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Enhancing molecularly imprinted polymer binding properties via controlled/living radical polymerization and reaction analysis

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Abstract

Fractional double bond conversion and associated template binding parameters of molecularly imprinted polymers were explored in this study in relation to initiator type and concentration, crosslinking monomer length, temperature, and solvent concentration. Controlled/living polymerization techniques were used to synthesize recognitive poly(methacrylic acid-*co*-ethylene glycol dimethacrylate) (poly(MAA-*co*-EGDMA)) networks which resulted in a 63% increase in the number of binding sites at approximately equivalent average binding affinity while retaining selectivity of the target molecule, ethyladenine-9-acetate. This is hypothesized to be attributed to a decrease in kinetic chain length and/or a more narrow dispersity of kinetic chains which leads to increased structural homogeneity and increased stability and integrity of binding sites. Reaction analysis of a typical poly(MAA-*co*-EGDMA) molecularly imprinted network measured via differential scanning calorimetry revealed low double bond conversion ($35 \pm 2.3\%$ at 0 °C to $54 \pm 1.9\%$ at 50 °C) which is due to severely constrained network formation; therefore, the final composition of imprinted polymers does not represent the initial formulation when using significant amounts of short bifunctional crosslinking monomer. Optimization of conventional photoinitiator was shown to lead to a small improvement in template selectivity at equivalent affinity and capacity. However, the use of controlled/living polymerization techniques within the field of imprinted polymers has the greatest potential to improve the structural homogeneity and drastically enhance the binding parameters. Polymerization reaction analysis and the use of controlled polymerization strategies will lead to a greater understanding of the imprinting mechanism, optimization of binding parameters, and an increase in the application potential of imprinted networks.

Keywords: Molecularly imprinted polymers; Polymer reaction analysis; Living radical polymerization

1. Introduction

For the past several decades, researchers have studied free-radical hetero/homopolymerization reactions of multifunctional monomers in the analysis of highly crosslinked polymeric materials. Due to dense crosslinking, which yields mechanical strength, rigidity, and low solvent penetration, highly crosslinked networks have been used as dental restorative materials, information storage materials, optical materials, etc. More recently, the field of macromolecular recognition and molecular imprinting has exploited porous, highly crosslinked heteropolymers as robust recognition matrices [1,2]. As specialized applications are pursued, such as point-of-care diagnostics [2], assays [3], and drug delivery carriers [2,4,5], the characterization and optimization of the polymer structure via reaction analysis are paramount.

Non-covalent complexation or covalent bonding between template or 'guest' molecules and functional monomers during polymerization can create networks with selective binding sites (Fig. 1). The concept of macromolecular recognition manifests itself from two major synergistic effects: (i) shape specific cavities that match the template molecule, which provide stabilization of the chemistry in a crosslinked matrix, and (ii) chemical groups oriented to form multiple

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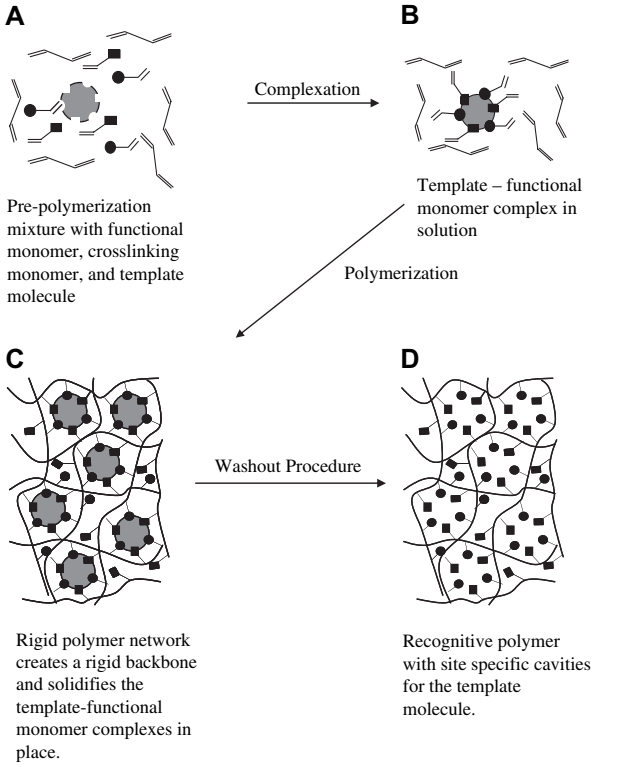

Fig. 1. Recognitive polymer synthesis. (A) Solution mixture of template, functional monomer(s) (squares and circles), crosslinking monomer, solvent, and initiator(s). (B) The pre-polymerization complex is formed via covalent or non-covalent chemistry. (C) The formation of the network (template mediated molecular imprinting process). (D) Wash step where original template is removed.

complexation points with the template. After the original template is washed out of the polymer, a rigid crosslinked network retains the three-dimensional size of specific cavity of the chemical functionality which is target molecule specific [1]. Solvent is used to produce pores in the structure, which significantly increases the mass transfer of template molecules.

The number of papers involving molecular imprinting has increased dramatically in the last decade, with most researchers using acrylate and methacrylate monomers (predominantly ethylene glycol dimethacrylate (EGDMA), a bifunctional vinyl crosslinking monomer, and methacrylic acid (MAA), a monofunctional vinyl monomer, respectively [2,6]). However, there is little progress within the field in the area of polymerization reaction analysis to optimize the binding affinity, selectivity, and number of binding sites within the polymer matrix, main properties that determine the imprinting effectiveness [7]. Since these properties are strongly dependent on the network structure, it becomes increasingly important to study the details of the reaction. For example, network structure of free-radical polymerizations of multifunctional monomers depends upon monomer/macromer size, flexibility, functionality, the amount of solvent and concentration of monomers and initiators, initiation methods and initiation rate, as well as diffusional reaction constraints of propagating polymer chains. For example, monomer double bonds may possess different reactivities that are influenced by conversion (i.e., pendant double bonds typically have reduced reactivity) and significantly affect structural characteristics of the polymer network.

Initiator-chain transfer molecules, iniferters [8,9], have been used to produce well-controlled block copolymers [10,11], polymers of low polydispersity [11], and graft polymers [12,13] as well as crosslinked polymer systems on surfaces [13]. Controlled/living radical polymerization, pioneered by Otsu and Yoshida [14] and the subject of a recent commentary by Matyjaszewski [15], has mostly involved mono-vinyl systems, but more recent work includes studying the polymerization of more homogeneous multifunctional monomer structures [16].

Recent work in the field of controlled/living polymerization has involved the building of specific block copolymers with highly controlled monomer segments within the polymer chain in order to create chains of low polydispersity with specific functionality [17–19]. For example, Johnson and coworkers have created degradable macromonomers via living polymerization techniques (atom transfer radical polymerization (ATRP)) which were covalently crosslinked to create structures with homogeneous crosslinking density and well-defined pore sizes (the macromonomer length was the determining factor of the molecular weight between crosslinks) [20]. Also, Wang and Zhu have studied ATRP methods to produce EGDMA crosslinked networks with a maximum of 10% crosslinking and demonstrated high crosslinking efficiency and increased homogeneity with controlled structures [21].

Within the field of molecular imprinting, recent work has involved the formation of molecularly imprinted polymers on surfaces using iniferters [22], but these investigators have not analyzed the polymerization reaction and subsequent influence on the binding parameters as a result of controlled/living polymerization.

Of utmost importance is the control over the polymerization and associated network structure, which depends on the dynamic equilibrium between active and dormant species [14,15]. Conventional free-radical polymerization is highly non-ideal and differences in theory and experimental data indicate heterogeneity within the network structure [21]. In this work, we are the first to explore the implications of controlling structural homogeneity and its influence on the associated binding parameters of a typical molecularly imprinted polymer system. In order to accomplish this task, we began with analysis of the double bond conversion for a poly(MAA-*co*-EGDMA) molecularly imprinted system that has previously been studied. A well-studied system was chosen from the literature [24–26] to probe the polymerization reaction and potential binding parameter optimization strategies.

2. Experimental

2.1. Ethyladenine-9-acetate recognitive network synthesis

The monomers, methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA), had inhibitors removed via inhibitor removal packing sieves or vacuum distillation prior to polymerization. The initiator (azo-bis(isobutyronitrile, AIBN), template

molecule (ethyladenine-9-acetate, EA9A), ethyl 2-amino-1,6-dihydro-6-oxo-4-pyrimidineacetate (EADOP), and iniferter (tetraethylthiuram disulfide, TED) were used as received. Monomers, inhibitor removal packing sieves, initiator, iniferter, template, and template analogue were purchased from Aldrich (Milwaukee, WI). HPLC grade solvents, acetonitrile and methanol, were used as received from Fisher Scientific (Pittsburgh, PA). The polymerization solvent was acetonitrile and the polymer wash solvent (to remove template and unreacted monomer) was acetonitrile/methanol at a 4:1 volume ratio.

A typical polymerization solution, which matched literature formulation [23] (resulting polymer designated as RP1), was made with 2.61 mL EGDMA (13.83 mmol), 0.16 mL MAA (1.87 mmol), 3.96 mL acetonitrile (704.30 mmol), 26.3 mg AIBN, and 35.4 mg EA9A. Solutions were placed in a sonicator for several minutes until all solids were dissolved. Control monomer solution was made exactly the same as RP1 except EA9A, template, was not added. Polymer RP2 was made by increasing the amount of AIBN to 157.6 mg, and polymer RP3 was made by addition of iniferter, 47.4 mg TED, with an increase of initiator, 236.4 mg AIBN. The molar ratio of the initiator to the iniferter in RP3 was 14.4:1.6; moreover, this ratio gave double bond conversions similar to the double bond conversions of RP2. For RP1, RP2, and RP3, the amount of monomer, solvent, and template added to mixtures was 0.16 mL MAA, 2.61 mL EGDMA, 3.96 mL acetonitrile, and 35.4 mg EA9A. For RP1, experimental materials and conditions such as the temperature of polymerization (0 °C ± 1 °C throughout exothermic reaction), template (ethyladenine-9-acetate, EA9A), functional monomer (methacrylic acid, MAA), crosslinking monomer (ethylene glycol dimethacrylate, EGDMA), solvent (acetonitrile), photoinitiator molecule and concentration (azo-bis(isobutyronitrile), AIBN), purge gas (nitrogen), and the UV light source (mercury arc source and intensity) were matched with the literature reference [23].

All polymerization reactions were carried out in a Q-100 differential photocalorimeter (DPC) TA Instruments (New Castle, Delaware). The DPC measures the heat flow from the sample relative to a reference pan. The heat evolved was measured as a function of time, and the theoretical reaction enthalpy of the monomer solution was used to calculate the rate of polymerization, R_p, in units of fractional double bond conversion per second. Integration of the rate of polymerization curve versus time yielded the experimental heat of reaction and along with the theoretical heat of reaction yields final double bond conversion. The assumptions in the copolymerization of multiple monomers (i.e., two types in this case, functional and crosslinking monomer) were that each monomer had equal reactivity and the theoretical reaction enthalpy derived for a co-monomer mixture was calculated by the summation of component mole fraction multiplied by the monomer heat of reaction. The theoretical enthalpy of methacrylate double bonds was equal to 13.1 kcal mol^{-1} [26,27]. Due to the overwhelming fraction of ethylene glycol dimethacrylate (EGDMA) in the system 88% crosslinking (i.e., 13.83 mmol of EGDMA and 1.87 mmol of MAA), the majority of the heat of reaction will be due to EGDMA double bonds reacting. EGDMA has two moles of double bond per mole of monomer which gives the number of double bonds that are attributed to EGDMA to be approximately 94% of all double bonds in solution. Therefore, this system can be considered to be EGDMA in acetonitrile with a dilute amount of MAA.

In a typical experiment involving RP1 networks, a recognitive polymer disk was produced by placing 12.5 μL of prepolymerization solution within an aluminum hermetic pan, which was placed within the cell of the DPC and allowed to purge with nitrogen for 5 min with a purge rate of 40 mL/min and at a temperature of 20 °C. However, to prevent possible evaporation of the solvent a small quartz plate was placed on top of the pan after the 5 min purge time. Also, since oxygen is a free-radical scavenger, separate oxygen inhibition experiments were conducted to assure adequate nitrogen purge times. Nitrogen continued to flow for the duration of the experiment at a purge rate of 40 mL/min. The solution was then cooled to the polymerization temperature of 0 °C and was held at 0 °C for 15 min. The shutter on the UV light source (Novacure 2100, Exfo, Canada, with a 100 W mercury arc light bulb) was opened and the solution was irradiated by 52.5 mW/cm^2 UV light for 17 min at which time the polymerization reaction was ensured to be over (i.e., the typical polymerization time was in the order of a few minutes). The temperature of the sample was held between 0 °C and 1 °C throughout the reaction, and the end point of each reaction was determined when the heat flow changed to less than 1%. Control and RP2 disks were made in the exact same manner while RP3 was exposed to UV light for 60 min due to a longer reaction time. It is important to note that in the literature match RP1 network, monomer solutions were purged with nitrogen and irradiated with UV light for much longer polymerization times (i.e., approximately 24 h). The extended cure time is due to the polymer mold yielding a significant amount of monomer solution along the axis of the light source. With a low transmittance of UV light through a bulk polymer solution [28], this would lead to longer cure times.

2.2. Evaluation of binding parameters

The polymer disks were removed from the DPC pans and washed by Soxhlet extraction with a solution of acetonitrile/methanol in a 4:1 ratio. Extraction was performed for 2½ weeks and confirmed by analysis of the template in the wash. The washing procedure was allowed to run until EA9A was no longer detected in the wash solution and absorbance was measured by a Synergy UV–vis spectrophotometer (BioTek Instruments, Winooski, Vermont). The disks were then taken out of the Soxhlet extraction device and allowed to dry in a fume hood at ambient temperature for a 24 h period, which reduced the sudden stress cracking by rapid evaporation of solvent, and then placed in a vacuum oven at 30 °C and 25 in of mercury vacuum. Typically, 80–90% of the original template was recovered in the wash with no clear trend associated between systems.

The average disk weight for each of the samples was 3.69 ± 0.29 mg. The disk diameter was 4 mm with a width of 0.5 mm on the outside of the disk with a concavity of

less than 0.1 mm in the center of the disk. All samples had a reaction signature that was within one standard deviation from the mean to maintain a high degree of quality control for the reaction analysis and resultant polymer networks.

Dynamic binding analysis was determined by placing disks in 200 μL of various concentrations of EA9A in acetonitrile (0.01–2.0 mM solutions). After equilibrium was reached, a 100 μL aliquot of the solution was taken and the absorbance measured at 265 nm using a Biotek UV–vis spectrophotometer. Binding parameters were calculated using various isotherms (e.g., Scatchard, Langmuir, Freundlich) and the best-fit isotherm was used to determine binding parameters. Once the equilibrium concentration was determined, a simple mass balance yielded the bound concentration.

Selectivity studies of the recognitive polymers were conducted in similar fashion to the rebinding studies. Disks were placed in a 2 mM solution and EADOP in acetonitrile and allowed to reach equilibrium. Once equilibrium was reached, a 100 μL aliquot of solution was sampled and the absorbance measured at a wavelength of 282 nm. The equilibrium concentration was calculated, and a mass balance was used to determine the bound concentration.

3. Results and discussion

The monomer to template ratio for the poly(MAA-*co*-EGDMA) molecularly imprinted system that has previously been studied (i.e., our RP1 network) was 11.79, and the degree of feed crosslinking in the system was 88 mol% (no. of moles of crosslinking monomer/no. of moles all monomers). Analysis of the total number of double bonds reacted via differential scanning photocalorimetry revealed a low level of fractional double bond conversion ($35 \pm 2.3\%$).

In order to study the effect of crosslinking monomer length upon the double bond conversion for this imprinted system an equivalent experiment was conducted using a slightly longer bifunctional crosslinking monomer (poly(ethylene glycol 200) dimethacrylate, PEG200DMA, where the average number of ethylene glycol groups is 4.5 as opposed to 1 with EGDMA). The double bond conversion for the PEG200DMA crosslinked polymer was $53 \pm 2.0\%$ which can be attributed to the increased diffusional mobility of the longer crosslinking monomer. Double bond conversions for similar systems using PEG200DMA have been well recorded in literature [28–32]. Therefore, for the molecular imprinting field these results highlight that a significant amount of EGDMA crosslinking monomer in the formulation results in a severely constrained network formation. Specifically, there is a decrease in the diffusional ability of pendant double bonds in the growing polymeric network to react or limited diffusion of radicals on the growing network which lowers conversion. More importantly, it also highlights that the final polymer composition does not represent the initial formulation when using significant amounts of short bifunctional crosslinking monomer (i.e., when intra-molecular distances between crosslinking monomer double bonds are short). This is of significance in the field of molecular imprinting since most groups use high amounts of EGDMA as crosslinking monomer to produce imprinted networks and report feed crosslinker compositions in relation to binding properties (affinity, capacity, and selectivity). Therefore, reaction analysis provides a basis for the accurate comparison of molecularly imprinted systems; furthermore, while double bond conversion has been studied in highly crosslinked networks [33] this is the first study to confirm low double bond conversion within highly crosslinked molecularly imprinted polymer systems and the associated effect on the binding properties.

Contrary to our results, ^{13}C NMR studies of poly(MAA-*co*-EGDMA) imprinted polymers with 83% feed crosslinker produced at a constant temperature of 25 °C estimated 83% final double bond conversion [34]. Within the molecular imprinting literature, to the best of our knowledge, this has been the only study of a highly crosslinked imprinted poly (MAA-*co*-EGDMA) network to analyze the double bond conversion. It gives an uncharacteristically high double bond conversion for dilute MAA in EGDMA (91% of the double bonds are attributed to EGDMA). Double bond conversions of pure EGDMA (non-imprinted) have been reported to be 69% at 60 °C [35]. Since the temperatures of reaction are different, additional experimental analysis was warranted to compare conversions and ascertain the effect of temperature on the rate of reaction and conversion for this system.

By using a differential photocalorimeter, temperature could be kept constant within ±1 °C during the course of the polymerization reaction. The temperature of reaction was set a 0 °C, 25 °C, and 50 °C which resulted in $35 \pm 2.3\%$, $51 \pm 1.2\%$, and $54 \pm 1.9\%$ final double bond conversion for our imprinted system, respectively (Fig. 2). For pure EGDMA, we experimentally found the double bond conversion at 0 °C to be $36 \pm 2.5\%$, very close in value to our imprinted system. Even if none of the MAA reacts in our system, which is highly unlikely, the double bond conversion at 0 °C is calculated to be $39 \pm 2.3\%$. Therefore, this confirms that the majority of the heat of reaction is due to EGDMA double bonds reacting. However, MAA is incorporated into the system as reflected in template binding analysis. Networks with only EGDMA as monomer showed very low levels of binding of the template and no specificity (data not shown). Studies which use extraction methods and subsequent

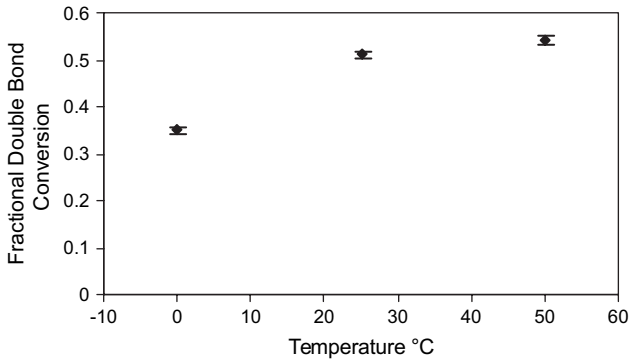

Fig. 2. Temperature influence on fractional double bond conversion of poly (MAA-*co*-EGDMA) recognitive network templated for EA9A (literature match recognitive polymer network, RP1).

measurement of unreacted monomers will overestimate conversion by not counting the crosslinking monomers that do not diffuse from the structure due to partial reaction that result in dangling, unreacted double bonds.

Since termination events are more frequent at higher temperatures with a small increase in the propagation constant, the result is shorter kinetic chain lengths. The temperature increase during the reaction increased the rate of reaction and overall conversion, but typically led to decreased affinity since hydrogen bonding and the formation of binding sites decreased with increasing temperature. This is in agreement with studies involving changes in polymerization temperature and associated binding parameters [24]. Therefore, for non-covalent imprinting within free-radical hetero/homopolymerization reactions of multifunctional monomers, the strength of template–monomer interactions is an important variable as are the network properties that influence the stability of the binding site (e.g., crosslinking density and homogeneity).

The effect of photoinitiator and solvent concentration on the fractional double bond conversion is presented in Figs. 3 and 4, respectively. An increase in the photoinitiator concentration from 0.4 wt% (literature match concentration, RP1) to 4.6 wt% increased the double bond conversion from $35 \pm 2.3\%$ to $48 \pm 2.1\%$ (Fig. 3). This can be attributed to an increase in the concentration of free radicals which provides an increased rate of chain initiating species and an increased rate of reaction (i.e., the rate scales to the square root of initiator concentration) that ultimately increases the conversion. After 2.4 wt% initiator, the fractional double bond conversion remained constant.

Increasing the solvent wt% decreased the double bond conversion (Fig. 4). This is due to a decreased concentration of initiator and a decreased concentration of monomers. The effect of solvent is important for recognitive polymer systems since increased amounts of solvent have been shown to increase matrix porosity which is beneficial for diffusional transport [36]; however, increasing the solvent wt% without a corresponding increase in photoinitiator concentration may negatively impact the double bond conversion and overall stability or fidelity of the binding sites (e.g., after 60 wt% solvent the double bond conversion decreases substantially). Since the solvent does not get incorporated into the growing polymer chains, the polymeric network must form around the solvent and this produces an accessible porous structure for adequate template diffusional transport.

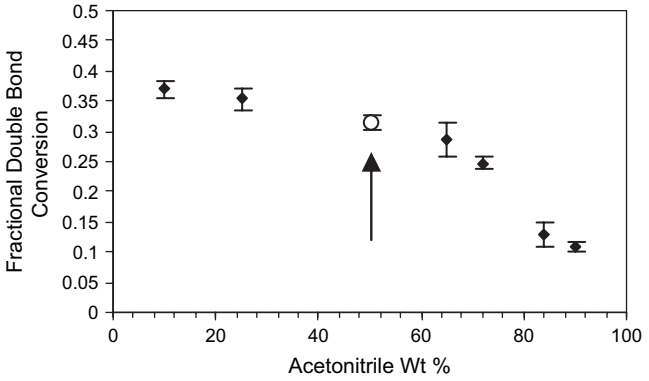

Fig. 4. Fractional double bond conversion of poly(MAA-*co*-EGDMA) recognitive networks: solvent wt% effect upon double bond conversion. Note: parameters such as feed crosslinking percent (88%), initiator wt% (0.4), and monomer template ratio (11.79) are held constant. Error bars represent standard deviation ($n = 3$). (Arrow indicates literature match, RP1.)

Equilibrium binding isotherms of the literature match recognitive polymer (RP1), associated control (i.e., no target molecule present in the formulation), recognitive polymer with 2.4 wt% initiator that demonstrated an increased double bond conversion of 48% (RP2) are shown in Fig. 5.

The average affinity of RP1 showed statistically good agreement with reported data in the literature $3.23 \pm 0.030\ \mathrm{mM}^{-1}$ [37]. The Freundlich isotherm was used as the basis for analysis of average binding affinity along with number of binding sites, since the Freundlich isotherm gave the best fit to the data, based

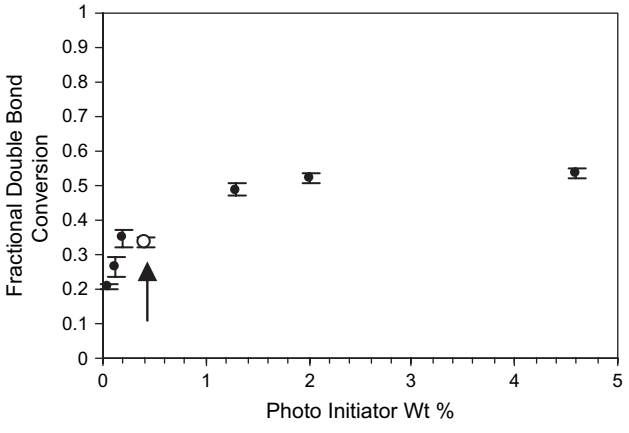

Fig. 3. Fractional double bond conversion of poly(MAA-*co*-EGDMA) recognitive networks: photoinitiator wt% effect upon double bond conversion. Note: parameters such as feed crosslinking percent (88%), solvent wt% (50), and monomer template ratio (11.79) are held constant. Error bars represent standard deviation ($n = 3$). (Arrow indicates the literature match, RP1.)

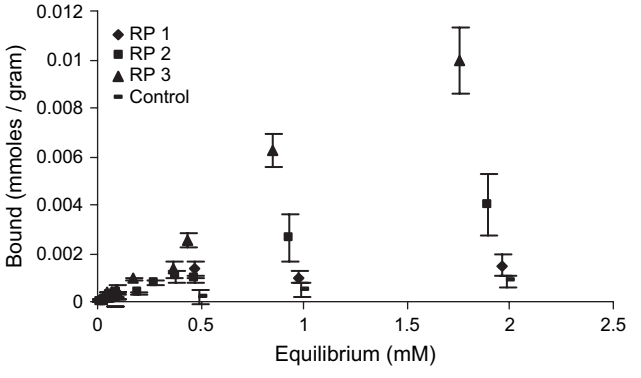

Fig. 5. Binding characteristics of poly(MAA-*co*-EGDMA) recognitive networks for ethyladenine (EA9A): equilibrium binding isotherm. RP1 is the literature match recognitive polymer, RP2 has the same formulation composition except it contains 2.4 wt% initiator which demonstrated an increased double bond conversion of 48%, RP3 is the recognitive polymer with iniferter. With RP2 a 37% increase in conversion above that of the original literature match (RP1) leads to a modest increase in the capacity of the recognitive polymer. Note: error bars represent the standard error ($n = 4$).

Table 1
Quantitative binding parameters of poly(MAA-*co*-EGDMA) networks (Freundlich isotherm analysis)

Polymer network	Binding affinity, K_{avg} (mM^{-1})	Capacity, no of sites (μmol/g)
RP1	3.12 ± 0.21	776 ± 54
RP2	2.63 ± 0.17	862 ± 60
RP3	2.61 ± 0.12	1421 ± 64

upon R^2 values. Statistically, RP2 binding capacity was within the standard deviation of RP1 values (Table 1, RP2 had a modest higher mean binding capacity at a slightly reduced affinity). It is important to note that average affinity values take into account the site sub-populations of varying affinity. While the concentration of initiator should not be overlooked in optimization, the increased conversion did not lead to improved binding affinity or capacity in this case. An increase in initiator concentration can theoretically lead to a decrease in the kinetic chain length which, we hypothesize with an increase in conversion, may result in increased binding site stabilization and increased structural homogeneity. The kinetic chain length represents the average number of monomers reacting with an active center from initiation to termination, and it is inversely proportional to the radical concentration and the rate of polymerization [38]. Thus, attempts to increase polymerization rate by increasing radical concentration produces smaller sized polymer chains [39].

In order to control the polymerization reaction further by altering the kinetic chain length and potentially increasing the homogeneity of the crosslink architecture, we investigated the use of initiator-chain transfer molecules, iniferters [8,9]. By using the iniferter, tetraethylthiuram disulfide (TED), the number of binding sites was dramatically increased at approximately equivalent binding affinity (RP3). The final double bond conversion for RP3 was 44% (maximum double bond conversion with iniferter was 48%), and the reaction was approximately 10 times longer which led to an increased binding capacity of 63% at a roughly equivalent binding affinity. This is hypothesized to be due to shorter kinetic chain lengths and/or a more narrow dispersity of kinetic chains, which leads to a more homogeneous network and potentially a more uniform crosslinking density. A smaller number of chains with a narrow size distribution would decrease the mesh size of the macromolecular structure and lead to a more uniform and higher population of appropriately sized imprinted macromolecular cavities (Fig. 6). Evidence in the literature of radical chain homopolymerization of multifunctional monomers using size exclusion chromatography and measurements of crosslinking density support this conclusion [39].

Iniferters used in this work decay into two dithiocarbamyl radicals (DTC); which are more stable compared to carbon radicals. The stability of the iniferter produced radical negates its significance on the initiation and propagation steps during the polymerization reaction, which in this particular case required the addition of carbon radicals, AIBN, to initiate the polymerization reaction. During termination steps of the polymerization reaction, the stable DTC radicals reversibly terminates with growing polymer radical chains which form a chain

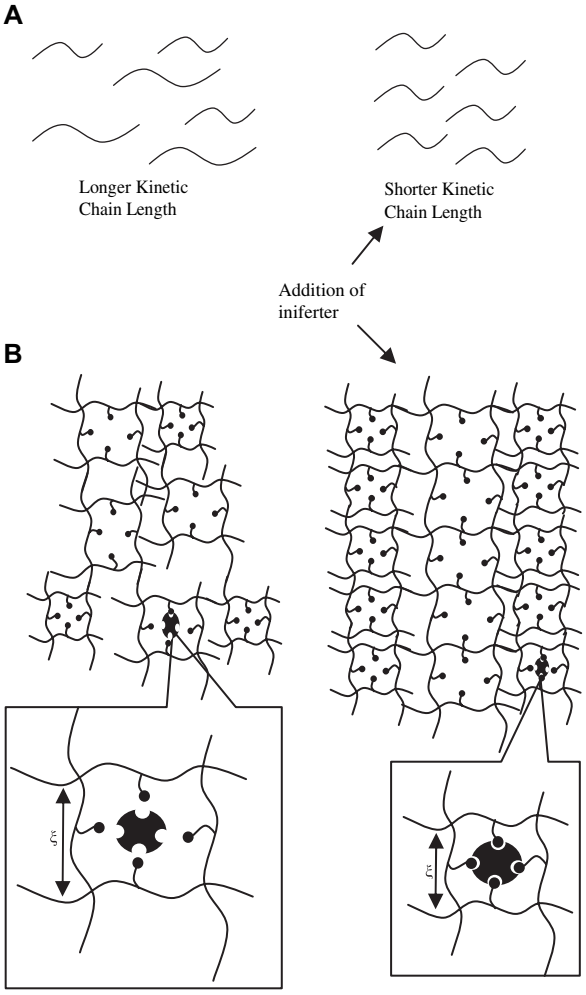

Fig. 6. Controlled/living polymerization and the effect on imprinted network structure. (A) In mono-vinyl polymerization, the use of iniferter yields a lower polydispersity of kinetic chains and decreased average chain length. (B) Within crosslinked networks, addition of iniferter leads to a more uniform and higher population of appropriately sized imprinted macromolecular cavities for the template. An optimal mesh size, ξ, gives the binding site a better functional configuration which leads to enhanced binding properties.

that can re-absorb UV light and decay back into a polymer radical and a DTC radical [8] (Fig. 7). The limitations and structural heterogeneity of radical polymerizations caused by fast termination reactions can be reduced since iniferters provide a reversible termination reaction. Recently, the use of iniferter has been shown to decrease the kinetic chain length for the homopolymerization of methacrylic anhydride [40].

Selectivity studies were performed using a molecule with similar chemical functionality of EA9A (Fig. 8A), ethyl 2-amino-1,6-dihydro-6-oxo-4-pyrimidineacetate (EADOP) (Fig. 8B). Binding capacity of EA9A and EADOP values for RP2 and RP3 are shown in Fig. 9; additionally, selectivity numbers (bound template/bound other molecule) at 2 mM concentration were 2.4 ± 1.0 and 1.9 ± 0.5 for RP2 and RP3, respectively. It is important to note that RP3 has a 63% increase in the number of binding sites while retaining a selective nature for the template. Additionally, selectivity numbers for RP2 and RP3 were found to be higher than that

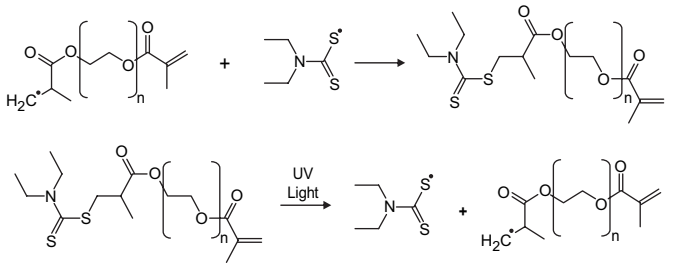

Fig. 7. The reversible termination reaction that occurs with the DTC radical and a growing polymer radical to form a macroiniferter. This macroiniferter can be irradiated with UV light to decay into a DTC radical and polymer radical to continue the reaction process. Note: n is the number of repeating ethylene glycol units.

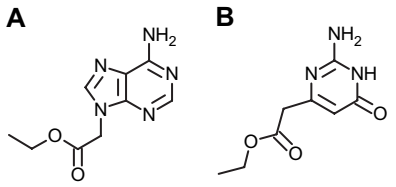

Fig. 8. Template and analogue molecular structure. (A) Template molecule, ethyladenine-9-acetate (EA9A). (B) Selectivity molecule, ethyl 2-amino-1, 6-dihydro-6-oxo-4-pyrimidineacetate (EADOP).

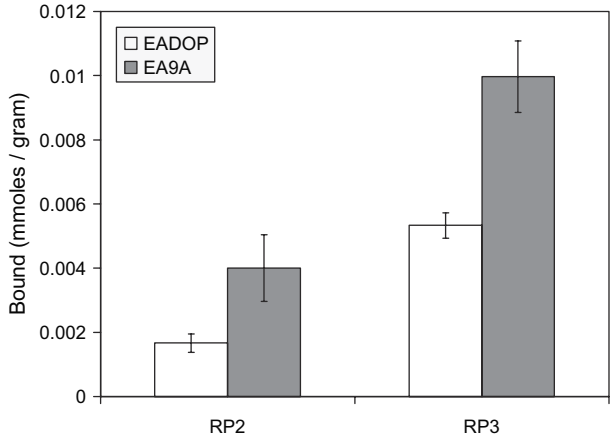

Fig. 9. Binding characteristics of poly(MAA-*co*-EGDMA) recognitive networks for ethyladenine (EA9A): selectivity study. Both RP2 and RP3 networks were more selective at a template concentration of 2.0 mM than that of the original literature match (RP1). Note: $n = 3$ and error bars represent the standard error.

of RP1 which was not selective for the template in our studies (the literature match does not report selectivity data). Therefore, the increased conversion for RP2 did not lead to improved binding affinity or capacity, but increased the selectivity compared to RP1. This is hypothesized to be due to a decrease in the kinetic chain length with increased binding site stabilization and increased structural homogeneity due to an increase in the initiator concentration. Therefore, even optimization of conventional photoinitiator can lead to a small improvement in binding parameters. However, the use of living polymerization techniques to create imprinted polymers has the greatest potential to enhance and optimize binding affinity, capacity, and selectivity.

4. Conclusion

This work indicates that reaction analysis of molecularly imprinted polymerization reactions has the potential to yield a greater understanding of the imprinting mechanism and associated binding parameters as related to the structural architecture of the polymeric network. In this work, living polymerization techniques were used to produce molecularly imprinted networks with a significant increase in binding capacity while retaining equivalent affinity and selectivity for the template molecule. Additional work with controlled polymerization strategies of molecularly imprinted polymers will inevitability lead to improved binding characteristics via a rationally optimized macromolecular structure. Since imprinted network applications depend implicitly on the extent of control of both the structural and binding characteristics, detailed reaction analysis is expected to yield promising new materials for sensors, point-of-care diagnostics, and drug delivery carriers.

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References

[1] Wulff G. Angew Chem Int Ed Engl 1995;34:1812–32.
[2] Hilt JZ, Byrne ME. Adv Drug Delivery Rev 2004;56:1599–620.
[3] Lavignac N, Christopher JA, Brain KR. Anal Chim Acta 2004;51: 139–45.
[4] Byrne ME, Park K, Peppas NA. Adv Drug Delivery Rev 2002;54: 149–61.
[5] Alvarez-Lorenzo C, Concheiro A. J Chromatogr B 2004;804:231–45.
[6] Alexander C, Andersson HS, Andersson LI, Ansell RJ, Kirsch N, Nicholls IA, et al. J Mol Recognit 2006;19:106–80.
[7] Yungerman I, Srebnik S. Chem Mater 2006;18:657–63.
[8] Kannurpatti AR, Lu S, Bunker GM, Bowman CN. Macromolecules 1996;29:7310–5.
[9] Piletsky SA, Mijangos I, Guerreiro A, Piletska EV, Chianella I, Karim K, et al. Macromolecules 2005;38:1410–4.
[10] Hutchison JB, Stark PF, Hawker CJ, Anseth KS. Chem Mater 2005;17:4789–97.
[11] Shen Y, Zhu S, Pelton R. Macromolecules 2001;34:3182–5.
[12] Liu P, Liu Y, Su Z. Ind Eng Chem Res 2006;45:2255–60.
[13] Ward JH, Bashir R, Peppas NA. J Biomed Mater Res 2001;56:351–60.
[14] Otsu T, Yoshida M. Makromol Chem Rapid Commun 1982;3:127–32.
[15] Matyjaszewski K. Macromol Rapid Commun 2005;26:135–42.
[16] Kannurpatti AR, Anderson KJ, Anseth JW, Bowman CN. J Polym Sci Part B Polym Phys 1997;35:2297–307.
[17] Cheng Z, Zhu X, Fu GD, Kang ET, Neoh KG. Macromolecules 2005; 38:7187–92.
[18] Qin Y, Sukul V, Pagakos D, Cui C, Jakle F. Macromolecules 2005; 38:8987–90.
[19] Sumerlin BS, Tsarevsky NV, Louche G, Lee RY, Matyjaszewski K. Macromolecules 2005;38:7540–5.
[20] Johnson JA, Lewis DR, Diaz DD, Finn MG, Koberstien JT, Turro NJ. J Am Chem Soc 2006;128:6564–5.
[21] Wang AR, Zhu S. Polym Eng Sci 2005;45:720–7.
[22] Tamayo FG, Titirici MM, Martin-Esteban A, Sellergren B. Anal Chim Acta 2005;542:38–46.

[23] Umpleby II RJ, Rushton GT, Shah RN, Rampey AM, Bradshaw JC, Berch Jr JK, et al. Macromolecules 2001;34:8446–52.
[24] Spivak D, Gilmore MA, Shea KD. J Am Chem Soc 1997;119:4388–93.
[25] Umpleby II RJ, Bode M, Shimizu KD. Analyst 2000;125:1261–5.
[26] Anseth KS, Wang CM, Bowman CN. Polymer 1994;35:3243–50.
[27] Ward J, Shahar A, Peppas NA. Polymer 2002;43:1745–52.
[28] Bowman CN, Peppas NA. Macromolecules 1991;42:2013–8.
[29] Scott RA, Peppas NA. Macromolecules 1999;32:6149–58.
[30] Elliot JE, Bowman CN. Macromolecules 1999;32:8621–9628.
[31] Anseth KS, Kline LM, Walker TA, Anderson KJ, Bowman CN. Macromolecules 1995;28:2491–9.
[32] Bowman CN, Peppas NA. Macromolecules 1991;24:1914–20.
[33] Lu H, Lovell LG, Bowman CN. Macromolecules 2001;34:8021–5.
[34] Sibrian-Vazquez M, Spivak DA. J Polym Sci Part A Polym Chem 2004;42:3668–75.
[35] Sun X, Chiu YY, Lee LJ. Ind Eng Chem Res 1997;36:1343–51.
[36] Spizzirri UG, Peppas NA. Chem Mater 2005;17:6719–27.
[37] Rampey AM, Umpleby II RJ, Rushton GT, Iseman JC, Shah RN, Shimizu KD. Anal Chem 2004;76:1123–33.
[38] Flory PJ. Principles of polymer chemistry. Ithaca: Cornell University Press; 1953.
[39] Odian G. Principles of polymerization. New York: John Wiley & Sons; 1991.
[40] Hutchison JB, Lindquist AS, Anseth KS. Macromolecules 2004;37:3823–31.