Available online at www.sciencedirect.com
SCIENCE DIRECT
Colloids and Surfaces A: Physicochem. Eng. Aspects 278 (2006) 60–66
COLLOIDS AND SURFACES A
www.elsevier.com/locate/colsurfa

Synthesis and characterization of polyion complex micelles between poly(ethylene glycol)-grafted poly(aspartic acid) and cetyltrimethyl ammonium bromide

Wei Chen, Haoran Chen, Jianhua Hu, Wuli Yang, Changchun Wang *

Department of Macromolecular Science and Key Laboratory of Macromolecular, Engineering of Polymers, Fudan University, Shanghai 200433, China

Received 24 August 2005; received in revised form 29 November 2005; accepted 30 November 2005
Available online 18 January 2006

Abstract

Graft copolymers, poly(ethylene glycol)-grafted poly(aspartic acid) (PEG-*g*-PAsp), were synthesized by ring opening reaction of polysuccinimide with α-methoxy-ω-amino-poly(ethylene glycol) (PEG-NH_2) and sodium hydroxide. Through changing the amount of PEG-NH_2, varying grafting degrees of PEG-*g*-PAsp could be obtained. Polyion complex micelles (PIC) were prepared by mixing PEG-*g*-PAsp with CTAB in water. When the carboxyl groups were exactly neutralized by CTAB, the formed micellar solution had maximal turbidity and minimal polydispersity. The CAC of polymer–surfactant complex was much smaller than the CMC of the pure surfactant CTAB. ξ-Potential of the complex micelles was studied by varying the ratio of PEG-*g*-PAsp and CTAB. The polyion complex micelles were spherical particles with clear core/shell structures. The composition of the graft copolymers had great influence on the particle size of the micelles. The higher the PEG content in the graft copolymer, the smaller the size of PIC micelles.

Keywords: Polyion complex micelles; Poly(ethylene glycol)-grafted poly(aspartic acid) (PEG-*g*-PAsp); Cetyltrimethyl ammonium bromide (CTAB)

1. Introduction

"Polyion complex (PIC) micelles", formed by electrostatic interaction in an aqueous medium, have been intensively investigated for many years [1–4]. This system has been widely used in gene and drug delivery systems [5–8]. Unlike polyion complexes formed from an oppositely charged pair of simple homopolymers, PIC micelles were formed by block copolymer, in which one part is charged segment and the other part is neutral polymer chain; the whole molecule is totally water-soluble and narrowly distributed [9].

Polymer–surfactant complexes could form typical PIC micelles, which have been attracting more and more attention recently [10,11]. The polymer always contains a nonionic water-soluble segment, e.g. poly(ethylene glycol) (PEG) and an ionic segment which can been neutralized by oppositely charged surfactant to form hydrophobic core. The electrostatic interaction between the ionic segments of the polyion and the surfactant head groups drives these segments from water soluble to insoluble, leading to a hydrophobic domain in situ. The nonionic water-soluble segments then form a water soluble shell to stabilize the hydrophobic domain and form PIC micells [12].

The self-assembly of the polyion complexes is usually from the polymer–surfactant complexes. It has been shown that the complexes between poly(ethylene oxide)-*block*-poly(sodium methacrylate) (PEG-*b*-PMANa) and cationic surfactants spontaneously formed small vesicles [13]. The complexes between poly(ethylene oxide)-*graft*-poly(ethyleneimine) (PEG-*g*-PEI) and alkyl sulfates could form different morphologies, from sphere to cylinder. It is possible that the branching in PEI segment and graft copolymer architecture confine the formation of continuous lamella-like structures, which is necessary to form the vesicles [14].

In this paper, a new type of biocompatible graft copolymers, the poly(ethylene glycol)-grafted poly(aspartic acid) (PEG-*g*-PAsp) with different grafting degrees, were synthesized, the complex micelles between the copolymer of PEG-*g*-PAsp and cationic surfactant cetyltrimethyl ammonium bromide (CTAB)

* Corresponding author. Tel.: +86 21 65642385; fax: +86 21 65640293.
E-mail address: ccwang@fudan.edu.cn (C. Wang).

0927-7757/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.colsurfa.2005.11.084

were studied. The properties of polyion complex micelles including polydispersity, hydrodynamic diameter, critical aggregate concentration (CAC), ξ-potential and morphology were studied. The successful complexing of PEG-*g*-PAsp with CTAB showed that this polymer could be further used in complexing with ionic drugs, enzyme and DNA.

2. Experiment

2.1. Materials

L-Aspartic acid was purchased from Shanghai biochemical reagent company, and was recrystallized three times from ethyl acetate before use. α-Methoxy-ω-amino-poly(ethy1ene glycol) 5000, (PEG-NH_2 5000), was purchased from Fluka, and was used without further purification. Cetyltrimethyl ammonium bromide was purchased from Nanjing Xuanguang technology company, and was used without further purification. *N,N*-Dimethylformamide (DMF) was distilled and dried via 4 Å molecular sieve before use, tetrahydrofuran (THF) was used as received.

2.2. Synthesis of poly(ethylene glycol)-grafted poly(aspartic acid) (PEG-g-PAsp)

Polysuccinimide (PSI) was synthesized from L-aspartic acid according to the procedure as published in ref. [15]. In order to prepare the graft copolymer, a suitable amount of PEG-NH_2 and PSI were dissolved in DMF, respectively, the PEG-NH_2 solution was slowly dropped into the solution of PSI, and the mixture was stirred at 60 °C for 8 h. The resultant was precipitated in the ice-cold ethyl ether, the precipitate was washed several times with THF to remove the unreacted PEG-NH_2, and the final product was dried under vacuum for 8 h. With the change of the ratio of PSI and PEG-NH_2, the copolymer with different grafting degrees could be prepared. Here we define the grafting degree (GD) as the ratio of the reacted pentacyclic ring of PSI with PEG-NH_2 to the total amount of pentacyclic ring of PSI. In order to open the residual pentacyclic ring on the copolymer chain, a certain amount of PEG-*g*-PSI was dispersed in water, then excess sodium hydroxide aqueous solution was dropped into the water solution. The mixture was stirred at room temperature for 3 h until the mixture became completely transparent. The pH of the mixture solution was adjusted to 10 (all carboxyl groups of the graft copolymer were ionized) [14] and the solution was diluted into a certain concentration for further use.

2.3. Preparation of polyion complex micelles between PEG-g-PAsp and CTAB

A suitable amount of CTAB solution was added to PEG-*g*-PAsp solution under stirring, the pH of the mixture solution was adjusted to 10, and all these solutions were filtrated by 0.45 μm syringe-type filtrator before further measurement. The concentration of the carboxyl groups of PEG-*g*-PAsp and the pH of the system were respectively maintained at 0.2 mM and 10 in the whole preparation and further measurement process, but the ratio of surfactant to carboxyl group was changed from 0 to 6 for further measurement.

2.4. Characterization techniques

^{1}H NMR analysis was carried out on Philips DMX500 Spectrometer with DMSO-d_6 as the solvent. Turbidity measurement was carried out on a Spectrumlab 22pc spectrophotometer at 420 nm. The turbidity data was recorded as $(100 - T)/100$, where T is transmittance (%). Dynamic light scattering (DLS) measurement was performed on a Malvern Autosizer 4700, and the laser wavelength (λ) applied in the measurements was 514.5 nm. Fluorescence measurement (steady-state) was carried on an Edinburgh Instruments FLS920 spectrophotometer using quartz cell. The excitation wavelength was 330 nm and the width of both excitation and emission slits was 5.0 nm. Pyrene was used as the fluorescent probe (excitation wavelength at 339 nm). ξ-Potential measurement was carried out on a Zetasizer Nano ZS Zeta Potential Analyzer. The morphology of the complex micelles was studied using a Hitachi H-600 microscope. The negative staining technique was used for the transmission electron microscopy (TEM) studies. A drop of sample solution was allowed to settle on copper grid for 1 min. Excess sample was wicked away with filter paper and a drop of 1% phosphotungstic acid was allowed to contact with the sample for 1 min.

3. Results and discussion

3.1. The synthesis of graft copolymer

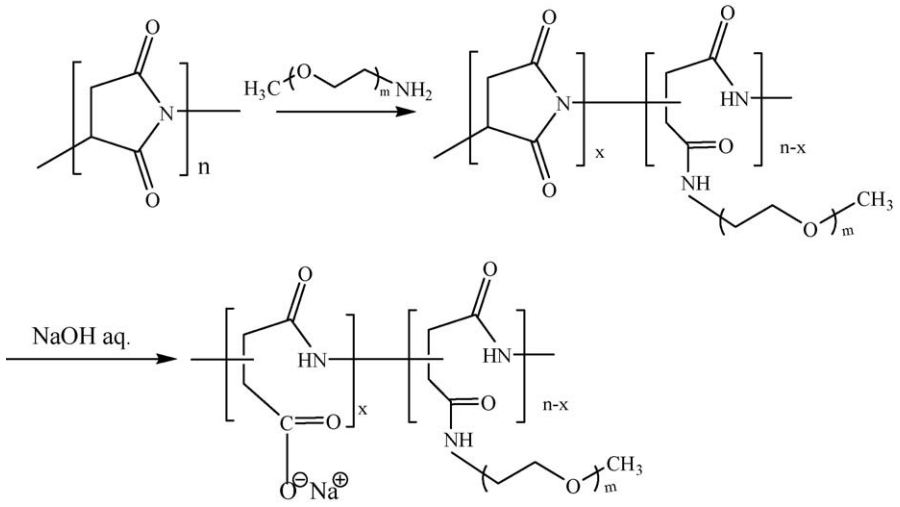
The synthesis procedure of poly(ethylene glycol)-grafted poly(aspartic acid) is showed in Scheme 1.

PEG-NH_2 was directly reacted with PSI to prepare the copolymers of PEG-*g*-PAsp (GD = 1.5 and 7.5%). The exact GD was calculated from ^{1}H NMR of PEG-*g*-PSI, which is shown in Fig. 1. The signal of methene proton (a) in main chain is located at 2.73–3.06 ppm, the signal of methene proton (b) of repeating PEG unit is located at 3.36–3.78 ppm, the signal of methyne proton (c) of the repeating succinimide unit is located at 5.14 ppm, the signal of methyne proton (d) of the repeating succinimide unit after grafting is located at 4.49 ppm. The sharp peaks at 2.7, 2.9 ppm correspond to DMF and the sharp peaks at 3.09, 3.24 ppm correspond to ethyl ether. The very sharp peaks at 2.5 and 3.3 ppm correspond to DMSO and water, respectively. From the integration area of proton in Fig. 1(b), GD could be calculated by the following equation:

$$\mathrm{GD} = \frac{S_d}{S_d + S_c}$$ (S is the integration area of corresponding peaks).

3.2. The turbidity and DLS study of polyion micelles solution

Here, the concentration of CTAB in the mixture was defined as C_t, the concentration of carboxyl groups of PEG-*g*-PAsp as C_i, $Z = C_t/C_i$. When $Z = 1$, the carboxyl groups were exactly neutralized by oppositely charged CTAB. When $0 < Z < 1$, there were excess carboxyl groups in the solution, oppositely, when



Scheme 1. The overall synthetic route of poly(ethylene glycol)-grafted poly(aspartic acid) (PEG-*g*-PAsp).

$Z > 1$, there was excess CTAB. The relation of turbidity and Z (GD = 1.5 and 7.5%) of the solution was showed in Fig. 2.

It can be concluded that when $0 < Z < 1$, the turbidity of the solution increased as the concentration of CTAB increased, indicative of the formation of polyion complex micelles. The gradually increasing trend levelled off when Z reached 1, which indicated that the carboxyl groups were completely neutralized by CTAB, hereafter, the continuous increase of the concentration of CTAB ($Z > 1$) would not change the value of the turbidity because carboxyl groups were completely complexed, suggesting no more polyion complex micelles were formed. The partly excess surfactant can form micelles in solution or absorb on the surface of PIC particles by interaction with PEG shell [14], both of which had little influence on the turbidity.

Moreover, when $Z = 1$, the complex solution of PEG-*g*-PAsp (GD = 1.5%)/CTAB was more turbid than that of PEG-*g*-PAsp (GD = 7.5%)/CTAB, due to the larger micelle size for the PEG-*g*-PAsp with a low GD.

The polydispersity of polyion complex micelles (DG = 1.5 and 7.5%) is showed in Fig. 3.

In Fig. 3, we can find that the polydispersity of the polyion micelles reached a minimum at $Z = 1$. At this composition, the carboxyl groups of PEG-*g*-PAsp were completely neutralized by CTAB, so there were no micelles formed from surfactant and no surplus carboxyl groups in the solution. Only polyion complexes present in the solution, indicated by the minimal polydispersity of the micelles. Moreover, from Table 1, the particle size of micelles of PEG-*g*-PAsp (GD = 7.5%)/CTAB was smaller than that of PEG-*g*-PAsp (GD = 1.5%)/CTAB in agreement with the turbidity measurement. The increased PEG content obviously resulted in the increased steric repulsion of micelles between the hydrophilic coronas, which can be balanced out only by the increase in the curvature of the surface of the hydrophobic core [14].

Table 1
Properties of the polyion micelles of PEG-*g*-PAsp with different GDs

Grafting degree[a] (%)	Hydrodynamic diameter (nm)[b]	Polydispersity[c]	Turbidity ($\lambda = 420$ nm)[d]
1.5	190.8	0.082	0.71
7.5	77.6	0.061	0.17

Note: $Z = 1$.
[a] The value was calculated from ^{1}H NMR spectra of PSI-*g*-PEG.
[b] The value was obtained from DLS measurement.
[c] The value was obtained from Fig. 3.
[d] The value was obtained from Fig. 2.

3.3. Study on the polyion micelles by fluorescence probe

Pyrene is a strongly hydrophobic probe and its solubility in water is very low. Fluorescence spectra of pyrene labels provide information about their local environments. The intensity ratio between the first and third highest energy (frequency) emission peaks, known as the I_1/I_3 ratio, has been shown to correlate well with solvent polarity. The I_1 peak, which arises from the (0,0) transition from the lowest excited electronic state, is a "symmetry-forbidden" transition that can be enhanced by the distortion of the π-electron cloud. On the other hand, the I_3 peak is not forbidden and thus is relatively solvent-insensitive. In a wide variety of aromatic hydrocarbons, forbidden vibronic bands in weak electronic transitions show marked intensity enhancements under the influence of solvent polarity. Thus, the ratio (I_1/I_3) serves as a measure of the polarity of the environment. In the presence of micelles and other aggregate systems, pyrene is preferentially solubilized in the interior hydrophobic regions of these aggregates. So pyrene was frequently used to examine the formation process of surfactant or polymer micelles [16,17]. When the concentration of the surfactant reached critical micelle concentration (CMC), there was a sharp decrease of the I_1/I_3 value (see Fig. 4(a)); the formation of micelles was caused by the formation of non-polar core, into which non-polar pyrene molecules preferred to reside, and the transfer of pyrene from polar environment to non-polar environment resulted in such a sharp decrease. For the polyion complex micelles system, the carboxyl groups in copolymer were neutralized by CTAB, in our experiment, we find that the critical association

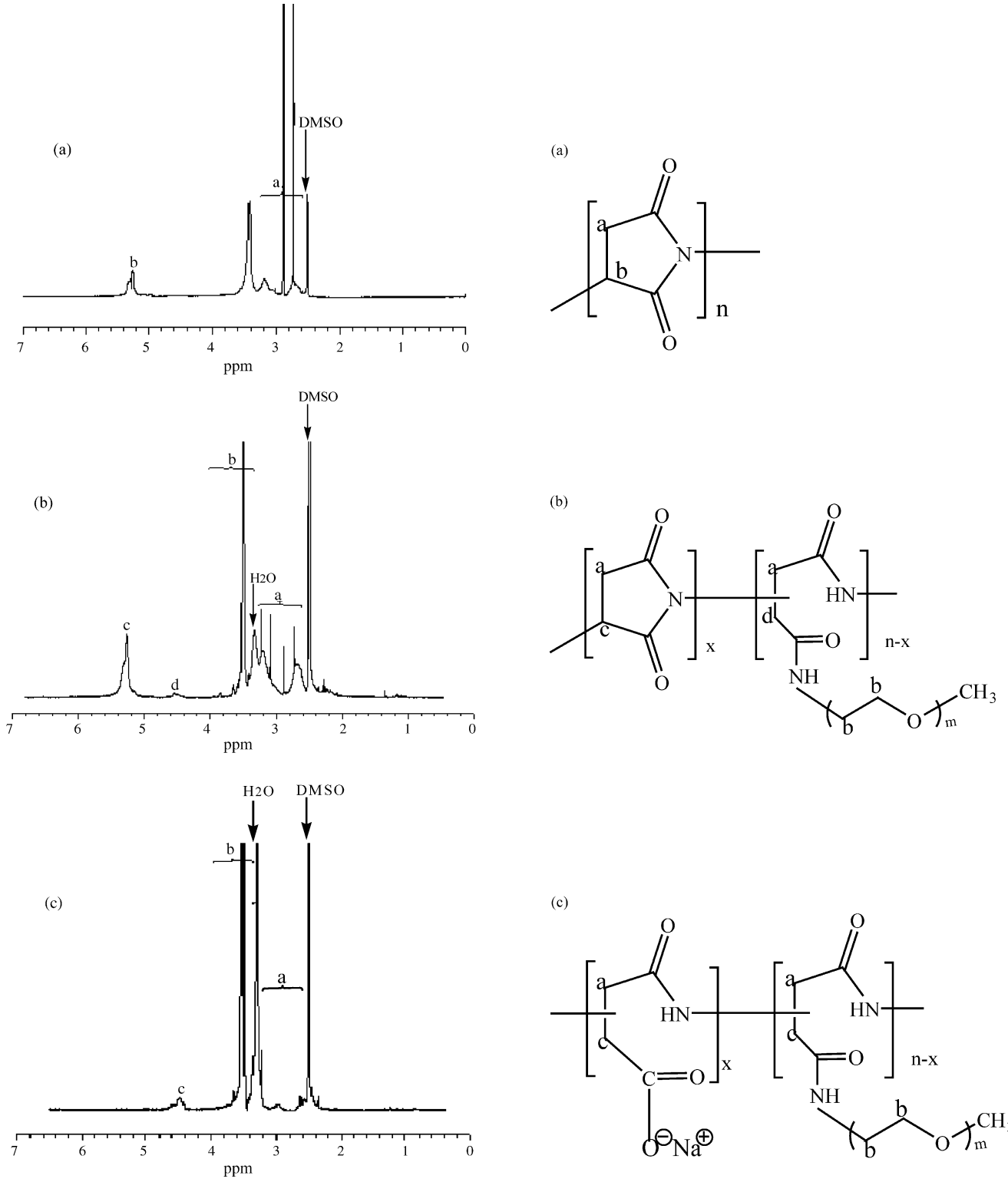


Fig. 1. ^{1}H NMR spectra of PSI (a), PEG-*g*-PSI (b) and full-opened PEG-*g*-PAsp (c).

concentration (CAC) of the complex solution was much smaller than the CMC of the pure surfactant CTAB solution, indicating the interaction between carboxyl group and CTAB is very strong. The combination of carboxyl group with CTAB led to the hydrophobic part [12], which resulted in such a low CAC value.

3.4. ξ-Potential study on the micelle solution

The ξ-potential of the complex micelle solution is presented in Fig. 5. With the increase of the CTAB amount added to the graft copolymer solution, the ξ-potential of the copolymer solution increased at $0 < Z < 1$, indicating a decrease of the negative

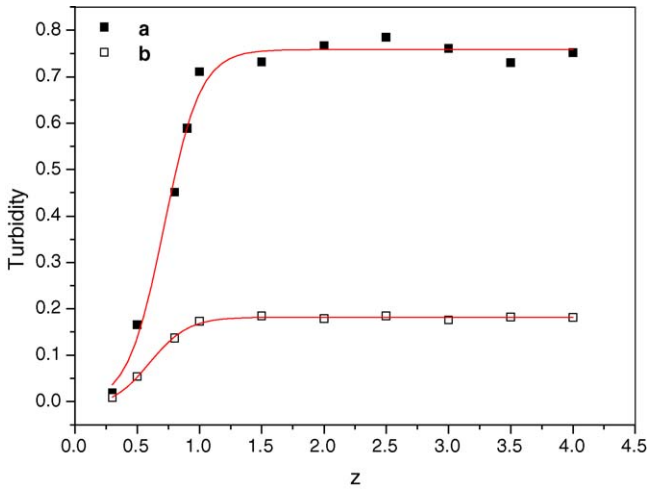


Fig. 2. The turbidity of the solution as a function of Z (a) (GD = 1.5%) and (b) (GD = 7.5%).

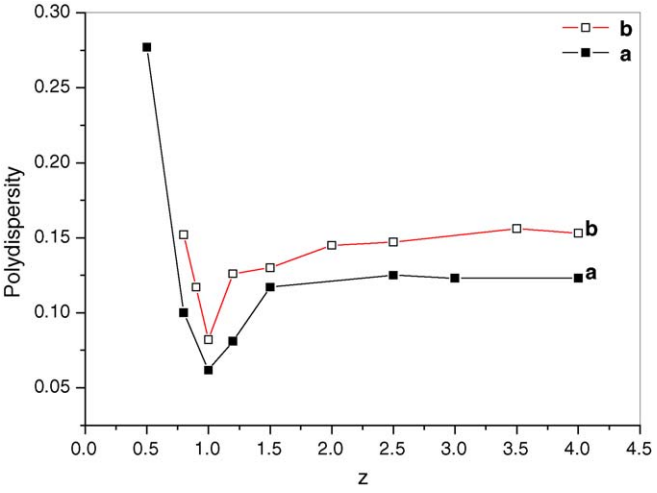


Fig. 3. The polydispersity of the micelle particles as a function of Z (a) (GD = 1.5%) and (b) (GD = 7.5%).

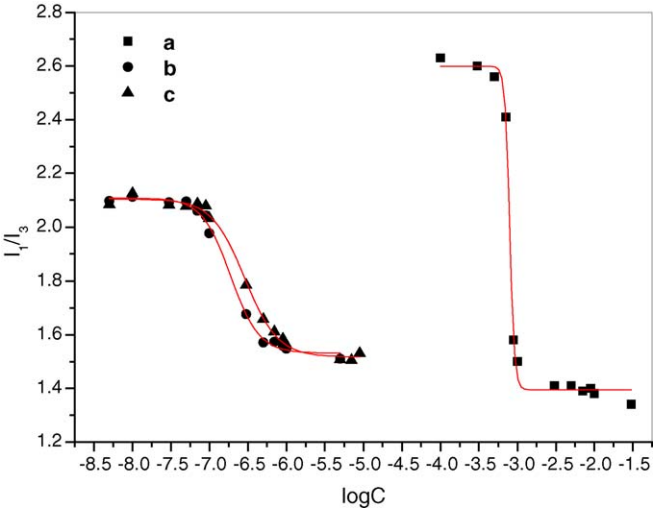


Fig. 4. I_1/I_3 value in steady-state fluorescence spectra as a function of the concentration of (a) pure CTAB, (b) PEG-*g*-PAsp (GD = 1.5%)/CTAB = 1 complex and (c) PEG-*g*-PAsp (GD = 7.5%)/CTAB = 1 complex using pyrene as molecular probe ([pyrene] = 6.00×10^{-7} M).

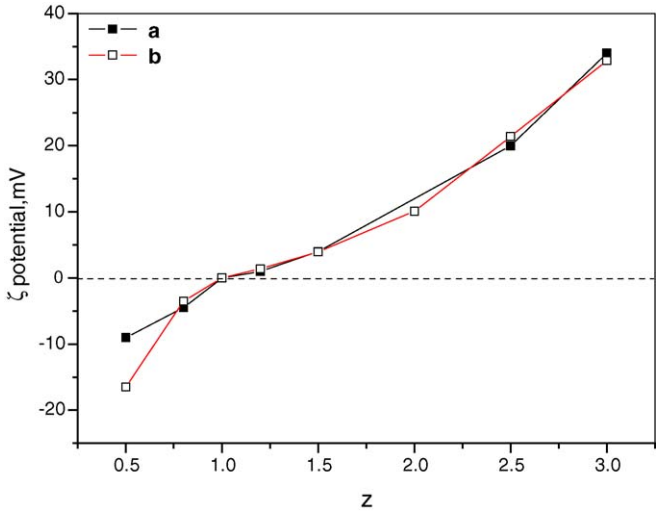


Fig. 5. ζ-Potential of the complex micelle solution as a function of Z (a) (GD = 1.5%) and (b) (GD = 7.5%) (pH 10).

charges on the micelle surface. This provided the evidence of the gradual neutralization of the anionic fragments of PEG-*g*-PAsp by the surfactant cations incorporated into the complex. At $Z = 1$, ξ-potential values were approximately zero, suggesting that the charges of the PAsp segments were completely neutralized by CTAB. This result was consistent with the assumption that all surfactant cations added to the PEG-*g*-PAsp solution form ionic bonds with the carboxyl group of PEG-*g*-PAsp at $Z = 1$. Oppositely, when $Z > 1$, ξ-potential changed from negative to positive and increased with the increase of the CTAB amount. The surplus cationic CTAB would partly interact with the PEG shell, partly form micelles and disperse in solution. Both aspects can induce the change and increase of the ξ-potential values [14].

3.5. Electron microscopy characterization

The morphologies of the complex micelles of PEG-*g*-PAsp and CTAB were investigated by transmission electron microscopy. All the samples were stained by phosphotungstic acid (negative staining). The shape of complex micelles was close to sphere, as seen in Fig. 6. The steric repulsion effects of PEG chains prevented stacking of hydrophobic core and formed a clear core-shell structure. The PEG layer cannot be stained by phosphotungstic acid and a clear white ring could be observed in Fig. 6. The dark core was the CTAB bonded PAsp. In Fig. 6, we also can find that the complex micelles of PEG-*g*-PAsp (GD = 1.5%)/CTAB have much bigger size than that of PEG-*g*-PAsp (GD = 7.5%)/CATB complex micelles, consistent with the result from DLS measurement.

From above experimental results, we propose the micelle formation procedure as showed in Scheme 2. At first, the comb-like copolymer of PEG-*g*-PAsp could be well dissolved in water, after adding a suitable amount of CTAB solution to above solution slowly at pH 10 under stirring, the micellization was induced in situ because of the strong interaction of carboxyl group and CTAB.

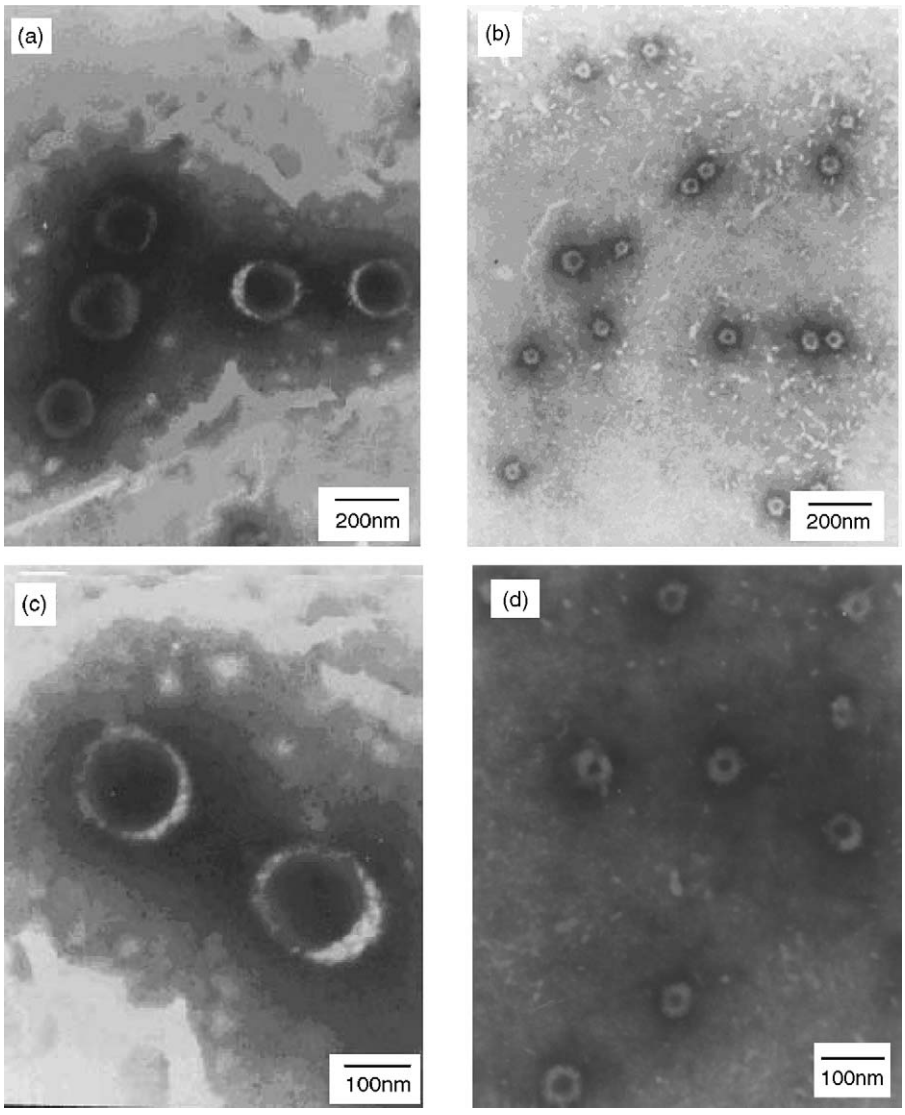
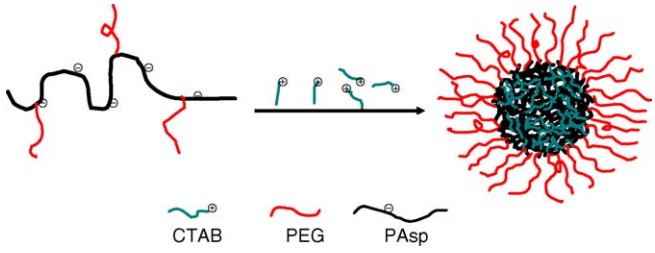


Fig. 6. TEM photographs of stoichiometric complex micelles using 1% phosphotungstic acid staining (a and c) (GD = 1.5%) and (b and d) (GD = 7.5%).



Scheme 2. The formation of polyion complex micelles.

4. Conclusion

The polymer–surfactant complex micelles between PEG-*g*-PAsp and CTAB were successfully prepared in this paper. The synthesis method provided a good model for the preparation of complex micelles containing drug, DNA or enzyme. Different GDs of PEG-*g*-PAsp could be synthesized by ring open reaction, and polyion complex micelles could be prepared by mixing of PEG-*g*-PAsp with CTAB in water conveniently. The stoichiometric complex micelles showed maximal turbidity and minimal polydispersity. The complexing of PEG-*g*-PAsp and CTAB resulted in a very low CAC of the complex solution compared with the CMC of pure CTAB solution. Although using different GDs of copolymers, the complex micelles formed similar morphology of sphere in different size which depended on the PEG content.

Acknowledgments

This work was supported by National Science Foundation of China (grant no. 20374012 and 50525310) and STCSM.

References

[1] A.V. Kabanov, T.K. Bronich, V.A. Kabanov, A. Eisenberg, Macromolecules 29 (1996) 6797.
[2] G.D. Zhang, A. Harada, N. Nishiyama, J. Controll. Rel. 93 (2003) 141.
[3] A. Harada, K. Kataoka, Langmiur 15 (1999) 4208.
[4] A. Harada, K. Kataoka, Macromolecules 31 (1998) 288.

[5] K. Itaka, K. Yamauchi, A. Harada, K. Nakamurak, Biomaterial 24 (2003) 4495.
[6] S. Katayose, K. Kataoka, Bioconj. Chem. 8 (1997) 702.
[7] M.L. Adams, A. Lavasanifar, G.S. Kwon, J. Pharm. Sci. 92 (2003) 1343.
[8] A. Kataoka, A. Harada, Y. Nagasaki, Adv. Drug Deli. Rev. 47 (2001) 113.
[9] A. Harada, K. Kataoka, Macromolecules 28 (1995) 5294.
[10] E. Castro, P. Taboada, V. Mosquera, J. Phys. Chem. B 109 (2005) 5592.
[11] K.Y. Mya, A. Sirivat, A.M. Jamieson, Macromolecules 34 (2001) 5260.
[12] T.K. Bronich, V.A. Kabanov, A.V. Kabanov, A. Eisenberg, Macromolecules 30 (1997) 3519.
[13] A.V. Kabanov, T.K. Bronich, V.A. Kabanov, A. Eisenberg, J. Am. Chem. Soc. 120 (1998) 9941.
[14] T.K. Bronich, T. Cherry, S.V. Vinogradov, A. Eisenberg, V.A. Kabanov, Langmuir 14 (1998) 6101.
[15] W. Xu, L. Li, W.L. Yang, J.H. Hu, C.C. Wang, S.K. Fu, J. Macromol. Sci: Pure Appl. Chem. 40 (2003) 511.
[16] I. Astafieva, X.F. Zhong, A. Eisenberg, Macromolecules 26 (1993) 7339.
[17] M. Suwa, A. Hashidzume, Y. Morishima, Macromolecules 33 (2000) 7884.