Macromol. Symp. ***150,*** *297–303 (2000)*

Preparation of thermosensitive latexes by copolymerization of N-isopropylmethacrylamide with a chelating monomer

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SUMMARY: Functional poly(N-isopropylmethacrylamide) (NIPMAM) copolymer latexes were prepared at 80°C using methylene-bisacrylamide (MBA) as the crosslinking agent, and potassium persulfate (KPS) as the initiator in the presence of N-(vinylbenzylimino)-diacetic acid (IDA). Adding functional monomer (IDA) was found to drastically affect particle size, but not size distribution as observed both by scanning electron microscopy and quasi elastic light scattering. However, performing the polymerization reaction at too high functional monomer concentration, led to enhanced the formation of water soluble polymers (WSP). The Lowest Critical Solubility Temperature (LCST) of cleaned latexes was measured by a turbidimetric method reflecting the thermosensitive behavior of the particles. The transition temperature was found to be around 43°C, and was only slightly dependent on the concentration of functional monomer.

Introduction

In recent years, many papers have reported on the preparation of hydrogel latexes containing thermosensitive polymers such as poly[N-isopropylacrylamide]$^{1,2)}$ (poly[NIPAM]) or other poly[alkyl(meth)acrylamide]$^{3-5)}$ derivatives, due to their increasing interest for use in biological applications. The aim of this study was to synthesize thermosensitive and functionalized latexes exhibiting a Lowest Critical Solubility Temperature (LCST) value higher than that of poly[NIPAM] (32°C), for direct immobilization of modified recombinant proteins bearing (6-histidine) tagged proteins by chelation. N-isopropylmethacrylamide (NIPMAM) was chosen as the main monomer since the corresponding homopolymer displays a LCST value of around 43°C$^{6,7)}$ in free solution. The functional monomer, N-(vinylbenzylimino)-diacetic acid (IDA), was selected due to its ability to form complexes with metal ions such as Zn^{2+}, Cu^{2+}, Ni^{2+}, etc. This immobilization method of recombinant protein via metals is a well-established method for protein purification (Immobilized Metal Affinity Chromatography, IMAC). It relies on the high affinity and selective interaction of transition-metal ions immobilized by chelation on a solid support and amino acid residues of recombinant tagged proteins.

CCC 1022-1360/00/$ 17.50+.50/0

Experimental

Preparation of microgel particles

NIPMAM and IDA monomers were first synthesized according to Netopilik et al.$^{6)}$ and Morris et al.$^{8)}$ respectively. Polymerizations were carried out in a 100 ml round-bottomed four-necked flask equipped with a glass anchor-shaped stirrer, condenser and nitrogen inlet. Monomers (NIPMAM, MBA, IDA) dissolved in boiled and deoxigenated water were then added. After temperature equilibrium (80°C), the solution was stirred for 30 minutes at polymerization temperature before introducing the initiator (KPS) dissolved in water. The solution was stirred at a constant rate under nitrogen during polymerization and the reaction was carried out for a duration of 6 hours. The recipe used in this study are given in table 1.

Table 1 : Latex recipe

Reagents	weight (g)
Water	50
NIPMAM	1
MBA (Crosslinker)	0.12
KPS (initiator)	0.012
IDA (functional monomer)	0 - 0.028

Results and discussion

Titration curves of the IDA monomer were used to study the ability of the iminodiacetic acid to complex metals. The titration curve of the functional monomer is shown in Figure 1. As can be seen, from the starting point to the first end point requires one equivalent of base and corresponds to the undissociated carboxyl group. A second equivalent of base titrates the proton of the ammonium nitrogen with an end-point at two moles of base per mole of functional monomer. On adding one mole of $Ni(Cl)_2$ per mole of functional monomer, the equivalent point was shifted to a higher NaOH volume, indicating the formation of a 1:1 chelate between the monomer and the metal.

Kinetics of solution polymerization were examined to estimate the ability of both monomers to polymerize in water. NIPMAM and IDA homopolymerizations were carried out at 70°C in deuterium oxyde (D_2O), using KPS as the initiator. Kinetics were followed directly by ^{1}H-NMR, which provide a quantitative assessment of the residual monomer amount. A high polymerization rate (R_p) is clearly observed (data not shown) in both cases (the reactions were complete within 60 minutes) Rp being significantly faster with NIPMAM monomer. These data were used to determine the so-called kp/kt$^{1/2}$ ratio using the well-known equation :

$$\ln([M_0]/[M]) = 2(k_p / k_t^{1/2})(2f[I_0]/kd)^{1/2}(1-e^{-k_d t/2})$$

Where $[M_0]$ and [M] are the monomer concentrations at times t=0 and t, respectively; $[I_0]$ is the initial initiator concentration; k_p and kt are the propagation and termination rate constants, respectively; kd and f are the decomposition rate and the initiator efficiency, respectively. A plot of ln ($[M_0]$/[M]) versus (1-e$^{-kdt/2}$) is illustrated in Figure 2, given that k_d=2.33.10^{-5} for the KPS at 70°C in water and f=0.5. The $k_p/k_t^{1/2}$ values, deduced from the slope of the obtained curves, are 1.36 and 0.85 (l.mol^{-1}.s^{-1}) for NIPMAM and IDA monomer, respectively. $k_p/k_t^{1/2}$ for NIPMAM is higher than that of the IDA monomer. This could be due to the high reactivity of methacrylamide derivatives compared to styrene, together with a lower termination constant.

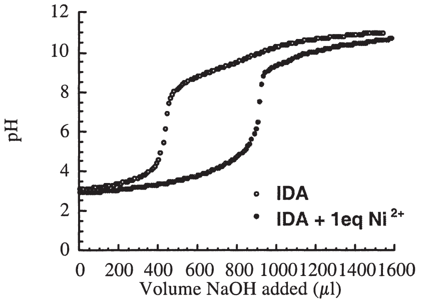

Figure 1: Potentiometric titration of IDA:IDA = 0.0125 g in 50 ml H_2O, [NaOH] = 10^{-2} mol.l^{-1}.

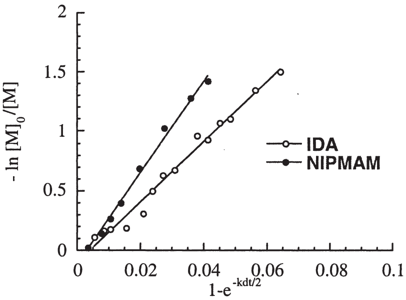

Figure 2: ln ([M0]/[M]) versus (1-e$^{-kdt/2}$) plots for NIPMAM and IDA in water.

In this study, the influence of the functional monomer (IDA) concentration was explored, all other parameters being kept constant as previously determined in a first paper$^{4)}$. All final latexes were cleaned by repetitive centrifugation in order to remove water soluble polymer and then latex were examined as regards particle size and size distribution by Scanning Electronic Microscopy. As illustrated in Figure 3, SEM micrographs of all samples show that all latexes are highly monodisperse whatever the concentration of functional monomer (IDA) used in the polymerization recipe. This suggests an extremely short nucleation step and a constant increase in particle size over time.

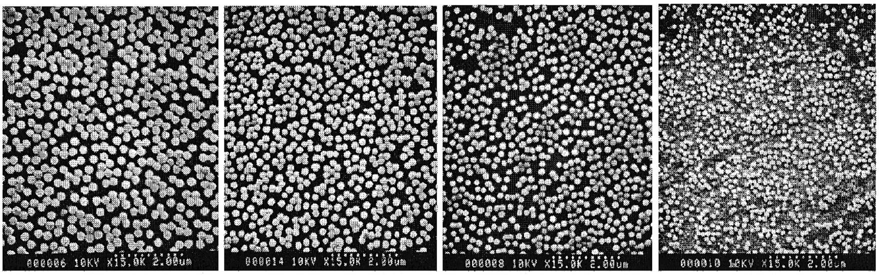

Figure 3: Scanning Electron Microscopy images of the final: 0, 0.6, 1.2 and 2% respectively

Figure 4 shows the effect of the IDA monomer on the final particle size measured by QELS at two temperatures (below and above the LCST). As observed in Figure 4, the particle size progressively decreases at both temperatures upon increasing the functional monomer from 0 to 2.8% (w/w). This observation shows that the number of particles increases when the functional monomer concentration is increased in the polymerization recipe. In fact, introduction of a small amount of IDA monomer should enhance the formation of more precursors and/or the charge coming from the IDA monomer increased the colloidal stabilization of the nucleated particles. These particles then rapidly become the polymerization loci and no new particles are formed.

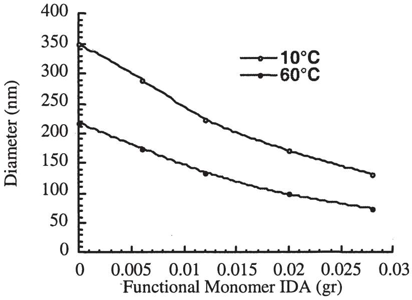

Figure 4: Effect of IDA on diameter of hydrodynamic particles at 10°C and 60°C.

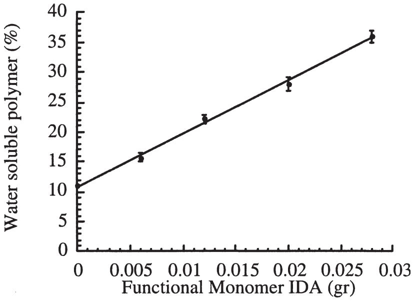

Figure 5: Influence of IDA concentration on water soluble polymer formation (WSP).

Water soluble polymer (WSP)

Figure 5 shows the amount of water soluble polymer determined by a gravimetric method after latex centrifugation. An important increase in the amount of WSP (from 10 to 35% w/w) is observed when the functional monomer concentration is increased. Moreover, there is a linear relationship between the WSP formation and the functional monomer concentration. This drastic effect might originate from : (i) the formation of NIPMAM-IDA copolymer that would not precipitate under polymerization conditions because of the polyelectrolyte character of the formed copolymer. (ii) IDA monomer should induce some transfer reaction producing some low Mw chains with low ability to precipitate. iii) poly(NIPAM) chains formed at higher conversion are only physically adsorbed.

Effect of temperature on particle size

The hydrodynamic diameter of the particles was measured by QELS as a function of temperature for each sample and the obtained results are reported in Figure 6. As expected a marked decrease in particle size is observed around the transition temperature of the non cross-linked poly(NIPMAM) homopolymer (i.e. 44°C). This decrease in particle size is more marked in the case of large particles due to the more pronounced size difference between the shrunken and the expanded state. In all cases, latexes exhibit the same curvature with a dramatic decrease in particle size between 40 to 50°C.

Swelling properties of the microgel particles

The swelling ratio was calculated from the hydrodynamic radius of the expanded and collapsed particle. The swelling capacity of microgel is drastically dependent on the IDA monomer concentration as reported in Figure 7, in which the swelling ratio is reported as a function of the IDA amount introduced in the polymerization recipe. The observed behavior may be attributed to the following phenomena : (i) the difference in the incorporated amount of the cross-linker agent from one sample to another, since the crosslinker indeed governs the internal structure of the particles. (ii) the presence of ionic groups coming from the functional monomer inside the particle may lead to an increase in the swelling factor of the particles. Considering the effect of ionic groups, Hirose et al.9) reported that increasing the ionic groups in the microgel particles leads to an increase in the transition temperature and increase the swelling ratio. The explanation was that when a given gel contains ionic groups, the contribution of the osmotic pressure due to the presence of counterions enhanced the swelling capability of the gel.

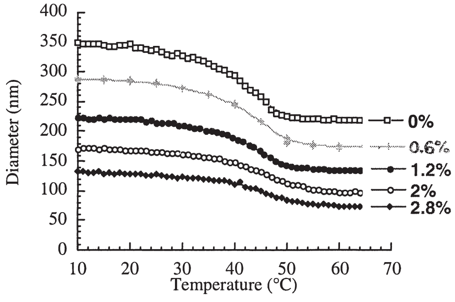

Figure 6 : Hydrodynamic diameter of latexes as a function of temperature.

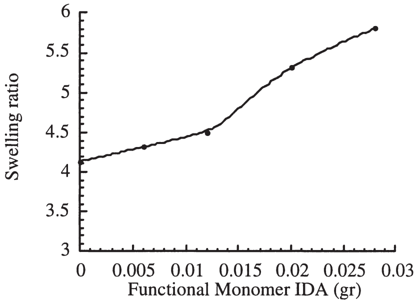

Figure 7: Swelling ratio (S_W) of poly-(NIPMAM-IDA) latexes as a function of IDA concentration.

These results are consistent with the swelling capability (S_W) values plotted in Figure 7, showing that S_W increases as the functional monomer (IDA) used in the polymerization is increased. Given that the crosslinker and the initiator concentration were constant in the polymerization, it can be concluded that the ionic groups introduced by the functional monomer induced some change in the S_W.

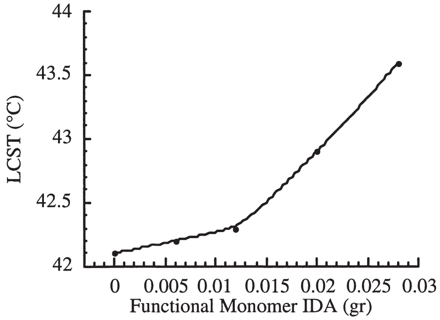

Figure 8: LCST of poly(NIPMAM-IDA] latexes as a function of IDA concentration.

Lower Critical Solubility Temperature (LCST) of the latexes

The transition temperature (LCST) of poly(NIPMAM-IDA) latexes was deduced by measuring the Optical Density (OD) variation as a function of temperature, the value being determined as the maximum of the (δOD/δT) curve versus temperature. From the obtained values reported in Figure 8, it appears that the LCST obtained in the case of the pure poly(NIPMAM) particle (no IDA) is very close to that of the corresponding polymer in free solution6,7). In addition, Figure 8 shows that the transition temperature slightly increased from 42.1 to 43.7 with the functional monomer concentration. The increase in the LCST and the swelling capability of the poly(NIPMAM-IDA) microgel reveal the incorporation of charges coming from IDA into the microgel.

Conclusion

Monodisperse and functionalized poly(NIPMAM) latexes were successfully prepared by batch polymerization using N-isopropylmethacrylamide, N-methylenebisacrylamide as the cross-linker and N-vinylbenzylimino-diacetic acid as the functional monomer. It was shown that the functional monomer (IDA) played a determinant role on the particle formation during the polymerization step which is reflected by a decrease in particle size and a significant increase in the WSP. It was then found that IDA monomer slightly increases both the LCST and the swelling ratio of the particles. This change in the colloidal properties of the poly(NIPMAM-IDA) microgel reveals the presence of charges into the microgel originating from the functional monomer and proves that the incorporation of IDA is effective.

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