Binary mixing of micelles using Pluronics for a nano-sized drug delivery system

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Abstract

Pluronics with different structural compositions and properties are used for several applications, including drug delivery systems. We developed a binary mixing system with two Pluronics, L121/P123, as a nano-sized drug delivery carrier. The lamellar-forming Pluronic L121 (0.1 wt%) was incorporated with Pluronic P123 to produce nano-sized dispersions (in case of 0.1 and 0.5 wt% P123) with high stability due to Pluronic P123 and high solubilization capacity due to Pluronic L121. The binary systems were spherical and less than 200-nm diameter, with high thermodynamic stability (at least 2 weeks) in aqueous solution. The CMC of the binary system was located in the middle of the CMC of each polymer. In particular, the solubilization capacity of the binary system (0.1/0.1 wt%) was higher than mono-systems of P123. The main advantage of binary systems is overcoming limitations of mono systems to allow tailored mixing of block copolymers with different physicochemical characteristics. These nano-sized systems may have potential as anticancer drug delivery systems with simple preparation method, high stability, and high loading capacity.

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1. Introduction

Amphiphilic block copolymers with hydrophilic and hydrophobic blocks that form multi-molecular aggregates in aqueous solution may have application in medicine, engineering, and biology [1–3]. The amphiphilic block copolymer, Pluronic, consisting of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) blocks with a PEO–PPO–PEO arrangement (often as EO\textsubscript{x}–PO\textsubscript{y}–EO\textsubscript{x}) have been studied for pharmaceutical applications such as solubilization excipients, drug carriers, immunoadjuvants, cryopreservation agents, and gene carriers [2,4–9]. These copolymers are commercially available from BASF and customized for applications and customer needs. Pluronics exist as different compositions and display abundant phases as self-assembling into polymeric micelles with various morphologies depending on the aqueous solvent quality, the critical micelle concentration (CMC), and critical micelle temperature (CMT) [10–13].

The aggregates have spherical, cylindrical, or lamellar structures depending on the relative length of both blocks, which changes the hydrophilic–lipophilic balance (HLB) of block copolymers. The spherical micelles are composed of a hydrophobic core providing a hydrophobic molecule pool and hydrophilic shell that provides aqueous stability. Therefore, the micelle can increase drug stability, metabolic stability, and circulation time. In addition, the lamellar and cylindrical morphology produces high solubilization capacity [8,13] and avoids phagocytosis by macrophages [14–16]. However, non-spherical structures are large, unstable, and show phase separation [8,17].

Binary systems may compensate for drawbacks of a mono system and control the physicochemical properties for smart drug delivery systems. For example, a pH-sensitive block copolymer, poly(histidine)–PEO (P[His]–PEO) developed for targeting extracellular tumor pH (pH 6.8–7.0) was controlled in early endosomal pH (pH 6.0–6.5) by mixing an amphiphilic block copolymer, poly(lactic acid)–PEO (PLA–PEO) [9,18,19]. In addition, a blend of Pluronic P105 (Pluronic P105 is designated as P105 throughout) and L121 was developed for multidrug resistance tumors by incorporation with paclitaxel [20]. A mixture of hydrophobic L61 and hydrophilic F127 has practical application in pharmaceutics for a doxorubicin delivery system [21], and selection of the mixture components produced physicochemical properties such as a high colloidal stability, formation of small particle size, and good solubilization capacity. In particular, F127/L121 systems produced nano-sized stable systems with several-fold higher loading capacity in hydrophobic agents compared to F127 micelles [8]. However, the system needs extra
energy input such as heating and ultrasonication to form the stable nano-sized particles due to the high CMT of F127 [8].

In this paper, we have tested blend systems with L121 and P123, which have low CMT. A lamellar-forming L121 was incorporated with spherical-forming P123 to increase thermodynamic stability and to produce small particles with high solubilization capacity. We evaluated size, morphology, CMC, and solubilization capacity of the blends using a model hydrophobic compound, Sudan III.

2. Materials and methods

2.1. Materials

Pluronics are commercially available from BASF Corporation (Parsippany, NJ) and were used without additional purification. The molecular characteristics of the block copolymers are presented in Table 1. Sudan III (Samchun Co., Korea) was used as a hydrophobic model drug for solubility studies. All other chemicals were of analytical grade.

2.2. Preparation of binary mixed systems

The stock solutions of L121 (2%, w/v) and P123 (5%, w/v) were prepared in distilled water and stored at 4 °C. Pluronic mixed systems were prepared from stock solutions and left at 4 °C overnight to produce unimers. The solutions were transferred to room temperature (23 °C) and incubated for more than 1 h before further experiments to form the aggregates. The samples with energy input were prepared by sonicating them in polystyrene tubes (FALCONR, Becton Dickinson, NJ) using a probe sonicator (Sonics VC505, Sonics & Materials, Inc. CT) at 20% of 500 W for 1 min.

2.3. Size measurements

The effective hydrodynamic diameter (D_{eff}) of the particles was measured by photon correlation spectroscopy using a “Zetazizer Nano-ZS” (Malvern Instruments, UK) equipped with the Multi Angle Sizing Option (BI-MAS). The sizing measurements were performed in a thermostatic cell at a scattering angle of 90°. Software provided by the manufacturer was used to calculate D_{eff} values. The averaged D_{eff} values were calculated from three measurements performed on each sample (n = 3).

2.4. Turbidity measurements

The turbidity experiments were performed by measuring the transmittance of the mixed samples using a Genesys 10UV (Thermo Sci., USA) at λ = 520 nm. Before the test, all micelle solutions (n = 3) were stabilized at room temperature for 1 h.

2.5. Morphology of the aqueous dispersion of Pluronics using hyperspectral imagery

The hyperspectral imagery (HSI) microscope system quantify particle abundance and cluster size using a high-intensity dark-field illuminator to view nanoscale structures [23]. This system consists of a Nikon microscope equipped with a visible-near infrared (VNIR) hyperspectral camera system, CytoViva dual mode fluorescence (DMF) module, and CytoViva high-resolution adapter (CytoViva, Auburn, AL). Illumination was controlled by a DC-stabilized 150-W halogen light source, lamp voltage was a constant 11 V, and the internal neutral density filter was used to decrease light intensity. To obtain images, we used HyperVisual Software from the Institute for Technology Development (ITD) (Stennis, Miss). Sample preparation for images analysis involved 1–2 drops of the prepared micelle-liquid on slide glass.

2.6. CMC analysis

All fluorescence measurements for CMC determination were performed using a Shimadzu RF-5301PC Spectrofluorometer equipped with polarizers for excitation and emission light beams. Pyrene was used as the fluorescent probe. Emission spectra of pyrene were recorded with a bandwidth of 3 nm for excitation and 3 nm for emission. For emission and excitation spectra, λ_{ex} and λ_{em} were 339 and 374 nm, respectively. The sample solutions were prepared by adding pyrene in acetone to empty vials. After acetone evaporation, aqueous solutions of Pluronic dispersions of copolymer (from 10^{-4} to 1%, w/v) were added to the vials. The final pyrene concentration was 6 × 10^{-7} M, slightly below the saturation concentration of pyrene in water at 25 °C. These samples were stirred overnight at room temperature. All measurements were performed at room temperature using air-equilibrated solutions. The CMC was estimated by plotting the ratio of I_1 (intensity of first peak) to I_3 (intensity of third peak) of the emission spectra profile against the log of the micelle concentration. The CMC was determined by the crossover point of low polymer concentrations on this plot [24].

2.7. Solubilization of water-insoluble dye

Ten microliters of Sudan III stock solution (10 mg/ml) in CHCl_3 was added to empty vials and the solvent was evaporated. Two milliliter aqueous solutions of P123 dispersions varying from 0.01 to 0.5 wt% with/without 0.1 wt% L121 were individually added to the vials and then allowed to equilibrate in the shaker (100 rpm) at room temperature for 1 day. Absorption spectra of Sudan III in aqueous solutions at 25 °C were recorded on a Genesys 10UV (Thermo Sci., USA). The data are reported as absorbance at 362 nm corresponding to the maximum in the Sudan III spectra.

3. Results and discussion

3.1. Pluronic mixed system

L121 (EO_{50}–PO_{68}–EO_{50}) aggregated with a lamella structure [8,13]. The hydrophobic block, PPO of L121, aggregated due to its hydrophobic interaction in aqueous solution, forming a supermacromolecular structure. However, the hydrophilic block PEO of L121 only has 10% of the whole MW, not providing sufficient steric hindrance to form a stable dispersion. In the previous study, F127 (EO_{100}–PO_{65}–EO_{100}), which has a long PEO chain and similar MW of PPO to L121, was admixed into L121 to prepare a stable micelle [8]. The F127 with long chains of PEO kinetically provided high stability and spherical structure for L121. However, the relatively low CMT of F127 required energy input to be miscible with L121 [8,25]. Therefore, P123 with an intermediate CMT may be a good candidate for the formation of a self-assembling dispersion with L121 without energy input. Table 2 shows the sizes and stability of dispersions. The 0.1% L121 aggregates were 1 μm as measured by the cumulated method in DLS, and the size distribution of particles was larger than 1 μm and showed a multimodal

Table 1

<table>
<thead>
<tr>
<th>Copolymer</th>
<th>MWa</th>
<th>HELBb</th>
<th>Cloud point in 1% solution (°C)c</th>
<th>CMC (M)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>L121</td>
<td>4400</td>
<td>1</td>
<td>14</td>
<td>1.0 × 10^{-6}</td>
</tr>
<tr>
<td>P123</td>
<td>5750</td>
<td>8</td>
<td>90</td>
<td>4.4 × 10^{-6}</td>
</tr>
</tbody>
</table>

a Average molecular weights provided by the manufacturer (BASF).
b HLB values of the copolymers were determined by the manufacturer.
c Provided by the manufacturer.
d Critical micelle concentration (CMC) were determined with a pyrene probe at 37 °C [22].
Table 2
Size and stability of particles for different Pluronic dispersions.

<table>
<thead>
<tr>
<th>Pluronic (wt%)</th>
<th>W/O L121</th>
<th>W/L 121 (0.1 wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W/O sonication</td>
<td>W/sonication</td>
</tr>
<tr>
<td>0</td>
<td>N/A</td>
<td>970 (×)</td>
</tr>
<tr>
<td>0.01</td>
<td>220 (○)</td>
<td>1014 (×)</td>
</tr>
<tr>
<td>0.05</td>
<td>134 (○)</td>
<td>679 (△)</td>
</tr>
<tr>
<td>0.1</td>
<td>90 (○)</td>
<td>197 (○)</td>
</tr>
<tr>
<td>0.5</td>
<td>45 (○)</td>
<td>79 (○)</td>
</tr>
</tbody>
</table>

$D_{eff}$ were calculated from three measurements ($n = 3$). Symbols in parenthesis indicate: ×, precipitation within 1 day; △, precipitation within 2 days; O, stable for more than 2 weeks.

distribution (Fig. 1), indicating that aggregates formed a lamellar structure.

For P123 (EO20–PO69–EO20), the micelle particles showed a stable, nano-sized dispersion of 50-nm (0.5%) particles with increased concentrations, putatively due to increased polymer density and formation of a tight aggregated core by increasing hydrophobic interactions between the hydrophobic PPO blocks and cooperatively coronal hindrances to prevent micelle adsorption. Mixtures of L121 (0.1%) with P123 showed lower dispersion sizes with increasing P123 concentrations (Fig. 2). In addition, the mixture dispersions at 0.1 and 0.5% P123 have high stability without changing sizes or showing precipitation for at least 2 weeks. All the mixtures prepared with energy or sonication showed smaller dispersions below 200 nm. However, the stability pattern of mixture dispersions was similar to those prepared without sonication. The size of dispersions prepared with sonication approximated those made without sonication within 24 h. As for L121/F127 mixtures, energy input increased the kinetic stability by changing CMT, resulting in a metastable dispersion with small particles [8]. However, the metastable dispersion of 0.1% L121/0.1 and 0.5% P123 (12–24 h) showed less kinetic stability but higher thermodynamic stability than the same composition of L121/F127 (preparation after 1 week).

Thus, hydrophilic–lipophilic balance (HLB) is important in the kinetic and thermodynamic stability of micelles. L121 with a lamellar structure showed less aqueous stability and precipitation at 0.1%. Adding hydrophilic Pluronics with the same PPO portion increased aqueous stability. Previous studies demonstrated that Pluronics with different MW PPO did not change the stability of the L121 dispersion and CMT plays an important role in forming a miscible micelle without any further modification [8]. Our results show that the P123 with a low CMT increased the thermodynamic stability due to tight hydrophobic interactions with hydrophobic blocks, while the Pluronic with long hydrophilic chain increased kinetic stability due to steric hindrance for micelle aggregation. This suggests that amphiphilic polymers with relatively low HLB increase the thermodynamic stability but do not affect the kinetic stability. Conversely, polymers with high CMT due to high HLB values did not have a hydrophobic core due to the high hydrophilicity of long PEO chains. Energy input, or increased temperature, changes CMT to kinetically produce a hydrophobic polymer and produce steric hindrance by incorporating long PEO chains into micelles to prevent secondary micellar aggregation by steric hindrance, forming a kinetically stable dispersion.

3.2. Turbidity and morphology of L121/P123 mixtures

The turbidity of the P123 dispersion did not change as a function of concentration and L121 formed a very cloudy dispersion.
(Fig. 3). However, the L121 solution cleared with increasing P123 concentrations as the lamellar structure of the L121 dispersion changed to a spherical structure. Micellar morphology at a specific temperature is difficult to measure for Pluronics because they show low critical solution temperature (LCST) behavior [26], where dispersion particle morphology may change at different temperatures. To evaluate morphology, we used high-resolution optical microscopy, which visualizes the structure of the aqueous state dispersion without additional treatment, as is needed for cryo-transmission electron microscopy (cryo-TEM). The hyperspectral imagery (HSI) microscope system can quantify particle abundance and cluster size using a high-intensity, dark-field illuminator to view nanoscale structures [23], particularly liquid samples.

The structure of 0.1% L121 dispersion was cloudy and did not allow clear pictures. However, the mixture of L121/P123 formed spherical micelle structures and short cylinders (Fig. 4). To understand whether the PPO of P123 was incorporated or adsorbed into the lamellar structure of L121, various MW (400, 1000, 2000, 4000, 8000, and 20,000) of PEO were added into the 0.1% L121 solution, but did not change size, turbidity, and stability of the solution (data not shown). The transmittance of different aqueous dispersions (Fig. 5) suggested that PEO itself cannot incorporate or attach into the Pluronic aggregates because there is no hydrophobic block. Therefore, the hydrophobic PPO block of Pluronics should blend with the lamella agglomerates to form a stable spherical structure. The effect of PPO block MW on cooperative aggregation [8,27,28] in the binary systems was under investigation.

The L121/P123 mixture formed a spherical micelle composed of a new mixed micelle core from the PPO of P123 and L121, and an external corona from PEO of P123. A physically mixed or adsorbed aggregated core was consistent with the results of L121/F127.

3.3. Critical micelle concentration

Micelle formation by the self-assembly of Pluronic mixtures was monitored by fluorometry in the presence of pyrene as a fluorescent probe. Pyrene is highly hydrophobic and preferentially migrates into the hydrophobic PPO microdomains in an aqueous solution. Pyrene in a non-polar environment shows strong fluorescence, but in a polar environment shows weak fluorescence intensity and a shift of the $I_1/I_3$ ratio in the emission peak [24,29]. Fig. 6 presents a typical dependence of the pyrene fluorescence intensity ratio $I_1/I_3$ versus the concentration of the dispersion phase of the Pluronics and mixture. The CMC of L121, P123, and mixture was 2.6, 6.8, 4.9 μM, respectively. The CMC values of L121 and P123 were slightly higher than previous reports due to different temperatures.
The solubilization capacity of the Pluronic dispersion was evaluated using a water-insoluble dye, Sudan III (log P = 5.737), that has a maximum absorbance at 362 nm. The UV/vis absorbance (λ = 362 nm) of Sudan III in Pluronic dispersions versus P123 concentration is shown in Fig. 7. The solubilization of Sudan III in P123 slightly increased with increasing Pluronic concentrations, but the solubilization capacity of P123 was lower than the mixed L121 (0.1%)/P123. The lamella-forming L121 provides a hydrophobic pool to increase in the solubilization capacity compared to the spherical micelle-forming P123. Increased P123 levels decrease the solubilization of Sudan III. Interestingly, the solubilization capacity of L121/P123 (0.1/0.1%) was 7-fold higher than L121/P123 (0.1/0.5%), which may be due to different core sizes providing a hydrophobic pool. The size of the L121/P123 (0.1%) dispersion was bigger than L121/P123 (0.5%). The incorporation of insoluble dye into L121/P123 (0.1 and 0.5%) aggregates did not change the particle size, and dispersions remained stable for several days (data not shown). The calculated weight-to-weight ratio of Sudan III to copolymer in 0.1% L121/0.1% P123 mixed aggregates is approximately 46.9 mg/g. Therefore, mixtures of hydrophobic and hydrophilic Pluronics can improve the solubilization of poorly soluble molecules.

3.4. Solubilization of binary Pluronics

The solubilization capacity of the Pluronic dispersion was evaluated using a water-insoluble dye, Sudan III (log P = 5.737), that has a maximum absorbance at 362 nm. The UV/vis absorbance (λ = 362 nm) of Sudan III in Pluronic dispersions versus P123 concentration is shown in Fig. 7. The solubilization of Sudan III in P123 slightly increased with increasing Pluronic concentrations, but the solubilization capacity of P123 was lower than the mixed L121 (0.1%)/P123. The lamella-forming L121 provides a hydrophobic pool to increase in the solubilization capacity compared to the spherical micelle-forming P123. Increased P123 levels decrease the solubilization of Sudan III. Interestingly, the solubilization capacity of L121/P123 (0.1/0.1%) was 7-fold higher than L121/P123 (0.1/0.5%), which may be due to different core sizes providing a hydrophobic pool. The size of the L121/P123 (0.1%) dispersion was bigger than L121/P123 (0.5%). The incorporation of insoluble dye into L121/P123 (0.1 and 0.5%) aggregates did not change the particle size, and dispersions remained stable for several days (data not shown). The calculated weight-to-weight ratio of Sudan III to copolymer in 0.1% L121/0.1% P123 mixed aggregates is approximately 46.9 mg/g. Therefore, mixtures of hydrophobic and hydrophilic Pluronics can improve the solubilization of poorly soluble molecules.

4. Conclusion

Pluronics are used for several applications, including drug delivery systems. We used a binary mixing system with L121/P123 to produce a stable carrier for hydrophobic agents. The lamellar-forming L121 was incorporated with P123, which had a relatively low CMT and spherical morphology. The binary systems showed nano-sized spherical morphology with a high thermodynamic stability in aqueous solution. The solubilization capacity of the binary system was higher than mono-systems of P123 because L121 has high solubilization capacity. The CMC of binary systems was intermediate to the CMC of each polymer, suggesting that favorable properties of drug delivery systems can be obtained by mixing block copolymers with different physicochemical characteristics. These nano-sized systems could be useful for anticancer drug delivery systems with simple preparation methods, high stability, and high loading capacity. Further studies are in progress to evaluate binary systems incorporated with anticancer agents such as doxorubicin and paclitaxel in vitro and in vivo.

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References


