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Selective Recognition of Endosulfan Pesticide in Environmental Matrix with Molecularly Imprinted Polymer Membrane

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Abstract

The excessive and uncontrolled uses of Endosulfan pesticide in crops have raised an alarm for its toxicity not only in crops but also in drinking water. So, it has become imperative to detect and separate the hazardous pesticide Endosulfan from contaminated water. In present studies, molecularly imprinted membrane has been fabricated for the specific recognition of Endosulfan pesticide, which is widely being used in controlling insects. The composite membranes have been prepared by using Methacrylic acid (MAA) as functional monomer and Ethylene Glycol Di Methyl Acrylate (EGDMA) as cross linker. MAA was chosen as a functional monomer, as it is capable of strong electrostatic interactions with Endosulfan. The molecular imprinting of Endosulfan on membrane matrix was confirmed by the Solid Phase Extraction (SPE), UV-Visible spectrophotometer, Fourier Transform Infra-Red (FTIR) Spectrophotometry and Scanning Electron Microscopy (SEM). This was followed by sensing of the herbicide by SPE measurements.

Keywords: Methacrylic acid (MAA), solid phase extraction, ethylene glycol di methyl acrylate (EGDMA), characterization, endosulfan pesticides.

Introduction

Today, farmers are using higher percentage of chemical fertilizers and pesticides to enhance the yield of crops to fulfill growing demands of food crops. If pesticides are not used, crop yield has been reported to reduce by about 10%[1]. It is well known that several pesticides used for agricultural purposes are associated with surface and ground water contamination[2-4]. Excessive and uncontrolled use of Endosulfan pesticides can leave harmful and environmentally unsafe residues in soil and water[5]. Public concern regarding pesticide exposure and good health is increasing day by day. Due to exposure to pesticides, children, pregnant women, farmers, farm workers and the elderly undergo negative health effects and can also cause acute poisoning, cancer, neurological damage, birth defects and reproductive as well as development harms. The Endosulfan pesticide exposure leads to symptoms like blue lips or fingernails, confusion, headache, weakness, dizziness, nausea, vomiting, diarrhea or unconsciousness. The World Health Organization (WHO) estimated in 1992 that 3 million pesticides poisonings occurs annually causing 2,20,000 deaths[6].

It, therefore, becomes the barest necessity to detect and separate these kinds of pesticides from the contaminated water. There are number of methods for sensing and separation of pesticides. Biosensors and sensors provide excellent tools for the detection of the pesticides[7]. Molecularly Imprinted Polymers (MIPs) are used for separation, which have artificially created receptor structures[8]. The first MIP employed for solid-phase extraction was reported by Sellergren in 1994[9]. Reproducible way of providing selectivity for analytical process is by incorporating MIPs into sample preparation steps[10-12]. Molecular imprinting, by Wulff and Sarhan[13], and Arshady and Mosbach[14], has produced materials with "antibody-like" selectivity[15]. MIP's are extensively cross-linked polymers having specific recognition sites with a predetermined selectivity for analytes of interest[16,17]. Heuristic techniques such as hierarchical imprinting method were used for making MIPs by Sellergren and Buechel for imprinting small target molecules[18]. Nematollahzadeh et al. developed 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[19]. There are many recent reports of molecularly imprinted polymer, which are used to develop detection systems for pharmaceuticals and environmental contaminants[20].

This study was aimed to develop a molecularly imprinted polymer composite membrane to selectively recognize the contaminant (Endosulfan) in water test samples. The selective membrane matrix will work a dual function of identification and removal of targeted analyte. In present attempt, composite membrane of MIPs has been synthesized and used for separation and sensing of the pesticides due to their unique stability and easy to adaptation using advanced instrumental techniques like UV-Visible spectrophotometer, Fourier Transform Infra-Red (FTIR) and Scanning Electron Microscopy (SEM).

Material and Methods

Preparation of Endosulfan template: A template of Endosulfan (1.005 gm) was taken in a 15 ml reaction vial and solvent (Acetonitrile, 5 ml), cross linker (EGDMA, 4.5 ml), functional monomer (Methacrilic 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 Endosulfan was also prepared. After that reaction vials were kept at 70-80˚C 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 was packed in cartridges with frits with the help of frit settling rod.

Solid Phase Extraction (SPE): Use of solid phase extraction (SPE) is a sample preparation method based on use of a solid separation material to extract specific compound(s) by retention allowing rapid sample clean-up or pre-concentration. Sample pre-concentration is done by liquid–liquid extraction or SPE[21]. Recently, advanced SPE materials like highly cross-linked polymers (MIPs) have been developed to allow more attractive extractions[22-27]. After conditioning of the packing material, the analyte of interest (or the interferences) are adsorbed onto the solid phase. A washing step allows selective removal of interfering components, followed by an elution step, where the analyte of interest is eluted with a different solvent and collected in fractions. MIPs are compatible with aqueous as well as non aqueous samples. Depending on the nature of the interactions involved in the rebinding process, non-specific binding or hydrophobic interactions contribute to the retention of molecules contained in aqueous samples due to non-specific adsorption to the polymer surface. Figure 1 shows washing with an organic solvent allows selective binding due to recognition of functionalities. As a final step, the analyte of interest is eluted with a suitable solvent.

Preparation of MIP Composite Membrane with Endosulfan Template: The reactive mixtures were prepared by mixing of selected monomer (Methacrilic acid, 0.5ml) with inert porogen (acetonitrile, 5 ml), suitable template (Endosulfan, 1.005 gm) and crosslinker (EGDMA, 4.5 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[28].

Removal of Template and Confirmation by UV-Visible Spectrophotometer: Specific binding is confirmed by SPE and UV-Visible spectrophotometer. The MIPs were washed five times with methanol and also water to remove the print molecules. All the extraction experiments were performed through 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 by using UV-Visible spectrophotometer.

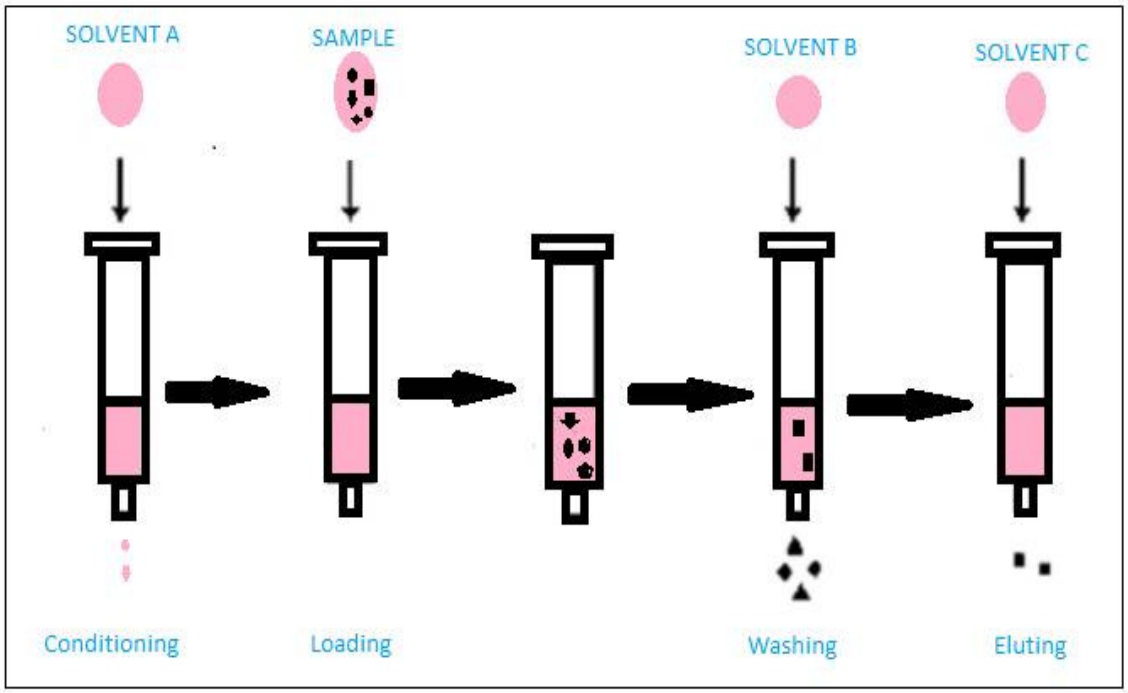

Figure-1
Washing and eluting process in solid phase extraction

Fourier Transform Infrared Spectroscopy (FTIR) 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.

Scanning Electron Microscopy (SEM): Prepared molecular imprinted composite membranes with and without template were categorized by using scanning electron microscopy (SEM, Model: JEOL JSM 840) and the micrographs were recorded of all the different samples of the membrane including non-imprinted and MIP composite membranes.

Results and Discussion

Characterization by Fourier Transform Infrared Spectroscopy: The FTIR spectra of imprinted polymers can be acquired and then applied to elemental micro-analysis to extract quantitative details of composition of the polymer. This method becomes more useful, when different chemical environments in the sample (e.g. arising from functional monomer and cross linker in an imprinted polymer) give rise to well resolved, diagnostic signals. FTIR can be used to probe non-covalent interactions, e.g. hydrogen bonds, although the insensitivity of the method sets limits on its utility in this regard. Figure 2 shows FTIR spectra of polymeric matrix with Endosulfan template. This matrix shows peak at the wave number 444 cm^{-1}, 464 cm^{-1}, 521 cm^{-1}, 664 cm^{-1}, 754 cm^{-1}, 816 cm^{-1}, 877 cm^{-1}, 963 cm^{-1}, 1167 cm^{-1}, 1267 cm^{-1}, and 1474 cm^{-1} indicating the additional peaks of template molecule.

Characterization of MIP Composite Membrane by Scanning Electron Microscope: The prepared MIP composite membrane is of different porosity due to polymerization of polymers with and without the template within the pores of membrane and on the surface as well. Since, the membrane was made wet with reaction mixture of polymers and this reaction mixture was well absorbed within the pores and also forms a very thin layer of porous polymer on the Millipore membrane, therefore, the study of surface morphology was essential in context with porosity and surface texture.

Figures 3-5 show the SEM micrographs of the MIP composite membrane imprinted by Endosulfan template, which were recorded at 500 X, 1.0 KX, 1.5KX magnifications and at a resolution of 30 µm. In figure 6, the SEM micrograph was recorded at 1.99 KX magnification and at a resolution 10 µm. The surface texture clearly exhibits the cavities on the surface and within the pores, which is a clear indication of change of porous behavior of MIP composite membrane after removal of template. This change in porous characteristic may be further correlated with its electrochemical behavior in thermo dynamical studies.

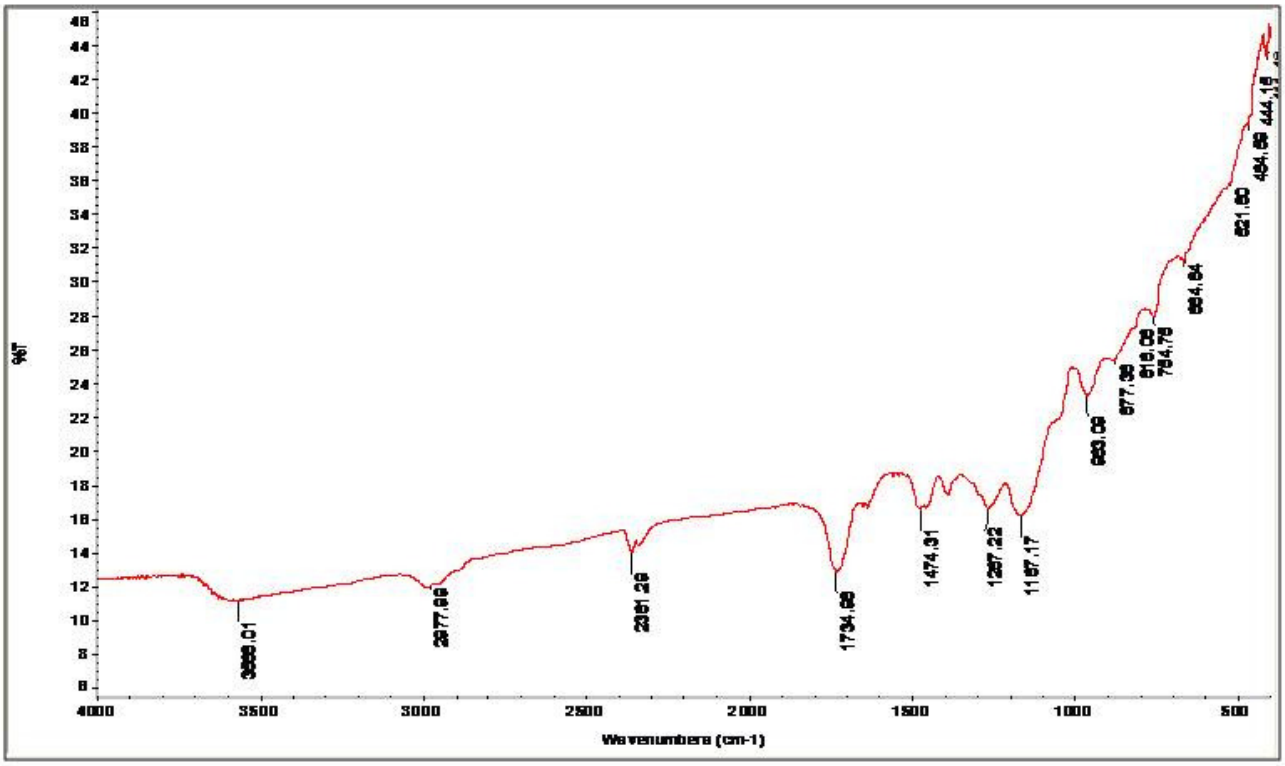

Figure-2
FTIR spectra of prepared polymer with Endosulfan template

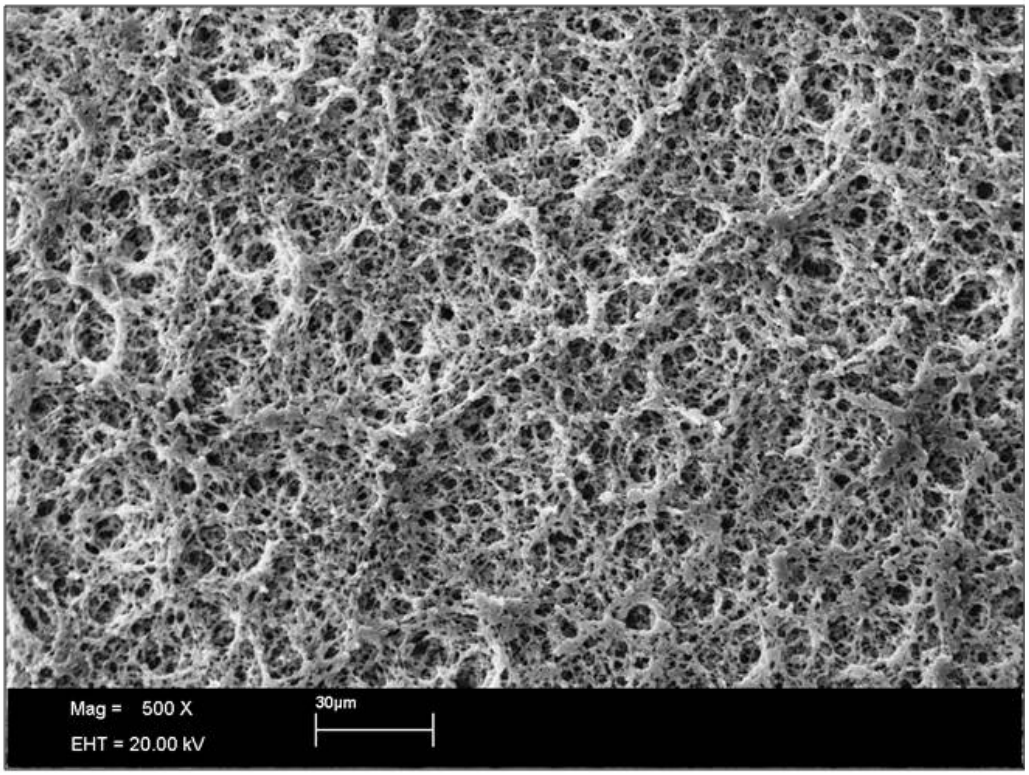

Figure-3
SEM micrograph of the MIP composite membrane imprinted by endosulfan template at 500 X magnification and 30 µm resolution

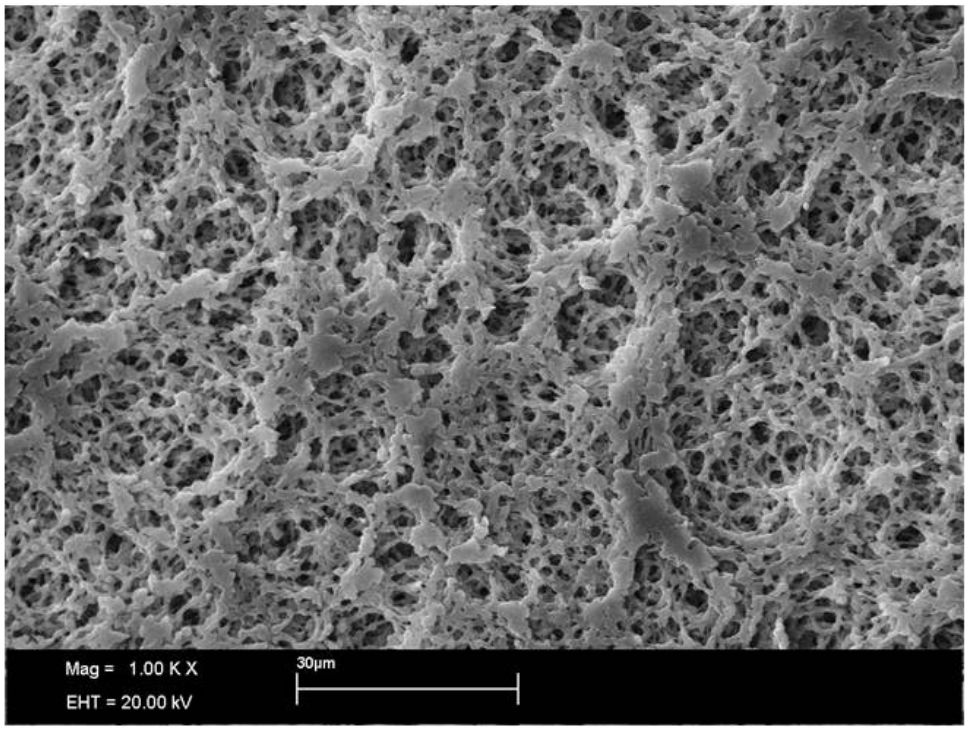

Figure-4
SEM micrograph of the MIP composite membrane imprinted by endosulfan template at 1.0 KX magnification and 30 µm resolution

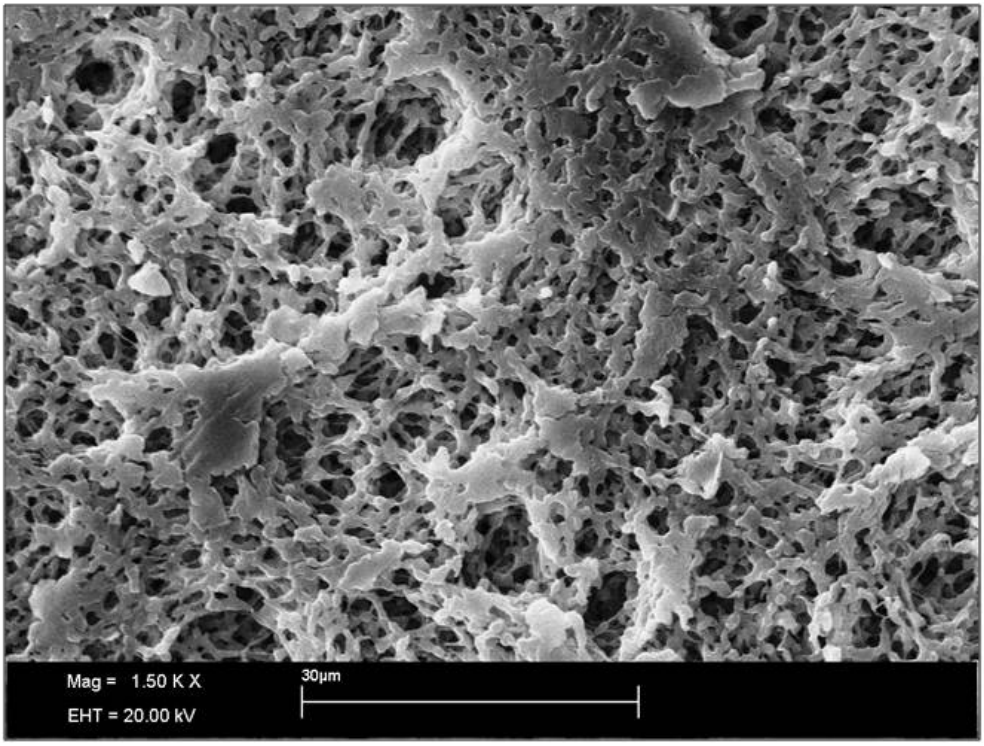

Figure-5
SEM micrograph of the MIP composite membrane imprinted by endosulfan template at 1.5 KX magnification and 30 µm resolution

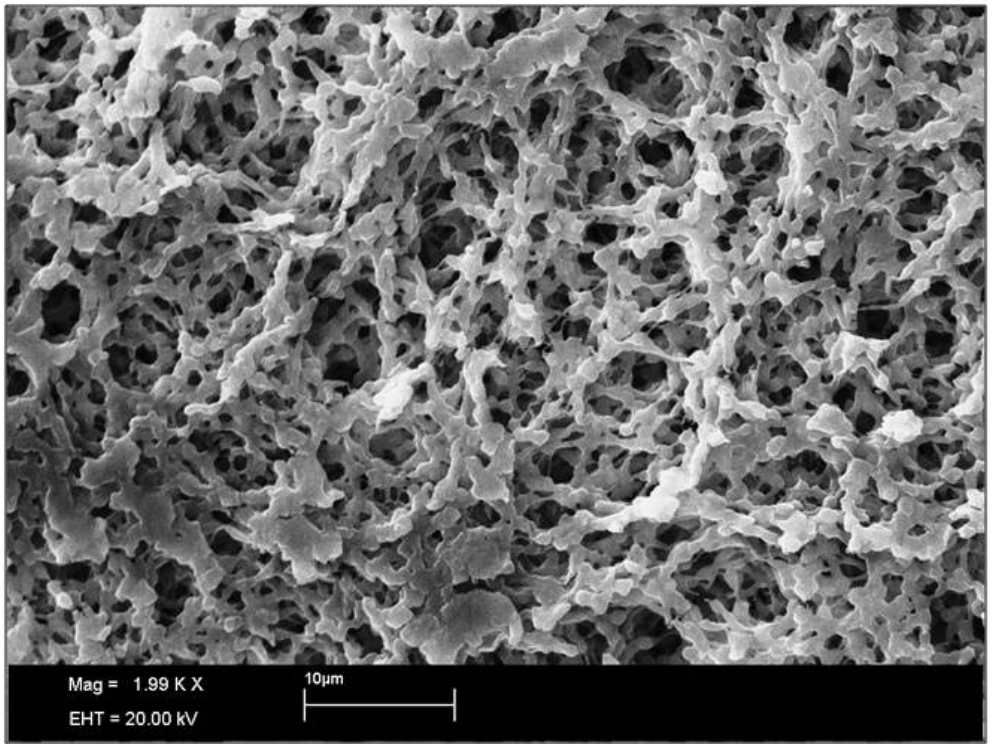

Figure-6
SEM micrograph of the MIP composite membrane imprinted by endosulfan template at 1.99 KX magnification and 10 µm resolution

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 7 shows the absorbance and wavelength graphs after first and fifth methanol and water washing of Endosulfan template.

Binding of Endosulfan 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 was confirmed by UV-Visible spectra of elutes. MIP elutes show lower absorbance than non-imprinted polymer. It confirms that MIP has sensitivity to template. Figure 8 is showing the graph between absorbance and wavelength of Endosulfan concentration passed from MIP and non-imprinted polymer.

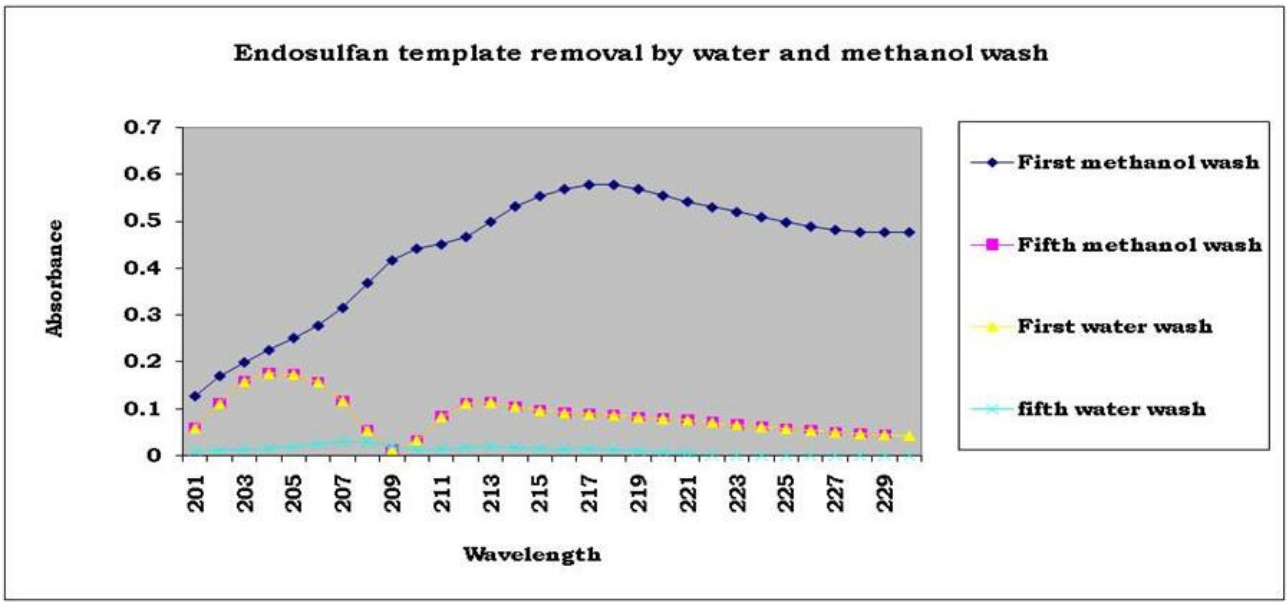

Figure-7
Endosulfan template removal by water and methanol wash Binding of Endosulfan template with MIPs

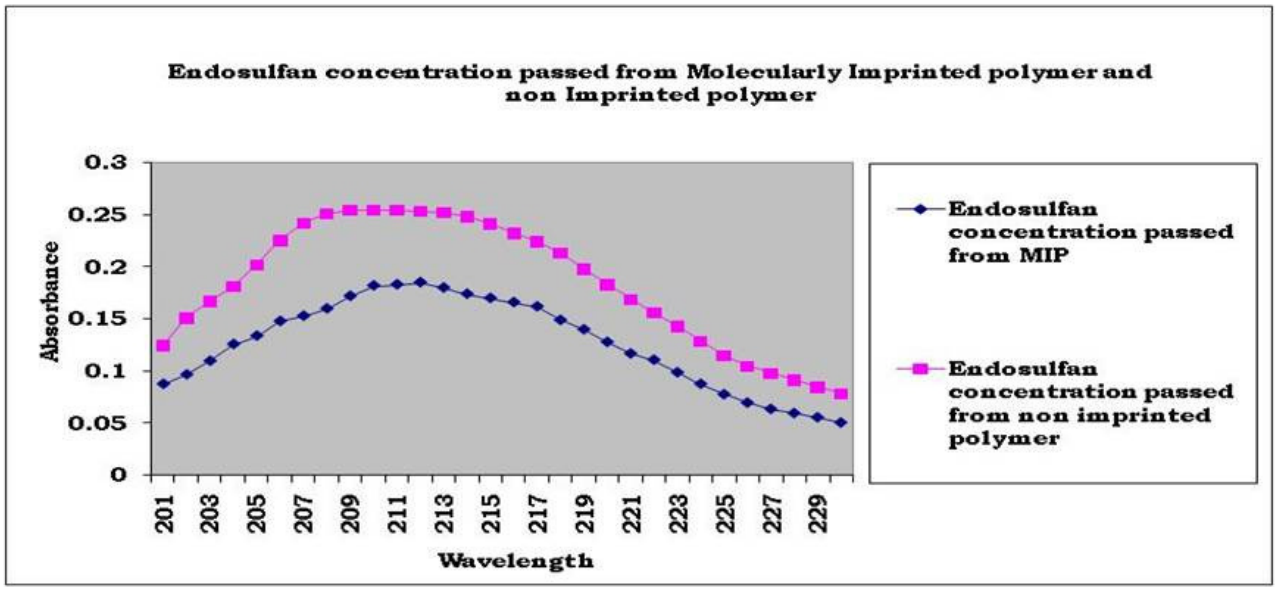

Figure-8
Endosulfan concentration passed from molecularly imprinted polymer and non-imprinted polymer

Endosulfan MIPs Sensing: When Endosulfan concentration was charged in SPE Cartridges, MIP with Endosulfan template shows absorbance 0.770 at wave number 201, 0.794 at 202, 0.793 at 203, 0.752 at 204, 0.660 at 205 and 0.546 at 206. It clearly shows that Endosulfan MIP has sensitivity to template Endosulfan.

Conclusion

The detection and separation of hazardous contaminants i.e. Endosulfan is a challenge to ensure the safe water availability in irrigated zones of India. The molecularly imprinted polymer matrices specific to Endosulfan pesticides were synthesized and used for the fabrication of MIP composite membranes. These MIP composite membranes were further characterized by using SPE, UV-Visible, FTIR and SEM study. Moreover, well characterized MIP composite membrane was also used for specific separation and recognition of hazardous Endosulfan as targeted analyte. In present studies, molecularly imprinted membrane has been fabricated for the specific recognition of Endosulfan pesticide, widely used in controlling insects. The sensing of the herbicide was done by SPE measurements. This safe, cost effective and reproducible method could be a good replacement of costly techniques of recognition and separation.

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