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Comonomer effects on binding performances and morphology of acrylate-based imprinted polymers

Yeliz Tunc [a], Nesrin Hasirci [b], Akgul Yesilada [a], Kezban Ulubayram [a,*]

[a] *Hacettepe University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, 06100 Ankara, Turkey*
[b] *Middle East Technical University, Department of Chemistry, 06531 Ankara, Turkey*

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Abstract

The objective of this study was to investigate the effect of different functional groups of molecularly imprinted polymers (MIPs) on the binding characteristics towards a specific template molecule by examining selectivity and recognition processes. Several non-covalent theophylline imprinted polymers (TIPs) were prepared by using only methacrylic acid (MAA), or MAA and 2-hydroxyethyl methacrylate (HEMA) comonomer, or MAA and acrylamide (ACM) comonomer. In all cases, a high amount of ethylene glycol dimethacrylate (EDMA) as crosslinker existed in the medium. The highest selective theophylline binding of TIPs was found to be 61%, 41% and 40% for MAA/EDMA, MAA/HEMA/EDMA and MAA/ACM/EDMA systems, respectively. The use of a comonomer (ACM or HEMA) reduced the binding performance of the MAA/EDMA polymer matrix, probably due to the monomer–monomer association and morphological differences. Results obtained from the batch binding experiments demonstrated that all of the TIPs have sites that have selective binding ability for theophylline, but not to another structurally similar molecule, caffeine. According to the Langmuir isotherm model, a heterogeneous distribution of binding sites was observed in the polymers. The maximum association constant and binding site density were computed as 2.3×10^2 mM^{-1} and 8.6 μmol/g, respectively, for copolymer of MAA/EDMA under the examined concentration range.

Keywords: Molecular imprinting; Molecular recognition; Comonomer

1. Introduction

Molecular imprinting has become an increasingly active field of study for the construction of new highly stable molecularly imprinted polymers (MIPs) that possess selective molecular recognition properties [1–4]. Selective recognition by the imprinted polymers is critically based on the strength of interactions between the template and the monomers. Therefore the choice of functional monomers is crucial to maintain stable monomer–template complexes during imprinting process. It is based on the functional group complementarity with the template. Generally, for the templates containing acid groups, monomers with basic functionality are preferably chosen. For example, for imprinting of a template, carrying carboxylic acid moieties, vinylpyridine (VP) is a particularly preferred monomer. Vice versa, methacrylic acid (MAA) is frequently used for basic templates.

Generally, in molecular imprinting, use of one kind of functional monomer is preferred by researchers, since the relative strengths of monomer–monomer and template–template interactions affect the monomer–template complex stability negatively [5]. The systems having more than one functional monomer are complex, because of the competition of the monomer–monomer interactions with the monomer–template interactions in the pre-polymerization step. The most successful non-covalent imprinting systems are based on MAA which is crosslinked with ethylene glycol dimethacrylate (EDMA)

* Corresponding author. Tel.: +90 312 305 2471; fax: +90 312 311 4777.
E-mail addresses: yeliz@hacettepe.edu.tr (Y. Tunc), nhasirci@metu.edu.tr (N. Hasirci), ayesilad@hacettepe.edu.tr (A. Yesilada), ukezban@hacettepe.edu.tr (K. Ulubayram).

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since the carboxylic acid groups of MAA serve as both hydrogen donor and acceptor [6]. Furthermore, MAA can form stable cyclic hydrogen bonds with the suitable templates [7]. It is also a suitable functional monomer for theophylline, because carboxylic acid groups can form hydrogen bonds with the functional groups on theophylline [8]. In the study of Pavel and Lagowski on molecular simulations of molecular imprinting of theophylline, it was stated that the functional monomer simulations show that carboxylic acid and double bonds tend to come closest to the ligands [9]. They observed that the simulations of polymers obtained from the monomers indicated that polymethacrylic acid was one of the polymers interacting strongest and preferentially with theophylline.

It was demonstrated that acrylamide (ACM) could be an alternative monomer to MAA in some cases, since it may form stronger hydrogen bonds with lower pK_b value (p$K_b = 14.5$) than that of MAA (p$K_b = 20$) even in polar solvents [10]. Trifluoromethyl acrylic acid [11], 2-(dimethylamino) ethyl methacrylate [12], 4-vinylpyridine [13], 2-vinylpyridine [14], and 2-hydroxyethyl methacrylate [15] are the other common functional monomers used for imprinting purposes and reported in the previous studies. Although, in conventional molecular imprinting, a single functional monomer is preferred, combinations of two or more functional monomers could also be used to improve the recognition capabilities in some cases [16–18]. One of the successful examples is the copolymer of MAA and vinyl-substitute zinc(II) porphyrin which showed higher binding ability for (−)-cinchonidine than MIPs prepared by using only MAA or zinc (II) porphyrin [18].

Although the methodology of molecular imprinting technique seems to be relatively easy, the rational design of MIPs is very complicated because of number of experimental variables, for example functional monomer(s), nature of template, ratio of functional monomer(s) to template, crosslinker(s), ratio of functional monomer(s) to crosslinker(s), solvent(s), initiator, methodology and polymerization parameters. The findings of research papers are helpful in this regard, however, molecular imprinting technology has not created a great commercial impact yet. Therefore detailed studies investigating the merits of various parameters and making comparison among them are needed for further progress.

The main objective of the present study is to examine the effects of different interactions on selectivity towards a template molecule by using various MIPs and to understand more about recognition properties. In this study, theophylline imprinted polymers (TIPs) were prepared by using various monomer combinations by non-covalent imprinting method. For this purpose MAA was used as main functional monomer with EDMA as crosslinker. Use of an acidic functional monomer with a basic one may cause strong interactions that compete with the template. To minimize the monomer–monomer interactions, relatively neutral monomers, ACM and 2-hydroxyethyl methacrylate (HEMA) were preferred as functional comonomers. These monomers individually have been investigated for molecular imprinting in various studies, but as comonomers, they have not yet been directly used and compared in parallel on the same systems. Theophylline, a bronchodilator, was chosen as suitable template in terms of its functional groups that easily interacts with the functional monomers through intermolecular interactions.

2. Experimental section

2.1. Materials

Theophylline (THE), caffeine (CAF) and theobromine (TBR) were supplied by Sigma (USA). Methacrylic acid (MAA), 2-hydroxyethyl methacrylate (HEMA), acrylamide (ACM) and ethylene glycol dimethacrylate (EDMA) were purchased from Aldrich (USA). Azobisisobutyronitrile (AIBN) was obtained from Merck (Germany). All monomers were purified by vacuum distillation prior to use to remove the inhibitors. Chloroform, methanol, tetrahydrofuran and acetonitrile were all HPLC grade and obtained from Riedel (Germany).

2.2. Spectrophotometric analysis of template–monomer interaction

UV–vis spectrophotometric analysis was performed to characterize the stability of complexes formed between THE and functional monomers in the solution prior to polymerization. A solution of 0.005 mM THE in chloroform was titrated with monomer solutions in the range of 2–20 mM. The spectra of the solutions were recorded by Agilent 8453E UV–vis spectrophotometer. The change in absorption (ΔA) or difference absorption spectra of these solutions were determined at 290 nm by using THE solution as reference. The average data of triplicate independent experiments were used for the plots of $\Delta A/b_o$ versus ΔA as described in literature [19,20].

2.3. Preparation of theophylline imprinted polymers

Theophylline imprinted polymers (TIPs) were prepared basically according to the method described in literature [8]. Briefly, known amounts of monomers were dissolved in chloroform with certain amount of template molecule, THE. The mixture was cooled on ice and degassed in a sonicating bath for 15 min, and then EDMA and AIBN were added into the mixture in a glass ampoule and sparged with N_2 for 5 min. The ampoule was sealed under vacuum and the polymerization was initiated thermally in water bath at 60 °C and continued for 24 h. The resultant polymeric monolith was then ground and sieved through number 53 μm mesh. Extraction of THE molecules from the matrix was performed by Soxhlet extraction, washing 2.0 g of polymer with 120 mL of methanol containing 10% acetic acid for 24 h and then the polymer was dried to constant weight under vacuum at 60 °C. The amount of the extracted template was monitored by UV–vis Spectrophotometer (Shimadzu UV-160A) at 271 nm. Non-imprinted polymers (NIPs) were prepared with the same procedure in the absence of THE. The compositions of the prepared polymeric matrices are given in Table 1.

Table 1
Composition and extraction efficiency of the prepared polymers

Polymer	MAA (mmol)	Comonomer (mmol)	EDMA (mmol)	THE (mmol)	Extracted THE (%)
NIP(MAA/EDMA)	9.41	–	42.4	–	–
TIP(MAA/EDMA)	9.41	–	42.4	2.35	87.6 ± 8.6
NIP(MAA/HEMA/EDMA)	4.71	HEMA (4.71)	42.4	–	–
TIP(MAA/HEMA/EDMA)	4.71	HEMA (4.71)	42.4	2.35	67.0 ± 4.2
NIP(MAA/ACM/EDMA)	4.71	ACM (4.71)	42.4	–	–
TIP(MAA/ACM/EDMA)	4.71	ACM (4.71)	42.4	2.35	80.2 ± 3.6

TIP: theophylline imprinted polymers, NIP: non-imprinted polymers.

2.4. Characterization of polymeric matrices

Thermal properties were investigated by a Differential Scanning Calorimeter (DSC, Dupont 2000) with a heating rate of 10 °C min^{-1} up to 350 °C. The average size and size distribution curves of polymer particles were obtained in chloroform by using a particle size analyzer (Malvern Instruments Ltd., Model Mastersize S). The pore structures of the polymer particles in the dry state were measured by Mercury Porosimeter (Autopore II 9220). Measurements were made with 130° contact angle, 2.76×10^8 Pa and 4.85×10^{-1} Nm^{-1} surface tension of mercury. The morphologies of the samples were examined by scanning electron microscope (SEM, Jeol 6400), after coating the samples with gold films.

2.5. Evaluation of imprinting efficiency

2.5.1. Batch rebinding assay

The binding efficiency of polymeric matrices towards THE was assessed in the batch rebinding experiments. Briefly, 25 mg polymer was placed in a 10 mL conical flask including a known concentration of THE (defined as $[THE]_{initial}$) in 5 mL chloroform and was mechanically shaken at room temperature for 24 h. Then the polymer was filtered by 0.45 μm syringe filter and the concentration of the substrate remaining in the solution (defined as $[THE]_{free}$) was determined by HPLC.[1] These rebinding experiments were repeated five times for each imprinted and non-imprinted polymeric matrices by using different concentrations of THE. The NIPs were used as control group to determine the non-specific binding so that binding and selective binding to the imprinted polymers can be calculated from the following equations:

$$\text{Binding}(\%) = [([\text{THE}]_{\text{initial}} - [\text{THE}]_{\text{free}})/[\text{THE}]_{\text{initial}}] \times 100$$

$$\text{Selective binding}(\%) = \text{binding to TIP}(\%) - \text{binding to NIP}(\%)$$

The analysis was modified from Schreiber-Deturmeny and Bruguerolle [21]. Elution was carried out by using acetonitrile/tetrahydrofuran/acetic acid/water (20:20:5:955 v/v) solution at a flow rate of 1 mL/min. Theobromine was used as internal standard. Calibration curve was obtained from standard solutions of THE and CAF between the concentrations of 0.0625 and 20 μg/mL. Detection was via UV adsorption monitoring at 273 nm. The within-day and between-day precisions of the HPLC method were investigated by using the sample of THE and CAF mixture at the concentrations of 2, 8 and 15 μg/mL. Concerning the within-day precision, five samples were analyzed on the same day. Concerning the between-day precision, five measurements were performed for 6 days.

2.5.2. Determination of association constant by Scatchard analysis

According to Langmuir isotherm model, the association constant and binding site density were estimated graphically from a linearised version of the binding isotherm. This was done by plotting the binding isotherm in a Scatchard format as bound/free template ratio (μmol/$mMg_{polymer}$) versus bound template (μmol/$g_{polymer}$). Each linear region of the binding isotherm is fitted with a straight line. The association constant and binding site density were calculated from the slope and the *y*-intercepts, respectively, according to the following equation [22]:

$$\frac{B}{F} = K_a Q_S - K_a B$$

where B is amount of the template bound to polymer, F is concentration of free template, Q_S is site density and K_a is association constant.

2.5.3. Selectivity of TIPs against caffeine

The selectivities of the TIPs and NIPs were evaluated by competitive binding studies using a solution of THE and CAF at the concentrations of 5 μg/mL of each. The free THE and CAF concentration remaining in the solution were determined by HPLC as mentioned in Section 2.5.1.

3. Results and discussion

3.1. Interaction between template and functional monomers

The strength and positioning of the monomer–template interactions are crucial to obtain polymers with good molecular recognition properties. THE is a suitable template to be used in imprinting process on account of its rigid structure and its high number of hydrogen bond acceptor and donor sites appropriate for non-covalent interactions with the functional monomers. Possible interactions between THE and the functional monomers that are used in this study are as shown in Fig. 1.

[1] Chromatographic studies were carried out by using HP Agilent 1100 system with μbondapak C18 column (250 × 4.6 mm $L \times$ ID).

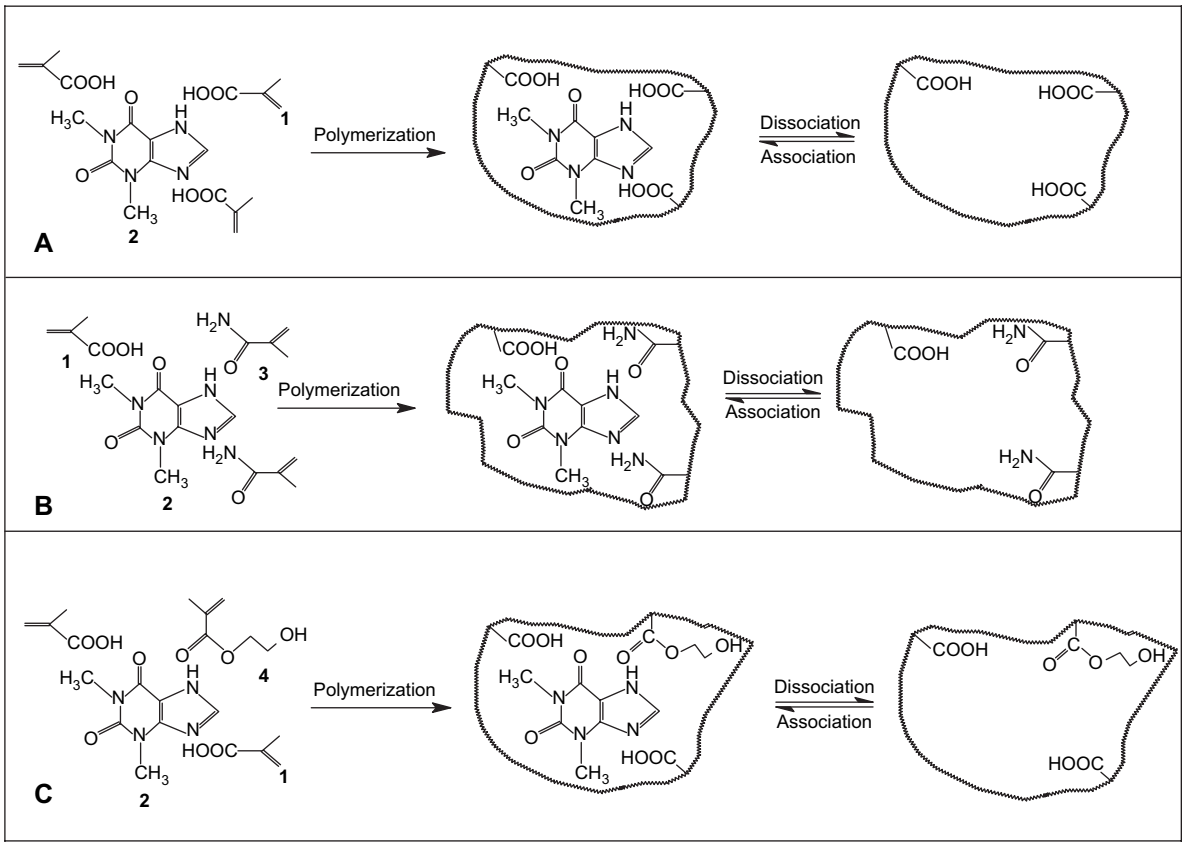

Fig. 1. Representation of possible interactions between theophylline and functional monomers to obtain a specific cavity (**1**. MAA, **2**. THE, **3**. ACM, **4**. HEMA). (A) TIP(MAA/EDMA), (B) TIP(MAA/ACM/EDMA), (C) TIP(MAA/HEMA/EDMA).

In the functional monomer structures, −COOH sites of MAA are hydrogen bond acceptor groups while −OH sites of MAA and HEMA and also $-NH_2$ sites of ACM are hydrogen bond donor groups.

Spectroscopic method is a valuable tool for investigating the strength of interaction between template and functional monomer [19,20]. In the present study the difference spectra of THE (0.005 mM) in chloroform were sensitive to the monomer concentration in the range of 2–20 mM. Observed red shifts of the first band of molecule in the difference spectra are due to the hydrogen bonding effects [20,23].

Generally the formation of the complex C between template A and functional monomer B can be expressed by the following reaction:

$$A + nB \overset{K_a}{\rightleftharpoons} C \tag{1}$$

where $n = 1, 2, 3,\ldots$, is the composition of the complex, and K_a refers to the association constant.

Calling the template concentration, a_o, complex concentration, c, and if the equilibrium concentration of B is approximated as b_o, if further $b_o \gg a_o$, K_a can be written as:

$$K_a = \frac{c}{b_o(a_o - c)} \tag{2}$$

After rearrangement of Eq. (2), the complex concentration, c can be calculated according to:

$$c = \frac{a_o b_o K}{1 + b_o K_a} \tag{3}$$

The absorbance of the mixture measured at a given wavelength can be expressed as:

$$A = [(a_o - c)\varepsilon_A + (b_o - nc)\varepsilon_B + c\varepsilon_C]l \tag{4}$$

where ε_A, ε_B and ε_C are the molar absorptivities of A, B and C, respectively.

When $b_o = 0$, the absorbance is:

$$A_o = a_o \varepsilon_A l \tag{5}$$

The absorbance difference measured is:

$$\Delta A = A - A_o = c\Delta\varepsilon l \tag{6}$$

where $\Delta\varepsilon = \varepsilon_C - \varepsilon_A$. Substituting Eq. (6) into Eq. (3) we get the equation:

$$\frac{\Delta A}{b_o^n} = -K_a \Delta A + K_a \Delta\varepsilon_C a_o l \tag{7}$$

now K_a and n can be obtained by plotting $\Delta A/b_o^n$ versus ΔA.

The appropriate plots of $\Delta A/b_o^n$ versus ΔA according to Eq. (7) give straight lines whose slopes give K_a (Fig. 2A–C). The K_a values are calculated for self assembly complexes as $1.5 \times 10^3\ M^{-1}$ and $4.9 \times 10^2\ M^{-1}$, $0.9 \times 10^2\ M^{-1}$ for MAA–THE, HEMA–THE, ACM–THE mixtures, respectively.

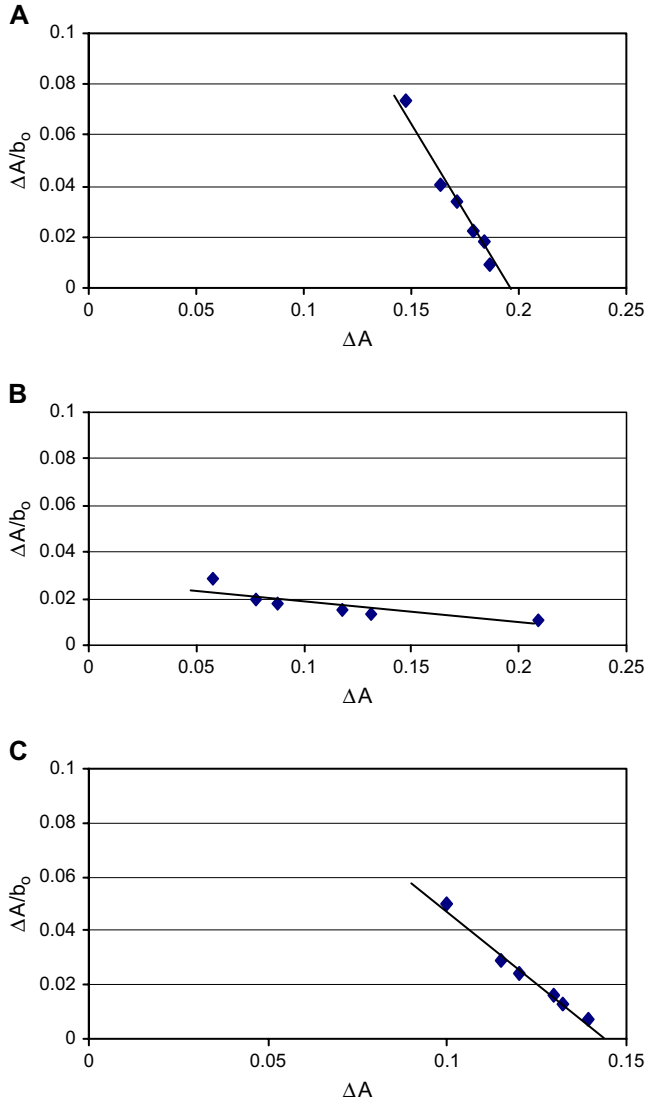

Fig. 2. Plot of $\Delta A/b_o$ versus ΔA at 290 nm. Plots obtained from the titration of THE against monomer solutions in 2–20 mM concentration range at room temperature. The concentration of THE was 0.005 mM. (A) MAA only, (B) [MAA]/[ACM]:1, (C) [MAA]/[HEMA]:1.

These results indicate that all monomers can be used to produce polymers for imprinting of THE, however, the complex formed between MAA and THE is more stable than those of others. As seen in Table 2, addition HEMA to THE–MAA mixture barely changes the association constant whereas the addition of ACM reduces the association constant by a factor of 10. That means ACM weakens the interaction between template and monomers in pre-polymerization stage. NH_2 groups in ACM as hydrogen bond donor that can interact with carboxylic acid group of MAA with hydrogen bonding. These association between monomers probably reduced the binding of THE to MAA/ACM.

Table 2
Association constants[a]

Functional monomer	K_a (M^{-1})
MAA	1.5×10^3
HEMA	4.9×10^2
ACM	0.9×10^2
MAA/HEMA	1.1×10^3
MAA/ACM	1.4×10^2

[a] Obtained from titration of THE with functional monomers.

The recognition properties of imprinted polymers depend on various factors as mentioned before, therefore the binding performance of the polymer matrix is also important and will be discussed in the following sections.

3.2. Theophylline imprinted polymers (TIPs)

Theophylline imprinted polymers were prepared as copolymer of MAA and EDMA as well as their terpolymers with HEMA and ACM by homogeneous solution–precipitation polymerization method which produced white polymer monoliths. Due to the involvement of many variables in molecular imprinting process, a systematic approach for the preparation and evaluation of these materials was made to exploit the potential of this technology. An important part of the optimization process is the stabilization of the monomer–template complex. This was achieved by high crosslinking so that 82% of the total monomer in the system was EDMA. The molar relationship between the functional monomer and the template had been found to be important with respect to the number and the quality of the recognition sites [24]. Low monomer–template ratios afford less than optimal complexation on account of insufficient functional monomer and too high monomer–template ratio yields non-selective polymer matrices. Polymers with two different monomer–template ratios, which were 4/1 and 5/1, were used in the preliminary studies. Since the polymer with 4/1 monomer ratio demonstrated better selectivity than the others, succeeded polymer preparations were achieved with this ratio. Selection of solvent is another important parameter in molecular imprinting. Polar solvents weaken the interactions between the template and the functional monomers. Chloroform, a poorly hydrogen bonding solvent (hydrogen bonding term of solubility parameter: 5.7), was found to be suitable in imprinting of THE.

The extraction efficiencies of TIP(MAA/EDMA) and TIP(MAA/ACM/EDMA) were found to be higher than 80%, which were sufficiently high to ensure the presence of selective cavities in the prepared polymer matrices. The lowest value for the extraction efficiency was obtained for TIP(MAA/HEMA/EDMA) matrix as 67% (Table 1).

3.3. Thermal analysis results

Differential scanning calorimetric results (Fig. 3) showed that all TIPs and NIPs were thermally stable up to approximately 300 °C and the endothermic peak around 300 °C was related to the process of rheological flow. Glass transition temperature was not observed due to the high level of crosslinking of the prepared polymers. A transition that was observed at 134 °C in thermogram of TIP(MAA/HEMA/EDMA) which contains THE can be explained as plasticizer effect of THE.

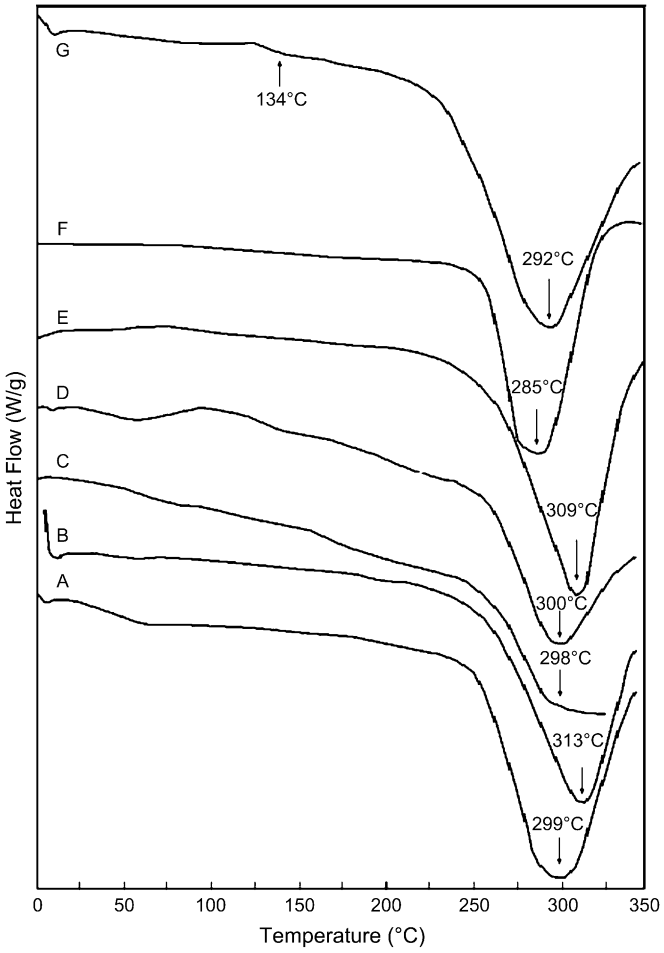

Fig. 3. DSC thermograms of imprinted and non-imprinted polymers (A) NIP (MAA/EDMA), (B) TIP(MAA/EDMA), (C) NIP(MAA/ACM/EDMA), (D) TIP(MAA/ACM/EDMA), (E) NIP(MAA/HEMA/EDMA), (F) TIP(MAA/HEMA/EDMA), (G) TIP(MAA/HEMA/EDMA) including THE.

3.4. Morphology of the polymeric matrices

The particle sizes of the grinded polymers were found to be in the range of 23–44 μm. Grinding produced irregular particles with limited control over particle size because of the nature of the polymer and the method used for grinding. On the basis of SEM microphotographs (Fig. 4), polymer particles demonstrated irregular shapes with heterogeneous surfaces. Presence of fine particles deposited on matrices is observed and this may cause an increase in non-specific adsorption of THE.

3.5. Porosity analysis results

It is now well known that a porous polymer networks form as a result of the phase separation during the free-radical crosslinking of vinyl and divinyl monomers in the presence of inert diluents. In order to obtain macroporous structures, a phase separation must occur during the course of the crosslinking process so that two-phase structure is fixed by the formation of additional crosslinks. Depending on the synthesis parameters, phase separation takes place on a macroscale or on a microscale [25].

The pore diameter of TIP(MAA/EDMA), TIP(MAA/HEMA/EDMA) and TIP(MAA/ACM/EDMA) was found as 1.72 μm, 0.0137 μm and 0.0135 μm, respectively, while that of the corresponding non-imprinted polymers was as 0.717 μm, 0.0136 μm and 0.0148 μm, respectively (Table 3). These results show that theophylline imprinted and non-imprinted copolymers of MAA/EDMA are macroporous, while the theophylline imprinted and non-imprinted terpolymers of MAA/HEMA/EDMA and MAA/ACM/EDMA are mesoporous. Porosity differences of the polymers are resulted from the effect of functional monomers on the solvation of the growing polymer chains. The larger the difference between the solubility parameter (δ) values of the copolymer (δ_2) and diluent (unreacted monomers–diluents mixture: δ_1), the stronger will be the phase separation effect between copolymer and diluent for microspherical agglomeration, resulting in higher pore volume [25,26]. However, a correct estimate of δ_2 for our polymers is difficult; firstly they are co- or terpolymers, secondly because they are highly crosslinked. In terms of site accessibility, large surface area and accessible meso- and macro-pores, which provide rapid mass transfer, are preferred [26]. While the copolymer of MAA/EDMA possesses higher average pore diameter, terpolymers of MAA/HEMA/EDMA and MAA/ACM/EDMA demonstrated higher surface areas. For a given pore volume, surface area increases with decreasing pore sizes, therefore it is difficult to compare the effectiveness of these morphological differences of polymer structures on recognition of THE. Nevertheless, a comparison can be made for theophylline imprinted and non-imprinted copolymers of MAA/EDMA in terms of the morphological differences. Higher pore diameter in the structure of imprinted one may enforce the binding to this polymer and so increases selectivity.

3.6. Binding characteristics of imprinted polymers

3.6.1. Effect of polymer concentration on binding performances of TIPs

The effects of polymer concentration on binding performance were studied by many researchers. Haupt studied the binding of 2,4-dichlorophenoxyacetic acid in the range of 0–2 mg/mL polymer concentration (poly(4-vinylpyridine-*co*-ethylene glycol dimethacrylate)) [27]. They observed that 2 mg/mL polymer could bind about 90% of the template while 0.15 mg/mL polymer concentration was enough to bind 50% of the template. On the other hand it was reported by Yılmaz et al. that equal amounts of different polymers show different bindings to the same template [28]. In our study, to determine the ideal amount of polymer for the rebinding process, incremental amounts of polymers in the range of 1.2–20 mg/mL were tested at constant THE concentration (5 μg/mL). As shown in Fig. 5, all TIPs demonstrated rapid increase in binding of THE with increasing polymer concentration up to 10 mg/mL. However, it also increased the binding to the NIPs, which represented ‘‘non-specific binding’’ in the polymer system. High non-specific binding reduces the ‘‘selective binding’’ of the polymer system [29]. Therefore, 5 mg/mL was chosen as the optimum value for polymer concentration to be used in

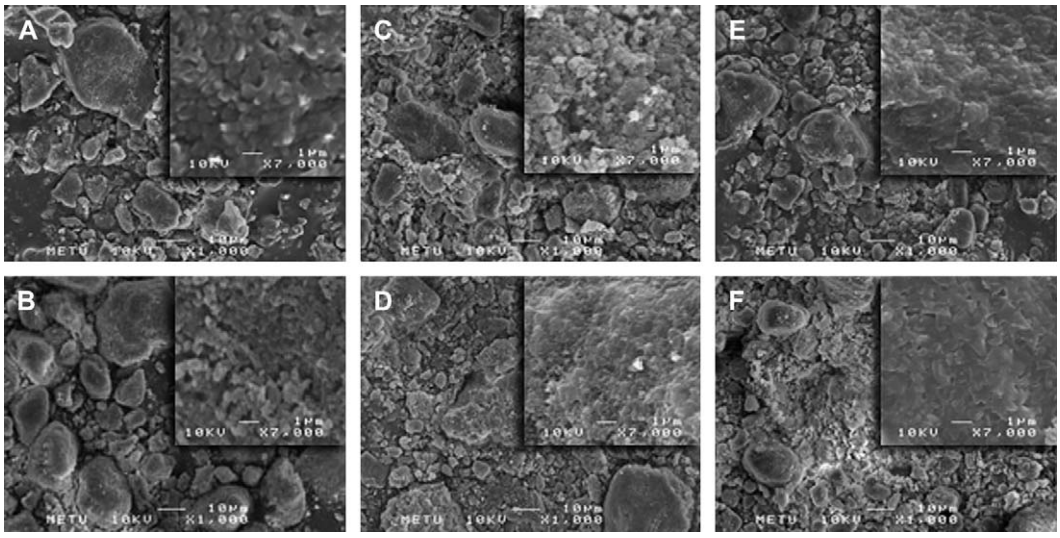

Fig. 4. Scanning electron micrographs of imprinted and non-imprinted polymers (A) NIP(MAA/EDMA), (B) TIP(MAA/EDMA), (C) NIP(MAA/HEMA/EDMA), (D) TIP(MAA/HEMA/EDMA), (E) NIP(MAA/ACM/EDMA), (F) TIP(MAA/ACM/EDMA).

Table 3
Porosity analysis results

Polymer	Specific surface area, S (m^2/g)	Average pore diameter, Dp (μm)
NIP(MAA/EDMA)	1.71	0.717
TIP(MAA/EDMA)	1.09	1.72
NIP(MAA/HEMA/EDMA)	119	0.0136
TIP(MAA/HEMA/EDMA)	118	0.0137
NIP(MAA/ACM/EDMA)	129	0.0148
TIP(MAA/ACM/EDMA)	123	0.0135

the following batch experiments to obtain maximum selective binding.

3.6.2. *Effect of theophylline concentration on binding performance of TIPs*

Binding performances of TIPs were assessed in the range of 0.5–125 μg/mL THE concentrations. The above range of THE concentration was selected according to the results reported in the literature. The maximum effect of theophylline, by avoiding the toxic side effects, is obtained with serum concentrations between 10 and 20 μg/mL [30]. In the study of Zydron et al. on HPLC analysis of methylxanthines in urine samples of asthma patients, the concentration of THE was determined as 9–30 μg/mL [31]. Schreiber-Deturmeny and Bruguerolle developed an HPLC method for the determination of THE and CAF in human plasma, in the range of 2–20 μg/mL [21].

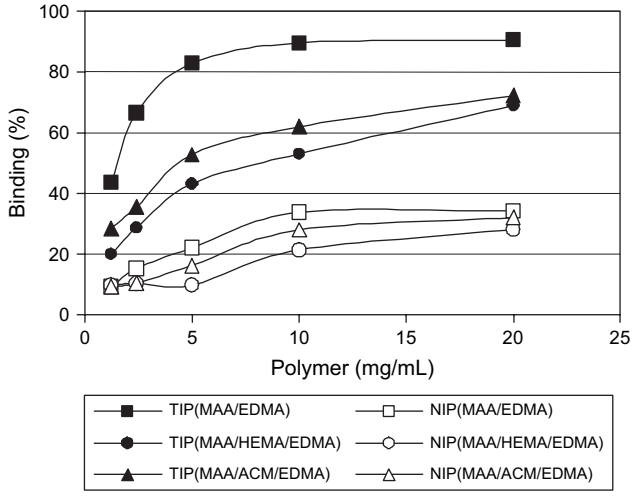

Fig. 5. Binding of theophylline by incremental amount of MIPs in the range of 1.2–20 mg/mL polymer concentration at THE concentration of 5 μg/mL.

The binding and selective binding of theophylline at equilibrium (for both imprinted and non-imprinted polymers) are given in Fig. 6(A–D). Binding results of TIP(MAA/EDMA) were found to be markedly higher than that of the others. THE binding to this polymer was increased from 20% to 92% with decreasing THE concentration from 125 μg/mL to 0.5 μg/mL value, while the binding to the non-imprinted polymer was increased from 8.1% to 37% under the same conditions (Fig. 6A). Therefore, the maximum selective binding was calculated as 61% at 5.8 μg/mL THE concentration. The results given in the literature spread in a large range of selective binding values for the similar systems. Mullet et al. studied this issue at a single THE concentration (20 μg/mL) and observed 98% THE binding to imprinted, 2.3% to non-imprinted polymers and obtained 96% of selective binding [32]. On the other hand, Allender et al. reported maximum 25% binding to imprinted and 12% to non-imprinted polymers, therefore 13% selectivity at THE concentration of 1.0 μg/mL in acetonitrile containing 5% acetic acid [33].

Terpolymer of MAA/HEMA/EDMA system demonstrated an increase in binding values from 13% to 60% for TIPs, and from 2.6% to 20% for NIPs with decreasing THE concentration (Fig. 6B). On the other hand, terpolymer of MAA/ACM/EDMA, exhibited an increase in binding value from 20% to 75% for TIPs and from 11% to 38% for NIPs under the similar concentrations of THE (Fig. 6C). The maximum selective binding values for MAA/HEMA/EDMA at 0.91 μg/mL THE concentration and for MAA/ACM/EDMA systems at 1.84 μg/mL THE concentration were obtained as 41% and 40%,

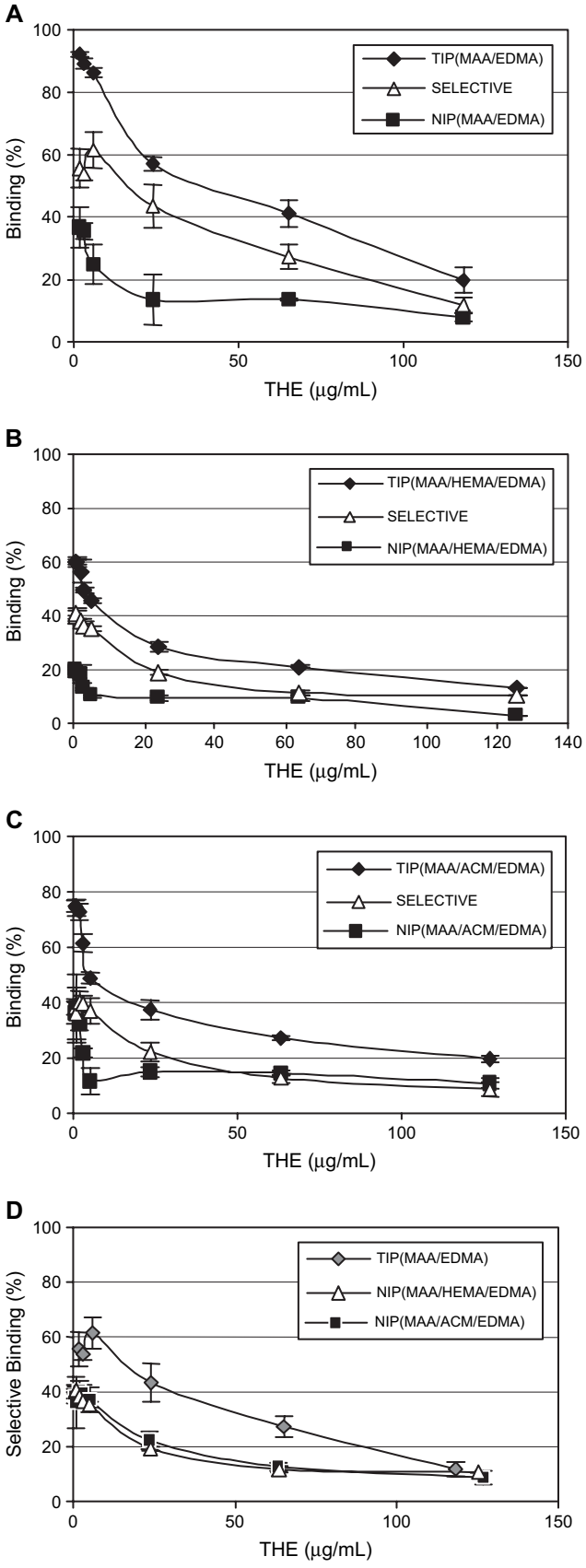

Fig. 6. Binding of theophylline by 5 mg/mL imprinted and non-imprinted polymers in the range of 0.5–125 μg/mL THE concentration (A) MAA/EDMA, (B) MAA/HEMA/EDMA, (C) MAA/ACM/EDMA, (D) Selective binding of theophylline by MIPs.

respectively. The selective binding efficiencies of TIP(MAA/HEMA/EDMA) and TIP(MAA/ACM/EDMA) terpolymers were lower than that of MAA/EDMA as it is seen in Fig. 6D.

In the matrix of MAA/HEMA/EDMA, the presence of hydroxyl groups of HEMA may achieve hydrophilic property and therefore, in rebinding step, may result in less solvation of the polymer in the non-polar solvent. This may cause less accessibility of THE to the recognition sites. In order to minimize this possible effect, a more polar aprotic solvent, acetonitrile (with 6.1 hydrogen bonding term solubility parameter) was used instead of chloroform (with 5.7 hydrogen bonding term solubility parameter) in the THE rebinding to TIP(MAA/HEMA/EDMA). The batch rebinding experiments were carried out by using 5 μg/mL of THE in acetonitrile with incremental amounts of increase in polymer in the range of 1.2–20 mg/mL. It was observed that maximum value of the selective binding of THE was reduced from 41% in chloroform to 1.0% in acetonitrile (Fig. 7). These results show that hydrogen bonding interactions between THE and functional monomers are important in rebinding of the template molecule. Acetonitrile, a more polar solvent than chloroform, weakens the interactions of monomer and THE in the rebinding step by preventing the hydrogen bond formation between the template and the monomer and therefore decreased the binding efficiency of THE. It is also reported that optimum recognition was observed when the same solvent was used in both the polymerization and rebinding steps due to the elimination of different swelling effects of solvents on the polymeric matrices [7].

3.6.3. Association constant and binding site density of TIPs

Obtained Scatchard plots are composed of two distinct sections that can be regarded as straight lines for all TIPs. These results show the existence of two classes of binding sites, one of which includes high affinity sites (Q_{S1}) and the other includes low affinity sites (Q_{S2}) (Table 4). In other words, the guest binding site in imprinted polymer is heterogeneous in nature. The amorphous nature of the polymer and the

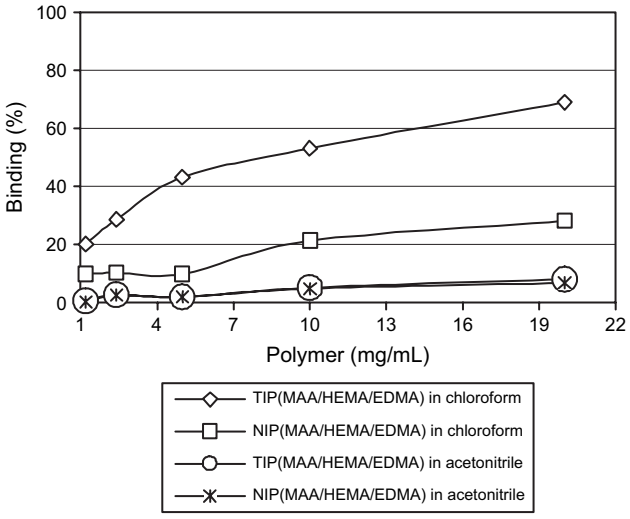

Fig. 7. Binding of 5 μg/mL theophylline by terpolymer of MAA/HEMA/EDMA in the concentration range of 1.2–20 mg/mL in chloroform and acetonitrile.

Table 4
Association constants[a] and number of binding sites of TIPs

Polymer	K_{a1} (mM^{-1})	Q_{S1} (μmol/g)	K_{a2} (mM^{-1})	Q_{S2} (μmol/g)
TIP(MAA/EDMA)	2.3×10^2	8.6	14	36
TIP(MAA/HEMA/EDMA)	0.8×10^2	4.4	4.6	26
TIP(MAA/ACM/EDMA)	1.7×10^2	4.0	3.8	40

[a] The association constant (K_a) have been determined from the linear region of the corresponding Scatchard plots.

incompleteness of the monomer–template association may reinforce this heterogeneity. The association constants for these two kinds of sites for TIP(MAA/EDMA) were determined to be 2.3×10^2 mM^{-1} for K_{a1} and 14 mM^{-1} for K_{a2}, respectively. For the other imprinted polymers, smaller association constants and lower number of binding sites were obtained. For more efficient molecular imprinting in the terpolymer systems, these binding constants may be increased by some further optimization studies carried out on the formulations and polymerization conditions.

As a result, the addition of HEMA or ACM to MAA/EDMA system as co-monomers resulted in lower binding values in both TIPs and NIPs. A probable explanation could be such that the functional groups of the resulting binding sites are arranged at more suitable positions for a stronger interaction with THE in MAA/EDMA copolymers. In addition macroporous structure of MAA/EDMA copolymers yields rapid mass transfer.

3.6.4. Selectivity of TIPs against caffeine

Selectivity analyses of TIPs were carried out by using mixtures of theophylline and caffeine solutions (both 5 μg/mL in chloroform) as shown Fig. 8. The binding values of TIP(MAA/EDMA) were obtained as 91% for theophylline and 16% for caffeine. Corresponding NIP(MAA/EDMA) matrices demonstrated binding values of 41% for theophylline and 1.8% for caffeine. Binding percentages of theophylline for TIP(MAA/HEMA/EDMA) and TIP(MAA/ACM/EDMA) were 56% and 73%, respectively, while binding percentages of caffeine were 5.2% and 7.9%, respectively. Corresponding values for the non-imprinted polymers were 22% and 38% for theophylline and 1.2% and 6.1% for caffeine. Caffeine demonstrated higher binding to TIP(MAA/EDMA) than NIP(MAA/EDMA) which may be due to porosity differences in-between imprinted and non-imprinted polymers.

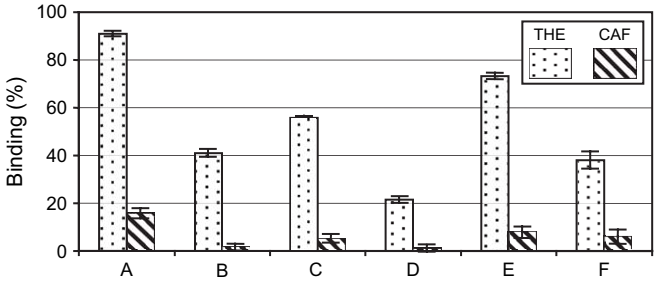

Fig. 8. Binding of 5 μg/mL THE and 5 μg/mL CAF by imprinted and non-imprinted polymers (A) TIP(MAA/EDMA), (B) NIP(MAA/EDMA), (C) TIP(MAA/HEMA/EDMA), (D) NIP(MAA/HEMA/EDMA), (E) TIP(MAA/ACM/EDMA), (F) NIP(MAA/ACM/EDMA).

The results of the selectivity of TIPs to THE in the presence of CAF showed that all TIPs exhibited high selectivity to theophylline with negligible binding to caffeine. The obvious difference of binding between theophylline and caffeine mainly results from the existence of $-CH_3$ groups on the 7-nitrogen on the structure of caffeine, instead of −H on the structure of THE. This may be the result of the loss of a hydrogen bond donor site of the molecule that interacts with the functional monomers in the recognition sites. The change in the solubility of the molecule also resulted in the higher solvent–template interaction and decreased the template–monomer interaction in the rebinding media.

4. Conclusion

Polymerization conditions and binding characteristics of molecularly imprinted polymers (MIPs) towards theophylline were established by preparing the polymeric matrices from various monomers with different functional groups. The maximum value for selective binding of TIPs was about 61% in the MAA/EDMA systems. Addition of co-monomers (ACM or HEMA) decreased the binding performance of the MAA system to about 40%. This is most probably due to the competition of the monomer–monomer interactions with the monomer–template interactions in the pre-polymerization step and morphological differences. The prepared theophylline imprinted polymers (TIPs) demonstrated an appreciable level of selectivity for the template molecule of THE when compared to non-imprinted polymers (NIPs). The matrices were also able to differentiate structurally similar molecules; such as THE and CAF.

The prepared TIPs reached to a maximum of 92% binding and 61% selective binding for THE. For these kinds of systems, it is very normal that there are different results reported in literature since there are large numbers of parameters that affect the systems. Therefore optimization studies of the systems should be achieved to include the effects of all the variables, which might need more detailed studies and longer time. Molecular simulation techniques can play important role in the designing of rational MIP systems and reduce the number of time consuming experiments. We believe those disagreements would be eliminated when molecular imprinting technology reaches to maturation point in the near future.

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