Study on the morphology and temperature-sensitivity of poly(N-isopropylacrylamide-co-acrylic acid) microgel by atomic force microscope

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Abstract. A group of crosslinked poly(N-isopropylacrylamide-co-acrylic acid) (P(NIPAM-co-AAc)) microgels with different amount of acrylic acid (AAc) were synthesized by surfactant-free emulsion polymerization. The morphology and environmental sensitivity were studied by atomic force microscope (AFM). It was founded that the microgel particles were monodispersed and the diameter of the dried microgel latex particles was about 800nm. Moreover, the volume of the microgel particles decreased as the environmental temperature increased from 15°C to 50°C, which revealed the temperature-sensitivity of P(NIPAM-co-AAc) microgel.

1 Introduction

Environmentally friendly microgels prepared from the temperature-sensitive monomer N-isopropylacrylamide (NIPAM) have been widely studied, and been applied in the controlled release drug system due to the outstanding biocompatibility, low toxicity and physicochemical sensitivity[1]. It is generally believed that the temperature sensitivity of NIPAM is related to the hydrophobic (-CH(CH₃)₂) and hydrophilic groups (-CONH-) present in its molecular chain. When the temperature is lower than the volume phase transition temperature (VPTT), the hydrophobicity of the polymer chain decreases. However, its hydrophilicity is enhanced, which is reflected in the expanding diameter and volume of the microgel. Conversely, when the temperature is higher than the VPTT, the hydrophilicity of the polymer chain decreases, while its hydrophobicity is improved, resulting in reduction in the diameter and volume of the particles. In addition to temperature, many other factors also contribute to the surface properties of the polymer, which enable it to respond to changes in the environment. These factors include the pH, the ionic strength and the medium, as well as the polymer composition, i.e., composition, charge density, degree of cross-linking, and monomer ratio.

Atomic force microscope (AFM) was developed in the 1980s and rapidly became a powerful tool for detecting the surface shape and structure of materials. AFM permits convenient sample preparation and efficient imaging in a natural state at the resolution of a nanometer. Scholars such as Harmon et al. [2] and Matzelle et al. [3] used AFM to investigate the different surface elastic moduli of the copolymer of NIPAM and poly(N-isopropylacrylamide) (PNIPAM) in different environments and materials. In

comparison, swollen and collapsed states of the poly (N-isopropylacrylamide-co-1-vinylimidazole) hydrogel nanoparticles at different pH values were visualized by AFM, reported by Kazakov and others[4].

This paper used AFM to investigate the morphology of hydrophilic modified poly(N-isopropylacrylamide-co-acrylic acid)(P (NIPAM-co-AAc)) microgels particles at varying temperatures, and used the AFM data analysis system to statistically analyse the AFM images, quantitatively and intuitively characterising it's sensitivity to temperature.

2 Experimental

2.1 Preparation of Microgels

The microgel is obtained through soap-free emulsion polymerization by mixing 5 g NIPAM with a suitable quantity of acrylic acid (AAc) and the cross-linking agent N, N'-methylenebisacrylamide (MBAAm). All of the compounds were dissolved in 350 ml of deionized water and stirred slowly in nitrogen at 70 °C. When equilibrium was achieved, 0.5 g of potassium persulfate (KPS) was dissolved in 50 ml of water and then added to the reaction to initiate polymerization. The reaction lasted for 6 h. After it was completed, the mixture was centrifuged, and the microgel sediment was obtained and re-dispersed with deionized water. This centrifugation step was repeated three times to remove unreacted monomers and initiators to obtain pure cross-linked P(NIPAM-co-AAc) microgels with AAc amount of 10% (1#) and 15% (2#), respectively.

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2.2 AFM Imaging

0.005 mg/ml samples of the microgel were dried on glass plates at either 15 °C or 50 °C. The microgel were then observed using an AFM at room temperature (25 °C) and at 50% humidity.

The AFM used in the present experiment was a SHIMADZU/SPM-9500J3 from Japan with an APS2-50 scanner in tapping mode. Its silicon nitride probe consists of a micro-cantilever, which is 180 μm in length, and has a force constant of 3.2 N/m. The image data was stored in a 256 $\times$ 256 lattice, and all images were automatically processed in the slow-scan direction by Flatten Auto to eliminate low-frequency noise.

3 Results and Discussion

3.1 Morphology of microgel

The morphology of the P(NIPAM-co-AAc) microgel scanned by AFM are shown in Figure 1. The results showed that the microgel particles were of the same size and had diameters of around 800 nm. In Figure 1 (c), the existence of some small particles between the microgel could be observed. They were approximately 150 nm in diameter and approximately 25 nm in height. The existence of these small particles may be explained by wear and tear of the probe as the surface on the tip of a worn probe is similar to a parabola, and it is also of the same size as the particles. Hence, in this experiment, when the tip of the probe scanned across the lower part of the microgel, the tail end would also move in a trajectory which was similar to the shape of the particle (as shown in Figure 1(d)).

Figure 1: AFM images of P(NIPAM-co-AAc) microgels (1#) at wide (a) and narrow ((b) and (c)) visual fields, illustration of the scanning process with the AFM probe(d)

Figure 2: AFM image of “scared” microgel

Some scratches and damage were caused to the surface of the samples by the tip of the probe during the experiments, as illustrated in Figure 2. This happens more frequently in AFM imaging of soft products such as collagen, cell culture media, microgels, and liposomes, etc. Rdler et al. [5,6] believed that the linear velocity of the progressively fast scanning of the tip was reduced when scanning a confined area. The shear stress of the tip and the relaxation of the scanned object were responsible for the damage. In contrast, if the area to be scanned was not limited, it would not lead to any damage. Zhao Tao and others[7] further proved the significance and relevance between the extent of such damage and the scanning range or direction of the tip. They proposed that the sample would be damaged when the tip moves along the Y-axis scanning from the bottom to the top of a small scanning range. Conversely, the tip would not cause any damage if the scanning was carried out along the Y-axis from the top down to the bottom.

3.2 Temperature sensitivities of microgel

The AFM images of microgel particles at different drying temperatures are shown in Figure 3.

Figure 3: AFM images of 1# and 2# P(NIPAM-co-AAc) microgels at 15 °C, and 50 °C respectively, (a)1#-15 °C; (b) 1#-50 °C; (c) 2#-15 °C; (d)2#-50 °C

At a low drying-temperature ($T = 15\text{ }^{\circ}\text{C}$), the particle boundaries were blurred (Figure 3). However, the same particles had a much clearer boundary at a high drying temperature ($T = 50\text{ }^{\circ}\text{C}$) and they were also significantly smaller (Figure 3). The drying process of the spherical

microgel particles on the glass plate is illustrated in Figure 4.

Figure 4: Illustration of the shape change of the microgel particle when it is transferred from a water solution to the glass plate

Table 1: The characteristics of 1# and 2# microgels at 15 °C and 50 °C from AFM images

Sample	Temperature /°C	Diameter /nm	High /nm	Volume / $\times 10^7 \text{ nm}^3$	Contact Angle $\theta / ^\circ$
1 (10%AAc)	15	900	216	7.37	51
	50	715	190	4.18	-
2 (15%AAc)	15	950	96	3.45	23
	50	875	75	2.27	-

The results confirmed that when the temperature of the specimen preparation process was increased from 15 °C to 50 °C, the diameter and height of the microgel particles 1# and 2# decreased (Figure 3, Table 1). The volume of particle 1# and 2# decreased from $7.37 \times 10^7 \text{ nm}^3$ to $4.18 \times 10^7 \text{ nm}^3$, and from $3.45 \times 10^7 \text{ nm}^3$ to $2.27 \times 10^7 \text{ nm}^3$, respectively. They declined in volume by 56.7% and 66.8% respectively. The results indicate that the same microgel might have different states at different temperatures and that it is sensitive to temperature.

The size of the microgel increased with increased amounts of AAc. Furthermore, based on the geometric relationship illustrated in Figure , the contact angle θ between the particle and the glass plate can be calculated as follows: $\theta = \angle 2 = 2 \angle 1 = 2 \arctg 2h/r$. The contact angles of the two particles were around 51° and 23° respectively. This indicates that the increased amount of AAc in the polymer enhances the hydrophilicity of the surface of the microgel particles, which led to the improvement of wettability between the particle and the slides.

4 Conclusion

AFM is a powerful tool for independently analysing the surface of structures. This paper used AFM to observe the form of temperature-sensitive microgel particles and measure their sizes. For a more detailed analysis, the hydrophilicity and temperature-sensitivity of the microgel particles were assessed based on calculations of their contact angles with a glass plate. However, this does not mean that there are no limitations to the present study. First, a worn probe may lead to a blurry image due to potential scratching or even damage to the surface of the samples. Second, the existences of many uncontrollable factors are not avoidable, such as the sharpness of the images, and the mode of data processing which may be influenced by both the scanner and the operator. Finally, micro-scanning may not be representative of the macro structure due to the differences in scale. In conclusion, further research is still required on the application of AFM in the field of quantitative analysis of the performance of

The average radius r (AB) and average height h (BC) of the “sphere” can be obtained from the AFM images, and the volume of the dried microgel particles can be accessed by the formula $V = \pi r h (3r^2 + h^2)/6$. The results of the analysis of the particles in Figure 2 are presented in Table 1.

microgel.

Acknowledgment

This research was financially supported by Research and Innovation Initiatives of WHPU (2020Y08)

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