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The pH influence of the yield and properties of photochemically synthesized poly(*N*-isopropylacrylamide) nanoparticles

Xiangli Qiao*, Zhenjia Zhang

Environmental Science and Engineering, Shanghai Jiaotong University, Shanghai 200240, China

Abstract

Poly(*N*-isopropylacrylamide), PNIPAm, nanoparticles (10%, m/m) are prepared from monomer aqueous solutions under the irradiation of UV rays, without initiator and crosslinker addition. H^+ plays an important role in the photopolymerization of PNIPAm nanoparticles: it accelerates polymerization, and determines size of the PNIPAm nanoparticles. The pH influence on the yield and properties of PNIPAm nanoparticles is investigated in detail. FTIR, DSC and swelling behaviors of the PNIPAm nanoparticles indicate that they are internally crosslinked macromolecules.
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Keywords: pH effect; Poly(*N*-isopropylacrylamide) nanoparticles; Size controlling; UV rays

1. Introduction

Poly(*N*-isopropylacrylamide), or PNIPAm particles, may be one of the most intensively studied subjects during last few decades, as they possess a well-known unique volume phase transition which undergo a dramatic reversible volume change at ca. 31 °C. This large volume transition in response to temperature makes PNIPAm particles be potential candidate for controlled drug delivery and gene delivery, etc. [1,2].

There is a growing interest in the preparation of tiny polymeric spheres (microparticles and nanoparticles), especially the latter having sub-micron dimensions, as the square of a linear dimension of the particles is proportional to the time required to its swell and collapse [3,4]. In addition, nanoparticles exhibit higher resistance to radical-inducing degradation, and they provide satisfactory performance as a carrier over a long period [5].

PNIPAm nanoparticles have been prepared by emulsion polymerization or precipitation polymerization. The presence of surfactants and chemical crosslinkers in these conventional systems influence the properties of the nanoparticles and result in inhomogeneous crosslinking densities [6], unsuitable for their biomedical uses. In recent years, radiation technique proves to

be a suitable tool for nanoparticles' preparation [7]: when a dilute, deoxygenated aqueous solution of a linear polymer is subjected to short, intense pulses of fast electrons, many radicals are generated simultaneously on each polymer chain, and the intra-molecular re-combinations lead to the formation of nanoparticles [8,9]. This method avoids the use of chemical initiator and crosslinker, the substrates containing only linear polymer and water, so the obtained nanoparticles are quite pure, needing no further treatment. In our previous work, initiator and crosslinker-free PNIPAm nanoparticles were synthesized from monomer aqueous solution under the irradiation of UV rays [10].

In the present work, we discuss in detail the effect of pH on the yield and properties of the PNIPAm nanoparticles. It is found that pH plays an important role in the photopolymerization. It accelerates the polymerization, improving the polymer yield, and size of the PNIPAm nanoparticles can be controlled by changing the pH value. FTIR, DSC, and swelling behaviors of the PNIPAm nanoparticles demonstrate that they are internally crosslinked macromolecules.

2. Experimental

2.1. Materials

NIPAm monomer is commercially available from Tokyo Kasei Ltd., and purified by recrystallization from hexane before use. Three-distilled de-ionized water is used as solvent for

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* Corresponding author. Tel.: +86 21 54747412; fax: +86 21 54747368.
E-mail address: qiao_xl@sjtu.edu.cn (X. Qiao).

NIPAm. Other reagents are all analytical grade, and used without further treatment.

2.2. Preparation of PNIPAm nanoparticles by UV technique

The irradiation condition and PNIPAm nanoparticles preparation procedure are described in detail in our previous report [10]. In the present study, varying amount of NIPAm is dissolved in 30 mL distilled water in 50 mL quartz flasks, which is stirred with a two-blade stirrer at 200 rpm. The solution pH is adjusted by adding HCl or NaOH. After saturated with N_2 for 30 min, the polymerization is carried out at 30 °C for 5 h upon the illumination of 500 W Xe-lamp (wavelength: 170–700 nm) under the protection of N_2 atmosphere. In our system, only a small fraction of UV rays from the lamp is converged at reaction flask, so temperature of the reaction mixture remains almost constant during the polymerization.

The obtained PNIPAm latex is filtered with sulfonated polyethersulfone (SPES) membrane (its molecular weight cut off value being 5 kDa) to remove the residual monomer.

2.3. Yield of PNIPAm nanoparticles

After treatment by SPES membrane, the PNIPAm nanoparticles are dried under vacuum and weighed. Yield of PNIPAm nanoparticles is defined as: $Y_{PNIPAm} = G_{PNIPAm}/G_{monomer}$ (G_{PNIPAm} and $G_{monomer}$ represent the weight of the PNIPAm nanoparticles and NIPAm monomer, respectively).

2.4. FTIR studies

The procedure of the sample preparation is described as follows: nanoparticles are dried under vacuum at 60 °C for 2 days. The dried PNIPAm is mixed and ground with KBr in the ratio of 1:25. KBr pellets with 1 mm thickness are formed by pressing the mixture at 15 tonnes for a few minutes under vacuum. FTIR-spectra of the samples are recorded on Nicolet 20SXC spectrometer.

2.5. Differential scanning calorimetry (DSC)

DSC curves of the dried PNIPAm nanoparticles are measured on DSC (Perkin-Elmer DSC-7 system) instrument. The samples are firstly preheated to 80 °C, and cooled to −20 °C under the continuous N_2 stream at a rate of 20 °C/min, then analyzed with temperature increasing rate of 20 °C/min.

2.6. Scanning electron microscope (SEM)

Morphology of the prepared PNIPAm nanoparticles is determined by Scanning Electron Microscope (SEM) LEO 1350 VP (Germany) employed at 5–10 kV.

2.7. Photon correlation spectroscopy (PCS)

In order to character the hydrodynamic radii of the PNIPAm nanoparticles, PCS ZetaSizer 3000HS (Malvern Instrument

Ltd.) fitted with a 5-mW He–Ne laser ($\ddot{I}$) 633 nm is employed. By adjusting pH to neutral, the PNIPAm nanoparticles are poured into a commercial cylindrical glass cell which is set inside a sample holder. The scattered light by the PNIPAm nanoparticles is detected by a photo-multiplier at a scattering of 90° under a temperature of 25 °C. Fluctuations in the intensity of the scattered light are analyzed through the use of first order and second order autocorrelation functions. The volume-weighed average diameter obtained by the manufacturer's software is used for the calculation of the average PNIPAm nanoparticles volume. Zeta potential of the PNIPAm nanoparticles is also measured by the instrument. Zeta potential is determined three times for each sample. Results are automatically calculated using the following Smoluchowski equation: $m = (ez)/h$, where z is the zeta potential, m the mobility, e the dielectric constant and h is the absolute viscosity of electrolyte solution.

3. Results and discussion

In our previous work [10], we proved the possibility of PNIPAm nanoparticles synthesis from dilute monomer solution without introduction of initiator and crosslinker under the irradiation of UV rays. In the present study, we increase the monomer concentration and study the pH influence on the properties and yield of the photochemically synthesized PNIPAm nanoparticles.

Firstly, we fix the monomer concentration at 9.08×10^{-1} mol/L (ca. 10%, m/m), and studies the yield of the PNIPAm nanoparticles during the photopolymerization at pH 2 and 6.5, respectively (see Table 1).

Monomer in pH 2 solution is rapidly synthesized to PNIPAm nanoparticles, and its corresponding yield increases up to ca. 88% in 2 h. In contrast, only 7% of monomer is converted to nanoparticles in 2 h at pH 6.5. After 5 h polymerization, the yields of PNIPAm nanoparticles in pH 2 and pH 6.5 solution are ca. 90% and 21%, respectively.

It can be observed that during the photopolymerization, the reaction solution of pH 2 quickly becomes viscous with time. However, for pH 6.5 system, no obvious phenomenon is found even after 5 h of polymerization. If we further increase the monomer concentration of pH 2, the reaction cannot go on smoothly as the corresponding viscosity increases rapidly with time, making agitation become very difficult.

In the following section, the influence of pH on diameter of the PNIPAm nanoparticles is further evaluated.

Curves a, b, and c in Fig. 1 respectively represent the pH influence on diameter of the PNIPAm nanoparticles prepared

Table 1
Yield of PNIPAm nanoparticles at different pH

	Polymerization time (h)				
	1	2	3	4	5
pH 2	52%	88%	90%	90%	90.5%
pH 6.5	2%	7%	13%	20%	21%

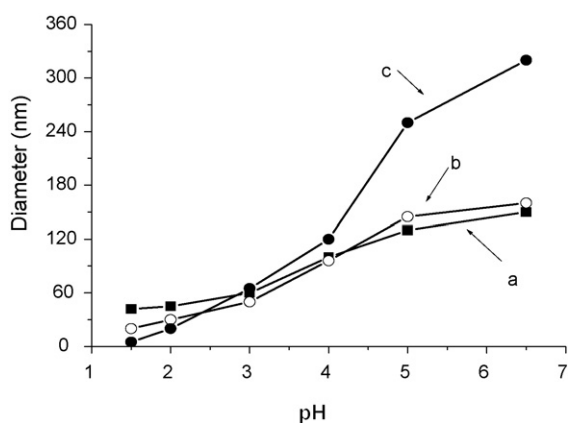Note: Monomer concentration is fixed at 9.08×10^{-1} mol/L.

Fig. 1. pH influence of diameters of the PNIPAm nanoparticles prepared at different monomer concentration. (a) 5.53×10^{-2} mol/L; (b) 9.06×10^{-2} mol/L; (c) 9.08×10^{-1} mol/L.

Fig. 3. Yield of the PNIPAm nanoparticles influenced by pH. (a) 5.53×10^{-2} mol/L; (b) 9.08×10^{-1} mol/L.

at 5.53×10^{-2}, 9.06×10^{-2}, and 9.08×10^{-1} mol/L monomer concentration.

A decrease in diameter of the PNIPAm nanoparticles with decreasing pH is observed in curves a, b, and c. For example, diameter of the PNIPAm nanoparticles decreases from ca. 151 nm at pH 6.5 to ca. 45 nm at pH 1.5 for 5.53×10^{-2} mol/L monomer concentration; and similar phenomena are observed for 9.06×10^{-2} mol/L, and 9.08×10^{-1} mol/L monomer concentration systems. The higher of the monomer concentration, the larger reduction in diameter of the corresponding PNIPAm nanoparticles. PCS data of the PNIPAm nanoparticles prepared at pH 1.5 and pH 6.5 are illustrated in Fig. 2 (monomer concentration being 9.08×10^{-1} mol/L).

Yield of the corresponding PNIPAm nanoparticles can also be improved by decreasing pH value of the solutions (see Fig. 3, monomer concentrations are fixed at 5.53×10^{-2} and 9.08×10^{-1} mol/L, respectively).

All the above data demonstrate that H$^+$ plays an important role in the photopolymerization of the PNIPAm nanoparticles.
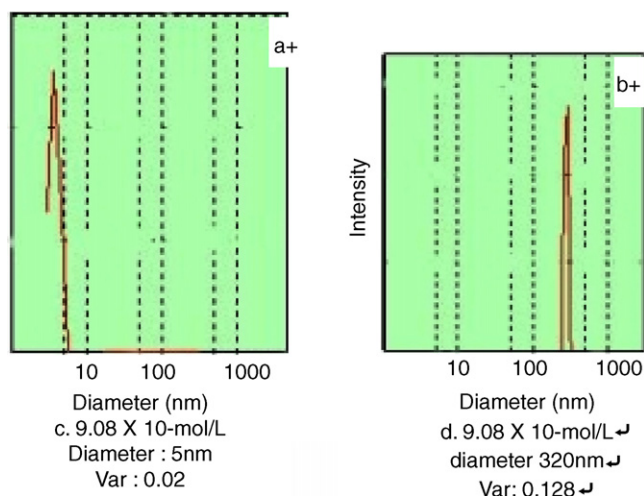
It not only accelerates the photo-polymerization, but determines diameter of the PNIPAm nanoparticles.

In an attempt to determine the influence of pH on the diameter measurement of the PNIPAm nanoparticles, we detect the diameter of the PNIPAm nanoparticles (prepared at pH 2) at different pH using PCS (see Fig. 4). Obviously, there is no significant change in the diameter of the PNIPAm nanoparticles within 2 and 6.5 pH range.

In our system, no initiator and crosslinker is added. NIPAm monomer is directly excited to its excited state under the irradiation of UV rays and generates active species [10]. These active species, such as triplet states and free radicals, attack the double bonds of NIPAm to initiate the photopolymerization:

$$M \xrightarrow{\text{UV}} [M]^* \xrightarrow{M} (M)_n{}^\bullet \rightarrow \text{polymer}$$

M represents NIPAm monomer.

In neutral system, the concentration of free radicals generated are too low to ensure high conversion and polymerization rate; however, a quite fast photopolymerization is observed resulting in a high monomer conversion and nanoparticle yield when H$^+$ is added, as the existence of H$^+$ accelerates the genera-

Fig. 2. Size distribution of the prepared PNIPAm nanoparticles at pH 1.5 (a) and pH 6.5 (b). Monomer concentration is fixed at 9.08×10^{-1} mol/L.
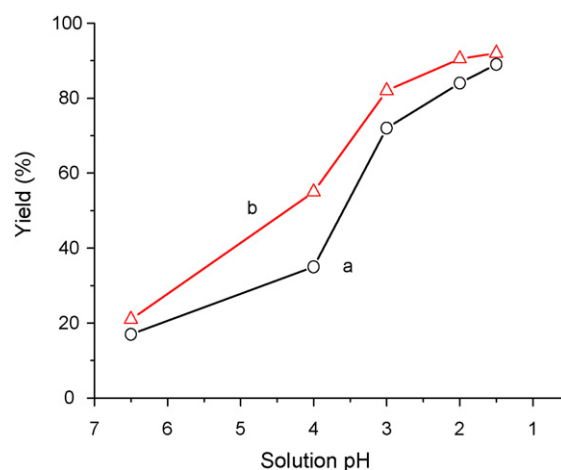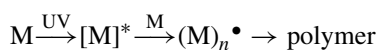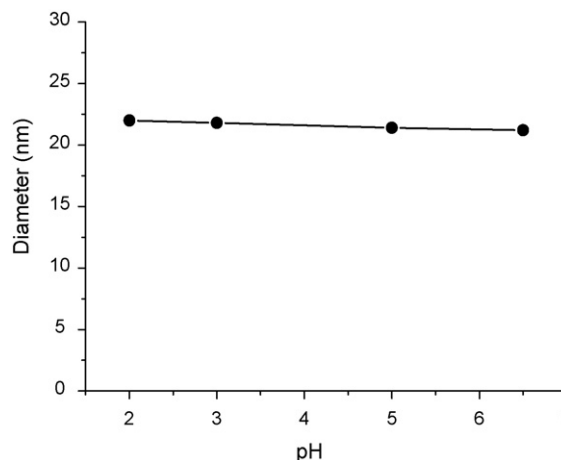

Fig. 4. Diameter of the PNIPAm nanoparticles measured at different pH (prepared at pH 2, C_{monomer} is 9.08×10^{-1} mol/L).

$$M \xrightarrow{\text{UV}} M^* \qquad \text{(excited state)} \qquad (1)$$
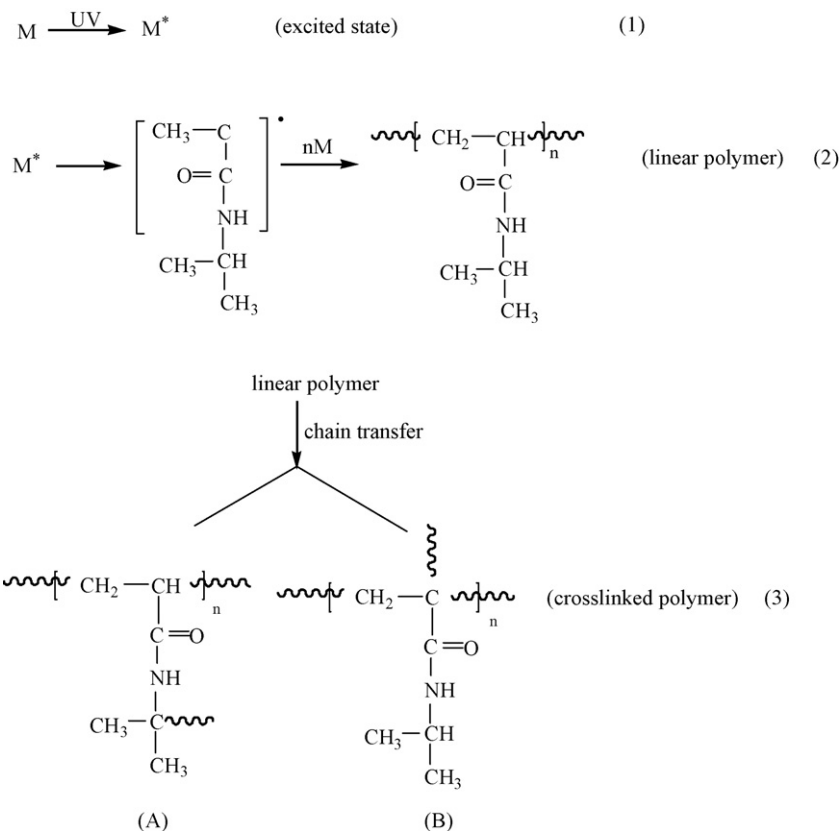

Fig. 5. Reaction mechanism in the photopolymerization of PNIPAm nanoparticles.

tion of free radicals in the reaction system (evidenced by ESR in [10]).

There are two modes of the structure of the prepared PNIPAm nanoparticles: one is the aggregation of a single polymer chain; the other is formed by crosslinking among polymer chains. We test the stability of the PNIPAm nanoparticles, and find out that no dissociation of these nanoparticles is observed even after stored at room temperature for more than half a year. The high stability indicates the possibility of the existence of crosslinked structure in the PNIPAm nanoparticles. Gao and Frisken [11] prepared chemically crosslinked PNIPAm nanoparticles with hundreds of nanometers initiated by potassium persulfate (KPS) without the addition of crosslinker and surfactant. They attributed the formation of crosslinked nanoparticles to the self-crosslinking of PNIPAm chains by chain transfer reaction during the free radical polymerization. There are two possible sites in PNIPAm structure for chain transfer: the hydrogen atom on the *tert*-C of the pendent isopropyl and on the *tert*-C of the main chain. They are both active and would like to be attacked by free radicals leading to the formation of *tert*-C free radicals [12].

In our system, NIPAm monomer can be excited to its excited state producing free radicals under the irradiation of UV rays [10]. Thus, it can be expected that chain transfer during the photopolymerization takes place, too. The *tert*-C free radicals produced may either react with double bonds or with another *tert*-C active atom on an adjacent PNIPAM chain to form crosslinking points. Both of these possibilities

lead to the crosslinking of PNIPAm. Its possible polymerization mechanism is illustrated in Fig. 5 (M represent monomer

). (A) and (B) in Fig. 5 are the crosslinked structures of the PNIPAm nanoparticles.

FTIR spectrum of the PNIPAm nanoparticles prepared at pH 2 is presented in Fig. 6.

Comparing with curve a (monomer), the peak at $2984\,\text{cm}^{-1}$ (A) assigned to $=C-H$ vibration in curve b is weakened, and the peak amplitude at $1450\,\text{cm}^{-1}$ (B) corresponding to stretching mode of $-C-H$ vibration is strongly strengthened. These facts prove that the double bond of NIPAm is initiated to polymerize. In addition, two peaks at 1385 and $1373\,\text{cm}^{-1}$ (C) with different
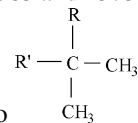
intensities are attributed to group vibration, which indicates that the existence of crosslinking points at the sites of *tert*-C of the pendent isopropyl and *tert*-C of the main chain in the PNIPAm nanoparticles as discussed above.

Size variation of the PNIPAm nanoparticles during the photopolymerization is measured using PCS, and the result is listed in Table 2. PNIPAm nanoparticles at different pH exhibit similar size variation trend: from dilute, large-size PNIPAm nanoparticles to thick, small-size ones. In general, diameters of the PNIPAm nanoparticles prepared at pH 2 are much smaller than that at pH 6.5.
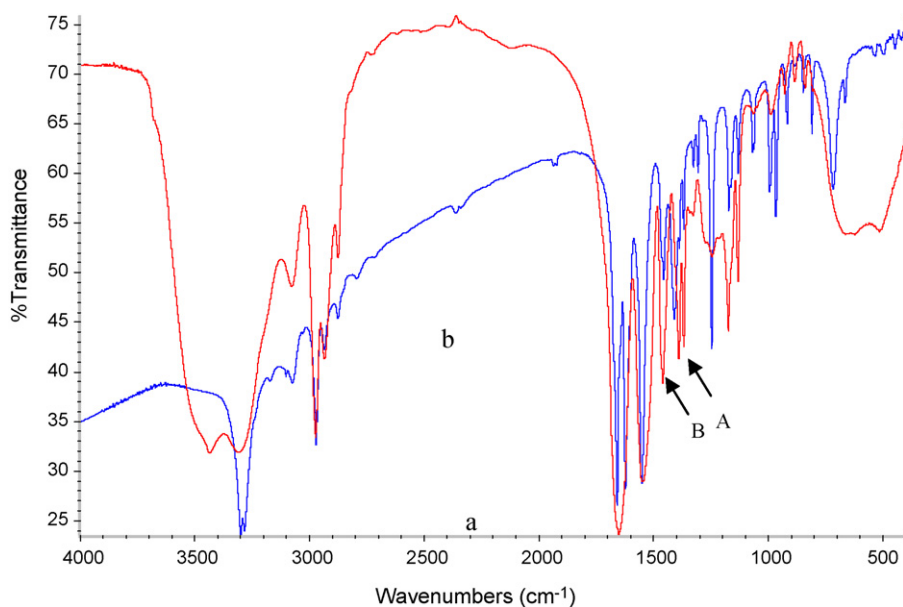
Fig. 6. FTIR spectrum of monomer (a) and the photosynthesized PNIPAm nanoparticles at low pH (b).
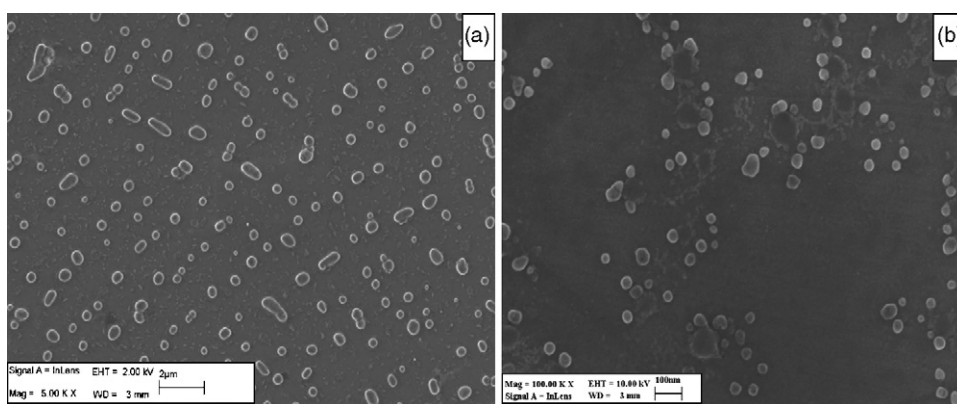

Fig. 7. SEM imagines of the PNIPAm nanoparticles prepared at pH 6.5 ((a) the scale bar is 2 μm) and pH 2 solution ((b) the scale bar is 100 nm). $C_{monomer} = 9.08 \times 10^{-1}$ mol/L.

SEM images of the PNIPAm nanoparticles prepared at pH 6.5 and pH 2 are presented in Fig. 7. The latter possesses a much narrow size distribution.

In these systems, except pH values, the photopolymerization conditions are the same, but their photopolymerization behaviors are very different (see data in Tables 1 and 2). The only explanation is the crucial role that H^+ plays in the polymerization. In our previous report, more free radicals are found to be generated in reaction mixture when H^+ is added. To explore the reason under it, we detect the UV–vis spectrum of NIPAm and

NIPAm plus HCl solutions (pH 2) and the result is demonstrated in Fig. 8. Comparing with NIPAm, there is a little absorption augmentation for NIPAm plus HCl (pH 2) in the UV–vis range (HCl has no absorption in the range), which indicates that some

Table 2
Size variation of the PNIPAm nanoparticles during the photo-polymerization

	Polymerization time (h)				
	1	2	3	4	5
pH 2	75 nm	52 nm	30 nm	25 nm	22 nm
pH 6.5	–	–	550 nm	362 nm	320 nm

Note: Monomer concentration is fixed at 9.08×10^{-1} mol/L. "–" means that no particles could be detected in the solution.
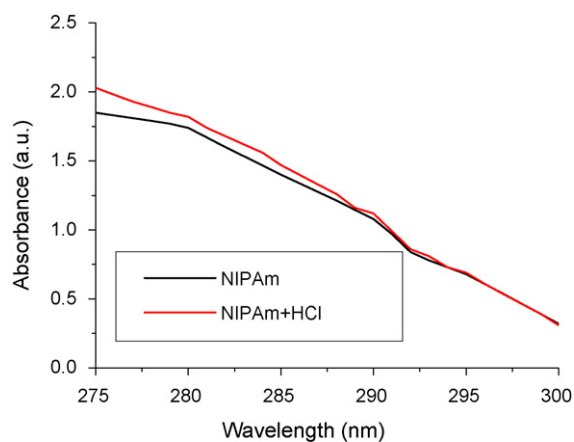

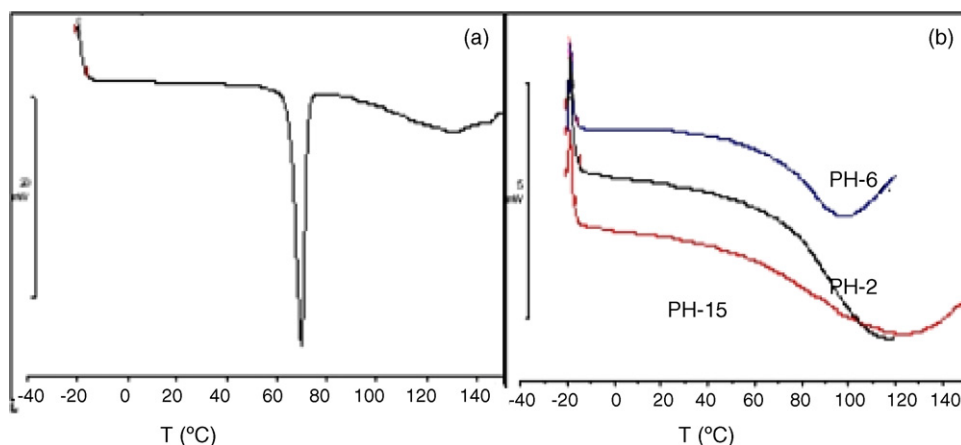Fig. 8. UV–vis spectrum of NIPAm and NIPAm plus HCl (pH 2).

Fig. 9. DSC curves of monomer (a) and the PNIPAm nanoparticles prepared at different pH (b).

more amount of photo is adsorbed by acidic reaction mixture. Obviously, more absorbed photo leads to a higher monomer conversion. However, the absorption augmentation observed in pH 2 is not proportional to the high conversion of monomer and high polymerization rate observed in pH 2 reaction solution (see Table 1 and Fig. 3). So, in addition to the increase of absorbed photo, a rise in photopolymerization rate at low pH may also result from the increase of photopolymerization efficiency of NIPAm with the addition of HCl.

Owing to the almost zero of zeta potential of NIPAm aqueous solution at pH 2 and neutral pH, we eliminate the possibility of the charging of the amine groups for NIPAm in low pH solution.

Thus, the increase of photopolymerization efficiency of NIPAm at low pH is attributed to different affinities of the monomer for the solvent, water. In acidic solution, NIPAm tends to aggregate together in a way which will result in the smooth process of free radical polymerization, with more free radicals generated. So, chain propagation and chain transfer of PNI-PAm would continue simultaneously, resulting in the crosslinked PNIPAm nanoparticles.

DSC curves of the PNIPAm nanoparticles prepared at low pH are measured, and the result is shown in Fig. 9.

NIPAm shows a sharp melting peak at 71.2 °C in the DSC curve (Fig. 9a). With the decreasing of solution pH, DSC exothermic peak corresponding to melting points of the PNIPAm nanoparticles shifts towards higher temperature, suggesting the PNIPAm nanoparticles prepared at low pH possess more compact or crosslinked structure than those prepared at high pH.

We also test the swelling behavior of the PNIPAm nanoparticles prepared at different pH. It was reported [13] that PCS is a reliable technique to measure diameter of polymeric nanoparticles precisely and swollen volume of polymer gels must be measured at completely equilibrium state. In general, the equilibrium time of swelling is proportional to the square of a linear dimension of the gel [14], the equilibrium time of the PNIPAm nanoparticles is estimated according to the following equation developed by Young and Young [14]:

$$\frac{(300\,\mu m)^2}{600\,min} = \frac{(d_m)^2}{t_c\,min} \quad (d_m : \text{linear dimension of a gel})$$

If the nanoparticle size is 300 nm, its maximum equilibration time is

$$t_c\ min = \frac{600\ min}{(300\,\mu m)^2} \times (0.3\,\mu m)^2 = 6 \times 10^{-4}\ min$$

So, equilibration time for our samples will not exceed 10 min. In this study, t_c is set as 30 min to ensure the PNIPAm nanoparticles to reach their equilibration states at certain temperature. Curves a and b in Fig. 10 illustrate the swelling behavior of the PNIPAm nanoparticles prepared at pH 3 and 5.

For both kinds of PNIPAm nanoparticles, a sharp decrease in diameter is observed in the temperature scope of 32 and 33 °C, which is in agreement with the swelling behavior of PNIPAm nanopartilces observed by Gao and Frisken [11], which exhibited a distinct shrinking over the same temperature scope. The PNIPAm nanoparticle prepared at pH 3 shows a sharper phase transition around 33 °C (see curve a in Fig. 10), while that prepared at pH 5 has a much broad phase transition scope. Even and dense crosslinking in the nanoparticles can accelerate the phase transition while loosely crosslinked structure will retard the phase transition process [15]. Thus, the former nanoparticles (prepared at pH 3) have a relative denser crosslinking than the latter one (prepared at pH 5). These swelling data of the nanopar-
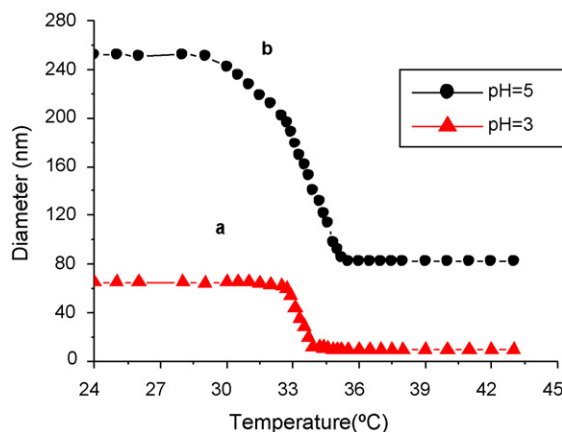

Fig. 10. Swelling behaviors of the PNIPAm nanoparticles prepared at pH 3 (a) and pH 5 (b).

ticles can be reproduced by repeating the temperature scope of 24 and 42 °C, which is also a good indication of the formation of crosslinked structure.

4. Conclusion

We investigate the pH influence on yield and properties of the PNIPAm nanoparticles. Experiment results demonstrate that H^+ plays an important role in the photo-polymerization of the PNIPAm nanoparticles. It accelerates the polymerization, improving the obtained yield, and determines the diameter of the PNIPAm nanoparticles. DSC, FTIR and the swelling behaviors of the PNIPAm nanoparticles indicate they have crosslinked structure. We attribute the increase of yield and photopolymerization rate of the PNIPAm nanosphere with the addition of H^+ to the combination of a rise in absorbed photo and increase of photopolymerization efficiency resulting from the aggregation of monomer in a way favoring the free radical photopolymerization. Furthermore, chain transfer leads to the crosslinked PNIPAm nanoparticles.

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