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Synthesis and Phase Behavior of Aqueous Poly(*N*-isopropylacrylamide-co-acrylamide-co-acrylic acid)

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Abstract

A thermo-responsive and biodegradable poly(N-isopropyl acrylamide-co-acrylic acid) (PNIPAM-AAc) and poly(N-isopropylacrylamide-co-acrylamide-co-acrylic acid) (PNIPAM-AAm-AAc), with various volume fractions of NIPAM γ, were synthesized by radical polymerization. The phase behavior of the polymers in water was investigated by means of optical transmittance and dynamic light scattering. The cloud point temperatures T_{cp} for PNIPAM-AAc were roughly constant irrespective of γ in the experimental range $0<1-\gamma<0.071$. The relationship between T_{cp} and $1-\gamma$ for PNIPAM-AAm-AAc with molar fraction of acrylic acid being 0.05 were observed on the same line as for PNIPAM-AAm; the reciprocal of T_{cp} decreased linearly with $1-\gamma$. Correspondingly, an increase of hydrodynamic radius of the copolymers around T_{cp} resulted from the aggregation of the globules of each copolymer was observed from dynamic light scattering.

Keywords: thermo-responsive polymers, N-isopropylacrylamide, random copolymer, albumin nanospheres, anti-cancer drug carriers

1. INTRODUCTION

The efficacy of chemotherapy is often limited by important side effects because the cancer fighting drugs are toxic to both tumor and normal cells. A strategy to overcome non-cellular and cellular based mechanisms of resistance and to increase selectivity of drugs towards cancer cells while reducing their toxicity towards normal tissues could be to associate anti-cancer drugs with colloidal nanoparticles [1]. At the tumor level, the accumulation mechanism of intravenously injected nanoparticles relies on a passive diffusion or convection across the leaky, hyperpermeable tumor vasculature [2]. However, the uptake of the nanoparticles can also result from a specific recognition in case of ligand decorated nanoparticles (active targeting) [3]. The following advantages of drug targeting are evident: (a) the drug administration protocols may be simplified; (b) the drug quantity required to achieve a therapeutic effect may be greatly reduced as well as the cost of therapy; (c) the drug concentration in the required sites can be sharply increased without negative effects on non-target compartments [4]. Active targeting nanoparticles are also called as intelligent nanoparticles, which have attracted many interests in biomedical applications due to their core-shell structures and properties [5]. The particle size, surface properties and environmental stimuli of nanoparticles can be manipulated through functional molecular devices and structure designs. To date, many distinctive intelligent nanoparticles, such as targeting agents-conjugated nanoparticles [6], pH- and temperature-responsive polymeric micelles [7-11], have been investigated in drug delivery applications.

In the preceding paper [12], we proposed an idea for the drug release using nanospheres containing anti-cancer drugs with the copolymer chains with the coil to globule transition around 40°C on the surface. In the paper we reported the synthesis, characterization and phase behavior of copolymers poly(N-isopropylacrylamide-co-acrylamide) (PNIPAM-AAm) in water with incorporation of different comonomers of AAm to NIPAM with a carboxylic group at the end, which plays the role of a stick on the nanospheres. It is, however, difficult to obtain high yield of nanospheres with the copolymers on the surface because the copolymers have only one functional carboxylic group at the end. In this paper, to enhance the yield, we synthesized PNIPAM-AAm-AAc, and studied the cloud point and aggregation behavior of PNIPAM-AAc and PNIPAM-AAm-AAc to examine the effect of AAc on the transition temperature. We expect that PNIPAM-AAm-AAc be a water-soluble polymer showing reversible hydration-dehydration changes in response to small solution temperature changes as PNIPAM [13].

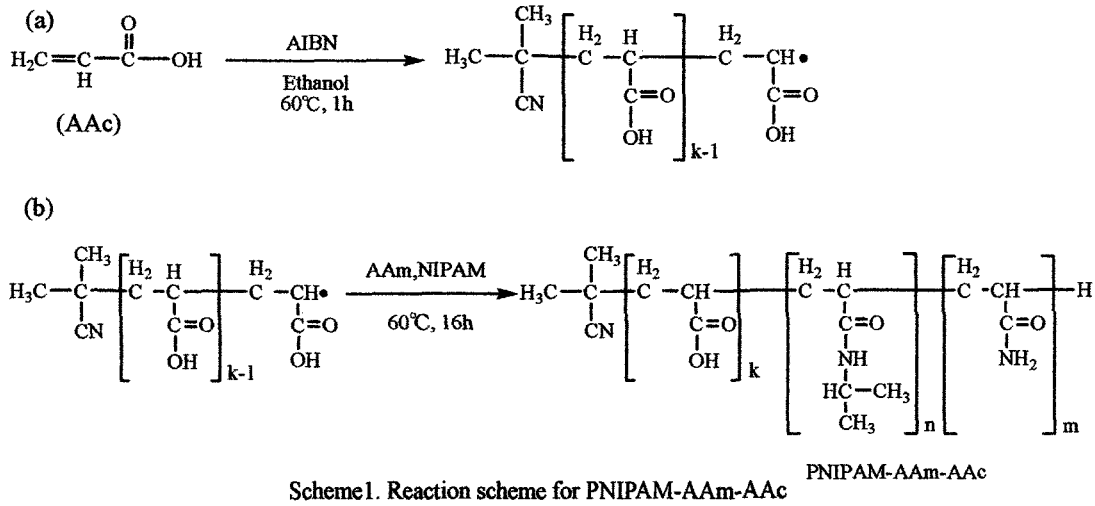
2. EXPERIMENTAL SECTION

2.1 Materials

N-isopropylacrylamide (NIPAM) and 2,2'-azobis(isobutyronitrile) (AIBN) were purchased from TCI (Tokyo, Japan). NIPAM was purified by recrystallization from *n*-hexane. Acrylamide (AAm) was purchased from SIGMA (USA). Acrylic acid (AAc) was purchased from Wako Pure Chemicals (Tokyo, Japan). Unless stated otherwise, all solvents were special grade chemicals, and used as received.

2.2 Synthesis of PNIPAM-AAc and PNIPAM-AAm-AAc

NIPAM with comonomer AAc were polymerized by free radical polymerization using AIBN as an initiator to obtain poly(*N*-isopropyl acrylamide-co-acrylic acid) (PNIPAM-AAc). Molar fraction x of NIPAM in the copolymers (for PNIPAM-AAm [12] and PNIPAM-AAc) was estimated from the molar fraction of NIPAM in the



Scheme1. Reaction scheme for PNIPAM-AAm-AAc

Table I Results of polymerization and T_{cp} in water for PNIPAM-AAc

Sample	x [a]	γ [b]	Yield (%) [c]	T_{cp} / °C
PNIPAM [d]	1.000	1.000	73	32.8
PNIPAM-AAc-1	0.953	0.974	86	33.7
PNIPAM-AAc-2	0.918	0.954	95	33
PNIPAM-AAc-3	0.875	0.929	92	32.9

[a] Molar fraction of NIPAM
[b] Volume fraction of NIPAM
[c] Diethylether insoluble fraction
[d] Reference 12

monomers approximately. x was chosen to be more than 0.85 for both copolymers. Volume fraction γ of NIPAM in each copolymer was evaluated from x and the densities of the pair of comonomers. 8.4 g of the mixed monomer and 0.05 g of AIBN were dissolved separately in 50 cm^3 of ethanol (total volume). Polymerizations were carried out at 60°C for 16 h under nitrogen atmosphere.

Poly(*N*-isopropylacrylamide-co-acrylamide-co-acrylic acid) (PNIPAM-AAm-AAc) were synthesized by two steps as shown in Scheme 1. Molar fraction x of NIPAM in PNIPAM-AAm-AAc was determined from the molar ratio of NIPAM to the sum of NIPAM and AAm. AAc was used for attaching carboxylic groups at the end of the copolymers which can be used to conjugate the polymers to the surface of nanospheres. Here, we note that we plan to use biodegradable albumin nanospheres with lots of amino groups on the surface. First, 1.45g of AAc, 0.25g of AIBN for PNIPAM-AAm-AAc-1,2 and 0.10g of AIBN for PNIPAM-AAm-AAc-3,4 and 100 cm^3 ethanol were added into a three-necked round-bottle flask with a magnetic stirrer. Molar fraction of AAc was 0.05 for these samples. The round-bottle flask was moved to an oil bath and stirred at 60°C for 1.0 h under nitrogen. Second, 42.0 g of mixed NIPAM and AAm with different NIPAM fractions were dissolved in 150 cm^3 of ethanol (heated to 40°C) and added into the reaction system. x was 0.919 for PNIPAM-AAm-AAc-1 and 0.893 for PNIPAM-AAm- AAc-2,3,4. The whole reaction mixture was stirred at 60°C for 16 h to obtain PNIPAM-AAm-AAc-1,2,3 and 24h to obtain PNIPAM-AAm-AAc-4 under nitrogen.

The resultant polymers designated as shown in Table I and II were separated and purified by reprecipitation into diethylether and then dried in vacuum. The polymers were dissolved in MilliQ water for optical transmittance and dynamic light scattering measurements.

2.3 Optical transmittance and dynamic light scattering

Optical transmittance (OT) of aqueous polymer solutions whose polymer concentration c was 5.00×10^{-3} g cm^{-3} was measured from lower to higher temperatures at 500 nm of wavelength with an optical spectrophotometer (Spectrum 721, SP-1105) with a temperature controller. A sample cell whose path length is 10 mm was used. Heating rate was 0.1°C/min. The cloud point T_{cp} of polymer solutions was determined as temperatures showing an optical transmittance of 50%.

Dynamic light-scattering (DLS) measurements were carried out at the scattering angle of 30° for aqueous PNIPAM-AAm-AAc-1 from lower to higher temperatures using a laboratory-made light scattering apparatus with a BI-9000AT correlator (Brookhaven) [14]. Vertically polarized incident light of 532 nm wavelength (a diode laser, BWT-50, B&W) was used. Polymer concentration was set to be 5.00×10^{-3} g cm^{-3} and the solutions were optically cleaned through a 0.45 μm membrane filter just before measurements. The solutions were equilibrated at given temperatures for 10 min before each measurement and then were heated to the next temperature for the next measurement. The DLS measurements were not made in the temperature range where the solutions were turbid because of phase separation. Hydrodynamic radius R_h was estimated from the obtained diffusion coefficient by using the Stokes-Einstein equation.

3.SURVEY OF THEORETICAL CONSIDERATION[12]

The interaction parameter χ of a solution composed of solvent and a two-species copolymer is expressed as

$$\chi=\frac{1}{2}\beta[\varepsilon_{00}-2\varepsilon_{10}\gamma-2\varepsilon_{20}(1-\gamma)+\varepsilon_{11}\gamma^2+2\varepsilon_{12}\gamma(1-\gamma)+\varepsilon_{22}(1-\gamma)^2] \quad (1)$$

Table II

Results of polymerization and cloud point temperature in water for PNIPAM-AAm-AAc

Sample	x	γ	AIBN (g)	Polymerization time (h)	Yield (%)	T_{cp} / °C
PNIPAM-AAm-AAc-1	0.919	0.958	0.25	17	74	39.5
PNIPAM-AAm-AAc-2	0.893	0.944	0.25	17	75	40.8
PNIPAM-AAm-AAc-3	0.893	0.944	0.10	17	91	40.4
PNIPAM-AAm-AAc-4	0.893	0.944	0.10	25	89	40.7

where $-\varepsilon_{00}/z$, $-\varepsilon_{10}/z$, $-\varepsilon_{20}/z$, $-\varepsilon_{11}/z$, $-\varepsilon_{12}/z$ and $-\varepsilon_{22}/z$ are the interaction energies of the nearest neighboring pairs of solvent-solvent, solvent-species 1 (NIPAM) segment of the copolymer, solvent-species 2, species1-species 1, species 1-species 2, and species 2-species 2, respectively, β is the "inverse temperature" given by $\beta = 1/(k_B T)$, k_B is the Boltzman factor; $1-\gamma$ is the volume fractions of species 2 in total copolymer and z is the coordination number of the lattice model.

Using the relationship $1-2\chi = 0$ at the coil to globule phase transition point for infinite molecular weight of polymer [15] and approximating $\varepsilon_{11} \cong \varepsilon_{12} \cong \varepsilon_{22} \cong \varepsilon_{pp}$, we have linear composition dependence of the transition inverse temperature β_c

$$\beta_c \cong \beta_0 + \beta_1(1-\gamma) \quad (2)$$

and

$$\beta_1 = \beta_0 \frac{\varepsilon_{10}(\beta_0) - \varepsilon_{20}(\beta_0)}{k_1 \beta_0 - \frac{1}{2\beta_0}} \quad (3)$$

where β_0 is the coil to globule transition inverse temperature for the polymer composed of only the species-1 segments, k_1 is an expansion coefficient of ε_{10} as a function of β around $\beta = \beta_* = 1/(k_B T_*)$, where T_* is room temperature;

$$k_1 \equiv d\varepsilon_{10}(\beta_*)/d\beta_* \quad (4)$$

Equation (3) indicates that the ratio β_1/β_0 (or T_0/T_1) is proportional to the interaction energy difference between the solvent-species 1 and the solvent-species 2.

4. RESULTS AND DISCUSSION

Table I and II summarize the results of polymerization. The yield determined as diethylether insoluble fraction for each polymer sample was between 64% and 93%.

Optical transmittance measured for PNIPAM, PNIPAM-AAc and PNIPAM-AAm-AAc aqueous solution (c = 5.00 × 10^{-3} g cm^{-3}) as a function of temperature is shown in Fig. 1. The optical transmittance of aqueous PNIPAM is almost unity at temperatures below 32°C. It decreases sharply with rising temperature between 32.7°C and 33.1 °C, and vanishes at higher temperatures. The similar transition behavior of optical transmittance was also found for PNIPAM-AAc and PNIPAM-AAm-AAc, as shown in Fig. 1. The transition temperature is roughly constant for different 1-γ for PNIPAM-AAc samples in the experimental range $0<1-\gamma<0.071$, but rises with increasing 1-γ for

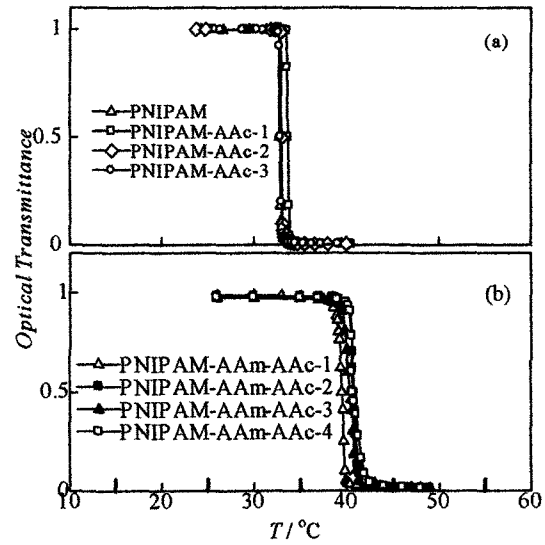


Fig.1. Temperature dependence of optical transmittance of indicated copolymers in water (c = 5.00 × 10^{-3} g cm^{-3}) for (a) PNIPAM and PNIPAM-AAc, (b) PNIPAM-AAm-AAc.

PNIPAM-AAm [12] and PNIPAM-AAm-AAc (molar fraction of AAc is 0.05) samples, as shown in Fig. 2. The cloud point of PNIPAM-AAm and that of PNIPAM-AAm-AAc are very close at the same γ. The sharpness of the transition was estimated from the temperature distance ΔT between 5% transmittance and 95% transmittance: ΔT was 0.4 K for PNIPAM, 0.4, 0.3 and 0.4 K for PNIPAM-AAc-1 to 3 and 1.3, 1.9, 2.2 and 2.1K for PNIPAM-AAm-AAc-1 to 4. Therefore, the temperature dependence of optical transmittance for PNIPAM-AAm-AAc is gentler than those of PNIPAM and PNIPAM-AAc. Comparing with PNIPAM-AAm-AAc-2, the amount of initiator AIBN is low and the reaction time is high for PNIPAM-AAm-AAc-3 and PNIPAM-AAm-AAc-4, meaning higher molecular weights. Nevertheless, the cloud point temperatures for PNIPAM-AAm-AAc-2, PNIPAM-AAm-AAc-3 and PNIPAM-AAm-AAc-4 are quite close to each other, as shown in Table II and Fig.1. This result illuminates that the cloud point temperature of PNINAM-AAm-AAc aqueous solutions is not highly dependent of molecular weight.

The cloud point temperatures T_{cp} are summarized at the fifth column of Table I and the seventh column of Table II and Fig. 2.

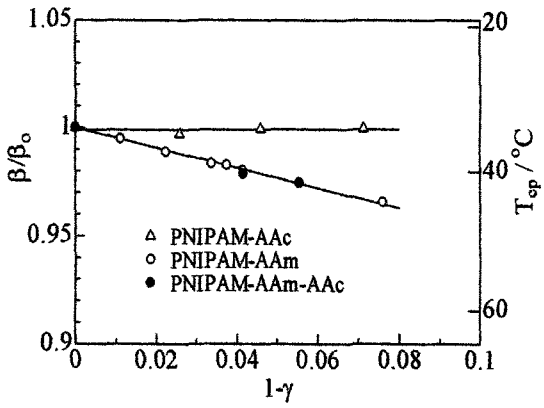


Fig.2. Reduced reciprocal cloud point temperature β/β_0 as a function of $1-\gamma$ for PNIPAM-AAc (empty triangles), PNIPAM-AAm (empty circles) and PNIPAM-AAm-AAc (filled circles) in water.

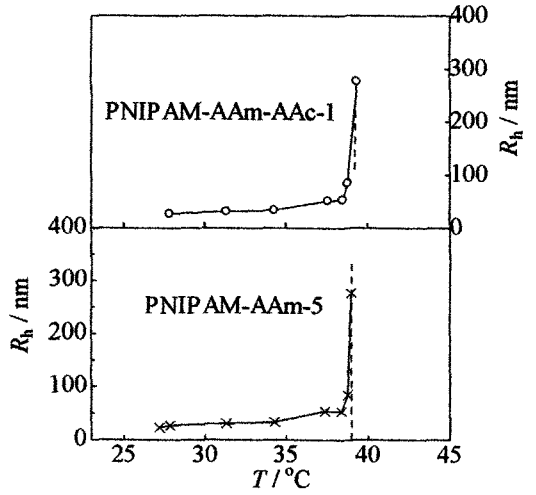


Fig.3. Temperature dependence of R_h for PNIPAM-AAm at γ= 0.958 and PNIPAM-AAm-AAc-1 in water (c = 5.00 × 10^{-3} g cm^{-3}). Dashed lines indicate the cloud point for each sample.

The temperature dependence of the hydrodynamic radius R_h of PNIPAM-AAm-AAc-1 and the corresponding copolymer PNIPAM-AAm-5 with the same volume fraction of NIPAM γ= 0.958 but without AAc groups is shown in Fig. 3, where the dashed lines indicate the cloud points given in Table I and II, respectively. R_h is almost independent of temperature at lower temperatures, and sharply increases near T_{cp}. The transition temperatures for PNIPAM-AAm-AAc-1 and PNIPAM-AAm-5 are very close to each other.

The copolymers appropriate for the current purpose need to change from hydrophilic type to hydrophobilic type around 40°C, and the change is very sensitive to temperature change. Because the transition temperature should coincide with the cloud point T_{cp}, these conditions can be satisfied for PNIPAM-AAm-AAc, as shown in Figs. 1-3. From the experimental results for the copolymers of PNIPAM-AAm-AAc, T_{cp} determined by optical transmittance is almost the same as the temperature where the aggregation of the copolymers occurs, probably due to the conformational change from coil state to globule state. The reduced temperature β/β_0 is linearly proportional to $1-\gamma$ as expected by the theoretical consideration, and the proportional coefficient is -0.4525. The optimum γ for this scheme can be obtained from Fig.2.

5. CONCLUSIONS

In this paper, we synthesized PNIPAM-AAc and PNIPAM-AAm-AAc with various volume fractions of NIPAM γ and studied cloud point and aggregation behavior using optical transmittance and DLS measurements. The reduced reciprocal cloud point temperature of PNIPAM-AAm and PNIPAM-AAm-AAc decreased linearly against $1-\gamma$ and that of PNIPAM-AAc was almost constant irrespective of $1-\gamma$. The temperature sensitivity of both optical transmittance and R_h at the transition temperature for the copolymers was fairly well. It is expected that PNIPAM-AAm-AAc can be used as the copolymer reacting on nanospheres and the yield of nanospheres with the copolymers on the surface be enhanced because of more carboxylic groups at the end.

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