Available online at www.sciencedirect.com
ScienceDirect
Progress in Natural Science 19 (2009) 1305–1310
Progress in Natural Science
www.elsevier.com/locate/pnsc

PEG-g-poly(aspartamide-co-*N*,*N*-dimethylethylenediamino aspartamide): Synthesis, characterization and its application as a drug delivery system

Dong Yang, Xiaohong Zhang, Li Yuan, Jianhua Hu *

Key Laboratory of Molecular Engineering of Polymers (Ministry of Education), Laboratory of Advanced Materials, Department of Macromolecular Science, Fudan University, Shanghai 200433, China

Received 1 January 2009; received in revised form 10 February 2009; accepted 16 February 2009

Abstract

PEG-g-poly(aspartamide-co-*N*,*N*-dimethylethylenediamino aspartamide) (PEG-DMEDA-PASP) was synthesized by two-step ring-opening reactions of polysuccinimide (PSI) with α-methoxy-ω-amino-poly(ethylene glycol) and *N*,*N*-dimethylethylenediamine. The polymer structure was confirmed by ^{1}H NMR and FT-IR. The resultant PEG-DMEDA-PASP with ammonium glycyrrhizinate (AMG) could form polymeric micelles in aqueous solution. The results of transmission electron microscopy (TEM) and dynamic light scattering (DLS) measurements revealed that these polymeric micelles were spherical particles with a narrow diameter distribution and that their average diameter was *ca*. 70 nm. These polymeric micelles had high-loading capacity (58%) and encapsulation efficiency (70%) for AMG. The results of *in vitro* release experiments showed that these polymeric micelles possessed sustained-release effects, with a release rate of 25% within 3 h and 90% within 24 h.

Keywords: PEG-g-poly(aspartamide-co-*N*,*N*-dimethylethylenediamino aspartamide); Polymeric micelle; Drug delivery system; Ammonium glycyrrhizinate

1. Introduction

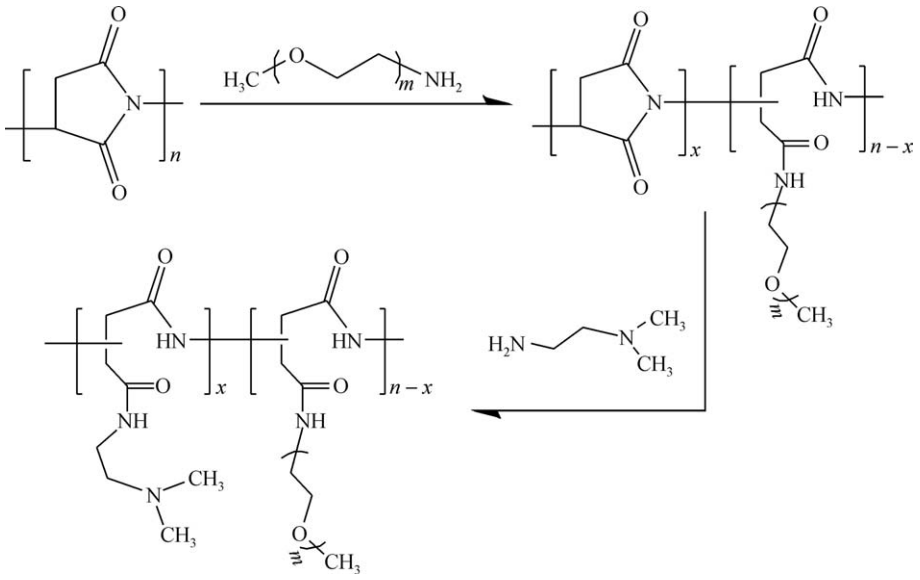
Poly(aspartic acid) (PASP) and its derivatives have attracted widespread concerns due to their low toxicity, excellent biocompatibility and biodegradability [1–4] and show even greater importance in biomedicine applications, such as gene delivery [5,6] and drug delivery [2,7].

PASP possesses multifunctional character and can afford a variety of modifications through simple chemical procedures [5–8]. It is convenient to purposefully synthesize PASP derivatives with different physicochemical properties, which make them a good candidate for obtaining polymeric micelle drug delivery systems. To date, many PASP derivatives have been synthesized and some of them have been applied to gene or drug delivery. Jiang et al. have reported the synthesis of PASP-g-octadecyl-g-PEG and investigated its drug delivery properties [7]. Previously, we have synthesized two series of PASP derivatives, poly(succinimide-co-*N*-propyl aspartamide) (PSI-PA) and poly(*N*-dodecyl aspartamide-co-*N*-propyl aspartamide) (PDDA-PA) and found that PSI-PA is suitable as a drug delivery system [9].

In this paper, we report the synthesis of a new type of PASP derivative, PEG-g-poly(aspartamide-co-*N*,*N*-dimethylethylenediamino aspartamide) (PEG-DMEDA-PASP), by two-step ring-opening reactions of polysuccinimide (PSI), as shown in Scheme 1. The resultant PEG-DMEDA-PASP with ammonium glycyrrhizinate (AMG) could form spherical polymeric micelles in aqueous

* Corresponding author. Tel.: +86 215 5665280; fax: +86 216 5649416.
E-mail address: hujh@fudan.edu.cn (J. Hu).

1002-0071/$ - see front matter
doi:10.1016/j.pnsc.2009.02.008



Scheme 1. The synthetic route of PEG-DMEDA-PASP.

solution, and these polymeric micelles had high-loading capacity and encapsulation efficiency for AMG. The *in vitro* release behavior of AMG in these polymeric micelles was also investigated.

2. Experimental

2.1. Materials

L-Aspartic acid was purchased from Shanghai Biochemical Reagent Company. α-Methoxy-ω-amino-poly(ethylene glycol) 5000 (MeOPEG-NH_2 5000) was purchased from Fluka. *N*,*N*-Dimethylethylenediamine (DMEDA, purity > 95%) was purchased from Sigma–Aldrich. Ammonium glycyrrhizinate (AMG), *N*,*N*-dimethyl formamide (DMF), tetrahydrofuran (THF) and ethyl ether were purchased from Shanghai Chemical Reagent Corporation. Polysuccinimide (PSI), with a molecular weight of Mn = 4.9×10^3, was synthesized according to the procedure as published in ref. [10].

2.2. Synthesis of PEG-grafted poly(aspartic acid) (PEG-PASP)

PEG-PASP was synthesized according to our previous work [11]. In a typical reaction, 2.0 g PSI was dissolved in 10 mL DMF under stirring. Then, a DMF solution containing 5.0 g MeOPEG-NH_2 was slowly added drop by drop to the PSI solution, and the mixture was stirred at 60 °C for 8 h. The resultant mixture was precipitated in ice-cold ethyl ether, the precipitation was washed four times with THF to remove the unreacted MeOPEG-NH_2, and the final product was dried under vacuum for 8 h.

2.3. Synthesis of PEG-g-poly(aspartamide-co-N,N-dimethylethylenediamino aspartamide) (PEG-DMEDA-PASP)

A suitable amount of PEG-PASP was dissolved in DMF in an ice-water bath and stirred. Then, an excessive dose of DMEDA was slowly added drop by drop to the solution of PEG-PASP and the mixture was stirred at 40 °C for 16 h. The resultant mixture was precipitated in ice-cold ethyl ether, the precipitation was washed four times with ethyl ether, and was then filtered to obtain PEG-DMEDA-PASP, a pale yellow solid powder. Finally, the powder was dried under vacuum for 8 h.

2.4. Preparation of the polymeric micelle of AMG and PEG-DMEDA-PASP

AMG (20 mg) was added to a 5-mL aqueous solution of 10 mg PEG-DMEDA-PASP. After sonication for 15 min, a blue dispersion was obtained. The dispersion was dialyzed for 72 h using a dialysis tubing and then transferred into a little bottle for further measurements.

2.5. Instruments and measurements

^{1}H NMR analysis was carried out on a Bruker DMX500 Spectrometer with DMSO-d_6 as the solvent. FT-IR spectroscopy was carried out on a Nicolet Magna 550 spectrometer using KBr pallets. Dynamic light scattering (DLS) measurement was performed on a Malvern Autosizer 4700 at an applied laser wavelength (λ) of 514.5 nm. GPC measurement was carried out on a HP series 1100 chromatograph equipped with Zorbax columns and RI/UV dual-mode detectors. The elution solution is 0.1 mol/L $NaNO_3$ solution, and the elution rate is 0.5 mL/min using standard PEG as calibration. Transmission electron

microscopy (TEM) images were obtained on a Hitachi H-600 transmission electron microscope. The UV–vis spectroscopic measurement was carried out on a Perkin-Elmer Lambda 35 UV/vis spectrometer. HPLC was performed on a Shimazu LC-4A high-performance liquid chromatograph.

3. Results and discussion

3.1. Synthesis and characterization of PEG-DMEDA-PASP

PEG-g-poly(aspartamide-co-*N*,*N*-dimethylethylenediamino aspartamide) (PEG-DMEDA-PASP) was synthesized by two-step ring-opening reactions of PSI. Fig. 1 shows the ^{1}H NMR spectra of the resultants of the two-step ring-opening reaction, *i.e.* PEG-PASP and PEG-DMEDA-PASP. As shown in Fig. 1(a), the ^{1}H NMR spectrum of PEG-PASP shows several distinct signals, which can be assigned as follows: $\delta = 5.3$ ppm, the signal of methyne proton (–CH–) of the repeating succinimide unit; $\delta = 4.7$ ppm, the signal of methyne proton (–CH–) of the repeating succinimide unit after ring opening by PEG. From the ratio of the peak area at 4.7 ppm to that at 5.3 ppm, the molar percentage of the ring opening of succinimide groups in PSI by MeOPEG-NH_2 can be evaluated as *ca.* 10%. It can be clearly seen from Fig. 1(b) that after the second ring-opening reaction by excessive DMEDA, the signal of methyne proton (–CH–) of the repeating succinimide unit at 5.3 ppm completely disappeared, indicating that all the residual succinimide units reacted with DMEDA. In addition, new signals of methyl and methylene protons of DMEDA appeared at $\delta = 2.1$–2.3 ppm.

The FT-IR spectra of PSI, PEG-PASP and PEG-DMEDA-PASP are shown in Fig. 2. After the ring-opening reaction of PSI by MeOPEG-NH_2, besides the carbonyl group (C═O) vibration peak centered at 1718 cm^{-1}, two new peaks at 1665 cm^{-1} (amide I) and 1540 cm^{-1} (amide II) appeared in the FT-IR spectrum of PEG-PASP, which indicated that partial succinimide units reacted with MeO-PEG-NH_2 to form an amido bond. In the FT-IR spectrum of PEG-DMEDA-PASP, the C═O peak at 1718 cm^{-1} completely disappeared, replaced by two much stronger characteristic peaks of an amido bond at 1665 cm^{-1} (amide I) and 1540 cm^{-1} (amide II), which suggested the complete ring opening of succinimide groups of PSI. This result was in agreement with that of ^{1}H NMR.

3.2. Application of PEG-DMEDA-PASP as a drug delivery system

Glycyrrhizin is of clinical interest for the treatment of chronic hepatitis C and inflammatory diseases [12–15]. As shown in Scheme 2(a), the structure of glycyrrhizin contains three carboxyl groups. Ammonium glycyrrhizinate (AMG) is the resultant, in which one of the carboxyl groups is neutralized by ammonia. The solubility of AMG in deionized water is relatively low, *ca.* 1 mg/mL at room temperature (25 °C). While AMG was added into the aqueous solution of PEG-DMEDA-PASP, the complex would be formed between the carboxyl groups in AMG and the quaternary amine groups in DMEDA. Thus, the complex of AMG and PEG-DMEDA containing hydrophilic PEG chains and hydrophobic AMG would self-assemble to form polymeric micelles, just like the behavior of amphiphilic block copolymers [16–18]; the schematic process of micelle formation is shown in Scheme 2(b).

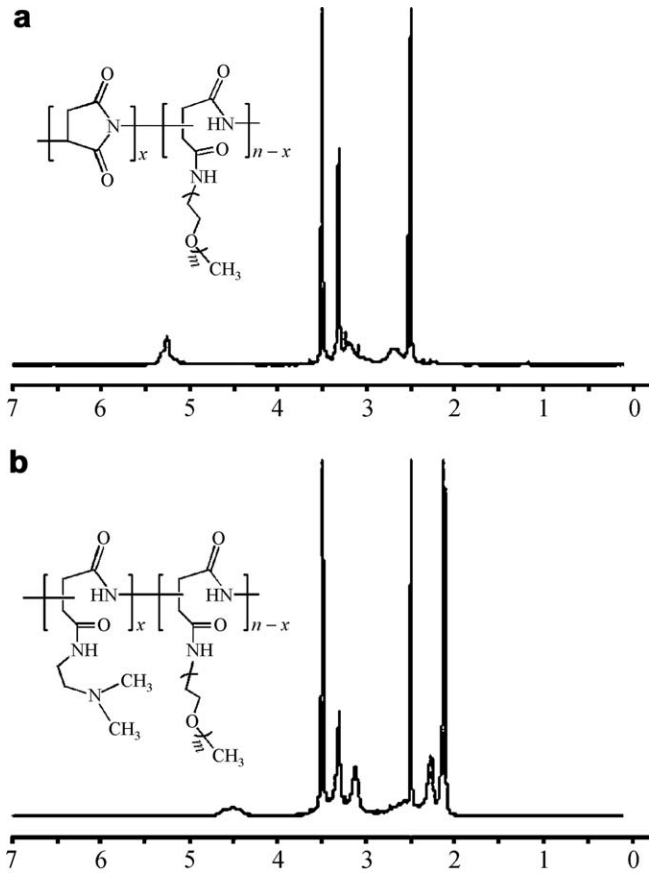


Fig. 1. ^{1}H NMR spectra of PEG-PASP (a) and PEG-DMEDA-PASP (b).

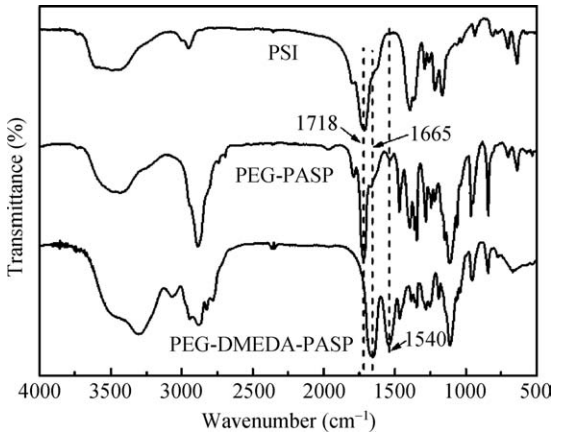
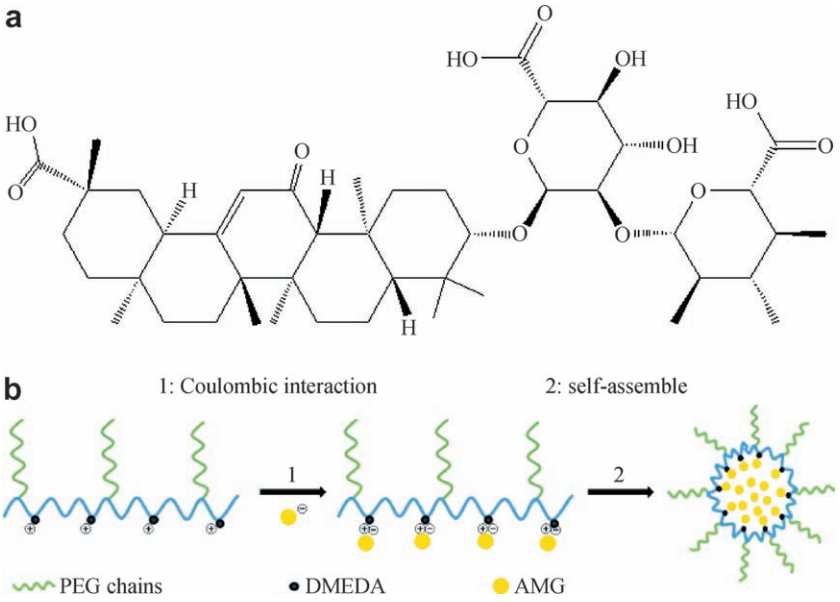


Fig. 2. FT-IR spectra of PSI, PEG-PASP and PEG-DMEDA-PASP.

The morphology of the polymeric micelle of PEG-DMEDA-PASP and AMG was investigated by TEM and DLS. As seen in Fig. 3(a), the shape of the polymeric micelle was close to a sphere, with an average diameter of about 70 nm, which is consistent with the result of DLS (Fig. 3(b)).

To measure the loading capacity and encapsulation efficiency of AMG, the following experiment was conducted. AMG (100 mg) was added to a 60-mL aqueous solution containing 50 mg PEG-DMEDA-PASP. After sonicating for 15 min and stirring for 1 h, the solution was filtered through an ultrafiltration membrane with a permeable molecular weight of 1000. The concentration of AMG in the filtrate measured by HPLC was 0.5 mg/mL, and the total mass of AMG in the filtrate was 30 mg. Thus, the amount of AMG in the polymeric micelle was 70 mg. Based on this, it could be evaluated that the loading capacity of AMG was 58% and the encapsulation efficiency of AMG was 70%.

The *in vitro* release behavior of AMG in the polymeric micelle was investigated. AMG (30.8 mg) was added to a 10-mL aqueous solution of 20 mg PEG-DMEDA-PASP. After sonication for 15 min, the solution was transferred into a dialysis tubing with a permeable molecular weight of 4000, and then the dialysis tubing was immersed in 200 mL of 0.01 mol/L phosphate buffer saline (PBS) to dialyze at 37 °C. The dialysate (1 mL) was collected at regular intervals, and then 1 mL of supplementary PBS was added



Scheme 2. (a) The molecular structure of glycyrrhizin and (b) the micelle formation process of AMG with PEG-DMEDA-PASP in water.

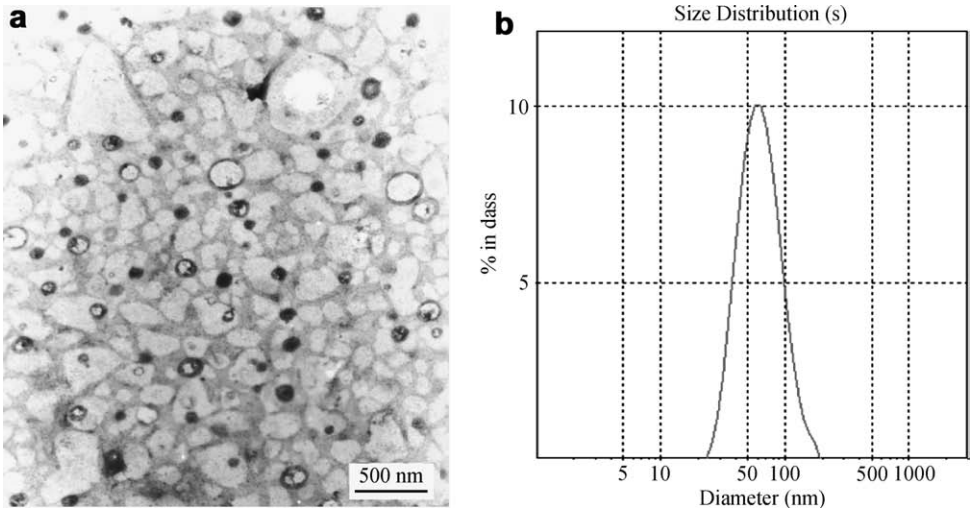


Fig. 3. (a) The TEM image and (b) DLS spectrum of the micelle of AMG and PEG-DMEDA-PASP. The TEM sample was stained by 1% phosphotungstic acid.

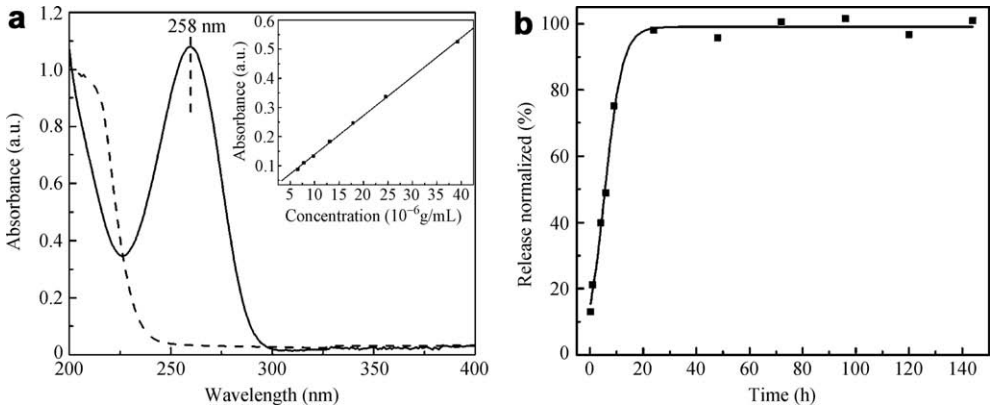


Fig. 4. (a) The UV–vis spectra of AMG (solid lines) and PEG-DMEDA-PASP (dashed lines), the inset is the calibration curve of AMG in aqueous solution. (b) *In vitro* release profile of micelles of AMG and PEG-DMEDA-PASP in PBS at 37 °C.

to keep the total volume of PBS to 200 mL. The collected samples were measured using UV–vis.

As shown in Fig. 4(a), AMG shows a strong absorption peak at 258 nm, while PEG-DMEDA-PASP has no absorption at this wavelength. So the wavelength of 258 nm was chosen to make the calibration curve of AMG in aqueous solution (Fig. 4(a) inset). From the release profile of Fig. 4(b), it could be found that the release rate of AMG was 25% within 3 h and 90% within 24 h. In the control experiment of *in vitro* release of AMG without PEG-DMEDA-PASP, the release rate of AMG was 90% within 3 h and 99.8% within 5 h [19], which was much faster than the situation of the polymeric micelle of AMG and PEG-DMEDA-PASP. Therefore, PEG-DMEDA-PASP was a suitable carrier for AMG for the sustained release of AMG and for efficiently exerting the drug effect.

4. Conclusions

In summary, a new type of PASP derivative, PEG-g-poly(aspartamide-co-*N*,*N*-dimethylethylenediamino aspartamide) (PEG-DMEDA-PASP), was synthesized by the ring-opening reaction of polysuccinimide (PSI). The prepared PEG-DMEDA-PASP could interact with ammonium glycyrrhizinate (AMG) to form polymeric micelles in aqueous solution. These polymeric micelles were spherical with a narrow diameter distribution, and the average diameter was *ca*. 70 nm. These polymeric micelles had a high-loading capacity (58%) and encapsulation efficiency (70%) for AMG. The results of *in vitro* release experiments showed that these polymeric micelles possessed sustained-release effects, with a release rate of 25% within 3 h and 90% within 24 h, which exhibited a great potential in its application as a drug delivery system.

Acknowledgements

This work was supported by the China Postdoctoral Science Foundation (Grant No. 20080440569), Shanghai Postdoctoral Scientific Program (Grant No. 09R21410900), the National Natural Science Foundation of China (Grant No. 50873029), and the Shanghai Scientific and Technological Innovation Project (Grant No. 08431902300).

References

[1] Frankel M, Berger A. Synthesis of poly-aspartic acid. Nature 1949;163:213–4.
[2] Xiong XB, Mahmud A, Uludag H, et al. Conjugation of arginine-glycine-aspartic acid peptides to poly(ethylene oxide)-b-poly(epsilon-caprolactone) micelles for enhanced intracellular drug delivery to metastatic tumor cells. Biomacromolecules 2007;8:874–84.
[3] Nakato T, Yoshitake M, Matsubara K, et al. Relationships between structure and properties of poly(aspartic acid)s. Macromolecules 1998;31:2107–13.
[4] Shogren RL, Willett JL, Westmoreland D, et al. Properties of copolymers of aspartic acid and aliphatic dicarboxylic acids prepared by reactive extrusion. J Appl Polym Sci 2008;110:3348–54.
[5] Yan-Ming W, Zhang ZF, Yu Q, et al. Properties of poly[L-(aspartic acid)-co-L-lysine] grafted with cholesterol conjugated low molecular weight poly(ethylenimine) for gene delivery. Chem J Chin Univ 2008;39:2011–4.
[6] Kakizawa Y, Miyata K, Furukawa S, et al. Size-controlled formation of a calcium phosphate-based organic-inorganic hybrid vector for gene delivery using poly(ethylene glycol)-block-poly(aspartic acid). Adv Mater 2004;16:699–702.
[7] Jiang TY, Wang ZY, Chen C, et al. Poly(aspartic-acid) derivatives as polymeric micelle drug delivery systems. J Appl Polym Sci 2006;101:2871–8.
[8] Wang Y, Wang YN, Wu GL, et al. pH-responsive self-assembly and conformational transition of partially propyl-esterified poly(alpha, beta-l-aspartic acid) as amphiphilic biodegradable polyanion. Colloids Surf B Biointerfaces 2009;68:13–9.
[9] Chen HR, Xu W, Chen TY, et al. 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. Polymer 2005;46:1821–7.
[10] Xu W, Li L, Yang WL, et al. Amphiphilic hexylamine modified polysuccinimide: synthesis, characterization, and formation of nanoparticles in aqueous medium. J Macromol Sci Pure Appl Chem 2003;A40:511–23.
[11] Chen W, Chen HR, Hu JH, et al. Synthesis and characterization of polyion complex micelles between poly(ethylene glycol)-grafted

poly(aspartic acid) and cetyltrimethyl ammonium bromide. Colloids Surf Physicochem Eng Aspects 2006;278:60–6.
[12] Ploeger B, Mensinga T, Sips A, et al. The pharmacokinetics of glycyrrhizic acid evaluated by physiologically based pharmacokinetic modeling. Drug Metab Rev 2001;33:125–47.
[13] Kimura M, Moro T, Okamoto H, et al. In vivo glycyrrhizin accelerates liver regeneration and rapidly lowers serum transaminase activities in 70% partially hepatectomized rats. J Pharm Sci 2008;106:257P.
[14] Kumada H. Long-term treatment of chronic hepatitis C with glycyrrhizin [stronger neo-minophagen C (SNMC)] for preventing liver cirrhosis and hepatocellular carcinoma. Oncology 2002;62:94–100.
[15] Nakata N, Kira Y, Yabunaka Y, et al. Prevention of venous thrombosis by preoperative glycyrrhizin infusion in a rat model. J Orthop Sci 2008;13:456–62.
[16] Savic R, Eisenberg A, Maysinger D. Block copolymer micelles as delivery vehicles of hydrophobic drugs: micelle–cell interactions. J Drug Target 2006;14:343–55.
[17] Aliabadi HM, Shahin M, Brocks DR, et al. Disposition of drugs in block copolymer micelle delivery systems – from discovery to recovery. Clin Pharmacokinet 2008;47:619–34.
[18] Humpolickova J, Prochazka K, Hof M, et al. Fluorescence correlation spectroscopy using octadecylrhodamine B as a specific micelle-binding fluorescent tag; light scattering and tapping mode atomic force microscopy studies of amphiphilic water-soluble block copolymer micelles. Langmuir 2003;19:4111–9.
[19] Zheng YL, Wu W, Yang WL, et al. Preparation, characterization, and drug release in vitro of chitosan–glycyrrhetic acid nanoparticles. J Pharm Sci 2006;95:181–91.