, to the stationary phase, greatly improving its performance in demanding separations. For this reason, MI-SPE protocols have been developed, and, in most cases, have successfully substituted traditional SPE sorbents, for analysis of environmental pollutants, clean-up of biological fluids, and analysis of pharmaceuticals and food samples.

Similar to conventional SPE, MI-SPE usually comprises of few steps, depicted in Figure- 1. Initially the column is conditioned with an appropriate solvent/buffer and the sample is loaded. The so-called ‘molecular recognition’ step follows, whereby the loaded column is washed with a solvent that will promote specific interactions between the stationary phase and the imprinted analyte, while disrupting nonspecific binding. An additional washing step is usually added, followed by elution of the selectively bound analyte(s) with a strong eluting system.

Apart from the traditional offline SPE protocol discussed above, column switching and pulsed elution have been implemented in inline SPE. The inline approaches have become

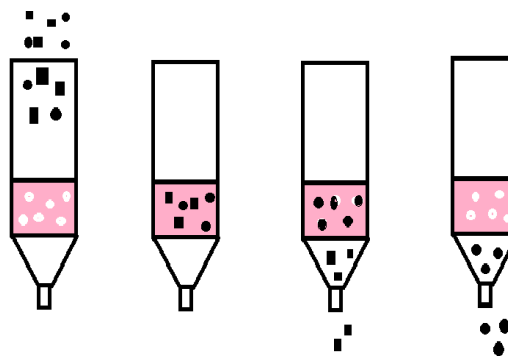


Figure 1: Principle of Solid phase extraction with an imprinted polymer. The sample is loaded on to the imprinted Polymer resulting in binding of both the analyte and contaminants (step 1). The first elution steps removes the contaminants and the analytes remains the specific binding site (step 2). The analyte is then eluted from the solution (step 3).

■ Other sample components ● analyte

increasingly popular, because they offer possibilities for automation of the whole combined sample pre-treatment/enrichment and analytical process. Here, an MI-SPE column is coupled with an analytical column via a switching valve. Initially, the MIP column is loaded with the sample of interest and all necessary intermediate washing steps are performed using a secondary valve and pump. The MIP column is then eluted and the analyte(s) of interest are transferred into a holding loop. After valve switching, the sample is injected into the analytical column for separation. The most widely used organic modifiers so far have been acetic acid or its analogue, trifluoroacetic acid. Once all the MISPE steps have been optimised, a problem can sometimes occur, especially when using highly sensitive detection systems such as MS. This is known as bleeding of the cartridge. This problem, as mentioned previously, is a consequence of an inefficient removal of the

template molecule used during the synthesis of the polymer once the MIP is obtained. Therefore, when the sorbent is used in a MISPE protocol, remaining template molecules might still elute from the cartridge, thus masking the final result obtained. All the above-mentioned stages are essential steps in any MISPE protocol, although, as stated previously, when loading the MIP with an organic solvent, the clean-up step can sometimes be avoided. There are basically two distinct protocols to perform all these stages: MISPE offline and MISPE on-line generally coupled to liquid chromatography (LC). MISPE off-line is the mostly used technique. In this case, the useful particles are generally suspended in a solvent and then poured into an empty polyethylene cartridge. As in the conventional SPE cartridge, the particles are held between two frits to avoid any losses. The cartridge is then connected to a SPE manifold and the sample is percolated through by negative pressure. The most widely used mass of sorbent for MISPE applications normally ranges from 40 to 200 mg of suitable particles. Their particle size and shape depends on the polymerisation approach taken during the synthesis of the MIP. In the synthetic protocols aiming to deliver spherical particles, the particles obtained are ready to be used, with no need for any further processing. In the case of traditional polymerisation, in which MIPs are obtained in a monolithic form, there is a need of further processing so that the particles for MISPE applications are in the range of between 20 and 60 μm . This range is the right balance because the particle size is low enough

to enable both a proper flow and mass-transfer of the analytes present in the mobile phase on to the sorbent.

Preparation of MIP Composite Membrane with Template

The reactive mixtures were prepared by mixing of selected monomer (Methacrylic acid, 0.5ml) with inert porogen (acetonitrile 5 ml), suitable template (Deltamethrin, 1.006 gm) and crosslinker (EGDMA, 4.8 ml). After addition of initiator [4, 4'- azo-bis (4-cyanovaleric acid)] (0.05 gm) reactants were mixed and degassed. The yield of properly created MIPs is limited by the capacity to effectively wash the substrate from the MIP once the polymer has been formed around it.

Removal of Template and Confirmation by UV-Visible Spectrophotometer

Specific binding is confirmed by SPE and UV-Vis spectrophotometer. The MIPs were washed five times with methanol and five times with water to remove the print molecules. All the extraction experiments were performed using a SPE vacuum unit. Imprinted template was eluted with methanol. The fractions eluted from each cartridge were collected separately. The removal of template was also confirmed with UV-Visible spectrophotometer.

Fourier Transform Infrared Spectroscopy Study

FTIR provides quantitative analysis of the binding modes of a substrate molecule to the polymer site by empirical calibration of FTIR. The technique gives a consistent representation in which the target analyte binds to the

polymer site. The analysis also provides an opportunity to quantify site isolation within the polymer and the fidelity of functionalized site is maintained by the network polymer.

Results

Characterization by FTIR Spectroscopy

The FTIR spectra of imprinted polymers can be readily acquired and then applied in a similar fashion to elemental micro-analysis to extract quantitative information on the composition of the polymer. The method is of particular value when the different chemical environments in the sample (e.g. arising from the functional monomer and cross linker in an imprinted polymer) give rise to well resolved, diagnostic signals. It is also possible to use FTIR to probe non-covalent interactions, e.g. hydrogen bonds, although the insensitivity of the technique sets limits on its utility in this regard.

Figure- 2 shows FTIR spectra of polymeric matrix with Deltamethrin template. This shows peak at the wave number 440 cm^{-1} , 460 cm^{-1} , 521 cm^{-1} , 656 cm^{-1} , 811 cm^{-1} , 873 cm^{-1} , 950 cm^{-1} , 1040 cm^{-1} , 1159 cm^{-1} , 1261 cm^{-1} , 1375 cm^{-1} , 1469 cm^{-1} , 1633 cm^{-1} , and 1727 cm^{-1} clearly showing bonds of template molecule and hydrogen bonding with polymer matrix. The template is surrounded by polymer matrix and can be easily resolved out by proper extraction with solvent methanol shown later. The FTIR spectra of polymer without template are prepared. The porogen used was the acetonitrile. The important peaks at 419 cm^{-1} , 1016 cm^{-1} , 1465 cm^{-1} , 1731 cm^{-1} , 2360 cm^{-1} , 2924 cm^{-1} and 3648 cm^{-1} clearly shows the polymerisation of

monomer. There is no sign of hydrogen bonding peaks, which shows the absence of template in the spectra. The FTIR spectra of the reference polymer i.e. blank polymer without template using the DMF as porogen are prepared. It shows peak at 3843 cm^{-1} , 2360 cm^{-1} , 1517 cm^{-1} , 1424 cm^{-1} , 1212 cm^{-1} , 1077 cm^{-1} , 1004 cm^{-1} , 971 cm^{-1} , 754 cm^{-1} , 668 cm^{-1} , 521 cm^{-1} , 480 cm^{-1} , and 435 cm^{-1} . These peaks exhibits the IR spectra of blank polymer matrix free of template.

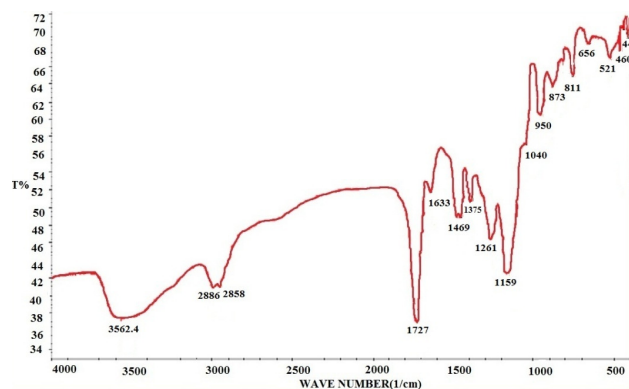


Figure- 2

Studies with SPE Column Preparation and Removal of Template

Removal of the template from the SPE cartridges was confirmed by UV-Visible spectrophotometer. The MIPs were washed five times with methanol and five times with water to remove the print molecules. UV-Visible spectra of elute was taken after first and fifth wash with methanol and first and fifth wash with water. Figure - 3 shows the absorbance and wavelength graph after first and methanol and water washing of Deltamethrin template.

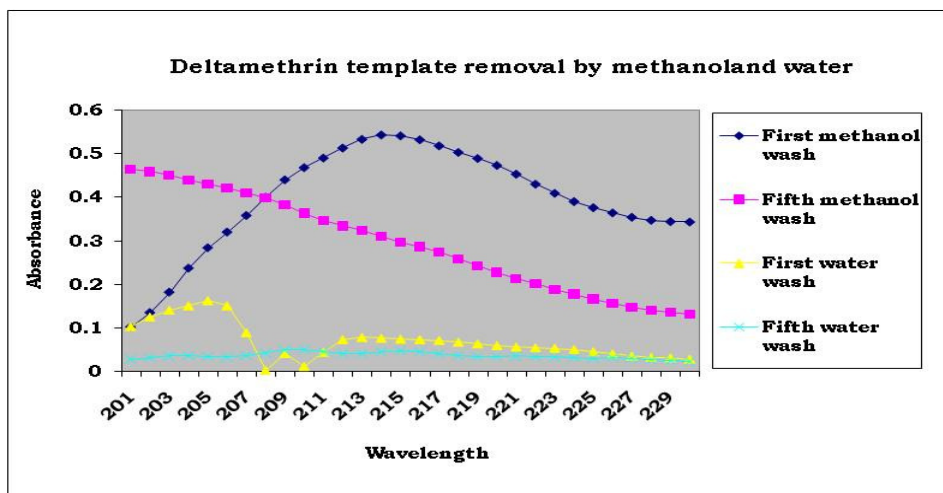


Figure - 3

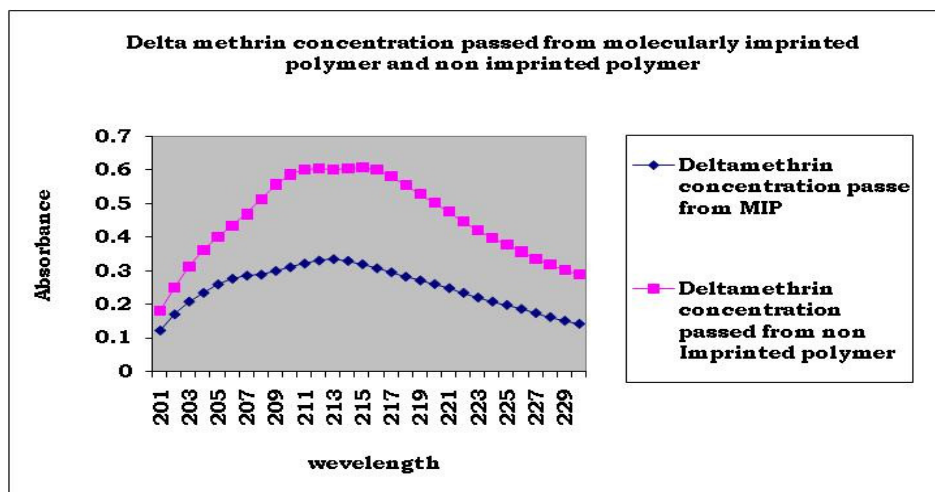


Figure - 4

Binding of Template with MIPs

Binding of the template was done by passing the concentration 20 µg/ml of template from imprinted polymer and non-imprinted polymer. MIP has specific cavities for template as concentration was passed from MIP, its shows sensitivity to template. Sensitivity of MIP confirmed by UV-visible spectra of elutes. MIP elutes shows lower absorbance than non-imprinted polymer. It confirms that MIP has sensitivity to template. Figure - 4 is showing the graph between absorbance and wavelength of Deltamethrin concentration passed from

MIP and Deltamethrin concentration passed from non-imprinted Polymer.

Deltamethrin MIPs sensing and separation

When Deltamethrin concentration was charged in SPE cartridges, MIP with template Deltamethrin shows absorbance 0.218 at wave number 201, 0.189 at 202, 0.154 at 203, 0.12 at 204, 0.93 at 205 and 0.078 at 206, which clearly shows Deltamethrin MIP has sensitivity to template Deltamethrin. After the Deltamethrin MIPs sensing in contaminated water its extraction is done by Solid Phase extraction as discussed above.

Conclusion

According to molecular imprinting approach, cross-linked polymers are formed around a template molecule. The template is then removed, thus leaving molecular cavities capable of binding the template molecules back. Moreover, the synthesis of MIPs is a straightforward and inexpensive procedure. The selectivity of the imprinted membrane was due to the formation of molecular cavities inside the polymer matrix and the molecular cavities capable of binding the template molecules back. The imprinted membrane was treated with methanol for the removal of template molecules and this confirmed by taking absorbance of eluted template in methanol and it also confirmed by UV-Visible spectrophotometer and FTIR by comparing the imprinted and composite membrane.

The next step of experiment was making sensor using the MIPs. For this, SPE unit was used. For sensing, concentration of the template Deltamethrin was prepared in methanol and water (methanol to water ratio 1:9). Deltamethrin concentration was loaded in the SPE cartridges and the fractions eluted were collected separately. UV-visible spectra of elute taken, the concerned MIP (MIP with template Deltamethrin) shows lower absorbance than others.

In the present study, the synthesis of molecularly imprinted polymer matrices specific to Deltamethrin pesticides were synthesized and these MIP matrices were also used for the fabrication of MIP composite membranes. The MIP matrices were also used

for specific separation and recognition of targeted analyte which are pesticide molecules with structurally related molecules. This study can resolve the problem of rising of natural receptor against these hazardous molecules by replacing with artificial receptor. These artificial receptor and equally specific and more durable than the natural were having characteristic of high stability in mild environment conditions. The identification, sensing and separation of these template molecules through SPE is a cost effective and easier way of commercial usage.

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