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Amphiphilic Hexylamine Modified Polysuccinimide: Synthesis, Characterization, and Formation of Nanoparticles in Aqueous Medium

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ABSTRACT

A series of hexylamine modified polysuccinimide (PSI–HA) copolymers were synthesized by aminolysis of polysuccinimide (PSI) with hexylamine. FTIR and ^{1}H NMR measurements confirmed the structure of the copolymers and the substitution degree of the N-hexyl aspartamide units ranged from 7 to 92 mol%. Stable nanoparticles formed when the DMF solution of PSI–HA copolymers was dropped into excess water, and the particle size reduced as increasing the substitution degree. ^{1}H NMR analysis indicated that hexyl chain and succinimide units interacted to form the hydrophobic core, while, the nanoparticles were stabilized by the amide groups which formed hydrogen bonds with the surrounding water molecules. The nanoparticles became more compact at higher temperature due to the break of hydrogen bonds between amide groups and water molecules. Dynamic light scattering (DLS) and scanning electron microscopy (SEM) results showed that the nanoparticles were nearly spherical. Larger nanoparticles formed when the dispersion concentration increased from 0.1% to 1.0% according to the DLS and steady-state fluorescence measurements. After the nanoparticles formed in water, a sequential dilution can't influence the particles size of the nanoparticles any longer.

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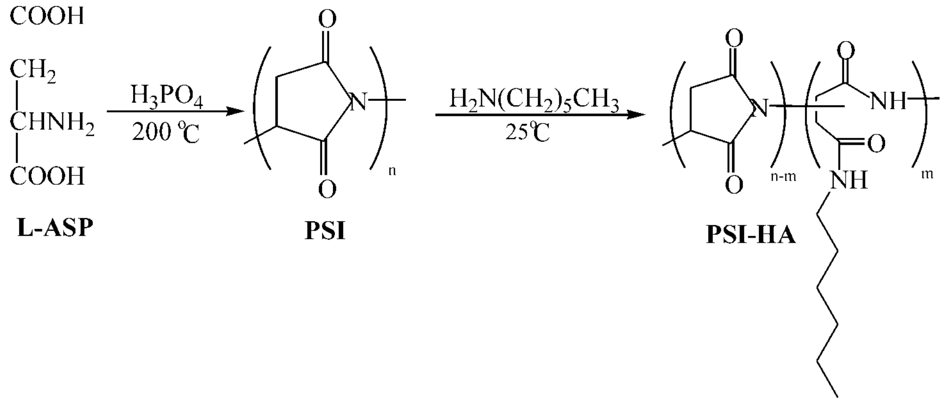
Key Words: Amphiphilic copolymers; Polysuccinimide; Hexylamine; Nanoparticles; Biodegradable.

INTRODUCTION

More attention has been paid during the last decades to the amphiphilic polymers containing both hydrophilic and hydrophobic units because of the biological importance and pharmaceutical applications. The extensively studied amphiphilic copolymers are the AB diblock copolymers or the ABA triblock copolymer,[1–4] because their association behaviors are easy to control, and the results are not very hard to explain. But for the random structure amphiphilic copolymers, some challenging problems will not be avoided, the association behaviors becomes more complicated,[5–7] and the influence parameter is hard to control. Naturally, this kind of copolymer also possesses more helpful properties, such as easy preparation, low price, especially for biodegradable amphiphilic copolymers.[8–10] As a representative, polysuccinimide attracts more attention because its derivatives, such as poly(aspartic acid) (PASP) and poly(hydroxyethyl aspartimide) (PHEA), are biodegradable,[11–13] and the backbone was easily modified to form amphiphilic copolymers. Recently, hydrophobic chain modified PASP with octadecyl chain and dodecyl chain was reported by Kim *et al.* and Nakato *et al.*,[14,15] respectively. They first modified the PASP by long chain alkylamine and then hydrolyzed the residual succinimide units to give the aspartic acid units, and studied the self-aggregation behaviors of the two amphiphilic random copolymers. The experimental results confirmed that the long chain alkyl units were hydrophobic and interacted to form intermolecular and intramolecular hydrophobic microdomains, and the hydrophilic main chain to stabilize the nanoparticles in water at the same time.[16,17]

In this paper, we prepared a new kind of random structure amphiphilic biodegradable copolymers, the main chain is hydrophobic and the side chain is partial hydrophilic (Sch. 1). These copolymers can form stable nanoparticles in aqueous medium when the DMF solution of PSI–HA copolymers dropped into excess water. The structure of the



Scheme 1.

nanoparticles was investigated by ^{1}HNMR and the morphology of the nanoparticles was determined by the DLS and SEM.

EXPERIMENTAL

Materials

L-aspartic acid was purchased from Shanghai Biochemical Reagent Company, hexylamine (≥98%) was purchased from Fluka and were used as received, N,N-dimethylformamide(DMF) was distilled and dried via 4 Å sieve before use, phosphoric acid (85%) and other reagents were used without further purification.

Synthesis of Polysuccinimide (PSI)

L-aspartic acid (30.0 g, 0.22 mol) and phosphoric acid (3.3 g, 29 mmol) were charged into a three-neck round bottom flask, and stirred under reduced pressure at 200°C for 2 h. Then the reaction mixture was cooled and dissolved in DMF. The solution precipitation in excess water and the precipitate was washed with water to remove the phosphoric acid. The final product was dried at 80°C under vacuum.

Synthesis of Hexylamine Modified Polysuccinimide (PSI–HA)

A typical procedure for the preparation of PSI–HA is as follows: to a solution of 2.0 g polysuccinimide in DMF (10 mL), 1.34 mL hexylamine was added at 0°C for 10 min, then the reaction flask was moved to a water bath at 25°C and stirring for another 8 h, the solution was precipitated in a 10-folded hexane/acetone (50%V/50%V) mixture. The precipitate was washed with hexane/acetone for several times and dried at 25°C under vacuum.

Preparation of PSI–HA Nanoparticles

Different DMF solutions of PSI–HA were prepared at first, then dropped them into an excessive amount of deionized water via rigorous stirring, respectively. The final solid content of PSI–HA in the dispersion was in the range of 0.1% to 1%. The solution was dialyzed over one week to remove the DMF and purified by passing through a 0.45 μm filter before carrying out further characterization.

Measurements

GPC measurements were carried out using a HP series 1100 Chromatograph equipped with Zorbax columns and RI/UV dual-mode detectors. The elution rate of 0.1 M $NaNO_3$ is

0.5 mL/min and standard PEG was used as calibration. FTIR spectra were obtained using Magna 550 spectrophotometer. A Philips DMX500 Spectrometer was used for ^{1}H-NMR analysis with DMSO-d_6 as the solvent. SEM photographs were obtained using Philips XL30 Scanning Electron Microscope. Steady-State Fluorescence spectra were recorded on a Cary Eclipse EL00063372 spectrophotometer using quartz cell. Excitation wavelength was at 330 nm and the width of both excitation and emission slits was 5.0 nm. Dynamic Light Scattering (DLS) measurements were performed in Malvern Autosizer 4700 spectrometer, and the laser wavelength (λ) applied in measurements was 514.5 nm.

RESULTS AND DISCUSSION

In order to obtain high molecular weight polysuccinimide with regular structures, an acid-catalyzed polycondensation method was used in our experiments. The ^{1}H NMR spectrum of polysuccinimide was showed in Fig. 1(a). The signals at 5.0–5.4 ppm were assigned to the methine proton (b) and at 2.5–3.3 ppm were assigned to methylene proton (a)

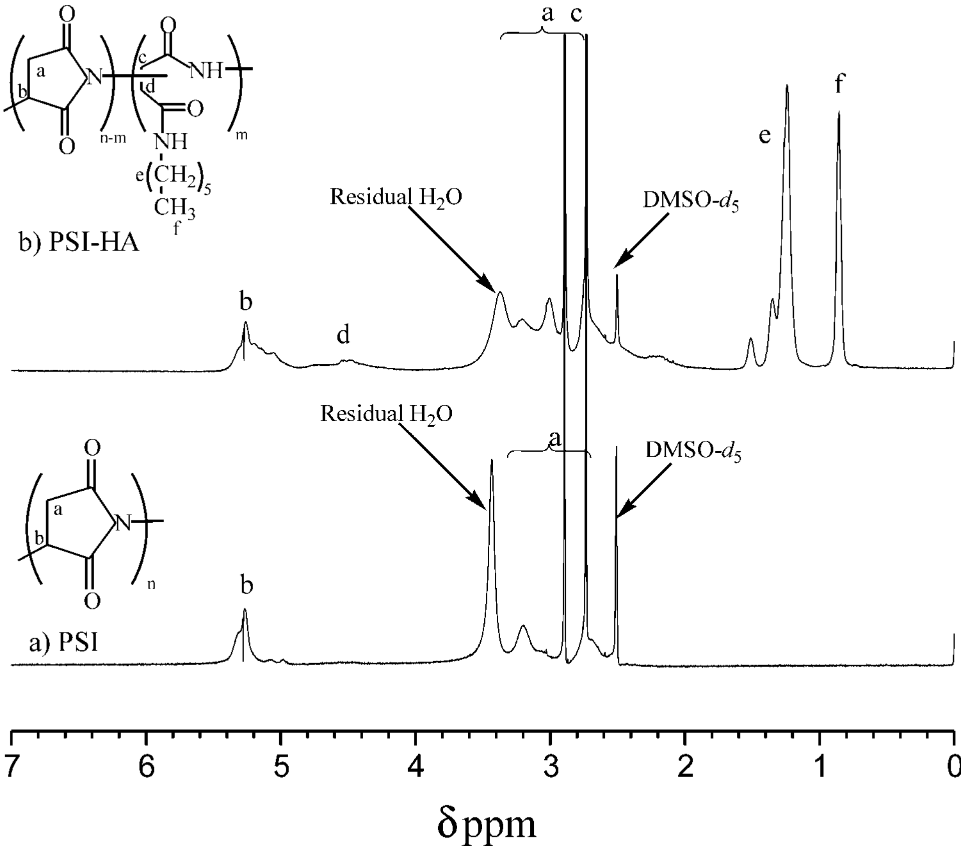


Figure 1. ^{1}H NMR spectra of a) PSI and b) PSI–HA-1 (DS = 7%) in DMSO-d_6.

the of the repeating succinimide unit, respectively.[21] In order to measure the molecular weight of PSI, the water soluble derivative poly (hydroxyethyl aspartamide) (PHEA) was prepared according to the procedure reported elsewhere.[22] The GPC measurement was carried out in 0.1 M $NaNO_3$ solution and indicated the molecular weight of PHEA, $M_w = 10,200$, therefore the molecular weight of PSI was calculated to be $M_w = 6,700$.

Because the ring structure of the succinimide unit was easily opened by an amiable reaction of PSI with hexylamine, a series of hexylamine modified polysuccinimides with different ring opening degrees were prepared by this aminolysis reaction. FTIR spectra of PSI–HA showed that new strong bands at 1649 cm^{-1} (amide I), 1545 cm^{-1} (amide II) and 3305 cm^{-1} (–NH–) appeared after aminolysis. ^{1}HNMR spectrum of PSI–HA in Fig. 1(b) showed that new signals from N-hexyl aspartimide units were observed at 0.84 ppm for methyl protons, 1.22, 1.36, and 1.50 ppm for methylene protons of hexyl chain. In addition, the ring opening of succinimide units resulted in the appearance of a new signal at 4.49 ppm assigned to the methine proton of the N-hexyl aspartamide units.[15] The ^{1}H NMR analysis results indicated that the aminolysis of PSI successfully, and PSI–HA was prepared. From ^{1}H NMR results, we can calculate that the mole ratio of the succinimide units to hexyl groups were from 7% to 92% (Fig. 2, Table 1).

In general, alkyl groups such as octadecyl and dodecyl chains were introduced into the water soluble poly(aspartic acid) as hydrophobic segments,[14,15] then the copolymer possesses amphiphilic properties. But in our system, we chose a short alkyl chain to the hydrophobic main chain, because the existence of a hydrophilic group of amide, the copolymer will also possess amphiphilic properties, the experimental results proved our idea. When the DMF solution of PSI–HA copolymers was dropped into excess water, however, no precipitates were observed and the SEM photograph in Fig. 3 showed that

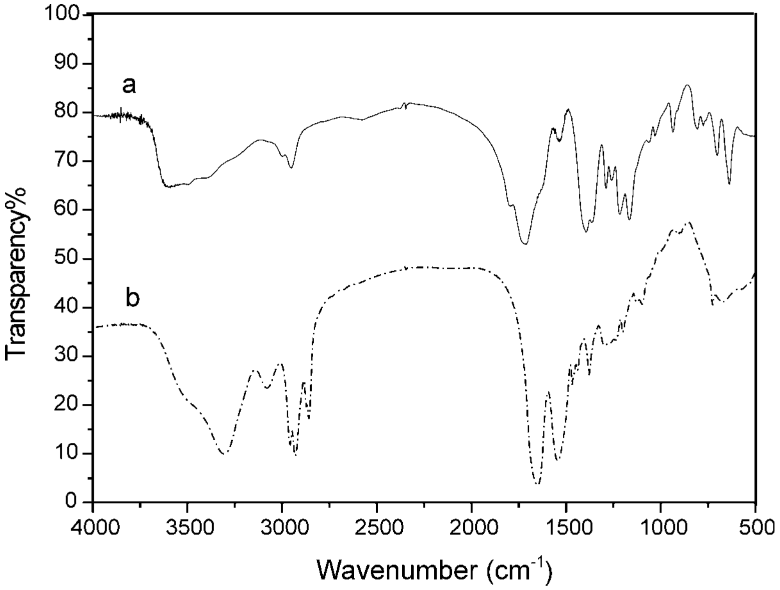


Figure 2. FTIR spectra of (a) PSI and (b) PSI–HA-6 (DS = 91%) by KBr pellets.

Table 1. The data of degree of substitution and hydrodynamic diameter in water of PSI–HA copolymers.

Sample	Degree of substitution[a]	Mole ratio (SI unit/HA-modified unit)	Hydrodynamic diameter (nm)[b]	Polydispersity
PSI–HA-1	7%	93/7	153.6	1.00
PSI–HA-2	10%	90/10	130.5	0.93
PSI–HA-3	28%	72/28	81.4	0.75
PSI–HA-4	62%	38/62	38.3	0.29
PSI–HA-5	81%	19/81	29.6	0.32
PSI–HA-6	92%	8/92	26.4	0.24

[a] Determined by ^{1}HNMR spectra.
[b] Measurements under the concentration of 0.25%.

these copolymers aggregated to form particles with nanoscale size. Table 1 gives the DLS results of the nanoparticles formed by PSI–HA copolymers with different substitution degrees. With the increase of the substitution, the hydrodynamic diameter of the nanoparticles reduced gradually. The DLS measurements also showed that the distributions of the hydrodynamic diameter of nanoparticles were not fairly narrow and decreased as the increase of the content of *N*-hexyl aspartimide units, all these results hint that the *N*-hexyl aspartimide units possess hydrophilic property.

Since the hexyl chain is shorter than the dodecyl and octacyl group, the amide group of *N*-hexyl aspartimide units in PSI–HA copolymers may show stronger hydrophilic property, the hydrogen bond formed between water and amide group may further improve its hydrophilic property. Therefore, when this copolymer solution is dropped into water, the hexyl chain and

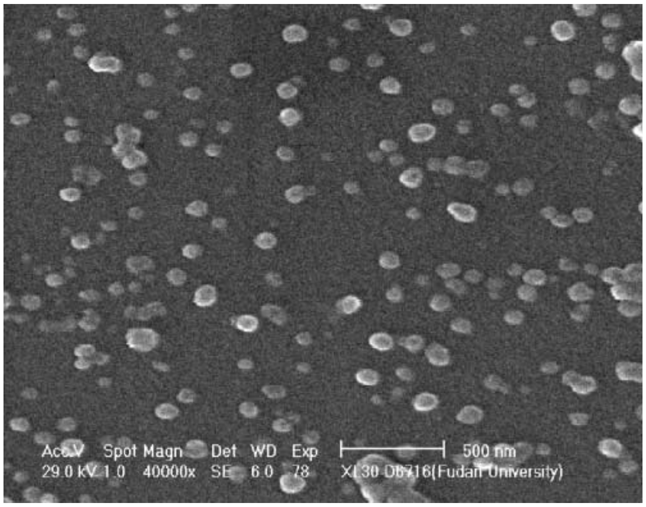


Figure 3. SEM photograph of nanoparticles formed by dropping DMF solution of PSI–HA-1 copolymers (DS = 7%) into excess water (concentration: 0.75%).

succinimide units may form hydrophobic microdomains and the amide groups tend to stay at the interface to stabilize the nanoparticles, ^{1}H NMR spectrum of PSI–HA dispersion in Fig. 4 shows supporting evidence for this explanation. In Fig. 4, we can see that the methine proton signal of the succinimide unit at 5.0–5.4 ppm reduced significantly when PSI–HA copolymers dispersed in D_2O, and the signals of methyl and methylene protons of hexyl groups turn to weak and broad (at 0.8 ppm and 1.2–1.5 ppm), respectively, all these indicated that the location of the succinimide units and the hexyl groups were rich in the hydrophobic microdomains. Figure 5 shows the dependence of hydrodynamic diameter of PSI–HA-6 nanoparticles with temperature. With the temperature rises from 25°C to 50°C, the hydrodynamic diameter of the nanoparticles decreases gradually. This result hints that the existence of hydrogen bonding between the amide groups and water, when the temperature increases, the hydrogen bonds will break up gradually and the thickness of the highly organized water layer around the polymer chains will reduce as a result.[18] According to the unfavorable change of the thermodynamic condition, nanoparticles begin to self-compress to reduce the surface area and result in more compact nanoparticles. No macroscopic phase separation or secondary aggregation of the nanoparticles was observed in our research, which indicated that the nanoparticles have enough stability to disperse individually at least in the temperature region of the current study.

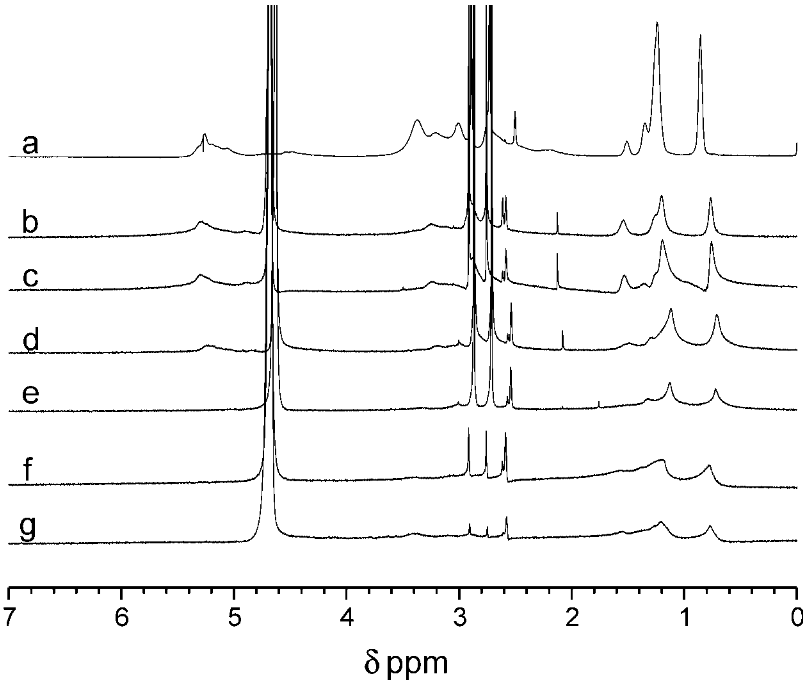


Figure 4. ^{1}H NMR spectra of a) PSI–HA-1 (DS% = 7%) in DMSO-d_6; b) PSI–HA-1 D_2O dispersion, the preparation method is to drop the DMSO-d_6 solution of PSI–HA into D_2O, (DMSO-d_6: D_2O = 1 : 10); c) PSI–HA-1 (DS% = 10%) in DMSO-d_6; d) PSI–HA-1 (DS% = 28%) in DMSO-d_6; e) PSI–HA-1 (DS% = 62%) in DMSO-d_6; f) PSI–HA-1 (DS% = 81%) in DMSO-d_6; g) PSI–HA-1 (DS% = 92%) in DMSO-d_6.

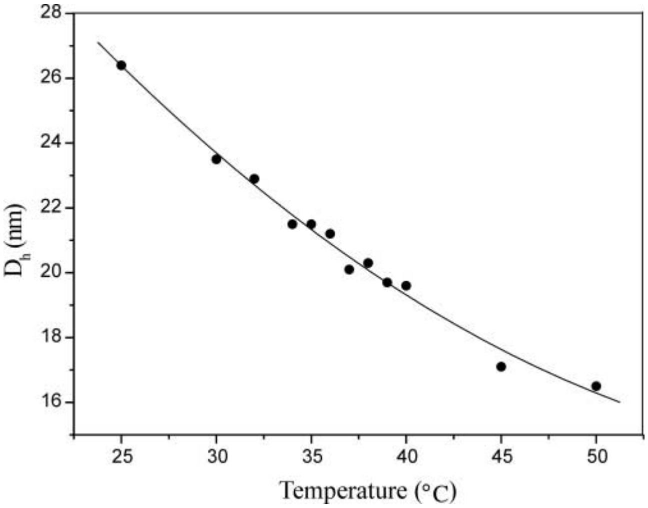


Figure 5. The influence of temperature on hydrodynamic diameter of the particles of PSI–HA-6 (DS = 92%) (Detected angle: 90°).

In order to investigate the shape of the nanoparticles in dispersion, angle-trace DLS measurements were performed at 45°, 60°, 90°, 120° and 145°. In colloidal or dispersion systems, diffusion coefficient D_T is related to the average line $\bar{\Gamma}$ which are based on the following equation:

$$\bar{\Gamma} = q^2 D_T \tag{1}$$

where q is the scattering vector. In dilute solution, the concentration dependence of the D_T can be expressed as:

$$D_T = D_0(1 + k_d C) \tag{2}$$

where D_0 is the D_T at infinite dilution, k_d is the diffusion second virial coefficient, C is the concentration. Corresponding hydrodynamic diameter D_h can be calculated with the Stokes–Einstein equation:

$$D_h = k_b T / 3\pi\eta D_0 \tag{3}$$

where k_b is the Boltzmann constant, T is the temperature, and η is the solvent viscosity.

For a spherical particle, diffusion coefficient and hydrodynamic diameter should be independent of the square of the scattering vector due to the undetectable rotational motion.[23] Figure 6shows the q^2 dependence of D_T and D_h of the nanoparticles. From this figure, we can't find the detection angle influence on the measurement results of D_T and D_h, these suggest that the nanoparticles are spherical, because of the negligible angular dependence of D_h, the following DLS measurements were performed at 90°.

Dropping of different initial concentrations of PSI–HA copolymer DMF solution in water, a series of dispersions were prepared, the dispersion concentration was from 0.1% to 1%. Figure 7 summarized the hydrodynamic diameter results, from this figure, we can

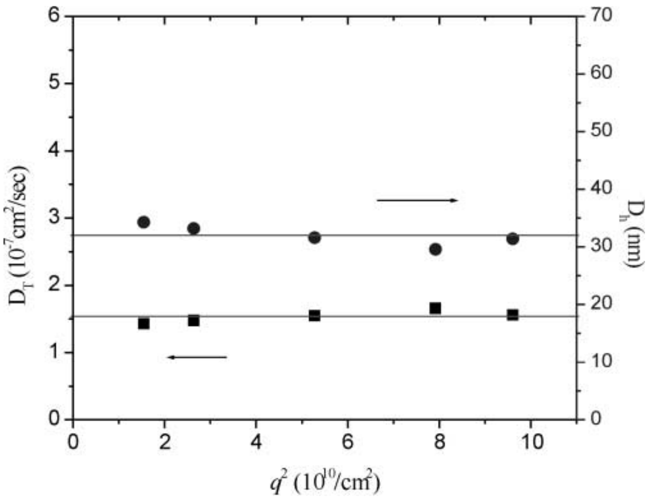


Figure 6. Diffusion coefficient (D_T) and hydrodynamic diameter (D_h) of the PSI–HA-5 (DS = 62%) particles as a function of the square of the scattering vector (q^2) (concentration: 0.25%, temperature: 25°C).

see that the particle size increases from 20.5 nm to 110.3 nm as increasing of the concentration. Steady-state fluorescence spectra of pyrene probe was used to explore the information about the nanoparticles. The intensity ratio of I_3/I_1 is sensitive to the polarity of the microenvironment where the pyrene exists: the larger the I_3/I_1, the less polar the microenvironment. Figure 8 shows the I_3/I_1 ratio increased with the concentration of the PSI–HA copolymers increased, which indicated that the polarity in the nanoparticles

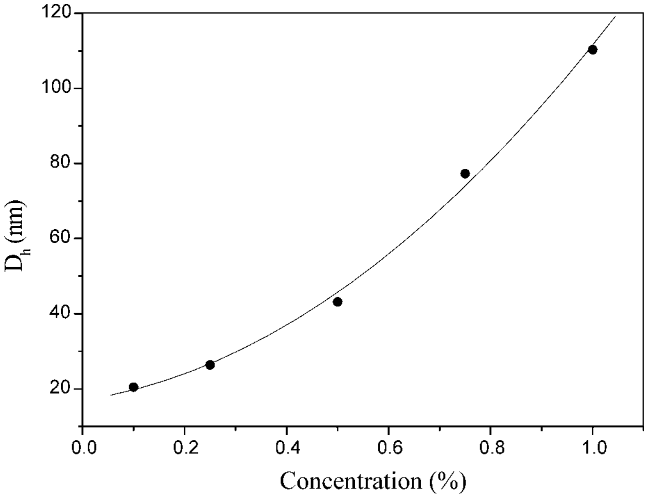


Figure 7. Hydrodynamic diameter of micelles of PSI–HA-6 (DS = 92%) as a function of the concentration (detected angle: 90°, temperature: 25°C).

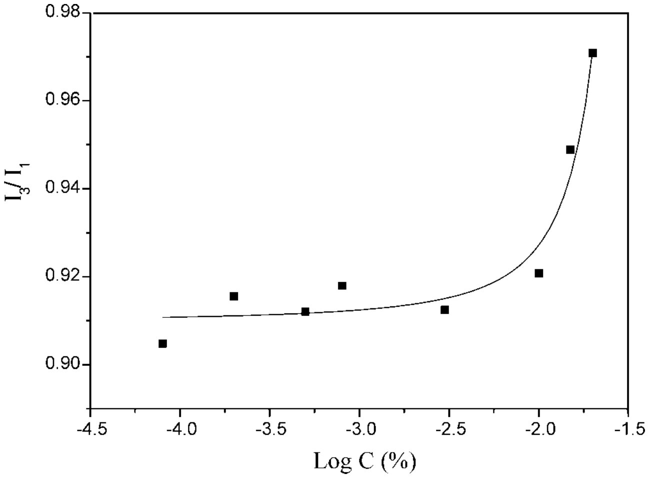


Figure 8. I_3/I_1 ratio in steady-state spectra of molecular pyrene probes ([pyrene] = 3.04×10^{-7} M) in the aqueous PSI–HA-4 (DS = 62%) dispersions as a function of the concentration of the copolymers.

decrease as the PSI–HA concentration increased. At a higher initial concentration, hydrophobic hexyl groups and succinimide units of PSI–HA copolymers have more chance to interact with each other to form larger particles, and the polarity of the big particle core will decrease.

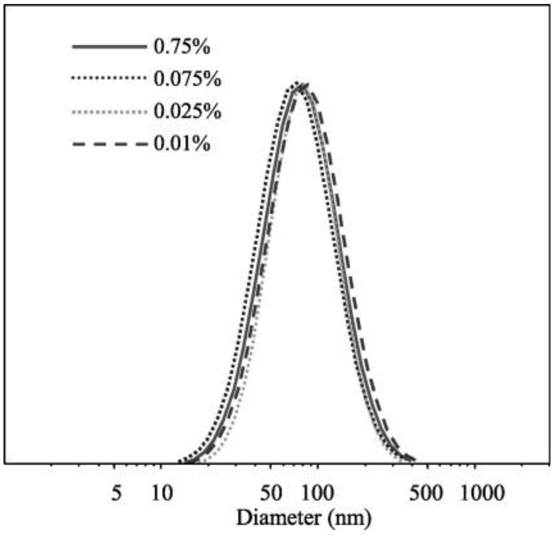


Figure 9. Hydrodynamic diameter curves of the PSI–HA-6 (DS = 92%) nanoparticles after a sequential dilution.

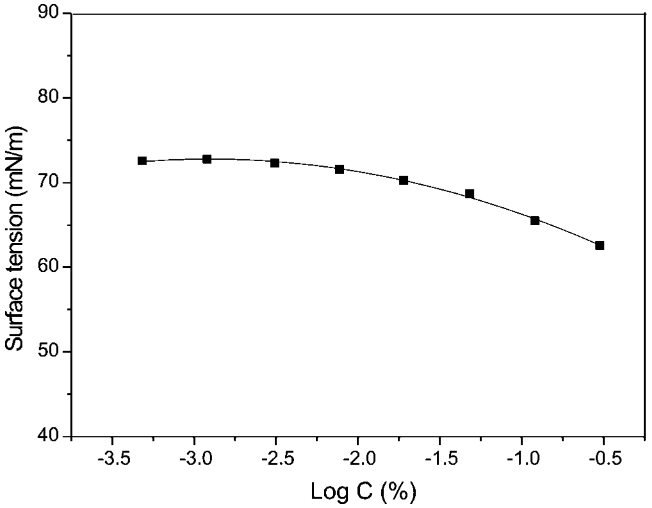


Figure 10. Surface tension of PSI–HA-4 (DS = 62%) as a function of dispersion concentration by sequential dilution.

In the Experimental section, we also find an interesting phenomenon, i.e., once the nanoparticles formed, a sequential dilution of the dispersion will not influence on the hydrodynamic diameter. Figure 9 shows the data of the hydrodynamic diameter after a sequential dilution, the hydrodynamic diameter almost kept constant even at the low concentration of 0.01%. This result indicated the nanoparticles are fixed once formed, the hydrophobic interaction between individual copolymer chains aggregated in the nanoparticles will be quite strong, otherwise the copolymer chains will disaggregate and escape from the nanoparticles and the hydrodynamic diameter of the nanoparticles will be changed as the dispersion diluted. A typical surface tension curve as a function of concentration of the PSI–HA aqueous dispersion is shown in Fig. 10. There is no critical dependence of surface tension on the concentration, which suggests that the PSI–HA copolymers prefer to aggregate in the nanoparticles rather than exist at the interface of air/water to lower surface tension. This property of the nanoparticles will be very useful for drug delivery system, because the nanoparticles can keep its shape perfectly even in a very dilute solution.

Nanoscale, biodegradable and dilute stable, this PSI–HA copolymer nanoparticles have great potential application in the drug delivery system. Investigation of applying this material into biodegradable drug deliver system is now under way in our laboratory.

CONCLUSION

Hexylamine modified polysuccinimide (PSI–HA) copolymers were synthesized by aminolysis of PSI with hexylamine. Due to the hydrogen bonds between amide groups and water molecules, PSI–HA copolymers exhibited good amphiphilic properties and formed stable nanoparticles by adding the DMF solution of these copolymers into excessive water via a dropwise fashion. Hydrodynamic diameter of the nanoparticles reduced with the temperature decreased due to the temperature dependence of the hydrogen bonds. The hydrodynamic diameters of the nanoparticles were strongly influenced by the substitution degree of the PSI–HA copolymers. The nanoparticles were nearly spherical according to the morphology investigation. The particle size of the nanoparticles depended on the initial concentration of the PSI–HA DMF solution. After the nanoparticles formed, a sequential dilution affect the stability and particle size of the nanoparticles.

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