JOURNAL OF Applied Polymer SCIENCE

Crosslinking Diversity on Network Morphology, Template Binding, and Template Transport of Molecularly Imprinted Polymers Prepared via Living Radical Polymerization

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ABSTRACT: The objective of this work is to study the effects of altering the chain building blocks and the reaction on template binding and transport parameters of imprinted polymer gels. The characterization of imprinted poly(diethylaminoethylmethacrylate-*co*-hydroxyethylmethacrylate-*co*-polyethyleneglycol(n)dimethacrylate) polymer gels prepared via free (FRP) or living (LRP) radical polymerization with varying crosslinking monomer lengths (number of ethylene glycol repeat units of 1, ~4.5, ~9) and concentrations (1, 5, 10, 50%) is presented. All imprinted networks prepared via LRP exhibited significantly higher template binding affinities and capacities as well as significantly lower template diffusion coefficients compared to those prepared via FRP. Synthesizing imprinted polymers via LRP results in much smaller and relatively constant dispersities of polymer chains compared to imprinted polymers prepared via FRP. Therefore, LRP has a profound structural effect on the imprinted polymer network leading to increased homogeneity in the mesh structure which enhances the molecular imprinting effect. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2013

KEYWORDS: living radical polymerization; crosslinking; molecular recognition

Received 14 March 2013; accepted 19 May 2013; Published online
DOI: 10.1002/app.39568

INTRODUCTION

Chemically crosslinked polymer gels are insoluble, polymer network structures composed of homo- or hetero-polymers, which have the ability to absorb significant amounts of solvent and retain their shape without dissolving. They can deform and respond as an elastic body when solvated at temperatures above their glass transition with the amorphous portions of the polymer in a rubbery state with significant mobility. Crosslinks can be covalent bonds, permanent physical entanglements, non-covalent interactions, or microcrystalline regions incorporating various chains and are primarily responsible for preventing the dissolution of the polymer in solvent.[1,2]

Macromolecular memory within heteropolymer gels is a relatively new method for additional control of therapeutic diffusion within gels.[2–8] Molecular imprinting techniques introduce "macromolecular memory" within gels and increase the tailorability of the macromolecular structure by producing networks with intrinsic affinity and capacity for a template molecule. As polymer gels have higher potential for mobility within the chains, macromolecular memory is used to better differentiate these systems compared to highly crosslinked imprinted polymers.[3] The advantage of tuning co-polymer network functionality is the ability to manipulate the macromolecular structure and chemistry on the scale of the therapeutic, thus providing better control over the drug transport.[2–8] For gels with covalent crosslinks, the crosslinker length (i.e., linear size), number of double bonds, concentration (i.e., the percent of crosslinking monomer reacted in the network or degree of crosslinking) influence imprinting effectiveness. When a dry gel is immersed in a thermodynamically compatible solvent, the solvent movement into the gel polymer chains leads to considerable volume expansion and macromolecular rearrangement depending on the nature and extent of crosslinking within the network.

Structural assessment of imprinted gels can be achieved by analysis of the following related parameters: the polymer volume fraction in the swollen state (i.e., the percentage of water absorbed by the gel), the average molecular weight between two adjacent crosslinking or junction points, and the average correlation distance between two adjacent crosslinking or junction points (i.e., the average mesh size or free space between the macromolecular chains available for transport). Typically, higher crosslinked imprinted gels have a tighter mesh structure and swell to a lesser extent than weakly crosslinked gels. Equilibrium

swelling and rubber elasticity theories have been used to characterize the structural parameters of polymer gels.[1,2]

For molecularly imprinted networks, one of the most important considerations in effective template recognition is to maintain the binding cavity produced via differing polymer chains close to the state when the original imprint was formed (i.e., close to the relaxed state of the polymer). In other words, the swollen or collapsed polymer volume at equilibrium conditions of use must not be too different from the relaxed polymer volume fraction. The thermodynamic compatibility of the polymer chains and solvent as well as the number of crosslinking points within the network determines the nature and extent of this transition. The expansion of polymer chains increases the free volume available for template transport, but it can decrease the effectiveness of the imprinting site created by multiple polymer chains. Variations in network structure itself have been demonstrated to influence template binding and control the size of the imprinted cavities.[5–12]

In this work, we studied the effects of altering the chain building blocks and the polymerization reaction on the imprinting effectiveness of polymer gels as well as template transport. The crosslinking junctions were altered by varying the concentration and length of the crosslinking monomer with imprinted gels prepared via both conventional free radical polymerization (FRP) and living radical polymerization (LRP) with the use of a chain transfer agent. While monomer reactivity is important, the primary issue with FRP is the mismatch between the rapid chain growth during polymerization and slow chain relaxation of the forming polymer which results in structural heterogeneity in polymer networks generated by FRP.[13] With LRP, the bimolecular termination between polymer chains is replaced with a macroradical iniferter termination reaction which is much slower and reversible making it thermodynamically favorable to the formation of a more homogeneous polymer network.[14–16] With imprinted networks, LRP could lead to improved binding properties.[17–22]

It is well documented that the kinetic chain length of a linear polymer directly impacts its polymerization kinetics and the final structural and mechanical properties of the polymer.[23,24] However, there is very little work highlighting the effect of kinetic chain length in crosslinking systems and even less relating to imprinted crosslinked systems. One reason for the lack of interest is the perceived unlikelihood that kinetic chain length is important in the kinetics of crosslinking systems that quickly form gels of infinite molecular weight. Though it may seem counterintuitive, recent research has shown that chain length does have a measurable effect on the polymerization kinetics of multi-functional methacrylates.[25,26] It has been demonstrated that chain length dependent termination is important in crosslinking systems. Adding a chain transfer agent to the system or increasing the initiation rate (i.e., decreasing the kinetic chain length) leads to a more mobile reaction environment and more rapid termination.[25,26] Additionally, the presence of a chain transfer agent alters the chemical identity of the radical fragment that begins a polymer chain. In chain transfer dominated systems, the initiator fragments are not responsible for initiating the majority of chains. Rather, the chain transfer agent fragment will reinitiate and begin a new polymer chain. In the presence of small amounts of chain transfer agents, it is unlikely that the chemical identity of this beginning fragment has any significant impact on the polymer properties or the network formation. Thus, the effect of chain transfer, even in these systems, is primarily on the kinetic chain length distribution.[25,26] As the double bond conversion increases and reaction diffusion controlled termination begins to dominate the kinetics, the effect of kinetic chain length on the polymerization kinetics diminishes. The studies illustrate that the kinetics of multi-functional methacrylate systems are significantly impacted by chain length and the termination environment is dominated by the more mobile radical species present in the system.[25,26]

The main findings of the work in this article demonstrate that imprinted polymers prepared via LRP exhibit a significantly reduced and relatively constant polydispersity, which may lead to a more homogeneous network structure. Imprinted polymer networks prepared via LRP exhibit significantly higher template binding affinities and capacities as well as significantly lower template diffusion coefficients compared to the corresponding polymers prepared via FRP.

EXPERIMENTAL

Poly(ethyleneglycol(200))dimethacrylate (PEG200DMA) and Poly(ethylenglycol(400))dimethacrylate (PEG400DMA) were used as received while ethyleneglycol dimethacrylate (EGDMA), (diethylaminoethyl)methacrylate (DEAEM) and (hydroxyethyl)-methacrylate (HEMA) had inhibitors removed via inhibitor removal packing sieves prior to polymerization. The initiator azo-bis(isobutyronitrile) (AIBN), template molecule (diclofenac sodium (DS)), and chain transfer agent (tetraethylthiuram disulfide (TED)) were used as received. Monomers (except PEG200DMA AND PEG400DMA), inhibitor removal packing sieves, initiator, chain transfer agent, and template were purchased from Aldrich (Milwaukee, WI). PEG200DMA and PEG400DMA were purchased for Polysciences Inc. (Warrington, PA). Deionized (DI) water was the template rebinding solvent, the wash solvent (to remove template and unreacted monomer), as well as the mobile phase in the HPLC system.

Synthesis of Poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) Gels

Poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels imprinted for DS were produced via two reaction schemes, FRP and LRP, varying mole percentage of crosslinking monomer (1, 5, 10, 50%) and crosslinking monomer (EGDMA, PEG200DMA, PEG400DMA). To highlight a representative example, poly(-DEAEM-*co*-HEMA-*co*-PEG200DMA) gels imprinted for DS with a 5 mol % crosslinking percentage were made with 0.336 mL of DEAEM (1.673 mmol), 3.659 mL of HEMA (30.118 mmol), 0.538 mL of PEG200DMA (1.673 mmol), 20 mg of AIBN (0.121 mmol), and 150 mg of DS (0.472 mmol). For all gels, the DEAEM functional monomer concentration was fixed at 5 mol % and the template (DS) to DEAEM functional monomer ratio was fixed at 0.3. The solutions were mixed and sonicated until all solids were dissolved. Non-imprinted polymers were prepared similarly except the template was absent. The poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) imprinted gel

prepared via living/controlled radical polymerization (LRP) was synthesized with 4.20 mg of TED (0.014 mmol) and 40 mg of AIBN (0.242 mmol). Solutions were transferred to an MBraun Labmaster 130 1500/1000 Glovebox (Stratham, NH), which provided an inert (nitrogen) atmosphere for free-radical UV photopolymerization. Then monomer solutions were pipetted between two 6″ × 6″ glass plates coated with trichloromethylsilane (to prevent strong adherence of the polymer matrix to the glass) and separated by 0.25 mm Teflon spacers. The solutions were left uncapped and open to the nitrogen atmosphere until the O_2 levels inside reached negligible levels (<1 ppm) as determined by an attached solid state O_2 analyzer. The polymerization reaction was carried out for 8 min for the poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) control and imprinted gels, whereas the reaction time was 24 min for the polymers prepared via LRP. Separate differential photocalorimetry (DPC) studies revealed exact reaction times. The intensity of light from a UV Flood Curing System (Torrington, CT) was 40 mW/cm^2 at 325 V and the temperature within the glovebox was held constant at 25°C. After polymerization, the glass plates were soaked in DI water and the polymers were quickly peeled off the plates and cut into circular discs using a size 10 cork borer (13.5 mm). The gels were washed in a well-mixed 2 L container of DI water for 7 days with a constant 5 mL per minute flowrate of DI water. Absence of detectable drug released from the polymer gel was verified by removing random gels, placing them in fresh DI water with adequate mixing, and sampling the supernatant via spectroscopic monitoring. The discs were allowed to dry under laboratory conditions at a temperature of 20°C for 24 h and then transferred to a vacuum oven (27 in Hg, 33–34°C) for 24 h until the disc weight change was less than 0.1 wt %.

Analysis of Kinetic Chain Length

The molecular weight distributions of uncrosslinked polymer chains were characterized by a modified HPLC system (Shimadzu, Columbia, MD) used for gel permeation chromatography (GPC). The GPC setup consisted of two PL Aquagel size exclusion columns in series (Varian LLC, Santa Clara, CA), for separation of various molecular weight fractions of the polymer which were detected using a RID10A refractive index detector (Shimadzu, Columbia, MD). DI water was used as the mobile phase for the system. Prior to running the samples, the system was calibrated using narrow molecular weight distribution poly(MAA) standards (Polymer Source Inc., Dorval, Quebec). The polymer chains obtained after early termination of polymerization were dissolved in the mobile phase to achieve a concentration of 5 mg/mL. A 50 μL aliquot of the solution was then injected into the system using a Rheodyne (Oak Harbour, WA) 7725(i) manual injection unit. Using the subsequent peaks obtained on the chromatograph, the weight average molecular weight (M_w), the number average molecular weight (M_n), and the polydispersity index (PDI) were calculated, where the molecular weight (M_i) for a particular weight fraction was described by the *x*-coordinate of the corresponding point on the chromatograph while the number of chains (N_i) was described by the *y*-coordinate. Mark-Houwink parameters for poly(HEMA) polymer chains were obtained from the literature[27] while those of the poly(MAA) chains were calculated using the standards calibration curve. It is important to note that the polymers produced in this work had relatively low monomer conversions and were dilute solutions in all other monomers with a preponderance of the single monomer (HEMA).

Analysis of Kinetic Parameters

A dark reaction was used to determine the kinetic reaction profile for the poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels.[28,29] For each polymerization reaction, the UV light was shut off at a specific time point during the reaction. The rate of polymerization was calculated via reaction analysis using the heat flow vs. time from the DPC, average molecular weight of the polymerization solution, and the theoretical heat of reaction for the methacrylate double bond. Fractional double bond conversion was determined by dividing the experimental heat of reaction by the theoretical heat of reaction. The experimental heat of reaction was determined by the area under the heat flow versus time curve from the DPC. The termination and propagation constants, k_t and k_p, were calculated from eqs. (1) and (2), and the derivation of these equations can be found in Flory[30] or Odian.[31]

$$\frac{k_p}{k_t^{0.5}} = \frac{R_p}{[\mathrm{M}](f I_o \epsilon [\mathrm{I}])^{0.5}} \tag{1}$$

where [M] is the monomer concentration, the initiator efficiency is *f*, I_o is the light intensity, ϵ is the extinction coefficient, and [I] is the initiator concentration. The unsteady state equation used to decouple the propagation constant and the termination constant is shown below

$$k_t^{0.5} = \frac{k_p / k_t^{0.5}}{2(t_1 - t_0)} \left[\frac{[M]t = t_1}{Rpt = t_1} - \frac{[M]t = t_0}{Rpt = t_0} \right] \tag{2}$$

where $t_1$and t_0 are the final and initial times, $[\mathrm{M}]_{t=t1}$ and $[\mathrm{M}]_{t=t0}$ are the corresponding monomer concentrations, respectively, and $R_{pt=t1}$ and $R_{pt=t0}$ are the rate of polymerization at final and initial times, respectively.

Template Binding Experiments and Analysis of Binding Parameters

A stock solution of 1 mg/mL of DS was prepared and diluted to five concentrations (0.05, 0.10, 0.15, 0.20, and 0.25 mg/mL). Initial absorbances of each concentration were measured using a Synergy UV–vis spectrophotometer (BioTek Instruments, Winooski, VT) at 276 nm, the wavelength of maximum absorption. After the initial absorbance was taken, a dry, washed poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) polymer disk was inserted in each vial and the vials were gently mixed until equilibrium. Separate dynamic studies were performed to assure equilibrium conditions were reached. After equilibrium was reached over a 7-day period, the solutions were vortexed for 10 s, and the equilibrium concentrations were measured. A mass balance was used to determine the bound amount of drug within the polymer gel. All gels were analyzed in triplicate, and all binding values are based upon the dry weight of the gel. The Langmuir–Freundlich isotherm was used to determine binding parameters because it gave the best fit to the experimental data.

Dynamic Template Release Studies and Diffusion Coefficient Determination

After binding studies, poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels, loaded with template at a concentration of 0.25 mg/mL,

were placed in 30 mL of DI water which was continuously agitated with an Ocelot orbital shaker (Cheshire, WA) at 375 rpm at 25°C. At various time points, the absorbance of the solution was measured using a Synergy UV–vis spectrophotometer (Bio-Tek Instruments, Winooski, VT) at 276 nm until the concentration did not change more than 1%. At each sample point, the DI water was replaced to maintain infinite sink conditions. Fractional template release profiles were calculated for all polymer gels by taking the amount of template released at specific times, M_t, divided by the maximum amount of DS released during the experiment, M_∞. The fractional template release profile, M_t/M_∞ vs. time, was determined for each gel. Template diffusion coefficients were calculated using Fick's law, which describes one-dimensional planar solute release from gels.[32] For polymer geometries with aspect ratios (exposed surface length/thickness) greater than 10, edge effects can be ignored and the problem can be approached as a one-dimensional problem.

Swelling Studies and Polymer Volume Fraction Determination

After polymerization, three gels of each polymer formulation were used for dry, swollen, and relaxed specific volume determination experiments. For the dry specific volume determination, gels were placed in the vacuum oven at a temperature of 30°C and pressure of 28 inches of Hg until the weight change was less than 0.1 wt %. Once dry, the gels were then taken out and the dry mass was measured on a Sartorius scale. Afterward, a density determination kit was installed on the Sartorius scale. The mass of the gel was then measured in heptane, a non-solvent (density of 0.684 g/mL at a temperature of 25°C). Once measurements were taken, Archimedes buoyancy principle was used to calculate the density of the dry polymer. The experiment was repeated for both the relaxed and swollen gel. The relaxed gel specific volume was calculated directly after the polymerization reaction without any additional solvent being introduced into the gel. The swollen gel specific volume was calculated after the gel reached equilibrium with the solvent for each system. The equilibrium volume swelling ratio, Q, was calculated as the ratio of the swollen polymer volume to the volume of the dry polymer.

Dynamic swelling studies of poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels were performed by measuring the initial gel dry weight to determine the dry mass of polymer. The gel was then placed in a 0.5 mg/mL DS solution. The gel was removed from solution, patted dry with Kimwipes®, and weighed. After the weight was measured, the gel was placed back in solution to continue swelling. The measurement was repeated once every 5 min for the first hour, once every 10 min for the second hour, and then every 30 min until the gel reached a constant mass which indicated equilibrium.

Mechanical Analysis and Calculation of Mesh Size

Mechanical analysis of DS imprinted poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels in the equilibrium swollen state (with DI water as solvent) was performed. Samples of each gel (1 mm × 3 mm × 10 mm strips) were removed and analyzed with a RSA III Dynamic Mechanical Analyzer (DMA), (TA Instruments, New Castle, DE) to obtain stress versus strain. Each experiment was conducted in controlled force mode with a force ramp from 0.001 to 0.3 N.

Polymer gel mesh size was calculated via data collected from the static experiments via a DMA and by using the theory of rubber elasticity. Equation (3) describes the tension of a polymer sample swollen until equilibrium with the solvent, but not prepared in solvent

$$\tau = RT\left(\frac{1}{\bar{v}\overline{M}_c}\right)\left(\alpha - \frac{1}{\alpha^2}\right)\upsilon_{2,s}^{1/3} \quad (3)$$

where R is the universal gas constant, T is the temperature, $\bar{v}$ is the specific volume of the polymer in the relaxed state, $\overline{M}_c$ is the average molecular weight between crosslinks, α is the deformation of a network structure by elongation which is equivalent to the stretched length over initial length ($\alpha = L/L_o$) and $\upsilon_{2,s}$ is the equilibrium swollen polymer fraction calculated by polymer dry volume, V_{dry}, divided by the polymer swollen volume, V_s. In deriving eq. (3), it is assumed that the average molecular weight between crosslinks is much smaller than the number average molecular weight (i.e., $\overline{M}_c << M_n$).

The stress and strain data obtained by the static experiments from DMA was plotted with the α term on the y axis and tension τ on the x axis to obtain the slope which gave the average molecular weight between crosslinks $\overline{M}_c$. To determine the actual mesh size, ξ of the polymer network, the relationship of ξ to $\overline{M}_c$ was used.[33,34]

$$\xi = Q^{1/3}\left(2C_n\left(\frac{\overline{M}_c}{M_r}\right)\right)^{1/2}l \quad (4)$$

where Q is the equilibrium volume swelling ratio, C_n is the characteristic ratio for the polymer (obtained from the molar average of the C_n from the homopolymers), and M_r is the effective molecular weight of the repeating unit (determined by a weighted average of the copolymer composition). It is important to note the equilibrium volume swelling ratio, Q, is the swollen volume of the gel divided by the dry volume of the gel or the reciprocal of the swollen polymer volume fraction. The C_n values used in this analysis were for polyethylene glycol dimethacrylate (C_n =3.8), and for the poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) a typical average value of the characteristic ratio (C_n = 11) was used.[35–38] The carbon–carbon bond length of the polymer backbone, which is equal to 1.54 Å is represented by length, l.

RESULTS AND DISCUSSION

Table I lists the calculated template binding affinities and capacities of all imprinted polymers studied in this work. Figure 1 shows equilibrium binding isotherms for imprinted polymer gels with varying amounts of the crosslinking monomer, PEG200DMA, ranging from 1 to 50 mol % of the total monomer content. Figure 1(A) highlights imprinted polymers prepared via FRP while Figure 1(B) highlights imprinted polymers prepared via LRP. To demonstrate effective imprinting, we first highlight these polymers prepared with 5% crosslinking PEG200DMA monomer in comparison to the corresponding non-imprinted polymers. For polymers prepared with 5% crosslinking monomer, the imprinted polymers prepared via FRP

Table I. Template Binding Capacities, Affinities, and Diffusion Coefficients for Poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) Imprinted Polymer Gels

Crosslinker (type of reaction)	K_a (mM^{-1})	Q_{max} (mg/g)	Diffusion coefficient (cm^2/s) × 10^{13}
5% EGDMA (FRP)	16.8 ± 0.43	10.7 ± 0.27	3.64 ± 0.02
5% EGDMA (LRP)	22.4 ± 0.20	20.8 ± 0.26	1.58 ± 0.02
5% PEG400DMA (FRP)	15.1 ± 0.20	13.3 ± 0.38	5.38 ± 0.07
5% PEG400DMA (LRP)	19.3 ± 0.20	16.7 ± 0.41	2.42 ± 0.02
1% PEG200DMA (FRP)	15.3 ± 0.27	17.1 ± 0.43	5.47 ± 0.05
1% PEG200DMA (LRP)	21.6 ± 0.20	24.5 ± 0.38	3.38 ± 0.01
5% PEG200DMA (FRP)	15.7 ± 0.12	16.7 ± 0.64	3.68 ± 0.05
5% PEG200DMA (LRP)	21.7 ± 0.17	23.9 ± 0.60	1.84 ± 0.02
10% PEG200DMA (FRP)	15.1 ± 0.24	11.6 ± 0.22	3.73 ± 0.04
10% PEG200DMA (LRP)	20.1 ± 0.57	18.4 ± 0.69	1.68 ± 0.03
50% PEG200DMA (FRP)	12.0 ± 0.18	6.1 ± 0.27	3.16 ± 0.08
50% PEG200DMA (LRP)	16.2 ± 0.31	12.3 ± 0.18	1.35 ± 0.02

demonstrate an increased binding affinity (K_a = 15.7 ± 0.12 mM^{-1}) and capacity (Q_{max} = 16.7 ± 0.64 mg/g) over the corresponding non-imprinted polymer networks prepared via FRP (K_a = 10.1 ± 0.20 mM^{-1}, Q_{max} = 9.6 ± 0.38 mg/g) indicating the successful creation of molecular memory in the imprinted networks. Thus, the imprinted polymer gels exhibited a 55%

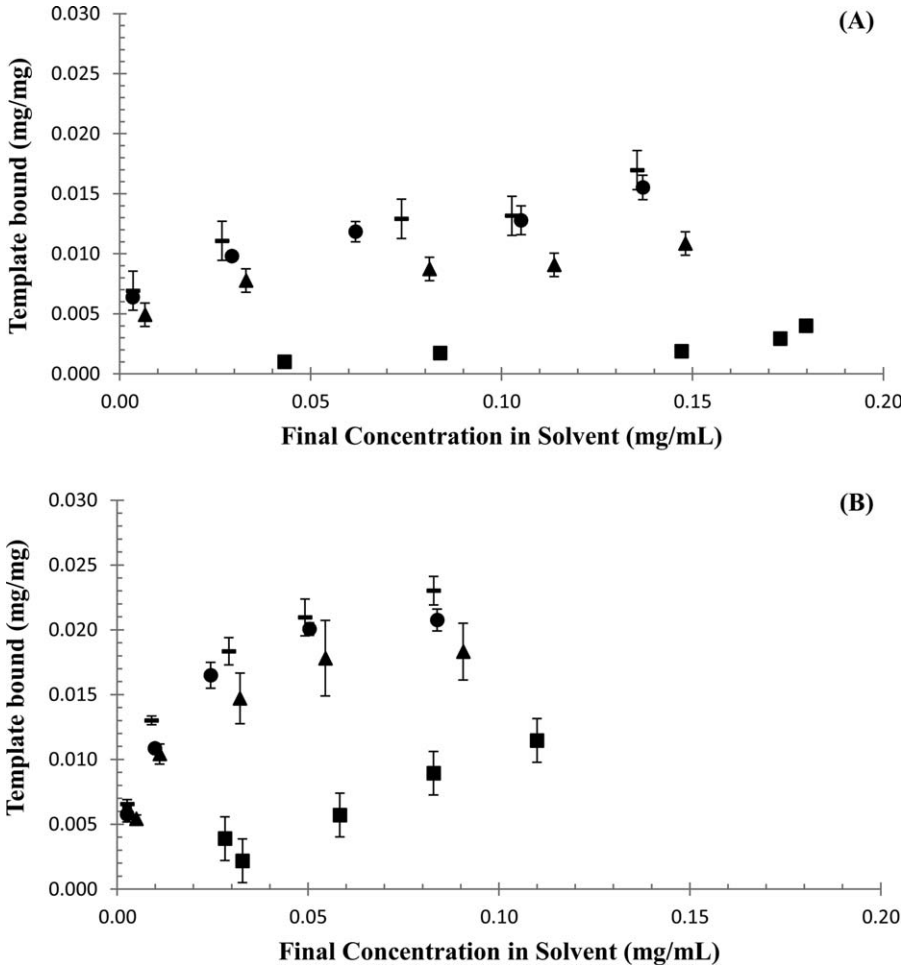

Figure 1. Equilibrium binding isotherms for DS binding by imprinted poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels prepared via (A) FRP and (B) LRP with varying concentrations of crosslinker. Polymers prepared with varying concentrations of crosslinker in feed: crosslinker content = 1%(▬); crosslinker content = 5%(●); crosslinker content = 10%(▲); crosslinker content = 50%(■). Error bars represent the standard error with n = 3.

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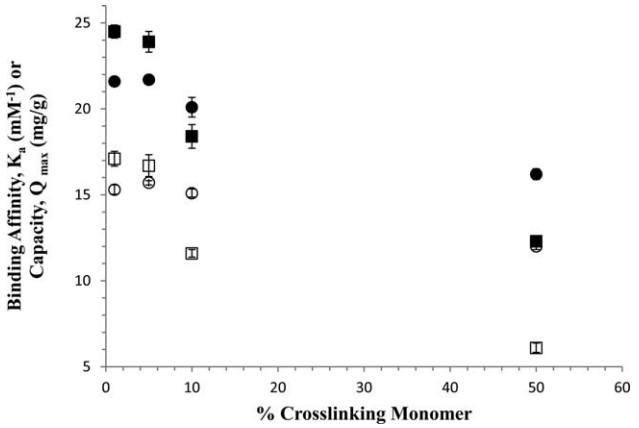

Figure 2. DS binding affinity and capacity of imprinted poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels prepared via FRP and LRP with varying concentrations of crosslinker. Binding affinity (●, ○) and capacity (■, □) of gels prepared via LRP (■, ●) and FRP (□, ○). Error bars represent the standard error ($n = 3$).

increase in binding affinity and a 74% increase in binding capacity. For all polymers studied, imprinted polymers demonstrated increased affinity and capacity compared to their non-imprinted polymers. Similarly, imprinted polymers networks prepared via LRP ($K_a = 21.7 \pm 0.17$ mM^{-1}, $Q_{max} = 23.9 \pm 0.60$ mg/g) demonstrated a 97% increase in binding affinity and a 130% increase in the number of binding sites (Q_{max}) over the corresponding non-imprinted polymer networks prepared via LRP ($K_a = 11.0 \pm 0.34$ mM^{-1}, $Q_{max} = 10.4 \pm 0.48$ mg/g). For all polymers studied, the use of LRP resulted in imprinted polymers with significantly higher template affinity and capacity compared to the corresponding imprinted polymers prepared with FRP (Figure 2). Non-imprinted gels prepared via LRP did not demonstrate a statistically significant enhancement of binding parameters over the non-imprinted gels prepared via FRP strongly indicating that LRP enhanced the template binding parameters of the polymers and the molecular imprinting effect.

From Figure 2, one can see that at low concentrations of PEG200DMA changing the extent of crosslinking (1–5%) does not have a statistically significant impact on either the template binding capacity or the binding affinity of the imprinted polymer gels. However, as the extent of crosslinking was increased, the template binding capacity was significantly decreased and template affinity decreased by a much lesser extent. For example, polymer gels prepared via FRP with 10% PEG200DMA concentration demonstrated a binding capacity of 11.6 ± 0.22 mg/g which was 31% lower than the binding capacity of polymer gels prepared with 5% PEG200DMA ($Q_{max} = 16.7 \pm 0.64$ mg/g). The template binding affinity showed a much smaller 4% decrease. This makes sense for capacity if one considers that an increase in crosslinker content means less functional monomer is added to the formulation at a fixed template to functional monomer ratio, thus less binding sites are formed. The average affinity is lower as crosslinking is increased, which is counterintuitive as less mobility in the polymer network is expected to lead to more stable binding sites. We hypothesize that the proportion of high affinity sites increases while the fraction of lower affinity sites also increases more due to a more heterogeneous structure.

A similar trend was observed for the corresponding polymer gels prepared via LRP. Polymers with higher crosslinking content demonstrated significantly decreased template binding capacity and a lesser decrease in the template affinity. For example, polymer gels prepared via LRP with 10% PEG200DMA concentration demonstrated a binding capacity of 18.4 ± 0.69 mg/g which was 23% lower than the binding capacity of polymer gels prepared with 5% PEG200DMA ($Q_{max} = 23.9 \pm 0.60$ mg/g). However, all imprinted polymer gels prepared via LRP at a given crosslinking percentage still demonstrated higher binding affinity and capacity when compared with the corresponding imprinted polymers prepared via FRP. This is consistent with the observed extension of chain propagation and delayed gel formation reported previously by our group[39] for imprinted polymers prepared via LRP compared to FRP. The percent improvement in the template binding capacity increased with increasing crosslinker content. For example, polymers prepared via LRP with 5% PEG200DMA exhibited a 43% increase in template binding capacity compared to polymers prepared via FRP with 5% PEG200DMA, while polymers prepared with 10% and 50% PEG200DMA demonstrated a 59% and 102% increase in binding capacity, respectively. The relationship between increasing crosslinker content and improved template binding affinity due to LRP was less significant. As the crosslinker content was increased from 5% PEG200DMA to 10% PEG200DMA to 50% PEG200DMA, template binding affinity increases due to LRP comparing FRP to LRP were 38%, 33%, and 35%.

From Figure 3(A), one can see that increasing the crosslinking monomer content resulted in slower template transport through the poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels. For polymers prepared via FRP, the diffusion coefficient decreased by 42% as the extent of crosslinking was increased from 1% to 50%. The slower template transport through the highly crosslinked polymers can be attributed to a decrease in the mesh size of the polymer network and a decrease in the free volume available for transport as the number of crosslinking points increased. Polymers prepared via FRP with 1% PEG200DMA were calculated to have a mesh size of 4.86 ± 0.32 nm while the corresponding polymers with 50% PEG200DMA were calculated to have a mesh size of 1.79 ± 0.19 nm. Polymers prepared via LRP exhibited a similar trend to that observed for polymers prepared via FRP. However, the diffusion coefficient decreased by more than the FRP prepared polymers. It decreased by 60% as the extent of crosslinking increased from 1% to 50%. In addition, at a given crosslinking percentage, all polymers prepared via LRP exhibited lower diffusion coefficients compared to their corresponding polymers prepared via FRP (Figure 4). This indicates decreased free volume for transport, and we hypothesize that this was due to decreased network heterogeneity.

Figure 3(B) shows the equilibrium volume swelling ratio of the imprinted gels versus the crosslinker content for poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) polymers prepared via FRP and LRP. It was observed that increasing the crosslinking content

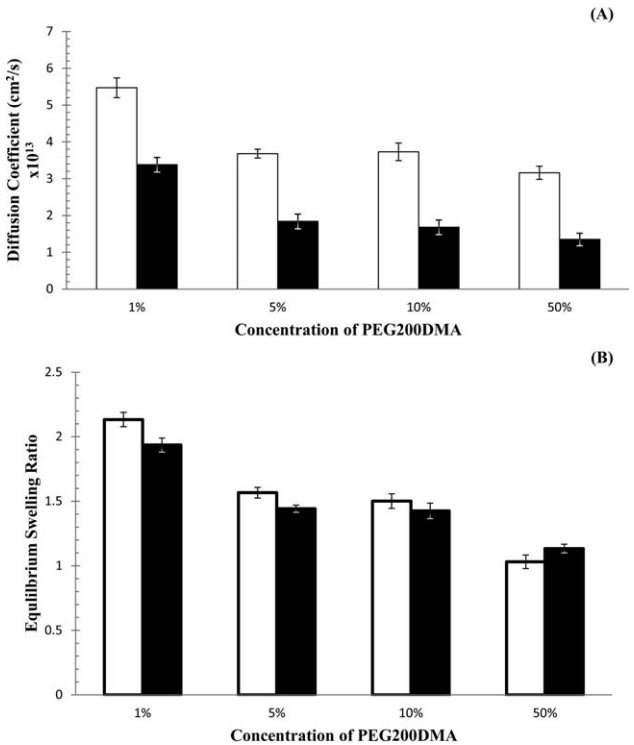

Figure 3. Effect of crosslinking monomer concentration on DS diffusion coefficient and polymer equilibrium volume swelling ratio for imprinted poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels prepared via FRP and LRP. Polymers prepared via FRP (□) and LRP (■) and error bars represent the standard error with $n = 3$.

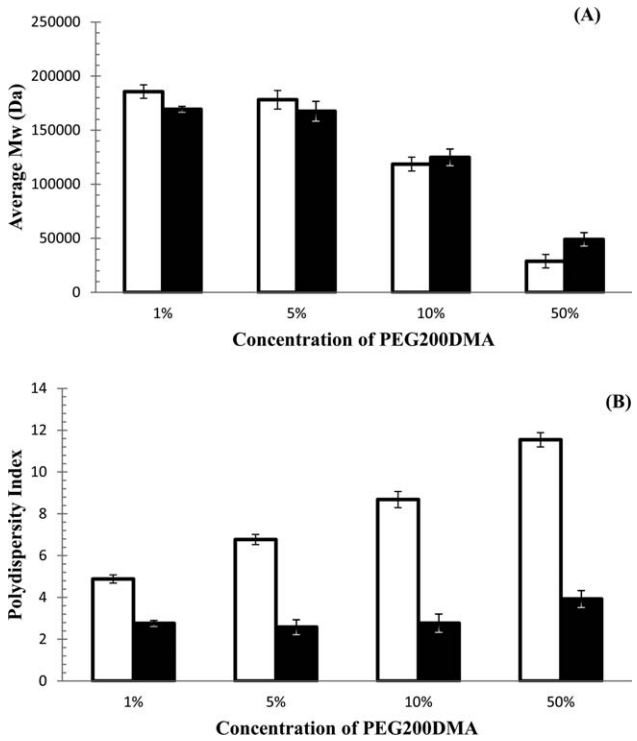

Figure 5. Effect of crosslinking monomer concentration on kinetic chain length and polydispersity index of polymer chains. Weight average molecular weight (A) and polydispersity index (B) versus functional monomer concentration for pre-gelation polymers prepared via FRP (□) and LRP (■). Error bars represent the standard error with $n = 3$.

generally resulted in polymers with lower equilibrium swelling ratios. The presence of crosslinking points has been demonstrated to restrict the ability of a polymer gel to solvate in a favorable solvent resulting in lower equilibrium swelling ratios. Since water is a favorable solvent for the poly(HEMA-*co*-DEAEM-*co*-PEG200DMA) gels, these results are consistent with other reported results.[32] As the equilibrium volume swelling ratio is the reciprocal of the polymer volume fraction in the swollen state, the polymer volume fraction increased as the crosslinking content was increased. The observed increase in polymer volume fraction as the extent of crosslinking was increased supports the decrease in diffusion coefficients for template transport through the polymer gels observed in Figure 3(A). The use of LRP also resulted in lower equilibrium swelling ratios and higher polymer volume fractions in the swollen state at low concentrations of crosslinker. Comparing polymers prepared by LRP and FRP, the polymer volume fractions in the swollen state are very close in comparison for a given crosslinking percentage, but there are significant differences in the template diffusion coefficients. The polymers prepared by LRP have much lower template diffusion coefficients with almost

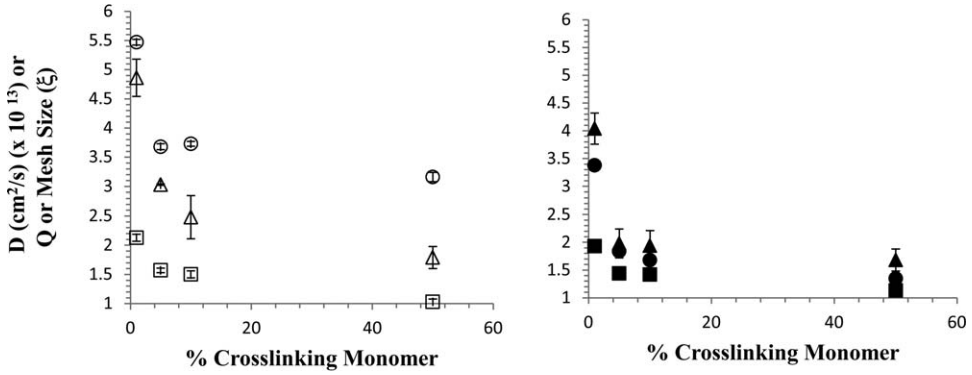

Figure 4. DS diffusion coefficient and network properties of imprinted poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels prepared via FRP and LRP with varying crosslinker content. DS diffusion coefficient (D) (●, ○), mesh size (▲, Δ), and equilibrium volume swelling ratio (Q) (■, □) for gels prepared via LRP (■, ●, ▲) and FRP (□, ○, Δ). Error bars represent the standard error ($n = 3$).

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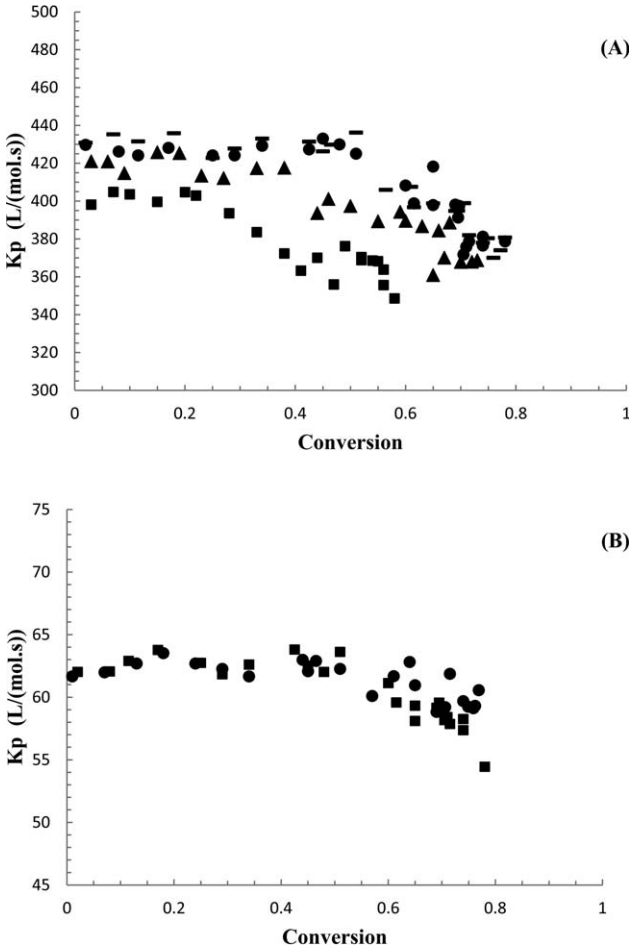

Figure 6. Effective propagation coefficient from kinetic analysis of imprinted polymer gels prepared via (A) FRP and (B) LRP at varying amounts of crosslinking content. Poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels prepared with 1% (▬); 5% (●); 10% (▲); and 50% (■) crosslinking monomer content.

equivalent polymer volume fractions (Figure 4). These results also support the hypothesis of decreased network heterogeneity in polymers prepared by LRP. Also, as the percentage of crosslinking content is increased, there is higher probability for network heterogeneity.

Figure 5 compares the average molecular weight and PDI of imprinted polymer gels prepared via FRP and LRP with varying concentration of crosslinking monomer. For polymers prepared via FRP, one can see that an increase in the crosslinker content resulted in a decrease in the kinetic chain length of the polymers as well as an increase in their PDI. This may be explained by an early transition to gelation during polymerization when the crosslinker content is increased. This is supported by the kinetic data for the polymerization reaction [Figure 6(A)], showing the apparent propagation coefficient decreasing at earlier conversions, indicating a transfer from the reaction-controlled to the diffusion-controlled stage of the polymerization. The early transition to gelation may result in the loss of available binding sites further explaining the observed decrease in template binding capacity as the extent of crosslinking was

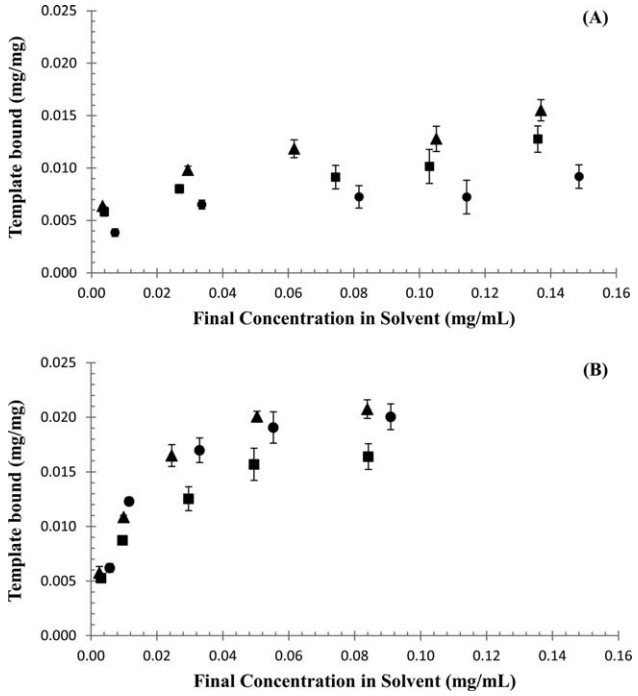

Figure 7. Equilibrium binding isotherm for DS binding by imprinted polymer gels prepared with different crosslinking monomers via (A) FRP and (B) LRP. Poly(DEAEM-*co*-HEMA-*co*-PEGnDMA) gels prepared with EGDMA (●); PEG200DMA(▲); and PEG400DMA(■) as crosslinking monomers. The crosslinking monomer concentration was 5% by mole and error bars represent the standard error with $n = 3$.

increased. For polymers prepared via LRP, an increase in the crosslinker content resulted in a lesser decrease in the kinetic chain length of the polymers compared to those prepared via FRP. Also, the PDI was significantly reduced and remained

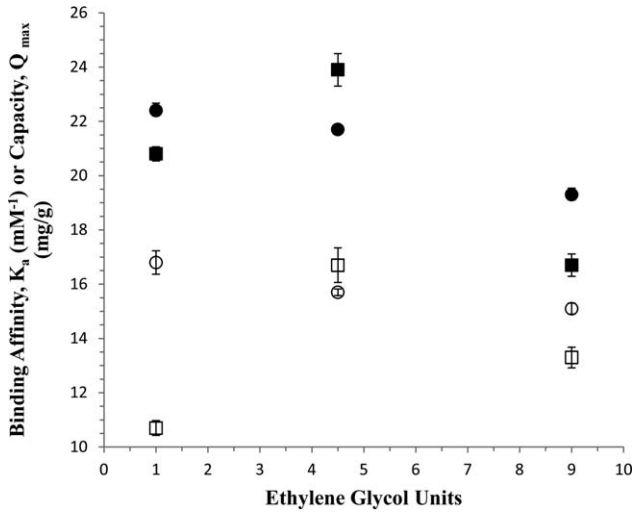

Figure 8. DS binding affinity and capacity of imprinted poly(DEAEM-*co*-HEMA-*co*-PEG200DMA) gels prepared via FRP and LRP with varying crosslinker ethylene glycol content. Binding affinity (●, ○) and capacity (■, □) of gels prepared via LRP (■,●) and FRP (□, ○). The crosslinking monomer concentration was 5% by mole and error bars represent the standard error with $n = 3$.

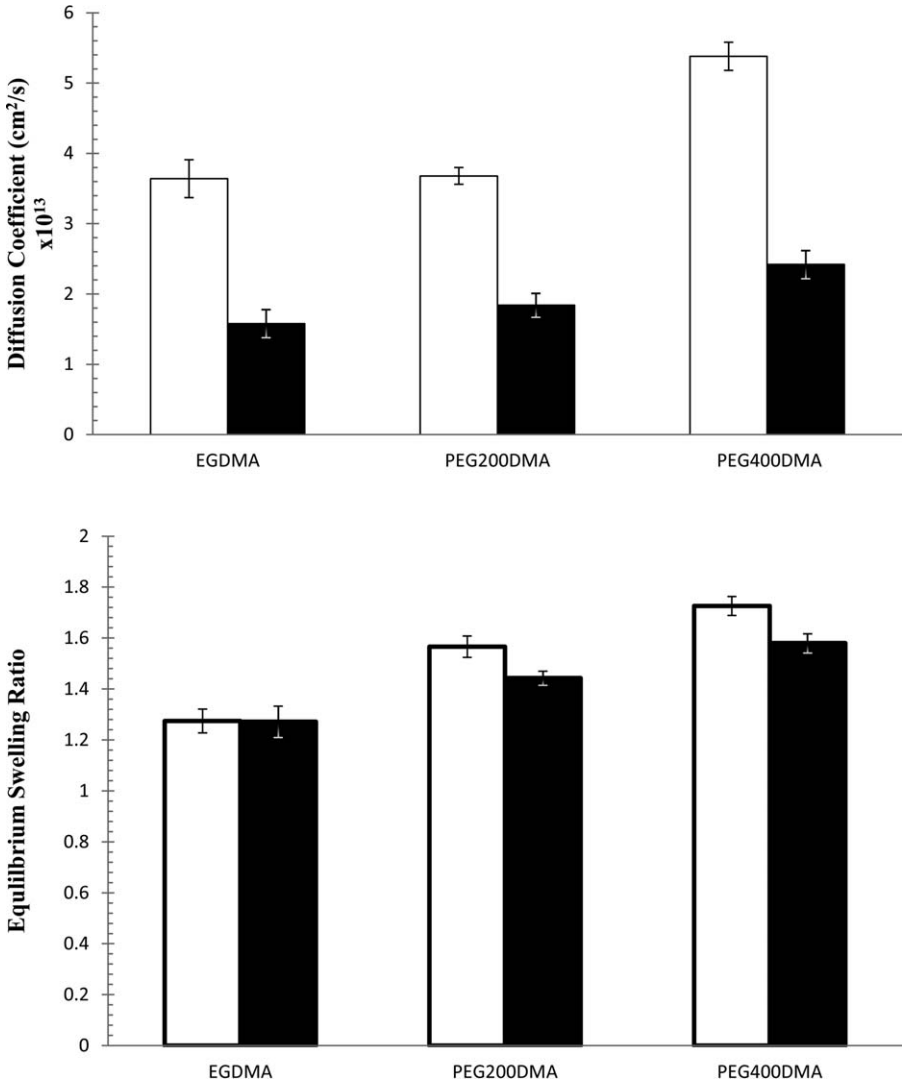

Figure 9. Effect of length of crosslinking monomer on DS diffusion coefficient and polymer equilibrium volume swelling ratio for imprinted poly(-DEAEM-*co*-HEMA-*co*-PEG200DMA) gels prepared via FRP and LRP. Polymers prepared via FRP (□) and LRP (■). The crosslinking monomer concentration was 5% by mole and error bars represent the standard error with $n = 3$.

constant as crosslinking content was increased. The use of LRP extended propagation and delayed gelation resulting in simultaneous growth of multiple polymer macroradicals in solution as described in previous reports.[39] The presence of the chain transfer agent in LRP controlled the kinetics of the reaction. As a result, the effective propagation constant versus conversion curves were quite similar despite the dramatic change in crosslinker content [Figure 6(B)]. The effect of LRP is demonstrated by the significant decrease in the PDI for the polymer chains. Thus, analyzing the polymer building blocks provides evidence that the hypothesis of increased homogeneity may be correct. The hypothesized increased homogeneity due to LRP leads to imprinted polymers with increased template binding affinity and loading capacity.

Figure 7 shows equilibrium binding isotherms for imprinted polymer gels with varying crosslinking monomer size (i.e., varying number of ethylene glycol units). The concentration of the crosslinking monomers was held constant at 5% of the total monomer concentration. The crosslinking monomers were chosen to vary the length of the chain between the two vinyl ends. PEG200DMA has an average of 4.5 ethylene glycol repeating units (average molecular weight = 200 Da) between the two vinyl groups. PEG400DMA has an average of nine ethylene glycol repeating units (average molecular weight = 400 Da) between the two vinyl groups while EGDMA has a single ethylene glycol unit between the two vinyl groups. Figure 7(A,B) shows polymers prepared via FRP and LRP, respectively. From Figure 8, we observe that polymers prepared with 5% PEG200DMA as crosslinker exhibit the highest template binding capacities (Q_{max} = 16.7 ± 0.64 mg/g) when compared with the corresponding polymers with EGDMA and PEG400DMA as crosslinkers ((Q_{max} = 10.7 ± 0.27 mg/g and 13.3 ± 0.38 mg/g respectively). Template binding affinity increased as the crosslinker length was reduced (K_a = 15.1 ± 0.20 mM^{-1}, 15.7 ± 0.12 mM^{-1}, 16.8 ± 0.43 mM^{-1} for PEG400DMA,

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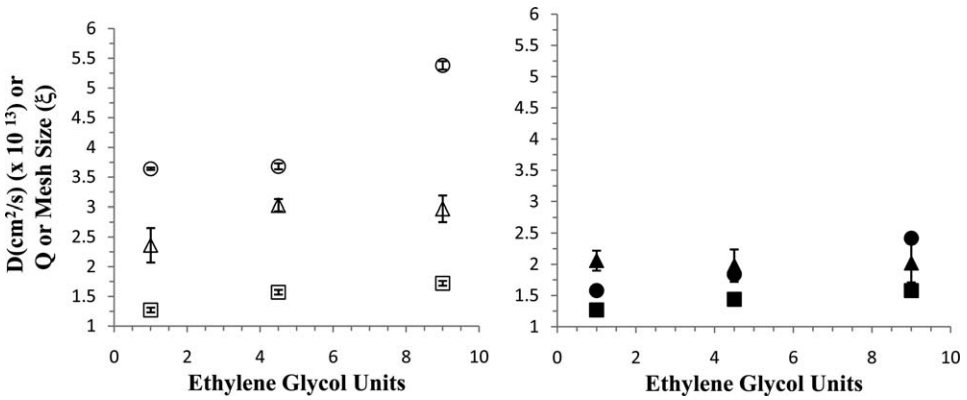

Figure 10. DS diffusion coefficient and network properties of imprinted poly(DEAEM-*co*-HEMA-*co*-PEGnDMA) gels prepared via FRP and LRP with varying crosslinker ethylene glycol content. DS diffusion coefficient (D) (●, ○), mesh size (▲, Δ), and equilibrium volume swelling ratio (Q) (■, □) for gels prepared via LRP (■, ●, ▲) and FRP (□, ○, Δ). Error bars represent the standard error ($n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

PEG200DMA, and EGDMA, respectively). Thus, PEG200DMA may be the ideal crosslinker for maximizing both binding capacity and affinity. The monomer may be ideal in terms of its increased reactivity and incorporation into the growing polymer network as well as producing a final network that it is more ideal in terms of the flexibility and orientation of polymer chains that produce the template binding site. Polymers prepared via LRP showed a similar trend. All polymers prepared via LRP demonstrated higher binding capacities as well as affinities when compared with the corresponding polymers prepared via FRP.

From Figures 9 and 10, one can see that polymers prepared with longer crosslinking monomers exhibited faster template transport. For polymers prepared via FRP, the diffusion coefficient increased from $3.64 \pm 0.02 \times 10^{-13}$ cm^2/s for polymers crosslinked with EGDMA to $5.38 \pm 0.07 \times 10^{-13}$ cm^2/s for polymers crosslinked with PEG400DMA. The slower template transport in polymers crosslinked with EGDMA is due to their smaller mesh sizes (2.36 ± 0.29 nm) and reduced free volume for transport. As crosslinking length was increased, the polymer volume swelling ratio increased and the polymer volume fraction in the swollen state decreased. Polymer gels prepared via LRP demonstrated similar qualitative template transport as the polymers prepared via FRP. The diffusion coefficient increased from $1.58 \pm 0.02 \times 10^{-13}$ cm^2/s for polymers prepared with EGDMA to $1.84 \pm 0.02 \times 10^{-13}$ cm^2/s for polymers prepared with PEG200DMA and $2.42 \pm 0.02 \times 10^{-13}$ cm^2/s for polymers prepared with PEG400DMA. Once again, the use of LRP resulted in significantly decreased template diffusion. We hypothesize that this is due to the improved structural homogeneity and decreased average mesh size leading to improved template binding affinity of these polymers compared to those prepared with FRP (Figure 10).

Figure 11 highlights the average molecular weight and PDI for imprinted gels prepared via FRP and LRP varying crosslinker ethylene glycol content with EGDMA, PEG200DMA, and PEG400DMA as the crosslinking monomers fixed at 5% molar concentration. The decreased flexibility of the shorter EGDMA favors faster transition to gelation [Figure 12(A)] and as a result the polymers formed are observed to have the shortest kinetic chain lengths. As the ethylene glycol content is increased, the polydispersity of both FRP and LRP polymers remained

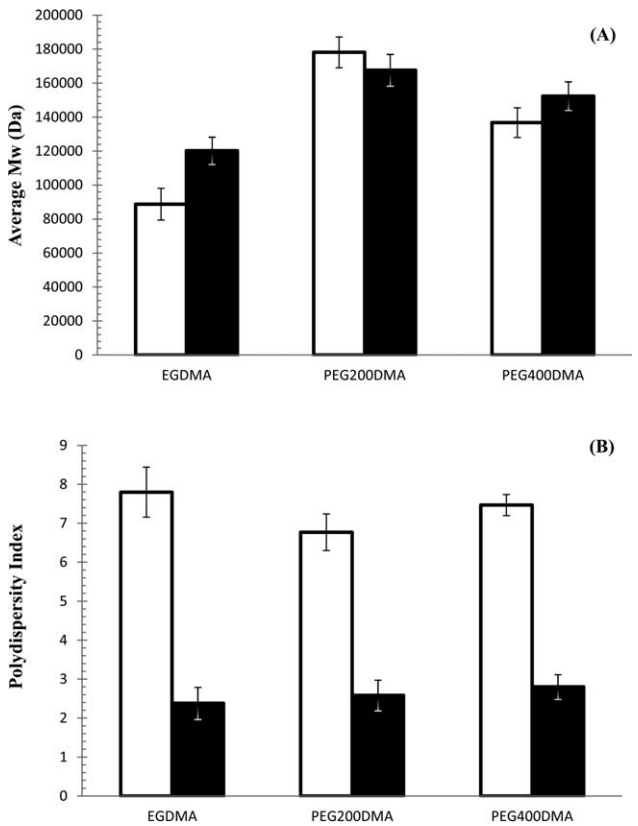

Figure 11. Effect of length of crosslinking monomer on kinetic chain length and polydispersity index of polymer chains. Weight average molecular weight (A) and polydispersity index (B) for polymers prepared via FRP (□) and LRP (■). The crosslinking monomer concentration was 5% by mole and error bars represent the standard error with $n = 3$.

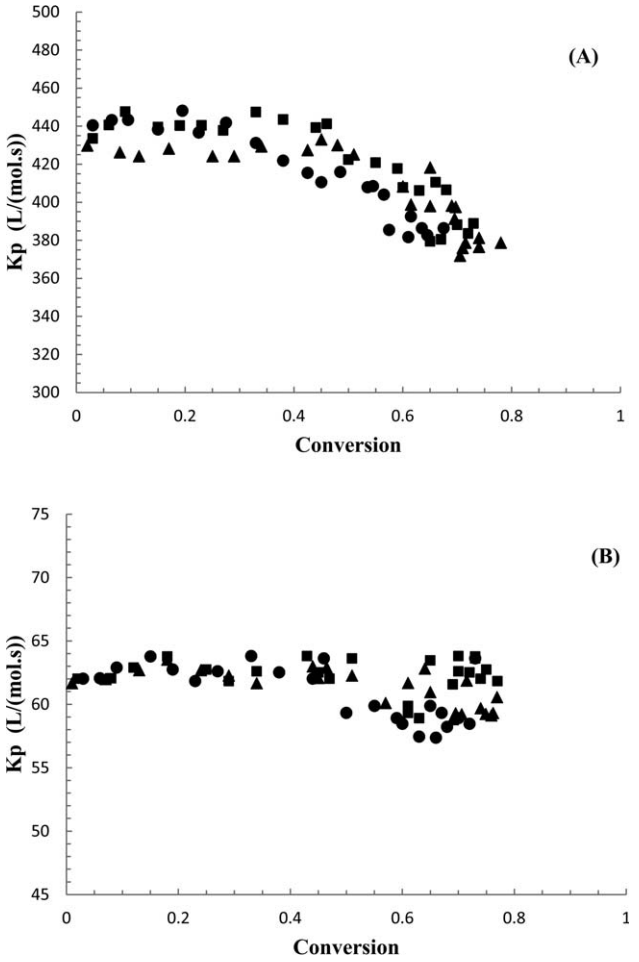

Figure 12. Effective propagation coefficient from kinetic analysis of imprinted polymer gels prepared with different crosslinking monomers via (A) FRP and (B) LRP. Poly(DEAEM-*co*-HEMA-*co*-PEGnDMA) gels prepared with EGDMA (●); PEG200DMA(▲); and PEG400DMA(■) as crosslinking monomers. The crosslinking monomer concentration was 5% by mole.

approximately constant. However, polymers prepared via LRP had significantly less dispersity. Thus, the increased homogeneity of chains may be leading to the improved binding affinity and capacity compared to polymers produced via FRP.

CONCLUSIONS

The work presented in this article examined the effects of crosslinker diversity on the structure of imprinted gels and subsequently their drug binding and transport properties. This was the first attempt to comprehensively examine the effects of crosslinker diversity in imprinted gels prepared via living radical polymerization. It was found that an increase in the extent of crosslinking in the polymer gels resulted in a decrease in template binding capacity and slower template transport with smaller reductions in template binding affinity. This corresponded with lower equilibrium swelling ratios, higher polymer volume fractions, and smaller mesh sizes of the solvated polymer gels. In addition, a decrease in the kinetic chain length of the polymers as well as a large increase in their polydispersity was observed. This may be explained by an early transition to gelation during polymerization. By using LRP, the dispersity of the polymer chains was decreased considerably, which correlated to significant increases in template binding affinity and capacity.

Increases in the length of the crosslinker resulted in decreased binding affinity and faster template transport through the polymer which corresponded with increased equilibrium swelling ratios, lower polymer volume fractions, and increased mesh size. All polymer gels prepared via LRP demonstrated significantly higher binding capacity as well as affinity when compared with the corresponding polymers prepared via FRP. The use of LRP also resulted in significantly decreased template transport through the imprinted polymer. The use of LRP also resulted in polymers with lower equilibrium swelling ratios, higher polymer volume fractions, and smaller mesh sizes of solvated gels. These results combined with the observed large decrease in the polydispersity also indicate increased homogeneity in the crosslinking structure of polymers prepared via LRP. Thus, for imprinted polymer networks with considerable probability for considerable heterogeneity, LRP can mitigate these issues leading to better properties.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Foundation (NSF-CBET-0730903, Grant G00003191).

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