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Design of 2,4-Dichlorophenoxyacetic Acid Imprinted Polymer with High Specificity and Selectivity

Kizhakekuthiathottil Mathew Annamma[1,2], Beena Mathew[1]

[1]School of Chemical Sciences, Mahatma Gandhi University, Kottayam, India; [2]Department of Chemistry, Devamatha College, Kottayam, India.
Email: beenamathewscs@hotmail.com

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ABSTRACT

A widely used herbicide 2,4-*dichlorophenoxyacetic acid* (2,4-*D*) *was imprinted on poly* (4-*vinylpyridine*) (4-*VP*) *using* (40%) *ethyleneglycol dimethacrylate* (*EGDMA*) *as crosslinking agent. The classical imprinting technology makes use of a high degree of crosslinking which does not allow the template molecules to move freely. So the binding sites, located in the central area of the three dimensional polymer matrix are hard to be accessed and the template molecules cannot be extracted totally. But here we propose a low crosslinked system with high specificity and selectivity. The imprinted and non-imprinted polymers were characterized by various spectroscopic techniques. The extent of binding was followed by batch equilibration method and compared with the respective non-imprinted polymer. Conditions for maximum specific rebinding were set by altering certain factors like template/monomer ratio, concentration of template solution, rebinding medium, mass of polymer and time of incubation. The selectivity of the imprinted polymer was investigated by comparing the binding with structural analogues of* 2,4-*D like*, *phenoxyacetic acid* (*POA*), 4-*chlorophenoxyacetic acid* (4-*CPOA*) *and* 2,4,5-*trichlorophenoxyacetic acid* (2,4,5-*T*). *The imprinted polymer exhibited high affinity towards the template molecule and was selectively rebound to the specific sites. The binding towards the structural analogues depends on the number of chlorine in the benzene ring.*

***Keywords*:** *Molecular Imprinting*, 2,4-*Dichlorophenoxyacetic Acid*, *Specificity*, *Selectivity*, *Separation Factor*

1. Introduction

The agricultural efficiency of the world depends on the effective control of a variety of diseases and pests, especially weeds. Herbicides are the chemicals that are widely used in agricultural field for controlling the growth of herbs, weeds and bushes. Thus the herbicides and their hydrolysis products are the most abundant pollutants found in the environment and in agricultural products. Concern about the health hazards connected with pesticide use has focused on 2,4-D and 2,4,5-T as suspected cancer-causing agents. Molecular imprinting is a technique for preparing synthetic polymers possessing recognition sites complementary to a template molecule [1-3]. MIPs can be used for the removal of pesticides, herbicides, endocrine disrupting compounds and heavy metals from waste and drinking water [4]. The high selectivity of MIPs for organic compounds, along with other useful properties opens up wide opportunities for the use of these materials in analytical chemistry [5-7]. Attempts were made previously to imprint herbicides in polymers and to use them in various applications [8-10]. But the traditional synthesis results in a highly crosslinked rigid structure, which does not allow the template molecules to move freely. So the binding sites, located in the central area of the three dimensional polymer matrix are hard to be accessed and the template molecules cannot be extracted totally. Hereafter, many imprinting strategies have been introduced to solve the problems faced by the traditional MIPs [11-15]. The reported techniques for herbicide detection using MIPs require high crosslinking, sophisticated instruments, skilled technician and longer analysis time. The most common methods of herbicide detection are gas chromatography with mass spectrometry detection (GC-MS) and HPLC. These methods require one or more preconcentration steps for trace level

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detection and quantification of herbicides due to insufficient sensitivity of these methods. In the present work high specific and selective recognition is given by low crosslinked polymers. Further the UV-visible spectrophotometric method is technically simple and can perform easily. The present paper deals with the synthesis of low crosslinked (40%) 2,4-dichlorophenoxyacetic acid (2,4-D) imprinted 4-vinylpyridine (4-VP) polymer, and the specificity and selectivity studies. The imprinted polymers were prepared by non-covalent method of imprinting polymerization in the presence of polar solvents like methanol and water. Non-imprinted polymers were also prepared without the template. Factors affecting specificity and selectivity were also discussed. The rebinding ability and selectivity performance of the polymer was followed by UV-vis. spectrophotometric method. The nature of binding sites was followed by Scatchard analysis.

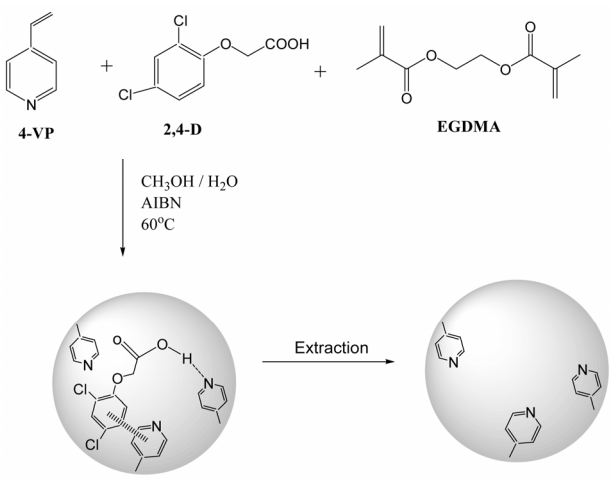

Figure 1. Synthesis of 2,4-D imprinted and non-imprinted polymers.

2. Experimental

2.1. Materials and Methods

4-chlorophenoxyacetic acid (4-CPOA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 4-vinylpyridine (4-VP) were obtained from Sigma-Aldrich (USA). Ethyleneglycol dimethacrylate (EGDMA) was from Merck (Germany). Phenoxyacetic acid (POA) and 2,2'-azobisisobutyronitrile (AIBN) were purchased from SRL (Mumbai). Methanol, acetonitrile and chloroform were obtained from Merck (India). 4-vinylpyridine was distilled under reduced pressure prior to use. Doubly distilled water was used throughout. The imprinted polymers were characterized by FT-IR (8400 S, Shimadzu), UV-vis. (Shimadzu 2450), and ^{1}H (BRUKER AMX-400) and ^{13}C CP-MAS-NMR (BRUKER DSX-300) spectrometer.

2.2. Synthesis of EGDMA-Crosslinked 2,4-D Imprinted and Non-Imprinted Polymers in Methanol/Water

EGDMA-crosslinked 2,4-D imprinted poly (4-vinylpyridine) with 1:1, 1:2 and 1:4 ratios of 2,4-D and 4-vinylpyridine were prepared (**Table 1**). The template 2,4-D (1 mmol), functional monomer (4-VP), (4 mmol), required amount of the crosslinking agent ethyleneglycol dimethacrylate and the initiator AIBN (0.32 mmol) were weighed into glass tubes and dissolved in10 ml solvent (methanol/water, 4:1, v/v). The resulting mixture was purged with nitrogen for 15 min. The tubes were then sealed and inserted in a water bath at 60˚C for 4 h, followed by 2 h at 70˚C (**Figure 1**). The resultant hard bulk polymers were ground in a mechanical mortar and wet-sieved in acetone through a 25 μm sieve. Non-imprinted polymers were also prepared using the same recipe but without the addition of the template.

2.3. Extraction of Imprinted Template: General Procedure

The fine polymer particles were carefully washed by incubation in methanol/acetic acid (7:3, v/v, 2x), acetonitrile/acetic acid (9:1, v/v, 2x), acetonitrile (1x), methanol (2x) for 2 h and Soxhlet extracted with methanol until no template could be detected under UV (λ_{max} = 284 nm) in the wash solution. The particles were then suspended in acetone and allowed to settle for 4 h. The solvent was removed by centrifugation and the particles were dried to constant weight in vacuum.

2.4. Swelling Studies

Fixed amount of the polymer particles were packed into sintered crucibles, which were filled with different solvents. After 24 h of equilibration, the excess solvent was removed from the polymer by applying reduced pressure for 1 min. and the weight of the swollen particles (W_w) was measured. Subsequently the particles were freeze-dried for 24 h and were weighed again to obtain the dry weight (W_d). The swelling ratio (SR) of the polymer in each solvent was calculated from the following equation: $SR = (W_w - W_d)/W_d$.

2.5. Rebinding of Template: General Procedure

The template-desorbed polymers were treated with solutions of the desorbed template and the extent of rebinding was followed by UV measurements at 284 nm. The polymer particles were put into sample tubes, and the template solutions of known concentrations were introduced and equilibrated for a period of time. After this incubation, the

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Table 1. Synthesis of 40% EGDMA-crosslinked MIPs and NIPs with varying 2,4-D/4 VP ratio.

Ratio of 2,4-D:4-VP	2,4-D (1 mmol)	4-VP	EGDMA	Yield (%)	
				MIP	NIP
1:1	0.221 g	0.11 ml (1 mmol)	0.14 ml (0.75 mmol)	35	45
1:2	0.221 g	0.22 ml (2 mmol)	0.28 ml (1.5 mmol)	65	68
1:4	0.221 g	0.44 ml (4 mmol)	0.56 ml (3 mmol)	72	75

polymer particles were filtered off, and the remaining concentrations of the template were determined spectrophotometrically. The amount of template bound to the polymer S_b (mM/g polymer) was calculated by the equation, $S_b = (C_o - C_t)/W$. Where C_o and C_t are the 2,4-D molar concentration in the solution at initial and after interval time 't', respectively. 'W' is the weight of the dry polymer used.

2.6. Optimization of the Conditions of 2,4-D Rebinding

The optimum conditions for maximum 2,4-D rebinding were set by changing certain factors like templatemonomer ratio, rebinding solvent, time, concentration of 2,4-D solution and mass of polymer.

2.7. Factors Affecting Rebinding

2.7.1. Concentration of 2,4-D Solution

The influence of concentration of template solution on rebinding was evaluated by batch binding experiments. From the difference in concentration of template solution before and after incubation, the amount of template bound by the polymer was determined.

2.7.2. Rebinding Solvent

The template solutions were prepared in different solvents and the rebinding in each solvent was determined spectrophotometrically.

2.7.3. Time of Rebinding

The time required for the saturation of binding sites was determined by measuring the absorbance at regular intervals of time till saturation was attained at 30˚C.

2.7.4. Mass of Polymer

Varying amounts of polymers were introduced into equal volume of template solution for a fixed time and rebinding was followed.

2.8. Selectivity Studies: General Procedure

The selectivity of imprinted polymers were evaluated by incubating template desorbed imprinted polymer in solutions of structural analogues for definite time and the rebinding was followed spectrophotometrically. Separation and selectivity factors were also determined.

3. Results and Discussion

3.1. Synthesis of EGDMA-Crosslinked 2,4-D Imprinted and Non-Imprinted Polymers with Varying Composition of Functional Monomers and Template

EGDMA-crosslinked 2,4-D imprinted and non-imprinted polymers with varying template-monomer ratio were synthesized by free radical polymerization (**Figure 1**).

3.2. Characterization of 2,4-D Imprinted and Non-Imprinted Polymers

3.2.1. FT-IR

The incorporation of EGDMA in both imprinted and non-imprinted polymers was supported by the IR spectra. The bands at 1396 and 1458 cm^{-1} correspond to C–H bend of CH_3 and CH_2 respectively. The C=O stretch of the EGDMA crosslinking was observed at 1647 cm^{-1}. The IR spectrum of 4-vinylpyridine showed an intense band at 1596 cm^{-1} due to the C=N stretching. The spectral characteristics of 4-vinylpyridine changed upon addition of 2,4-D. The 2,4-D/4-vinylpyridine complex revealed a new band at 1546 cm^{-1} within the C=N mode. The peaks at 1596 and 1546 cm^{-1} were assigned to monomeric 4-vinylpyridine and H-bonded 4-vinylpyridine respectively. The C=O and O–H stretching of the carboxyl group of 2,4-D at 1735 and 3263 cm^{-1} were also shifted and broadened on complexation with 4-vinylpyridine. These changes strongly suggest the formation of intermolecular H-bonding between the carboxylic group of 2,4-D and the nitrogen of 4-vinylpyridine.

3.2.2. ^{1}H NMR

The pre-polymerisation complex formation during the preparation of molecular imprinted polymers can be investigated by ^{1}H NMR. Due to the low solubility of 2,4-D in chloroform, the ^{1}H NMR titration studies of the 2,4-D/4-VP system were performed in CD_3OD. The study was conducted by adding increasing amounts of 4-VP into 2,4-D solution and the spectra were recorded after each addition. Because of the rapid exchange between labile proton of the template and solvent deute-

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rium atom, the signal due to acidic proton of the carboxylic acid is not seen in the spectrum. Therefore the change in chemical shifts of the aromatic and CH_2 protons of 2,4-D as well as aromatic protons of 4-VP were investigated (**Figure 2**). As a result of ion-pair formation between basic nitrogen and acidic proton, the chemical environment of H2 and H6 (8.8 ppm), H3 and H5 (7.6 ppm) of 4-VP changes and the peaks corresponding to them were shifted upfield. An upfield shifting of the peaks corresponding to the aromatic protons of 2,4-D (H3 - 7.7, H5 - 7.5, H6 - 7.2 ppm) was also observed and attributed to the participation in π-π stacking interactions. The signals due to CH_2 protons of 2,4-D (4.9 ppm) was broadened along with upfield shifting and gradually the peaks disappeared due to complex formation with 4-VP. The results confirmed the existence of a 1:3 (2,4-D/4-VP) complex prior to polymerisation.

3.2.3. ^{13}C CP-MAS-NMR

The incorporation of crosslinking agent and functional monomer 4-VP in the polymer backbone of imprinted polymer was confirmed by ^{13}C NMR spectrum (**Figure 3**). The ester carbonyl of the EGDMA crosslinking was characterised by the strong peak at 178.8 ppm. The small peak at 65.0 and the one at 16.7 ppm correspond to the OCH_2 and CH_3 carbons of EGDMA. The intense peak at

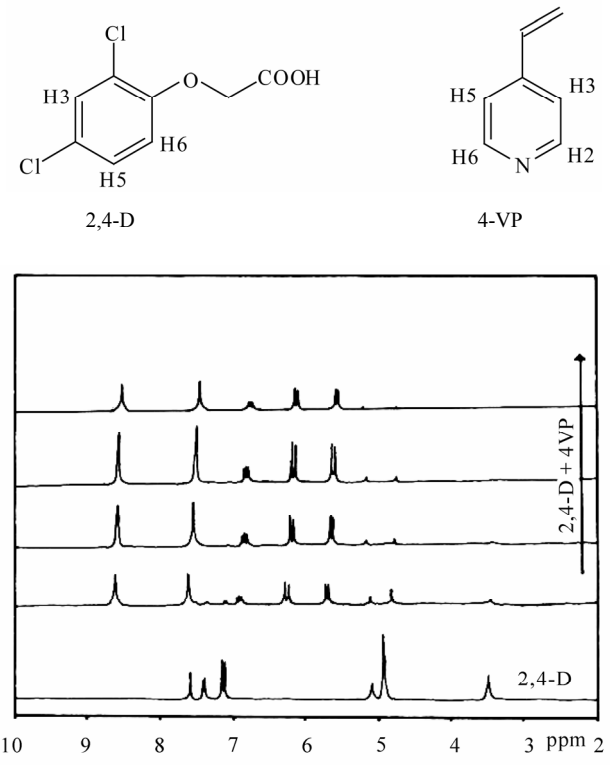

Figure 2. ^{1}H NMR spectra of 2,4-D with increasing concentration of 4-VP.

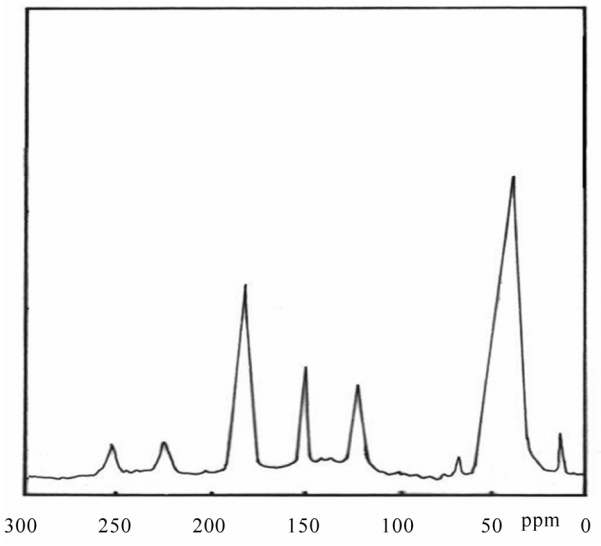

Figure 3. ^{13}C NMR spectrum of EGDMA-crosslinked imprinted polymer.

42.8 ppm corresponds to CH_2 carbon of the polymer backbone.

3.3. Determination of Swelling Ratio (SR)

In order to determine the swelling ratios of 2,4-D imprinted and non-imprinted polymers, the polymers were immersed in four different solvents such as acetonitrile, methanol, methanol/water and water. The dispersed polymeric particles were recovered by vacuum filtration and the swollen weight (W_w) of the particles was measured. Subsequently, the particles were dried and weighed again to obtain the dry weight (W_d). The swelling ratio [16] of the polymer was calculated as given below and summarised in the experimental part.

The polymer exhibited higher swelling in methanol-water, the porogen. Here the swelling ratios of non-imprinted polymers are higher than that of the imprinted polymers. This is due to the reluctance of the crosslinked polymer matrix to expand from the designed geometry around the template molecule. Whereas there is no such complementary cavity and framework in the non-imprinted polymer and so it could undergo extensive swelling. The swelling ratio of both imprinted and non-imprinted polymer in water is very high compared to other solvents. This anomalous behaviour in water is due to the possibility of extensive H-bonding with the nitrogen in 4-vinylpyridine.

3.4. 2,4-D Rebinding Studies

The methodology of molecular imprinting induces the formation of recognition sites with predetermined selectivity into a synthetic matrix. After removal of the tem-

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Table 2. Swelling ratios of 2,4-D imprinted and non-imprinted polymers.

Solvent	Swelling Ratio	
	MIP	NIP
CH_3CN	0.15	0.28
CH_3OH	1.72	0.97
CH_3OH/H_2O	2.25	4.63
H_2O	7.09	4.30

plate, complementary binding sites are revealed and a molecular memory is introduced into the polymer, capable of rebinding the template with high specificity and selectivity [3,4,17]. According to this principle of molecular imprinting, the 2,4-D imprinted polymers must specifically rebind the print molecule than the non-imprinted polymers. The imprinted and non-imprinted polymers were investigated for their rebinding capacity by incubating a fixed amount of each polymer in template solution of known concentration and equilibrated for a period of time 't'. The concentration of template solution before and after incubation was determined spectrophotometrically. The amount of template bound to the polymer $[S]_b$ (mM/g) was calculated as discussed earlier.

Specificity in 2, 4-D Rebinding by Imprinted Polymers

The 2,4-D imprinted polymers showed high specificity towards the template than the non-imprinted polymer. The investigation was done by comparing the specific binding of imprinted polymer (MIP) with a non-imprinted polymer (NIP) of same crosslinking (40%). Equal amounts of MIP and NIP were introduced into 0.9 mM solution of 2, 4-D for a definite time and the amount of 2,4-D bound was determined. From the difference in binding by the MIP and NIP, it is evident that the imprinted polymer has a significant affinity towards 2,4-D than the non-imprinted polymer. Further, the specificity shown by the imprinted polymer supports the memory of the polymer towards the print molecule (**Figure 4**).

3.5. Template-Monomer Ratio on 2,4-D Rebinding

It has been found that the molar relationship between the template and functional monomer influences the number and quality of MIP recognition sites. Hence 2,4-D imprinted and non-imprinted polymers were prepared in 1:2 and 1:4 template-monomer ratio. To investigate the influence of this ratio on rebinding, imprinted and non-imprinted polymers were incubated in equal volume of 2,4-D solutions of definite concentration for a fixed time. After filtration the 2,4-D binding was followed spectrophotometrically. The results are summarised in **Figure 5**.

The 1:4 system offered high specific binding compared to the 1:2 system. In order to accomplish highly efficient imprinting, the template-monomer adduct must exist in large excess with respect to free template and functional monomer. Otherwise non-selective polymerisation would occur concurrently and diminish the efficiency of molecular imprinting. Thus the 1:4 system is more specific than the 1:2 system. Hence for further studies the imprinted and non-imprinted polymers in the 1:4 template-monomer ratios were used.

3.6. Optimisation of the Conditions of 2,4-D Rebinding

There is the need to optimise the conditions for maximum template rebinding by the imprinted polymers. The total

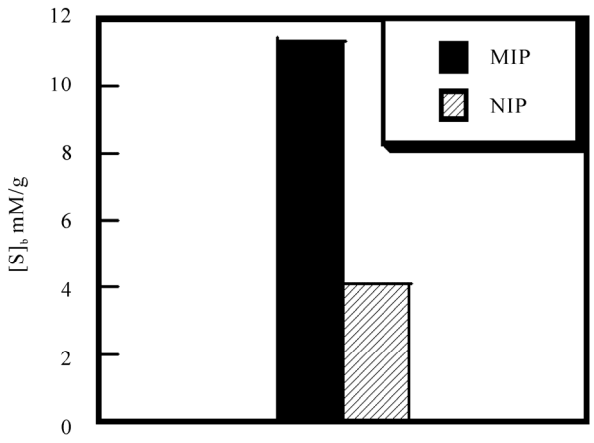

Figure 4. Specificity in 2,4-D rebinding by 40% EGDMA-crosslinked MIP and NIP.

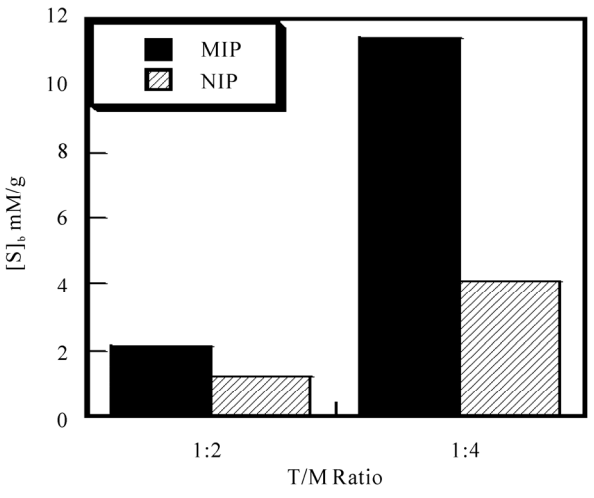

Figure 5. Effect of template-monomer ratio on 2,4-D rebinding by MIP and NIP.

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rebinding to a MIP is the sum of specific binding to the imprinted binding sites and non-specific binding to the polymer. The non-specific binding to the polymer is measured as the binding, under identical conditions to a non-imprinted polymer. If this non-specific binding to the polymer is dominant, specific binding to the imprinted sites will be obscured. Thus the ratio of the specific to non-specific binding should be optimised to observe the binding at the imprinted sites.

3.6.1. Effect of Concentration of 2,4-D Solution

To evaluate the variation of rebinding with concentration of 2,4-D solution, batch methods were carried out using stock solutions of 2,4-D with concentrations ranging from 0.3 to 1.5 mM. Definite amount of MIPs were incubated in these solutions for a fixed interval of time and the amount of 2,4-D bound at the saturation point $[S]_{b,}$ was calculated as described earlier. It was plotted against initial concentration C_o and is given in **Figure 6**.

The extent of 2,4-D binding increased with concentration up to 0.9 mM and then remained constant. Thus the binding attained a saturation point at 0.9 mM. The individual cavities in the polymer vary in their affinity and selectivity towards the template [18]. The binding of the template molecules to high affinity sites concentrated inside the polymeric domains led to the increase in specific binding, which later resulted in shape change of the polymer network. Binding sites on the surface of the polymer bound the template without causing much reorganisation of the polymer structure.

3.6.2. Determination of Binding Parameters and Guest Binding Constants

In the molecular imprinting technique, Scatchard model was often used to evaluate the binding characteristics of the imprinted polymers [19,20]. The binding data obtained were treated with Scatchard equation $[S]_b/[S]_f = (S_{max} - [S]_b)/K_D$, where K_D is an equilibrium dissociation constant of the binding site, S_{max} an apparent maximum number of binding sites and $[S]_b$ is the amount of 2,4-D bound to MIP. $[S]_f$ is the fraction of 2,4-D left in solution. The plot of $[S]_b/[S]_f$ against $[S]_b$ is shown in **Figure 7**. The Scatchard plot is not linear and composed of two straight lines indicating that the binding sites in the polymer matrix are heterogeneous in respect to the affinity for 2,4-D. Thus two classes of binding sites are mainly produced in the studied template concentration. From the slope and intercept of the straight lines obtained, the values of K_D and S_{max} are determined, where K_{D1} (50 M/L) and K_{D2} (201 M/L) are the dissociation constants, and S_{max1} (12.5 mM/g) and S_{max2} (19 mM/g) are the apparent maximum number of binding sites at high and low affinity sites. Since the dissociation constant at high affinity site (K_{D1}) is lower than that at low affinity site, guest binding at high affinity site is stronger.

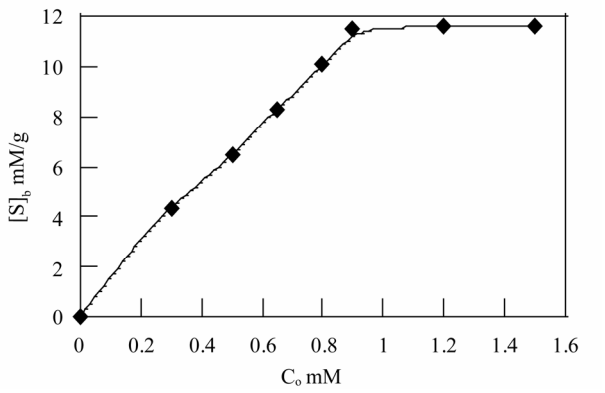

Figure 6. Binding isotherm for the binding of 2,4-D by MIP.

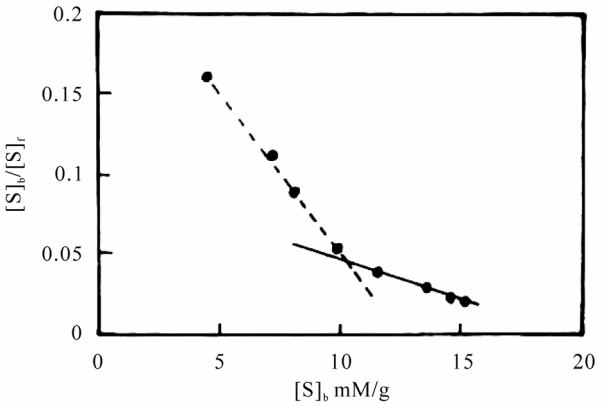

Figure 7. Scatchard plot to determine the nature of binding sites.

3.6.3. Effect of Rebinding Medium

Molecular imprinted polymers utilise a solvent in polymerisation to provide porosity within the network polymer. Binding properties of molecular imprinted polymers are influenced by the type of solvent, or porogen, used in the polymer synthesis and the solvent used in the particular application of the MIP [21]. A number of reports in the literature indicated that the best recognition of imprinted polymer occurs when the rebinding medium and the porogen used are the same [22,23]. The ability of the rebinding medium to recreate the binding site dimensions formed during the polymerisation determines the binding performance of the imprinted polymer in that medium. To determine the effect of binding medium on specific binding properties, imprinted and non-imprinted polymers were equilibrated with solutions of 2,4-D in acetonitrile, methanol, methanol/water, and water. The EGDMA cross-linked polymer offered maximum specificity in methanol/water, the porogen. The high binding observed in water is due to a partitioning effect of the lipophilic 2,4-D into the hydrophobic polymer matrix. The results are given

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in **Figure 8**.

3.6.4. Effect of Time

To optimise the time taken for maximum binding of 2,4-D by MIP and NIP, definite amounts of the polymer was equilibrated with 2,4-D solution of known concentration and the binding was followed spectrophotometrically at definite intervals of time (**Figure 9**). The imprinted polymers took more time for saturation of binding sites compared to the non-imprinted polymers. The imprinted polymers possessing shape complementary binding sites took more time to attain saturation, since the template molecules have to penetrate through the highly cross-linked polymer network to access the imprinted cavities for rebinding. In non-imprinted system, there is no such specific arrangement of the binding sites, and non-specific interactions occur only at the surface of the polymer, the binding of template is rather fast and random. The MIP attained saturation within 4-6 h. So it is not necessary to equilibrate the polymers for 24 h for getting the imprinted sites saturated.

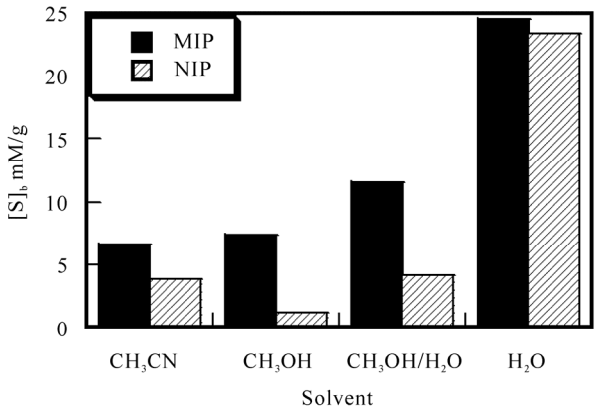

Figure 8. Effect of solvent on 2,4-D rebinding by EGDMA-crosslinked MIP and NIP.

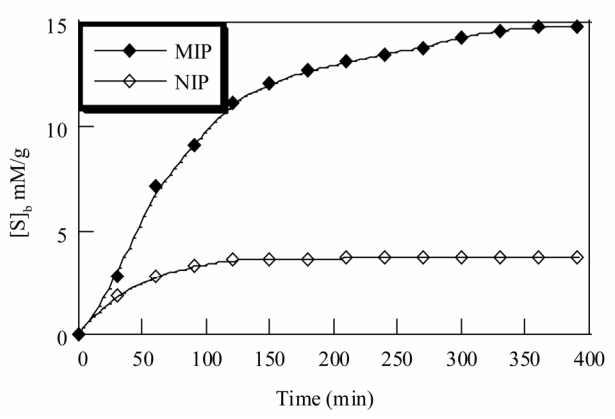

Figure 9. Time dependence on 2,4-D rebinding by MIP and NIP.

3.6.5. Amount of Polymer

Increasing the amount of polymer is expected to provide more binding sites for a substrate and as a result the amount of the substrate bound is also expected to increase. The effect of the mass of polymer on rebinding was observed by incubating varying amounts of MIP and NIP in fixed volume of 0.9 mM 2,4-D solution. As the amount of polymer is increased, the amount of bound substrate also increased (**Figure 10**). When higher amount of polymer was used the substrate bound to the higher affinity binding sites in preference to the low affinity binding sites. The increase in the number of binding sites with increase in mass of the polymer leads to the increase in specificity. But the substrate bound per gram decreased. This is due to the possible changes in binding parameters with the different amounts of the polymers used in the batch rebinding studies. The non-imprinted polymers bound signifycantly less template than the imprinted polymers.

3.7. Selectivity of 2,4-D Imprinted Polymers

3.7.1. Template Selectivity

The selectivity of MIP is based on the configuration of the binding sites and the orientation of the functional groups in the sites. Three structural analogues of 2,4-D, viz. phenoxyacetic acid (POA), 4-chlorophenoxyacetic acid (4-CPOA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were used to evaluate the selectivity of the imprinted polymer. The molecular structures of these compounds are given in **Figure 11**.

The molecules related to 2,4-D in the acidic part and different in the aromatic part were recognised by the imprinted polymers, but to a lesser extent than the template (**Figure 12**). Since the molecules exactly matching the template molecule in the carboxylic substructure can be recognised by the imprinted polymer, it is clear that this interaction is strongly governed by the ion pair formation. It is also observed that molecules resembling 2,4-D in the

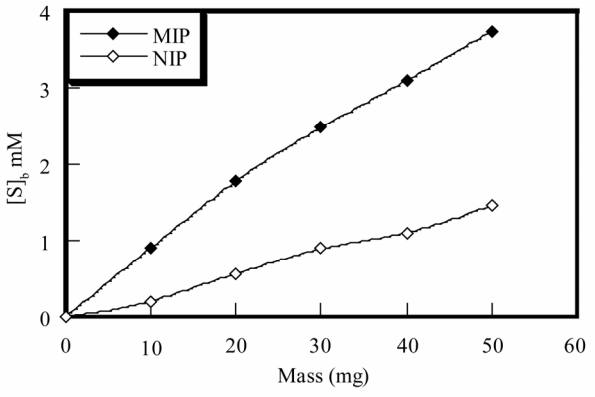

Figure 10. Effect of the mass of polymer on 2,4-D rebinding by MIP and NIP.

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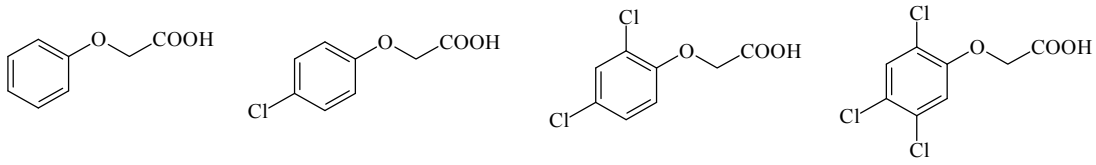

Figure 11. Molecular structures of 2,4-D and its structural analogues.

Table 3. Separation factor of MIP and NIP to 2,4-D.

EGDMA (%)	MIP			NIP			Separation factor $\alpha_{2,4\text{-D}} = K_{MIP}/K_{NIP}$
	2,4-D bound $\times 10^{-4}$ M	2,4-D free $\times 10^{-4}$ M	K_{MIP}	2,4-D bound $\times 10^{-4}$ M	2,4-D free $\times 10^{-4}$ M	K_{NIP}	
40	2.87	6.12	0.468	1.03	7.97	0.128	3.65

Table 4. Selectivity factors of EGDMA-crosslinked MIP.

$\alpha_{2,4\text{-D}}$	α_{POA}	α_{CPOA}	$\alpha_{2,4.5\text{-T}}$	$\alpha_{2,4\text{-D}}/\alpha_{POA}$	$\alpha_{2,4\text{-D}}/\alpha_{MCPOA}$	$\alpha_{2,4\text{-D}}/\alpha_{2,4.5\text{-T}}$
3.65	2.00	3.12	3.40	1.82	1.20	1.15

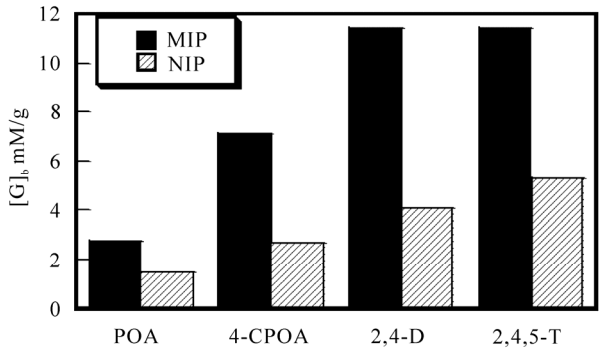

Figure 12. Selectivity between MIP and NIP.

carboxylic substructure, but differ in the substitution on the aromatic ring are recognised proportionally through the similarity with 2,4-D. 4-CPOA with only one chlorine atom on the ring is more recognised than POA with no chlorine atom on the ring. The polymers bound 2,4,5-T nearly as well as the original template 2,4-D. This may be due to the accommodation of 2,4,5-T molecule in the flexible EGDMA-crosslinked networks. The increase in binding with increasing number of chlorine atoms on the aromatic ring is reported [24]. The existence of weak secondary interactions between the ring chlorine and hydrogens of 2,4,5-T with 4-VP is suggested by modelling [25].

3.7.2. Separation and Selectivity Factors of 2,4-D Imprinted Polymers

A complete secondary screen for binding and selectivity was performed and binding of the template to the imprinted and non-imprinted polymers was compared in terms of separation factor [26]. The high separation factor ($\alpha_{2,4\text{-D}}$) of 40% EGDMA-crosslinked polymer proved the high efficiency of the imprinted system (**Table 3**).

$$\text{Separation factor } (\alpha_{2,4\text{-D}}) = K_{MIP}/K_{NIP}$$

$$\text{Selectivity factor [27]} = \alpha_{2,4\text{-D}}/\alpha_{\text{analogue}}$$

The imprinted system has a selectivity factor greater than unity in all cases and so this system can be used for the selective recognition of 2,4-D (**Table 4**).

4. Conclusions

The technique of molecular imprinting leads to highly stable synthetic polymers possessing selective molecular recognition properties. In the present work priority is given to the design of imprinted polymers of the herbicide 2,4-D with maximum specificity and selectivity. Among the polymers prepared in 1:1, 1:2 and 1:4 template-monomer ratios, the 1:4 system exhibited better specificity and selectivity. The present results proved that imprinted polymers can very well retain the imprinted site at moderate extent of crosslinking and can recognise the template with significant specificity. A crosslinking of 40% is sufficient for 2,4-D imprinted polymers in methanol/water. The amount of template binding increased regularly with increasing concentration of the template solution and attained saturation. From the Scatchard plots the binding sites are found to be heterogeneous in nature with respect to the affinity towards the template in the studied concentration range. The MIPs took more time for saturation of binding sites than NIPs

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and overnight equilibration is not necessary for getting the sites saturated. Selectivity studies with structural analogues revealed that in addition to complementarity in size and shape, optimal spatial fit also had a role in binding interactions. The present work in molecular imprinting provided an alternative method against the conventional analytical methods. In the present work high specific and selective recognition is given by low cross-linked polymers. Further the UV-visible spectrophotometric method is technically simple and can perform easily. The attempts made in this work led to the successful design of 2,4-D imprinted polymers with high specificity and selectivity.

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