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Fabrication and Optimization of Chiral Receptor Sites for D-MA Using Molecular Imprinting Technology

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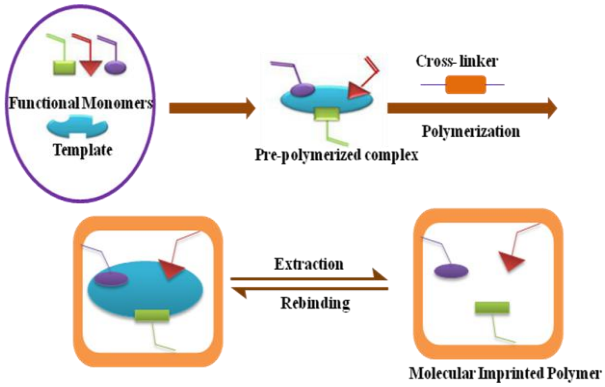
ABSTRACT :
A novel chiral receptor sites for D-mandelic acid (D-MA) on a polymer matrix is fabricated using molecular imprinting technique. The imprinted and non-imprinted polymers of D-MA, based on 4-vinylpyridine (4-VP) as the functional monomer and ethylene glycol dimethacrylate (EGDMA) and divinyl benzene (DVB)as cross linking agent (40-80 mol%), were synthesized. All polymers were prepared by heat-induced polymerization using AIBN as an initiator. In this work, a comparative study of EGDMA and DVB were done to know which one give better selectivity towards template molecule. Fourier transform infrared spectroscopy (FT-IR), ^{1}H-NMR titration study and UV-Vis. spectroscopic analysis were performed to characterize the complexes formed between functional monomers and template molecule in the solution prior to polymerization. The binding specificities of the obtained polymers were followed and optimized the conditions of maximum binding. The binding characteristics of the imprinted polymers were explored in various solvents using equilibrium binding experiments. Selectivity of the imprinted polymers was investigated using its enantiomer L-MA. To get an insight into the role of MIP, non-imprinted polymers without template were prepared and examined. Imprinted polymers showed specific and selective binding of D-MA which varied with the degree of EGDMA cross linking.

KEYWORDS: Cross linking, molecular imprinting, templates- monomer interaction, selectivity, swelling.

I. INTRODUCTION:

Molecular recognition involves both the selection and recognition of a ligand by a given receptor structure and is the basis for most biological processes such as legend-receptor binding, enzyme-substrate interaction, translation and transcription of the genetic code. Molecular imprinting is a method of inducing sites of specific molecular recognition capabilities for a particular target molecule in synthetic polymers similar to that of natural receptors [1-4]. A schematic representation of molecular imprinting process is given in Scheme 1. This technique allows the formation of tailor made recognition sites exhibiting enantioselectivity, substrate selectivity and catalytic activity. These molecularly imprinted polymers (MIPs) have been widely employed for diverse applications (e.g., in chromatographic separation, drug screening, chemo sensors, catalysis, immunoassays etc.) owing to their specificity towards the target molecules and high stability against physicochemical perturbations [5-7].



Scheme 1: Schematic representation of molecular imprinting process

Molecular imprinting is achieved by the interaction, either noncovalent or covalent, between complementary groups in the target molecule (template) and functional monomer units through polymerization or poly condensation. Among these approaches, MIPs synthesized by non-covalent interaction can be considered the best one due to easy removal of template, applicable for a variety of molecules, economical and easy method.

Separation of chiral molecule from its mixture is a necessary step involved in pharmacology and biology [8-10]. Hence it is important to fabricate practical and rapid available methods for the chiral recognition and separation of enantiomers. The role of MIPs in chiral separation of racemic mixtures have got much more relevance in separation science. One of the α-hydroxy carboxylic acids, mandelic acid (MA) is a significant chiral analogue of amino acids in the pharmaceutical synthetic industry [11-17]. Here a simple and efficient receptor system is designed for enantios elective recognization of D- mandelic acid (D-MA) using molecular imprinting technique. For the synthesis of MIPs, compounds with functional groups reciprocal to those of the template are selected as functional monomer and are used to form a scaffold around the chosen template. Here 4-vinylpyridine (4-VP) was selected as the functional monomer because of its basic functionality and commercial availability which could preassociate with D-mandelic acid through several non-covalent processes including electrostatic attraction, hydrogen bonding, and π-π stacking interaction. The complex formed between 4-VP and D-MA is preserved within a matrix to form an imprint that is chemically and sterically complementary to the template. To know about the better cross linking agent, a comparative study had done in which MIPs were prepared using both ethylene glycol dimethacrylate (EGDMA) and divinylbenzene (DVB). To get an insight into the role of MIP in selective and specific binding towards target D-MA, non imrinted polymer (NIP) were also prepared and analyzed.

II. EXPERIMENTAL:

2.1 MATERIALS:

L-mandelic acid (L-MA) and D-mandelic acid (D-MA) were from SRL, Mumbai. Ethylene glycol dimethacrylate (EGDMA) and divinylbenzene (DVB) were from Sigma-Aldrich, USA. 4-Vinylpyridine (4-VP) was from Alfa Acer and 2,2'-azo-bis-isobutyronitrile (AIBN) was from Merck, Germany. Chloroform and methanol were from Merck, India. Other chemicals used were analytical grade and obtained from commercial sources. 4-VP was stabilized with hydroquinone and was destabilized by distillation under reduced pressure.

2.2 SYNTHESIS OF MIP AND NIP:

D-mandelic acid imprinted polymers were synthesized using 4-vinylpyridine (4-VP) as functional monomer and EGDMA/DVB as cross linking agent having T/FM ratio 1:2, 1:4, and 1:8 keeping the extent of cross linking constant (40%). Porogen generally acts dual roles as a solvent and a pore forming agent in the polymerization process. To maximize the likelihood of complex formation between

functional monomer and template the general procedure is to choose the least polar solvent in which the template is dissolved completely. Here the imprinted polymer was synthesized in acetonitrile, which act dual role as a solvent and a pore forming agent in the polymerization process. Among the above mentioned polymers the one having the 1:4 T/FM ratios possesses the highest specific binding. Because of the very low specificity of the DVB-cross linked system the rest of the binding studies are mainly conducted using the EGDMA-cross linked system. For a further understanding of the effect of the crosslink density on the binding properties, polymers having different percentage of EGDMA cross linking (40-80 mmol%) keeping the T/FM ratio 1:4 were prepared. To evaluate the molecular recognition properties of the product polymers non-imprinted polymers (NIPs) were fabricated using the same polymerization protocol and post polymerization treatment without the template molecule

2.3 CHARACTERIZATION OF D-MA IMPRINTED AND NON-IMPRINTED POLYMERS:

Fourier Transform Infrared spectrometer 8400s (DIN 206-72400), Shimadzu, Japan was used to record FT-IR spectra of samples. The binding studies were carried out on a Shimadzu-UV-vis Spectrophotometer model 2450. ^{1}H-NMR spectra were, recorded on Bruker BZH Spectrometer operating at 500MHz at 298K, used to analyse the interaction of pre-polymerization complex. For the morphological studies of the polymer sample Scanning Electron Microscope, JEOL-JSM-6390 A microscope, was used.

2.4 Characterization Of Complexes Formed Between Template Molecule (D-Ma) And Functional Monomer (4-Vp) Prior To Polymerization:

The principle of molecular imprinting lies in the preservation of the host guest structure in the polymerization solution into a polymer matrix. So the formation of the prepolymerization complex prior to polymerization is crucial, since the structure of the resulting assemblies defines the subsequently formed binding sites, thereby affecting the recognition properties of the materials for the template molecule [18-19]. The higher the stability of the complex, the higher the fidelity of the resulting recognition sites, and stronger will be the recognition ability of the MIPs. Spectroscopic analysis has been used for the elucidation of chiral recognition mechanism on a molecular level. Here we characterized the interactions between the template (D-MA) and functional monomer (4-VP) at the pre-polymerization stage by ^{1}H NMR, FT-IR and UV-Vis spectroscopic analysis [20]. These techniques also provide the fundamental analytical basis for rationalizing the mechanisms of recognition during the imprinting process.

2.5 BINDING ANALYSIS, FACTORS INFLUENCE ON BINDING AND OPTIMIZATION OF CONDITIONS:

Binding analysis were done by weighing out definite amount of MIP and NIP having the same crosslink density was incubated with D-mandelic acid standard solution (1.2 x10^{-2} M) and allowed to reach equilibrium in the assay solvent. The mixture was oscillated in a constant temperature bath oscillator at room temperature for 1 h. After incubation, the samples were filtrated and concentration of the resulting supernatants was determined by UV-vis spectrometry (λ_{max} : 258 nm). The binding process can be written as

$[\text{Template}]_f + [\text{imprinted sits}]_f \rightleftharpoons [\text{Template imprinted sites}]_b$

where subscript f and b indicates free and bound states respectively. The amount of D-MA adsorbed, S_b (mmol g^{-1}) onto the polymer was determined according to the following equation,

$$S_b = \frac{(C_i - C_f)V}{m}$$

Where where C_i (mg/ml) and C_f (mg/ml) represent the initial and final D-MA solution concentration respectively V (ml) is the sample's volume and m(g) is the mass of the polymer. The data obtained from adsorption experiment was further processed with the Scatchard equation to measure the binding

parameters of the MIP [21]. Scatchard plot was constructed by plotting the ratios of bound amount to free D-MA concentration against the bound concentration. Scatchard equation is,

$$[S]_b / [S]_f = \frac{(S_{max} - S_b)}{K_D}$$

Where K_D is equilibrium dissociation constant, $[S]_b$ is amount of template bound to the polymer and $[S]_f$ is amount of free template in solution.

For the selectivity studies, to the template desorbed polymer, equal volume of the solutions of template and the compound with structural analogue (L-MA) having equal concentration were added in different tubes and the difference in the extent of binding was estimated spectrophotometrically. The imprinting factor or separation factor (α), which represents the effect of the imprinting process, is the ratio of the amount of substrate bound by the MIP to that bound by the corresponding NIP [22].

Separation factor,

$$\alpha_{Template} = \frac{K_{MIP}}{K_{NIP}} \qquad K = \frac{Template_{Bound}}{Template_{Free}}$$

The selectivity of the imprinted polymers towards the template was calculated in terms of selectivity factor (a).

Selectivity factor, $$a = \frac{\alpha_{Template}}{\alpha_{Analogue}}$$

Factors which affect the rate of binding such as nature of the functional monomer, template to functional monomer ratio, extent of cross linking were examined via binding analysis. Optimized conditions of D-MA binding were investigated by knowing the effect of concentration, solvent and time.

III. RESULTS AND DISCUSSION:

3.1 Characterization of pre-polymerized complexes of D-MA & 4-VP

The interaction between the template and the functional monomer was investigated by FT-IR, ^{1}H-NMR and UV-vis. spectrometric measurements.

3.1.1 FT-IR

The FT-IR spectrum of 0.3mmol L^{-1} D-MA solution and that of D-MA mixed with 4-VP is given in Fig 1. In spectrum A, symmetric stretching and deformation peak of O-H in D-MA appeared at 3441 and 930 cm^{-1} respectively. Also C=O stretching and C-H stretching vibration peak appeared at 1713 and 2906cm^{-1} respectively. These peaks transferred to 3243, 918, 1738 and 2253cm^{-1} respectively after mixing with 4-VP in spectrum B. In addition to these, a peak at 1375cm^{-1} in spectrum B indicate C=N stretching in ring. Hydrogen bonds have formed between D-MA and 4-VP causes the blue shifts of peaks. The formation of hydrogen bond decreases the electron cloud density of O-H and resulted in decrease in frequency of vibration.

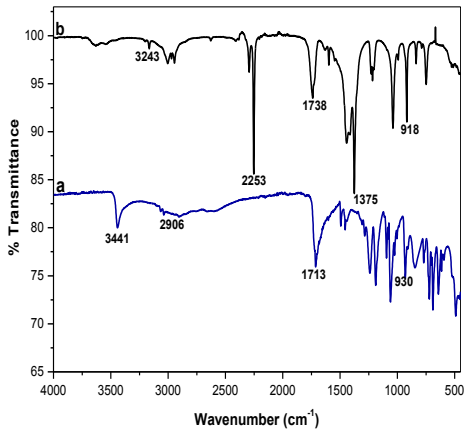


Figure 1: FT-IR spectra of a) D-MA and b) D-MA,4-VP complex

3.1.2 UV-vis. Analysis:

The complexes formed between template molecule and functional monomers can be generally expressed as:

$$T + nM \underset{}{\overset{K}{\rightleftharpoons}} T\,Mn$$

where K refers to association constant, n = 1, 2, 3,. . . , q.

In order to study the stability of the complexes formed between the template and the functional monomers, UV-vis spectrum analysis was often used. In this system the difference spectra of D-MA (0.2 mmolL^{-1}) in acetonitrile is fairly sensitive to the presence of small amounts of 4-VP as shown in Figure 2. Here 4-VP analytic concentration (B_0) greater than that of D-MA (A_0).

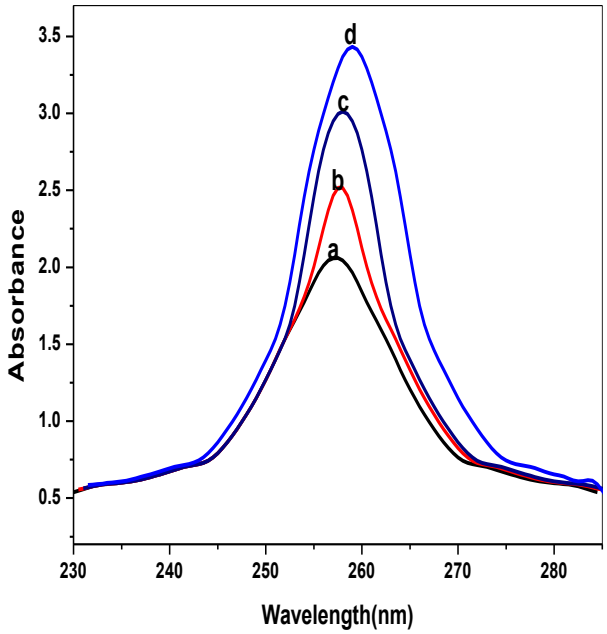


Figure 2 Difference absorption spectra of D-MA in the presence of 4-VP in acetonitrile

A_0= 0.2 mmol L^{-1}, B_0= 0.2040 (a); 0.4081(b); 0.6121(c) and 0.816mmol L^{-1}(d)

The results indicated that the maximum absorption wavelength of D-MA shifted remarkably to longer wavelength in the presence of 4-VP and the maximum absorbance of D-MA increases with the addition of 4-VP. The red-shift of the absorption band is typical for the hydrogen bonding effect on the π-π* absorption band of a molecule whose chromospheres acts as a proton donor.

3.1.3 ^{1}H-NMR analysis:

^{1}H-NMR was used to study the H-bond interaction between D-MA and 4-VP. ^{1}H-NMR titration studies were performed in CD_3CN due to the solubility of D-MA in acetonitrile. Samples were prepared with a fixed concentration of D-MA (0.3mmol L^{-1}) and various concentrations of 4-VP (0.2, 0.4 mmol L^{-1}) in CD_3CN. TMS was used as an internal standard. All spectra were recorded at 20 ^{o}C. As shown in Fig 3, the peaks correspond to pyridine ring protons appeared at 8.7ppm. On addition of 4-VP to D-MA solution, the signals due to D-MA were suppressed gradually on complexation with 4-VP. Aromatic nature of both template and functional monomer offered π-π* interaction, which is revealed from the changes in the peak corresponding to the aromatic proton on complexation.

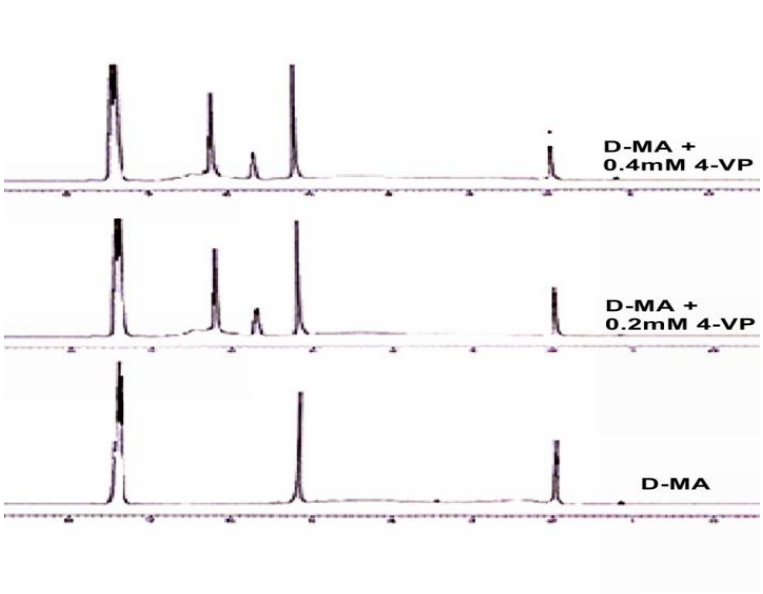


Figure 3: Variation of ^{1}H-NMR spectrum of D-MA with increasing concentration of 4-VP

3.2.1 CHARACTERISATION OF SYNTHESIZED POLYMERS:

3.2.2 FT-IR

The FT-IR spectrum of EGDMA- cross linked MIP and NIP are shown in Fig 4. There are vibrational peaks corresponding to 2955 cm^{-1} (-C-H stretching), 1728 cm^{-1} (-C=O stretching), 1043 cm^{-1} (-C-N stretching), 1455 cm^{-1} (-C-O symmetric stretching in ester), 1144 cm^{-1} (-C-O asymmetric stretching in ester), 957 cm^{-1} (-O-H Bending), and some overlapped peeks appearing in these spectra. The spectrum of the MIP becomes comparable to that of the NIP. This indicates that almost all D-MA are removed from the precursor, which therefore presents an advantage for the study of binding behaviors.

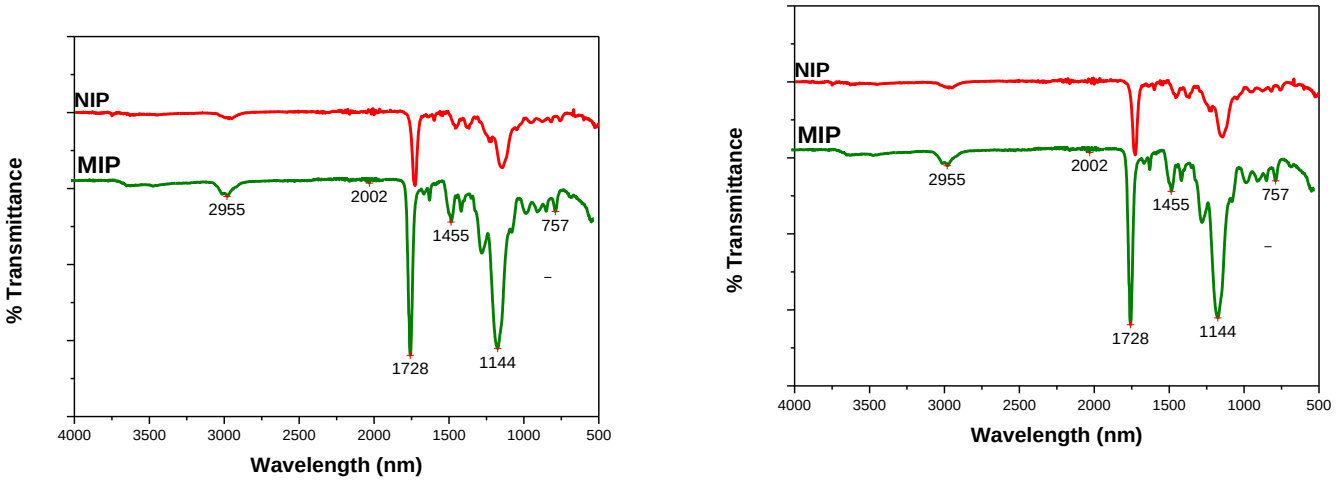


Figure 4: FT-IR spectrum of MIP and NIP

3.2.2 Scanning Electron Microscopy:

The SEM photographs of EGDMA-cross linked D-MA imprinted and non-imprinted polymers are given in Fig 5. NIP displays a relatively smooth surface. There are definitely some speckles and cavities appearing in the MIP, these can be a consequence of imprinting. The imprinting embeds the framework of template within the polymer, thereby leaving behind these tracks.

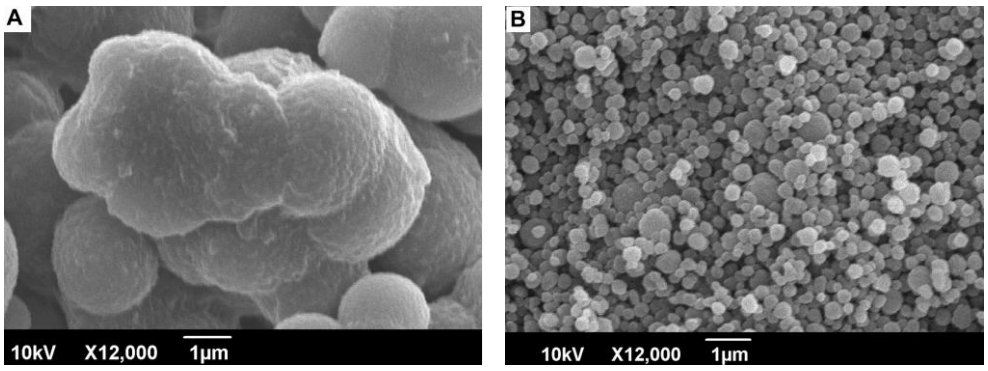


Figure 5 SEM images of EGDMA-cross linked D-MA imprinted (A) and non-imprinted polymers (B)

3.3 D-MANDELIC ACID BINDING STUDIES:

3.3.1 SPECIFICITY IN D-MANDELIC ACID BINDING BY IMPRINTED POLYMERS:

Definite weight of MIP and NIP having the same crosslink density is incubated with D-mandelic acid standard solution (1.2 $x10^{-2}$ M) and allowed to reach equilibrium in the assay solvent. The mixture was oscillated in a constant temperature bath oscillator at room temperature for 1 h. After incubation, the samples were filtrated and concentration of the resulting supernatants was determined by UV-vis spectrometry (λ_{max} : 258 nm).

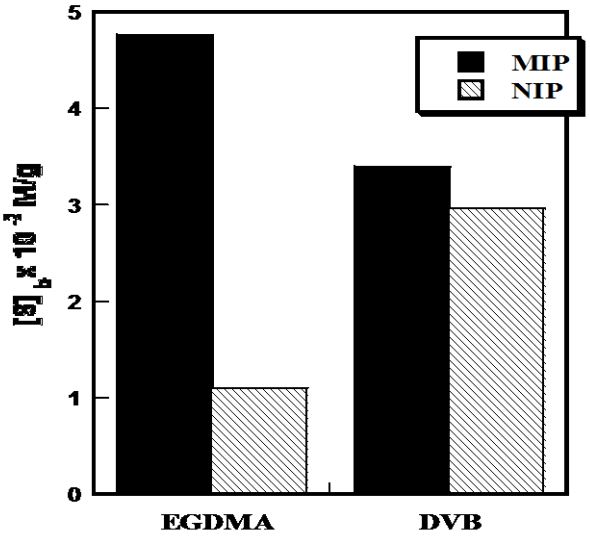


Figure 6 Specificity in D-mandelic acid binding by EGDMA- and DVB-crosslinked imprinted and non-imprinted polymers

The EGDMA-cross linked polymer exhibits higher specificity compared to the DVB-cross linked system. This could be explained by the rigidity of the DVB-cross linked polymer preventing the cavity having sufficient flexibility to orient so that less binding occurs within the polymer matrix. The reference polymer exhibits a lower degree of template binding compared to the imprinted polymer due to the lack of D-MA binding sites in the polymer matrix.

3.3.2 SWELLING STUDIES:

Definite weight of imprinted and non-imprinted polymer is immersed in different solvents like acetonitrile, methanol and water for 24h in a sintered crucible. After 24 h the excess of solvent was removed from the polymer by applying reduced pressure for 1min and the weight of the swollen polymer was measured. From the weight of the dry polymer and the amount of solvent sorbed, the swelling ratio was calculated **(**Table I).

The lower swelling of the imprinted polymers is due to the reluctance of the polymer network to expand from the designed geometry sculpt around the template molecule. In the non-imprinted polymer there is no such frame work cavity left by the template molecule. Hence the non-imprinted polymer network could undergo swelling. The high swelling of the 40 and 55% EGDMA-crosslinked imprinted polymer supports the high binding of template molecule by these systems. The high swelling ratio in water by the EGDMA-cross linked polymer accounts for the high template binding by these imprinted systems in aqueous medium.

Table 1 Swelling ratios of EGDMA-cross linked D-mandelic acid imprinted and non-imprinted polymers

EGDMA Solvent	40		55		70		80	
	MIP	NIP	MIP	NIP	MIP	NIP	MIP	NIP

CH_3CN	2.4	4.7	3.9	7.2	2.2	2.3	2.6	0.4
CH_3OH	1.9	5.7	4.5	7.7	2.9	3.5	3.3	1.6
H_2O	5.5	8.0	3.8	9.1	6.2	6.4	8.5	4.2

3.4 FACTORS AFFECTING BINDING:

3.4.1 NATURE OF THE FUNCTIONAL MONOMER:

To study extent of interaction strength between the template and functional monomer on imprinting effect, the MIPs using methacrylic acid (MAA) and 4-VP as the functional monomers were synthesized. All polymers were analyzed for the binding of template using equilibrium binding experiments. The binding data of MIPs compared to the corresponding NIPs are given in the Fig 7.

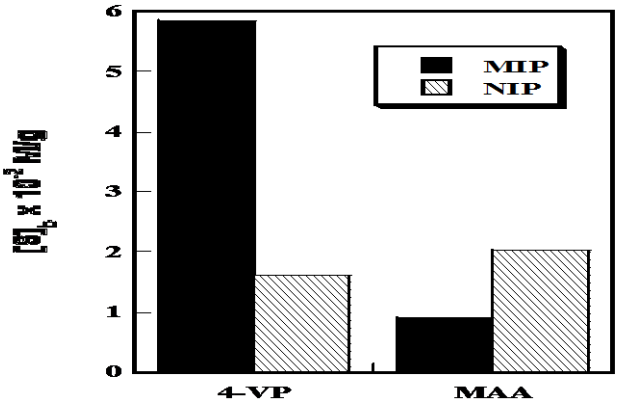


Figure 7 Dependence of the nature of the functional monomer on binding of the template by the 40% EGDMA-cross linked imprinted and non-imprinted polymers

It could be seen that among the MIPs prepared using the different types of functional monomers, 4-VPbased polymers show higher binding affinity for the template. The MIPs prepared with MAA asfunctional monomer exhibited low binding for the template obviously because of the absence of significant interaction between these functional monomer and the template molecule. 4-VP is a basic functional monomer and may form typical interaction with the carboxyl and hydroxyl groups of template by hydrogen bonding and/or ionic bonding. Also the possibility of π stacking interactions with the aromatic rings of the imprint molecule and 4-VP in addition to the electrostatic interaction and H-bonding leads to high affinity. Thus a stable host-guest complex between template and functional monomer is formed in the imprinting process. The existence of such a complex leads to the formation of well-defined specific binding sites in imprinted polymers. Consequently 4-vinylpyridine imprinted polymers show a higher molecular imprinting effect. Compared with 4-VP, MAA owing to its acidic property is unfavorable for the direct interaction with the -COOH groups on template. Therefore MAA based MIPs show poor recognition effect for the template.

3.4.2 TEMPLATE TO FUNCTIONAL MONOMER RATIO:

Template-monomer molar ratio has been found to be important with respect to the number and quality of MIP recognition sites. In order to produce a sufficiently selective polymer and find the optimum condition for the particular template, the imprinted polymers and the corresponding non-imprinted polymers were synthesized at different template to functional monomer ratio (1:2, 1:4, 1:8) keeping the cross linking density same. The binding data of MIPs compared to the corresponding NIPs are summarized in Fig 8.

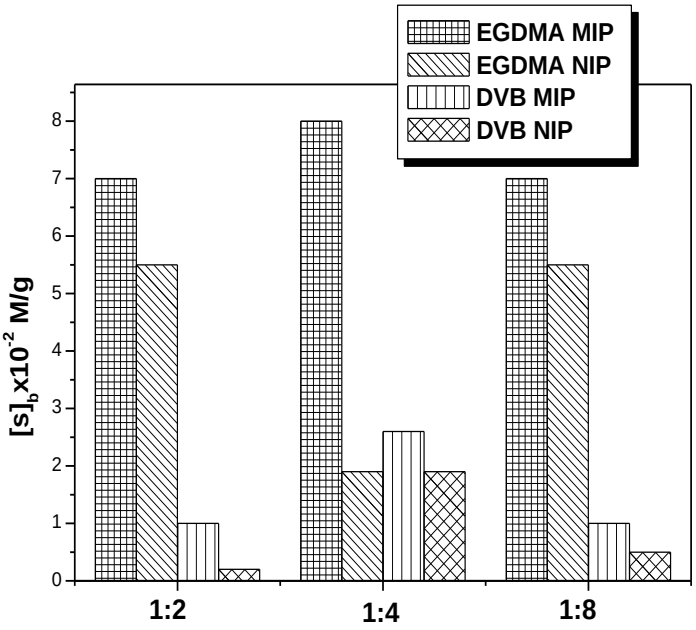


Figure 8 Effect of T/FM ratio on D-MA binding by 40% EGDMA- and DVB-crosslinked MIP and NIP

The specific binding amounts of these imprinted polymers toward substrate changes with T/FM ratios. In both EGDMA- and DVB-cross linked systems the specific adsorption of the 1:4 polymers is the highest. Based on these, the optimum T/FM ratio 1: 4 was chosen for preparing MIPs. Because of the very low specificity of the DVB-cross linked system the rest of the binding studies are mainly conducted using the EGDMA-cross linked system.

3.4.3 EXTENT OF CROSSLINKING:

The effect of cross linker concentration in the MIP on D-MA binding was investigated by using polymers having different percentage of crosslink ling. Cross linker concentrations were varied from 40 to 80%. As seen in Fig 9, the binding properties of MIPs are clearly affected by a change of the cross linker content. Increasing cross linking density of polymers reduces flexibility and decreases the contribution of specific binding to imprint receptor sites. This is not surprising considering the decrease of the affinity sites in unit weight with rising cross linker concentration in polymeric mixture, although a high cross linking is helpful to stabilize the recognition sites of imprinted polymers.

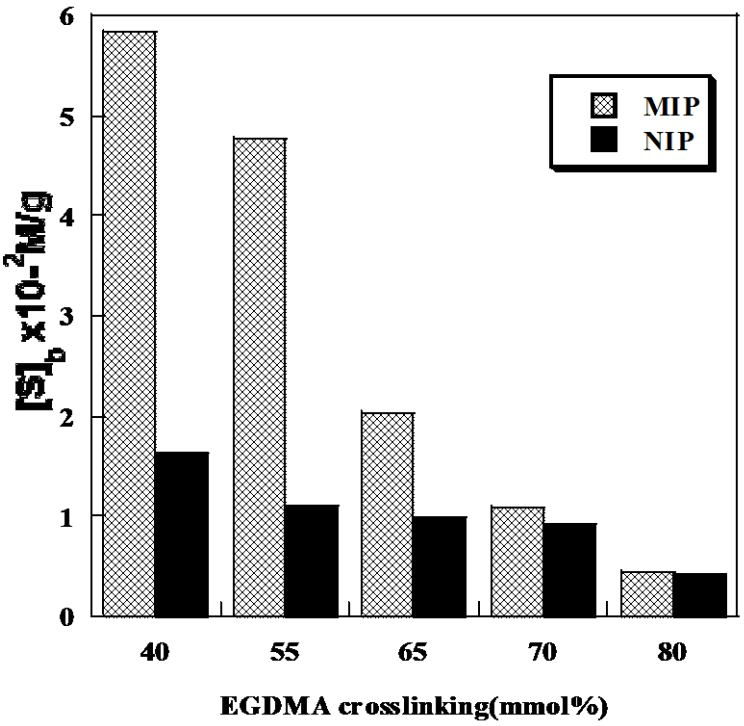


Figure 9 Dependence of EGDMA cross linking on D-MA binding by imprinted and non-imprinted polymers

Usually rigid structure of polymers favors selectivity by enabling the cavities to retain their shape even after the removal of the template. Here the decreased binding by the higher cross linked polymers indicates the increased rigidity of the polymer. The specificity is maximum for the 40% cross linked system and it shows a regular decrease with increase in cross linking.

3.5 OPTIMIZATION OF THE CONDITIONS OF D-MA BINDING:

3.5.1 EFFECT OF CONCENTRATION: MEASUREMENT OF BINDING CONSTANT: SCATCHARD ANALYSIS:

To estimate the binding capacity of the given polymer, saturation studies were performed with the polymer in the assay solvent. The equilibrium binding experiments were carried out by using template solutions of different concentration ranging from $4x10^{-3}$ M to $1.2x10^{-2}$ M in acetonitrile and is incubated with fixed amount of 40% EGDMA-cross linked polymer having 1:4 T/FM ratio. The binding is increased gradually with concentration of template and ultimately reaches a stable value (Fig 10).

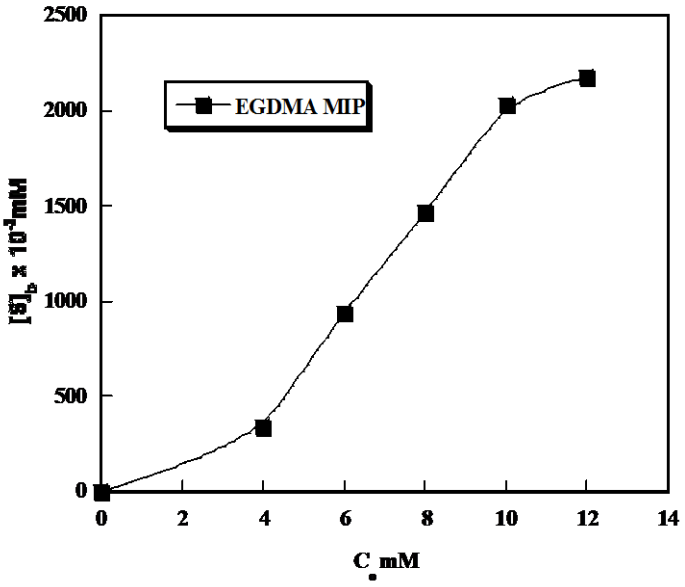


Figure 10 Binding isotherm for the binding of D-MA by 40% EGDMA-crosslinked imprinted polymer

The obtained binding data were plotted according to the Scat chard equation **(**Fig 11). On plotting $[S]_b/[S]_f$ verses $[S]_b$ two straight lines revealed the heterogeneous binding sites with different binding affinities. From the slope and X- intercept, value of K_{D1}, K_{D2} and Smax were determined, which is summarized in Table 2. K_{D1} and K_{D2} are the dissociation constants at high and low affinity binding sites respectively. Lower the value of K_D higher will be the guest binding.

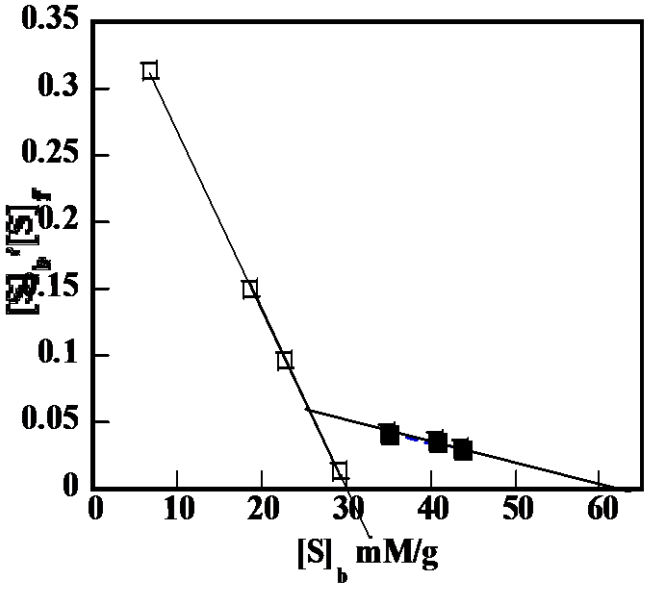


Figure 11 Scatchard plot to estimate the binding nature of EGDMA-crosslinked D-mandelic acid imprinted polymer

Table 2 Binding parameters of the D-MA imprinted EGDMA--cross linked polymers

Crosslinking	High affinity sites		Low affinity sites	
	K_{D1} M/L	S_{max} mM/g	K_{D2} M/L	S_{max} mM/g
EGDMA	74.29	30	726	61.75

3.5.2 EFFECT OF SOLVENT:

The D-MA binding of the MIPs and NIPs were evaluated using template solutions in different solvents like chloroform, acetonitrile and water. Usually the best results are obtained when the binding is conducted in the same solvent which is used as proven because under such conditions the loss of template-polymer conformational adaptation will be a minimum. The EGDMA-cross linked polymer showed maximum binding in water (Fig 12). The high swelling ratio accounts for the high template binding by these imprinted systems in aqueous medium. An obvious increase upon total binding to the polymers in water arises from increased hydrophobic interaction between D-MA molecule and polymer matrix.

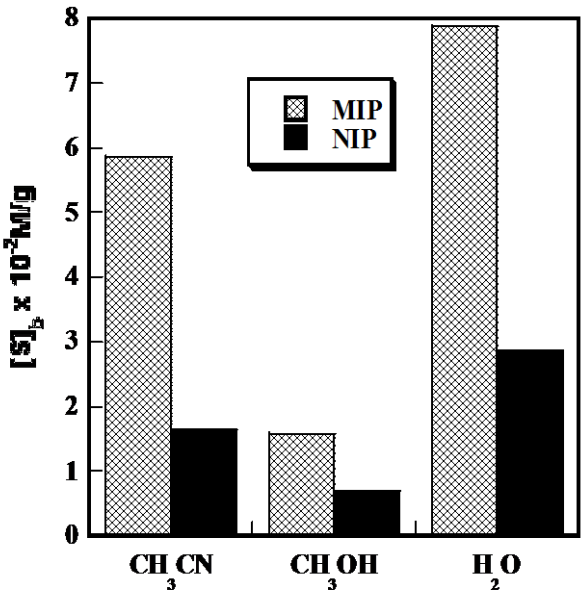


Figure 12 Solvent effect on D-mandelic acid binding by the 40% EGDMA-crosslinked imprinted and non-imprinted polymers

3.5.3 EFFECT OF TIME:

To optimize the time taken for maximum binding of D-MA by imprinted and non-imprinted polymers definite amount of the polymers were equilibrated with template solution of definite concentration and the binding was followed at regular intervals of time. At the initial stage a large number of imprinted cavities existed on the polymer matrix, so D-MA molecule was easy to reach the specific binding sites. When the recognition cavities were filled up, the rate of adsorption dropped significantly and then the adsorption process achieved equilibrium. The imprinted polymer took more time for saturation than the non-imprinted polymer (Fig13).

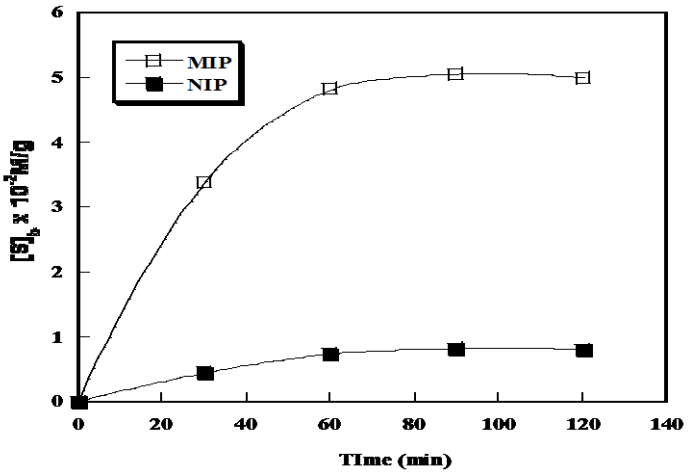


Figure 13 Dependence of time on the D-MA binding by MIP and NIP

3.6 ENANTIOSELECTIVITY OF THE D-MANDELIC ACID IMPRINTED POLYMERS:

Possibly more important than the binding potential of these polymers is their ability to stereo differentiates the enantiomers of D-MA. The binding amount bound to MIPs and NIPs was determined by batch method. As the cross linking percentage increases selectivity decreases and the 40% polymer is found to have the highest selectivity (Figure 14).

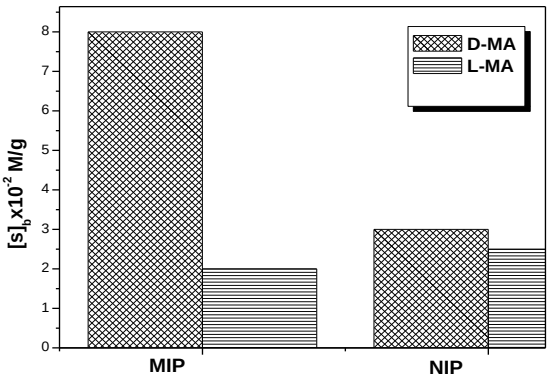


Figure 14 Evaluation of selectivity of the D-MA imprinted polymer

3.7 SEPARATION/SELECTIVITY FACTORS OF EGDMA-CROSSLINKED (40-80%) D-MA IMPRINTED POLYMERS:

The affinities of the template molecule towards the imprinted and non-imprinted polymers were compared based on separation factor. In this system the EGDMA system is proved successful in the specific and selective recognition of the template molecule.

Table 3 Batch binding of D-MA to EGDMA- cross linked MIPs and NIPs

EGDMA (%)	MIP			NIP			α D-MA
	Bound ($x10^{-4}$) M	Free ($x10^{-4}$) M	K_{MIP}	Bound ($x10^{-4}$) M	Free ($x10^{-4}$) M	K_{NIP}	
40	17.5	102.5	0.17	4.88	115.1	0.04	4.25
55	14.32	105.7	0.14	4.38	115.6	0.04	3.5
65	6.11	113.9	0.05	2.94	117.0	0.03	1.6
71	3.27	116.7	0.02	2.78	117.2	0.02	1.0
83	1.33	118.8	0.01	1.28	118.7	0.01	1.0

As given in Table 3, D-MA imprinted polymers exhibit a higher K for D-MA than the corresponding non-imprinted polymers. It can be explained by the synergistic effect of spatial complementarities and the interaction between the functional group of the template and those on the imprinted polymer. This indicates that the recognizing cavities have been created in resultant MIP owing to the addition of template molecules during polymerization. The orientation of the functional group inside the cavities and the shape of the cavities in the imprinted polymers are matching with the template molecules. Three polymers having selectivity factor greater than one shows enantioselective binding which might be attributed to a correlation with binding affinity **(**Table 4). These results suggest that the templates are successfully imprinted in the polymer matrix.

Table 4 Selectivity factors of EGDMA-crosslinked MIPs

EGDMA (%)	$\alpha_{D\text{-}MA}$	$\alpha_{L\text{-}MA}$	Selectivity factor
40	4.25	3.04	1.39
55	3.6	2.63	1.36
65	2.0	1.6	1.25
70	1.0	2.0	0.50
80	1.0	1.7	0.58

IV. CONCLUSIONS:

The success of using molecular imprinting technology for tailoring imprinted polymers with predetermined enantioselective binding properties by employing the enantiomers of interest as binding-site-forming templates is evident from the above discussions. The nature of the template as well as the monomer determines the quality of imprinting. Also it is evident that by the suitable selection of monomers and cross linking agents it is possible to tailor imprinted polymers with selectivity towards a particular target molecule. From the studies it can be concluded that using EGDMA as cross linking agent and 4-VP as functional monomer D-MA can be successfully imprinted in the synthetic polymer. EGDMA exhibits superior selectivity compared to the rigid DVB-cross linked system. Study also showed that with the systematic and careful optimization of the conditions it is possible to design imprinted polymers with maximum specificity and selectivity.

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