

Polymerization Control of Templated Recognitive Structures

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Abstract

This work highlights the rational design of recognitive networks via polymerization reaction analysis and detailed kinetic analysis for application in novel diagnostic or robust point-of-care devices. Non-covalent complexation interactions between template or 'guest' biomolecules and functional monomers during polymerization can create networks with selective binding sites for biomolecules within polymeric films. The concept of macromolecular recognition manifests itself from two major synergistic effects, (i) shape specific molecular cavities that match the template biomolecule and (ii) structured chemical groups oriented to form multiple complexation points with the template molecule. The resulting polymer networks are selective due to the particular chemistry of the binding site, the orientation and stabilization of the chemistry in a crosslinked matrix, as well as by the size and shape of the site for the template biomolecule.

Polymerization reactions of acrylate and methacrylate based templated systems were analyzed to increase the ability to tailor the functional design of networks with specific interest in template affinity, selectivity, loading, and diffusional properties. This work highlights that the final polymer composition does not represent feed compositions when using significant amounts of short bi-functional crosslinking monomer (i.e., intramolecular distances between crosslinking monomer double bonds are short). Additionally, this work highlights the use of living polymerization techniques for the formation of recognitive polymeric structures. Living free radical polymerization has shown to dramatically increase the loading capacity of recognitive systems while retaining selectivity for a specific biomolecule.

Introduction

Researchers have studied, for the past several decades, free radical hetero/homopolymerization reactions of multifunctional monomers in the analysis of highly crosslinked polymeric materials. Typically, dense crosslinking gives the resulting polymer network mechanical strength, rigidity, and low solvent penetration. Macromolecular recognition, molecular imprinting, has developed using similar highly crosslinked materials that are "trained" to have a specific recognition to a target molecule [1, 2], while at the same time exhibiting a porous structure to allow diffusion of the template molecule. These materials have a promising future in specialized applications such as point-of-care diagnostics [2], assays [3], and drug delivery carriers

[2,4,5]. For the future development of these specialized devices the characterization and optimization of the polymer structure via reaction analysis is critical.

Controlled/living polymerization with the use of initiator-chain transfer molecules, iniferters [6], have been used to create linear polymers of low polydispersity, linear controlled specific block copolymers [7], and have been used to graft crosslinked polymer networks upon surfaces[8]. Otsu [9] was the pioneer in this controlled/living radical polymerization. Producing imprinted networks with controlled/living polymerization techniques will allow additional control over the network structure.

Results and Discussion

For the studied imprinted polymer system, the monomer to template ratio of the poly(MAA-co-EGDMA) network was 11.79 with the feed crosslinker percentage of 88.3% (mole crosslinking monomer per mole all monomers). After a detailed study of the double bond conversion via a differential photo calorimeter (DPC), the double bond conversion for this system at 0°C was 35 +/- 2.3%. The low double bond conversion indicates 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. Additional experiments were conducted that show a direct correlation between crosslinking monomer length and double bond conversion (i.e., longer crosslinking monomer increases the flexibility of the growing macromolecular structure which in turn lowers the number of pendant double bonds and leads to a higher double bond conversion). With a poly(ethylene glycol(200) dimethacrylate) crosslinking monomer, double bond conversion was found to be 53 +/- 2.0% at 0°C. Most research groups characterize binding properties in relation to feed crosslinking compositions; however, with low double bond conversion of these highly crosslinked systems, the feed compositions are not indicative of the final polymer structure. The use of reaction analysis can give a basis for a more accurate comparison of imprinted polymer networks in relation to affinity, capacity, and selectivity. Our group

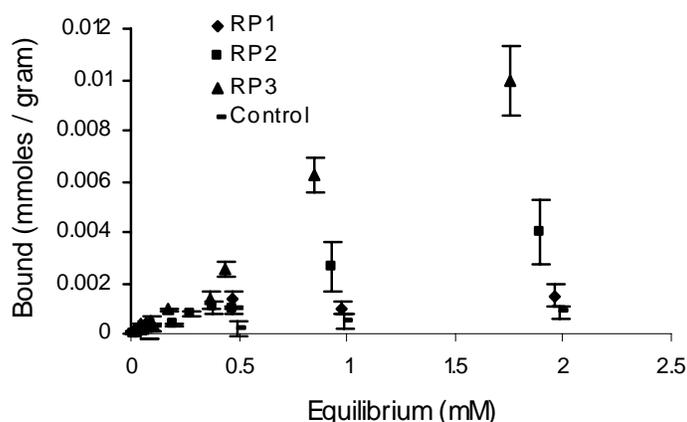


Figure 1 Binding Characteristics of Poly(MAA-co-EGDMA) Recognitive Networks for Ethyladenine (EA9A): Equilibrium Binding Isotherm. RP1 is the literature match recognitive polymer[11], RP2 has the same formulation composition except it contains 2.4 wt % initiator which demonstrated an increased double bond conversion of 48%, RP3 is 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).

has been the first to confirm low double bond conversion within highly crosslinked cognitive polymers and the resultant effect upon binding properties.

Equilibrium binding isotherms for the literature match cognitive polymer (RP1), control (i.e., no target molecule present in the formulation), cognitive polymer with 2.4 wt % initiator with an increased double bond conversion of 48% (RP2), and a cognitive polymer synthesized using controlled/living polymerization techniques with a 44% double bond conversion (RP3) are shown in Figure 1. Calculation of binding affinities and number of binding sites using the Freundlich isotherm which gave the best fit to the data resulted in $(3.12 \pm 0.21 \text{ mM}^{-1}$ with $776 \pm 54 \text{ } \mu\text{mole/g}$, $2.63 \pm 0.17 \text{ mM}^{-1}$ with $862 \pm 60 \text{ } \mu\text{mole/g}$, and $2.61 \pm 0.12 \text{ mM}^{-1}$ with $1421 \pm 64 \text{ } \mu\text{mole/g}$) for the affinity and binding capacities values for RP1, RP2, and RP3, respectively.

The most significant result of the equilibrium binding studies demonstrates that the use of a controlled/living polymerization reaction that produces a 63% increase in the number of binding sites at roughly equivalent affinity values. 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 crosslink 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 (Figure 2). Evidence in the literature of radical chain homopolymerization of multifunctional monomers using size exclusion chromatography and measurements of crosslink density support this conclusion [10].

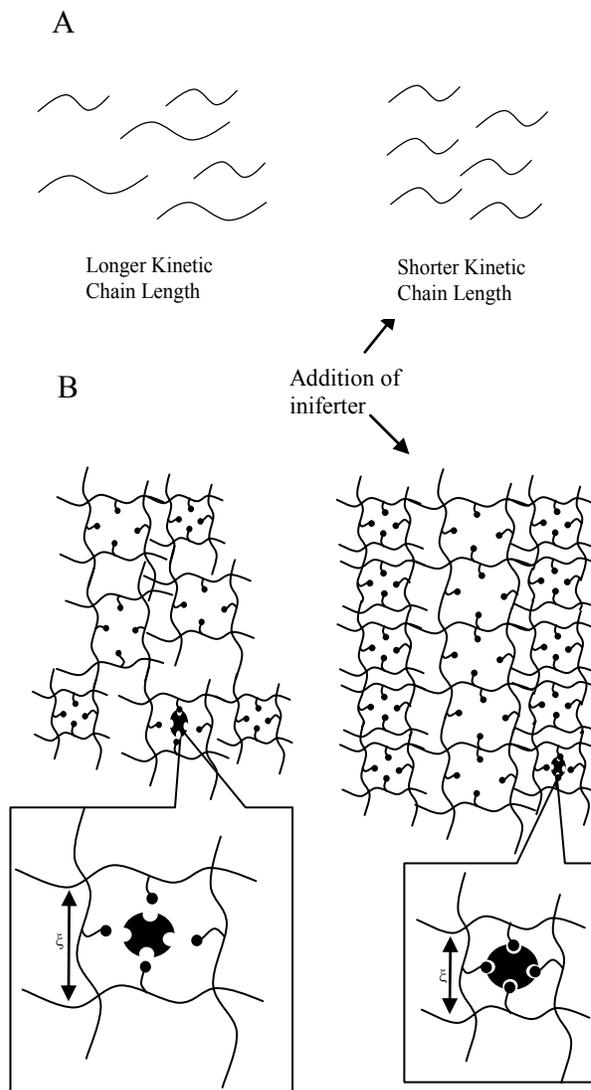


Figure 2 Controlled/Living Polymerization and the Effect on Imprinted Network Structure. A. In monovinyl polymerization, the use of iniferter yields a lower polydispersity of kinetic chains and decreased average chain length. B. Within crosslinked networks, addition of iniferter leads to a more uniform 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.

Selectivity studies were performed using a molecule with similar chemical functionality of ethyl adenine-9-acetate (EA9A), the target molecule, which was ethyl 2-amino-1,6-dihydro-6-oxo-4-pyrimidineacetate (EADOP). Binding capacity of EA9A and EADOP values for RP2 and RP3 are shown in Figure 3. RP2 and RP3 selectivity values were found to be higher than that of RP1. Increasing the conversion for RP2 did not lead to improved binding affinity or capacity, but

an increase in the selectivity compared to RP1. Again, 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. Furthermore, even optimization of conventional photo-initiator 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.

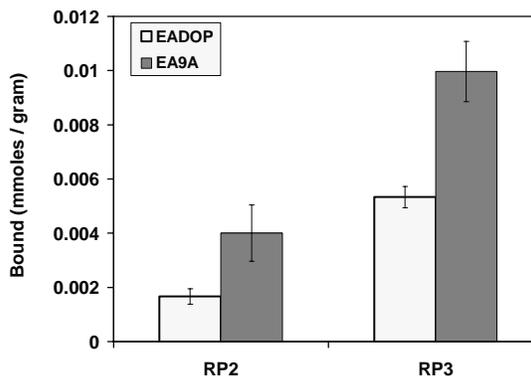


Figure 3 Binding Characteristics of Poly(MAA-co-EGDMA) Recognitive Networks for Ethyladenine (EA9A): Selectivity Study. Both RP2 and RP3 networks were more selective at a template concentration of 2.0 mM than that of the original literature match (RP1). Note: $n = 3$ and error bars represent the standard error.

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 macromolecular 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 molecular imprinted polymers will inevitably lead to improved binding characteristics via a rationally optimized macromolecular structure.

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