Caffeine Selectivity of Divinylbenzene Crosslinked Polymers in Aqueous Media

FREDERICK A. VILLAMENA, ARMAH A. DE LA CRUZ

National Exposure Research Laboratory, U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, Ohio 45268

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ABSTRACT: The selective binding of caffeine to polymers is of interest in developing caffeine-specific sensors. The influence of the nature and quantity of crosslinking agents and functional monomers on the selectivity and binding affinity of a polymer to caffeine is reported. A high binding affinity and selectivity of divinylbenzene (DVB) crosslinked polymers toward caffeine was exhibited by the binding competition of caffeine with several dimethylated and chlorinated xanthines and *N*-methylated uric acids in aqueous media. To understand the nature of the caffeine–polymer interaction, we performed binding studies with solvents of different polarities and ionic strengths. The binding properties of DVB-based polymers containing different functional monomers were compared with Amberlite® XAD resins. Analytes with hydrophilic and electron-withdrawing groups lowered their binding affinity with the polymer in comparison with caffeine and its dimethylated derivatives. The caffeine–polymer interaction appeared to be predominantly a hydrophobic π–π interaction but partly due to the presence of caffeine-specific sites. The reversibility of the caffeine–polymer binding was investigated, and the dissociation constants were approximated to be 27 and 6 m*M*. Dipole moments of caffeine and related molecules were estimated theoretically and were correlated with their corresponding B/T ratio, which is defined as the fraction of caffeine bound to the polymer. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 82: 195–205, 2001

Key words: molecular imprinting; π–π interaction; divinylbenzene; caffeine; polymers

INTRODUCTION

The application of conventional microbial methods to the detection of human fecal contamination in water is limited by the following factors: the extreme variability of coliform survival under various environmental conditions, the poor correlation of indicator bacteria with health effects in swimmers, and the inability of coliforms to indicate whether their source is animal or human excreta. Caffeine was used by the U.S. Geological Survey as a marker of human waste in assessing the water quality of the Mississippi River because the source of caffeine in surface waters was thought to be largely due to human contamination.[1] The use of caffeine as a fecal source marker has been hampered by the absence of a rapid, sensitive, and inexpensive method to detect its presence in water. Active research on molecularly imprinted polymers (MIPs) has shown their potential application in the fields of molecular rec-

Correspondence to: F. A. Villamena (villamena.frederick@epamail.epa.gov).

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ognition, separation, and sensor technologies.[2–5] These molecular molds have been previously found to effectively remove molecules from chemical or pharmaceutical bulk materials. In addition, they have also been used in chromatography,[6–8] radio immunoassay,[9,10] solid-phase extraction,[11,12] synthesis and catalysis,[13] and recognition systems in chemical sensors.[7,14,15] Most studies involving molecular imprinting employ organic solvents that limit their application for the environmental monitoring of water quality. Like enzymes and antibodies, MIPs have high specific binding sites that are arranged in a definite orientation. The binding sites are composed of different functional groups that can covalently or noncovalently (i.e., hydrogen bonds) bind with an analyte. Syntheses of MIPs specific to caffeine and theophylline have been reported,[9,12,16,17] but few applications of MIPs in aqueous media have been reported.[18–22] Most of these polymers use ethylene glycol dimethylacrylate (EDMA) as a crosslinking agent and methacrylic acid (MAA) as a functional monomer. Polymer selectivity in aqueous solutions is desirable because of the potential applications of these polymers in assays involving biological substances or in the development of sensors that can be used to assess water quality. The competition between water and analytes for hydrogen bonding in the recognition sites in the polymer may be overcome through the use of hydrophobic interactions between the analyte and polymer. Our work involves a preparation similar to MIP preparation, which includes the preassembly of template molecules with functional monomers, the copolymerization of monomers to form a highly crosslinked polymer, and the removal of print molecules. Caffeine selectivity was observed in aqueous solutions, but not in acetonitrile (MeCN), which was used as the porogen during polymer preparation. Another preparation involved no preassembly of template molecules with the monomer, and selectivity to caffeine was observed in aqueous solutions. Nonimprinted polymers (prepared without a template) with a specific affinity to certain molecules are very uncommon, but we observed that polymers of divinylbenzene (DVB)–MAA showed unexpected specificity to caffeine in aqueous media.

This study explored the chemistry behind this peculiar phenomenon. Although poly(styrene–divinylbenzene) copolymers (e.g., Amberlite® XAD-4) are commonly used for the decaffeination of some beverages, there has been no systematic study so far that demonstrates the chemistry behind this phenomenon. The nature of the polymer–caffeine interactions was examined via binding studies with different crosslinker molecules, functional monomers, methods of polymer preparation, solvent systems, pHs, and ion concentrations. Competition studies with other xanthine and uric acid derivatives were performed. Dipole moments of the various analytes were calculated and correlated with their binding affinity. The possible role of π–π interactions between caffeine and the polymer is discussed.

EXPERIMENTAL

Reagents

All reagents were used as purchased from Sigma–Aldrich (St. Louis, MO). The crosslinking agents EDMA, trimethylolpropane trimethacrylate (TRIM), and DVB (80% tech) and the functional monomers MAA, acrylic acid (AA), 1-vinylimidazole (VI), 2-acrylamido-2-methyl-1-propane sulfonic acid (AMPSA), itaconic acid (IA), 2-(trifluoromethyl)acrylic acid (TFMAA), and 4-vinylpyridine (VP) were used in the polymer syntheses. 2,2′-Azobisisobutyronitrile (AIBN) was used as a radical initiator. The standards used for the binding study were 8-chlorotheophylline; 8-chloroxanthine; 1,3-dimethylxanthine, 1,7-dimethylxanthine, and 3,7-dimethylxanthine; uric acid; 1,3-dimethyluric acid, 1,7-dimethyluric acid, and 3,7-dimethyluric acid; and 1,3,7-trimethyluric acid. All solvents were reagent-grade or HPLC-grade and used without further purification. A phosphate buffer (0.01*M*, pH 7.2) was used in the binding study.

Polymer Synthesis

A typical synthesis of a polymer composed of 85% DVB and 15% MAA (based on the molar percentage ratio) involved dissolving caffeine (8.5×10^{-3} mol, 1.65 g) in a mixture of MAA (3.4×10^{-2} mol, 2.93 g) and MeCN (23 mL). The crosslinker (0.202 mol, 32.81 g) and AIBN as an initiator were added (5.2×10^{-3} mol, 0.846 g) and purged with dry N_2 gas. The mixture was allowed to polymerize at 60°C for 16 h (or photochemically under UV irradiation of 365 nm at 4°C). The polymer was ground to less than 20 μm with a Fritsch vibratory ball mill grinder and washed extensively with 10% acetic acid in methanol (MeOH) and then with pure MeOH for 4–5 h with a Soxhlet

extractor. Similar polymers were also synthesized in the absence of caffeine before polymerization. The BET surface area and pore size distribution analyses were performed by Particle Technology Labs, Ltd. (Illinois).

Binding Study

Two-milliliter solutions of mixture I, with 0.1 m*M* concentrations of caffeine, 1,3-dimethylxanthine, 1,7-dimethylxanthine, 3,7-dimethylxanthine, 8-chlorotheophylline, and 8-chloroxanthine, were added to five 3-mL vials containing 10, 20, 30, 40, and 50 mg (or 10, 25, 35, and 50 mg) of polymer. Mixture II, with 0.1 m*M* concentrations of uric acid, 1,3-dimethyluric acid, 1,7-dimethyluric acid, 3,7-dimethyluric acid, and 1,3,7-trimethyluric acid, was also added to another set of vials containing the same amounts of polymer. The mixtures were shaken at room temperature for 16 h and filtered through a 0.10-μm syringe filter. The filtered solutions were analyzed by HPLC with a Waters Alliance 2690, a reverse-phase column (Waters NovaPak C_{18}; 4 μm, 50 mm $\times$ 3.9 mm i.d.), and a PDA detector (Waters 996). Good separation was achieved for mixtures I and II with 85:15 and 95:5 solvent systems (pH 7.41 for phosphate/saline buffer:MeOH), respectively. A flow rate of 1.00 mL/min and a temperature of 25°C were employed for both analyses. Mixture I was analyzed at 273 nm, whereas Mixture II was analyzed at 290 nm. Blank solutions of the mixtures (without an added polymer) were used as standards. The amounts of bound analyte versus the amounts of polymer were plotted.

Reversibility of Caffeine Binding

The caffeine binding studies consisted of four experiments with the DVB–MAA (85%/15%) blank. Each of the experiments mentioned next involved 11 vials, each containing equivalent amounts of polymer suspended in a solution containing a known concentration of caffeine. Vials were shaken for a predetermined period of time (0, 15, 30, 45, 60, 105, 120, 150, 1110, 1170, and 1230 min). Samples were then filtered, and the filtrates were analyzed by HPLC as mentioned previously:

i. The first experiment involved 5 mg/mL of polymer in 6 m*M* caffeine.
ii. For the second experiment, 10 mg/mL of polymer was suspended in a 3 m*M* caffeine solution.
iii. In the third experiment, 20 mg of polymer was suspended in 1 mL of 6 m*M* caffeine. The caffeine concentration was similar to that of mixture i, but the polymer concentration was higher. This mixture was shaken for 60 min and then diluted with 1 mL of a phosphate buffer to make the final caffeine and polymer concentrations similar to those of mixture ii.
iv. In the final experiment, the mixture consisted of 10 mg of polymer suspended in 1 mL of 3 m*M* caffeine. The caffeine and polymer concentrations were similar to those of mixture ii. The solution was shaken for 60 min, and 1 mL of 9 m*M* caffeine was added to make the final caffeine and polymer concentrations similar to those of mixture i.

Scatchard Analysis

The dissociation constant (K_D) and maximum number of binding sites (B_{max}) in the polymer could be extracted from the Scatchard equation $B/[F] = (B_{max} - B)/K_D$, where B is the amount of caffeine bound to the polymer and [F] is the concentration of free caffeine.[23] A plot of $B/[F]$ versus B was constructed and curve-fitted to give K_D and B_{max} values from the estimated slope and intercept, respectively. To generate the Scatchard plot, we used an assay with DVB–MAA nonimprinted polymer. Two milliliters of 2.0, 2.5, 3.0, 3.5 and 4.0 m*M* caffeine solutions in a phosphate buffer was added to each of the five vials containing 10 mg of polymer. The mixtures were shaken overnight and filtered, and the concentrations were determined by HPLC.

Geometry Optimization

The program used for this study was HyperChem 5.1 from Hypercube, Inc. (Gainesville, FL).[24] Dipole moments were estimated for the xanthine and uric acid molecules and their corresponding tautomers via AM1 and MNDO semiempirical quantum chemistry methods. Additional dipole moments were estimated at the *ab initio* level with a minimum basis set of ST0-3G. The Polak–Rebiere (conjugate gradient) algorithm method was employed for all calculations at an RMS gradient of 0.1 kcal/(Å mol). Dipole moments from these calculations were compared and correlated with the B/T ratio of the corresponding molecules, the ratio of the amount of caffeine bound (B) to the total amount (T) added to the test tube.

Table I Binding Ratios of Xanthine Derivatives with Various Polymers

			B/T[a,b]			
Entry	% Crosslinker	% Monomer	Caffeine	3,7-	1,7-	1,3-
T-1	95% TRIM	5% MAA	0.16	0.04	0.07	0.09
T-2	80% TRIM	20% MAA	0.75	0.41	0.56	0.61
E-1	91% EDMA	9% MAA[c]	0.46	0.08	0.32	0.35
E-2	91% EDMA	9% MAA	0.34	0.03	0.27	0.21
E-3	80% EDMA	20% MAA	0.35	0.11	0.20	0.22
D-1	89% DVB	11% MAA	0.75	0.08	0.25	0.35
D-2	85% DVB	15% MAA[c]	0.95	0.50	0.59	0.66
D-3	85% DVB	15% MAA	0.96	0.53	0.63	0.69
D-4	76% DVB	24% MAA	0.97	0.72	0.82	0.82

[a] Defined as the fraction of caffeine bound to the polymer equilibrated for 16 h in 0.01*M* phosphate buffer at pH 7.2 with 10 mg of the polymer.

[b] 3,7-, 1,3-, and 1,7- refer to the dimethylated xanthines. Caffeine is 1,3,7-trimethylxanthine. All polymers were synthesized by thermal polymerization at 40°C in the absence of caffeine.

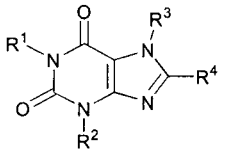
[c] Prepared in the presence of caffeine prior to polymerization.

RESULTS AND DISCUSSION

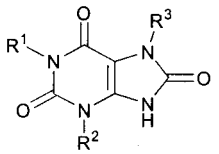
Elucidation of Binding Mechanism

Optimization of Experimental Conditions

The optimization of polymer selectivity and binding affinity to caffeine was achieved with various crosslinkers, functional monomers, crosslinker–functional-monomer ratios, polymerization conditions, and porogen natures. Table I shows the B/T ratio of various nonimprinted polymers for compounds **1**–**4**:



xanthine



uric acid

1 $R^1 = R^2 = R^3 = Me, R^4 = H$
2 $R^1 = R^2 = Me, R^3 = R^4 = H$
3 $R^1 = R^3 = Me, R^2 = R^4 = H$
4 $R^1 = R^4 = H, R^2 = R^3 = Me$
5 $R^1 = R^2 = Me, R^3 = H, R^4 = Cl$
6 $R^1 = R^2 = R^3 = H, R^4 = Cl$
7 $R^1 = R^2 = Me, R^3 = (CH_2)_2OH, R^4 = H$

8 $R^1 = R^2 = R^3 = Me$
9 $R^1 = R^2 = Me, R^3 = H$
10 $R^1 = R^3 = Me, R^2 = H$
11 $R^1 = H, R^2 = R^3 = Me$

MAA was copolymerized with TRIM, EDMA, and DVB as crosslinking agents. Higher binding affinity is indicated by a larger B/T ratio, and selectivity was inferred from the highest B/T ratio of caffeine to xanthine derivative. In general, binding to the polymers of compounds **1**–**4** gave considerably higher B/T values than binding to chlorinated xanthines **5** and **6** and uric acid derivatives **8**–**11**, which all showed poor binding to most polymers, with B/T ratios of almost 0 (see Fig. 1). Binding studies on polymers prepared with dichloromethane or MeCN as a porogen showed no significant difference in the binding affinity and selectivity to caffeine. Polymers containing low crosslinker concentrations showed better binding affinity to caffeine, but their selectivity to caffeine decreased. For example, compare T-2 and T-1: T-2 was 80% crosslinked and bound 75% of the caffeine in solution, whereas T-1, which was 95% crosslinked, had a 16% binding with caffeine. However, better selectivity was observed for T-1 with a $B/T_{1,3\text{-dimethylxanthine}}/B/T_{caffeine}$ value of

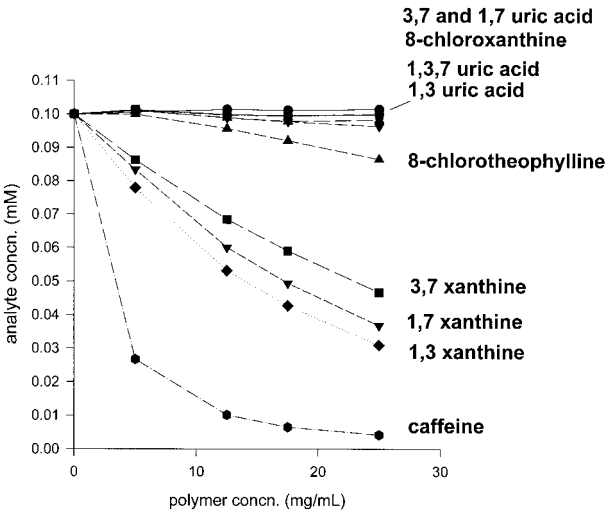


Figure 1 Binding profile of D-3 with caffeine and structurally related compounds. Unbound analytes were measured after being allowed to equilibrate with various amounts of polymer in a 0.01*M* phosphate buffer (pH 7.2) at ambient temperature.

0.56; the value for T-2 was 0.81. This lesser selectivity to caffeine may be the result of poor rigidity of the polymeric matrix[4] and may allow the cavities to be more accessible to other analytes. Lower crosslinker concentrations could also increase the number of carboxyl functional groups that could hydrogen-bond with caffeine and other analytes.

Binding Properties of DVB–MAA Polymers

The DVB-based polymers D-1 to D-4 gave the highest binding affinity for caffeine, with D-1 exhibiting the lowest affinity for caffeine. However, selectivity was compromised for high caffeine affinity for polymers D-2 to D-4 because the B/T ratios for dimethylated xanthines were higher than the ratio for D-1. No significant difference in the binding affinity for caffeine was observed between D-3 and D-4. The binding capacities observed for D-3 and D-4 were surprisingly high, with a B/T ratio reaching almost 1.0, but selectivity was again compromised with a higher MAA content for D-4. This lowering of selectivity toward caffeine with increasing MAA content may be due to the presence of more carboxyl groups available for hydrogen bonding with the analytes. The slope of the curve for caffeine (Fig. 2) is also much higher for the DVB-based polymers than the slopes observed for EDMA and TRIM polymers, indicating more favorable binding with caffeine. The rationale behind this unexpected binding specificity of nonimprinted DVB–MAA polymers to caffeine may be due to the influence of weak intermolecular interactions between the analyte and the polymer. Similar observations were also reported by Hosoya et al.[25] on nonimprinted EDMA polymers with an unusual recognition ability toward certain polyaromatic hydrocarbons due to the inherent nature of the polymers prepared.

Comparison of Binding Properties of Imprinted and Nonimprinted DVB-Based Polymers

The similarities in the binding affinity and selectivity for caffeine of both D-2 and D-3 prepared in the presence and absence of caffeine, respectively, may suggest that imprinting may not be the major factor in the adsorption process. To further determine whether such binding behavior is due to imprinting or changes in polymer morphology resulting from the presence of foreign compounds during polymerization, we prepared a DVB polymer in the presence of 1,3,7-trimethyl uric acid (**8**). The result showed a significant improvement, with a B/T ratio of 0.28 for **8** with the DVB polymer prepared in the presence of **8** compared with a B/T ratio of almost 0 for **8** with the DVB polymer prepared in the presence of caffeine (Fig. 1). However, caffeine B/T ratios in both **8** and caffeine

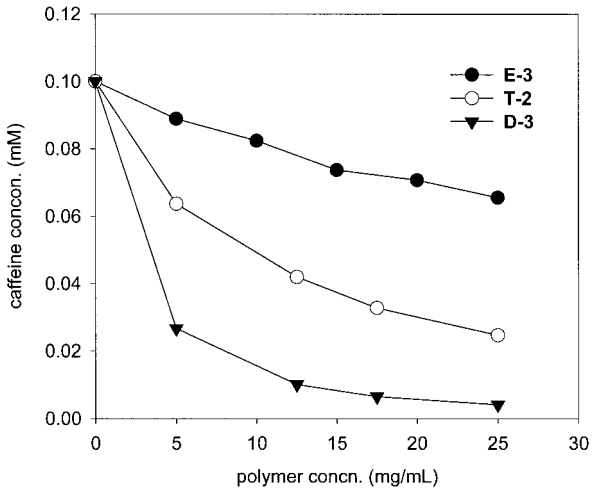


Figure 2 Binding profile of caffeine (**1**) with various crosslinker molecules: (●) E-3 (80% EDMA and 20% MAA), (○) T-2 (80% TRIM and 20% MAA), and (▼) D-3 (85% DVB and 15% MAA). Unbound analytes were measured after being allowed to equilibrate with various amounts of polymer in a 0.01*M* phosphate buffer (pH 7.2) at ambient temperature.

Table II Surface Area and Pore Analysis of Selected Polymers

Polymer	Surface Area[a] (m^2/g)	Pore Volume[b] (mL/g)	Pore Diameter[c] (Å)
D-2	416	0.28	25
D-3	169	0.13	32

[a] Determined with a BET model on a three-point linear plot.
[b] Total pore volume for pores smaller than 3848 Å except for D-2 of 6395 Å.
[c] BJH adsorption average pore diameter (4 × pore volume/surface area) of pores between 12 and 2400 Å except for D-2 (between 12 and 3700 Å).

MIPs remained unchanged. This suggests that the imprinting process plays a minor role in the DVB polymer binding affinity and selectivity to caffeine, and these characteristics are more likely due to the polymer morphology, including porosity and surface area, which favors caffeine binding (see Table II). Moreover, the presence of caffeine during polymer preparation affects the polymer morphology, as indicated by a higher surface area and pore volume, but no significant effect on the caffeine selectivity or affinity was observed within the range presented in Table II. To further demonstrate the selectivity of the polymers toward caffeine, we carried out a microscale experiment involving the extraction and rebinding of caffeine from the polymers. The rebinding of caffeine was done after the exhaustive extraction of caffeine from the polymers but showed no significant rebinding of caffeine. This indicates that the solvation of caffeine by MeCN inhibits its affinity toward the polymers. However, the recovery percentage data obtained with MeCN, which was employed during polymerization, may provide some insights into the formation of caffeine-imprinted sites. As demonstrated in a previous work on the recovery of tertbutylazine from various MIPs,[26] polymers with caffeine-imprinted sites should yield relatively low caffeine concentrations in the supernatant during extraction, and polymers with poor affinity should give caffeine concentrations close to the initial caffeine concentration if all the caffeine goes into the solution. Table III shows the polymers tested for selectivity and their corresponding yields of caffeine in solution after extraction. Yields lower than 80% were observed in polymers without functional monomers and those that had MAA, TFMAA, IA, and AA. The presence of caffeine-imprinted sites from hydrogen bonding with the carboxyl groups, from π–π interaction from DVB, or from both may be responsible for the high retention of caffeine in these polymers in MeCN. This further supports the presence of caffeine-imprinted sites when the polymers are formed in the presence of caffeine. These results as a whole indicate that polymers prepared in the presence of caffeine have an inherent affinity toward caffeine that may be partly due to adsorption from molecular imprinting.

Comparison of Binding Properties of DVB-Based Polymers and Resins

The binding properties in aqueous solutions of DVB-based polymers were compared with those of the Amberlite® resins XAD-4 and XAD-7, which are commonly used for industrial caffeine-extraction processes (see Table IV). The results suggest that the binding affinity of the polystyrene–DVB resin XAD-4 is comparable to that of the DVB polymers but has relatively lower selectivity; however, the acrylic–ester-based resin XAD-7 gave poor binding affinity and selectivity toward caffeine. This demonstrates the role of DVB in caffeine selectivity due to the possible π–π interaction. The π–π interaction, hydrogen bonding, or both may be the modes of interaction between caffeine and DVB polymers. To investigate the

Table III Total Recovery of Caffeine from the MIPs After Extraction

MIP	% Yield
DVB	74.6
DVB–MAA	52.1
DVB–TFMAA	80.0
DVB–VI	110.7
DVB–IA	69.2
DVB–VP	91.5
DVB–AA	51.8
EDMA	70.9
EDMA–MAA	60.1
EDMA–TFMAA	63.6
EDMA–VI	87.2
EDMA–IA	75.7
EDMA–VP	94.0
EDMA–AA	80.8

Prepared in the presence of caffeine during polymerization. After polymerization, 1 mL of MeCN was added to each vial and sonicated for 1 hr at 40°C. The supernatant was analyzed for the amount of caffeine by HPLC. The concentration of free caffeine was calculated with reference to an external standard, and the percent yield was calculated based on the theoretical concentration of 12.5 m*M*.

Table IV Binding Ratios of Xanthine Derivatives with Various DVB-Based Polymers

			B/T[b,c]			
Entry	% Crosslinker	% Monomer[a]	Caffeine	3,7-	1,7-	1,3-
M-1	100% MAA		0.07	0.00	0.04	0.02
XAD-4	Polystyrene-DVB copolymer		0.94	0.74	0.84	0.79
XAD-7	Acrylic ester		0.59	0.26	0.42	0.38
D-5	100% DVB		0.94	0.35	0.49	0.60
D-6	85% DVB	15% AA	0.98	0.68	0.76	0.78
D-7	85% DVB	15% VI	0.99	0.49	0.70	0.73
D-8	85% DVB	15% AMPSA	0.96	0.83	0.85	0.64
D-9	85% DVB	15% IA	0.99	0.88	0.97	0.93
D-10	85% DVB	15% TFMAA	0.97	0.73	0.82	0.78
D-11	85% DVB	15% VP	0.99	0.42	0.60	0.63

[a] All polymers were nonimprinted and were prepared by thermal polymerization.
[b] Defined as the fraction of caffeine bound to the polymer equilibrated for 16 h in 0.01*M* phosphate buffer at pH 7.2 with 10 mg of the polymer.
[c] 3,7-, 1,3-, and 1,7- refer to the dimethylated xanthines. Caffeine is 1,3,7 trimethylxanthine.

predominant interaction present in the system, we prepared pure D-5 and M-1 polymers (Table IV). M-1 gave almost a 0 B/T ratio for caffeine and dimethylxanthines, whereas D-5 polymer showed a higher B/T ratio and selectivity toward caffeine (Table IV). The higher binding affinity in D-5 suggests that π–π interaction is the dominant mode of interaction between caffeine and the DVB–MAA polymer because pure DVB does not contain any carboxyl group to produce hydrogen bonding. Aside from a typical aromatic π–π stacking interaction that usually occurs between π-electron-deficient and π-electron-rich ones, the caffeine–DVB polymer interaction may also involve a CH–π type of interaction known to occur in some systems.[27] This supports the hypothesis that the binding and specificity of the DVB polymer to caffeine are governed primarily by hydrophobic interactions rather than purely by the presence of caffeine-specific sites.

Effect of the Nature of the Functional Monomers

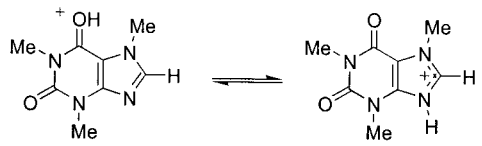
To investigate the effect of the nature of the functional monomers on the polymer binding, we prepared several nonimprinted DVB polymers with different functional monomers and performed binding studies on the polymers. Table IV shows the B/T ratios of various DVB polymers (D-6 to D-11) with different functional monomers. Although almost all of the DVB polymers exhibited comparatively high B/T ratios for caffeine, some showed poor selectivity, as demonstrated in polymers D-6, D-8, D-9, and D-10. D-7 and D-11 had better binding selectivity with respect to the rest of the polymers. This high selectivity of the polymers further demonstrates how additional π–π interactions from imidazole and pyridine moieties can improve polymer selectivity in aqueous media.

Effect of the Nature of the Solvents

Binding studies on D-3 were performed at various pHs (4, 7, and 10) and in organic solvents such as MeCN, CH_2Cl_2, hexane, acetone, MeOH, and ethanol. Results showed that caffeine binding to the polymer is inhibited at pH 4, which may be due to the disruption of the π system due to protonation (Scheme 1) and increased hydrophilicity of caffeine. However, no inhibition was observed at pHs 7 and 10. Binding was also inhibited in all of the organic solvents, probably because of solvation of the caffeine molecule or the polymeric surface, thus preventing weak hydrophobic π–π interactions.

Effect of Ionic Strength

In contrast to binding in nonpolar solvents, the effect of the ion concentration on the binding



Scheme 1 Protonation of caffeine.

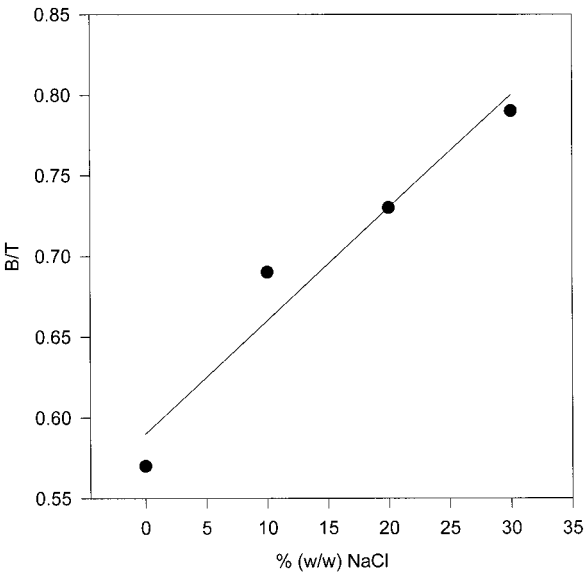
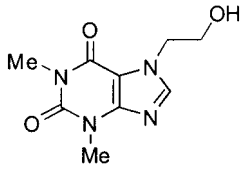


Figure 3 Effect of the NaCl concentration on the B/T ratio with 10 mg of D-3 in a 0.1 m*M* caffeine aqueous solution equilibrated for 16 h at 25°C.

property of D-3 showed an increasing B/T ratio for caffeine with increasing ionic strength (Fig. 3). The presence of ions increases the polar character of the solvent, thus enhancing the hydrophobic interaction of the solute and polymer. These results on the effect of the ion concentration further support the hydrophobic nature of interactions between the caffeine and polymer.

Effect of Analyte Structure

The presence of hydroxyl groups in the analyte molecule affects its binding to the polymer. The hydrophilic interaction of the hydroxyl group in β-hydroxyethyl theophylline (BHET; **7**) with water may be competing with the hydrophobic π–π interaction of the purine moiety with the polymer. As demonstrated in Figure 4, compound **7** had a lower binding affinity than caffeine despite their structural similarities, with substitutions at positions N-1, N-2, and N-3. This binding points to the importance of π–π interactions in caffeine binding:



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Estimating the Magnitude of π–π Interactions

The results of the experimental binding studies point to the importance of π–π interactions for caffeine binding. For the purpose of potential applications, it is useful to gain insight into the magnitude of the π–π interactions that appear to govern caffeine binding. To do this, we performed kinetic binding studies and calculated estimated dipole moments.

Reversibility of the Caffeine Binding and Scatchard Plot

Kinetic studies show that the binding of caffeine with the D-3 polymer is reversible (Fig. 5). This reversibility indicates that caffeine is not degraded in the presence of the polymer and shows potential for these polymers in the development of sensors for caffeine. As shown in Figure 6, the Scatchard plot exhibits nonlinearity, suggesting the presence of heterogeneous binding sites in D-3. Two straight lines can be drawn within the plot that can be interpreted as the polymer having two binding sites, each with distinct binding properties. The plot gave K_D's of 27 and 6 m*M* and respective B_{max}'s of 22 and 11 μmol g^{-1} of dry polymer. Vlatakis et al.[9] reported K_D's of approximately 0.4 and 65 μM and respective B_{max}'s of 0.016 and 1.3 μmol g^{-1} from the molecularly imprinted sorbent assay for theophylline with ED-

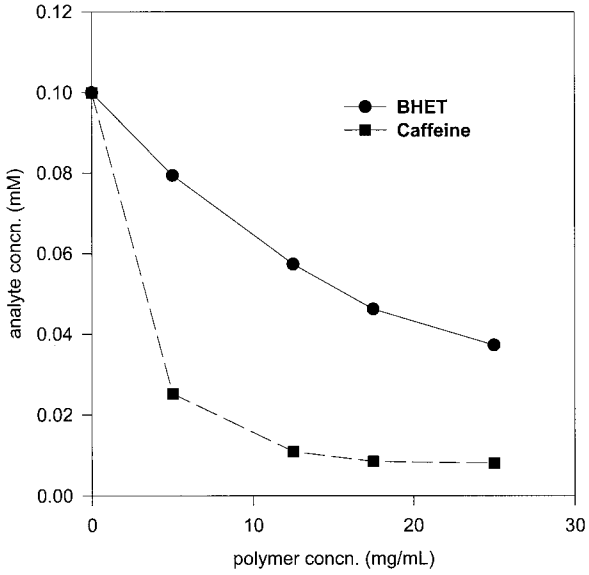


Figure 4 Binding profile of caffeine (**1**) and BHET (**7**) for several polymer concentrations. Analyte concentrations were measured after equilibration in a phosphate buffer (pH 7.0) with various amounts of D-3.

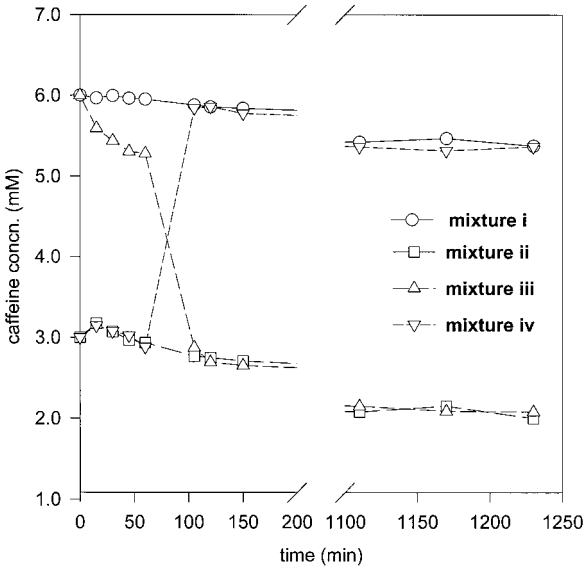


Figure 5 Graph of the reversible nature of caffeine binding to D-3: (i) 10 mg of polymer with 2 mL of 6 m*M* caffeine (5 mg/mL); (ii) 20 mg of polymer with 2 mL of 3 m*M* caffeine (10 mg/mL); (iii) 20 mg of polymer with 1 mL of 6 m*M* caffeine, with the caffeine concentration similar to that of mixture i, except for the polymer concentration (note the absorption of caffeine due to excess polymer), and with the mixture then diluted with 1 mL of a phosphate buffer after 60 min of equilibration to make the final caffeine and polymer concentrations similar to those of mixture ii; and (iv) 10 mg of polymer with 1 mL of 3 m*M* caffeine, with caffeine and polymer concentrations similar to those of mixture ii, and with 1 mL of 9 m*M* caffeine added after 60 min of equilibration to make the final caffeine and polymer concentrations similar to those of mixture i.

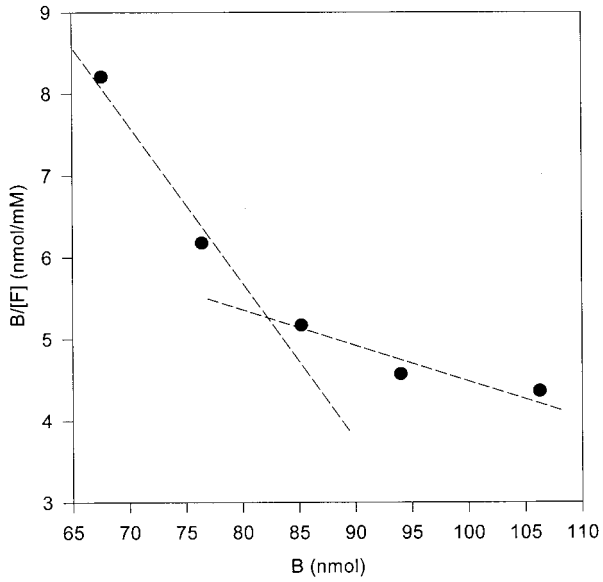


Figure 6 Scatchard plot for the estimation of K_D and B_{max} for the binding of caffeine to D-3.

Scheme 2 Keto-enol form of a xanthine derivative.

MA–MAA polymer. The calculated values can provide a rough estimate of the degree of affinity to caffeine achievable with these types of polymers.

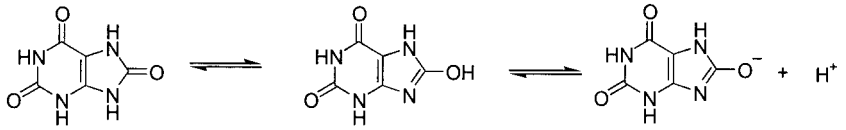
Correlation of the Calculated Dipole Moments with the Affinity of the Analytes

Dipole moments may be indicative of π–π interactions because the calculation uses π–π terms.[24] To calculate the dipole moment, we must know the tautomeric form of the molecules in an aqueous medium. Xanthine derivatives can undergo tautomerization, as shown in Scheme 2; however, ^{1}H-NMR[28] and *ab initio*[29] computational studies (*in vacuo* and in an aqueous environment) show that the keto form is preferred. All dipole moments of xanthines are based on their keto forms, as shown in Table V. However, for uric acids, the enol form is predominantly present in solution with a pK_a value of 5.22[30] (Scheme 3), and the dipole moments were calculated with the enol form. Estimates of the dipole moments of the analytes based on theoretical calculations on the semiempirical and *ab initio* levels reveal that the

Table V Estimated Dipole Moments for Various Analytes

	Dipole (debye units)		
Entry	MNDO	AM1	*Ab initio*
1	3.939	3.676	3.2445
2	3.580	3.293	2.8562
3	4.077	3.921	3.3454
4	4.499	4.204	3.4466
5	2.255	2.425	1.2437
6	2.963	3.175	1.5943
7	3.601	1.999	2.7460
8	3.658	3.191	2.9390
9	3.867	3.301	2.6783
10	4.668	4.270	2.9227
11	4.292	3.848	3.3130

Calculations for compounds 1–7 were based on the keto form, whereas calculations for 8–11 were based on the enol form (Scheme 3).



Scheme 3 Keto-enol form of uric acid.

chlorinated xanthines **5** and **6** gave comparatively lower dipole moments than the nonchlorinated xanthines. The presence of the electron-withdrawing Cl group can inductively lower the electron π density within the molecule, thereby enhancing its binding affinity with the π electron-rich DVB polymer. However, this was not the case for **5** and **6** because they exhibited low binding affinity. Compounds **1**–**4** gave higher dipole moments than compounds **5** and **6** but showed no linear correlation with their corresponding B/T ratio. For example, **1** did not give the highest dipole moment, although it had the highest B/T ratio of the analytes. These results indicate that the presence of caffeine-specific sites in the DVB polymer may be a contributing factor in the magnitude of the B/T ratio. However, as expected for dimethylated and trimethylated uric acids, **8**–**11** gave relatively high dipole moments and showed poor affinity for the polymer. This may also be due to the formation of the enol tautomer (Scheme 3) in solution, in which the hydrophilic interaction with water predominates.

CONCLUSION

This study demonstrate the nonspecific yet selective adsorption of caffeine by some polymers to caffeine. DVB as a crosslinker with the MAA monomer showed the highest affinity and selectivity toward caffeine in comparison with other polymers with different crosslinking agents. The nature of the caffeine–DVB polymer interaction appears to be a synergy between the predominant π–π interaction and the presence of caffeine-specific sites. These interactions can be affected significantly by the solvent polarity and the nature of the functional monomers and analytes. This study further demonstrates how the selectivity for certain analytes by some polymers can be affected by both hydrophobic and structural-type interactions.

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