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Aggregation of biodegradable amphiphilic poly(succinimide-*co*-*N*-propyl aspartamide) and poly(*N*-dodecyl aspartamide-*co*-*N*-propyl aspartamide) in aqueous medium and its preliminary drug-released properties

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Abstract

Two series of biodegradable amphiphilic copolymers, poly(succinimide-*co*-*N*-propyl aspartamide) (PSI-PA) and poly(*N*-dodecyl aspartamide-*co*-*N*-propyl aspartamide) (PDDA-PA) were synthesized by partial and total aminolysis of polysuccinimide (PSI), respectively. PSI-PA copolymers could self-aggregate in water directly under ultrasonication at room temperature. Differing from PSI-PA copolymers, the aggregates of PDDA-PA need to add PDDA-PA DMF solution into an excessive amount of water. The aggregative properties of PSI-PA and PDDA-PA copolymers have been investigated by dynamic light scattering (DLS) and surface tension measurements. Hydrophilicity of these two copolymers was attributed to the *N*-propyl aspartamide segments. Due to the stiff structure, succinimide segments preferred to form irregular hydrophobic microdomains, and some aggregates of PSI-PA are bimodal size distribution in water medium, while the more flexible PDDA-PA copolymer chains preferred to form monodispersed spherical aggregates. Elevated temperature could reduce the aggregate size of both PSI-PA and PDDA-PA copolymers due to the breaking of the hydrogen bonding and the releasing of the bonded water molecules. PSI-PA copolymers were surface active, while the surface tension of PDDA-PA copolymers was independent on concentration. The drug-loaded aggregates of PSI-PA also have been prepared and the preliminary release properties have been studied in vitro.

Keywords: Biodegradable amphiphilic copolymers; Micelle-like aggregates; Poly(succinimide-*co*-*N*-propyl aspartamide)

1. Introduction

In the past decades, the aggregative behaviors of amphiphilic polymers in aqueous medium have attracted considerable attention for both academic research and industrial application. Variety of amphiphilic polymers have been synthesized and the aggregative properties were studied, such as block polymers prepared by living/controlled polymerization from hydrophilic and hydrophobic monomers sequentially [1–4], random copolymers prepared by copolymerization of hydrophilic monomers with hydrophobic monomers [5], grafted copolymers prepared by hydrophobic modification of water-soluble polymers or hydrophilic treatment of hydrophobic polymers [6–8]. Although the amphiphilic block copolymers have clear molecular structure and well-controlled aggregative behavior, amphiphilic statistical copolymers still attracted much attention due to the convenient synthesis route, especially for full biodegradable copolymers [9–11]. Due to the full biodegradable properties and its potential application in biological and pharmaceutical area, polysuccinimide and its derivatives, such as poly(aspartic acid) (PASP) and poly(hydroxyethyl aspartamide) (PHEA), attracted much attention [12,13]. Kataoka and co-workers prepared PEO-PASP block copolymers, and through the modification of the PASP segment by anticancer drug adriamycin, they obtained an amphiphilic block copolymers, which could form nanoparticles in aqueous medium and were applied in drug delivery system [14]. Now, many groups [15–18] reported the hydrophobic modification of PASP by grafting

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hydrophobic octadecyl chains and dodecyl chains onto the backbone of PSI polymers with aminolysis procedure. Through the investigation of self-aggregation behaviors of these amphiphilic random copolymers, they suggested that the long chain alkyl units could interact each other to form intermolecular and intramolecular hydrophobic microdomains in water. In our lab, the aggregative properties of poly(succinimide-*co*-*N*-hexyl aspartamide) (PSI-HA) copolymers, which were prepared by adding the DMF solution of PSI-HA copolymers into an excessive amount of water, have been studied extensively [19].

In this paper, we want to elucidate the aggregative behaviors of two new types of PSI derivatives, PSI-PA and PDDA-PA. Different methods were used to prepare these micelle-like aggregates based on their amphiphilicity. The stability and size distribution of the aggregates have been investigated in detail, and the drug-loaded aggregates of PSI-PA also have been studied.

2. Experimental section

2.1. Chemicals

L-aspartic acid was purchased from Shanghai Biochemical Reagent Company. Propylamine ($\geq$98%) and dodecylamine ($\geq$98%) was purchased from Fluka and were used as received. *N*,*N*-dimethylformamide (DMF) was distilled and dried via a 4 Å molecular sieve before use. Phosphoric acid (85%) and other reagents were used without further purification.

2.2. Synthesis procedure

2.2.1. Synthesis of PSI

L-aspartic acid (30.0 g, 0.22 mol) and phosphoric acid (3.3 g, 29 mmol) were charged into a round-bottom flask and stirred under reduced pressure at 200 °C for 2 h. Then the reaction mixture was cooled and DMF was added to dissolve this product. Then the solution was precipitated in excessive water and the precipitate was washed with water to remove the residue phosphoric acid. The final product was dried at 80 °C under vacuum. ^{1}HNMR (DMSO-d_6) analysis confirmed the PSI structure (Scheme 1): δ=2.68 and 3.20 ppm (a) (–CH_2–, s); δ=5.28 ppm (b) (–C*H*–, t). Molecular weight of the PSI polymer was obtained from its water soluble derivative poly(hydroxyethyl aspartamide) (PHEA) prepared by the procedure reported elsewhere [20]. The GPC measurement indicated the molecular weight of PHEA $M_w=1.02\times10^4$ and the molecular weight of the original PSI was calculated to be $M_w=6.7\times10^3$.

2.2.2. Synthesis of PSI-PA

PSI (2 g) was dissolved in 10 mL DMF in a round-bottom flask, then appropriate amount of propylamine was slowly added at 0 °C, after that, the reaction flask was moved to a water bath at 25 °C. After stirring for 4 h, the solution was precipitated in 10-fold hexane/acetone (1/1, v/v) mixture. The precipitate of PSI-PA was washed with hexane/acetone mixture for three times and dried at 25 °C in vacuo. ^{1}HNMR (DMSO-d_6) of PSI-PA: δ=0.84 ppm (g) (–CH_3, t); δ=1.22 ppm (f) (–CH_2–CH$_3$,s); δ =1.50 ppm (e) (–NH–CH_2–,s); δ=2.68 and 3.20 ppm (a and c) (–CH_2–, s); δ=4.49 ppm (d) (–C*H*–, t); δ=5.28 ppm (b) (–C*H*–, t) (See Scheme 1).

2.2.3. Synthesis of PDDA-PA

PSI (2 g) was dissolved in 10 mL DMF, the appropriate amount of dodecylamine was slowly added at 0 °C, then the reaction flask was moved to a oil bath at 70 °C. After stirring for 8 h, the solution was precipitated in 100 mL deionized water. The precipitate of PSI-PDDA was washed with deionized water for several times and dried at 25 °C in vacuo.

PSI-PDDA 2.0 g was dissolved in 10 mL DMF, excessive amount of propylamine was slowly added at 0 °C, then the reaction flask was moved to a water bath at 25 °C and stirred for another 4 h. The solution was precipitated in 100 mL acetone. Using suction filtration, the precipitate of PDDA-PA was washed with acetone for three times and dried at 25 °C in vacuo.

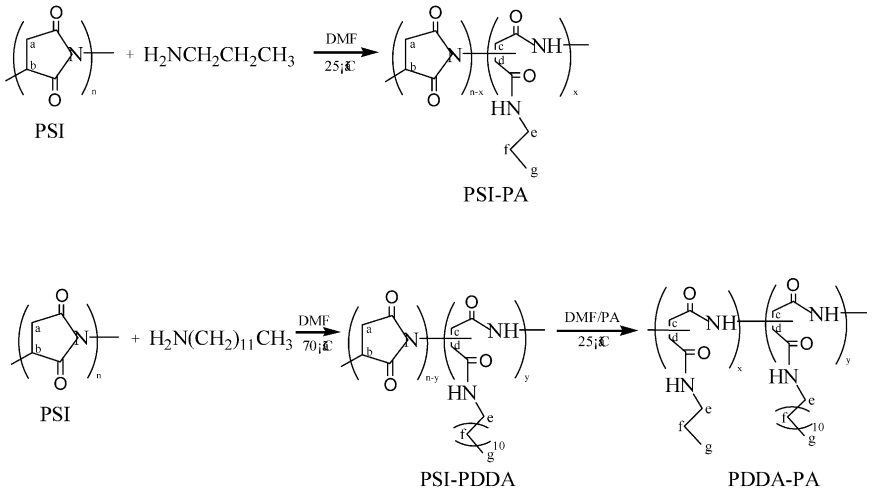
2.3. Preparation of the micelle-like aggregative sample

PSI-PA was added into deionized water under stirring and then was sonicated for 30 min at 25 °C to obtain a clear solution. Prepared solution was stored overnight and purified by passing through a 0.45 μm filter before carrying out the characterization. Solutions with different pH values were adjusted by adding 1.0 mol/L of HCl or NaOH solutions.

The typical preparation procedure of PDDA-PA aggregates is as following: a certain amount of dried PDDA-PA was dissolved in 1 mL DMF firstly, and then the PDDA-PA DMF solution was dropped stepwise into 10 mL deionized water via vigorous stirring to form the micelle-like aggregative solution. After 15 min stir, the aggregative solution was put into dialytic bag and carefully dialyzed it in deionized water for 1 week to remove the DMF completely. After dialyzed, the aggregative solution was purified by passing through a 0.45 μm filter before carrying out the characterization.

2.4. Drug release experiment in vitro

A certain amount of freezing dried drug loaded PSI-PA sample dispersed in 20 mL phosphate buffer saline (0.1 M, PBS). Under 37 °C, the solution was kept on shaking table (120 rpm), take 1 mL solution form the solution at predetermined time and following separation by ultracentrifuge, the supernate was measured by UV–Vis, from the



Scheme 1.

absorption at 480 nm to determine the amount of the released drug.

2.5. Measurements

^{1}HNMR analysis was carried out on a Philips DMX500 Spectrometer using DMSO-d_6 as solvent. FTIR spectra were obtained on a Magna 550 spectrophotometer. DSC measurements was carried out on a Pyris DSC-1 calorimeter to get T_g under nitrogen atmosphere at a heating rate of 20 °C/min. Dynamic Light Scattering (DLS) measurements were performed on a Malvern Autosizer 4700 spectrometer, and the laser wavelength (λ) applied in measurements was 514.5 nm. Surface tension measurements were carried out on a JYW-200A tensiometer at 25 °C. UV–Vis measurement was carried out on a Perkin Elmer Lambda 35 UV/Vis spectrometer.

3. Results and discussion

Poly(succinimide-*co*-*N*-propyl aspartamide) (PSI-PA) copolymers were synthesized by acid catalysized polycondensation of L-aspartic acid [21], followed by aminolysis with different amounts of propylamine (see Scheme 1). FTIR spectra of PSI-PA showed that strong bending vibration bands of –CONH– at 1649 cm^{-1}(amide I), 1545 cm^{-1}(amide II) and stretching vibration band at 3305 cm^{-1} appeared after aminolysis. ^{1}H NMR spectrum (Fig. 1) of PSI-PA showed that the signal at 0.84 ppm is for methyl protons (g), 1.22 and 1.50 ppm are for methylene protons (e, f) (see Scheme 1). In addition, the ring opening of succinimide units resulted in a new signal at 4.49 ppm assigned to the methine proton (d) of the *N*-propyl aspartamide units [16]. The ^{1}H NMR results indicated that PSI-PA was successfully prepared by aminolysis of PSI with propylamine.

Poly(*N*-dodecyl aspartamide-*co*-*N*-propyl aspartamide) (PDDA-PA) copolymers were synthesized by sequential aminolysis of PSI with dodecylamine and propylamine. FTIR analysis showed the existence of the amide bending vibration bands at 1649 and 1545 cm^{-1}, the stretching vibration band at 3300 cm^{-1} and the strong C–H stretching vibration bands at 2850 and 2953 cm^{-1}, indicates the successful grafting of alkyl chains. ^{1}H NMR spectrum of PDDA-PA is shown in Fig. 1. The signals can be assigned as following: $\delta = 0.92$ ppm (g) (–CH_3, t); $\delta = 1.22$ ppm (f) (–CH_2–CH$_3$, s); $\delta = 1.50$ ppm (e) (–NH–CH_2–, s); $\delta = 2.68$ and 3.20 ppm (a and c) (–CH_2–, s); $\delta = 4.49$ ppm (d) (–CH–, t) (See Scheme 1). From Fig. 1, we can see that the signals at 5.0–5.4 ppm of the methine protons in the succinimide rings disappeared in the spectrum of PDDA-PA, hints the ring in the main chain has been opened completely.

In this paper, the degree of substitution (DS) was defined as the mole ratio of the *N*-propyl aspartamide units to the

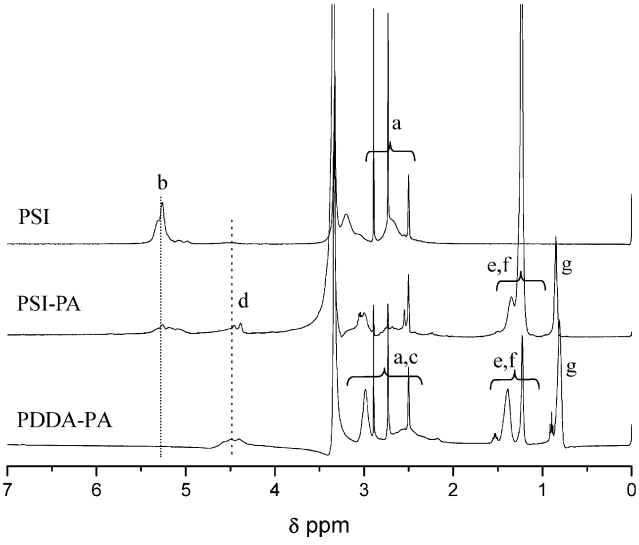


Fig. 1. ^{1}H NMR spectra of PSI, PSI-PA (DS = 76%) and PDDA-PA(DDA/PA = 24:76) in DMSO-d_6 at 25 °C.

original succinimide units in PSI. The DS of the PSI-PA copolymers can be obtained from the integrate area ratio of the signals at 4.49 ppm to that at 5.28 ppm. For PDDA-PA copolymers, the DDA/PA ratio could be calculated from the integrate area ratio of 5.28 to 4.49 ppm in the PSI-PDDA spectra since the residual succinimide units have be totally replaced by *N*-propyl aspartamide units in the followed aminolysis reaction. The summary data of these two series copolymers are shown in Table 1.

Dynamic light scattering (DLS) measurement was applied to investigate the aqueous solution of PSI-PA copolymers with different DS (see Table 1). In Table 1, we can find that PSI-PA copolymers could form aggregates in water and the particle size decreased with the DS increase from 47 to 100%. In our studies in [19], we found that PSI-HA copolymers could not self-aggregate in water directly by ultrasonication; the aggregates must be prepared by dropwise addition of the PSI-HA DMF solution into excessive amount water. When hexyl side chains of PSI-HA copolymer replaced by propyl chains, the hydrophilicity of the copolymers was dramatically increased, and nano-size aggregates could be formed in water in situ.

In this experiment, we also found that bimodal size distribution of the aggregates of PSI-PA copolymers could be obtained at some conditions. In aqueous medium, the aggregative behavior of the amphiphilic copolymers will be affected by hydrogen bonding and the ratio of hydrophilic and hydrophobic segment in non-ionic amphiphilic copolymers [22,23]. Bimodal size distribution can be obtained when the copolymers possess high T_g hydrophobic segments [24] or too large mole fraction of hydrophobic segments [25], and also dependent on the preparation procedure. When the process involves the hydrophobic segments with high T_g and stiff structures, however, the formed aggregates are more likely under kinetic control rather than thermodynamic control, and broaden size distribution of the aggregates will be formed. PSI has a high T_g up to 260 °C due to its stiff ring structure, ring-opening by aminolysis could dramatically reduce the rigidity of PSI and the T_g, but the T_g was still higher than 130 °C according to the data in Table 1. It is believed that the residual stiff succinimide segments in PSI-PA are difficult to bend and form the hydrophobic microdomains by intramolecular association. So the succinimide segments tend to contact each other by intermolecular association and to form the PSI-PA aggregates with hydrophobic microdomains, and if the primary aggregates are not stable, they will tend to form secondary aggregates at room temperature. The bimodal size distribution maybe attribute to the presence of secondary aggregates and the primary aggregates. In our system, bimodal size distribution not always could be obtained, suitable amphiphilic copolymer could get aggregates with monomodal size distribution. Such as from PSI-PA-1 and PDDA-PA-4, we could get very good monomodal size distribution (see Fig. 3), this may due to the primary aggregates cannot be stable by themselves or they are stable enough. When the temperature increased, as shown in Fig. 2, the average size of the aggregates decreased. The changes of the aggregative size versus temperature are considered to originate from the adjusting of the intermolecular association between the hydrophobic succinimide segments and the reconstitution of secondary aggregates. On the other hand, hydrogen bonding between the amide groups and water molecules will break up when the temperature increases, then the thickness of the highly organized water layer around the aggregates will reduce by releasing the bounded water [26], which will result in the shrinking of the aggregate size.

The PDDA-PA copolymers cannot dissolve in water directly by ultrasonication as PSI-PA, indicating that the grafted dodecyl chain improved the hydrophobicity of the amphiphilic copolymers. The aggregates of PDDA-PA were prepared by dropping PDDA-PA DMF solution into an excessive amount of water, followed by dialysis against distilled water. As we can see from Table 1, the particle sizes of the aggregates of PDDA-PA copolymers are smaller than those of PSI-PA copolymers. The possible reasons may be relate to the flexible polymer chains. Although the T_gs of

Table 1
Characteristics of micelle-like aggregates of PSI-PA and PDDA-PA copolymers formed in water at different substitution degrees

Sample	DS[a]	T_g (°C)	Hydrodynamic diameter (nm)	Polydispersity
PSI-PA-1	100%	136	112[b]	0.16
PSI-PA-2	83%	142	154[b]	0.20
PSI-PA-3	70%	150	181[b]	0.28
PSI-PA-4	47%	196	190[b]	0.34
	(DDA/PA)[c]			
PDDA-PA-1	10:90	138	34[d]	0.55
PDDA-PA-2	19:81	142	14[d]	0.54
PDDA-PA-3	24:76	146	18[d]	0.52
PDDA-PA-4	48:52	163	32[d]	0.50

[a] Determined by ^{1}HNMR spectra.
[b] Measurements carried out in the 0.40 wt% PSI-PA dispersions prepared by ultrasonication of PSI-PA copolymers in water.
[c] The DS of PDDA-PA is 100%.
[d] Measurements carried out in the 0.25 wt% PDDA-PA dispersions prepared by dropping DMF solution of PDDA-PA copolymers into water.

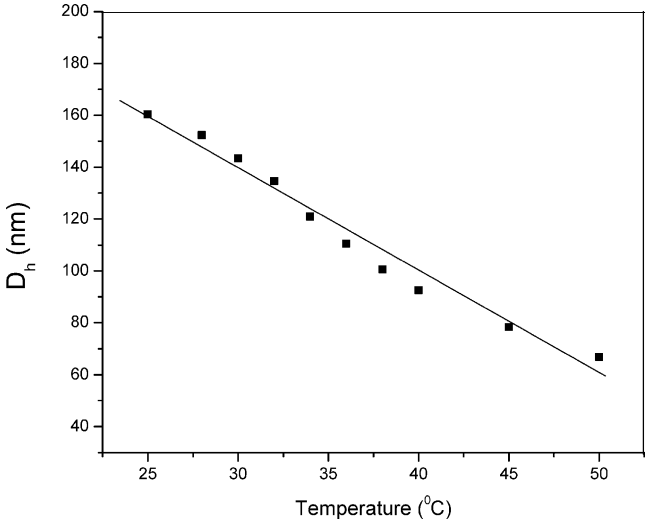


Fig. 2. The influence of temperature on hydrodynamic diameter of the PSI-PA-3 (DS=70%) aggregates in water medium (Detected angle: 90°).

PDDA-PA are almost same as PSI-PA with similar DS, the side chain of DDA is much free than succiminde unites, then the DDA side chains preferred to intramolecular association when the copolymer was dispersed in the aqueous medium, and led to small stable primary aggregates with strong hydrophobic association [18,23]. Another characteristic of the aggregation of PDDA-PA copolymers was that there was a minimum particles size value with the increase of DS, the minimum value is about 14 nm. With DDA/PA ratio changes from 19:81 to 48:52, the PDDA-PA copolymers become more and more hydrophobic and the particles size of the aggregates increases.

When the temperature of the aqueous dispersions of PDDA-PA copolymers increased, the average particle size also decreased as shown in Fig. 3. This result indicates that the aggregates of PDDA-PA copolymers are stable and the decrease of the particle size with temperature attributes to same reasons as we discussed above.

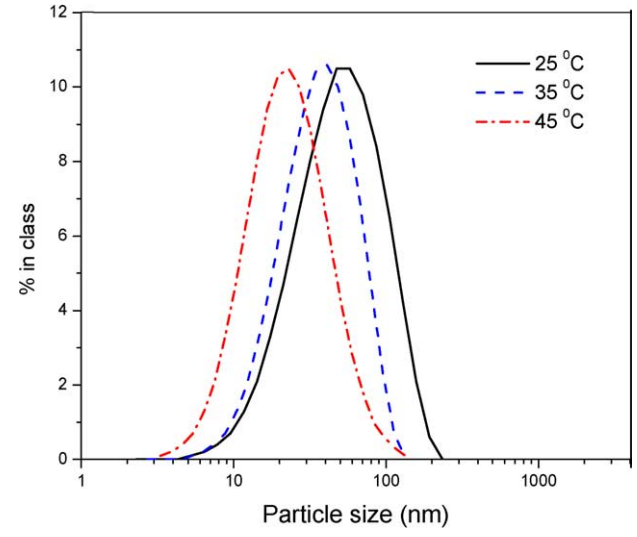


Fig. 3. Size distribution of PDDA-PA-4 (DDA:PA=48:52) in 0.25% aqueous solution at deferent temperatures.

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

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

where q is the scattering vector. In dilute solution, the concentration and detection angle dependence of $\bar{\Gamma}$ can be expressed as:

$$\bar{\Gamma}/q^2 = D_0(1 + k_D C)(1 + f\langle Rg^2\rangle q^2) \tag{2}$$

where D_0 is the D_T at infinite dilution, k_D is the diffusion second virial coefficient, C is the concentration, f is the parameter related to the polymer configuration, intramolecular movement and solvent properties, $\langle Rg^2\rangle$ is the mean square gyration radius of the polymers.

For spherical particles, diffusion coefficient should be independent on the square of the scattering vector due to the undetectable rotational motion [23]. Fig. 4 shows the aggregate diffusion coefficient D_T dependence upon the scattering vector q^2. It is obvious that the detection angle affects D_T of PSI-PA aggregates, which suggested that the aggregates were non-spherical. Also, the non-spherical morphology of the aggregates was originated from the rigid succinimide segments. On the contrary, the D_T of PDDA-PA aggregates are independent on the scattering vector, indicating the spherical aggregates of PDDA-PA copolymers were formed.

Fig. 5 shows that the diffusion coefficient D_T of PSI-PA aggregates decrease with the concentration of the PSI-PA copolymers increases. Due to the more opportunities for

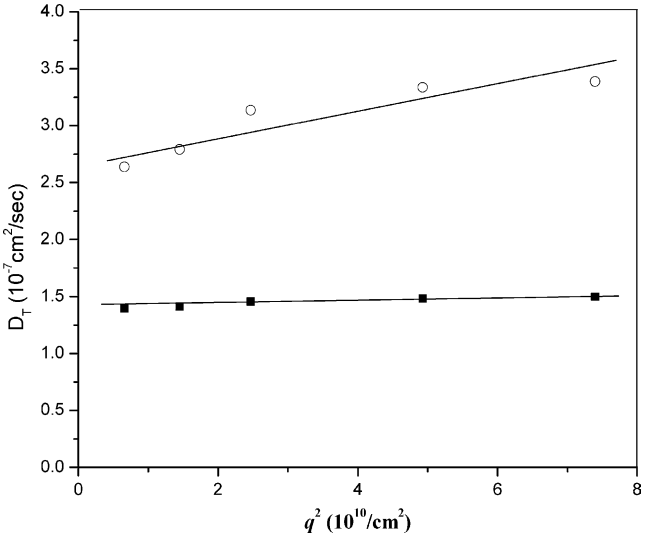


Fig. 4. Diffusion coefficient D_T of PSI-PA-3 (DS=70%, ○) and PDDA-PA-4 (DDA:PA=48:52■) micelle-like aggregates as a function of the square of the scattering vector (q^2) (concentration: 0.10%, temperature: 25 °C).

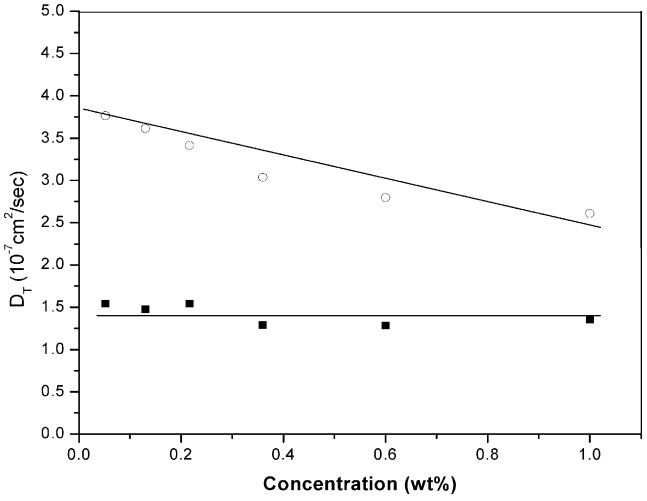


Fig. 5. Diffusion coefficient D_T of PSI-PA-3 (DS = 70%, ○) aggregates and PDDA-PA-4 (DA:PA = 48:52 ■) in water solution as a function of the concentration (detected angle: 90°, temperature: 25 °C).

intermolecular association of the succinimide segments in high solid solution of PSI-PA copolymers, then the aggregate size will increase, and the diffusion coefficient D_T of the aggregates will decrease. But, the diffusion coefficient D_T of PDDA-PA aggregates is independence on the change of the concentration.

In general, amphiphilic copolymers are surface active in water like surfactant molecules. Surface tension measurements were carried out to study the surface activity of the PSI-PA and PDDA-PA copolymers. The surface tension as a function of logarithmic concentration of PSI-PA was shown in Fig. 6. No sharp transition of surface tension vs. the concentration has been observed in PDDA-PA solution, this result suggests that the PDDA-PA copolymers preferred to form the aggregates rather than exist at the interface of air/water to lower surface tension. Typical surface tension curve like surfactant molecule was obtained when the PSI-PA copolymers dissolved in water, which suggested that the PSI-PA copolymers could exist in the water–air surface to lower the surface tension. CAC (critical aggregative concentration) can be obtained from the intersection point of the sharp decreasing region and the flat region in the plot, it is about C = 10^{-5} g/mL. Below CAC, the amphiphilic PSI-PA copolymer chains seldom contact each other and it is hard for the succinimide segments to form hydrophobic domains by intermolecular association. When the concentration increases to CAC, stable aggregates will formed and the surface tension reduce to minimum value. When acid added into the solution of PSI-PA copolymer to reduce pH value from 7.04 to 5.21, the critical aggregation concentration did not change a lot. This is helpful when these biocompatible and biodegradable copolymers are applied in vivo as drug carrier.

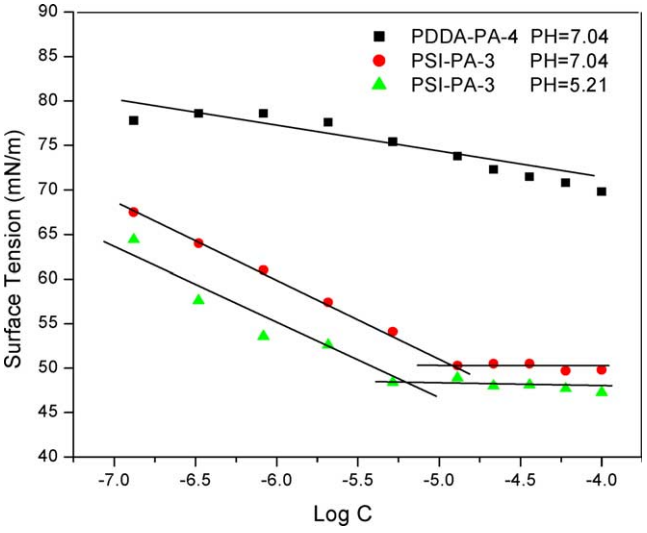


Fig. 6. Surface tension of PDDA-PA-4 (DDA:PA = 48:52) at pH = 5.21 and PSI-HA-3 (DDA:PA = 30:70) at pH = 5.21 and pH = 7.04 as a function of concentration by sequential dilution.

In our lab, drug release experiment of the drug-loaded PSI-PA nano-particles has been done. The preliminary result showed that PSI-PA nano-aggregates can load up to 20 wt% doxorubicin inside. The drug-released test in vitro indicated that the doxorubicin-loaded nanoparticles could prolong the drug release time, 85% doxorubicin could be released after 170 h dialysis in 0.1 M in phosphate buffer solution.

4. Conclusion

Amphiphilic PSI-PA and PDDA-PA copolymers were synthesized and the aggregative behaviors were studied. PSI-PA copolymers could disperse in water directly by ultrasonication at room temperature and form aggregates spontaneously. Some aggregates exhibit bimodal size distribution due to the existence of stiff succinimide segments. PDDA-PA copolymers could form stable aggregates when PDDA-PA DMF solution was added into excessive amount of water. The PDDA-PA aggregates possess monomodal size distribution and spherical morphology due to the strong intramolecular association of dodecyl chains. Particle size of both PSI-PA and PDDA-PA aggregates were reduced with the temperature increased, this may due to the breaking of bonded water. The PDDA-PA aggregates were independent on the concentration. Surface tension study indicated that the PDDA-PA copolymers were no surface active while the PSI-PA copolymers were surface active. The preliminary results showed that PSI-PA copolymers are suitable for drug delivery system.

Acknowledgements

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