Pluronic® P123/TPGS and Pluronic® F127/TPGS Mixed Micelles for the Entrapment of Itraconazole

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ABSTRACT

Mixed micelles of Pluronic® P123 (P123) or Pluronic® F127 (F127) and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) at different mass ratios (4:0, 3:1, 2:2, and 1:3) and various total polymer concentrations (5, 10, and 20 %w/v) were prepared to incorporate itraconazole (ITZ), an aqueous solubility problematic antifungal drug. The results revealed that the critical micelle concentrations of all mixed micelles were lower than that of the single-component (P123 or F127) polymeric micelles. The particle size of P123/TPGS and F127/TPGS mixed micelles ranged from 8-17 nm and 9-28 nm, respectively, depending on polymer concentration and mass ratio. The increasing polymer concentration and TPGS mass ratio declined the particle size of mixed micelles. After drug loading, the polymeric mixed micelles became slightly larger. The surface charge of all polymeric mixed micelles was negative and closed to zero when increasing polymer concentration. The loading efficiency of polymeric mixed micelles for ITZ could be enhanced by increasing polymer concentration and TPGS mass ratio in the mixed micelles.

Keywords: itraconazole, d-α-tocopheryl polyethylene glycol 1000 succinate, pluronic® P123, pluronic® F127, polymeric mixed micelles

1. INTRODUCTION

Itraconazole (ITZ) is a broad spectrum triazole antifungal agent. It is a poorly water-soluble drug and is classified in Biopharmaceutical Classification System class II [1]. The commercial oral formulation of ITZ is available in solution form using hydroxypropyl-β-cyclodextrin (HP-β-CD) as a complexing agent to solubilize ITZ. However, the use of HP-β-CD could potentially result in the in vivo toxicity after administering for long period [2]. For the long term treatment by ITZ, the use of other delivery systems may thus be an alternative for ITZ administration. However, from the formulation point of view, the solubility of ITZ is still a major obstacle. One approach to overcome the solubility problem of ITZ is polymeric micelles. Polymeric micelles consisting of hydrophobic inner core and hydrophilic corona shell can self-assembly form in an aqueous environment. The hydrophobic micellar core creates a space...
available for hydrophobic drug whereas the corona shell stabilize the micelles in an aqueous environment. Hence, the advantages of micelles are solubilizing water-soluble problematic drugs, increasing in vitro and in vivo stability profiles, and prolonging drug release. The spherical micellar diameter generally ranges from 10 to 100 nm [3]. Based on the composition of polymeric micelles, this system can be basically divided into single-component micelles and binary mixed micelles. The binary mixed micelles exhibit the synergistic properties in addition to the single-component micelles such as enhancing drug loading efficiency and micellar stability. It has been reported that the binary mixing system of Pluronic® P105 and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was more stable and efficient in solubilization of camptothecin because an aromatic ring of TPGS may form stronger hydrophobic interactions with the drug [4]. Another report established that the mixed micelles of Pluronic® F127 (F127) and TPGS at the molar ratio of 5:5 provided higher doxorubicin entrapment efficiency than the F127 micelles [5]. In addition, the Pluronic® P123 (P123)/Pluronic® F68 mixed micelles could enhance the curcumin loading efficiency and sustain the release of curcumin [6]. As a result, the binary polymeric mixed micelles may improve the entrapment efficiency for hydrophobic drugs and the stability of micelles.

Pluronic block copolymers consist of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) blocks. A wide array of Pluronic® is available depending on the PPO/PEO ratio and molecular weight [7]. P123, composed of PEO20-PPO68-PEO20, and F127, containing PEO100-PPO69-PEO100, have been extensively used for the development of drug delivery system including polymeric micelles. It has been reported that ITZ solubility could be enhanced by P123 polymeric micelles [8]. In addition, Singh-Joy and McLain indicated that when Pluronics are introduced into the body, they have a rapid clearance from the body and no risk of reproductive and/or developmental toxicity [9].

TPGS, a derivative of vitamin E and poly(ethylene glycol) 1000, is approved by U.S.FDA for human use as a solubilizer, absorption enhancer and drug delivery vehicle [10]. It has been found that TPGS could enhance the solubility of various hydrophobic drugs by micellar solubilization such as estradiol and docetaxel [10,11].

Therefore, the objective of this study was to develop polymeric mixed micelles as nanocontainers for ITZ. The polymeric mixed micelles were prepared using P123/TPGS and F127/TPGS at various polymer mass ratios and concentrations. The obtained polymeric mixed micelles were evaluated for their size, zeta potential, critical micelle concentration, %drug loading, and %entrapment efficiency.

2. MATERIALS AND METHODS

2.1 Materials

ITZ was purchased from Megafine Pharma Ltd., (Mumbai, India). P123 and pyrene were purchased from Sigma-Aldrich, (Missouri, USA). F127 and TPGS were received as gift samples from BASF, (Ludwigshafen, Germany). High-performance liquid chromatography (HPLC) grade methanol and ammonium acetate were bought from Burdick & Jackson (Ulsan, Korea) and Loba Chemie (Mumbai, India), respectively. All other chemicals were of analytical grade. Deionized water was used throughout this work.

2.2 Preparation of Polymeric Mixed Micelles

The polymeric mixed micelles were prepared by solvent diffusion evaporation method as reported elsewhere [12-15]. The polymers used in this experiment were TPGS, F127, and P123. The different formulations are summarized in Table 1. Briefly, various amounts of polymer
were weighed and dissolved in 3 mL of tetrahydrofuran (THF). In case of drug-loaded polymeric micelles, 80 mg of ITZ was added in polymeric solution. The organic phase was added drop-by-drop in 8 mL of deionized water under magnetic stirring. THF was evaporated at room temperature under continuously magnetical stirring for 1 h. After that, the final volume was adjusted to 8 mL with deionized water. Finally, the colloidal dispersion was filtered through 0.45 µm nylon membrane to eliminate unloaded drugs and aggregates.

2.3 Determination of Critical Micelle Concentration (CMC)

Pyrene fluorescence method was often used to determine the onset of micellization in polymer solution [16-19]. Pyrene probe is sensitive to the polarity level of environment. In water or other polar solvents, the first emission peak shows the maximum intensity at the wavelength of 374 nm \(I_{374}\) while the third emission peak gives the minimum intensity at 384 nm \(I_{384}\). When the polarity of environment decreases, the \(I_{374}\) value decreases whereas the \(I_{384}\) value increases gradually. Therefore, the intensity ratio of \(I_{374}/I_{384}\) diminishes with the reducing environment polarity. In the experiment, a stock solution of pyrene \((1.2 \times 10^{-7} \text{ M})\) was prepared in ethanol. A 25 µL aliquot of pyrene solution was transferred into clean bottles. The solvent was then evaporated resulting in a thin film of pyrene. A series of polymer solution \((0.002-1.000 \text{ mg/mL})\) in deionized water were added to rehydrate the thin film of pyrene. The mixture was incubated in the dark at 25°C with thermostatically controlled water bath shaker (Hetofrig, Birkerod, Denmark) for 24 h. Free pyrene was removed by filtration through 0.45 µm nylon membrane filter. Fluorescence absorbance measurement was carried out by FP-6200 Jasco spectrofluorometer (Tokyo, Japan) using a 10-mm path length quartz cuvette. The excitation and emission wavelengths were 335 and 350-460 nm, respectively. The scan rate and excitation slit were fixed at 60 nm/min and 5.0 nm, respectively [15]. The intensity ratio of \(I_{374}/I_{384}\) was plotted against polymer concentrations. The CMC value was determined by the concentration of polymer at which a sharp decrease in the intensity ratio of \(I_{374}/I_{384}\) occurred [6].

2.4 Particle Size Analysis

The mean particle size (z-ave) and polydispersity index (PDI) of freshly prepared micelles were measured by light scattering method using Zetasizer NanoZS (Malvern instruments, Malvern, UK) at a scattering angle of 173°, 25°C [20, 21]. The sample was placed into a quartz cuvette and measured in triplicate.

Table 1. Compositions of polymeric mixed micelles.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polymer ratio (w/w)</th>
<th>Total concentration of polymers (%w/v)</th>
<th>Initial concentration of itraconazole in micellar solution (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P123/TPGS</td>
<td>4:0, 3:1, 2:2, 1:3</td>
<td>5</td>
<td>10</td>
</tr>
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<td></td>
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<td>10</td>
<td>10</td>
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<td></td>
<td></td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>F127/TPGS</td>
<td>4:0, 3:1, 2:2, 1:3</td>
<td>5</td>
<td>10</td>
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<td></td>
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<td></td>
<td></td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>
2.5 Zeta Potential Analysis

The surface charge of micelles was determined using Zetasizer NanoZS (Malvern instruments, Malvern, UK). The measurement was repeated three times.

2.6 Determination of Drug Loading and Entrapment Efficiency

The drug loading and entrapment efficiency of P123/TPGS and F127/TPGS mixed micelles were determined by direct method as previously reported [6, 21]. The micellar solution was suitably diluted with the mixture of methanol and 0.5% w/v ammonium acetate (80:20 v/v) prior to HPLC determination. The drug loading (%DL) and entrapment efficiency (%EE) of polymeric micelles were calculated according to equations 1 and 2.

\[
%DL = \left( \frac{\text{analyzed weight of drug in micelles}}{\text{weight of the feeding polymer and drug}} \right) \times 100\%
\]  

(1)

\[
%EE = \left( \frac{\text{analyzed weight of drug in micelles}}{\text{weight of the feeding drug}} \right) \times 100\%
\]  

(2)

2.7 HPLC Analysis of ITZ

The amount of ITZ in polymeric micelles was determined by HPLC method using Shimadzu HPLC machine (Shimadzu Corporation, Tokyo, Japan) equipped with UV-Vis detector. ITZ was eluted through Zorbax® SB-C18 column, 5 µm, 150 mm 4.6 mm (Agilent Technologies Inc., California, USA) used as a stationary phase. The mixture of methanol and 0.5% w/v ammonium acetate (80:20 v/v) was used as a mobile phase [22]. A flow rate was 1.0 mL/min and a detector wavelength was 263 nm. The HPLC performance was validated in terms of linearity, precision, and accuracy. The amount of drug was calculated from a calibration curve over the concentration range of 0.5-10 µg/mL with R² of at least 0.9995. The relative standard deviation of inter- and intraday precisions was less than 2%. The %recovery of ITZ was in the acceptable range of 98.5-101.5% [23].

2.8 Statistical Analysis

The data are expressed as the mean ± standard deviation (S.D.) from at least three measurements. The t-test or one-way analysis of variance (ANOVA) was used to compare two or multiple groups, respectively. All analyses were determined using SPSS program (SPSS 17.0). The p-value less than 0.05 (p-value < 0.05) is considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Blank Polymeric Mixed Micelles

The physicochemical characteristics of blank polymeric mixed micelles are shown in Figure 1.

Effect of polymer type (P123 and F127): The z-ave and PDI values of all ratios of P123/TPGS mixed micelles were found in the ranges of 8-17 nm and 0.021-0.286, respectively. Meanwhile those values of all F127/TPGS mixed micelles ranged from 9 to 28 nm and from 0.094 to 0.537, respectively. The P123/TPGS mixed micelles showed smaller particle size and narrower size distribution compared to the F127/TPGS mixed micelle. This result was attributed to more hydrophobicity of P123 than F127. Reportedly, the PPO/PEO ratio of polymer affected the micelle size [24]. At the same length of PPO segment of P123 and F127, longer PEO chain of F127 led to more hydrophilic property of F127 and thus enlarged the micellar size. The zeta potential of both P123/TPGS and F127/TPGS mixed micelles showed the negative charged surface.

Effect of polymer concentration: At the same mass ratio, an increase in polymer concentration significantly reduced the particle size but gained the PDI value of
both P123/TPGS and F127/TPGS mixed micelles ($p$-value < 0.05). This result is in consistent with the previous report establishing that the increasing polymer concentration led to the coexistence of unimers, micelles, and large aggregates [24]. However, the F127/TPGS mixed micelles at 4:0 mass ratio and 20% polymer concentration could not be prepared since it turned to gel at this concentration. The zeta potential of P123/TPGS and F127/TPGS mixed micelles became almost zero when increased the polymer concentration because of the adsorption of nonionic polymer on the particle surface [24]. The reduction of absolute zeta potential was due to the residual unimers of nonionic polymers adsorbed on the surface of micelles. Furthermore, the adsorption of unimers may sterically stabilize the micelles [13, 25].

Figure 1. The particle size (z-ave, A and D), polydispersity index (PDI, B and E), and zeta potential (C and F) of blank polymeric mixed micelles (Left column: P123/TPGS mixed micelles and right column: F127/TPGS mixed micelles). An error bar indicates the standard deviation from at least three measurements. **No significant difference comparing among all polymer concentrations at the same mass ratio. ***No significant difference comparing among various polymer mass ratios of mixed micelles at the same concentration of polymer.
Effect of polymer mass ratio:

The increasing TPGS mass ratio of both P123/TPGS and F127/TPGS mixed micelles led to the significant decrease in particle size ($p$-value < 0.05) except for the F127/TPGS mixed micelles at 2:2 and 1:3 mass ratios. The PDI value of P123/TPGS mixed micelles slightly decreased when the proportion of TPGS increased. Interestingly, TPGS significantly reduced the PDI value of F127/TPGS mixed micelles. As seen from the size distribution curve in Figure 2, the F127 micelles exhibited two distinct peaks and became unimodal when the P123/TPGS mixed micelles showed unimodal peaks at all mass ratios. This result was attributed to higher hydrophobicity of F127/TPGS mixed micelles with the increment of TPGS proportion. However, the increasing proportion of TPGS in mixed micelles had no effect on the zeta potential of both P123/TPGS and F127/TPGS mixed micelles.

3.2 Critical Micelle Concentration (CMC)

CMC is a parameter indicative of the in vitro and in vivo stability of micelles. In this report, it was measured by fluorescence technique using pyrene as a fluorescence probe. The obtained CMC values of all polymeric mixed micelles are summarized in Table 2. The CMC values of all mixed micelles were lower than those of the single-component (P123 and F127) micelles ($p$-value < 0.05). The CMC value decreased with the increasing TPGS fraction. As a result, the mixed micelles may

![Figure 2](image)

**Figure 2.** Size distribution curves of blank 10 %w/v polymeric mixed micelles at various polymer mass ratios (Left column: P123/TPGS mixed micelles and right column: F127/TPGS mixed micelles).
exhibit higher physical stability upon dilution than P123 and F127 micelles. This result was probably due to the increasing hydrophobic interactions between PPO segment of Pluronic® and vitamin E portion of TPGS in the inner core of micelles [4].

3.3 ITZ-loaded Polymeric Mixed Micelles

The z-ave, PDI and zeta potential values of ITZ-loaded polymeric mixed micelles are shown in Figure 3. After drug loading, most ITZ-loaded polymeric micelles exhibited larger particle size and wider size distribution than the blank micelles (Figures 3 and 1, respectively). The incorporation of ITZ did not change the zeta potential of polymeric micelles as compared to the blank micelles (p-value > 0.05) except for the F127/TPGS mixed micelles 5 %w/v polymer concentration for all mass ratios. The %DL and %EE of polymeric mixed micelles are illustrated in Figure 4. Both P123/TPGS (Figure 4A and B) and F127/TPGS (Figure 4C and D) mixed micelles gave the maximum %DL and %EE of around 0.08-0.1% and 1.6%, respectively. Although, the %DL and %EE were rather low, the solubility of ITZ could dramatically be enhanced by 80-1200 folds as compared to the solubility of ITZ in water (approximately 50 ng/mL, [25]). Nevertheless, several factors may affect the loading capacity of this system such as the initially added amount of drug and other types of polymers in combination with TPGS [26].

### Effect of polymer type (P123 and F127):

The %DL and %EE of P123 micelles (4:0 P123/TPGS mass ratio) were higher than those of F127 micelles comparing at the same mass ratio. This was due to the fact that F127 (HLB 22) is more hydrophilic than P123 (HLB 8)[7], hence the F127 micelles could incorporate less amount of ITZ than P123 ones. Moreover, the addition of TPGS to F127 micelles made the F127/TPGS mixed micelles more hydrophobic and thus it could improve the drug entrapment efficiency. In contrast, the mixed micelles of P123/TPGS exhibited more hydrophilic but the %DL and %EE were still higher than those of P123 micelles. The enhancing drug loading and entrapment efficiency of P123/TPGS mixed micelles was a result of their lower CMC values and stronger hydrophobic interactions in the micellar core [20, 21].

### Effect of polymer concentration:

After drug loading, the increasing polymer concentration decreased the z-ave and absolute zeta potential values of almost all P123/TPGS and F127/TPGS mixed micelles. Nevertheless, no relationship between polymer concentration and PDI value was observed for both series of polymeric mixed micelles. Regarding drug loading and entrapment efficiency, the augmentation of polymer concentration of single-component polymeric micelles did no further increased the %DL and %EE of ITZ (p-value > 0.05) except for 20 %w/v polymer.

### Table 2. CMC values of polymers (n=3).

<table>
<thead>
<tr>
<th>Polymer mass ratio</th>
<th>P123/TPGS (w/v)</th>
<th>F127/TPGS (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td>0.0104 ± 0.0002</td>
<td>0.2515 ± 0.0002</td>
</tr>
<tr>
<td>3:1</td>
<td>0.0082 ± 0.0002</td>
<td>0.0197 ± 0.0013</td>
</tr>
<tr>
<td>2:2</td>
<td>0.0074 ± 0.0003</td>
<td>0.0185 ± 0.0007</td>
</tr>
<tr>
<td>1:3</td>
<td>0.0064 ± 0.0003</td>
<td>0.0102 ± 0.0006</td>
</tr>
</tbody>
</table>

* The reported values from literatures [21, 19] for P123 and F127, respectively.
Figure 3. The particle size (z-ave, A and D), polydispersity index (PDI, B and E), and zeta potential (C and F) of ITZ-loaded polymeric mixed micelles (Left column: P123/TPGS mixed micelles and right column: F127/TPGS mixed micelles). An error bar indicates the standard deviation from at least three measurements. *No significant difference comparing between blank and ITZ-loaded mixed micelles at the same polymer mass ratio and polymer concentration. **No significant difference comparing among all polymer concentrations at the same mass ratio. ***No significant difference comparing among various polymer mass ratios of mixed micelles at the same concentration of polymer.

At all mass ratios of ITZ-loaded polymeric mixed micelles, when the polymer concentration reached 20 %w/v, %DL and %EE gradually increased due to more number of micelles formed at higher concentration [28].

Effect of polymer mass ratio:

For both series after drug loading, the reduction of z-ave related to the increase in TPGS mass ratio except for 5 %w/v P123/TPGS mixed micelles at 2:2 mass ratio as shown
in Figure 3A. Again, no correlation between P123/TPGS mass ratio and PDI value was noticed. However, the increasing TPGS fraction in ITZ-loaded F127/TPGS mixed micelles tended to decrease the PDI value as shown in Figure 3E. The absolute zeta potential of all mixed micelles was not affected by the amount of TPGS except for the F127/TPGS mixed micelles at low polymer concentration. As low as 5% w/v polymer concentration, the absolute zeta potential slightly decreased with the change in polymer mass ratio. The improvement of %DL and %EE related to the increment of TPGS proportion in mixed micelles. The maximum %DL and %EE of both P123/TPGS and F127/TPGS mixed micelles were attained when the mixed micelles were prepared at 1:3 polymer mass ratio and 20% w/v polymer concentration. The addition of TPGS in the mixed micelles improved %DL and %EE because of the hydrophobic π-π and van der Waals interactions between the aromatic ring and hydrocarbon chain of TPGS, the PPO segment of Pluronic®, and the aromatic and hydrocarbon rings of ITZ in the inner core of micelles. As previously described, these
interactions contributed to the increase in cohesive force in the inner core and thus led to the enhancement of physical incorporation of drug [29, 30].

4. CONCLUSION
The polymeric mixed micelles were successfully prepared by P123/TPGS and F127/TPGS polymers and primarily aimed to improve drug loading and entrapment efficiency for ITZ by incorporating ITZ into the core of polymeric micelles. From the results, the F127/TPGS mixed micelles exhibited larger particle size and higher PDI value than the P123/TPGS mixed micelles. The particle size of both P123/TPGS and F127/TPGS mixed micelles gradually decreased as the TPGS mass fraction or the total polymer concentration increased. The zeta potential of all polymeric mixed micelles was negative and closed to zero when increasing polymer concentration. The CMC value of polymeric mixed micelles mainly depended on the polymer mass ratio. Higher proportion of TPGS lowered the CMC value of mixed micelles. In addition, the %DL and %EE for entrapment of ITZ could be improved by increasing TPGS mass ratio and polymer concentration.

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