

Physicochemical Properties of Binary Pluronic Systems for Reversal of Multi-drug Resistant (MDR) Cancers

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ABSTRACT – Pluronic as pharmaceutical excipients are listed in the US and British Pharmacopoeia. In particular, 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 composition of structure, and hydrophilic-lipophilic balance (HLB). Pluronics were also known as a P-gp modulator, which was exploited as a reversal molecule of multi-drug resistant (MDR) cancers. We selected a lamella forming Pluronic L92 which has high hydrophobicity and relatively long PEO block among L series of Pluronics. The dispersion of L92 showed great size particles and low stability. To increase the stability and to decrease the particle size, secondary Pluronics (F68, F88, F98, F127, P85, P105, and P123) with relatively long PEO chain were added into 0.1 wt% Pluronic L92 dispersion. The stability of binary systems was increased due to incorporated long PEO chain. Their particle sizes slightly decreased to over 200~400 nm and their solubilization capacity of binary systems didn't change except Pluronic L92/P123 mixtures. The L92/P123 systems showed *ca.* 100 nm sizes and lowest turbidity among the all systems. The solubilization capacity of 0.1 wt% L92/0.1 wt% P123 was slightly increased compared to 0.1 wt% L92 mono system and other binary systems. These nano-sized binary systems may have potential as alternative drug delivery systems with simple preparation method and overcome the drawbacks of mono systems such as low stability and loading capacity.

Key words – Pluronic, binary system, nanosized, MDR, drug delivery system, solubilization

Recently, polymer-based drug delivery and drug targeting systems have a tremendous influence on development of new drug therapies (Langer 1998). Soluble amphiphilic block copolymers can self-assemble into supramolecular structures, which were studied for nano-carriers of anticancer drugs, proteins, and genes (Kwon and Kataoka 1995; Allen et al., 1999; Kataoka and Kabanov 1999), as well as imaging agents (Torchilin 1999). Pluronic, consisting of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) blocks with a PEO-PPO-PEO arrangement (often as EO_x-PO_y-EO_x) have been studied for pharmaceutical applications such as solubilization excipients, drug carriers, immunoadjuvants, cryopreservation agents, and gene carriers (Allison and Byars 1986, 1990; Alakhov et al., 2001; Batrakova et al., 2001; Kabanov and Alakhov

2002; Oh et al., 2004; Oh et al., 2008). This arrangement results in an amphiphilic copolymer in which the number of hydrophilic EO_x and hydrophobic PO_y units can be altered to vary the size, morphology, hydrophilicity, and lipophilicity.

A defining property of Pluronic is micellization defined as a process to form aggregation from individual block copolymer molecules, unimers at concentration of Pluronic above the critical micelle concentration (CMC) and critical micelle temperature (CMT). Pluronic micelles (above 30% PEO content) are commonly pictured as spheres composed of a PPO core and a PEO corona. However, cylindrical and lamellar structures are also included in micelle morphologies (Nagarajan, 1999). This morphology is affected by several factors such as Pluronic composition, concentration, temperature, and quality of the solvent (Allen et al., 1999; Kabanov and Alakhov 2002).

These copolymers such as Pluronic L92 (Pluronic L92 is designated as L92 throughout), P105, and F127 are commercially available from BASF and customized for applica-

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tions and customer needs. In the trade names, the letters stand for solid (F), paste (P), or liquid (L). The last digit of the number approximates the weight content of PEO block in tens of weight percent. The remaining first one or two digits encode the molecular mass of the central PPO block which can be obtained by multiplying the corresponding number by 300. This nomenclature is widely used and helpful for predicting their behavior in solution.

Pluronic has used to interrupt the P-gp mediated drug efflux pump (Batrakova et al., 1999b; Kabanov et al., 2002). Pluronic formulations with anticancer drugs, below their CMC, have been claimed to be effective in treating MDR tumors by their crossing the plasma membrane and suppressing ATP production. The P-gp modulation and cytotoxicity for MDR cells increased with increase in the copolymer hydrophobicity (Batrakova et al., 1999b). This effect has also been linked with gene modulation by Pluronic block copolymers (Batrakova et al., 1999b; Kabanov et al., 2002). In addition, binary systems with two Pluronic have been researched to compensate for drawbacks of a mono system and control the physicochemical properties for smart drug delivery systems (Alakhov et al., 1999; Lee et al., 2003; Lee et al., 2005; Lee et al., 2010; Oh et al., 2004; Oh et al., 2008; Wang et al., 2007). For example, a mixture of hydrophobic L61 and hydrophilic F127 has practical application in pharmaceuticals for a doxorubicin delivery system (Alakhov et al., 1999), 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, L121/F127 and L121/P123 systems produced nanosized stable systems with several-fold higher loading capacity in hydrophobic agents compared to F127 and P123 micelles (Oh et al., 2004).

In this paper, we have tested blend systems with L92 and several secondary Pluronic (Table I) (BASF; Kozlov et al., 2000). L92 has high hydrophobicity and is one of the Pluronic with relatively long PEO block among L series of Pluronic. L92 showed the inhibition of the P-gp efflux systems and high solubilization capacity. However, the hydrophobic Pluronic have a low aqueous stability. We tried to overcome the drawbacks of L92 such as low stability and relatively huge aggregations by addition of intermediate and hydrophilic Pluronic which have relatively long PEO chains compared to L92. We evaluated size, stability, turbidity and solubilization capacity of the blends using a model hydrophobic compound, Sudan III.

Experimental

Materials

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

Preparation of Binary Mixed Systems

The stock solutions (2~10 wt%) of Pluronic were prepared in distilled water and stored at 4°C. 0.01~0.05 wt% L92 dispersion were prepared by diluting the cold stock solution (2 wt%) with distilled water and stored in the refrigerator overnight. In case of the binary systems, the concentration of hydrophobic L92 was kept constant at 0.1 wt% while secondary Pluronic (F68, F88, F98, F127, P85, P105, and P123) was varied from 0.01 to 0.1 wt%. Pluronic binary systems

Table I. Physicochemical Characteristics of Pluronic Used in this Study

Copolymer	MW ^a	Average no. PEO units (x) ^b	Average no. PPO units (y) ^b	HLB ^c	Cloud point in 1% w/v aqueous solution(°C) ^c	CMC(M) ^d
F68	8400	152.73	28.97	29	>100	4.8×10 ⁻⁴
P85	4600	52.27	39.66	16	85	6.5×10 ⁻⁵
F88	11400	207.27	39.31	28	>100	2.5×10 ⁻⁴
L92	3650	16.59	50.34	6	26	8.8×10 ⁻⁵
F98	13000	236.36	44.83	28	>100	7.7×10 ⁻⁵
P105	6500	73.86	56.03	15	91	6.2×10 ⁻⁶
P123	5750	39.20	69.40	8	90	4.4×10 ⁻⁶
F127	12600	200.45	65.17	22	>100	2.8×10 ⁻⁶

^aAverage molecular weights provided by the manufacturer (BASF Co, Parsippany, NJ) (BASF).

^bThe average number of PEO and PPO units were calculated using the average molecular weights.

^cHydrophilic-lipophilic balance (HLB) values of the copolymers and the cloud points were determined by the manufacturer.

^dCritical micelle concentration (CMC) values were determined previously using pyrene probe (Kozlov et al., 2000).

were prepared from stock solutions in the proportions indicated, and left at 4°C overnight to produce unimers. The solutions were transferred to room temperature (23°C) and incubated for more than 1 hour to form the aggregates.

Size Measurements Using Dynamic Light Scattering (DLS)

The effective hydrodynamic diameter (D_{eff}) of the particles was measured by photon correlation spectroscopy using a “Zetasizer 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 ($n=3$).

Turbidity Measurements

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

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 binary mixture dispersions prepared were individually added to the vials and then allowed to equilibrate in the shaker (100 rpm) at room tem-

perature 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.

Results and Discussion

Formation of Binary System Using Pluronics

Amphiphilic block copolymers have various phases depending on their nature, structure, and composition. Pluronic, symmetric triblock copolymers, displayed spherical, cylindrical, and lamellar aggregates according to relative length of PEO and PPO. L92 ($\text{EO}_8\text{-PO}_{50}\text{-EO}_8$) with approximately 20% PEO block and 80% PPO block have *ca.* 0.03% w/v CMC at 37°C (Batrakova et al., 1999a). L92 forms a supra-macromolecular structure with a lamellar morphology because 20% PEO of the whole MW cannot provide sufficient steric hindrance of spherical formation. The lamella aggregation has high solubilization capacity but low aqueous stability (Nagarajan 1999). The size of L92 particle was average *ca.* 600 nm at 0.05% wt and very unstable. The data of DLS measurement showed size fluctuation and multimodal size distribution. As increase of L92 concentration, the aqueous dispersion turned cloud and precipitation in the dispersion was observed even though the size of particle appeared somewhat decreased and size distribution showed bi-modal distribution (Figure 1 and 2, (a)). Although the particle size in L92 dispersion prepared in this study appeared decreased and less fluctuated, the results that may be due to measurement of dispersion phase after precipitation. It

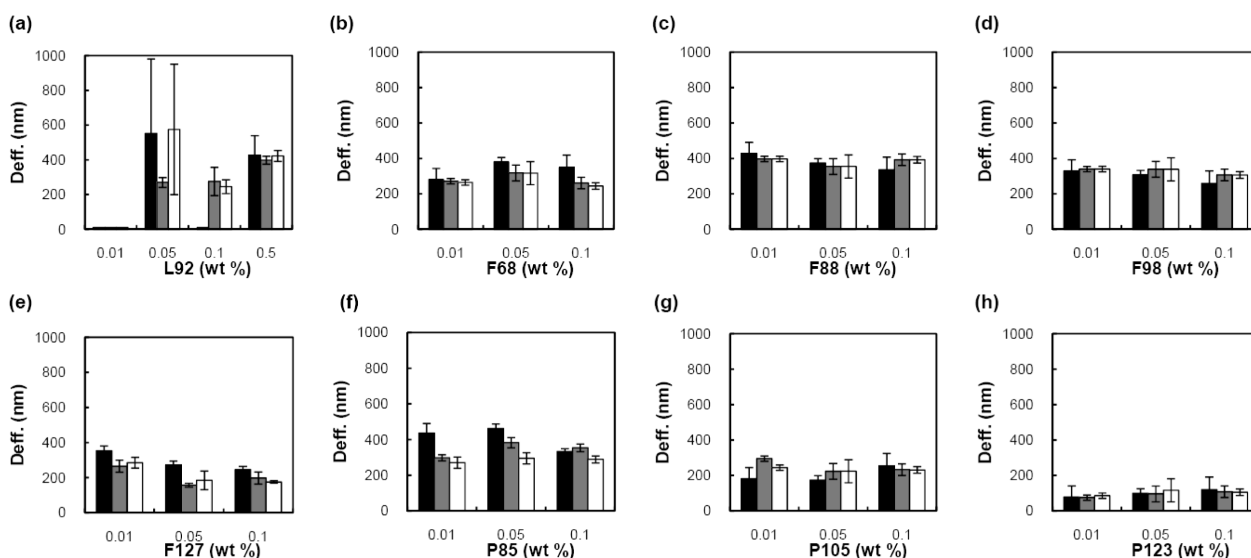


Figure 1. The particle size of different Pluronic dispersions as a function of the concentration at 1 hour (black bar), 12 hours (grey bar), and 24 hours (white bar): (a) mono system of L92 (b)–(h) binary systems of 0.1 wt % L92 admixed with (b) F68 (c) F88 (d) F98 (e) F127, (f) P85 (g) P105 and (h) P123. Each point represents average \pm SD ($n=3$).

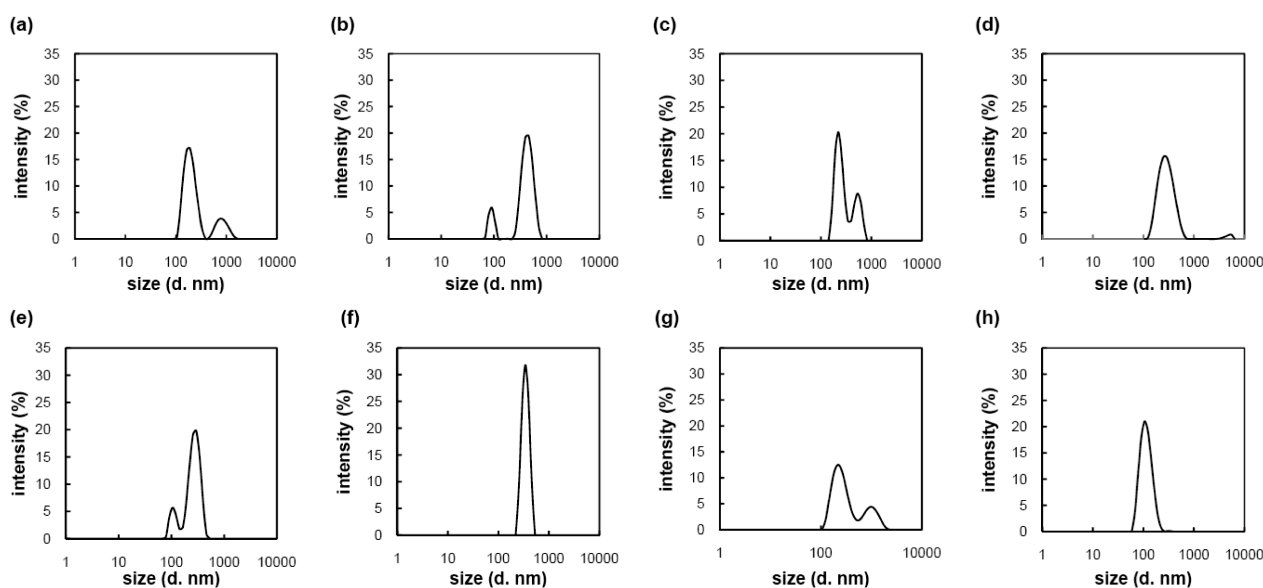


Figure 2. The particle size distribution of (a) 0.1 wt% L92 and mixtures of 0.1 wt% L92 and 0.1% of (b) F68 (c) F88 (d) F98 (e) F127 (f) P85 (g) P105 and (h) P123.

indicated that the kinetic stability in particle of L92 displayed low.

To form a stable dispersion, other Pluronic with relatively long PEO chain was admixed into L92. Figure 1 ((b) ~ (h)) represents the particle size of binary mixtures as a function of concentration of Pluronic. The concentration of hydrophobic L92 was kept constant at 0.1 wt %, which showed less stability with fluctuation and bimodal distribution while the concentration of other Pluronic was varied from 0.01 to 0.1 wt%. In case of Pluronic with long PEO chain, F68, F88, F98 and F127, the addition of hydrophilic Pluronic led to formation of 300 nm to 400 nm aggregation and increase of stability without precipitation for 24 hours (Figure 1, (b) ~ (e)). The size distribution of binary systems was still broad and showed a changed bi-modal distribution at mixture with F68, F88, and F127 (Figure 2). Interestingly, the binary system with 0.1% L92 and 0.1% F98, however, demonstrated the uni-modal distribution (Figure 2 (d)). This suggested that Pluronic with similar MW of PPO block can be blended and form a cooperative binding and those with different PPO block cannot form non-cooperative aggregation (Chaibundit et al., 2007; Gaisford et al., 1997; Oh et al., 2004). In addition, the increased stability of binary systems may be due to addition of hydrophilic Pluronic which can bind into PPO of L92 and provide the steric hindrance by increasing the corona forming PEO (Oh et al., 2004).

Addition of P85, P105, and P123 with intermediate hydrophilic-lipophilic balance (HLB) to L92 similarly resulted in decrease of particle size and increase of aqueous stability (Fig-

ure 1. (f) ~ (h)). The size distribution of L92/P85 and L92/P123 were uni-modal and narrow while L92/P105 showed bimodal size distribution despite of 200 nm particle size (Figure 2. (f) ~ (h)). Notably, L92/P123 systems represented less than 100 nm aggregates in all ranges of concentrations. This may be the facts of that P123 with most long PPO chains tightly self-assembles into a thermodynamically stable micelle ($D_{\text{eff}} = 50$ nm, data not shown) and L92 with very short PEO is incorporated into P123 micelle core led to increase the particle size to 100 nm. Similarly, F127 with same PPO of P123 formed *ca.* 200 nm particles in aqueous solution. The micelle mixed with L92 increased the core size of micelle by adding the PPO of L92, resulting in *ca.* 300 nm.

Turbidity of Binary Systems

The turbidity of L92 dispersion and binary systems including L92 (0.1%) presents in Figure 3. The dispersion of the L92 below 0.1 % was transparent and the turbidity didn't change. The turbidity of 0.5% L92 dispersion was drastically increased and slightly decreased after 24 hours due to precipitation (data not shown). The binary systems of L92 (0.1%) including F68, F88, and F98 (0.01~0.1 wt%) increased the turbidity. This may be due to non-cooperative binding and those secondary aggregations. However, the binary dispersions of L92 (0.1%) with P85, F127, P123 and P105 (0.01~0.1%) decreased the turbidity as increase of secondary Pluronic's concentration. Particularly, L92/P123 showed less turbidity and high stability compared to other binary systems.

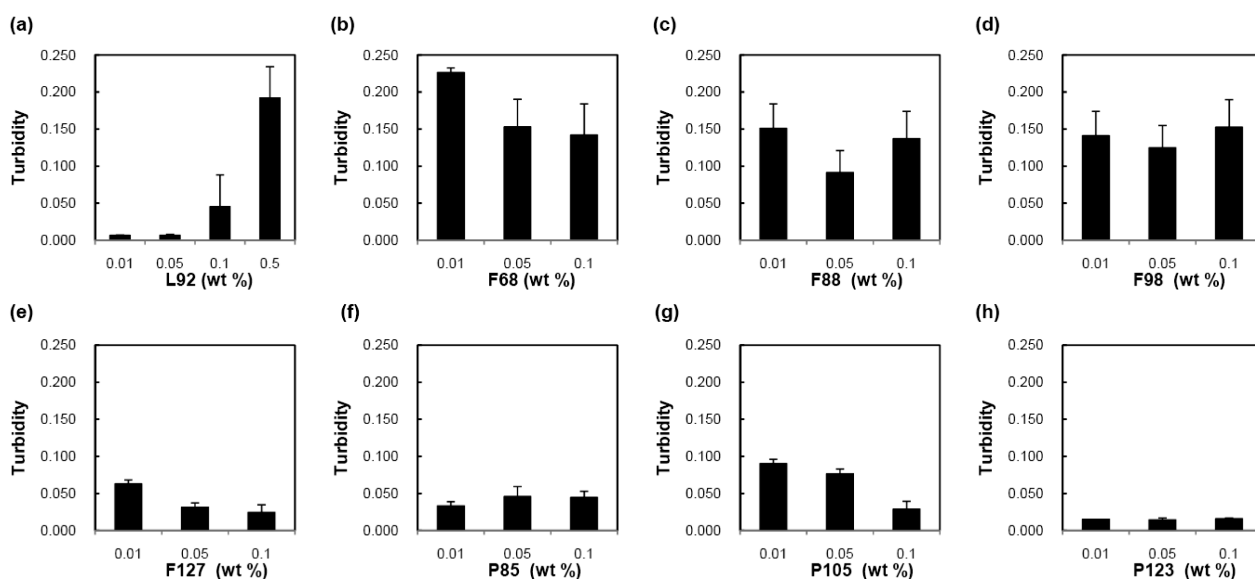


Figure 3. Turbidity of dispersion solutions of (a) mono system L92; and (b)–(h) binary systems of 0.1 wt% L92 admixed with (b) F68 (c) F88 (d) F98 (e) F127 (f) P85 (g) P105 and (h) P123 as a function of the concentration. Each point represents average \pm SD ($n=3$).

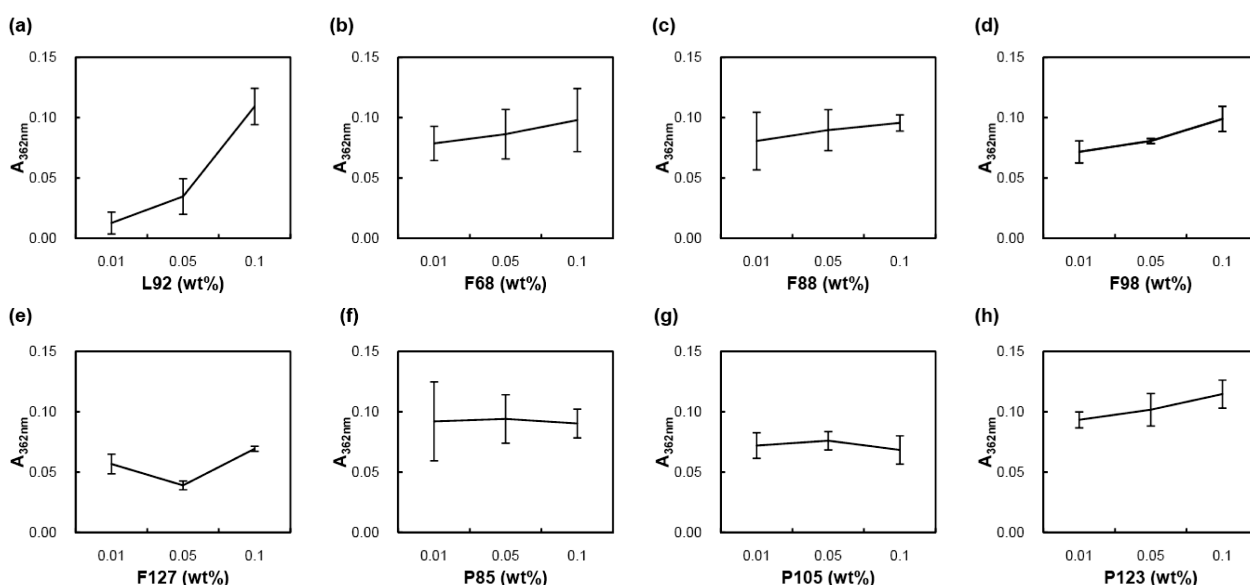


Figure 4. Solubilization of Sudan III in (a) mono system L92; (b)–(h) binary systems of 0.1 wt% L92 admixed with (b) F68 (c) F88 (d) F98 (e) F127 (f) P85 (g) P105, and (h) P123 as a function of the concentration. Each point represents average \pm SD ($n=3$).

Solubilization of Binary Pluronic

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 ($\lambda = 362$ nm) of Sudan III in Pluronic dispersions versus L92 concentration is shown in Figure 4 (a). The solubilization of Sudan III in L92 was increased with raising L92 concentrations but the particle sizes of L92 was also increased by solubilizing Sudan III. The lamella-forming L92 provides a hydrophobic pool to increase in the solubilization

capacity compared to the spherical micelle-forming Pluronic such as F68 and F127 (data not shown). The solubilization capacity of binary systems including L92 prepared in this study was slightly decreased or similar (Figure 4 (b) ~ (h)). However, the particle sizes of the systems didn't change after solubilizing the insoluble dye (data not shown). Interestingly, L92/F127 mixtures drastically decreased the solubilization capacity, which suggested that this combination would not be proper to increase the solubilization of insoluble molecules. On the contrast, the 0.1% L92/0.1% P123 presented slightly

increased solubilization of Sudan III. The incorporation of insoluble dye into the L92/P123 aggregates did not change the particle size, and dispersions remained stable for several days (data not shown). Hence, when the secondary polymer was selected, its cooperative binding to primary polymer, composition of blocks, length of PEO and PPO block, and compatibility between drug and systems should be considered to develop a stable nanocarrier.

Conclusion

Pluronic are used for pharmaceutical applications such as drug delivery systems, excipients for formulation, and nano-sized targeting systems. L92 is hydrophobic, huge aggregation to form the lamellar structure, and unstable in aqueous solution. We tried to develop better systems with L92 by mixing the hydrophilic Pluronic with relatively long PEO block. Among the binary systems, the aggregation of L92/P123 showed less than 100nm size and low turbidity. The dispersion of L92/P123 also presented high stability without change of their size and turbidity for a few weeks. The solubilization capacity of most binary systems didn't increase compared to mono system, L92. Only 0.1% L92/0.1% P123 slightly increased the solubilization capacity. This system can be a possible candidate for anticancer nano-sized carriers to reverse the MDR cancer. These nano-sized systems could be useful for anticancer drug delivery systems with simple preparation methods, high stability, and high loading capacity.

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