Controlled synthesis and association behavior of graft Pluronic in aqueous solutions

Y. Zhang, Y.M. Lam *

School of Material Science and Engineering, Nanyang Technological University, Nanyang Drive, 639798 Singapore

Received 19 June 2006; accepted 27 October 2006
Available online 11 December 2006

Abstract

Poly(vinyl pyrrolidone) (PVP) was grafted onto Pluronic F127 (PEO–PPO–PEO) to produce novel amphiphilic PVP–g–F127 graft copolymers. A controlled synthesis method was used to graft PVP onto different parts of F127. Two types of graft polymers were obtained: one has PVP grafted onto the PEO part of F127 and the other has PVP grafted onto the PPO part of F127. The association behavior of the two modified polymers was examined using differential scanning calorimetry, surface tension measurements, and dynamic light scattering.

© 2006 Elsevier Inc. All rights reserved.

Keywords: Pluronic; Graft copolymer; Poly(vinyl pyrrolidone); 1H NMR; Surface tension (ST); Dynamic light scattering (DLS)

1. Introduction

Poly(ethylene oxide)–b–poly(propylene oxide)–b–poly(ethylene oxide) triblock copolymers are of commercial importance and also excite academic interest on account of their association properties in aqueous solution. Pluronic or Poloxamer (BASF) and Synperonic (ICI) polyols are the commercial names that find widespread industrial applications as emulsifying, wetting, thickening, coating, solubilizing, stabilizing, dispersing, lubricating, and foaming agents [1–5]. The association characteristics of Pluronic, such as critical micellization concentration, critical micellization temperature, association number, micelle size, and thermodynamic properties [6–10], in the dilute regime have been widely and deeply investigated by many researchers.

In recent studies, researchers have paid more attention to the modification of Pluronic to improve its properties and obtain broader applications. One of the reasons that chemical modifications of Pluronic block copolymers are carried out is its critical micelle concentration. A good example is F127, which has a relatively high CMC due to the low hydrophobicity of PPO blocks. The Pluronic copolymers have been hydrophobically modified with polycaprolactone (PCL) [11,12]. The copolymers PCL–Pluronic–PCL are synthesized by the ring-opening polymerization of the monomer ε-caprolactone using the Pluronic copolymers as the initiator and stannous octoate (Sn(Oct)2) as the catalyst. The PCL–Pluronic–PCL copolymers can self-assemble into micelles in water at lower CMC than Pluronic copolymer. The size of micelles decreases with increasing temperature. This may be attributed to the hydrophobicity of the PPO block, which increases with increasing temperature. Hence, the interaction between the PCL block in the core and the PPO block in the shell is strengthened. Bromberg [13,14] has synthesized PEO–PPO–PEO block copolymers with poly(acrylic acid) (PAA), thereby imparting a dual responsive property (i.e., pH and temperature) to the polymer by dispersion/emulsion polymerization. The PEO–PPO–PEO–g–PAA graft polymer has a high molecular weight and extreme temperature sensitivity. Compared with Pluronic copolymers, the graft copolymers can form reversible gels with significant elastic moduli in water at lower concentration above a certain temperature. Apart from these few studies, little research has been conducted on the chemical modification of Pluronic block copolymer.

PVP is a well-known water-soluble, biocompatible, and relatively amphiphilic polymer. The highly polar amide confers hydrophilic and polar attracting properties on the polymer, while the apolar methylene group in the backbone and the methine
group in the ring contribute to its hydrophobic properties [15]. Since PVP was discovered, the applications of PVP have increased tremendously. Murnper and Alain [16] reported the use of PVP as a protective agent during plasmid delivery to muscle. Ziebell et al. [17] studied the extent of the binding affinity of PVP toward small molecules in aqueous solution. Other applications [18] took advantage of certain PVP properties, such as water solubility and formation of complexes with anion and neutral organic molecules.

Pluronic micelles, which are used to solubilize low-molecular-mass drugs, such as polypeptides, are being actively investigated as a potential drug delivery system [19–23]. Since PVP is itself a lyoprotectant, it should self-assemble in micelles, with excellent storage properties, and should be able to incorporate a variety of drugs into the inner core and the outer shell, with a high loading capacity.

The objective of the present study was to synthesize such novel graft copolymers and compare the solution behavior of the novel graft copolymers to that of the ungrafted polymers. The novel graft copolymer, F127–g–PVP, is designed to integrate the benign nature of the two polymers. It will have excellent properties when interacting with various additives.

2. Experimental

2.1. Materials

Pluronic F127 was purchased from Fluka and used without further purification. It has the formula EO$_{100}$PO$_{65}$EO$_{100}$ and a nominal molecular weight of 12,600. The molecular weight of the PPO segment is 3780 and 70 wt% of the chain is made up of PEO. N-vinyl pyrrolidone (VP) obtained from Fluka was distilled at 100°C before use.

Lauroyl peroxide (LP) (97%, redox initiator) and ammonium persulfate (98%) were obtained from Lancaster. Heptane and cyclohexane (HPLC grade) were purchased from Fisher company and used without further purification. Acetone (HPLC grade) and THF (HPLC grade) were obtained from Baker and were used as received. Poly(vinylpyrrolidone–co-1-hexadecene) (Ganex V216) (dispersion stabilizer) was supplied courtesy of International Specialty Products (Wayne, NJ) and used as received.

2.2. Synthesis of F127–g–PVP graft copolymers

Vinyl pyrrolidone and dodecane were first distilled at 100°C and then kept in sealed bottles. The rest of the chemicals were used as received unless specified. Dodecane was distilled to eliminate impurities that may have negative effects on polymerization.

Two methods of graft polymerization were used. 

Method 1. F127(PO)–g–PVP was first synthesized by dispersion polymerization. Synthesis was carried out on a laboratory scale. Pluronic (6 g) was dissolved in vinyl pyrrolidone (13 ml) under constant nitrogen purging. Lauroyl peroxide (25 mg) and 4,4′-azobis(cyanovaleric acid) (25 mg) were dissolved in 2 ml of vinyl pyrrolidone and added to the resulting solution. The resulting solution was deaerated by nitrogen bubbling for 0.5 h and added to a 250-ml three-necked flask containing 60 ml of 1 wt% solution of Ganex V-216 in dodecane. The flask was vigorously stirred by a magnetic stirrer and deaerated by constant nitrogen purging from the bottom. Then the flask was heated to 70°C using an oil bath and kept at that temperature under constant stirring and nitrogen purging. The reaction was continued for 6 h. Then the reaction was stopped, and the contents of the reactor were filtered using Whatman filter paper (retention size 10 µm) in heptane and cyclohexane. The products were repeatedly washed in acetone and THF and dried under vacuum.

Method 2. F127(PEO)–g–PVP, F127 (6 g), in aqueous solution (60 ml) was heated to 70°C using an oil bath and kept at that temperature under constant stirring and nitrogen purging. At the same time, vinyl pyrrolidone (13 ml) was heated to 70°C and transferred quickly to a 250-ml three-necked flask containing an aqueous solution of F127. Ammonium persulfate was dissolved in 2 ml of vinyl pyrrolidone and then added into the resulting solution. The reaction was kept at 70°C under constant stirring and nitrogen purging for 6 h. After that, the solution containing the product was freeze-dried. The white powder was washed using THF and acetone and dried under vacuum.

2.3. Sample preparation

Pluronic F127–g–PVP copolymers were dissolved in distilled water under gentle stirring. The solutions were centrifuged to remove any contaminant and allowed to equilibrate for more than 24 h.

3. Characterization

3.1. Fourier transform infrared spectrometer (FTIR)

A Perkin–Elmer System 2000 Fourier transform infrared spectrometer was used to characterize the polymer. First, a background spectrum was run. The copolymer sample was then thoroughly mixed with KBr powder with careful grinding and then the mixture was pressed into pellets. The spectrum of the mixed pellet was collected against the spectrum of the background. A total of 10 scans at a resolution of 4 cm$^{-1}$ (in the Mid IR region of 4000–400 cm$^{-1}$) were obtained to achieve a good signal-to-noise ratio.

3.2. Nuclear magnetic resonance (NMR)

The $^1$H NMR studies were carried out on a Bruker instrument operating at 400 MHz. CDCl$_3$ was used as the solvent and TMS was used as the internal zero reference.

3.3. Differential scanning calorimetry (DSC)

Thermal studies were performed using a DSC (DSC 2920, TA Instruments, USA), equipped with a refrigerated cooling
The aqueous solutions of copolymers (5–15 mg), were hermetically sealed into aluminum pans under dry conditions. To test the thermal responsiveness of the sample, the sample was cooled to 2°C and equilibrated for 10 min. The sample was then heated to 50°C at 5°C/min. Following that, the sample was cooled to 2°C at 5°C/min.

3.4. Surface tension (ST)

The surface tension measurement was carried out using a First Ten Angstroms system with an automatic gain control camera. The surface tension was automatically computed using the Bashforth–Adams technique. The CMC values of block and graft copolymers in aqueous solution at room temperature were obtained using surface tension measurements. The CMC of mixture of polymeric surfactants can also be obtained using ST measurements.

3.5. Dynamic light scattering (DLS)

Dynamic light scattering experiments were performed with a Brookhaven Instruments BI-200SM goniometer (Brookhaven Instruments Corporation), a BI-9000 correlator, and a Spectra Physics He–Ne Model 127 laser operating at scattering angles of θ = 45°, 90°, and 135° and an incident light wavelength of λ = 633 nm at a power of 35 mW. A 10-µl quartz scattering cell was thermostated using refractive index matching silicone oil, and the temperature was 39°C and controlled to within 0.02°C.

4. Results and discussion

4.1. Synthesis of Pluronic graft copolymers

The novel graft copolymer F127–g–PVP was synthesized by simultaneous free radical polymerization of the monomer vinyl pyrrolidone and chain transfer of the PVP radicals to F127. The mechanism of the reaction was discussed by Bromberg [24]. Systematic studies using other initiators such as AIBN (2,2′-azobisisobutyronitrile), BPO (benzyl peroxide), and V52 (2,2′-azobis(2,4-dimethylpentanenitrile) for the synthesis of PVP/F127 copolymers have been carried out. It was found that the initiator lauroyl peroxide with 4,4′-azobis(cyanovaleric acid) and ammonium persulfate appears to have the advantage of producing a polymer of higher molecular weight with better yield [25]. It is important to note that the concentration of initiator added also has a significant effect on the polymerization. Although the polydispersity of the polymer obtained is narrower with a low initiator concentration, an insufficient amount of initiator would lead to failure in the polymerization.

The polymer composition, structure, and molecular weight were characterized using FTIR and NMR. Fig. 1 shows the IR spectra for F127, homopolymer PVP, and Pluronic F127–g–PVP graft copolymer. The VP carbonyl peak occurs at around 1650 cm⁻¹. The ether bands of the Pluronic F127 can be seen near the 1100 cm⁻¹ spectral region. Since the graft copolymer Pluronic–g–PVP has both carbonyl bonds from VP and ether bonds from F127, peaks in both areas can be observed in the spectra.

An investigation as to whether the materials obtained were graft copolymers with covalent bonds or mere blends where the two polymers are physically mixed is important. A mixture of PVP and F127 was dissolved and cast from chloroform solution at room temperature. This blend was characterized before and after the extraction procedure. The extraction procedure consists of acetone extraction at room temperature. The spectra obtained for the graft copolymer were compared to the spectra obtained for the blend of PVP and F127 before and after the acetone extraction, as shown in Fig. 1. A strong reduction of the carbonyl peak of PVP after the acetone extraction for the blend can be observed. These findings strongly indicate that when there is no covalent bond connecting the PVP and F127 copolymer chain, as in the case of the blend, both components of the system could easily be separated by a selective solubilization procedure. However, the carbonyl peak for the graft copolymer was not reduced significantly.

The 1H NMR spectrum was obtained using 1% solutions of F127–g–PVP graft copolymers in CDC1₃ at 295 K as shown in Figs. 2 and 3. For the practical consideration of signal/noise, the methyl resonances of the propylene oxide segment in the PPO block and the methylene of the ethylene oxide segment in the PEO blocks were used as intrinsic probes. The major features of the NMR spectra of Pluronic F127 and F127–g–PVP copolymers in CDC1₃ are shown in Tables 1, 2, and 3 [26,27].

As mentioned previously, to control the grafting site of PVP on F127, two methods were employed. In method 1, F127 and VP monomer were reacted in dodecane at 70°C using LP and 4,4′-azobis(4-cyanovaleric acid) as initiators for 6 h; while in method 2, the solvent and initiator were replaced with water and ammonia persulfate and the other reaction conditions kept the same.

First, a characteristic peak from PVP (3.1–3.3 ppm), representing P₅, can be clearly seen in Figs. 2 and 3, confirming successful graft polymerization of PVP on F127 in both methods. To verify the grafting position of PVP on F127, we will
have to look at another peak. The peak around 3.8, which appears in Fig. 3 but not in Fig. 2, provides an indication of the different grafting sites of PVP on F127 in the two methods. This peak is split from the –CH2– in the EO part of F127 due to the grafting of PVP on its adjacent carbon atom. The emergence of this peak indicates that in method 2, PVP prefers to graft onto the EO block of F127. While the peak at the same site is quite weak in Fig. 2, it indicates that most of the PVP is grafted onto the PO block of F127. Hence, the analysis of the NMR spectra confirms that the grafting site of PVP on F127 can be well controlled by selecting different solvents and initiators. Using method 1, PVP is specifically grafted on the PPO block of F127 and using method 2, PVP is specifically grafted on the PEO blocks of F127. Although it is possible to control the PVP graft to be either at the PEO or PPO blocks, it is not possible to determine what is the proportion of PVP grafted on each of the EO blocks in F127.

The intensity ratio of P5 to the methyl group in PPO for the graft copolymer F127(PPO)–g–PVP can be obtained from Fig. 2:

\[
\frac{P_5}{CH_3} = 1.5 \times \frac{I_{CH_2CO}}{I_{CH_3}} = 5.25:1.
\]

F127 can be taken as the internal standard, since the molecular weight of F127 is known \((M_w \approx 12,600)\). Hence, the molecular weight of F127(PPO)–g–PVP can be estimated to be about 52,400.

The intensity ratio of P5 and methyl groups in PPO for the graft copolymer F127(PEO)–g–PVP can be obtained from Fig. 3:

\[
\frac{P_5}{CH_3} = 1.5 \times \frac{I_{CH_2CO}}{I_{CH_3}} = 7.5:1.
\]

Hence, the molecular weight of F127(PEO)–g–PVP can be obtained. The value is about 70,000.
4.2. Micellization process

The self-assembly of Pluronic copolymer in aqueous solution can be initiated either by a change in concentration or by a change in temperature. Hence, the critical micelle concentration is one of the most important parameters for characterizing the association behavior of copolymers. The CMC is defined as the copolymer concentration above which the formation of micelles becomes increasingly significant. The critical micelle concentration can be determined by surface tension measurement [28,29].

Fig. 4 shows the effect of concentration on the surface tension of the copolymer. In general, the surface tension decreases with an increase in the concentration of the surfactant. This is because the surfactants tend to orient on the surface of water so that the hydrophilic end is directed toward the water phase and the hydrophobic end is oriented toward air. This surface orientation property changes the surface properties of the water phase by decreasing the surface tension. When the surface tension does not change with a further increase in the surfactant concentration, this indicates that any increase in the concentration of the surfactant in the solution will result in the formation of micelles. Hence the break in the surface tension curve at higher concentration corresponds to the formation of micelles. A further increase in the concentration of copolymer solution will result in the formation of more micelles in the bulk solution. The activity of the copolymer remains constant, and the surface tension does not change. Other studies also verified that the break in the surface tension curve at higher concentration corresponds to the CMC by showing good agreement with the values obtained from dye solubilization [30] and its strong dependence on the temperature [31].

However, we find that there is more than one break in the surface tension curve with increasing concentration of F127 in aqueous solution, as shown in Fig. 4. A similar observation was also reported by other researchers [29]. When the concentration increased, the surface tension dropped sharply at first and then decreased slowly. As described before, F127 molecules tend to go to the interface of the solution and air. The polymers at the interface help to reduce the surface tension. With increasing concentration, the amount of F127 molecules at the interface increased correspondingly. Hence the surface tension decreases at the beginning.

The most probable explanation for the break at lower concentration is provided by Alexandridis et al. [32]. They proposed that this break is not related to the molecular weight distribution and impurities in the copolymer, but instead is related to the formation of “monomolecular micelles” in the bulk aqueous phase and a “phase separation” of the copolymer at low concentration. At low concentration, the polymer molecules exist as an extended configuration when they are adsorbed on the interface between air and water. The surface tension decreases with increasing concentration. In the concentration range $10^{-6}$ – $10^{-3}$%, the constant surface tension attained is due to the proposed orientations of PEO chains located at the air/water interface. When the concentration was increased to above $10^{-3}$%, this configuration changes to be more compact and expels water around it. The surface tension decreases with an increase in the bulk concentration but with a slower rate. After that, any further increase in the concentration is attributed to the micellization and the surface tension remains constant.

The CMCs of the two kinds of graft copolymers synthesized are larger than that of F127 in weight concentration, as shown in Fig. 4. Comparing the surface tension curves of F127, F127(PPO)–g–PVP, and F127(PEO)–g–PVP, we can see both breaks show narrower and slower changes. One possible way to explain the changes is that the effective volume of the polymer becomes very large after grafting the PVP, which is a five-ring structure. The larger steric hindrance of the copolymers attributed to PVP grafted onto F127 make a bigger inverted ‘U’
Fig. 5. Effect of the concentrations of polymers in aqueous solutions on $T_{CMT}$.  

Table 4  
Influence of the different parts of F127 grafted by PVP on hydrodynamic radius  

<table>
<thead>
<tr>
<th>Percentage of grafting (%)</th>
<th>$R_h$ (nm)</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F127</td>
<td>-</td>
<td>9.6</td>
</tr>
<tr>
<td>F127(PPO)-g-PVP</td>
<td>76</td>
<td>37.8</td>
</tr>
<tr>
<td>F127(PEO)-g-PVP</td>
<td>81</td>
<td>18.7</td>
</tr>
</tbody>
</table>

at the air/water interface. With a further increase in the concentration of copolymer molecules, the surface tension attains a steady value that does not change with increased copolymer concentration. This corresponds to the micellization concentration. Beyond this concentration, the surface tension does not change with increased copolymer concentration. The CMC of F127(PEO)-g-PVP and F127(PPO)-g-PVP is larger than that of F127 in weight percent concentration. This is expected, since the molecular weights are much larger than that of F127.

The critical micelle temperature at which micelles start to form is between 10 and 30 $^\circ$C. The graft copolymer has a higher CMT than F127, as shown in Fig. 5. The critical micellization temperature of the graft copolymer increases with decreasing concentration in aqueous solutions. It is generally believed that the dehydration of the PPO chain with increasing temperature is responsible for the micelle formation. This explains why the graft copolymer shows strong temperature dependence of its solution properties. The graft copolymer shows more hydrophilic behavior due to the presence of the amphiphilic PVP group.

The hydrodynamic radii of PVP–g–F127 graft copolymers can be successfully examined by DLS. In general, the average hydrodynamic radius of F127 is about 9.6 nm. After modification of Pluronic F127 using PVP, the hydrodynamic radius of the graft copolymer is larger than that of Pluronic F127. Referring to Table 4, the most probable reason is that the steric repulsion due to the branching structure of the graft copolymer becomes stronger whether PVP is mainly grafted to the PEO or to the PPO block of F127. Such steric repulsion makes both the compact inner core of PPO and the soluble corona of PEO larger. PVP may also form another layer in the micelle formed or may form smaller micelles existing in both core and corona of F127. This shows that the modification of Pluronic F127 using PVP also has a good size distribution of the aggregation in aqueous solution. It is known that good polydispersity of the copolymer used in pharmacological applications, especially in the drug release system, is very important.

5. Conclusions

Two different F127–g–PVP graft copolymers have been synthesized successfully using two different reaction schemes. It is confirmed that using a selective solvent for either the PEO or the PPO block in F127 will result in controlled grafting for either the PEO site or the PPO site. The association behavior of the graft copolymers in aqueous solutions was studied in detail using surface tension measurements and DSC. The CMC of the PVP graft copolymers were lower than that of F127 by mole fraction but higher in weight fraction. The reason for this is due to the increase in molecular weight of the grafted chains. The CMTs of the graft copolymers were nearly all higher than that of F127. The diameters of F127–g–PVP graft copolymers were larger than that of F127. From this work, it can be concluded that the PVP can be grafted in a controlled manner onto F127 without compromising the F127 solution behavior. With the presence of PVP in the chains, the storage properties of the F127 micelles can be improved.

Acknowledgment

The authors thank Professor Gan Leong Huat from the National Institute of Education, Nanyang Technological University for his help in carrying out the NMR measurements.
References