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Development of Molecularly Imprinted Polymers for 2, 4-Dichlorophenoxyacetic Acid

Sabike Noobia¹, K.S.Lokesh², H.K.Manonamani³

Research scholar, Department of Environmental Engineering, S.J.C.E, Mysore, Karnataka, India¹

Professor, Department of Environmental Engineering, JSS S & T University, Mysore, Karnataka, India²

Principal Scientist, FPIC Department, CFTRI, Mysore, Karnataka, India³

ABSTRACT: Specific material based on molecularly imprinted polymers (MIPs) was made and applied in the removal of 2,4-D in simulated waste. Various parameters influencing MIP formation and binding of 2,4-D were optimized for the selective binding of the herbicide. Under the optimized conditions, the developed MIP exhibited good binding capacity of the herbicide up to 30µg. Developed MIP was very specific to 2,4-D. The developed method was successfully applied for the removal of 2,4-D from simulated waste water with 98% removal of added herbicide.

KEYWORDS: Molecular imprinted polymer, 2,4-D, herbicide, simulated waste water, binding capacity

I. INTRODUCTION

Phenoxyacetic acids are extensively applied in agriculture as herbicides or hormone mimics. Phenoxyacetic acid group includes 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid, (MCPA), 4-chlorophenoxyacetic acid (4-CPA), 2,4,5-trichlorophenoxyacetic acid etc. 2,4-D has been used as a pesticide since the 1946, in 1986 was registered with the EPA and re-reviewed in 2005 (2,4-D Chemical Fact Sheet, 2012). 2,4-D is applied to ward off broadleaf weeds in several places such as: vegetable crops and fruits, turf, lawns, forestry sites and aquatic sites (USEPA, Oct 31, 2016). Currently, more than 46 million pounds of 2,4-D have been used annually, with the international market place for this herbicide accounting for more than US\$300 million (EPA RED Facts, 2005 and PAN-UK, 1997). Due to their polarity, residues of these compounds are of concern in soil, ground water, and plants. In general population groups exposures to 2,4-D arises not only via trace residues present in the diet, through drinking water and direct contact with recently 2,4-D treated lawns (USEPA 2005). 2,4-D is quickly taken into the body by inhalation, skin contact ingestion and is swiftly expelled, largely in the urine in unchanged form (Kohli et al 1974, Sauerhoff et al., 1977). Current studies that are well-documented indicate a link between the agricultural application of the 2,4-dichlorophenoxyacetic acid (2,4-D) and the risk of non-Hodgkin's lymphoma (Irons 1985). 2,4-D has been grouped under possibly carcinogenic to humans (IARC, group 2B) (IARC 1998). Additionally, incessant usage of 2,4-D might bring in soil percolation and groundwater contamination (Harris et al 1992). Maximum contaminant level (MCL) of chlorophenoxy acid herbicides in drinking water has been in the range of 10-70 ng mL⁻¹ (USEPA 2009, Hamilton et al., 2003).

Methods classically employed to estimate these herbicides and their metabolites in environmental samples depend on extraction and subsequent analysis by gas chromatography (Garrison et al., 1996, Nilsson et al., 1998, Eitzer and Chevalier 1999). But, owing to the chemical and physical properties of these compounds, they make it to be difficult to isolate from complex sample matrices. Hence, substance that specifically distinguishes these compounds would be advantageous.

Recently, molecularly imprinted polymers (MIPs) have been used for very efficient separation of different analytes in composite environments (Jing et al 2010, Behbahani et al 2014, Hoshina et al 2009, Behbahani et al 2013, 2014, Omidi et al 2014), depending on their exceptional imprinting and recognition properties. MIPs establish great

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prospective for clean-up of sample in very complex matrix (Wang et al 2011). Molecular imprinted polymers (MIPs) are a group of compounds, where in the cross linking and functional monomers are co-polymerised in the existence of a target analyte (imprint molecule), which act as a molecular template. The functional monomer molecules are arranged and fixed around the template molecule during course of the entire mechanism of polymerization. In an imprinted polymer the chemically active components of the target molecules are placed in position by the highly cross linked polymeric networks. Consequent elimination of the imprint molecule indicates the binding sites which are complimentary in shape and size to the imprinted template, are comparable to that of antibodies (Vlatakis et al 1993, Ramstrom et al 1996) creating a molecular memory in the polymer.

In this manuscript we describe the synthesis and characterization of MIPs specific for 2,4D and establish the removal of 2,4-D using MIPs in environmental samples.

II. MATERIALS AND METHODOLOGY

All reagents used in this work were procured from standard chemical companies. 2,4-D was recrystallized from chloroform. Methacrylic acid (MAA), 4 vinyl pyridine (4 VP), Azobis-Isobutylnitrile (AIBN), ethylene glycoldimethacrylate (EDMA), 2,4- Dichlorophenoxyacetic acid (2,4- D) toluene, chloroform and hexanewere from Sigma-Aldrich chemical company. The solvents used were distilled, to remove stabilizer before use. EDMA was washed with equal amount of NaOH (10%) solution, separated with a separating funnel, followed by drying with Na_2SO_4 . Dimethyl form amide (DMF) Methanol, Ethanol, Acetonitrile were from Merck. All other chemicals used were of analytical grade, and solvents used were of HPLC grade.

Preparation of Non-imprinted Polymers and Imprinted Polymers

1 mM of 2,4-D dissolved in 2 ml of DMF (referred to as template) was dissolved in 8 ml of methanol and 2 ml of ultrapure water in a glass tube. To this was added 200mM of EDMA (Cross Linker), 4 mM of 4-VP (Functional polymer), 20 mM of AIBN (Polymerization initiator). They were taken in 30 ml of stoppered test tube, the contents were mixed thoroughly. Mixture was sonicated, purged with nitrogen, capped tightly and polymerization was initiated in a thermostat water bath at 65°C for 24 hrs. The polymer formed was filtered and dried under vacuum at 400 C. The polymers referred to as Molecularly Imprinted polymers were ground. Particles were acquired by sieving (25-45 μm sieve) and decanted in acetonitrile and methanol. Then ethanol wash was given to remove template to remove fine particles Control polymers (Non Imprinted polymers) were made employing the same procedure but without the presence of template. In order to take out the template embedded within the polymer matrix, 5g of polymer was extracted over 24 hours with ethanol (5 times) before oven-dried for 6 hours (70 °C).

Choice of the porogen

Different porogens toluene, methanol, acetonitrile and chloroform were used as porogens in independent polymer preparations and yields of polymers were observed. The best solvent yielding more polymer was chosen.

Scanning electron microscopy

All images were recorded on a Scanning Electron Microscope.

Fourier-transform infrared spectroscopy (FTIR)

FTIR was done to define the functional groups in MIP before and after the template removal stage and also in NIP employing the KBr pellet method.. Approximately 0.1 % MIP was mixed well into 200 mg fine KBr powder and then finely ground and put into a pellet-forming die. Care was taken to eliminate air and moisture from the KBr powder. The pellet was used to measure the spectrum. Only KBR was taken to nullify background.

HPLC analysis

Chromatographic analysis were carried out using a Waters HPLC system (WATERS No 1525 system USA) equipped with a UV WATERS 2489 detector and WATERS HPLC 1325 binary pump and and a 20 μL injector. The mobile phase was made of acetonitrile: water (75: 25) under Isocratic conditions. Analysis was done using C18 column with (4.6 mm id x 250 mm length, particle size of 5 μm) using flow rate of 1 mL/ min. Detection of compounds was at 228 nm. Capacity of template removed MIP in

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holding 2,4-D was studied by using different concentrations between 100ng to 3mg. Stock and working solution of the compound were diluted in Acetonitrile. The contact time required for binding 2,4-D was done by incubating 2,4-D with template removed MIP for different periods of time such as 1 hr, 2hr, 4hr and 6 hr. The specificity of the polymer prepared was investigated using 2,4-D, dieldrin, Aldrin, monochrotophos.

Molecular imprinted solid phase Clean-up of simulated 2,4-D contaminated water body.

Template removed MIP was loaded in to a small reactor column (plastic syringe cartridges) and washed with ethanol. 2,4-D spiked water was passed through the column @ 1 ml per min. with a contact time of 1h. The effluent was collected and analysed for residual 2,4-D by HPLC.

III. RESULTS AND DISCUSSIONS

A. Choice of the solvent

The identification of the best solvent system that acted as the most appropriate porogen is important to obtain best MIP. Of the different porogenic solvents used, toluene was observed to be the best in producing a good, suitably brittle polymer monolith for the purpose of grinding and packing into glass columns (Table. 1).The yield of polymer was good with toluene as solvent.

Table.1: Yield of polymer prepared from different solvents

Solvent used	Yield of polymer (per 10mL)
Toluene	1.47g
Methanol	0.87g
Acetonitrile	0.47g
Chloroform	0.52g

B. Analysis of polymer

Scanning electron microscopy - The SEM pictures indicated a rough surface of MIP showing irregular order. A rough and porous surface is anticipated to augment the extraction efficacy of MIP as observed by Song et al. (2009). The size of MIP was between 1 – 4 μ m.(fig.1)

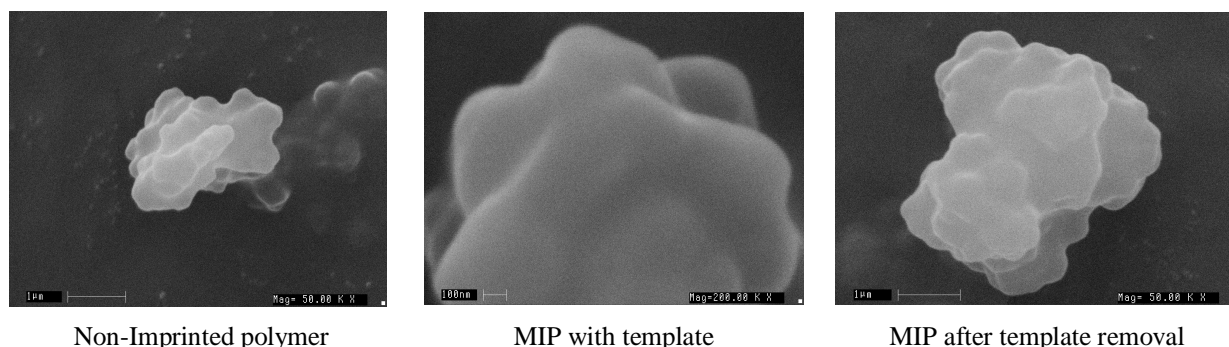


Fig. 1 Scanning electron microscopic picture of MIPs

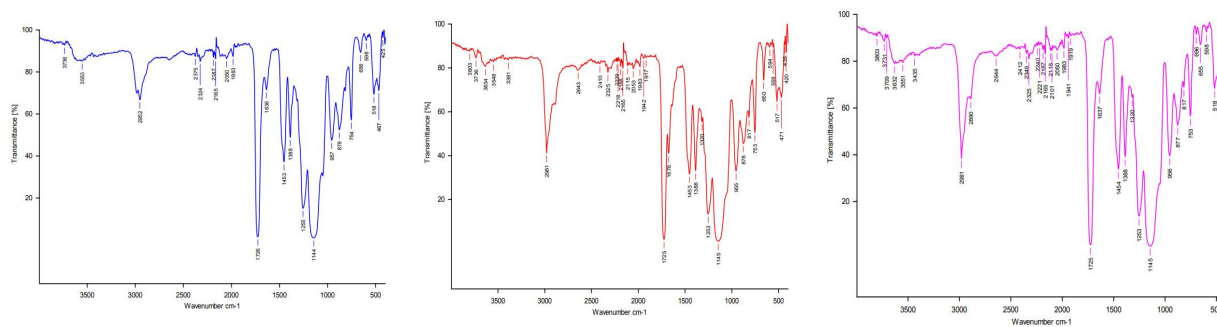
FTIR spectral analysis - The FT-IR spectra of MIP with its corresponding NIP were given in fig. 2. Peaks at 1650 cm^{-1} and 1560 cm^{-1} originated from OH and N-H bending vibrations. The broad peaks at ca. 3250 cm^{-1} is owing to the stretching vibrations of O-H, N-H and C-H. It can be perceived that the spectrum of NIP is nearly close to that of its MIP, demonstrating that the templates 2, 4-D has been absolutely removed (fig. 2)

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Non-Imprinted polymer

MIP with template

MIP after template removal

Fig. 2 FTIR spectra of MIP with and without template

C. Template removal

The template, 2,4-D could be removed completely from MIP using ethanol. The recovery from each wash is given in Fig 3. 2,4-D could be completely recovered from MIP by 5h.

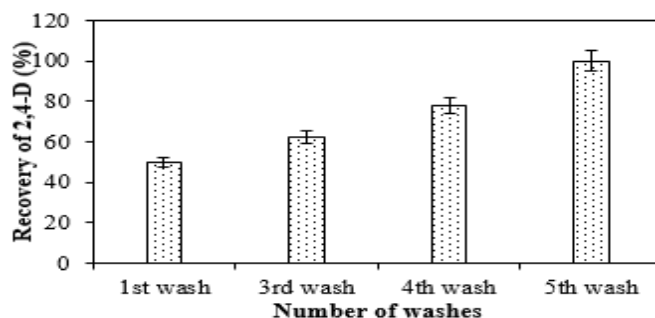


Fig 3 Template (2,4-D) removal from MIP

D. The quantity of 2, 4-D

The amount of 2, 4-D has some influence on the execution of MIPs (Fig 4). At low concentrations of 2, 4-D, the binding capacity of MIPs was 21.87%, 14.10 %, 10.375% and 4.91 % respectively. With increase in concentration of 2,4-D, the binding capacity of MIPs did not increase much after 30 µg. The binding was 98.24%. When the molar ratio value of the monomers to the template adsorption capacity of MIPs increased up to 30 µg and remained same thereafter.

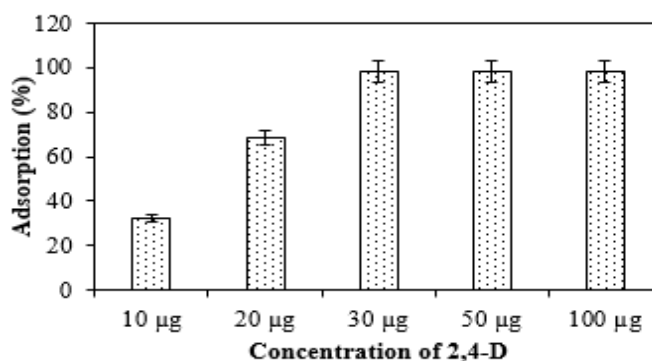


Fig 4 The 2, 4-D, the adsorption capacity of MIP template

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E. Binding capacity of MIP with time

Table 2 shows that the amount of 2, 4-D binding to MIP with respect to time. Template removed MIP was exposed to 30 µg 2,4-D and incubated at different intervals of time. The supernatant was analysed for residual 2,4-D. It was observed that, the binding increased with time. In the 1st hour, there was nearly 78% of binding of 2,4-D and by 2nd hour only 7% was bound. The binding capacity did not increase in an alarming rate with time.

Table 2. Binding of 2,4-D in relation to contact time

Contact Time	2,4-D (%) observed in supernatant (as analysed by HPLC)
1 hr	21.87 %
2 hr	14.10 %
4 hr	10.37 %
6hr	4.91 %

F. Specificity of MIP

2, 4-D, Aldrin, Dieldrin and Monochrotophos were chosen and the specificity of template removed MIP was explored in binding experiments. The result (figure 6) indicated that MIP prepared using for 2, 4-D as template was very specific to 2, 4-D. Binding of other pesticides used was very low. This indicated that the polymers possess good specificity (Fig. 6).

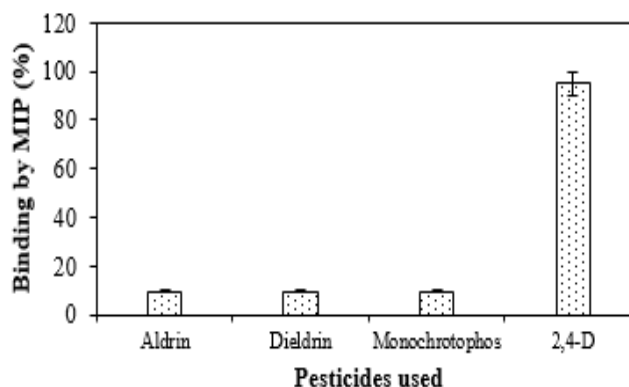


Fig 6 Specificity of MIP

G. Purification of simulated 2,4-D contaminated effluent

A column reactor of 54mm dia X 300 mm H was loaded with template removed MIP and 2,4-D spiked water was allowed to trickle slowly through MIP in the column. Contact time of 4h was maintained. Effluent was analysed for residual 2,4-D. It was observed that 98% of the added 2,4-D was removed in the spiked water.

Discussions

Molecular imprinted polymers (MIPs) are a group of compounds, where in the cross linking and functional monomers are copolymerised with the existence of a target analyte (imprint molecule), that behaves as a molecular template. A vital prerequisite for the preparation of these polymers is the genesis of a stable pre-polymerisation complex between the monomer and the template (Selligren 1997, Mayersand Mosbach 1997). During polymerization the functional monomer molecules are arranged and fixed around the template molecule. In a molecular imprinted polymer the chemically active groups of the template are kept in position by the highly cross linked polymeric networks. Consequent to the removal of the template binding sites that are similar in size and shape to the imprinted template are

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revealed which can be compared to that of antibodies (Vlatakis et al 1993, Ramstrom et al 1996) creating a molecular memory in the polymer. Through molecular imprinting, it is possible to develop tailor made polymers that are specific for different compounds. This technique permits the development of specific recognition and catalytic sites in macromolecules by employing templates (Haupt 2003, Alexander et al 2003, Haginaka 2004)

The MIPs contains two of the vital features of the biological receptors – the capacity to recognize and attach specific target molecules. Unlike biological molecules (antibodies), MIPs are stable to physical and chemical treatment. The MIPs have been employed in cumulative number of utilizations where molecular binding trials are of interest. The low cost and stability of MIPs frame them advantageous for number of applications besides in industrial scale application and production (Mosbach and Haupt 1998, Haupt 2003, Kandimalla and Ju 2004).

In our studies, MIP was prepared using EDMA (Cross Linker), 4-VP (Functional polymer), AIBN (Polymerization initiator). Toluene (porogen) and 2,4-D was used as template. The MIP was characterized by SEM and FTIR. Conditions were optimized for maximum removal of template 2,4-D. The formed polymer was selective for 2,4-D.

It is possible that the template molecule (2,4-D) might have been brought together with monomer (MAA) *via* hydrogen bonding with hydroxyl group in the course of the making of MIP before washing. But, after the analyte removal, a broad and strong stretching vibration absorbance peak of hydroxyl group from monomer is clearly observed because of the absence of any disruption from hydrogen bond. Similar results were observed by Brune et al.(1999). The reduction in intensity designates that the template has-been washed out by the washing step. The size of the MIP was between 1 – 4 μm as determined by SEM.

The analytical performance of the developed imprinted polymer was confirmed by determining of repeatability, sensitivity, limit of quantification (LOQ) using HPLC prior to simulated sample analysis. The spiked water sample could be made free of 2,4-D in a MIP based column reactor.

A diverse range of substances including sugars, polymers and amino acid derivatives have been imprinted. Metal ion imprinted polymeric beads are successfully applied in analytical applications (Saatcilar et al 2006). First reports of imprinting of bacterial cells on the polymer surface have been reported (Aherne et al 1996). Specific protocols have been proposed for the imprinting of proteins (Guo et al 2005).

IV. CONCLUSIONS

A molecularly imprinted polymer (MIP) was synthesized successfully with 2,4-dichlorophenoxyacetic acid as template. The synthesized MIP exhibited a very high degree of selectivity and adsorption ability toward the 2,4-dichlorophenoxyacetic acid template. The MIP was able to remove 2,4-dichlorophenoxyacetic acid completely from simulated effluent. These remarkably stable MIPs could be used effectually for the removal of specific compounds from contaminated/waste water. This is beneficial due to the high level of selectivity exhibited by the MIPs. Since the synthesis of MIPs is simple and straight forward, the synthesis of these polymers can be achieved in the most economical way and the application of such polymers can result in high suitability in removing pollutants from contaminated water sources. Another attractive feature is that, the technology does not need highly skilled personnel to synthesize these polymers as the procedure is simple and it doesn't involve complex steps.

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