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Synthesis of amphiphilic polyaspartamide derivatives and construction of reverse micelles

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A series of amphiphilic graft copolymers based on biodegradable and biocompatible poly(aspartic acid)s were synthesized by a successive aminolysis reaction of polysuccinimide using octadecylamine/dodecylamine, and ethylenediamine-β-cyclodextrin/ethanediamine. The chemical structures of the copolymers were confirmed by FT-IR and ^{1}H NMR spectroscopy. Large compound reverse micelles consisting of numerous small reverse micelles with polar cores and hydrophobic shells were formed in octanol solution. The reverse micelles showed various particle sizes based on the different length of hydrophobic alkyl chains and molecular weight of polysuccinimide, as determined by dynamic light scattering. Interestingly, the particle size of micelles showed temperature dependence, the diameter decreased continuously with increasing temperature. Their morphology and assembly properties were characterized using scanning electron microscopy, transmission electron microscopy and fluorescence spectroscopy. The reverse micelles were extremely efficient in extracting Congo red from water into octanol, exhibiting a potential application as delivery vehicles in the pharmaceutical and cosmetic fields, and as nanocontainers for separation of inorganic molecules as well.

Introduction

The self-assembly behaviour of amphiphilic copolymers in aqueous media has been extensively investigated during the past decades due to their special physicochemical properties and potential applications in target and transdermal drug delivery systems, catalysis, and microelectronics.[1–5] Amphiphilic graft copolymers, in which the hydrophilic and hydrophobic moieties are tunable, have been widely studied because of their various applications and relatively easy preparation methods.[6–9] Graft copolymers possess additional complexity in self-assembly due to their complicated and confined structures,[10–12] which can provide more information about the control of micellar morphologies and the design of new nanomaterials.[13,14]

Poly(amino acid)s and their derivatives have been known to exhibit interesting biochemical properties on account of their superior biocompatible, biodegradability and non-toxicity.[15–17] They are used in various fields including biomedicine, pharmaceuticals, cosmetics and agrochemicals.[18–21] Poly(succinimide) (PSI) is a reactive precursor for synthetic poly(amino acid) derivatives because PSI can be converted easily to hydrophilic biodegradable poly(amino acid) derivatives such as poly(aspartic acid), poly(aspartamide)s, poly(hydroxyethyl aspartamide)s, *etc.*[22–26] The self-assembly behaviour of the amphiphilic graft poly(amino acid) derivatives have been investigated to form regular micelles in water.[27–32] However, to the best of our knowledge, investigations about reverse micellization of them have not been reported. RMs has been widely used as nanospace microreactors and soft template for preparing inorganic nanoparticles,[33,34] carriers for drugs,[35–37] and they can be used to selectively enrich biomarker peptides and protein fragments from human serum and selectively extract/fractionate peptides based on their isoelectric points.[38,39] In this paper, the amphiphilic graft copolymers of poly(aspartic acid) (PASP) derivatives were synthesized by a successive aminolysis reaction of PSI using octadecylamine (ODA)/dodecylamine (DDA) as hydrophobic moieties, ethanediamine-β-cyclodextrin (CDen)/ethanediamine (EDA) as hydrophilic moieties. Spherical large compound reverse micelles (RMs), which consist of numerous small reverse micelles with polar cores and hydrophobic shells in octanol were prepared by using the single-solvent dissolving method. The reverse micellization behaviour was explored effectively by using the polar fluorescence compound *N*-(1-naphthyl)ethylenediamine dihydrochloride (NEAH) as a polar fluorescence probe.[40] The effect of microstructure of PASP derivatives in octanol on the reverse micellization behaviour, temperature-responsive behaviour, as well as on the extraction efficiency for a hydrophilic dye, were investigated.

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Experimental sections

Material

L-aspartic acid (L-Asp, 99%) was purchased from Alfa Co., Ltd. (Tianjin, China). Octadecylamine (ODA) and dodecylamine (DDA) were purchased from Heowns biochem technologies Co., Ltd. (Tianjin, China). Tetramethylene sulfone was purchased from J&K Scientific Co., Ltd. (Beijing, China). Mesitylene, β-cyclodextrin (β-CD, 98%), ethanediamine (EDA), dimethylformamide (DMF) and NEAH were purchased from Tianjin Chemical Reagent Co. Ltd. (Tianjin, China). β-CD was used after recrystallization from water and drying under vacuum. DMF and EDA were distilled before use.

Synthesis of mono(6-(2-aminoethyl)amino-6-deoxy)-β-cyclodextrin (CDen). Mono-6-deoxy-6-(*p*-tolylsulfonyl)-β-cyclodextrin (Mono-6-OTs-β-CD) was synthesized according to the method reported in the literature.[41] CDen was synthesized as described in the previously reported method.[42] Briefly, 5.0 g of mono-6-OTs-β-CD was reacted with excess amount of EDA at 75 °C for 4 h. After the reaction was completed, the mixture was allowed to cool to room temperature, followed by addition of 30 mL of cold acetone, resulting in immediate formation of white precipitate. After the precipitate was collected by suction filtration, the crude product was repeatedly dissolved in 30 mL of water–methanol mixture, and poured into a large amount of cold acetone several times for the removal of unreacted EDA. The sample obtained was dried at 50 °C for 3 d in a vacuum oven, and 4.3 g of CDen were obtained (yield: 80%).

Preparation of poly(succinimide) (PSI). PSI was synthesized according to the method reported in the literature.[43] Briefly, L-aspartic acid (25 g, 0.188 mol) was suspended in the mixed solution (mesitylene/sulfolane = 56 g/24 g) in the presence of *o*-phosphoric acid (2 g, 17.34 mmol) and stirred at 180 °C under N_2 atmosphere. Water formed in the reaction process was continuously removed using the Dean–Stark trap. The reaction was processed for 4.5 h. After the reaction mixture was cooled down, the solvent was removed and DMF was added to dissolve the polymer, and precipitated into excess methanol twice. After filtration, the precipitate was washed several times with distilled water to remove the phosphoric acid. The product was finally dried at 80 °C in vacuum for three days to obtain PSI in white powder (yield: 80%).

Synthesis of octadecylamine/dodecylamine and CDen-grafted-polysuccinimide (PASP-ODA/DDA-CDen). The preparation of PASP-ODA-CDen was according to the synthesis method of PASP-ODA and PASP-CDen.[6,44] Briefly, PSI (97 mg, 1 mmol) was dissolved in DMF (10 mL), followed by addition of ODA (135 mg, 0.5 mmol) dissolved in DMF (5 mL) rapidly. The reaction mixture was stirred continuously under a N_2 atmosphere at 70 °C for 24 h. Excess CDen dissolved in DMF was then added to the solution to react with the remaining succinimide units. After stirring at 60 °C for another 48 h, the product was precipitated in excess ether twice to remove the residual ODA. The precipitate was filtered and dissolved in DMF, after which the product solution was dialyzed against ethanol, then dialyzed against distilled water using a dialysis membrane (molecular weight cut-off = 10–12 kDa) for three days to remove the residual ODA and CDen, and then freeze-dried (yield: 74–85%). PASP-DDA-CDen was prepared similarly. The reaction was performed at 100 °C for 7 h to obtain PASP-DDA, according to the reference,[22] then excess CDen was added and the reaction continued as above (yield: 80–85%).

Synthesis of octadecylamine/dodecylamine and EDA-grafted–polysuccinimide (PASP-ODA/DDA-EDA). To obtain PASP-ODA/DDA-EDA, excess EDA was then added to the PASP-ODA/DDA solution to react with the remaining succinimide units. After stirring at 75 °C for 6 h, the product was purified as described above in "Synthesis of PASP-ODA/DDA-CDen" (yield: 80–85%).

Characterization of polymers

The FT-IR spectra were recorded using KBr discs on a Bio-Rod 6000 spectrometer (Thermo Electron, USA) at room temperature. The ^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer (400 MHz, Bruker, Freemont, CA) at room temperature with D_2O or DMSO-d_6 as solvents, respectively. The molecular weight of PSI was estimated in DMF containing LiBr (20 mmol L^{-1}) by gel permeation chromatography [GPC; column, PL gel 5 mm MIXED-C X 2 (Polymer Laboratories Ltd., U.K.); detector, refractive index; standard, polystyrene].

Preparation of RMs

RMs of PASP-ODA/DDA-CDen/EDA in octanol were prepared by using the single-solvent dissolving method. The polymer (5 mg) was dissolved in octanol (5 mL), sonicated at 100 W for 4 min in an ice bath, and agitated until visibly clear solutions were obtained.

Particle size analysis and morphology examination

The mean particle size and size distribution of the polymer aggregates were investigated in octanol at 20 °C by dynamic light scattering measurements (DLS) on a Zetasizer Nano ZS90 (Malvern, Worcestershire, U.K.).

Transmission electron microscopy (TEM, JEOL, Japan) was conducted on a Jeol 1400 instrument with an accelerating voltage of 100 kV. Samples were prepared by dropping two drops of the RM solutions (0.1 mg mL^{-1}) onto a carbon coated copper grid and drying for 1 week in the fume cupboards.

Scanning electron microscope (SEM, JEOL, Japan) was conducted on a JSM-6700F type instrument with an accelerated voltage of 10 kV. Samples were prepared by dropping two drops of the RM solutions (0.5 mg mL^{-1}) on coverslip. The samples were dried and coated with a thin layer of gold.

Aggregation behaviour and critical aggregation concentration (CAC)

The self-assembly behaviour of PASP derivatives in octanol was explored by using the polar fluorescence compound NEAH as a polar fluorescence probe. The prepared copolymer micellar solution was diluted step by step in the range of 1×10^{-1} to 5×10^{-6} mg mL^{-1} to obtain octanol solutions of PASP derivatives

with continuously varying concentrations. After stirring for half an hour, 10 mL of the solutions was taken and added into a conical flask of 50 mL, respectively, and 6 μL of methanol solution of NEAH (2 mg mL^{-1}) was added into these conical flask using a microinjector to obtain a solution with NEAH concentration of 4.6×10^{-6} mmol mL^{-1}. After stirring for 15 min, the fluorescence emission of these solutions was measured by the excitation at 320 nm using a spectrofluorophotometer (F-7000, Hitachi, Japan). The CACs were determined as the concentration where the fluorescence intensity substantially changed.

Temperature sensitivity of RMs

The temperature responsibility of the RMs was investigated using DLS performed from 20 °C to 75 °C in octanol solution at a temperature interval of 5 °C.

Thermodynamic investigation of RMs

5 μL of deionized water was added into 1.5 mL (0.5 mg mL^{-1}) of RMs each time under stirring. After stirring for 1 h, DLS was used to measure the size of the RMs. The volume was recorded as the critical volume when micro-phase separation occurred.

Extraction of Congo red from water

The aqueous solution of Congo red with a concentration of 0.25 mg mL^{-1} was equilibrated with equal volume of polymer solutions in octanol (1 mg mL^{-1}) for 2 h. The amount of dye extracted into the organic layer was determined by the final dye concentrations in water, which can be estimated by monitoring the absorbance changes at the wavelength of maximum absorption (496 nm) using a UV-vis spectrophotometer (TU-1900, Persee, Beijing).

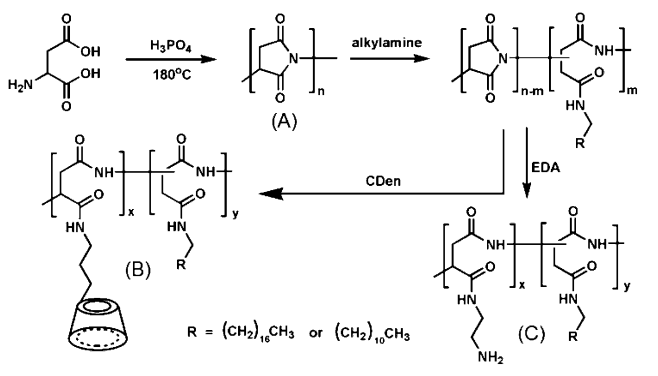
Cell viability assays

Cell viability was detected using Cell Counting Kit-8 (CCK8) assay. Mouse fibroblast cells (L929) were cultivated in a humidified 5% carbon dioxide atmosphere at 37 °C on a 96-well microplate, with 5000 cells immersed in complete growth medium per well. The cells were allowed to attach for 24 h. Subsequently, the copolymer suspensions with the concentrations of 2.5, 2.0, 1.5, 1.0, and 0.5 mg mL^{-1} were added to 96-well plates at 10 μL per well and incubated for 24 h, respectively. The solution was then removed and replaced with 100 μL of RPMI-1640. Then, CCK-8 solution was added to 96-well plates at 10 μL per well and incubated for 24 h, and the resulting solution was analyzed at 450 nm by means of a plate reader with a background correction using a Bio-Tek FLx800 Fluorescence Microplate Reader. This process was repeated for eight times in parallel. The results are expressed as the relative cell viability (%) with respect to control wells containing culture medium.

Results and discussion

Synthesis and characterization of the polymers

Scheme 1 shows the reaction scheme for the synthesis of PASP-DDA/ODA-CDen/EDA. These copolymers were synthesized from PSI through a successive aminolysis reaction with quantitative



Scheme 1 Synthesis of PSI (A), PASP-DDA/ODA-CDen (B) and PASP-DDA/ODA-EDA (C).

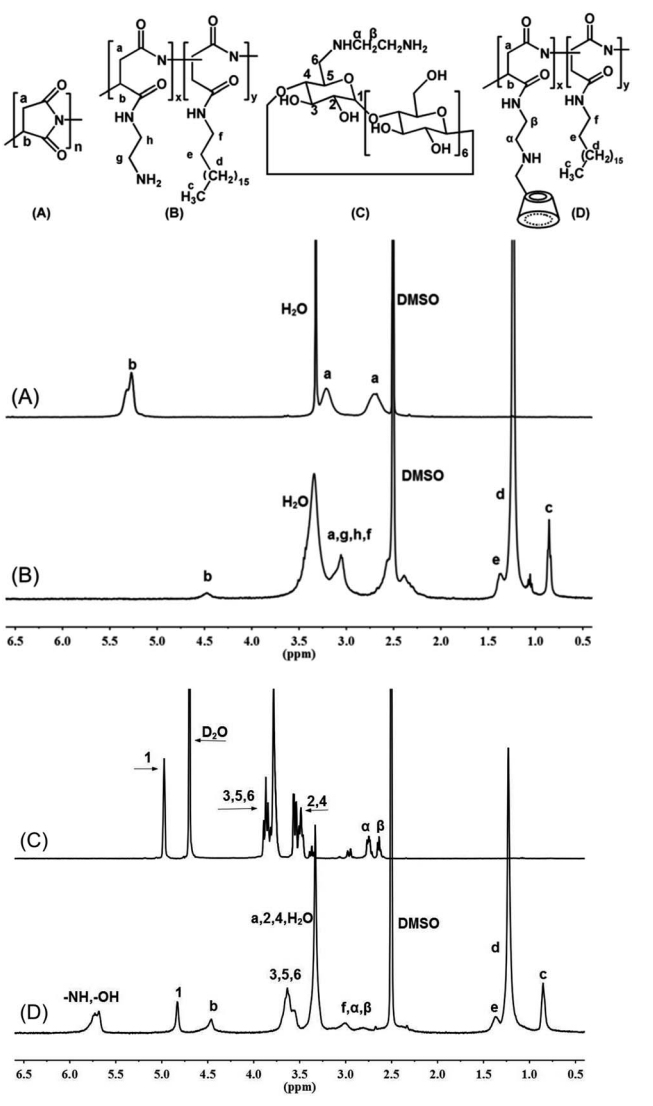


Fig. 1 ^1H NMR spectra of PSI (A), PASP-ODA-EDA (B) in DMSO-d_6, CDen (C) in D_2O and PASP-ODA-CDen (D) in DMSO-d_6.

DDA/ODA, and CDen/EDA. The weight average molecular weight of PSI was determined by GPC as 25 kDa and 45 kDa respectively. Their chemical structures were characterized by ^{1}H NMR spectroscopy (Fig. 1). The signals at 5.0–5.4 ppm and 2.5–3.3 ppm were assigned to methine proton (b) and methylene proton (a) of PSI. While in the ^{1}H NMR spectrum of PASP-DDA/ODA-CDen, new signals from the *N*-dodecyl/octadecyl aspartimide units were observed at 0.8 ppm for methyl protons (c), at 1.2–1.50 ppm for methylene protons (d, e) and at 3.0 ppm for methylene protons (f) adjacent to amino group of the dodecyl/octadecyl chain. In addition, the ring opening of succinimide units caused the appearance of a new signal at 4.5 ppm assigned to the methine proton of the *N*-dodecyl/octadecyl aspartamide units. The absence of the peak at 5.0–5.4 ppm related to the methine proton in succinimide units clearly indicated that the ring opening reaction of PSI completed. The peaks "1" at 4.80 ppm and "2–6" at 3.20–3.80 ppm could be assigned to the protons on C1 and on C2–C6 of the CDen unit. The peaks "α" and "β" at 2.5–2.8 ppm arose from the methylene protons of ethanediamine groups in CDens. In addition, the peaks located at 5.5–6.0 ppm assigned to active hydrogens (–NH, –OH) was observed. The ^{1}H NMR results indicated that the PASP-DDA/ODA-CDen was synthesized successfully *via* aminolysis of PSI. The ^{1}H NMR spectrum of PASP-DDA/ODA-EDA was shown in Fig. 1B. The peaks "g" and "h" were methylene protons of EDA. Other peaks could be well assigned as above.

The aminolysis degree (AD) is the mole percent of the attached unit with alkyl group per total succinimide unit. Table 1 shows AD of DDA/ODA and CDen according to feed molar ratio. The AD of each side chain was determined by comparing the integral ratio of peaks assigned to the methyl protons (c) of *N*-dodecyl/octadecyl aspartimide units, the protons on C1 (1) of the CD unit with the integral peak assigned to the methine proton (b) of the polyaspartamide backbone in ^{1}H NMR. The AD of CDen-contained polymers was much lower than that of ODA/DDA-contained ones due to the steric hindrance of CDen unit.

As shown in Fig. 2, the FT-IR spectra of PSI showed the stretching vibration of –NH– located at about 3430 cm^{-1}, and the carbonyl C=O from cyclic imides stretching located at 1710 cm^{-1}. The peaks located at 3380 and 1030 cm^{-1} were attributed to multihydroxy of the CDen moiety in PASP-DDA-CDen.

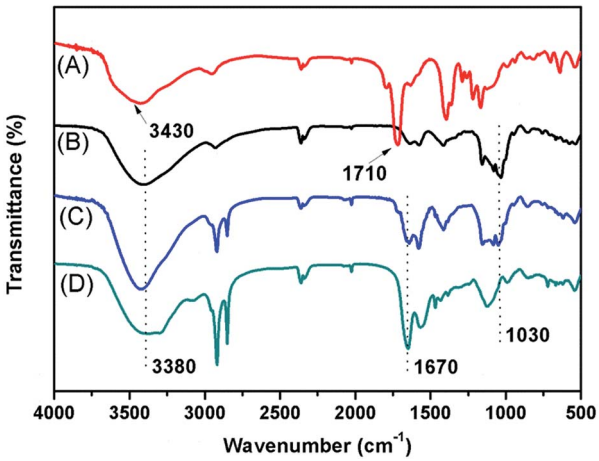


Fig. 2 FT-IR spectra of PSI (A), CDen (B), PASP-DDA-CDen (C), and PASP-DDA-EDA (D).

Especially, the carbonyl C=O from cyclic imides stretching located at 1710 cm^{-1} was absence in Fig. 2C and D. A new peak located at 1670 cm^{-1}, assigned to the stretching vibration of carbonyl in the amido group on the PASP-DDA-CDen/EDA appeared, indicating that the aminolysis of PSI completed.

Characterization of RMs

The amphiphilic PASP derivatives were then employed to prepare RMs. The DLS graph of the PASP(45K)-ODA-CDen RMs with narrow particle size distribution was shown in Fig. 3. Their particle size and distribution were measured by DLS (Table 1). The average diameters of nanoparticles were around 300–400 nm for the PASP derivatives, and increased with increasing alkyl length of the alkyl chain and the molecular weight of PSI. The longer alkyl chain led to the lower ratio of the hydrophilic and hydrophobic segments, thus the size of PASP(45K)-ODA-CDen was larger than that of PASP(45K)-DDA-CDen. On the other hand, a larger space volume produced from the copolymers with higher molecular weight, thus the PASP(45K)-based polymers exhibited larger size than PASP(25K)-based ones. Most of PDI of these assemblies was lower than 0.2, indicating the

Table 1 Characteristics of PASP derivatives

Sample	AD[a] (%)	AD[b] (%)	Diameter (nm)	PDI	Extraction of Congo red[c] (%)
PASP(25K)-DDA-CDen	45.7	19.7	305	0.163	53.5
PASP(45K)-DDA-CDen	48.3	15.1	331	0.171	63.0
PASP(25K)-ODA-CDen	43.0	21.4	310	0.145	59.8
PASP(45K)-ODA-CDen	47.3	20.6	362	0.143	66.3
PASP(25K)-DDA-EDA	43.0	—	315	0.148	92.3
PASP(45K)-DDA-EDA	46.0	—	376	0.103	95.0
PASP(25K)-ODA-EDA	40.0	—	350	0.149	93.0
PASP(45K)-ODA-EDA	36.3	—	423	0.252	97.0

[a] The aminolysis degree of the *N*-dodecyl/octadecyl aspartimide units, defined as the molar ratio of the *N*-dodecyl/octadecyl aspartimide units to the succinimide unit in original polysuccinimide, and was determined by ^{1}H NMR spectra (mol%). [b] The aminolysis degree of the CDen units, defined as the molar ratio of the CDen units to the succinimide unit in original polysuccinimide, and was determined by ^{1}H NMR spectra (mol%). [c] The initial concentration of Congo red in water is 0.25 mg mL^{-1}.

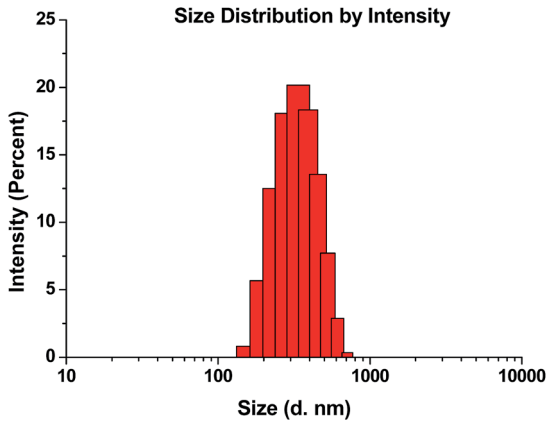


Fig. 3 DLS graph of the PASP(45K)-ODA-CDen RMs.

narrow distribution of the RMs. Only PASP(45K)-ODA-EDA exhibited a relative high PDI. Its larger diameter probably led to a wider dispersion.

Fig. 4A shows a typical SEM image of PASP(45K)-DDA-CDen RMs formed in octanol solution (0.1 mg mL^{-1}) at 25 °C. Spherical particles with a diameter of approximately 200 nm were observed. The TEM image (Fig. 4B) also shows the presence of spherical micelles with a diameter of 200 nm. The size observed from TEM and SEM was smaller than that measured by DLS (331 nm) because the samples were in the dried state when observed using microscopy. Here, the size of spherical micelles (300–400 nm) from DLS was much larger than the maximum length of the repeating unit of copolymers estimated by ChemOffice software. The enlarged TEM image (Fig. 4C and D) also showed multi-cores of the micelle. This kind of micelle was named as a large compound reverse micelle consisting of

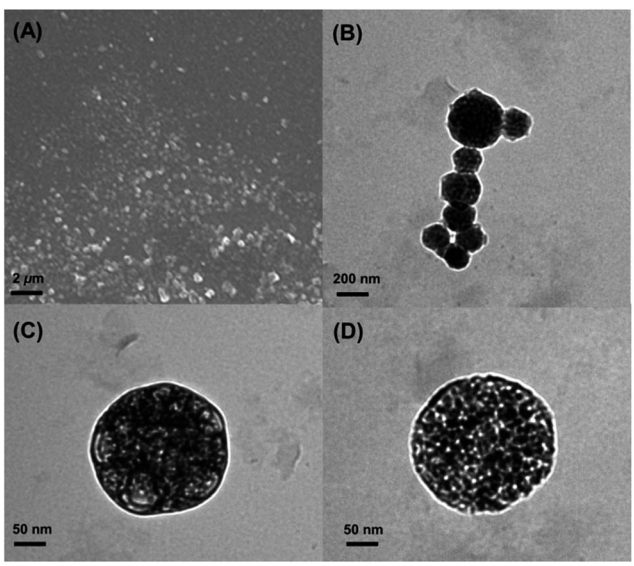
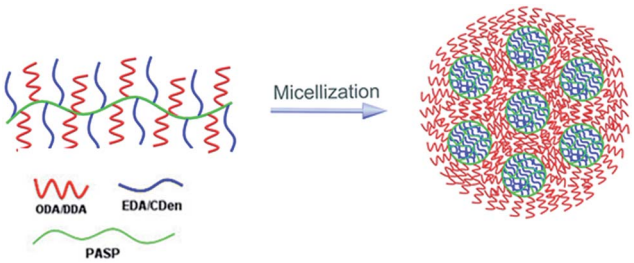


Fig. 4 SEM image (A), TEM image (B) of PASP-DDA-CDen and enlarged TEM of PASP(45K)-DDA-CDen (C) and PASP(45K)-DDA-EDA (D) in octanol solution at 25 °C.



Scheme 2 Formation of large compound reverse micelles by PASP derivatives.

numerous small reverse micelles with polar cores and hydrophobic shells as shown in Scheme 2. We then examined the assembly properties of these micelles using a polar fluorescence probe, and found that reverse micelles with hydrophilic cores and hydrophobic shells were formed (Fig. 5).

Determination of the CAC

Fig. 5 shows the varied fluorescence emission of NEAH at different copolymer concentrations in octanol. The variation in fluorescence intensity was similar for these four polymers. When the concentration of copolymers was lower, the fluorescence emissions of NEAH decreased slightly with the increased concentrations of copolymers. While as the concentrations of copolymers increased to a certain value, the fluorescence emission of NEAH decreased sharply, and then the emissions remained constant. The abrupt changes of the fluorescence emissions of NEAH evidenced the formations of RMs, indicating that NEAH can be used as a polar fluorescence probe, and it can be used to explore macroscopically the reverse micellation behaviour of the amphiphilic graft copolymers of PASP derivatives in octanol. PASP derivatives could self-assemble into RMs at a certain concentration in octanol, consisting of hydrophilic PASP-CDen/EDA units as core and hydrophobic alkyl chain as shell. The CACs of the four copolymer samples were 5 μg mL^{-1} for PASP(25K)-ODA-CDen (Fig. 5A), 1 μg mL^{-1} for PASP(25K)-DDA-CDen (Fig. 5B), 5 μg mL^{-1} for PASP(45K)-ODA-CDen (Fig. 5C) and 10 μg mL^{-1} for PASP(45K)-DDA-EDA (Fig. 5D). By comparing these data, it could be found that for the copolymer samples with the same length of PASP unit but different length of alkyl chain (ODA, DDA), the longer the alkyl chain is, the lower ratio of the hydrophilic and hydrophobic segments is, and the higher the CAC is. Therefore, the CAC was 5 μg mL^{-1} for PASP(25K)-ODA-CDen and 1 μg mL^{-1} for PASP(25K)-DDA-CDen. For polymer PASP(45K)-DDA-EDA with EDA as the hydrophilic chain, which is shorter than CDen units, had the highest CAC (10 μg mL^{-1}). PASP(25K)-ODA-CDen and PASP(45K)-ODA-CDen had the same CAC (5 μg mL^{-1}), indicating that the molecular weight of the polymers did not influence on the CACs. The experimental results are in agreement with general rule that the CAC of the amphiphilic copolymer in the selective solvent depends greatly on the length of the insoluble segments, and the copolymer with longer insoluble segments has a lower CAC.[40]

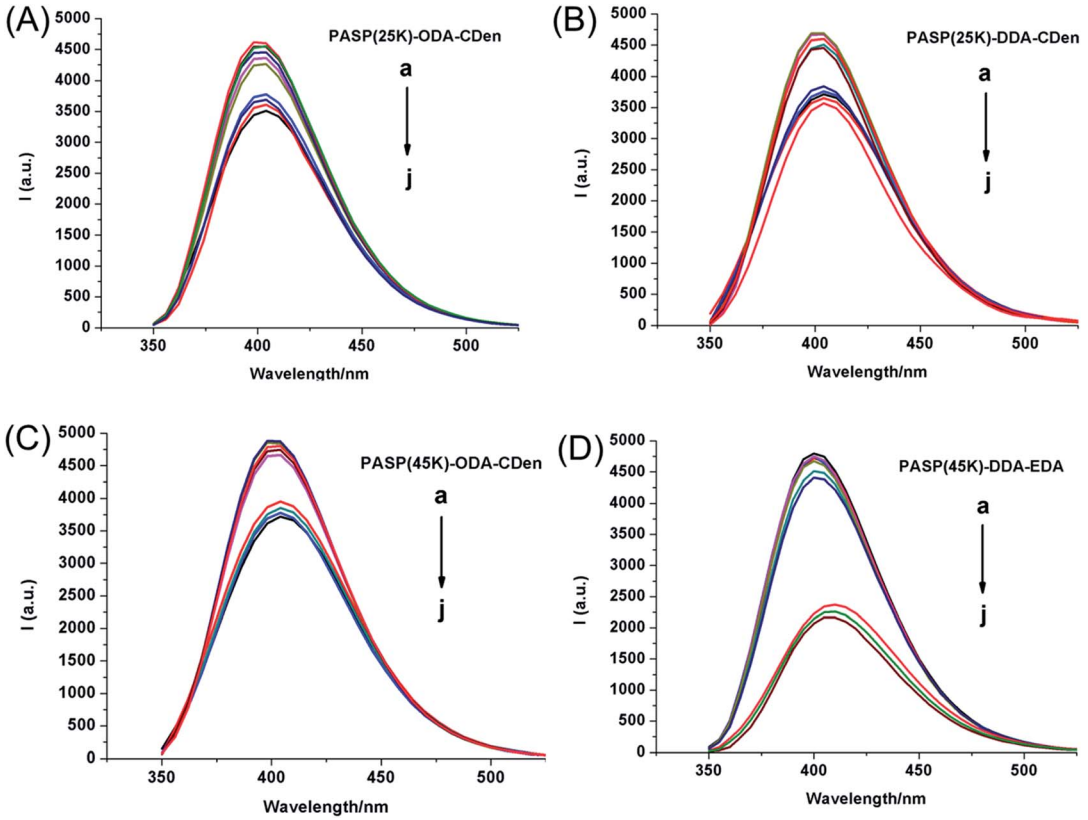


Fig. 5 Varying fluorescence emission of NEAH in copolymer octanol solution with copolymer concentration (mg mL^{-1}) for different copolymers: a, 5×10^{-6}; b, 1×10^{-5}; c, 5×10^{-5}; d, 1×10^{-4}; e, 5×10^{-4}; f, 1×10^{-3}; g, 5×10^{-3}; h, 1×10^{-2}; i, 5×10^{-2}; j, 1×10^{-1}.

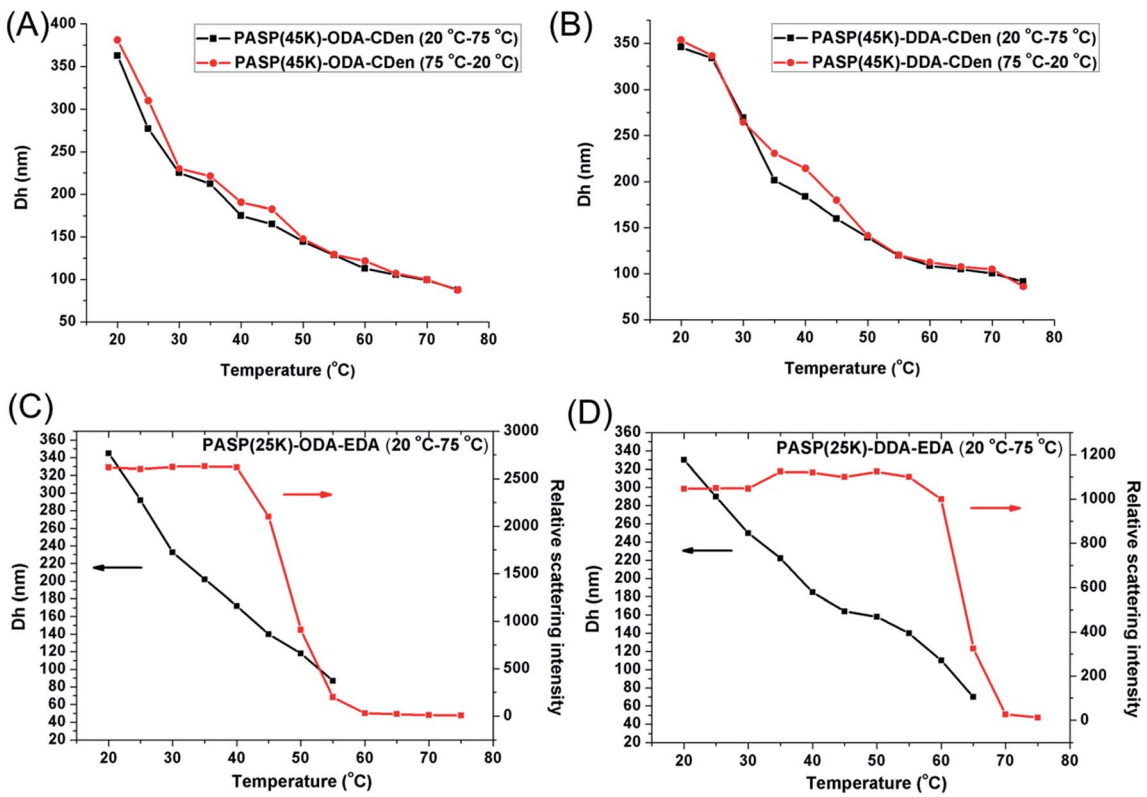


Fig. 6 Effects of temperature on average diameter (A–D) and relative scattering intensity (C and D) of different RMs.

Table 2 The maximum water content in RMs

Sample	V_{water} (μL)	$V_{water}/V_{octanol}$
PASP(25K)-ODA-CDen	20	0.013
PASP(45K)-ODA-CDen	30	0.020
PASP(25K)-ODA-EDA	40	0.026
PASP(45K)-ODA-EDA	60	0.040

Temperature sensitivity of RMs

Fig. 6 shows temperature dependence of the diameter of different RMs. The size of the polymers decreased upon increasing temperature at a uniform speed. The polymer chain shrinked with increasing temperature, which probably led to the decrease in particle size. The size variation upon temperature changes was reversible for PASP-ODA/DDA-CDen (Fig. 6A and B). However, the particle size variation was irreversible for PASP(25K)-ODA/DDA-EDA (Fig. 6C and D) with EDA as the hydrophilic chains and the RMs could not be reconstructed upon decreasing temperature. This is probably because that the relative shorter hydrophilic EDA chain, compared with CDen unit, as the driving force to form hydrophilic core is lower. To check the existence or dissolution of micelles, we measured the relative scattering intensity. As for PASP(25K)-ODA-EDA, when the temperature was higher than 55 °C, the intensity decreased sharply to zero. While for PASP(25K)-DDA-EDA the transition temperature was 65 °C, which is higher than that of PASP(25K)-ODA-EDA. The longer alkyl chain (ODA) made it easier dissolve in octanol solution, leading to a disaggregation.

Thermodynamic investigation of RMs

A similar method was employed to explore the thermodynamic characteristic of the RMs as reported by Eisenberg[45] and Cheng.[46] Table 2 showed the critical water volume in 1.5 mL RMs before generating micro-phase separation. The polymer solutions of PASP(25K)-ODA-EDA and PASP(45K)-ODA-EDA containing EDA unit displayed higher water content than PASP(25K)-ODA-CDen and PASP(45K)-ODA-CDen The larger sizes of EDA-contained polymers (Table 1) endowed them with larger inside water cores to accommodate more water. Additionally, a larger space volume probably produced from the copolymers with higher molecular weight. Therefore, The critical water volume of PASP(45K)-ODA-CDen/EDA was higher than that of PASP(25K)-ODA-CDen/EDA.

Encapsulation of Congo red in RMs

Fig. 7 shows the differences in color intensity of dye solution when different polymers were used for the transfer, exhibiting the difference in loading capacity of polymeric micelles. We distinguished the differences between these copolymers by comparing an initial Congo red concentration with the final dye concentration in water after the transfer, measured by optical spectroscopy. The extraction rate of Congo red by different PASP derivatives was shown in Table 2. The extraction rate of PASP derivatives with CDen unites was around 60%, and 90% for the polymers without CDen unites. Analysis of the dye concentration in aqueous revealed that vast majority of the dye molecules was transferred to the octanol. The polymers without CDen units were more efficient to transfer the Congo red. The larger size of PASP-ODA/DDA-EDA may be suitable to accommodate more Congo red molecules. However, the RMs of PASP-ODA/DDA-EDA was less stable during storage than PASP-ODA/DDA-CDen probably due to their larger sizes.

Cell viability assays

The *in vitro* cytotoxicity of the polymers is an extremely important factor for their potential application as delivery vehicles in the pharmaceutical and cosmetic fields. In this work, the *in vitro* cytotoxicity of the polymers of PASP(45K)-ODA-CDen and PASP(45K)-ODA-EDA were evaluated using CCK8 assays against L929 cells as a function of the concentrations (Fig. 8). All the samples retained high cell viability (>70%) after 24 h of incubation at all tested concentrations up to 0.25 mg mL^{-1}. Some average cell viabilities were a little bit larger than 100% (about 110%) in Fig. 8, probably due to the experimental error of the data, with standard deviation of ~10%. Their low cytotoxicity further highlighted the polymers could be used as biomaterials.

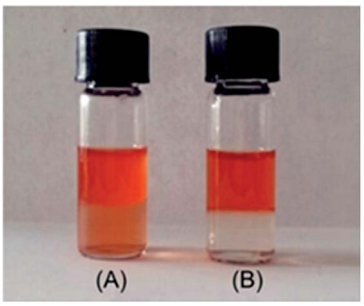


Fig. 7 The transfer of Congo red by PASP(45K)-ODA-CDen (A) and PASP(45K)-ODA-EDA (B) micellar solutions. The upper is the octanol layer, the lower is the water layer.

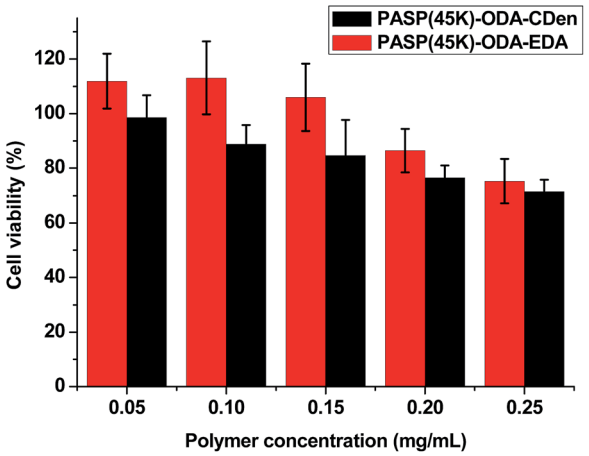


Fig. 8 Cell viabilities of polymers against L929 cells. The cells were treated with increasing concentrations of polymers, incubated for 24 h before analysis by CCK8 assay.

Conclusions

In this study, a series of amphiphilic PASP derivatives were synthesized and characterized. All copolymers could form RMs in octanol solution using the single-solvent method. The size of the RMs decreased upon heating and it can be reversible when cooling for copolymers containing CDen units. The microstructures and CACs of the RMs were explored using NEAH as a polar fluorescence probe. Their low cytotoxicity and the ability to efficient extraction of Congo red from water into octanol may be particularly interesting for the application of sustained drug delivery systems and nanocontainers.

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Notes and references

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