Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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C. Mercy Philip[ab]; Beena Mathew[a]
[a] School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India [b] Department of Chemistry, St. Stephen's College, Kottayam, Kerala, India

To cite this Article Philip, C. Mercy and Mathew, Beena(2008) 'Design of EGDMA-Crosslinked Theophylline Imprinted Polymer with High Specificity and Selectivity', Journal of Macromolecular Science, Part A, 45: 4, 335 — 343
To link to this Article: DOI: 10.1080/10601320701865978
URL: http://dx.doi.org/10.1080/10601320701865978

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry (2008) **45**, 335–343

ISSN: 1060-1325 print/1520-5738 online
DOI: 10.1080/10601320701865978

Design of EGDMA-Crosslinked Theophylline Imprinted Polymer with High Specificity and Selectivity

C. MERCY PHILIP and BEENA MATHEW

School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India

Received January, 2007, Accepted September, 2007

An attempt has been made to design theophylline selective polymers with maximum selectivity and specificity, and to relate the rebinding capacity of the polymers with the degree of crosslinking, as well as with the template-monomer ratio. The theophylline imprinted and non-imprinted polymers based on methacrylic acid as the functional monomer and ethylene glycol dimethacrylate (EGDMA) as crosslinking agent (35–80 mol%) were prepared. The developed imprinted polymers were characterized by FT-IR, ^{1}H and ^{13}C-NMR spectra. Equilibrium binding of theophylline by the imprinted and non-imprinted polymers were investigated and optimized the conditions. Imprinted polymers showed specific binding of the template theophylline. Selectivity of the imprinted polymers was investigated towards caffeine and nicotine. Imprinted polymers showed specific and selective binding of theophylline, which varied with the degree of EGDMA crosslinking. Equilibrium rebinding experiments proved that imprinted polymer with moderate (70%) crosslinking with 1:2 template-functional monomer ratio is ideal with maximum specificity and selectivity.

Keywords: molecular imprinting; crosslinking; templates; swelling; selectivity

1 Introduction

Molecular imprinted polymers (MIPs) have the ability to recognize target molecules specifically and thus have been used in a variety of molecular recognition based applications (1–3). In molecular imprinted polymers, the functional and crosslinking monomers are copolymerized in the presence of a target analyte (imprint molecule), which act as a molecular template. The selective removal of the template leaves memory for the target template molecule. Thus, molecular imprinting is a technique for the synthesis of polymeric materials with predetermined ligand selectivity (4–7). The stability and low cost of molecularly imprinted polymers make them advantageous for use in analysis, as well as in industrial scale production and application.

Theophylline is used as a bronchodilator, but its toxicity continues to be a commonly encountered clinical problem. Theophylline is iatro-genic and can cause seizures and convulsions either by acute overdose or chronic use. In this study, theophylline imprinted polymers were prepared using methacrylic acid as the functional monomer along with a different amount of crosslinking agents, and their specific rebinding capacity for theophylline was followed spectrophotometrically. The present paper describes the synthesis of EGDMA-crosslinked theophylline imprinted and non-imprinted polymers with optimum selectivity and specificity. Polymers with varying template-functional monomer ratios and with varying crosslink density (35–80 mmol%) were synthesized by following the routine method of synthesis (8). Characterization of the polymers, rebinding studies, investigation of the various parameters on the rebinding, and specificity and selectivity studies are detailed in this paper.

Address correspondence to: Beena Mathew, School of Chemical Sciences, Mahatma Gandhi University, Kottayam, 686 560, Kerala, India. E-mail: beenamathewscs@hotmail.com
Present address: Department of Chemistry, St. Stephen's College, Uzhavoor, Kottayam, Kerala, India.

2 Experimental

2.1 Materials and Methods

Ethylene glycol dimethacrylate (EGDMA) was purchased from Sigma-Aldrich (Germany), methacrylic acid (MAA), 2,2′-azobisisobutyronitrile (AIBN), chloroform and nicotine from Merck (Germany), caffeine and theophylline were from Fluka (Switzerland). MAA and EGDMA, were purified by distillation under reduced pressure. All solvents were of HPLC grade and used as such. Fourier Transform Infrared Spectrophotometer 8400 S Shimadzu, Japan was used to record FT-IR spectra of pre-polymerization

complex solutions, as well as for characterizing the MIP and NIP using KBr pellets. UV-Vis spectral measurements were done to follow the rebinding studies on a Shimadzu UV-Vis Spectrophotometer model 2450. ^{1}H-NMR spectra of the pre-polymerization complex in $CDCl_3$ were recorded on a Bruker BZH spectrometer operating at 500 MHz at 298 K. ^{13}C-NMR spectra of the solid polymer particles were recorded on AMX-400 NMR spectrometer.

2.2 Synthesis of EGDMA-Crosslinked Theophylline Imprinted and Non-imprinted Polymers

2.2.1 Synthesis of EGDMA-Crosslinked Imprinted (MIPs) and Non-imprinted Polymers (NIPs) with Varying Composition of Functional Monomers and Template

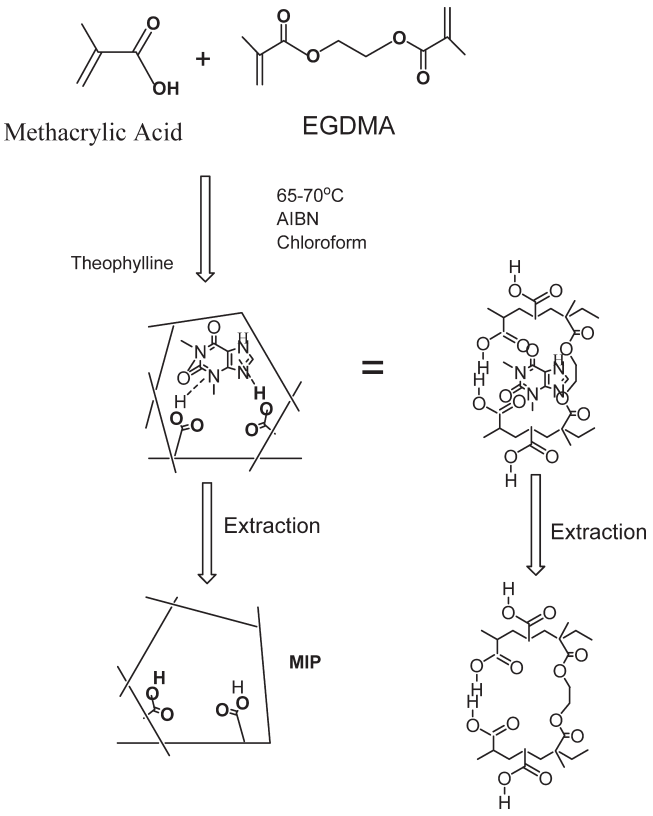
Polymethacrylic acid with EGDMA crosslinking (70%) in 1:1, 1:2 and 1:4 ratios of theophylline and methacrylic acid were synthesized. The composition of the monomer, crosslinking agent and template are given in Table 1. Required amounts of theophylline, methacrylic acid and EGDMA in mmoles were mixed with AIBN (50 mg) and dissolved in chloroform (15 ml). Heating at 65°C under nitrogen in a water bath for 24 h afforded the imprinted polymer. The solid bulk polymer monolith obtained was ground and sieved. Non-imprinted polymers without theophylline were also prepared.

2.2.2 Synthesis of 35–80% EGDMA-Crosslinked Theophylline Imprinted and Non-Imprinted Polymers

For the preparation of EGDMA-crosslinked imprinted polymers with template-functional monomer in 1:2 ratio, the template theophylline (1 mmol), methacrylic acid (2 mmol), the required amount of EGDMA and initiator 2,2′-azobisisobutyronitrile (0.24 mmol) were weighed into a 25 mL round-bottomed flask and dissolved in chloroform. It is kept in a water bath at 65°C for 24 h (Scheme 1). The resulting bulk polymers were ground to pass through a 100 μm sieve. Non-imprinted polymers without the template theophylline were also synthesized by the same procedure.

Table 1. Synthesis of (70%) EGDMA-crosslinked MIPs and NIPs with varying composition of functional monomer and theophylline

EGDMA-Polymer	Theophylline (1 mmol)	MAA	EGDMA	Yield (%) MIP	Yield (%) NIP
1:1	0.36 g	0.17 ml (1 mmol)	0.94 ml	71	75
1:2	0.36 g	0.34 ml (2 mmol)	1.88 ml	75	72
1:4	0.36 g	0.68 ml (4 mmol)	3.76 ml	70	83



Sch. 1. Synthesis of EGDMA-crosslinked theophylline imprinted polymers.

2.3 Desorption of the Bonded Template: General Procedure

The polymer particles were washed and Soxhlet extracted with a mixture of methanol-acetic acid (8:2, v/v) for 2 h, followed by ethanol and with chloroform, until the template could no longer be detected under UV ($\lambda_{max} = 276$ nm). The washed particles were suspended in acetone for 4 h and allowed to settle. The solvent was removed by centrifugal separation, and the particles were dried to a constant weight at 60°C in vacuum.

2.4 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 276 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 polymer particles were filtered off, and the remaining concentrations of the template are 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 theophylline molar concentration in the solution initially and after the interval time 't', respectively. W is the weight of the dry polymer used.

2.5 Optimization of the Theophylline Rebinding Conditions

In order to optimize the conditions of theophylline rebinding by MIP and NIP factors affecting rebinding such as the extent of crosslinking, effect of solvent, time, concentration of theophylline solution and polymer mass on the binding of theophylline were followed using 70% crosslinked MIP and NIP as detailed earlier.

2.6 Factors Affecting Rebinding

2.6.1 Extent of Crosslinking

Equal amounts of the polymers of varying degree of crosslinking were introduced into an equal volume of template solutions of known concentrations for a fixed time. The specificity in rebinding was followed spectrophotometrically.

2.6.2 Concentration of Template Solution

The batchwise guest-binding experiments were used to evaluate the imprinting efficiency. Similar rebinding experiments at various concentrations were carried out, the binding isotherms were obtained and the data were analyzed by the Scatchard method (9–12).

2.6.3 Rebinding Solvent

Equal amounts of the MIP and NIP were introduced into solutions of the templates in different solvents for a definite time and the extent of template bound were determined. The solvent with maximum rebinding specificity was used for further investigations.

2.6.4 Time of Rebinding

To investigate the time taken for saturation of binding sites, the different sets of polymers were incubated in a template solution for different time intervals at 30°C. The template binding was followed spectrophotometrically.

2.6.5 Mass of Polymer

Different masses of the polymers were introduced into template solutions of the same concentrations for the same time interval. The variation of the rebinding capacity with mass was followed spectrophotometrically

2.7 Swelling Analysis

A fixed amount of the polymer was packed into a sintered crucible and was filled with the solvent. 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 polymer was measured. The swelling factor S_x of the polymer was calculated from the following equation (13):

$$S_x = \frac{m_s - m_o}{m_o}$$

where m_s is the mass of the swollen polymer and m_o the mass of dry polymer.

2.8 Selectivity (cross reactivity) Studies: General Procedure

To the template desorbed polymer, the solutions of compounds having structural resemblance to that of the template were added and the bindings were estimated spectrophotometrically.

3 Results and Discussion

3.1 Synthesis of EGDMA-Crosslinked Theophylline Imprinted and Non-imprinted Polymers with Varying Composition of Functional Monomers and Template

The molar relationship between the functional monomer and template (M/T) has been found to be important with respect to the number and quality of recognition sites in molecular imprinted polymers (14–17). EGDMA-crosslinked (70%) polymethacrylic acid with 1:1, 1:2 and 1:4 ratios of theophylline and methacrylic acid were synthesized to investigate the system with optimum rebinding capacity (Scheme 1). The polymers were obtained in 70–80% yield.

3.2 Synthesis of 35–80 mmol% EGDMA-Crosslinked Theophylline Imprinted and Non-Imprinted Polymers

In order to synthesize polymers with optimum selectivity and specificity, the 1:2 (template: functional monomer) polymers of EGDMA with varying crosslink density (35–80%) were synthesized by following the routine method of synthesis. The polymers were obtained in an appreciable yield. The polymers were characterized using FT-IR, ^{1}H and ^{13}C NMR spectra.

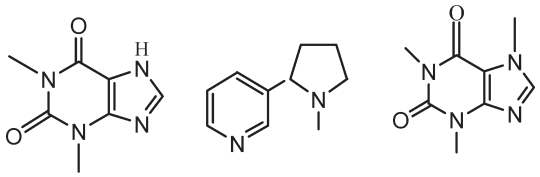
3.3 Characterization of EGDMA-Crosslinked Theophylline Imprinted and Non-Imprinted Polymers

3.3.1 FT-IR Spectra

For the EGDMA-crosslinked polymers, >C-H bend of -CH_3 (1388 cm^{-1}) and of >CH_2 (1454 cm^{-1}) are clear. The >C═O stretch of EGDMA was observed at 1724 cm^{-1}. The broad band at 3452 cm^{-1} indicates the hydrogen bonding between methacrylic acid and template theophylline in EGDMA-crosslinked polymer during rebinding.

IR spectra of the theophylline solution in chloroform before and after the addition of MAA proved the formation of a pre-polymerization complex. The broad band at 3409 cm^{-1} indicates complexation through hydrogen bonding with theophylline. The shifting of >C═C< and >C═N ring stretch from 1666 and 1477 cm^{-1} to 1662 and 1438 cm^{-1} respectively, proved the complexation of theophylline with methacrylic acid.

3.3.2 ^{1}H-NMR



The pre-polymerization complex formation is clear from the ^{1}H-NMR spectra of the theophylline-MAA complex. Interactions between functionalities on the template and the functional monomers can result in a chemical shift that can be used to identify and interpret ligand polymer interactions. The shift in the >N-H proton (12.6 ppm) followed by the disappearance and reappearance on the sequential addition of methacrylic acid show the interaction of the acid with the basic group of theophylline (Figure 1). The significant change in the magnetic environment of the adjacent H_8 due to the complexation is also clear (shift at 7.85 ppm). The steric effect restrict the >N-CH_3 of the pyrimidine ring from complexation with the acid which led in the least shifts at 3.48 and 3.65 ppm.

3.3.3 ^{13}C-CP-MAS-NMR

The incorporation of EGDMA and MAA in the polymer backbone can be confirmed by ^{13}C-NMR spectra. The well-defined peak at 176.31 ppm corresponds to the >C═O group of both -COOH and the ester groups between methacrylic acid and EGDMA. The small peaks at 60.59 ppm and 17.27 ppm were of the -OCH_2 of EGDMA, and -CH_3 of MAA, respectively. The intense peak at, 44.32 ppm corresponds to -CH_2 of the polymer backbone (Figure 2).

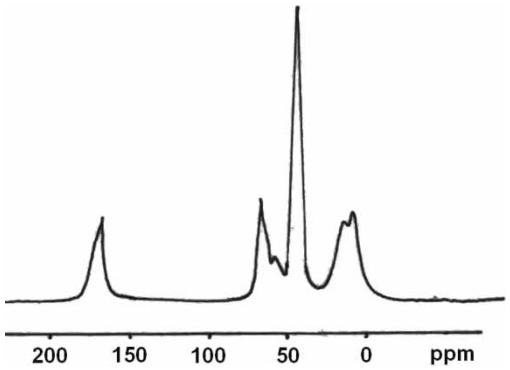


Fig. 2. ^{13}C-NMR spectrum of EGDMA-crosslinked imprinted polymer.

3.4 Swelling Studies

The efficiency of a functional polymer is governed by the accessibility of the reactive functional groups anchored on it, which in turn depends upon the extent of swelling and solvation (18). A good solvent brings the crosslinked polymer to a state of complete solvation and the polymeric network can expand to form a gel. The rate of diffusion of a reagent into the polymer matrix mainly depends on the extent of swelling (19). Thus, swelling is an important parameter, which controls the success of rebinding. The most effective solvent can carry out the rebinding reaction very effectively.

Swelling studies of various crosslinked, theophylline imprinted polymers with a 1:2 template to functional monomer ratio and non-imprinted polymers were carried out in chloroform, dichloromethane and methanol (Table 2). In all crosslinked systems, the swelling of the imprinted polymers are higher due to the accommodation of the solvent molecules within the cavities left by the theophylline molecules. For non-imprinted polymers, the ratio of swelling

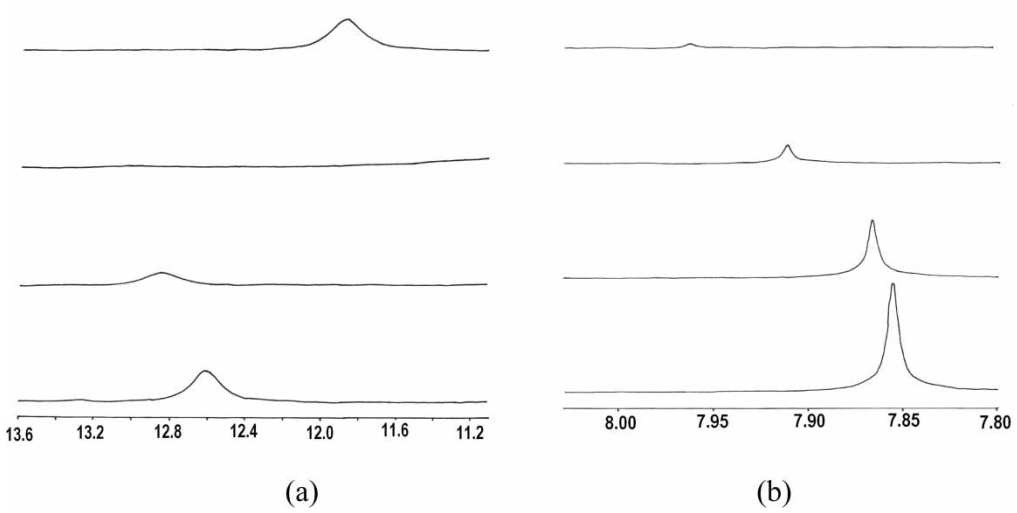


Fig. 1. Portion of the ^{1}H-NMR spectra of the theophylline showing changes in chemical shifts of protons upon subsequent addition of methacrylic acid shift in the (a) >N-H proton (b) H_8.

Table 2. Swelling ratios of theophylline imprinted and non-imprinted EGDMA-crosslinked polymers

	Crosslinked polymer/swelling ratio									
	1:2:1 (35% EGDMA)		1:2:2 (45% EGDMA)		1:2:3 (55% EGDMA)		1:2:5 (70% EGDMA)		1:2:10 (80% EGDMA)	
Solvent	MIP	NIP	MIP	NIP	MIP	NIP	MIP	NIP	MIP	NIP
$CHCl_3$	1.54	0.45	0.83	0.41	0.92	0.40	0.92	0.35	0.44	0.37
CH_2Cl_2	0.86	0.46	0.79	0.46	0.89	0.33	0.90	0.39	0.67	0.38
CH_3OH	0.28	0.23	0.27	0.22	0.47	0.40	0.57	0.24	0.08	0.04

decreases with increasing crosslink density. For imprinted polymers such a regular decrease in swelling with increasing crosslinking is not obtained. The 70% EGDMA-crosslinked imprinted polymers gave maximum swelling in all the solvents that proved the relation between the swelling and binding capacity of the imprinted polymers. The high swelling of the system in chloroform, which is due to the optimum swelling in the like solvent, also supports the high theophylline binding in chloroform.

3.5 TheophyllineRebinding Studies

Theophylline imprinted EGDMA-crosslinked polymers have high specificity to the template molecule. In order to investigate the specific rebinding of theophylline by the imprinted polymer, a control polymer without theophylline with the same crosslinking density (70%) was also prepared. Definite weight of the MIP and NIP were introduced into 3×10^{-4} M theophylline stock solution for a definite time interval and the amount of theophylline bonded was determined spectrophotometrically. The theophylline-imprinted polymer has specificity for theophylline, which is clear from the difference in binding by the imprinted and non-imprinted polymer This implies the memory of the molecular imprinted polymer for the imprinted nicotine molecule.

3.6 Factors Influencing Theophylline Binding

3.6.1 Template-Monomer Ratio

In order to optimize the template-functional monomer ratio for optimum rebinding of the template, imprinted and non-imprinted polymers in 1:1, 1:2, 1:4 theophylline-methacrylic acid ratio were synthesized, and theophylline binding was followed. Theophylline imprinted and non-imprinted polymers were incubated with equal volume of theophylline stock solution of definite concentration for a fixed time interval. The extent of theophylline binding was followed spectrophotometrically and the results are summarized in Figure 3.

For the EGDMA-crosslinked imprinted 1:1 and 1:2 systems, there is considerable specificity in theophylline binding with a slight higher specificity for a 1:2 system. The 1:4 EGDMA-crosslinked systems exhibit considerable non-specific binding. For further studies, EGDMA-

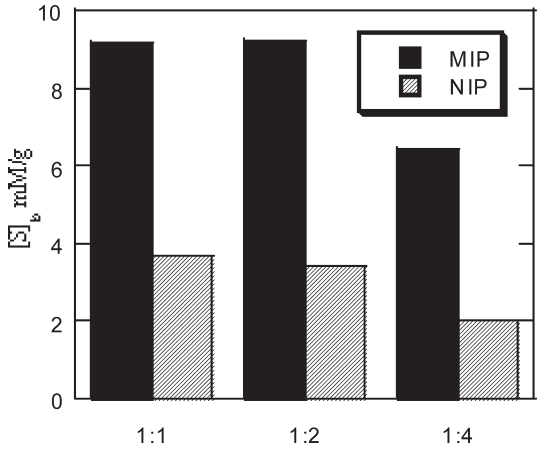


Fig. 3. Effect of template-monomer ratio on theophylline rebinding.

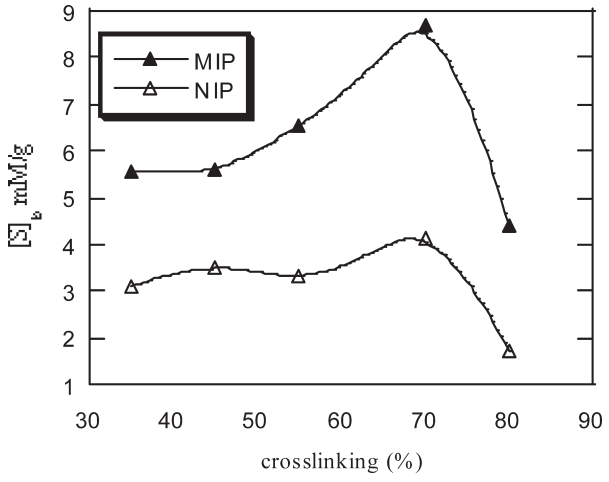


Fig. 4. Dependence of EGDMA crosslink density on theophylline binding by imprinted and non-imprinted polymers.

crosslinked theophylline imprinted polymers were prepared with a 1:2 template to functional monomer ratio.

3.6.2 Degree of Crosslinking

Theophylline imprinted polymers having the ideal template-monomer ratio (1:2) and the corresponding non-imprinted polymers were prepared with varying extents of EGDMA crosslinking (35–80%), and the binding of theophylline was followed in chloroform (Figure 4). In all cases, the binding by imprinted polymers is higher than the non-imprinted polymers. Specificity in binding increased with increasing crosslink density from 35 to 70% and then decreased. Thus, a 70% crosslinked system has a significant specific rebinding of theophylline. As the intensity of crosslinking increases, the polymer acquires the required rigidity to maintain the cavities left by theophylline to allow the template to access the site. Below the optimum percentage crosslinking, the polymer cannot hold the template due to the lack of stability of the network.

3.6.3 Effect of Concentration of Theophylline Solution

A definite amount of the 70% crosslinked MIP and NIP were introduced into the theophylline stock solution of different concentrations ranging from $1^{-4} \times 10^{-4}$ mM for the same time interval and the amount of theophylline bonded $[S]_b$ was calculated. The performance of the MIP and NIP was plotted against the initial concentrations of theophylline (C_o) in chloroform and is given in Figure 5. The extent of theophylline rebinding increased with increasing concentration of theophylline solution initially, which then tend toward a constant value. The cavities in the polymer have a varying degree of affinities and selectivity similar to those of polyclonal antibodies (20). 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 the deformation of the polymer network.

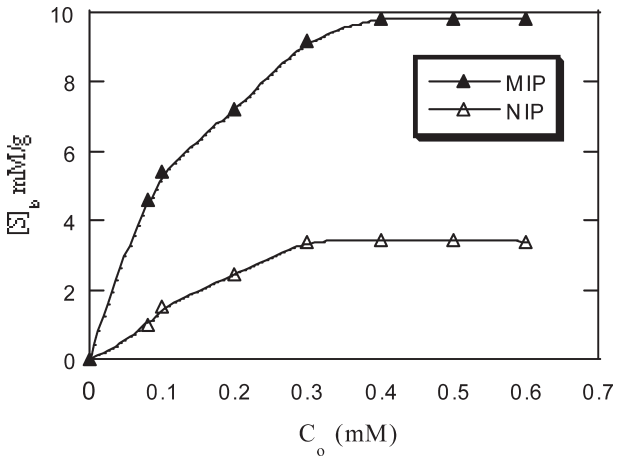


Fig. 5. Binding isotherm for the binding of theophylline by theophylline imprinted EGDMA-crosslinked polymer.

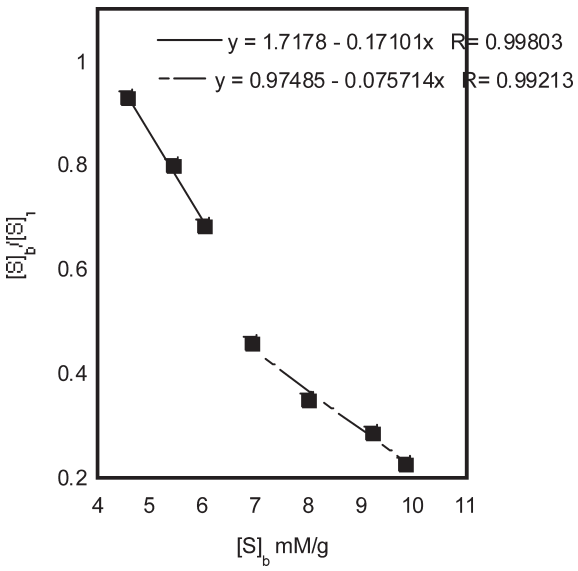


Fig. 6. Scatchard plots to estimate the binding nature of the imprinted polymer.

3.6.4 Determination of Binding Parameters and Guest Binding Constants

The obtained binding data in the batch methods were further processed with the Scatchard equation (9–12) $[S]_b/[S]_f = (S_{max} - [S]_b)/K_D$ to evaluate the binding parameters of theophylline imprinted polymer where K_D is an equilibrium dissociation constant S_{max} an apparent maximum number of binding sites and $[S]_b$ is the amount of theophylline bound to MIPs and $[S]_f$ the amount of theophylline remaining unbound at equilibrium. The plot of $[S]_b/[S]_f$ against $[S]_b$ is shown in the Figure 6. The plot is not linear, and is composed of two straight lines, indicating that the binding sites are heterogeneous with respect to the affinity for theophylline in the studied concentration range of theophylline. From the slope and intercept of the straight line obtained, the values of K_D and S_{max} can be determined. The values of K_D and S_{max} for the higher affinity binding sites were calculated to be 13 M/L and 10.5 mM/g of dry polymer; the values of K_D and S_{max} for the low affinity binding sites

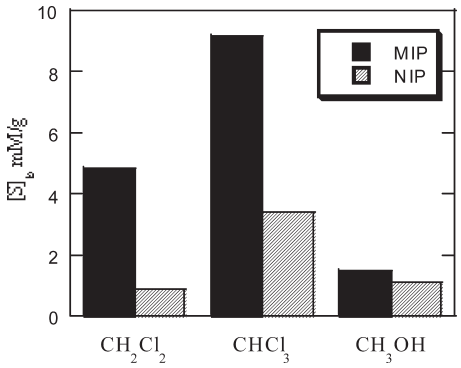


Fig. 7. Effect of solvent on theophylline rebinding by 70% EGDMA-crosslinked MIP and NIP.

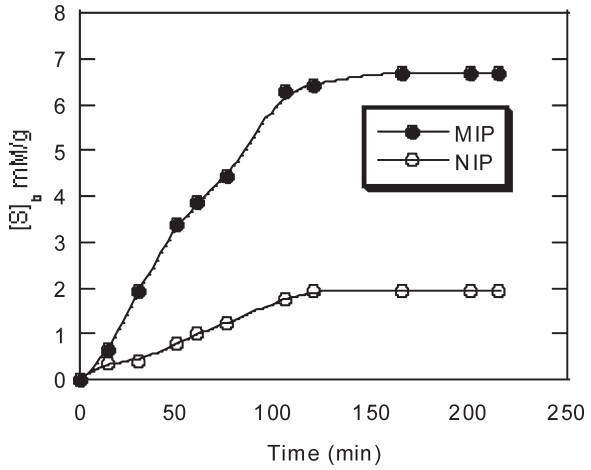


Fig. 8. Time dependence of theophylline binding in chloroform by MIP and NIP.

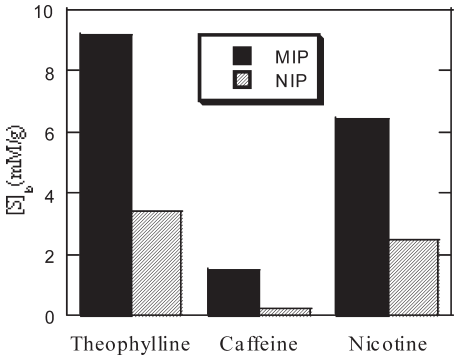


Fig. 10. Selectivity of EGDMA-crosslinked theophylline imprinted polymer.

were 5.8 M/L and 9.0 mM/g of dry polymer. The two classes of binding sites are produced due to template effects in the imprinting process. Apparently, in this imprinted polymer, highly effective guest binding sites are formed from two (or more) carboxylic acid groups, which cooperatively bind the guest.

3.6.5 *Effect of Solvent in Rebinding*

The porogen have an important impact on the molecular recognition properties of MIPs during rebinding; a fact that is often neglected in MIP studies. A number of reports in the literature indicate that the best recognition of the polymer occurs when the rebinding medium and the porogen were the same (21–24). To investigate the effect of solvent in rebinding, theophylline rebinding studies were carried out in different solvents like chloroform, dichloromethane and methanol. The results support the fact that the best recognition of the polymers occurs when the rebinding medium and the porogen used in the polymerization were the same (Figure 7). The low solvent template interaction in chloroform also favors the effective rebinding between template and the functional monomer. Being polar in nature, methanol cannot act as a good rebinding medium as it suppresses hydrogen bonding.

3.6.6 *Effect of Time*

To optimize the time taken for maximum binding of theophylline by MIP and NIP, a definite amount of the polymers were equilibrated with theophylline solution (3×10^{-4} M) and the binding was followed spectrophotometrically at a definite time interval (Figure 8). For imprinted polymers, the time taken for saturation of binding sites is much higher compared to the non-imprinted polymers. The theophylline molecules have to penetrate through the highly crosslinked polymer network to access the imprinted sites for rebinding. In a non-imprinted system, there is no specific arrangement of the binding sites, which leads to a fast random binding of theophylline.

3.6.7 *Effect of Amount of Polymer*

The relationship between mass of polymer with bonded concentration of theophylline was studied by incubating a fixed

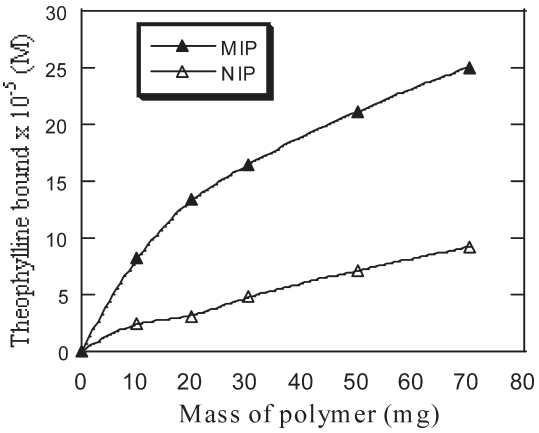


Fig. 9. Dependence of the amount of polymer on theophylline binding.

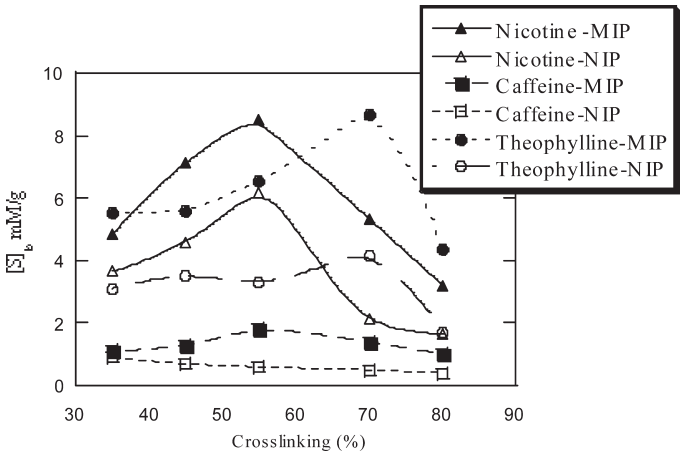


Fig. 11. Effect of the degree of EGDMA crosslinking on selectivity.

Table 3. Batch binding of theophylline to EGDMA-crosslinked theophylline imprinted and non-imprinted polymers in chloroform

	MIP			NIP			
EGDMA (%)	Bounded theophylline $\times 10^{-5}$ M	Free theophylline $\times 10^{-3}$ M	K_{mip}	Bounded theophylline $\times 10^{-5}$ M	Free theophylline $\times 10^{-3}$ M	K_{nip}	α_{theo}
35	5.54	0.245	0.226	3.10	0.269	0.115	1.96
45	5.60	0.244	0.229	3.50	0.265	0.132	1.74
55	6.55	0.235	0.279	3.32	0.267	0.124	2.24
70	8.68	0.213	0.408	4.13	0.259	0.160	2.55
80	4.38	0.256	0.172	2.72	0.273	0.099	1.72
Caffeine (70)	1.50	0.285	0.053	1.23	0.288	0.043	(α_{caf}) 1.23
Nicotine (70)	6.43	0.236	0.273	3.30	0.267	0.124	(α_{nico}) 2.20

volume of 3×10^{-4} M theophylline solution in chloroform with a different amount of MIP and NIP. A gradual decrease in binding per gm of the polymer was observed. The deviation from a regular increase proved that the binding parameters change with the different amount of polymers used in the binding studies. For the EGDMA-crosslinked polymer, the amount of polymer for binding 50% of free theophylline (25) (IC_{50} value) was 3.3 mg/mL for 3×10^{-4} M theophylline solution (Figure 9). The control polymer bound significantly less theophylline than the imprinted polymer.

3.7 Selectivity of the Nicotine Imprinted Polymers

MIP has specific selectivity to the template molecule, which is based on the interaction between the template and the MIP. Two structurally similar molecules, caffeine and nicotine were chosen as comparative molecules to study the selectivity of the 70% EGDMA-crosslinked theophylline imprinted polymers. The difference in performance between the MIP and the reference polymer towards theophylline and the comparative molecules proved the selectivity of the theophylline imprinted polymer (Figure 10). Both the specific, as well as non-specific binding towards caffeine were negligible, whereas less specificity in binding was observed towards nicotine.

To study the effect of the degree of EGDMA crosslinking on selectivity, definite amounts of the EGDMA-crosslinked polymers of varying crosslink density ranging from 35 to 80 mmol% were treated separately with the solutions of theophylline, nicotine and caffeine (Figure 11). In the moderately flexible EGDMA-crosslinked system, high specificity and selectivity in binding was observed with a 70% system. This is due to the required rigidity acquired by the 70% crosslinked system from the EGDMA crosslinks. Thus, an imprinted system with high specificity and selectivity can be tailored with a moderate amount of crosslinking. The region of maximum specificity is shifted from polymers with 70% crosslinking to 55% for the comparative molecules

The small nicotine molecule could enter freely through the network and imprinted cavities resulting in high binding, but with less specificity. Caffeine binding was very low compared to nicotine and theophylline. The methyl substituted nitrogen atoms hinder the effective binding of caffeine. The selectivity displayed by the imprinted polymer indicates that hydrogen bonding may not be the only interaction responsible for rebinding of template. Hydrophobic interaction and shape selectivity may be significant as well. Thus, in conclusion, EGDMA-crosslinked theophylline imprinted polymers showed high selective and specific rebinding of theophylline.

3.8 Molecular Recognition Selectivity of EGDMA-crosslinked Theophylline Imprinted Polymers

A complete secondary screen for binding and selectivity was performed for all the polymers. The rebinding of the template to imprinted and non-imprinted polymers were compared in terms of separation factor (26).

$$\text{Separation factor } \alpha_{theo} = K_{mip}/K_{nip}$$

where K = Bounded theophylline/Free theophylline

$$\text{Selectivity factor (27)} = \alpha_{theo}/\alpha_{comp.}$$

The high α_{theo} for the 70% EGDMA-crosslinked polymer compared to the others, proved their high efficiency among 35–80% polymers (Table 3). The high separation factors of 70% EGDMA-crosslinked theophylline imprinted polymer for theophylline in comparison with caffeine and nicotine, proved the high selectivity of the polymer for theophylline (Table 4).

Table 4. Selectivity of 70% EGDMA-crosslinked theophylline imprinted polymers

$\alpha_{theophylline}$	$\alpha_{caffeine}$	$\alpha_{nicotine}$	$\alpha_{theophylline}/\alpha_{caffeine}$	$\alpha_{theophylline}/\alpha_{nicotine}$
2.55	1.23	2.20	2.07	1.16

4 Conclusions

Theophylline imprinted polymers were prepared using methacrylic acid and EGDMA along with theophylline. Imprinted polymers with a 1:2 theophylline-methacrylic acid ratio showed high specificity. Extraction of the template allowed polymers to recognize and rebind theophylline. ^{1}H and ^{13}C-NMR, and FT-IR spectra provide information about the chemical content of the polymer, as well as the interaction between the functional monomer and the template. The rebinding of the template depends on the template to monomer ratio, porogen used for polymerization and the degree of crosslinking in the polymer matrix. The Scatchard plot suggests the heterogeneity of the binding sites. The rebinding of the template, as well as its selectivity, increased with increasing crosslinking till 70% and decreased further. These polymers showed enhanced selectivity to the template than other structurally related molecules. Thus, the design of theophylline selective polymers with a moderate degree of crosslinking can function as effective sorbents with enhanced specificity and selectivity.

5 Acknowledgment

MPC is grateful to the University Grants Commission (UGC) for the award of a Teacher Fellowship.

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