Published in final edited form as:

J Phys Chem B. 2011 April 14; 115(14): 3761–3764. doi:10.1021/jp111634k.

Network Deconstruction Reveals Network Structure in Responsive Microgels

Michael H. Smith, Emily S. Herman, and L. Andrew Lyon*

School of Chemistry & Biochemistry and the Petit Institute for Bioengineering & Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA

Abstract

Detailed characterization of hydrogel particle erosion revealed critical physicochemical differences between spheres, where network decomposition was informative of network structure. Real-time, in situ monitoring of the triggered erosion of colloidal hydrogels (microgels) was performed via multiangle light scattering. The solution-average molar mass and root-mean-square radii of eroding particles were measured as a function of time for microgels prepared from *N*-isopropylacrylamide (NIPAm) or *N*-isopropylmethacrylamide (NIPMAm), copolymerized with a chemically-labile cross-linker (1,2-dihydroxyethylene)bisacrylamide (DHEA). Precipitation polymerization was employed to yield particles of comparable dimensions but with distinct topological features. Heterogeneous cross-linker incorporation resulted in a heterogeneous network structure for pNIPAm microgels. During the erosion reaction, mass loss proceeded from the exterior towards the interior of the polymer. In contrast, pNIPMAm microgels had a more homogeneous network structure, which resulted in a more uniform mass loss throughout the particle during erosion. Although both particle types degraded into low molar mass products, pNIPAm microgels were incapable of complete dissolution due to the presence of non-degradable cross-links arising from chain transfer and branching during particle synthesis. The observations described herein provide insight into key design parameters associated with the synthesis of degradable hydrogel particles, which may be of use in various biotechnological applications.

Keywords

polymer erosion; microgel; multiangle light scattering; responsive polymer; pNIPAm; pNIPMAm

Introduction

Responsive hydrogel particles (microgels) are a versatile class of materials with potential utility in the encapsulation and delivery of therapeutic compounds.¹⁻⁵ As a result of their proposed use *in vivo*, microgels possessing degradable properties have gained significant attention in recent years.⁵⁻⁶ By enabling polymer decomposition into low molar mass components, the clearance of the vehicles may be facilitated, whereas erosion might also be used to modulate drug release from the polymer matrix.

Although microgel drug delivery systems are gaining increased visibility, several hurdles remain before they can be discussed as competent delivery vehicles for clinical applications. In the context of degradable microgels, assessment of erosion rate, erosion mechanism, and

*Corresponding author: lyon@gatech.edu.

Supporting Information Available: Experimental procedures, instrument validation, and additional discussion. This material is available free of charge via the Internet at <http://pubs.acs.org>.

products of the degradation reaction are essential for a complete understanding of the carrier's behavior. As seen with other erodible polymers, these characteristics are likely to influence polymer network diffusivity and drug release rates,⁷ and the stability of particles in biological media (as a result of size and topological changes).⁸⁻⁹ In an ongoing effort to investigate microgel erosion dynamics, we and others have employed a variety of techniques including atomic force microscopy (AFM),¹⁰⁻¹¹ multiangle light scattering (MALS),^{10, 12} and other optical methods.¹³⁻¹⁵

In this work we reveal how the characterization of microgel network structure can be facilitated by the detailed observation of that network's deconstruction. To illustrate the approach, we employed microgel particles composed of two different thermoresponsive polymers: poly(*N*-isopropylacrylamide) (pNIPAm) and poly(*N*-isopropylmethacrylamide) (pNIPMAm).¹⁶⁻¹⁷ Microgel erosion was enabled through copolymerization with a chemically labile cross-linker,¹⁸ (1,2-dihydroxyethylene)-bisacrylamide (DHEA), via precipitation polymerization. The DHEA cross-linker contains a vicinal diol that is rapidly cleaved in the presence of periodate, resulting in destruction of the polymer network.¹⁴ Using MALS in conjunction with a controlled reagent delivery and mixing device (Scheme S1), we measured the real-time changes in microgel solution-average root-mean-square radius (r_{rms}) and molar mass (M_w) during erosion, thereby revealing distinct differences in network erosion rates and pathways between the two microgel types.

Results and Discussion

The distinct structural features of both microgel types are graphically depicted in Scheme 1. Although the microgels had comparable r_{rms} values, the divergent hydrodynamic radii (r_h) suggest topological differences between the spheres. The topology may be inferred from the ratio of the r_{rms} (the particle dimension weighted by the mass distribution about the center of mass) and the r_h (inferred from the particle diffusion coefficient) determined through dynamic light scattering (DLS).¹⁹ As shown in Scheme 1 this ratio for the pNIPAm-DHEA microgels is $r_{rms}/r_h = 0.63$, which indicates a radially inhomogeneous mass distribution, as observed previously for other microgel chemistries.²⁰ In contrast, the calculated ratio for pNIPMAm-DHEA microgels ($r_{rms}/r_h = 0.78$) is indicative of a smooth sphere with an even distribution of mass throughout the particle. Particles composed of pNIPMAm are also significantly denser than pNIPAm spheres, suggesting a higher degree of cross-linking overall.

The architectural differences between the microgels resulted in distinct erosion profiles (**Figures 1 and 2**). Microgel erosion was monitored in real-time via MALS in the presence of excess periodate. In a typical erosion experiment, microgels and sodium periodate were pumped and mixed into the MALS flow cell with constant monitoring of the M_w and r_{rms} values. Constant M_w values were observed under flow conditions as the reactants were repeatedly replenished in the MALS flow cell. A sharp decay in the measured M_w values was then observed when the flow was stopped ($t = 0$ min). The molar mass for both the pNIPMAm and pNIPAm microgels reached equilibrium after ~80 min under the given experimental conditions. The measured molar mass of the scattering species at that time point (**Figures S2 and S3**) was approximately 2-3 orders of magnitude smaller than that of the original microgels. Simultaneous monitoring of the particle sizes revealed that the r_{rms} of the pNIPMAm microgels increased by ~15% to ~185 nm early in the erosion, with a subsequent decay to slightly smaller spheres and eventually into linear chains with poorly defined angular scattering functions (**Figure 1 and Figure S2**). This erosion pathway is expected in cases where the network is very homogenous in density and uniform in connectivity. Cross-linker degradation results in a decrease in network connectivity, allowing the network to swell. As the network connectivity continues to decrease, the

microgel eventually dissolves into a collection of oligomers with low scattering cross-sections.

Very different erosion behavior was observed in the case of pNIPAm-DHEA microgels (Figure 2). As described above, the combined MALS and DLS data were suggestive of a radial distribution of polymer mass within the microgels, with the greatest polymer connectivity being present in the particle interior. This structure implies that fewer cleavage events would be required to liberate polymer chains near the sphere periphery relative to the particle core. Thus, mass loss is favored from the exterior of the network, eventually proceeding towards the interior. As shown in Figure 2, pNIPAm-DHEA spheres demonstrated non-linear molar mass decay throughout erosion. In contrast to the swelling and network extension observed for pNIPMAm spheres, pNIPAm particles instead showed an immediate decrease in their r_{rms} upon erosion, which is associated with a large amount of mass loss proceeding from the exterior of the particle toward the interior. In other words, the erosion proceeded from regions of low connectivity (the particle periphery) to regions of high connectivity (a highly cross-linked core).

However, unlike pNIPMAm spheres, the pNIPAm microgels did not completely dissolve, even after ~80 min of incubation with periodate. Erosion products at that time point continued to display angle-dependent scattering, which is strongly indicative of the presence of remnant spheres following DHEA degradation (Figure S3). This dimensional stability is likely imparted by non-degradable cross-linking sites arising from self-cross-linking that occurs during precipitation polymerization.^{11, 21} NIPAm is prone to chain transfer reactions via the hydrogen atom on the tert-C of the polymer main chain, forming non-degradable connectivity (cross-links) in the network. Chain transfer on the main chain is preferred over the hydrogen on the pendent isopropyl group. Thus, microgels composed of pNIPMAm (lacking the tert-C on the main chain) would not be expected to show significant self-cross-linking, a hypothesis that is supported by the complete dissolution of the pNIPMAm spheres in the presence of periodate. Erosion products from pNIPMAm experiments showed loss of angle-dependent scattering, suggesting complete polymer dissolution and destruction of the particle (Figure S2).

The kinetics of microgel network deconstruction provided additional insight into network structure. The pNIPMAm microgels showed an exponential decrease in M_w during erosion, as indicated by the linearity in the M_w decay when plotted on a logarithmic scale (Figures 1 and 3). This exponential decay is characteristic for bulk eroding polymers, where the erosion rate generally decreases as polymer is depleted from the particle structure.⁷ Decay constant values, λ , for microgel erosion were determined through nonlinear least squares fitting of the exponential decay function, where $M_w(t) = M_{w0}e^{-\lambda t}$. As the concentration of periodate was decreased, the value of λ decreased in a linear fashion (Figure 3). In contrast, pNIPAm microgels (having an inhomogeneous mass distribution throughout the structure) showed very different kinetic behavior. For these polymers, mass loss proceeds from the exterior regions first (containing less connectivity) towards the interior (containing enhanced connectivity). As a result, the kinetics of molar mass loss decelerates during the erosion reaction (Figure 2), where reaction velocities are highest early in the reaction, as the peripheral, low-connectivity chains are lost from the structure.

A direct relationship between the microgel structural and the erosion behavior was observed in this study. The observed differences are likely caused by several factors, including monomer reactivity and cross-linker incorporation differences during polymerization. Heterogeneities in microgel structures are often caused by mismatched reactivity ratios for monomer and cross-linker (r_1 and r_2 , respectively) during precipitation polymerization, where the reactivity ratio is a measure of the propensity of a monomer to propagate by

reacting with itself or a different monomer in the synthetic mixture. For instance, microgels cross-linked with *N,N'*-methylenebis(acrylamide) (BIS) typically show a radial distribution of cross-links within the particle, with the highest cross-linker concentrations residing at the microgel interior.^{19, 22} This uneven incorporation of BIS has been attributed to a high reactivity ratio for the cross-linker relative to the monomer (e.g. acrylamide $r_1 = 0.57$, BIS $r_2 = 3.36$).²³ The divergent microgel structures used in this study were also likely caused by differential incorporation of DHEA in microgels composed of NIPAm relative to those composed of NIPMAm. However, unlike the case of BIS, the observed differential incorporation likely arises from a combination of multiple factors. First, NIPAm has been reported to undergo polymerization faster than NIPMAm, which would result in differential reaction rates between those two monomers and the cross-linker.²⁴ Secondly, DHEA is a more hydrophilic molecule than BIS, which would result in poorer incorporation of DHEA-rich polymer segments into the hydrophobic polymer globules during particle growth; this effect has been observed for a number of other co-polymer microgels synthesized by precipitation polymerization.²⁴⁻²⁵ We have previously found, for example, that DHEA tends to be inefficiently incorporated into pNIPAm microgels during precipitation polymerization as compared with BIS, resulting in a more loosely cross-linked network in the case of DHEA.¹⁴

Thus, the heterogeneous mass distribution found for pNIPAm-DHEA microgels may be attributed to preferential incorporation of the NIPAm monomer over DHEA during synthesis due to both the rapid homopropagation of NIPAm and the higher solubility of any DHEA rich-segments. As discussed previously, there are additional features of pNIPAm polymerization that can contribute to microgel heterogeneity (i.e. self-cross-linking), which might serve to exacerbate the observed structural differences. As shown Scheme 1, pNIPAm microgels were of significantly lower density than pNIPMAm spheres, suggesting a lower overall cross-linking density (poor incorporation of DHEA). This result, combined with the presence of remnant spheres following erosion, suggests that self-cross-linking contributes a significant fraction of cross-linking sites throughout the structure. In contrast, spheres composed of pNIPMAm showed significantly higher density and a smooth sphere topology. This is likely the result of even incorporation of reactants during synthesis, as NIPMAm reacts more slowly, which would permit a more statistically random incorporation of the DHEA cross-linker. The more random co-polymers will also be less likely to suffer from poor incorporation due to solubility issues, as described previously for polyelectrolyte copolymer microgel synthesis.²⁵

In summary, analysis of microgel network deconstruction using the in situ monitoring method revealed critical physicochemical differences between the particles. Although the two microgels were produced under similar synthetic conditions and were of comparable dimensions (i.e. their r_{rms} values), their reaction kinetics and the products of erosion were very different. Importantly, the presence of intrinsic self-cross-linking sites within pNIPAm-DHEA microgels limited the dissolution of the network. This result suggests that the formation of completely degradable pNIPAm microgels may require alternative synthetic strategies that limit parasitic chain transfer. The investigation described above was informative as we pursue the synthesis and application of thermoresponsive and degradable hydrogel particles, enabling a detailed assessment of microgel erosion. We anticipate that similar methods will assist in the study of other micro/nanoparticles, where rapid response events may enable specific biomedical applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was partially supported by the National Institutes of Health (1 R01 GM088291-01). Funding for MHS and ESH was provided by U.S. Department of Education GAANN awards. MHS thanks the Georgia Tech Center for Drug Design, Development and Delivery and the Georgia Tech TI:GER® program for additional support.

References

1. Raemdonck K, Demeester J, De Smedt S. *Soft Matter*. 2009; 5(4):707–715.
2. Nayak S, Lyon LA. *Angew. Chem., Int. Ed.* 2005; 44(47):7686–7708.
3. Oh JK, Drumright R, Siegwart DJ, Matyjaszewski K. *Prog. Polym. Sci.* 2008; 33(4):448–477.
4. Dickerson E, Blackburn W, Smith M, Kapa L, Lyon LA, McDonald J. *BMC Cancer*. 2010; 10(1): 10. [PubMed: 20064265]
5. Murthy N, Xu M, Schuck S, Kunisawa J, Shastri N, Fréchet JM. *Proc. Natl. Acad. Sci. USA*. 2003; 100(9):4995–5000. [PubMed: 12704236]
6. De Geest B, Déjugnat C, Sukhorukov G, Braeckmans K, De Smedt S, Demeester J. *Adv. Mater. (Weinheim, Ger.)*. 2005; 17(19):2357–2361.
7. Tamada JA, Langer R. *Proc. Natl. Acad. Sci. USA*. 1993; 90(2):552–556. [PubMed: 8421690]
8. Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, Nilsson H, Dawson KA, Linse S. *Proc. Natl. Acad. Sci. USA*. 2007; 104(7):2050–2055. [PubMed: 17267609]
9. Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA. *Proc. Natl. Acad. Sci. USA*. 2008; 105(38):14265–14270. [PubMed: 18809927]
10. Huang X, Misra GP, Vaish A, Flanagan JM, Sutermeister B, Lowe TL. *Macromolecules*. 2008; 41(22):8339–8345.
11. South AB, Lyon LA. *Chem. Mater.* 2010; 22(10):3300–3306.
12. Smith MH, South AB, Gaulding JC, Lyon LA. *Anal. Chem.* 2009; 82(2):523–530. [PubMed: 20000662]
13. Goh SL, Murthy N, Xu M, Fréchet JM. *Bioconjugate Chem.* 2004; 15(3):467–474.
14. Nayak S, Gan D, Serpe M, Lyon L. *Small*. 2005; 1(4):416–421. [PubMed: 17193466]
15. Singh N, Lyon LA. *Chem. Mater.* 2007; 19(4):719–726.
16. Schild HG. *Prog. Polym. Sci.* 1992; 17(2):163–249.
17. Tang Y, Ding Y, Zhang G. *J. Phys. Chem. B*. 2008; 112(29):8447–8451. [PubMed: 18582006]
18. Metz N, Theato P. *Macromolecules*. 2008; 42(1):37–39.
19. Stieger M, Richtering W, Pedersen JS, Lindner P. *J. Chem. Phys.* 2004; 120(13):6197–6206. [PubMed: 15267506]
20. Saunders BR. *Langmuir*. 2004; 20(10):3925–3932. [PubMed: 15969381]
21. Gao J, Frisken BJ. *Langmuir*. 2003; 19(13):5212–5216.
22. Wu X, Pelton RH, Hamielec AE, Woods DR, McPhee W. *Colloid & Polymer Science*. 1994; 272(4):467–477.
23. Baselga J, Llorente MA, Nieto JL, Hernández-Fuentes I, Piérola IF. *Eur. Polym. J.* 1988; 24(2): 161–165.
24. Duracher D, Elaïssari A, Pichot C. *J. Polym. Sci., Part A: Polym. Chem.* 1999; 37(12):1823–1837.
25. Xiaobo Hu ZT, Andrew Lyon L. *Colloid Polym. Sci.* 2010 in press.

Figure 1.

Erosion of pNIPMAm-DHEA microgels results in changes in particle molar mass (blue) and r_{rms} (red). The erosion process is schematically represented at different time points (blue spheres). Reactions were monitored at 25 °C in the presence of excess periodate (100 mM). Error bars (black) represent one standard deviation about the mean of measurements performed in triplicate.

Figure 2.

Erosion of pNIPAm-DHEA microgels results in the decay of particle molar mass (blue) and r_{rms} (red). The erosion process is schematically represented at different time points (blue spheres). Reactions were monitored at 25 °C in the presence of excess periodate (100 mM). Error bars (black) represent one standard deviation about the mean of measurements performed in triplicate.

Figure 3.

First-order erosion kinetics are observed for pNIPMAm spheres throughout the majority of the erosion reaction. Regions showing exponential molar mass decay (red) were fit via nonlinear least squares fitting of the exponential function (dotted blue lines). The decay constant, λ , scaled linearly with periodate concentration.

Scheme 1.

Molar mass, density, and proposed microgel topology from light scattering characterization. Topological differences were observed for the spheres with comparable r_{rms} but with divergent r_h values.