Preparation and Binding Evaluation of Histamine-Imprinted Microspheres via Conventional Thermal and RAFT-Mediated Free-Radical Polymerization

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Supporting Information

ABSTRACT: Elevated histamine (HTM) levels are closely linked to food poisoning as well as to pathophysiological allergic diseases. In this study, HTM-imprinted, solution-processable microspheres were prepared via high-dilution conventional thermal polymerization (CTP) and controlled radical polymerization (CRP) using ethylene glycol dimethacrylate (80 or 90 wt %) and methacrylic acid at 60 °C in acetonitrile and evaluated as recognition materials for sensing applications. The polymers were selective to HTM in binding studies, cross-rebinding, and competitive binding assays against the HTM analogues histidine, imidazole, and tryptamine. The selective binding capacity was significantly higher with CTP-80 (on the basis of mass: 21.0 μmol/g and surface area: 8.08 × 10−2 μmol/m2) than that with both CTP-90 (8.47 μmol/g, 4.49 × 10−2 μmol/m2) and CRP-80 (9.00 μmol/g, 1.19 × 10−2 μmol/m2).

INTRODUCTION

Molecularly imprinted polymers (MIPs) are synthetic materials with recognition properties that have been used in separation, catalysis, chemical sensing, and drug-discovery applications.1,2 The first generation of MIPs was prepared using traditional polymerization processes, in which polymer chain propagation and termination were hard to control. This often resulted in irregularly shaped, monolithic polymers that required grinding and sieving before use.3 In addition, these polymers were insoluble in most solvents and required exhaustive extraction for removal of the template. The presence of heterogeneous pockets within the polymer network structures also sometimes compromised the binding of the material with the template, resulting in low-affinity or nonselective binding. Over the years, the development of traditional radical precipitation polymerization (TRPP) allowed the preparation of MIPs in nano/submicrosphere formats. This process involved reaction under highly dilute conditions, where soluble branched oligomers were continuously captured from the solution, leading to the growth of the particles. Progressive growth occurred until the particles were no longer soluble in the reaction medium. Unlike that in emulsion or surfactant polymerization, no extra stabilizer was needed.4,5

Recently, controlled radical polymerization (CRP) techniques, namely, atom transfer radical polymerization,6,7 reversible addition fragmentation transfer (RAFT),8 nitroxide-mediated9 and photochemical-initiferter-induced10 polymerization, have been found to allow the generation of reactive chains that can undergo reversible propagation/termination cycles. The process involves a reversible chain transfer agent (CTA; typically a dithioester for RAFT), where there is a dynamic equilibrium between the active propagating radical species and the inactive thiocarbonylthio-terminated species. When a RAFT agent is introduced during precipitation polymerization, the RAFT mechanism can be imparted to the reaction. The controlled nature of RAFT polymerization is attractive for the preparation of MIPs, providing polymers with increased structural homogeneity and improved properties in comparison with those of the MIPs prepared via TRPP.11–13 In addition, functional polymer microspheres with tailor-made recognition sites and functional dithioester surface end groups can be produced. These dithioester groups facilitate further reaction or surface modification by reinitiation via RAFT polymer-
Our investigation focused on the in-binding data expressed with respect to mass versus surface area. 

The motivations of this study were as follows: to increase our understanding on the use of CTA in RAFT polymerization, to prepare processable MIPs that can be used as recognition elements for optical-based HTM sensing, and to analyze the binding data expressed with respect to mass versus surface area. Our investigation focused on the influence of the initiation/polymerization method on the binding performance of these HTM-imprinted microspheres.

HTM (1), the template of choice for this study, is one of the biogenic amines associated with food spoilage\textsuperscript{26,27} and pathophysiological conditions related to allergy.\textsuperscript{28} HTM is found naturally in foods, such as vegetables, fruits, fish, and cheese, in small quantities. Elevated levels of HTM occur as foods spoil, which when ingested can result in food poisoning. Hence, HTM levels are used to monitor and assess the safety and quality of food products to ensure that the concentration does not go beyond its safe threshold (i.e., 50 ppm, 5 mg/100 g).\textsuperscript{31} HTM is also found in human tissues at relatively low concentrations (0.1–20 μg/g). It is stored primarily in mast cells in tissues and basophils in blood, where it is tightly bound with heparin in membrane-bound granules. Upon exposure of cells to an antigen or a wide range of drugs, HTM can be released.\textsuperscript{32} Thus, there is a need to develop assays to measure HTM levels in food, urine, and tissues.

**EXPERIMENTAL SECTION**

**Materials and Reagents.** HTM (1), l-histidine (HTD, 2), imidazole (IDZ, 3), and tryptamine (TTM, 4) were purchased from Sigma-Aldrich and used as received. Methacrylic acid (MAA, 5) and ethylene glycol dimethacrylate (EGDMA, 6) were purchased from Sigma-Aldrich and purified by passing through basic alumina (Al\textsubscript{2}O\textsubscript{3}) columns. AIBN was obtained from Sigma-Aldrich and recrystallized in ethanol twice before use. Carbon disulfide and 1-butanol (both from Sigma-Aldrich) were used as received. MCEBTTC (7) was synthesized according to the procedure outlined below. High-performance liquid chromatography (HPLC)-grade solvents, such as dimethyl sulfoxide (DMSO) (Sigma-Aldrich), acetonitrile (MeCN) (Honeywell Burdick & Jackson), and methanol (Fisher Scientific), were used without further purification. Na\textsubscript{3}PO\textsubscript{4} (BDH Chemicals), NaH\textsubscript{2}PO\textsubscript{4}·2H\textsubscript{2}O (AJAX Chemicals), KH\textsubscript{2}PO\textsubscript{4} (Sigma-Aldrich), and H\textsubscript{3}PO\textsubscript{4} (85%) (Sigma-Aldrich) were used as received (Figure 1).

**Synthesis of RAFT Agent MCEBTTC, 7.** MCEBTTC was synthesized according to the procedure in the literature.\textsuperscript{32} Briefly, carbon disulfide (6.18 mL, 0.103 mol) dissolved in dichloromethane (DCM) (50 mL) was added to a stirred solution of 1-butanol (10 mL, 0.093 mol) and triethylamine (14.3 mL, 0.103 mol) in DCM (100 mL) at 0 °C under N\textsubscript{2} over 30 min. This reaction mixture was stirred for 1 h, followed by the addition of 1-methylbromopropionate (11.5 mL, 0.103

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**Figure 1.** Structures of HTM (1), HTD (2), IDZ (3), TTM (4), MAA (5), EGDMA (6), and MCEBTTC (7).
mol) in DCM (50 mL), and this mixture was stirred for 2 h. After the reaction, DCM was removed and the residue was dissolved in diethyl ether. This solution was then washed with cold 10% HCl solution (3 × 50 mL) and MilliQ water (3 × 50 mL) and then dried over anhydrous MgSO₄. The ether was removed under vacuum, and the residual yellow oil was purified by column chromatography (9:1 petroleum ether/ethyl acetate on silica). Subsequently, a yellow RAFT agent was obtained. ¹H NMR (CDCl₃): δ = 0.92 (t, J = 7.5 Hz, 3H, CH₃), 1.43 (mult, J = 7.5 Hz, 2H, CH₂), 1.62 (d, J = 7.5 Hz, 3H, CH₃), 1.65 (quin, J = 7.5 Hz, 2H, CH₂), 3.36 (t, J = 7.5 Hz, 2H, CH₂), 3.73 (s, 3H, CH₃), 4.84 (q, J = 7.5 Hz, 1H, CH); ¹³C NMR (CDCl₃): δ = 13.55, 16.91, 22.02, 29.89, 36.94, 47.68, 52.82, 171.6 [CH−C(=O)−O], 221.9 [S−C(=S)−S].

Synthesis of Microspheres: Determination of Critical Monomer Concentration for Precipitation (cmpc) and Solution Processability. Microspheres were prepared using an MAA/EGDMA (functional monomer/crosslinker) ratio of 30:70, 20:80, or 10:90 wt%. The monomer concentration was varied from 1 to 10 wt% of the total solution and diluted with an appropriate solvent. The initiator, AIBN (3 wt% with respect to the monomer concentration), was added to the reaction mixture and subsequently purged with N₂ for 5 min. Thermal polymerization was carried out with stirring at 300 rpm at 60 °C for 24 h. After polymerization, the microspheres were isolated by solvent evaporation, for those prepared in DMSO. Once dried, the microspheres were washed with diethyl ether (20 mL) at least three times to remove the unreacted monomers. Using the above procedure, different formulations for microsphere preparation were derived (Table 1).

| Sample | Feed conc. (wt%) | Run time (h) | Yield (%) | Processability |
|--------|-----------------|--------------|-----------|----------------|
| N90    | 4               | 3            | ~40       | Processable in all solvents tested |
|        | 6               | 8            | ~80       | DMSO, DMF, THF, MeCN |
|        | 12              | ~80          |           |                 |
|        | 24              | >90          |           |                 |
| N80    | 4               | 24           | >80       | DMSO, DMF, THF, MeCN |
|        | 5               | 24           | >90       | DMSO, DMF, THF, MeCN |
| N70    | 4               | 24           | >80       | DMSO, DMF, THF, MeOH |
|        | 5               | 24           | >90       | DMSO, DMF, THF, MeOH |

"Ability of the microgels to dissolve or form stable dispersions in a solvent. "Test solvents: H₂O, MeOH, DMSO, dmf, MeCN, tetrahydrofuran (THF), CHCl₃, DCM, diethyl ether. "Yield was based on the approximate amount of solid residue collected after drying over the initial weight of the monomer feed used during thermal polymerization at 60 °C.

Purification of the microspheres was performed using the following solvents of different polarities: water, methanol (MeOH), DMSO, DMF, MeCN, THF, chloroform (CHCl₃), DCM, and diethyl ether. Approximately 1 mg of microsphere was added with incremental amounts of solvent until 5 mL.

Synthesis of HTM-Imprinted Microspheres. MIMs were synthesized in the presence of HTM. In the case of CTP, polymerization was carried out upon the addition of 3 wt% AIBN to a monomer feed concentration of ~4 wt%, containing EGDMA (80 or 90 wt%) and a 1:4 mole ratio of HTM/MAA (20 or 10 wt%) in MeCN. The monomers were allowed to react at 60 °C for 2 h. A non-imprinted, that is, no template added, equivalent (NIM) was also prepared vis-à-vis each MIM formulation.

CRP MIMs were prepared using the same formulation and polymerization conditions as those above except with the addition of 3 mol% with respect to the total monomer concentration (25 mg in 480 mg of feed) of RAFT agent 7 and 20 mol% (3 mg) AIBN with respect to the amount of RAFT agent.

The template (HTM) was removed by stirring the polymers in 10% acetic acid in MeOH (~30 mL) for at least 60 min. Subsequently, the microspheres were washed with MeOH (5 × 30 mL) with stirring (~5 min) and subjected to centrifugation for 10 min at 4000 rpm. Finally, the microspheres were washed in diethyl ether prior to drying in a vacuum oven at 40 °C.

Template Rebinding Studies. Sorption of HTM onto the polymer was determined by suspending an appropriate amount of microspheres in a known concentration of HTM aqueous solution for the desired time and measuring the difference in the concentrations of HTM before and after sorption. Typically, a rebinding assay is carried out using 2 mg of microspheres suspended in a 0.10–2.0 mM HTM (1 mL) solution, buffered using 25 mM Na₂HPO₄/NaH₂PO₄ at pH 7 (the buffer was prepared by dissolving 239.2 mg of Na₂HPO₄·2H₂O and 137.3 mg of NaH₂PO₄ in 100.0 mL of reverse-osmosis water, 15.33 and 9.67 mM, respectively). After centrifugation and filtration of the microspheres, the supernatant was analyzed on a Shimadzu HPLC (LC-20AD) fitted with an EconosphereTM C18 5 μm column (Grace), and the mobile phase was composed of 20% MeCN and 80% buffer solution (25 mM KH₂PO₄ with 10 mM triethylamine, adjusted to pH 3.0 using 85% H₃PO₄). The volume of sample injected was 10 μL, with a run time of 5.0 min. The flow rate was set to 0.7 mL/min, using a detection wavelength of 215 nm. The retention time of HTM was around 3.0 min. The calibration curve for HTM was generated using the concentration range of 0.200–5.00 mM. Data were processed using LC software.

Figure S5A,B (or Figure S7A,B in the Supporting Information) was plotted by taking the values obtained from the equilibrium concentrations of bound (B), C_b, and free (F), C_f HTM in the heterogeneous solution. The data were expressed in μmol/g, on the basis of the mass of the polymer, or in μmol/m², with respect to the surface area. The log form (eq 2) was utilized for deriving the two factors together with eqs 3 and 4 were subsequently used for the affinity distribution plots (Figure S5C,D or Figure S7C,D).

The number of binding sites, N, in Table 3 was obtained using eq 3. K is the inverse of the concentration of the free template (C_f F_min or F_max) in the suspending solution (eq 4). The AD expression can be plotted for any range of binding affinities within the concentration range of the experimental binding isotherm.

$$B = aF^m$$

$$\log B = m \log F + \log A$$

$$N = 2.303am(1 - m^2)K^{-m} = N_{tetra}K^{-m}$$
$K_{\text{max}} = \frac{1}{F_{\text{min}}} \quad \text{and} \quad K_{\text{min}} = \frac{1}{F_{\text{max}}}$  \hspace{1cm} (4)

Selectivity and Competitive Binding Studies. Selectivity rebinding tests were carried out on 2 mg of microspheres with structural analogues HTD (2), IDZ (3), and TTM (4) using 1 mL of 1 mM aqueous solution of each analyte, buffered at pH 7, similar to the procedure above for the HTM-binding assay. Competitive binding was performed by suspending 2 mg of microspheres in 1 mL of aqueous solution (buffered at pH 7) containing 1 mM of each of the three competitors and HTM.

Morphology Studies. The morphology of the microspheres was determined using a Sigma Field Emission Scanning Electron Microscope (Zeiss FESEM and Bruker EDS). The samples were prepared by drying the microspheres in vacuum at 40 °C for 2–3 h; they were then transferred to the surface of the sample holder. Scanning electron microscopy (SEM) analyses were subsequently conducted, with the chamber pressure set at 1.01 × 10⁻⁵ Torr. The samples were directly analyzed on a carbon background and scanned using a 1.50 kV electron beam with a secondary electron detector. The images were viewed at 15 000× and 33 000× magnifications. The particle size, that is, the diameter, was estimated using ImageJ software by randomly selecting and averaging the sizes of at least 100 particles.

Particle-Size Determination. Particle sizing was performed at 20 °C using a dynamic light scattering (DLS) Malvern Zetasizer Nano ZS equipped with a He–Ne laser system tuned at 632.8 nm. The runs were carried out with a detection angle of 175° (backscatter). The measurement setting was adjusted using polyethylene glycol dimethacrylate as the reference material, with a refractive index of 1.51. DMSO was used as a dispersant.

The microspheres (∼0.200 mg) were dispersed in 1 mL of DMSO and sonicated for 20 min. An aliquot of 100 µL (dispersed microspheres) was diluted with 1.5 mL of DMSO. Measurements of the filtered dispersions were taken at 4.65 mm and an attenuation value between 6 and 10. The hydrodynamic particle size was expressed as peak mean ($D_h$); the particle size distribution was indicated as the polydispersity index (PDI) and determined using Cumulants analysis with the Zetasizer v6.12 software.

Surface Area and Porosity. Gas adsorption analysis was carried out using a Micrometrics ASAP 2020 Accelerated Surface Area and Porosity instrument (Norcross, GA). The samples (100 mg) were degassed at 110 °C under vacuum for at least 12 h, after which adsorption isotherms were obtained using nitrogen as the adsorbate at a temperature of 500 °C, covering a partial pressure ($P/P_0$) range from 1 × 10⁻¹ to 1.0. The specific surface area and pore size distribution of each sample were determined from the adsorption data using the linearized Brunauer–Emmett–Teller (BET) and Barrett, Joyner, and Halenda (BJH) models, respectively.

RESULTS AND DISCUSSION

Determination of cmcpc and Processability Tests. The microspheres used for this study were prepared via high-dilution conventional radical polymerization, employing MAA as the monomer and EGDMA as the crosslinker. MAA and EGDMA were included in the formulation because the microspheres will subsequently be utilized for the preparation of HTM-imprinted MIMs. MAA with EGDMA as the crosslinker has been shown to be an effective functional monomer for HTM-imprinted polymers in previous studies.\(^{25,33}\)

Initially, MeCN and DMSO were chosen as polymerization solvents on the basis of the solubilities of the components of the reaction (MAA and EGDMA) as well as of HTM. Stock EGDMA/MAA monomer feed mixtures containing 90 (N90), 80 (N80), and 70 (N70) wt % EGDMA were diluted with a fixed amount (0.5 mL) of solvent (MeCN or DMSO) to obtain various monomer feed concentrations between 1 and 10 wt %.

In the case of DMSO, reaction mixtures of various monomer feed concentrations (1–10 wt %) and compositions (N90, N80, and N70) were polymerized at 60 °C for 24 h to determine the critical oligomer degree of polymerization for precipitation (as per IUPAC nomenclature).\(^{34}\) Herein referred to as the cmcpc, that is, the highest monomer concentration that does not result in precipitation or gel formation during polymerization.\(^{20}\) The apparent cmcpc was observed to decrease with increasing EGDMA content and found to be 2 wt % for N90 and 4 wt % for both N80 and N70, with yields >80%. After isolation and drying, the processability of the polymers was tested in a number of organic solvents of varying polarities, including water, MeOH, DMSO, DMF, MeCN, THF, CHCl₃, DCM, and diethyl ether. As the CTP polymers were prepared under dilute precipitation polymerization conditions, particle growth takes place, as low-molecular-weight oligomers are continuously captured, leading to the formation of bigger and more rigid microgels. When these microgels are big enough, their solubilities in the reaction medium can vary from insoluble precipitates to colloidal suspensions. Polymer precipitates are usually difficult to disperse in a given solvent (nonprocessable). However, the polymers prepared in this study can be dispersed in common organic solvents. Hence, we define processability as the ability of a polymer to form stable dispersions in a given solvent. All polymers obtained above the apparent cmcpc (i.e., ≥2 wt % for N90 and ≥4 wt % for N80 and N70) were not processable in the test solvents, even at a high dilution, that is, a 0.15% w/v polymer suspension (27 times lower than the estimated cmcpc), and even after being subjected to continuous shaking and heating at 60 °C for 1 h. This observation suggests that the polymers obtained above the apparent cmcpc may no longer be microspheres/microgels. Stöver and co-workers\(^{35,36}\) have also observed microgel to macrogel transition at high volume fractions of good solvents (i.e., >85 vol % MEK) in a similar crosslinked polymeric system. This transition is favorable in a good solvent (DMSO in this case), as it allows the nanoparticles to swell such that each microgel particle is close to the others, enhancing the interaction between microgels.\(^{35}\) Upon removal of the solvent, the interconnected microspheres become more compact and are strongly aggregated such that it is difficult to redissolve the material. Alternatively, the material can assume the space-filling gel (macrogel) form. Dissolution of this macrogel is impossible, as the microgel–microgel interaction is very strong and can neither be compensated for by the energy of solvation upon addition of solvent nor be compensated for by the application of external energy, for example, heating or sonication.

In the case of the microspheres prepared in MeCN, gelation was not observed even at a monomer feed concentration of 10 wt % after 24 h of polymerization (see Figure S1). However, all reaction mixtures turned milky, with no significant change in the solution viscosity. This indicates that stable colloidal particles were formed from high-dilution precipitation polymerization,\(^{25}\) which is quite different from the TRPP, in which

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there is phase separation. In the absence of an apparent cmcp, processability tests were conducted using solvents of varying polarities to retroestimate the cmcp (Table 1). N90 microspheres prepared from a 5 wt % monomer feed concentration were not processable in all test solvents; the particles aggregated such that they would settle at the bottom of the solution (data not shown). However, microspheres that were processable in DMSO, DMF, THF, or MeCN were obtained when the feed concentration was lowered to 4 wt % (N90-4) in ≥6 h. On the other hand, the N90-4 microspheres obtained after 3 h were processable in all solvents tested but provided a yield of only 40%. Thus, subsequent microsphere syntheses were performed for 24 h (see Figure S2 for TEM images after polymerization) to optimize the yields (≥90%). Microspheres prepared at lower crosslinker contents, N70 and N80, using 4 and 5 wt % monomer feeds, respectively, were also found to be solution-processable. The processability of the microspheres increased with decreasing crosslinker content (i.e., N70 > N80) and decreasing feed concentration (i.e., 4 wt % > 5 wt %).

**Synthesis of MIM. Template–Monomer Interactions.** The interaction between the template, HTM, and the functional monomer, MAA, is evident in the Fourier transform infrared (FTIR) spectra of HTM, MAA, and a 1:1 mole ratio of the HTM–MAA mixture in MeCN (see Figure S3). Whereas the MAA spectrum showed a strong peak at 1690 cm\(^{-1}\) due to C=O stretching, the intensity of this peak markedly reduced upon addition of HTM, accompanied by the appearance of peaks at 1531 and 1400 cm\(^{-1}\), consistent with the asymmetric and symmetrical stretching frequencies of the carboxylate anion, which is indicative of an acid–base interaction between MAA and HTM, that is, deprotonation of MAA with concomitant proton transfer to HTM.\(^{37}\) This has also been previously observed between ephedrine and acrylic acid.\(^{38}\)

**MIMs by CTP.** HTM-imprinted microspheres were first prepared by CTP with the use of AIBN under the optimum conditions presented in the previous section, that is, ≤4% (w/w) monomer feed concentration, 80% crosslinker, and a HTM/MAA mole ratio of 1:4 in MeCN at 60 °C for 24 h. Previous reports on HTM-imprinted polymer systems have utilized HTM/MAA ratios of 1:2 (bulk polymer for HTM detection in the nM to μM range),\(^{33}\) 1:5 (bulk polymer selective to HTM against putrescine but not to spermine and spermidine),\(^{32}\) and 1:10 (bulk polymer for HTM detection in the mM range).\(^{39}\) However, our results showed that the amount of HTM employed in the preparation of MIMs greatly influenced their cmcp and processabilities. An HTM/MAA ratio of 1:2 resulted in excessive precipitation (and premature cmcp) during the early stage of polymerization (<1 h); this was not observed in the corresponding non-imprinted microspheres. The template molecules, HTM in this case, effectively increase the cross-linking percentage by binding to multiple monomers. Therefore, the addition of template results in a polymer that acts like it has a higher percentage of crosslinker, enhancing particle growth where bigger microspheres are obtained. Ye has also reported on the significant effect of template on the size of the imprinted particle.\(^{30,41}\) Early precipitation was circumvented by increasing the HTM/MAA ratio to 1:10. This polymer retained a processability comparable to that of NIM; however, both MIM and NIM exhibited similar HTM-binding performances. This is due to the fact that the polymerization carried out under high dilution does not favor the formation of the monomer–template complex required in the imprinting process. Hence, at a 1:4 mole ratio of HTM/MAA, at which precipitation was minimal, the imprinting effect was evident, MIMs were obtained in high yields (>90%), and the polymers were solution-processable, was chosen for this work.

Previous studies have shown that for MIPs to be highly selective, the formulation should contain at least 70 wt % crosslinker.\(^{20}\) Nevertheless, we found that using a 70% EGDMA (with 30% MAA) formulation (M70) also resulted in early precipitation when a 1:4 HTM/MAA formulation was used. Conversely, no precipitation occurred, and the solution turned milky only after 3 h, with the same formulation in the absence of HTM (N70). In addition, no early precipitation was observed on using 80% (M80) and 90% crosslinker (M90) reaction mixtures after 1 h of polymerization. In these systems, the MAA feed was lower (20 and 10 wt %, respectively) than that in M70 (30 wt % MAA) and the amount of HTM added was also less, in keeping with the 1:4 HTM/MAA ratio. Thus, to minimize changes in the particle size and processability of MIMs, further MIM synthesis by CTP was only conducted using the M80 and M90 formulations.

FTIR (Figure 2A; unextracted MIM) confirmed the presence of HTM within the MIM after polymerization. The peak at ~1550 cm\(^{-1}\) is indicative of the imidazole ring stretching and asymmetric bending of the amino group of HTM,\(^{42,43}\) whereas

![Figure 2](https://example.com/figure2.png)
the peak around 900–800 cm\(^{-1}\) is attributed to the HTM imidazole ring in-plane bending. These peaks were not visible in the non-imprinted microspheres (Figure 2). In addition, these peaks disappeared after exhaustive extraction of HTM and reappeared upon HTM rebinding, indicating successful creation of HTM-specific recognition cavities within the MIM.

Incorporation of the template onto the polymer, that is, creation of imprints, has been demonstrated to occur during the early stage of polymerization,\(^{41}\) such that controlling the
particle growth of the MIM up to its cmcp is not expected to affect the imprinting efficiency.

**MIPs by CRP.** In addition to that by CTP, MIMs were also prepared by CRP using the RAFT technique. Utilization of CRP in the preparation of highly crosslinked polymers has been reported to provide improved polymer binding performance compared to prepared using the TRPP method. Another advantage of CRP MIMs is the ability of the reactive chains to be reversibly cleaved, allowing further polymerization, which, in our case, is essential for future grafting of MIMs onto polymerizable substrates (e.g., vinyl-silylated substrates) for subsequent sensor fabrication. The mechanism of initiation via RAFT CRP has been thoroughly discussed in the literature.

In this study, a minimal amount of conventional azo initiator (AIBN) was employed to the reaction mixture to form initiating radicals that can react with the RAFT agent. The RAFT agent, MCEBTTC, employed in the synthesis is an efficient and commonly used RAFT initiator for styrenic and acrylic and acrylamide systems, but to the best of our knowledge, this is its first use in MIM/MIP synthesis and with methacrylic monomers.

Preliminary binding studies conducted on the M80 and M90 CTP MIMs showed the M80 formulation to exhibit a better binding behavior than that of M90. Thus, CTP MIMs were only synthesized using the M80 formulation at a 4% (w/w) monomer feed concentration in MeCN, at 60 °C for 24 h, keeping the HTM/MAA mole ratio of 1:4. As with CTP MIMs, premature phase separation of MIMs due to the presence of template was minimal with this formulation, and highly solution processable MIMs were obtained in high yields (>90%).

**Physical Properties of CTP and CRP MIMs.** SEM micrographs of microspheres prepared using CTP and CRP are shown in Figure 3, together with their particle sizes, estimated from SEM (D_{SEM} collapsed dry state), and PDIs, estimated from DLS (D_{D(MPSO)} swollen state in DMSO), and surface areas obtained from BET. The CTP polymers exist as a network of aggregated particles (Figure 3A,B). The TEM images of the microspheres (CTP-M90 and CTP-N90) obtained after the polymerization step in Figure S2 also showed similar results. Conversely, the SEM images show that the CRP microspheres are clustered (Figure 3C). The D_{D(MPSO)} and D_{SEM} of most microspheres are comparable, with the exception of those of CTP-M80, CTP-M90, and CTP-N90. The observed differences in the D_{D(MPSO)} (bigger) and D_{SEM} of these polymers could be attributed to particle aggregation in solution, which, particularly for CTP-M80, was observed to be enhanced in MeCN (D_{MeCN} = 609 nm).

The presence of the template clearly affected the size of the resulting particles, as demonstrated by CTP-M90/N90 (Figure 3A) and CTP-M80/N80 (Figure 3B). In both cases, the MIMs are ~2–3 times larger than the NIMs (from D_{b} and D_{SEM}), consistent with an earlier observation (during synthesis) of enhanced particle growth, leading to bigger particles for polymerizations conducted in the presence of HTM. In fact, this effect seems to be more pronounced in CTP-M90, for which a small number of bigger particles (843 nm) are evident from its SEM image. These bigger particles were most likely filtered out from the dispersion and hence not detected in DLS. The formation of a small number of bigger particles in CTP-M90, evident from its SEM image, is clearly due to the template increasing the crosslinking percentage by binding to multiple monomers (as discussed earlier) and is exacerbated by the high crosslinker content. This triggers enhanced particle growth, resulting in particles that are bigger than expected. In general, there seems to be no marked difference in particle size between CTP-M90 and -M80 and CTP-N90 and -N80. The PDIs from DLS measurements also show the CTP MIMs to be more polydisperse than the corresponding NIMs, which indicates that the presence of the template may have provided variable rates of particle growth (between monomer clusters interacting with HTM and monomer clusters not interacting with HTM), resulting in a broader particle-size distribution.

Unlike that in CTP microspheres, the presence of the template does not affect the particle growth of CRP microspheres, as both CRP-M80 and CRP-N80 exhibit comparable D_{b} and D_{SEM}. This is not surprising, as it is well known that chain propagation, and hence polymer growth, is slow and controlled in CRP. It is notable that CRP-M80 is less polydisperse compared to CTP-M90, CTP-M80, and its CRP-N80 counterpart. This is different from the result obtained using CTP polymers, where the imprinted polymers are more polydisperse.

As expected, the BET surface areas of the CTP polymers are inversely related to their sizes, such that the surface areas of CTP-M90 (48 m²/g) and CTP-M80 (31 m²/g) are much lower (2 and 4 times, respectively) than those of their corresponding NIMs (CTP-N90 = 92 m²/g and CTP-N80 = 137 m²/g). Both CTP-N90 (pore volume = 17 cm³/g) and CTP-N80 (pore volume = 27 cm³/g) are 4 and 3 times, respectively, more porous than their corresponding MIMs (pore volume = 4 and 9 cm³/g, respectively); this may be due to the presence of bigger pores, that is, >3 nm (Figure S4). These results suggest that polymerization under NIP conditions gives a higher surface area and pore volume. This is in contrast to that in polymers prepared in the presence of template, in which the presence of HTM significantly altered the polymerization process. Conversely, higher surface areas and larger pore volumes were observed from CRP-M80 compared to those from CRP-N80, where CRP MIM and NIM surface areas (138 and 123 m²/g, respectively) and pore volumes (40 and 25 cm³/g, respectively) were obtained. This observation is indicative that the nature of the polymerization process, particularly the CRP technique, could circumvent the template effect.

**Binding Studies.** HTM-binding tests were conducted in aqueous solutions using 2 mg of CTP-M80/N80 polymers at varying times from 15 to 240 min. Binding saturation for both polymers was achieved after 120 min (Figure S5), where the incubation was performed using a horizontal shaker. The data not shown), which hindered the accessibility of the binding sites. Nevertheless, the MIM was found to exhibit a higher binding capacity than that of the NIM, indicating that the imprinting process successfully created HTM-selective binding sites within the MIM.

**Optimization of HTM Binding Conditions: Effect of Buffer Concentration and pH.** The effect of buffer ionic strength on HTM sorption was determined by varying the concentration of the phosphate (Na₂HPO₄−NaH₂PO₄) buffer solution from 2 to 100 mM at pH 7. At this pH, HTM exists in protonated forms HT⁺⁺ and HT⁺ (Figure S6), which can readily interact (by electrostatic interaction) with the deprotonated MAA units (pKₐ = 6–7) randomly distributed along the polymeric network, thereby enhancing HTM sorption. HTM sorption was observed to decrease with increasing buffer concentration.
Figure 4. HTM binding on CTP-M80/N80 microspheres at (A) pH 7, with different phosphate buffer concentrations between 2 and 100 mM, and (B) at different pH’s, with a fixed phosphate buffer concentration of 25 mM.

Figure 5. (A) Freundlich binding isotherms, (B) linearized log–log Freundlich binding isotherms, (C) Freundlich affinity distribution expressed in the $N$ vs log $K$ format, and (D) Freundlich linearized affinity distribution expressed in the log $N$ vs log $K$ format, using calculations based on mass. $N$ and $K$ were obtained from the slope ($m$) and $y$ intercept $a$ of (B) (see Table 2). HTM-binding results were obtained between the 0.10 and 1.0 mM HTM concentration range (aqueous solution, 25 mM buffer, pH 7) using 2 mg of MIMs and NIMs. Affinity distributions have been generated using the equation $N(K) = 2.303αm(1 - m^2)K^{-m}$ over concentration ranges $K_{min} = 1/F_{max}$ and $K_{max} = 1/F_{min}$.
Batch binding studies were carried out to evaluate the binding performances of the different MIM and NIM samples at pH 7. The binding data were expressed in two manners: with respect to the mass of the MIP (Figure 5) and with respect to the surface area (Figure S7). All of the MIMs were shown to exhibit higher binding than their corresponding controls (Figures 5A and S7A). Because the binding characteristics of CTP and CRP microspheres toward HTM were studied at concentrations well below saturation binding (0.20–1.0 mM), as such, these binding isotherms have been fitted to, and found to conform with, the Freundlich model, with all log plots (Figures 5B and S7B) obtaining regression correlation coefficients ≥0.91.52 These linear log forms yield two fitting parameters, a (y intercept) and m (slope) (Table 2), which can be used to generate the corresponding affinity distribution over the concentration range studied (i.e., $K_{\text{min}} = 1/F_{\text{max}}$ and $K_{\text{max}} = 1/F_{\text{min}}$) that relates the number of binding sites, N, for each region of binding sites having an association constant K via the equation $N(K) = 2.303am(1 - m^2)K^{-m}$. The affinity distribution is presented in two formats: N versus log K (Figures 5C and 5D) and log N versus log K (Figures 5E and 5F). The N versus log K format gives the number of binding sites within the range of association constants, that is, the area under the distribution, whereas the linear log N versus log K format measures the heterogeneity (i.e., the ratio of the number of high-affinity to low-affinity sites) of the binding sites by virtue of slope m, such that the flatter the slope, that is, lower the m, the higher the concentration of high-affinity sites.53,54

From Figure 5C, the binding capacities, N (over the concentration range studied prior saturation), for the CTP MIMs are shown to be higher than those for their corresponding NIMs, indicative of the imprinting effect. Whereas CTP-M90 has a lower number of binding sites, its m is flatter (0.62) than that of CTP-M80 (m = 0.76), suggesting a higher ratio of high-affinity to low-affinity binding sites. We can deduce that a higher crosslinker content (in CTP-M90) can enhance the formation of higher-affinity binding sites, consistent with the observations of other groups. The amounts of MAA and HTM template added to CTP-M90 were also less consistent with the observations of other groups. The amounts of MAA and HTM template added to CTP-M90 were also less consistent with the observations of other groups. The amounts of MAA and HTM template added to CTP-M90 were also less consistent with the observations of other groups. The amounts of MAA and HTM template added to CTP-M90 were also less consistent with the observations of other groups.

### Table 2. Binding Parameters for CTP and CRP MIMs and NIMs Estimated from Freundlich Isotherms with Respect to Mass and Specific Surface Area

| Binding parameters | CTP-90 | CTP-80 | CRP-80 |
|--------------------|--------|--------|--------|
|                    | M90    | N90    | M80    | N80    | M80    | N80    |
| Calculations Based on Mass |
| $a^m = N_0 (\mu\text{mol/g}) + K$ | 128    | 78     | 431    | 112    | 285    | 189    |
| $m^a$              | 0.62   | 0.70   | 0.76   | 0.76   | 0.68   | 0.67   |
| $R^2$              | 0.98   | 0.98   | 0.97   | 0.91   | 0.99   | 0.99   |
| Calculations Based on Surface Area |
| $a^m = N_0 + K$    | 0.42   | 0.067  | 1.12   | 0.012  | 0.32   | 0.19   |
| $m^a$              | 0.62   | 0.72   | 0.73   | 0.90   | 0.68   | 0.67   |
| $R^2$              | 0.98   | 0.99   | 0.98   | 0.98   | 0.99   | 0.99   |

*From eq 2; see Figure 5 plots.*

concentration up to 25 mM, at which there is minimal change for both MIM and NIM at pH 7 (Figure 4A). Our findings corroborate those of Trikka et al.,25 who also observed that increasing the ionic strength (using NaCl) decreased the amount of HTM sorbed onto MAA-based bulk MIPs. In the presence of the buffer solution, the electrostatic interactions between the microspheres and HTM could be reduced due to competing interactions with the counterions from the buffer salt. In particular, Na+ ions could mask the negative charge density of microspheres. As the buffer concentration increases, more Na+ ions are also available to screen the surface charge of the microspheres, thereby diminishing the effect of nonspecific electrostatic interactions between the microspheres and HTM. In fact, the difference in HTM binding between the microspheres and NIM, that is, selective binding attributed to the creation of imprints over the buffer concentration range tested, had not changed significantly, indicating that buffer concentration only affects nonspecific superficial HTM sorption.

Employing the optimized phosphate buffer concentration of 25 mM, HTM rebinding tests were also conducted at pHs 5 and 9, at which MAA and HTM exist in different ionic forms (Figure S6). At pH 5, HTM is mostly double-protonated (HTM+++ and HTM++), whereas MAA in the microspheres is largely unprotonated. Such conditions do not promote the interaction between HTM and MAA. Thus, as shown in Figure 4B, the MIM exhibited a low affinity toward HTM. At pH 9, HTM+ would have been predominantly and MAA, fully deprotonated; HTM binding on the MIM improved as the interaction between the template and functional monomer was more favorable. At pH 7, however, optimum MIM binding (234 μmol/g) was observed. At this pH, HTM would have been completely protonated (mostly as HTM++) and some HTM+, whereas MAA in the microspheres was predominantly deprotonated, creating the most favorable condition for HTM sorption. Whereas MIM binding was significantly affected by pH, NIMs behaved similarly, giving comparable HTM bindings across the pH range tested. The difference in binding behavior between the MIM and NIM and the loss of binding selectivity within the MIM as a function of pH indicated that binding in MIM was more selective and primarily governed by favorable interactions between HTM and functional monomer MAA.

**Binding Characteristics.** Batch binding studies were carried out to evaluate the binding performances of the different MIM and NIM samples at pH 7. The binding data were expressed in two manners: with respect to the mass of the MIP (Figure 5) and with respect to the surface area (Figure S7). All of the MIMs were shown to exhibit higher binding than their corresponding controls (Figures 5A and S7A). Because the binding characteristics of CTP and CRP microspheres toward HTM were studied at concentrations well below saturation binding (0.20–1.0 mM), as such, these binding isotherms have been fitted to, and found to conform with, the Freundlich model, with all log plots (Figures 5B and S7B) obtaining regression correlation coefficients ≥0.91.52 These linear log forms yield two fitting parameters, a (y intercept) and m (slope) (Table 2), which can be used to generate the corresponding affinity distribution over the concentration range studied (i.e., $K_{\text{min}} = 1/F_{\text{max}}$ and $K_{\text{max}} = 1/F_{\text{min}}$) that relates the number of binding sites, N, for each region of binding sites having an association constant K via the equation $N(K) = 2.303am(1 - m^2)K^{-m}$. The affinity distribution is presented in two formats: N versus log K (Figures 5C and 5D) and log N versus log K (Figures 5E and 5F). The N versus log K format gives the number of binding sites within the range of association constants, that is, the area under the distribution, whereas the linear log N versus log K format measures the heterogeneity (i.e., the ratio of the number of high-affinity to low-affinity sites) of the binding sites by virtue of slope m, such that the flatter the slope, that is, lower the m, the higher the concentration of high-affinity sites.53,54

From Figure 5C, the binding capacities, N (over the concentration range studied prior saturation), for the CTP MIMs are shown to be higher than those for their corresponding NIMs, indicative of the imprinting effect. Whereas CTP-M90 has a lower number of binding sites, its m is flatter (0.62) than that of CTP-M80 (m = 0.76), suggesting a higher ratio of high-affinity to low-affinity binding sites. We can deduce that a higher crosslinker content (in CTP-M90) can enhance the formation of higher-affinity binding sites, consistent with the observations of other groups. The amounts of MAA and HTM template added to CTP-M90 were also less than those added to CTP-M80; hence, it is not surprising that the N value for CTP-M80 is greater than that for CTP-M90. In addition, the bigger particles (see Figure 3) from CTP-M90, presumed to result from "template-enhanced particle growth", were separated from the batch used in the binding studies. Conversely, CTP-N90 (m = 0.70) has a lower number of binding sites and a higher ratio of high-affinity to low-affinity binding sites than those in CTP-N80 (m = 0.76). In the case of CRP microspheres, even though both imprinted CRP-M80 and non-imprinted CRP-N80 exhibited comparable heterogeneities (i.e., m CRP-M80 = 0.68, m CRP-N80 = 0.67) (Figure 5D), an imprinting effect was still evident. Typically, prepared MIPs have lower m values (more heterogeneous) due to the presence of template in the polymerization step. However, this effect was
not observed in CRP microspheres. A closer look at the affinity distribution plots reveals CRP-80 to possess a higher population of higher-affinity imprinted sites than that in CTP-80 (m CTP-M80 and m CTP-N80 = 0.76). However, CTP-M80 exhibits a higher binding capacity than that of CRP-M80, whereas CTP-N80 has a much lower binding capacity than that of CRP-N80.

The data for the calculations based on mass (Figure 5, Table 2) appeared to be comparable to that obtained from the surface area (Figure S7, Table 2). As the MIMs (80 and 90) prepared using CTP have a smaller surface area compared to that of the NIM counterpart, it is not surprising that higher binding capacity values are obtained for MIM than those for NIM. The ratio of the binding capacity between the MIM and the corresponding NIM with respect to the surface area can be exaggerated compared to that from calculations based on mass, that is, the ratio of M80 and N80 by CTP calculated on the basis of the surface area provided 93, whereas the calculation
based on mass gave 3.8 (Table 2). For CRP, as there is a small difference between the binding capacities and surface areas of the particles, the calculated binding capacities between the MIMs and the corresponding NIMs are also comparable for both calculations.

A quantitative comparison of the binding performances of the microspheres on the basis of N, with K = 25 mM$^{-1}$ (i.e., log K = 1.4), the highest affinity sites across the concentration range studied, is given in Table 3. Among the MIMs, CTP-M80, which contains twice the amount of functional monomers and HTM, recorded the highest binding (calculations by mass, 28.2 μmol/g; calculations by surface area, 8.11 × 10$^{-2}$ μmol/m$^2$), almost twice that for CTP-M90 (15.1 μmol/g, 5.01 × 10$^{-2}$ μmol/m$^2$). CRP-M80 provided 27.2 μmol/g and 2.99 × 10$^{-2}$ μmol/m$^2$, whereas CRP-N80 gave 18.2 μmol/g and 1.80 × 10$^{-2}$ μmol/m$^2$, with a binding capacity more than 2 times higher compared to that of both CTP-N80 (7.18 μmol/g; 0.03 × 10$^{-2}$ μmol/m$^2$) and CTP-N90 (6.63 μmol/g; 0.52 × 10$^{-2}$ μmol/m$^2$). Thus, the difference in binding capacities (ΔN) between MIM and NIM, imparted by the imprinting process, is significantly higher in CTP-80 (21.0 μmol/g; 8.08 × 10$^{-2}$ μmol/m$^2$) than that in both CTP-90 (8.47 μmol/g; 4.49 × 10$^{-2}$ μmol/m$^2$) and CRP-80 (9.00 μmol/g; 1.19 × 10$^{-2}$ μmol/m$^2$). These results suggest that CRP can provide polymers with better structural homogeneity; however, the selectivity is lower compared to that of CTP-80.

MIP particles synthesized via precipitation polymerization are usually porous. It is also well documented that these pores carry the recognition sites in each particle. Thus, the template–MIP binding calculations based on per gram polymer considered the template bound to the template-specific cavities, template-nonspecific cavities, as well as the surface of the particles. Conversely, the calculations based on surface area precluded the presence of porous recognition sites in the particles.

On the basis of the results in Figure 3, the alternative calculations generated considering the surface area should not affect the calculated results for CRP-80 as much as those for CTP-80 and -90. CRP polymers were prepared using slow and controlled polymer growth; hence, the size and surface area of the MIMs and NIMs synthesized are comparable. However, for polymers prepared using CTP, the sizes of the CTP MIMs are larger than those of the NIMs, whereas the surface areas of the CTP-NIMs are larger those of the MIMs. If the polymers were prepared in the presence of template molecules, the template could effectively affect the rate of polymer particle growth (vide supra) and alter the surface area of the particle.

Selectivity Studies. The selectivity of the MIMs was investigated via noncompetitive and competitive binding assays, with equimolar amounts of HTD (2), IDZ (3), and TTM (4) (Figure 1) using CTP-80, the best performing MIP, and CRP-80. The results after a 2 h incubation time are shown in Figure 6. It is obvious that CRP-80 and CTP-80 are selective toward HTM for both noncompetitive cross-binding and under competitive conditions.

Although HTM and HTD are of similar sizes, both noncompetitive cross-binding and competitive binding tests with CTP-80 yielded negative binding for HTD. With the presence of an additional COOH group in HTD, the molecule exists as a stable zwitterion at pH 7. Hence, under this condition, HTD would not favor binding or interaction with the microspheres. This result is similar to that obtained by Ye, where negative template binding was observed for polymers suspended in phosphate buffered at pH 6 and positive binding, for phosphate buffered at pH 7.

The smaller IDZ molecule exhibited noncompetitive cross-binding toward CTP-M80. This result is also mirrored in the competitive binding study for both CTP-M80 and CRP-M80, in which the binding capacity of IDZ increased, while maintaining low NIM binding. We surmised that the ability of IDZ to bind more to CTP-M80 in the presence of other analytes is enhanced by its small size (0.42 nm in length), allowing it to fit into the smaller pore cavities, which are not occupied by the bigger analytes. As the pKₐ of IDZH⁻ (i.e., N-3 is protonated) is 6.99, both neutral and protonated forms of IDZ exist at pH 7, these species are capable of interacting with the MAA (pKₐ = 6–7)-based cavities. However, the observed binding capacity of HTM was reduced in the presence of competitors HTD, IDZ, and TTM (Figure 6B).

In both noncompetitive cross-binding and competitive binding tests, TTM exhibited the highest binding to polymers prepared by CTP and CRP surpassing HTM. TTM recorded a CTP-M80 binding of 295 μmol/g, slightly higher than that of HTM (234 μmol/g). This binding preference is markedly pronounced in competitive tests, in which TTM (314 μmol/g, CTP-M80; 259 μmol/g, CTP-N80) recorded a selective binding (i.e., M80–N80) higher than (5 and 7 times, respectively) that of HTM (61.5 μmol/g, CTP-M80; 37.6 μmol/g, CTP-N80). For CRP MIMs and -NIMs, TTM exhibited the highest binding for both cross-binding and competitive tests at >300 μmol/g for MIM and NIM. However, selectivity was observed for HTM, where 195 μmol/g for CRP-M80 and 141 μmol/g for CRP-N80 were bound. Under competitive conditions, less HTM was bound, at 60.4 and 36.3 μmol/g for CRP-M80 and CRP-N80, respectively. At pH 7, the amino groups of both HTM and TTM are protonated (pKₐ’s = 10) and hence interactions (electrostatic) with the functional monomer (MMA) moieties within the binding cavities are expected to be comparable. CTP-M80 also has a higher volume of pores between 0.7 and 3 nm, which can easily fit either a HTM or a TTM molecule. In addition, TTM molecules can interact with each other via π–π stacking of the phenyl rings, thereby potentially increasing the number of TTM molecules along the binding sites and enhancing their binding capability. Although high TTM binding was observed for CTP and CRP polymers, these interactions are not specific.

Perhaps the most significant difference with the two calculations can be observed between the HTM and TTM bound to the MIM versus NIM for CTP (Figure 6). As the value of the surface areas for CRP-M80 (138 m²/g), CRP-N80 (123 m²/g), 825 and CTP-N80 (137 m²/g) are close to each other, their HTM and TTM binding performances are similar. This is in contrast to that in CTP-M80 (31 m²/g), where the results for the noncompetitive cross-binding and competitive binding for HTM and TTM were higher compared to those for the NIM counterpart.

Despite the competition from TTM, it is worth noting that the potential application targeted for these HTM-selective MIMs is for sensing the presence of HTM in fish, as an indicator of fish spoilage, or in tissue or urine. Among the three analytes tested, only HTD, which did not exhibit affinity toward the HTM MIMs, is a potential competitor in the real situation of testing for fish spoilage.
CONCLUSIONS

The solution-processable HTM-imprinted microspheres used in this study were prepared via high-dilution CTP and CRP using the RAFT technique. The microspheres obtained below the apparent cmcp in MeCN were found to be processable in a number of organic test solvents of varying polarities, whereas those prepared in DMSO were not, even at a high dilution. Processable HTM CTP MIMs were prepared in high yields (>90%), with the optimal 4% (w/w) monomer feed concentration in MeCN containing a 1:4 HTM/MAA ratio and 80 or 90 wt % (M80 or M90, respectively) EGDMA at 60 °C for 24 h. CRP MIMs were synthesized using only 80 wt % (CRP-M80) EGDMA.

The presence of the template, HTM, has been observed to promote binding to multiple monomers and to increase the effective crosslinking percentage. This effect of the template on the physical properties of the resulting microspheres was evident in the CTP systems and not in the CRP systems. The CTP MIMs were bigger, with lower surface areas, and more polydispersed than the corresponding NIMs, which indicated the presence of template-introduced particle growth. However, CTP-NIMs were more porous than CTP MIMs. Conversely, the presence of the template did not seem to affect the particle growth of CRP microspheres, as both the MIM and NIM exhibited comparable sizes, expected in RAFT polymerization. However, contrary to the results obtained using CTP, the CRP MIMs exhibited a higher porosity and had a larger surface area than CRP-NIMs.

The data obtained form the binding studies fitted to the Freundlich model, where the binding isotherms and affinity distribution (over the concentration range studied) showed the binding capacity, $N_f$ for the CTP MIMs to be higher than that of their corresponding NIMs, indicative of the imprinting effect. Whereas $N_f$ for CTP-M80 is twice that for CTP-M90, CTP-M90 exhibited a flatter slope, $m$, demonstrating a higher ratio of high-affinity to low-affinity binding sites. This suggested that a higher crosslinker content enhances the formation of HTM-binding sites. In the case of the CRP microspheres, both imprinted CRP-M80 and non-imprinted CRP-N80 exhibited comparable heterogeneities, suggesting that the binding sites are as good as those obtained from CTP-90. Among the MIMs, CTP-M80, which contained twice the amounts of MAA and HTM compared to those in CTP-M90, recorded the highest $N$ (28.2 μmol/g), almost twice those for CTP-M90 (15.1 μmol/g) at $K = 25$ mM$^{-1}$, the highest affinity point across the concentration range studied. Conversely, CRP-M80 provided an $N$ value of 27.2 μmol/g, comparable to that of CTP-M80, whereas the value for CRP-N80 (18.2 μmol/g) was 2 times higher than that for CTP-N80 (7.18 μmol/g) and CTP-N90 (6.63 μmol/g). As a result, the selective binding capacity ($N_{MIM} - N_{NIM}$) imparted by the imprinting process was significantly higher in CTP-80 than that in both CTP-90 and CRP-80. From these results, the binding capacity calculations for CTP based on surface area can be exaggerated, especially if there is a smaller surface area, compared to the calculations based on mass. For CRP, as there is a small difference between the calculated binding capacities and the surface areas of the two particles, the difference obtained between the MIMs and the corresponding NIMs is also small.

Noncompetitive and competitive binding assays against HTM analogues HTD, IDZ, and TTM showed CTP MIMs and CRP MIMs to be selective to HTM under cross-binding and competitive conditions, although a reduction in HTM binding was observed in the latter test. TTM was also found to preferentially bind to both CTP-M80 and CRP-M80 over HTM in both noncompetitive cross-binding and competitive binding tests. We attributed this to the ability of TTM to easily compete with HTM for access to binding sites by virtue of their similarity in size, functionality, charge, and interaction through their phenyl rings, thereby enhancing TTM’s access to binding sites.

This study has demonstrated successful creation of imprints in solution-processable MIMs, allowing an alternative synthetic pathway toward the fabrication of MIP-based materials. Although previous studies have shown that polymers obtained under controlled free-radical precipitation conditions gave a better binding performance compared to that of CTP, in this study, CRP-M80 prepared with MCEBTTTC gave a lower binding capacity but a higher ratio of high-affinity to low-affinity sites compared to those of CTP-M80. These processable MIMs can be appropriately functionalized (demonstrated to be possible by CRP-RAFT) to facilitate further reactions in solution, such as attachment to supports and immobilization on a substrate to produce membranes/thin films for HTM recognition.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.6b00144.

Photographs of CTP-80 and CRP-80 microspheres after polymerization; sample TEM micrograph (CTP-90), FTIR spectra of HTM, MAA, and the HTM–MAA mixture, pore size distributions of MIMs and NIMs, HTM sorption of CTP-M80 and -N80 at different binding times, structural forms of HTM and microspheres at different pHs, and Freundlich binding isotherm plots based on surface area (PDF)

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Notes

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