Investigation of the Formation Process of PNIPAM-Based Ionic Microgels

Rui Chen, Xin Jin,* and Xinyuan Zhu*

School of Chemistry and Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

ABSTRACT: The formation process of poly(N-isopropylacrylamide) (PNIPAM)-based ionic microgels was investigated in this work. Different from the traditional formation process of covalent bond cross-linked PNIPAM-based microgels, a disassembling and reassembling process for PNIPAM-based ionic microgels was observed. During the formation process, loose microgels were first formed in a short time, and then, these loose microgels disassembled into smaller nanogel pieces. Meanwhile, the nanogel pieces reassembled into microgels by electrostatic interaction. After reassembling, large amounts of nanogels could be seen clearly in the interior of PNIPAM-based ionic microgels. The thermo-sensitivity and composition of the final prepared microgels were characterized by dynamic light scattering, infrared spectroscopy, and X-ray photoelectron spectroscopy.

INTRODUCTION

Microgels as soft materials have been applied in many areas such as drug delivery systems,1,2 catalysts,3,4 and nanotechnology.5,6 Owing to their wide applications, many types of microgels are prepared including polyacrylamide,7,8 poly(N-isopropylacrylamide) (PNIPAM),9−11 and poly(acrylic acid) microgels.12,13 Among them, the most studied is PNIPAM-based microgels for their thermo-sensitivity. Generally, they are covalent bond cross-linked with the cross-linker N,N′-methylenebisacrylamide (MBAA). However, sedimentation always occurs during the formation process. This is because the cross-linker MBAA also participates in the polymerization as a co-monomer and copolymerizes with NIPAM. However, with the development of polymer science, a new type of PNIPAM-based microgel is reported without the participation of MBAA.14−16 A second tertiary amine monomer which provides cross-linking sites and a dibromoalkane compound which is used as a cross-linker are applied in this system. The cross-linker here does not participate in the polymerization process. The cross-linking reaction is quaternarization of the tertiary amine monomer and the dibromoalkane compound, which happens after the polymer chains are formed. Thus, ionic bonds are produced which differ from the traditional covalent ones. Considering that the mechanism for forming PNIPAM-based ionic microgels becomes necessary and significant.

As far as we know, kinetic study of the microgel formation process is rare in these years. Understanding the essence of the formation process for a new material will provide us robust evidence for further scientific research. As a fundamental study, we investigated the formation process of the recently reported PNIPAM-based ionic microgels in this work. Different from the traditional covalent bond cross-linked PNIPAM-based microgels,17−19 the formation process for PNIPAM-based ionic microgels was a disassembling and reassembling process. In this process, loose microgels were first formed in a short time and then disassembled into smaller nanogel pieces. Meanwhile, the nanogel pieces reassembled into microgels by electrostatic interaction. After reassembling, large amounts of nanogels could be seen clearly in the interior of PNIPAM-based ionic microgels. The thermo-sensitivity and composition of the final prepared microgels were characterized by dynamic light scattering, infrared spectroscopy, and X-ray photoelectron spectroscopy.

RESULTS AND DISCUSSION

PNIPAM-based ionic microgels (encoded as PNI microgels) were prepared by surfactant-free emulsion polymerization as shown in Scheme 1. The formation process of PNI microgels
has been presumably reported in Du’s group.14 Their description for the formation process gave us a brief impression, which was easy to understand, as shown in the following. Because of the existence of NIPAM units, the early formed poly(N-isopropylacrylamide-co-1-vinylimidazole) (p-(NIPAM-co-VIM)) polymer chains in the polymerization process were thermo-sensitive and aggregated into hydrophobic nanospheres. As the cross-linker was also hydrophobic, it penetrated into these nanospheres and chemically cross-linked them by quaternization. With the polymerization going on, the latter formed p(NIPAM-co-VIM) polymer chains adsorbed onto the surface of the chemically cross-linked nanospheres and then were cross-linked by the cross-linker. The nanospheres grew larger and larger until monomers were exhausted. After microgels were formed, they became smaller and smaller in size on a small scale because of the further penetration of the cross-linker (Scheme 2, route 1).

However, the detailed formation process for PNI microgels was quite different from the above reported ones. We investigated the formation process in detail as shown in Figure 1. According to our investigation, the formation process of PNI microgels could be divided into two stages: (i) PNI microgels were first formed in a loose state within 1 h. They were in a loose state as they could change their shape after dehydration (Figure 1a). This was because only a small amount of cross-linkers penetrated into microgels in this state, resulting in a low degree of cross-linking. Then, the diameter of PNI microgels became smaller and smaller in a short time (Figure 1b). This was due to the further penetration of cross-linker into PNI microgels. They became hard with cross-linking, so that they were in a spherical shape after dehydration. In this stage, there were still linear p(NIPAM-co-VIM) polymer chains forming and aggregating into nanospheres outside these early formed PNI microgels. However, most of the cross-linker had already penetrated into the early formed microgels because of the hydrophobic interaction. Concentration difference of cross-linkers existed between the early formed PNI microgels and the latter formed nanospheres. Thus, redistribution of cross-linker happened between them. The redistribution of cross-linker led to the disassembling of PNI microgels which entered into stage (ii) as show in Figure 1c,d. In this stage, PNI microgels split into smaller nanogel pieces gradually with the exudation of cross-linker from PNI microgels to nanospheres. Meanwhile the nanogel pieces became smaller and smaller due to the penetration of cross-linker. As could be seen in Scheme 1, cross-linking was a reaction of quaternization in which organic salt was formed. Thus, negatively charged bromide ions could dissociate from the nanogel pieces. Similarly, nanogel pieces...
were positively charged after dissociation of bromide ions. So, these freely charged ions and nanogels reassembled into microgels with the disassembling of PNI microgels as shown in Figure 1e,f. During the whole process, disassembling and assembling were simultaneous. This investigated process was depicted in the route 2 of Scheme 2.

To verify our investigation, confirmatory experiments were performed by controlling the monomer concentration. As had been reported, with increasing the monomer concentration, the diameter of PNI microgels increased. This conclusion did not conflict with our results as shown in Figure 2. However, according to our proposed formation process, stage (ii) could disappear by lowering the monomer concentration. In this situation, once the PNI microgels were formed in the early stage, monomers were consumed immediately when the monomer concentration was super low. Thus, no latter formed linear p(NIPAM-co-VIM) polymer chains appeared and competed for the cross-linker with the early formed loose PNI microgels. As verification, the diameter of the PNI microgels prepared at low monomer concentration increased dramatically as shown in Figure 3. In fact, some nanogel pieces were formed outside the PNI microgels as shown in Figure 4a. But they could not split the PNI microgels at the early stage by forming concentration difference as they were in a small amount. They just adsorbed onto the surface of PNI microgels as shown in Figure 4b.

In the formation process of PNI microgels, monomers could not be consumed in a short time when the initial monomer concentration was relative high. In this situation, the latter formed p(NIPAM-co-VIM) polymer chains could split the PNI microgels for the concentration difference existed. We carefully examined the scope above which the concentration difference was strong enough to split PNI microgels. This scope was in the range of 10⁻¹⁵ mM based on the concentration of NIPAM. It was understandable that the diameter of PNI microgels increased with increasing the monomer concentration when it was higher than 15 mM. By controlling the molar ratio of monomers between NIPAM and VIM, no obvious difference of the formation process was observed. But no microgels were obtained when the molar percentage of VIM was higher than 25% compared to NIPAM. This was due to the fact that p(NIPAM-co-VIM) lost their thermo-sensitivity when the content of VIM was too high, which prevented the cross-linking process.

In a statistic analysis of the formation process of PNI microgels characterized by dynamic light scattering (DLS), the diameter variation of PNI microgels was in accordance with TEM (Figure 5). The PNI microgels were first formed in a loose state within 1 h. Then, the diameter decreased for the penetration of cross-linkers in a short time. However, a jump of the diameter at 120 min could be seen clearly. This was the time point when the PNI microgels began to disassemble. During this time, cross-linker began to ooze from the PNI microgels, which led to the increase of diameter of the PNI microgels. As had been mentioned above, disassembling and

---

**Figure 2.** PNI microgels with different diameters prepared by varying the monomer concentrations. (a) Monomer concentration: 60 mM of NIPAM, 9 mM of VIM, and 9 mM of DBB. (b) Monomer concentration: 40 mM of NIPAM, 6 mM of VIM, and 6 mM of DBB. (c) Monomer concentration: 20 mM of NIPAM, 3 mM of VIM, and 3 mM of DBB.

---

**Figure 3.** Comparison of the hydrodynamic diameter of PNI microgels formed at different monomer concentrations. For each recipe, the concentration of VIM and DBB relatively increased or decreased in proportion compared to NIPAM (black line: 20 mM of NIPAM, 3 mM of VIM, and 3 mM of DBB; red line: 40 mM of NIPAM, 6 mM of VIM, and 6 mM of DBB; blue line: 60 mM of NIPAM, 9 mM of VIM, and 9 mM of DBB; green line: 10 mM of NIPAM, 1.5 mM of VIM, and 1.5 mM of DBB).

---

**Figure 4.** PNI microgels were formed when the monomer concentration was low enough. Monomer concentration: 10 mM of NIPAM, 1.5 mM of VIM, and 0.75–3 mM of DBB. (a) Nanogel pieces could be seen clearly. (b) Nanogel pieces adsorbed onto the surface of PNI microgels.
reassembling was a dynamic process. During this process, the cross-linking reaction happened all the time which made the diameter of PNI microgels smaller and smaller as a whole.

The inner structure of the prepared ionic microgels was investigated by high resolution transmission electron microscopy (HR-TEM) as shown in Figure 6. As could be seen, lots of nanogels existed within the PNI microgels which further verified our hypothesis mentioned above. It meant that the formation process for PNI microgels was related to a process of disassembling and reassembling. However, nanogels could not be seen when the monomer concentration was low enough. This indicated that it could not go through the disassembling and reassembling process when the monomer concentration was very low.

The thermo-sensitivity of the prepared PNI microgels was characterized by DLS, as shown in Figure 7. Because of the large amount existence of NIPAM units in the backbone, the prepared PNI microgels were thermo-sensitive. With increase of the temperature, the diameter of PNI microgels decreased. However, because of the insertion of co-monomer VIM in the PNIPAM polymer chains randomly, the thermo-sensitivity of PNI microgels was quite different from the reported ones which had a sharp transition temperature at about 31°C.20 It was in a wide temperature transition range from 35°C to 60°C. In addition, the thermo-sensitivity also verified that the nanogel pieces within the PNI microgels were cross-linked during the reassembling process, because the PNI microgels were stable when increasing or decreasing the temperature.

According to the DLS data, we could see that both NIPAM and VIM existed in the backbone of PNI microgels. However, to further demonstrate the composition of PNI microgels, infrared spectroscopy (IR) and X-ray photoelectron spectroscopy (XPS) were used to characterize the detailed composition of PNI microgels, as shown in Figure 8. The peak of νC=O at 1645 wavenumbers demonstrated the existence of NIPAM as shown in Figure 8a. The overlapped peaks of νC=C and νC=N at 1453 wavenumbers indicated the existence of VIM. The appearance of the Br element in XPS data demonstrated that the cross-linker of DBB also existed in the PNI microgels, as shown in Figure 8b.

CONCLUSIONS

In conclusion, the formation process of PNI microgels was observed and discussed for the first time in our work. Different from the formation process of the covalent bond cross-linked microgel system, a disassembling and reassembling process was revealed for PNI microgels. This process was related to their cross-linking mechanism in which cross-linking happened in a hydrophobic environment. During this process, redistribution of the cross-linkers from the early formed PNI microgels to the latter formed p(NIPAM-co-VIM) polymer chains (they aggregated into nanospheres) was the driving force in disassembling of PNI microgels for the existence of concentration difference of the cross-linker. This discovery would provide us guidance for the future study relating to PNI microgels.

EXPERIMENTS

Materials. N-isopropylacrylamide (NIPAM), 1, 4-dibromo-butane (DBB), and 1-vinylimidazole (VIM) were purchased from Adamas Co. 2,2′-azodiisobutyramidine dihydrochloride (AIBA) was purchased from Accela Co.

Instrumentation. Dynamic light scattering (DLS) was used to detect the particle size of PNI microgels (Malvern Instruments Ltd, ZS90). Transmission electron microscope (TEM) was used to characterize the morphology of PNI microgels (120 kV, FEI, Tecnai G2 Spirit Biotwin). High resolution transmission electron microscope (HR-TEM) was used to characterize the inner structure of PNI microgels (Talos F200X/TALOS F200X). Infrared spectrometer (IR) was used to characterize the composition of PNI microgels (Spectrum 100, PerkinElmer, Inc). X-ray photoelectron spectroscopy (XPS) was used to characterize the elements of PNI microgels (AXIS UltraDLD).
During the formation process of PNI microgels, puriﬁed samples were characterized by TEM and DLS directly, without any staining. For characterization by TEM, the solution of PNI microgels was ﬁrst diluted by 100× (adding 10 μL of this solution into 990 μL of water). Then, 20 μL of this diluted solution was added on one side of Formvar-coated copper grids. After drying the grids at room temperature, they were characterized by TEM and HR-TEM. The sample was rapidly cooled down by putting the vial containing the sample into liquid nitrogen to stop the reaction. After the sample was restored to room temperature, they were characterized by TEM and DLS directly, without any staining.

Preparation of PNI Microgels. PNI microgels were synthesized by surfactant-free emulsion polymerization. 2 mmol of NIPAM, 0.3 mmol of VIM, and 0.3 mmol of DBB were added into a 100 mL three-necked round-bottom ﬂask containing 45 mL of water. This ﬂask was equipped with a magnetic stirrer, a condenser, and a nitrogen gas inlet. The reaction was allowed to proceed for at least 6 h and then stopped by cooling down to room temperature. The stirring speed was kept at 750 rpm during the whole polymerization process. This reaction was initiated by chemically injecting 0.5 mL of the AIBA solution containing 45 mL of water. This above solution was bubbled for 15 min with nitrogen gas to purge the oxygen. Then, the temperature of this solution was increased to 70 °C with stirring. After the temperature was stable, 25 mg of AIBA was dissolved in 5 mL of water and then injected into the above solution to initiate the polymerization. The stirring speed was kept at 750 rpm during the whole reaction process. This reaction was allowed to proceed for at least 6 h and then stopped by cooling down to room temperature. The obtained solution of PNI microgels was centrifuged at 9000 rpm for 30 min to remove uncross-linked p(NIPAM-co-VIM) macromolecules. The centrifuged PNI microgels were dispersed into water by ultrasonic treatment. This centrifugation/redispersion process was repeated for at least three times. The ﬁnal puriﬁed PNI microgels were stored in water for further characterization.

Investigation of the Formation Process of PNI Microgels. During the formation process of PNI microgels, 0.5 mL of the solution was sampled at preset time points. The sample was rapidly cooled down by putting the vial containing the sample into liquid nitrogen to stop the reaction. After the sample was restored to room temperature, they were characterized by TEM and DLS directly, without any puriﬁcation process.

Characterization. For characterization by TEM and HR-TEM, the solution of PNI microgels was ﬁrst diluted by 100× (adding 10 μL of this solution into 990 μL of water). Then, 20 μL of this diluted solution was added on one side of Formvar-coated copper grids. After drying the grids at room temperature, they were used for observation by TEM. The samples were not stained before observation. For characterization of diameter of PNI microgels, the solution of PNI microgels was diluted by 10× (adding 100 μL of this solution of PNI microgels into 900 μL of water). After dilution, 1 mL of this diluted solution was used to characterize the size by DLS. For characterization by IR and XPS, puriﬁed PNI microgels were freeze-dried before characterization.

**ORCID**

Xin Jin: 0000-0003-1779-6407
Xinyuan Zhu: 0000-0002-2891-837X

**Notes**

The authors declare no competing ﬁnancial interest.

**ACKNOWLEDGMENTS**

This research was supported by the National Basic Research Program of China (2015CB931801), National Natural Science Foundation of China (51503122, 51690151), and Shanghai Rising-Star Program (17QC1401100).

**REFERENCES**

1. Zhou, X.; Nie, J.; Wang, Q.; Du, B. Thermosensitive Ionic Microgels with pH Tunable Degradation via In Situ Quaternization Cross-Linking. Macromolecules 2015, 48, 3130–3139.
2. Campbell, S.; Maitland, D.; Hoare, T. Enhanced Pulsatile Drug Release from Injectable Magnetic Hydrogels with Embedded Thermosensitive Microgels. ACS Macro Lett. 2015, 4, 312–316.
3. Truong, V. X.; Li, F.; Forsythe, J. S. Versatile Bioorthogonal Hydrogel Platform by Catalyst-Free Visible Light Initiated Photopolymerization of Anthracene. ACS Macro Lett. 2017, 6, 657–662.
4. Hu, Y.; Pérez-Mercader, J. Controlled Synthesis of Uniform, Micrometer-Sized Ruthenium-Functionalized Poly(N-Isopropylacrylamide) Gel Particles and Their Application to the Catalysis of the Belousov-Zhabotinsky Reaction. Macromol. Rapid Commun. 2017, 38, 1600577.
5. Abisol, R.; Cakir, S.; Gabriele, S.; Dubois, P.; Barner-Kowollik, C.; Prez, F. D.; Mes pouille, L. Click reactive microgels as a strategy towards chemically injectable hydrogels. Polym. Chem. 2016, 7, 6752–6760.
6. Li, X.; Gao, Y.; Serpe, M. J. Responsive Polymer-Based Assemblies for Sensing Applications. Macromol. Rapid Commun. 2015, 36, 1382–1392.
7. Chen, R.; Chen, X.; Jin, X.; Zhu, X. Morphology design and control of polymer particles by regulating the droplet flowing mode in microﬂuidic chips. Polym. Chem. 2017, 8, 2953–2958.
8. Hu, J.; Hiwatashi, K.; Kurokawa, T.; Liang, S. M.; Wu, Z. L.; Gong, J. P. Microgel-Reinforced Hydrogel Films with High Mechanical Strength and Their Visible Mesoscale Fracture Structure. Macromolecules 2011, 44, 7775–7781.
9. Jones, C. D.; Lyon, L. A. Synthesis and Characterization of Multiresponsive Core–Shell Microgels. Macromolecules 2000, 33, 8301–8306.
10. Heskins, M.; Guillot, J. E. Solution Properties of Poly(N-isopropylacrylamide). J. Macromol. Sci., Chem. 1968, 2, 1441–1455.
11. Pelton, R. H.; Chibante, P. Preparation of aqueous latices with N-isopropylacrylamide. Colloids Surf. 1986, 20, 247–256.
(12) Echeverria, C.; López, D.; Mijangos, C. UCST Responsive Microgels of Poly(acrylamide−acrylic acid) Copolymers: Structure and Viscoelastic Properties. *Macromolecules* **2009**, *42*, 9118−9123.

(13) Wang, X.; Xu, J.; Li, L.; Wu, S.; Chen, Q.; Lu, Y.; Ballauff, M.; Guo, X. Synthesis of Spherical Polyelectrolyte Brushes by Thermocontrolled Emulsion Polymerization. *Macromol. Rapid Commun.* **2010**, *31*, 1272−1275.

(14) Zhou, X.; Zhou, Y.; Nie, J.; Ji, Z.; Xu, J.; Zhang, X.; Du, B. Thermosensitive Ionic Microgels via Surfactant-Free Emulsion Copolymerization and in Situ Quaternization Cross-Linking. *ACS Appl. Mater. Interfaces* **2014**, *6*, 4498−4513.

(15) Zhou, X.; Wang, J.; Nie, J.; Du, B. Poly(N-isopropylacrylamide)-based ionic hydrogels: synthesis, swelling properties, interfacial adsorption and release of dyes. *Polym. J.* **2016**, *48*, 431−438.

(16) Zhou, X.; Nie, J.; Du, B. 4-(2-Pyridylazo)-resorcinol Functionalized Thermosensitive Ionic Microgels for Optical Detection of Heavy Metal Ions at Nanomolar Level. *ACS Appl. Mater. Interfaces* **2015**, *7*, 21966−21974.

(17) Chen, S.; Jiang, X.; Sun, L. Reaction Mechanisms of N-isopropylacrylamide Soap-Free Emulsion Polymerization Based on Two Different Initiators. *J. Macromol. Sci., Part A: Pure Appl.Chem.* **2014**, *51*, 447−455.

(18) Wu, X.; Pelton, R. H.; Hamielec, A. E.; Woods, D. R.; McPhee, W. The kinetics of poly(N-isopropylacrylamide) microgel latex formation. *Colloid Polym. Sci.* **1994**, *272*, 467−477.

(19) Zhang, Y.; Zha, L. S.; Fu, S. K. Kinetic analysis of poly(N-isopropylacrylamide-co-dimethylaminoethyl methacrylate) microgel latex formation. *J. Appl. Polym. Sci.* **2004**, *92*, 839−846.

(20) Suzuki, D.; Kobayashi, C. Raspberry-Shaped Composite Microgel Synthesis by Seeded Emulsion Polymerization with Hydrogel Particles. *Langmuir* **2014**, *30*, 7085−7092.