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Research paper

Preparation and characterization of polymeric micelles for solubilization of poorly soluble anticancer drugs

Zerrin Sezgin, Nilufer Yüksel *, Tamer Baykara

Department of Pharmaceutical Technology, School of Pharmacy, Ankara University, Tandogan, Ankara, Turkey

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Abstract

The objective of this study is to investigate the solubilization of poorly water-soluble anticancer drugs, octaethylporphine (OEP), meso-tetraphenyl porphine (mTPP) and camptothecin (CPT), in Pluronic and polyethylene glycol–distearoylphosphatidylethanolamine (PEG–DSPE) polymeric micelles. Three different Pluronic and PEG–DSPE polymers with various chain lengths were chosen and micelle formulations were prepared by using various drug:polymer ratios. Formulations were characterized by critical micellization concentration (CMC) values of copolymers, micelle particle size and distribution, zeta potential, loading efficiency and stability. Polymers formed very stable, low CMC micelles with smaller sizes than 100 nm. It was shown that drug loading efficiency highly depends on the polymer type, drug type and their ratios. The most efficient drug loading was obtained by loading mTPP in PEG_{2000}–DSPE and Pluronic F127 micelles. This result is attributed to phenyl groups in mTPP might lead to attraction between alkyl groups in the polymer and increase drug incorporation. PEG–DSPE formulations had higher zeta potential values indicating that they would be more stable against aggregation than Pluronic micelles. From the drug assay aspect Pluronic micelles remained more stable in 3-month long stability test. These results showed that besides their solubilizing effects, polymeric micelles could be useful as novel drug carriers for hydrophobic drugs.

Keywords: Polymeric micelles; Anticancer drugs; Pluronics; Polyethylene glycol–distearoylphosphatidylethanolamine (PEG–DSPE); Critical micellization concentration (CMC)

1. Introduction

In the pharmaceutical technology, many drug-releasing and drug-targeting systems have been developed to reduce drug degradation, improve the bioavailability and enhance the amount of drug in the area of interest [1]. Among those, polymeric micelles are very promising drug carrier systems, which received growing scientific attention in recent years [2,3]. In aqueous media, amphiphilic block copolymers containing hydrophilic and hydrophobic chains self-assemble into polymeric micelles at or above the critical micelle concentration (CMC) [4]. Amphiphilic copolymers form polymeric micelles that are much more stable than micelles prepared from detergents due to low CMC. Many important active agents such as anticancer active agents have poor solubility. In pharmacological respect, hydrophobicity is beneficial for drug–tissue relation but formulation, solubilization and stabilization of these agents is a problem that should be solved. Micelles have a core-shell structure which enables the system to incorporate poorly soluble drugs thus improve their bioavailability and protect from inactivation in biological media. Due to their small particle size (<100 nm), targeting ability, long circulation and easy production, these systems exhibit many advantages on administration and effective delivery of drugs [5]. Incorporation into micellar core may be carried out by chemical conjugation or physical entrapment [6,7]. Depending on steric properties and interrelations of drug and polymer,

* Corresponding author. Present address: Department of Pharmaceutical Technology, School of Pharmacy, Ankara University, 06100 Tandogan, Ankara, Turkey. Tel.: +90 312 212 6805/2409; fax: +90 312 213 1081.
E-mail addresses: zsezgin@pharmacy.ankara.edu.tr (Z. Sezgin), nyuksel@pharmacy.ankara.edu.tr (N. Yüksel).

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a micelle core may/may not be suitable for one drug. For this reason, in this study three different drugs with various hydrophobicities and six polymers with various polymer chains were used to see the factors affecting the incorporation.

As known, a variety of block polymers may be used to form polymeric micelles [8]. Among those, conjugates of poly(ethylene glycol) and diacyllipids, such as phosphatidylethanolamine (PEG–DSPE), are able to form polymeric micelles [9]. Two long hydrophobic fatty acyl groups in the polymer structure lead PEG–DSPE to form stable micelles (Fig. 1). It was previously shown that various hydrophobic, hydrophobized and diagnostic agents were successfully incorporated into PEG–DSPE micelles [10,11]. Pluronic polymers are also known to self-assemble into polymeric micelles in their aqueous solutions [12]. Pluronic polymers have A–B–A basic structure formed by hydrophobic propylene oxide (PO) and hydrophilic ethylene oxide (EO) blocks (Fig. 1). Transfer of the hydrophobic compounds to hydrophobic PO core is described as solubilization.

There are studies on the treatment of malign neoplasms by porphyrins as photosensitizer [13–16]. It is known that porphyrins have the ability of destroying tumor cells when combined with a fixed-frequency laser light. This type of cancer treatment is called photodynamic therapy (PDT). PDT is especially effective in the treatment of gastric, esophageal, colorectal and bronchial tumors. In PDT studies, hydrophilic and hydrophobic photosensitizing agents show different effectiveness. Hydrophobic agents were found to be more effective in killing the tumor cells by penetrating the membranes and destroying the organelles by Jezek et al. [17]. At this point, formulation of hydrophobic porphyrin derivates came out to be a problem. Thus in this study it was aimed to examine the solubilization of two porphyrin derivates, octaethylporphine (OEP) and meso-tetraphenyl porphine (mTPP) (Fig. 2), with different micelle forming Pluronic and PEG–DSPE polymers (Table 1). Micellar solubilization studies were also done in another anticancer drug camptothecin (CPT) in order to see the factors effecting micellar loading.

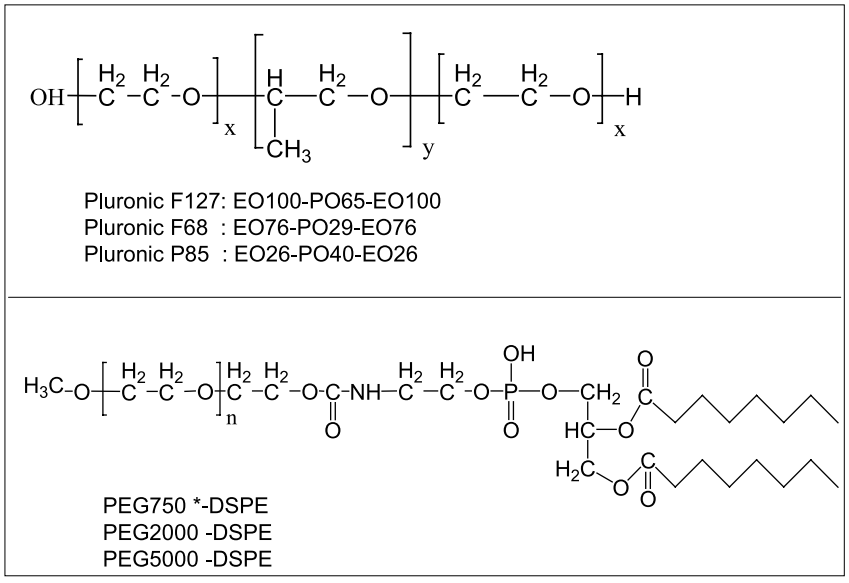

Fig. 1. Chemical structures of Pluronic and PEG–DSPE polymers (EO: ethylene oxide, PO: propylene oxide; PEG: polyethylene glycol, DSPE: distearoylphosphatidylethanolamine, *molecular weights of PEG chains).

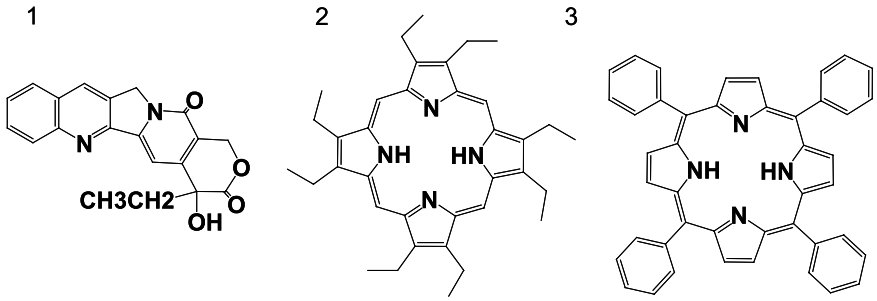

Fig. 2. Chemical structures of **1**: camptothecin (CPT), **2**: octaethylporphine (OEP) and **3**: meso-tetraphenyl porphine (mTPP).

Table 1
Properties of polymers used in micelle preparation

Polymers	Molecular weight	HLB value	CMC (M)	Micelle size (nm)
PEG_{750}–DSPE	1528	n.a	1.0×10^{-5}	5.7
PEG_{2000}–DSPE	2806	n.a.	1.2×10^{-5}	13.9
PEG_{5000}–DSPE	5801	n.a.	1.4×10^{-5}	21.6
Pluronic P85	4600	26	2.3×10^{-4}	1.5
Pluronic F68	8400	29	1.6×10^{-4}	1.3
Pluronic F127	12600	22	6.9×10^{-5}	3.3

Polymers were characterized in terms of CMC to make sure that micellar structure will stay intact upon dilution in body fluids. Drug-loaded micelles were prepared by film formation and dialysis method. Particle size distribution, zeta potential and stability of polymeric micelles were investigated.

2. Materials and methods

2.1. Materials

Diacyllipid-PEGs (PEG_{750}–DSPE, PEG_{2000}–DSPE and PEG_{5000}–DSPE) were purchased from Avanti Polar-Lipids (Alabaster, AL/USA), where 750, 2000 and 5000 are the molecular weight of PEG chains. Pluronics (Pluronic F127, Pluronic F68 and Pluronic P85) were kindly provided by BASF (Mount Olive, NJ, USA). Meso-tetraphenyl porphine (mTPP) and octaethylporphine (OEP) were purchased from Frontier Scientific (Logan, UT, USA). Camptothecin (CPT) was obtained from Sigma (St Louis, MO, USA) and Pyrene was purchased from Aldrich (Milwaukee, WI, USA). HEPES free acid was obtained from ICN Biomedicals, Inc. Dialysis membranes (MWCO: 12–14,000 and MWCO: 3000) were purchased from Spectra/Por (Houston, TX, USA). All other chemicals and components for buffer solutions were of analytical grade preparations.

2.2. Determination of critical micellization concentration (CMC)

Pyrene fluorescence method was used for CMC determination of Pluronic and PEG–DSPE polymers [18–20]. From a stock solution of 2.6 mg/ml pyrene dissolved in chloroform, 19.23 μl aliquots was transferred with a micropipette into a series of clean, dry test tubes and the solvent allowed to evaporate under vacuum by protecting from light to get 50 μg of dry pyrene. A series of polymer solutions (0.0195–10 mg/ml) in HEPES buffer solution (pH 7.4) were added to dry pyrene. Pyrene concentration in each tube was 6.18×10^{-5} M. The mixtures were shaken in dark for 24 h at 25 °C and then filtered through 0.6 μm membrane filter for separation of undissolved pyrene crystals. The concentration of solubilized pyrene in micellar phase was determined spectrofluorometrically at wavelengths of excitation (λ_{ex}) 339 nm and emission (λ_{em}) 390 nm.

2.3. Preparation of drug-loaded polymeric micelles

Dialysis method was used to incorporate CPT in micelles [20,21]. Twenty milligrams of PEG–DSPE film was formed in a round-bottomed flask by evaporating chloroform from 20 mg/ml polymer solution in chloroform. To assure the removal of the organic solvent, extra vacuum was applied for 3 h in lyophilizator. Solutions of CPT in dimethylsulfoxide (DMSO) with different concentrations were added to polymer film to obtain ratios given in Table 2. To load CPT in Pluronic micelles, 10 mg/ml solutions of Pluronics and CPT in DMSO were used to obtain ratios given in Table 2. Samples were mixed by vortexing for 15–20 min and incubated in the shaker overnight. Mixtures were dialyzed against HEPES buffered saline (HBS), pH 7.4, for 3 days to remove DMSO (MWCO = 12–14,000). The CPT concentration was estimated by measuring its fluorescence at $\lambda_{ex} = 360$ nm and $\lambda_{em} = 430$ nm after dilution of samples in 55% methanol solution, pH 5.5.

Two porphyrin derivatives OEP and mTPP were loaded into micelles by film formation method [10,21]. OEP 2.5 mg/ml or mTPP 10 mg/ml solutions in chloroform were added into 20 mg/ml solution of polymers in chloroform to obtain polymer:drug (w/w) ratios in Tables 3 and 4. Organic solvents were removed under vacuum by rotary evaporator and a drug–polymer film was obtained. Micelles were formed by an extensive vortexing of this film in HBS, pH 7.4. Nonincorporated drug was separated by filtration of micelle suspension through a 0.2 μm filter. The OEP concentration in filtrates was estimated by measuring its fluorescence at $\lambda_{ex} = 400$ nm and $\lambda_{em} = 625$ nm after extracting drug into chloroform. For estimation of mTPP concentration, fluorescence was measured at $\lambda_{ex} = 514$ nm and $\lambda_{em} = 650$ nm after extracting drug into toluene.

Table 2
Polymer:drug ratios used in CPT loading in polymeric micelles, incorporated drug percent and drug weight in micelle (%)

Polymers	Polymer: CPT ratio	Drug incorporated (%)	Drug weight in micelle (%)
PEG_{2000}–DSPE	10:1	0.202	0.0184
PEG_{2000}–DSPE	10:5	0.145	0.0484
PEG_{2000}–DSPE	10:10	0.131	0.0653
Pluronic F127	10:1	0.156	0.0142
Pluronic F127	10:5	0.195	0.0650
Pluronic F127	10:10	0.103	0.0516

Table 3
Polymer:drug ratios used in OEP loading in polymeric micelles, incorporated drug percent and drug weight in micelle (%)

Polymers	Polymer: OEP ratio	Drug incorporated (%)	Drug weight in micelle (%)
PEG_{2000}–DSPE	10:0.5	4.70	0.230
PEG_{2000}–DSPE	10:2	11.9	1.98
Pluronic F127	10:0.5	0.340	0.0160
Pluronic F127	10:2	1.20	0.200

Table 4
Polymer:drug ratios used in mTPP loading in polymeric micelles, incorporated drug percent, drug weight in micelle (%), micelle sizes after drug incorporation and zeta potentials

Polymers	Polymer:mTPP ratio	Drug incorporated (%) ± SE[a]	Drug weight in micelle (%)	Micelle size (nm)[b]	Zeta potential (mV) ± SE
Pluronic F127	10:0.5	90.1 ± 1.74	4.29 ± 0.083	5.60	−11.2 ± 0.560
	10:1	7.59 ± 0.162	0.690 ± 0.015	168	−7.25 ± 1.18
	10:2	1.80 ± 0.126	0.300 ± 0.021	33.8	−3.21 ± 0.650
Pluronic P85	10:0.5	4.87 ± 0.852	0.232 ± 0.041	21.9	−10.0 ± 0.930
	10:1	2.64 ± 0.484	0.240 ± 0.044	20.6	−11.8 ± 1.12
	10:2	0.810 ± 0.189	0.135 ± 0.031	20.3	−15.5 ± 1.25
Pluronic F68	10:0.5	4.12 ± 0.789	0.196 ± 0.038	20.5	−12.6 ± 1.41
	10:1	2.82 ± 0.310	0.257 ± 0.029	29.9	−8.54 ± 1.60
	10:2	2.13 ± 0.113	0.355 ± 0.019	79.7	−13.1 ± 1.00
PEG_{750}–DSPE	10:0.5	0.340 ± 0.020	0.0162 ± 0.0010	12.9	−27.8 ± 0.920
	10:1	1.04 ± 0.288	0.0942 ± 0.026	10.9	−22.9 ± 1.33
	10:2	0.244 ± 0.010	0.0406 ± 0.0017	13.5	−31.2 ± 1.13
PEG_{2000}–DSPE	10:0.5	93.3 ± 2.26	4.44 ± 0.11	38.5	−34.5 ± 0.570
	10:1	95.4 ± 7.80	8.68 ± 0.71	29.5	−13.7 ± 1.39
	10:2	24.3 ± 0.502	4.05 ± 0.084	41.0	−23.1 ± 1.37
PEG_{5000}–DSPE	10:0.5	82.1 ± 2.27	3.91 ± 0.11	11.1	−10.9 ± 1.59
	10:1	19.5 ± 0.447	1.77 ± 0.041	19.3	−13.9 ± 0.680
	10:2	8.16 ± 0.226	1.36 ± 0.038	19.9	−12.0 ± 0.680

[a] SE is standard error.
[b] Mode values.

After estimating the drug contents spectrofluorometrically, incorporated drug (%) and drug weight (%) in micelles were calculated using Eqs. (1) and (2):

$$\text{Drug incorporated } (\%) = \frac{a}{b} \times 100 \quad (1)$$

$$\text{Drug weight in micelle} = \frac{a}{b+c} \times 100 \quad (2)$$

where a is the amount of drug loaded in micelle (g), b the amount of drug used in micelle preparation (g) and c the amount of polymer used in micelle preparation (g).

2.4. Determination of micelle size

Micelle hydrodynamic diameters with or without drug were measured using dynamic light scattering with a Coulter N4 nanosizer (Coulter Scientific Instruments, Miami, FL, USA). The analyses was performed with 10 mW He–Ne Laser (633 nm) at scattering angle of 90 ° at 20 °C [20,22]. For each sample, the mean diameters were calculated as mode values from particle size distribution results according to below mentioned formula [23]:

$$M_0 = L_{M0} + \left(\frac{d_1}{d_1 + d_2}\right) w$$

where L_{M0} is the lower limit of mode class, d_1 the frequency of the modal class-frequency of the previous class, d_2 the frequency of the modal class-frequency of the following class and w the width of the interval.

2.5. Determination of zeta potential

Zeta potential was measured using PALS (phase analysis light scattering) Zeta Potential analyzer (Ver. 326, Brookhaven Ins. Coop., Holtsville, NY, USA). Before the measurements, the micelle formulations were dialyzed against 5 mM HEPES solution to remove NaCl ions. Particle sizes of the micelles were measured to assure that micellar structure was intact. Zeta potential was determined six times for each sample. Results were automatically calculated by the analyzer using the following Smoluchowski equation:

$$m = \frac{e.z}{h} \quad (3)$$

where z is the zeta potential, m the mobility, e the dielectric constant and h the absolute viscosity of electrolyte solution.

2.6. Stability studies

Stability test of micelles consisted of visual control and analytical measurement of drug content. For this purpose, lyophilized and aqueous solution forms of mTPP-loaded micelles were placed in a climatic chamber at 25 ± 2 °C, 60% relative humidity for 3 months. Lyophilized forms were placed in 2 ml Eppendorf tubes and solution forms were placed in 5 ml colored bottle with a lid. Samples were taken to determine the drug content at the beginning and at the end of 3 months. Drug content was measured spectrophotometrically after extracting drug into toluene and UV spectrums were also checked.

3. Results and discussion

3.1. Determination of CMC

In CMC, determination pyrene method was chosen due to its functional, versatile and easy application. Pyrene prefers to participate in hydrophobic microenvironment. For

CMC determination of copolymers, the fluorescence intensity of pyrene vs. logarithm of the copolymers concentration in HBS (pH 7.4) was plotted.

At low polymer concentration, low pyrene intensity was observed but beyond a specific polymer concentration pyrene intensity increased extremely. Thus a graph with two linear segments having different slopes was formed. The intersection point of these two segments gave CMC value. The graph used for CMC determination of PEG_{750}–DSPE copolymer is given in Fig. 3.

The CMC values of polymers in aqueous solution are presented in Table 1. CMC values are well associated with hydrophilic/lipophilic block lengths. In PEG–DSPE polymer, the length of PEG chain increases as 750, 2000 and 5000 and the CMC values were 1.0×10^{-5}, 1.2×10^{-5} and 1.4×10^{-5} M, respectively. It is obviously seen that CMC values were increased by decreasing hydrophobic part ratio in polymer. These results are in good agreement with other researchers' findings [24–26]. It is known that, onset of micellization is determined mainly by the nature and the length of hydrophobic block, where the nature of soluble hydrophilic blocks has only a slight dependence on the onset of micellization [24].

In different Pluronic copolymers, the lengths of both hydrophilic EO and hydrophobic PO chains change. Chemical structure, molecular weight and HLB values of Pluronics are given (Fig. 1) (Table 1) [12,27]. Different CMC values are given for Pluronics in several literatures. Depending on the CMC determination method, 3–10 times deviations can be seen in CMC values. Although CMC is given as a specific single value, micellization and transformation into micelle structure can be observed in a wide concentration range close to CMC or above CMC [12,28]. In our results, we observed that CMC values were influenced by hydrophobic PO chain length, not by molecular weight or HLB value of the polymer (Table 1). For Pluronic F127 with the longest PO length, CMC was the lowest (1.0×10^{-5}). The finding of lower CMC for Pluronic F68 (PO length: 29) than Pluronic P85 (PO length: 40) is not compatible with above result. This may be due to changing hydrophilic chain length besides hydrophobic chain length in Pluronic polymers. Approximate EO lengths for Pluronic P85 and Pluronic F68 are 52 and 152, respectively.

When Pluronics and PEG–DSPE were compared, low CMC of PEG–DSPE was connected to high hydrophobic interaction between two acyl chains in PEG–DSPE structure (Fig. 3).

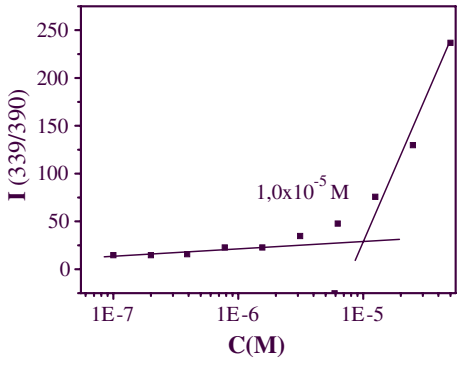

Fig. 3. Plot of fluorescence intensity of pyrene vs. logarithm of the PEG_{750}–DSPE copolymer in HBS (pH 7.4).

3.2. Loading of drugs in polymeric micelles

Hydrophobic micelle core works as a cargo to improve solubility and stability of lypophilic drugs. The capacity of this cargo space is limited, for example, Pluronic P85 has a cargo which is 13% of the whole micelle weight [12]. Drugs loaded into micelle core by hydrophobic interactions and covalent/non-covalent bond formation. Consequently, depending on both hydrophobic interactions and steric properties it is not possible to load all kind of drugs into polymeric micelles. Several of the major factors which influence the loading capacity and loading efficiency of block copolymer micelles are nature of the solute, nature of the core forming block, core block length, total copolymer molecular weight, solute concentration and to a lesser extent, the nature and block length of the outer micelle shell (corona) [8]. For reasons, we studied these three different drugs and six different micelle-forming copolymers.

Dialysis method was used to load CPT in polymeric micelles. In both polymer types, no loading efficiency was obtained. Changing the drug:polymer ratios did not contribute to loading (Table 2). It is pointed out that copolymer type and employed solvents are very effective on the success of dialysis method. When drug–drug or drug–solvent interactions are higher than drug–polymer interaction, loading efficiency decreases [6].

OEP was loaded into micelles by thin film method. The maximum loading efficiency of 11.9% was obtained by loading OEP into PEG_{2000}–DSPE. Loading percent into Pluronic F127 micelles was less than PEG_{2000}–DSPE micelles (Table 3). With further increase in polymer–drug ratio from 1:0.5 to 1:2, there was an increase in loading efficiency of OEP with both polymers. This might show that amount of added OEP was not exceeding the drug-carrying capacity of the core in these micelles.

By using the same film formation method, loading efficiency of mTPP was higher than that of OEP (Table 4). A loading of 90.1% was obtained in Pluronic F127 micelles, which has the longest PO chain. As seen in Fig. 4 high mTPP loading efficiencies of 95.4 and 82.1% were obtained by loading mTPP into PEG_{2000}–DSPE and PEG_{5000}—DSPE, respectively. The high drug-loading efficiency obtained when PEG_{2000}–DSPE polymer is used shows that this polymer is optimum in terms of micelle stabilization. Contrary to OEP, in mTPP loading study no regular increase on loading efficiency was observed with increasing drug ratio. The difference might come from the presence of possible precipitation or aggregate formation in the media caused by increasing drug concentration. It

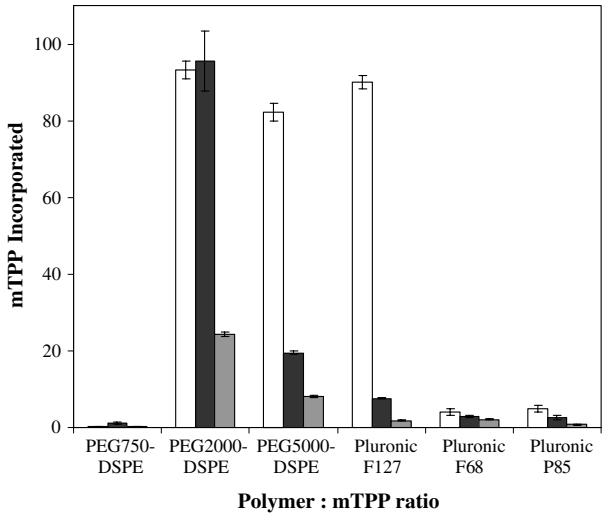

Fig. 4. mTPP incorporation into Pluronic and PEG–PE polymeric micelles prepared at 10:0.5 (□), 10:1 (■) and 10:2 (■) polymer:drug ratios.

was previously shown that increasing drug concentration causes aggregate formation which leads to a decrease in loading efficiency [29].

Due to phenyl groups in its structure mTPP loads into micelles more efficiently than other drugs. It is thought that micellar incorporation was provided by the forces assembled via the attraction between alkyl groups in the polymer and aromatic groups in mTPP. The asymmetric electron density distribution of atoms in the non-polar parts of the molecules forms temporary dipoles which lead to assembly of electrostatic van der Waals forces. Attraction between both PEG–DSPE and mTPP and molecules themselves may also affect the loading [30]. In the hydrophobic PO chain of Pluronics, there is only one $-CH_3$ per monomer while there are two saturated acyl chains with 15 carbon atoms in the PE position of PEG–DSPE polymer. This may lead to more interaction sites for mTPP with polymer thus leading to higher loading into PEG–DSPE micelles.

3.3. Determination of micelle size

Dynamic light scattering method was used to measure the hydrodynamic diameter of polymeric micelles [12,28]. Mean diameters for plain micelles in HEPES buffer are given (Table 1). Figs. 5A and B shows the size distribution of Pluronic F127 and PEG_{2000}–DSPE micelles. Mean size of the plain micelles is between 1.3 and 21.6 nm. Sizes of PEG–DSPE micelles were found to be larger than those of Pluronic micelles. In case of PEG–DSPE micelles, the particle size was increased as the PEG chain gets longer. According to literature, the length of PO and EO chains and their ratios are associated with the mean diameter of Pluronic micelles [12]. For the polymers that we used both the length of PO and EO chains and their ratios are not suitable to make such a comparison. But the micelles size results are compatible with the literature data [12].

In general, plain micelle sizes were smaller than drug-loaded micelles (Table 4). The sizes of two micelles with the highest drug loading, Pluronic F127 and PEG_{2000}–DSPE, are given in Figs. 5C and D. After drug incorporation, the mean size was raised from 3.3 to 5.6 nm in Pluronic F127 micelles (at a polymer:mTPP ratio of 10:0.5) and from 13.9 to 29.5 nm in PEG_{2000}–DSPE micelles (at a polymer:mTPP ratio of 10:1) (Fig. 6). The characteristic particle size for micelles is between 10 and 100 nm. The particle size measurements ensure the formation of polymeric micelles. In some formulations, such as mTPP-loaded Pluronic micelle at 10:1 polymer:drug ratio, high particle sizes such as 168 nm were obtained. This may be due to micelle aggregation.

3.4. Zeta potential

Zeta potential of mTPP-loaded micelles was measured as in Table 4. It was shown that formulations have negative surface charges. Zeta potential of Pluronic and PEG–DSPE micelles was within the ranges from −3 to −13 MV and from −11 to −34 MV, respectively. As the PEG chain length increases in PEG–DSPE polymers, the absolute value of zeta potential decreases. The zeta potential of PEG–DSPE micelles showed that they are more stable against aggregation than Pluronic micelles.

3.5. Micelle storage stability

For comparison, mTPP-loaded micelles prepared from two different polymer types with the highest loading efficiency were used in stability study. Micellar structure is strongly affected by temperature. The temperature at which micelles are formed is known as critical micellization temperature (CMT). For most copolymers this value is 25–40 °C. If micelles were kept in refrigerator, temperature would fall lower than CMT, thus micelles would lose their intact structure and drug would precipitate [12]. For this reason, stability test was performed at 25 °C. No sedimentation or phase separation was observed in the 3-month stability test. The spectrophotometric spectrums stayed the same in 3 months, showing that no chemical degradation took place. Drug content of mTPP-loaded Pluronic micelles did not change in 3 months showing the high storage stability of this formulation.

In the aqueous form of mTPP-loaded PEG–DSPE micelles, a 13.45% decrease in mTPP content was observed. This may be due to degradation of PEG–DSPE micelle structure. The commercial PEG–DSPE polymers are solutions in chloroform and they are kept at −20 °C or less. For this reason, keeping PEG–DSPE micelles at 25 °C in aqueous solutions was not appropriate with respect to storage stability. It was demonstrated that PEG–DSPE polymers were more stable in lyophilized form. In general, Pluronic formulation was more stable than PEG–DSPE formulation both in lyophilized and aqueous forms.

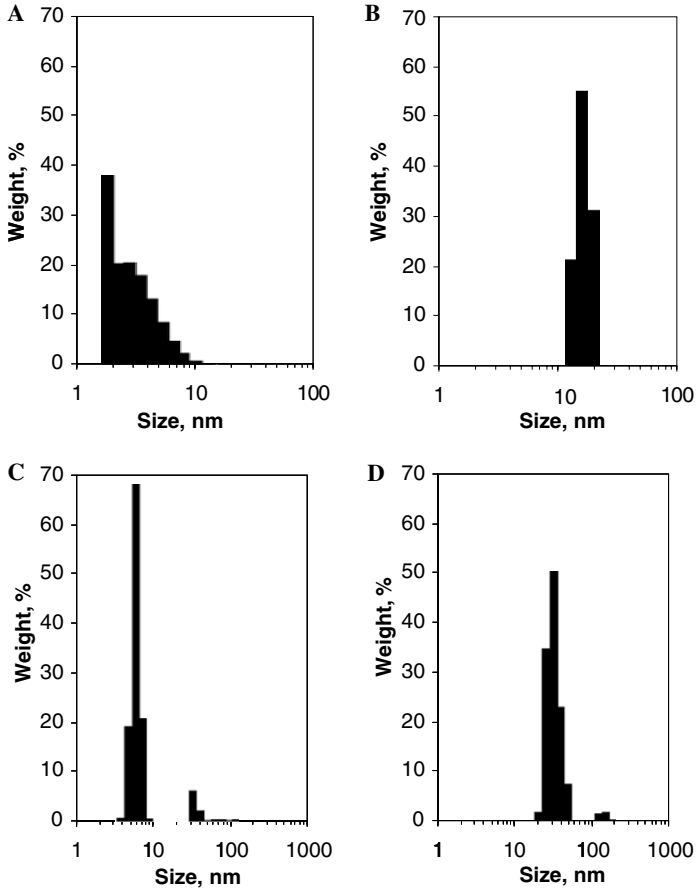

Fig. 5. Particle size distribution of Pluronic F127 and PEG_{2000}–DSPE micelles (A: empty Pluronic F127 micelles, B: empty PEG_{2000}–DSPE micelles. C: mTPP loaded Pluronic F127 micelles, D: mTPP-loaded PEG_{2000}–DSPE micelles).

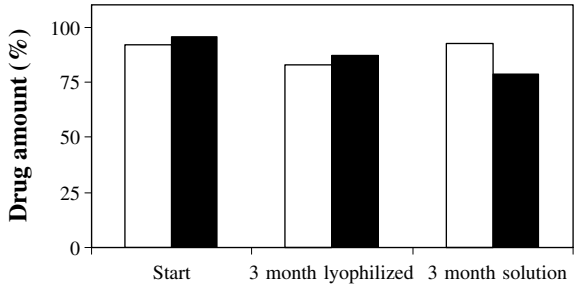

Fig. 6. Stability of lyophilized and solution forms of Pluronic F127 micelles prepared at 10:0.5 polymer:drug ratio (□) and PEG_{2000}–DSPE micelles prepared at 10:1 polymer:drug ratio formulations (■).

4. Conclusions

In this study, Pluronic and PEG–DSPE micelles were prepared. The low CMC values of both polymers showed that they form stable micellar forms and keep their intact structure upon dilutions with body liquids thus they would protect the drug until the target site. PEG–DSPE conjugates are introduced to control drug release area as liposome surface modifiers. They have amphiphilic property and they are relatively new polymers in micelle preparation. PEG–DSPE polymers showed lower CMC values than Pluronics. This is due to diacyllipid chains which might lead to an increase in hydrophobic interactions between the polymer chains in the micelle's core and provide some advantages in particle stability compared to Pluronic polymers [5]. The sizes of prepared micelles were generally between 10 and100 nm and show a narrow distribution. This small size of micelles provides targeting, decreases in emboli risk, encourage the particular absorption in GI system (peptide–protein agents, oral vaccine applications, etc.) and simple sterilization by filtration. For this reason, small particle size is a unique property of micelles. Drug loading studies demonstrated that a micelle core may be suitable for one drug while being unsuitable for another. For a specific application and drug, it is necessary to adjust the copolymer structure and properties such as hydrophilic–lipophilic block length and ratios, hydrophobicity degree and polarity.

A hydrophobic drug mTPP was successfully loaded in PEG_{2000}–DSPE and Pluronic F127 micelles efficiently. The results of this study showed that polymeric micelles can be used as drug carrier systems for many poorly soluble or non-soluble drugs and especially for anticancer drugs.

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