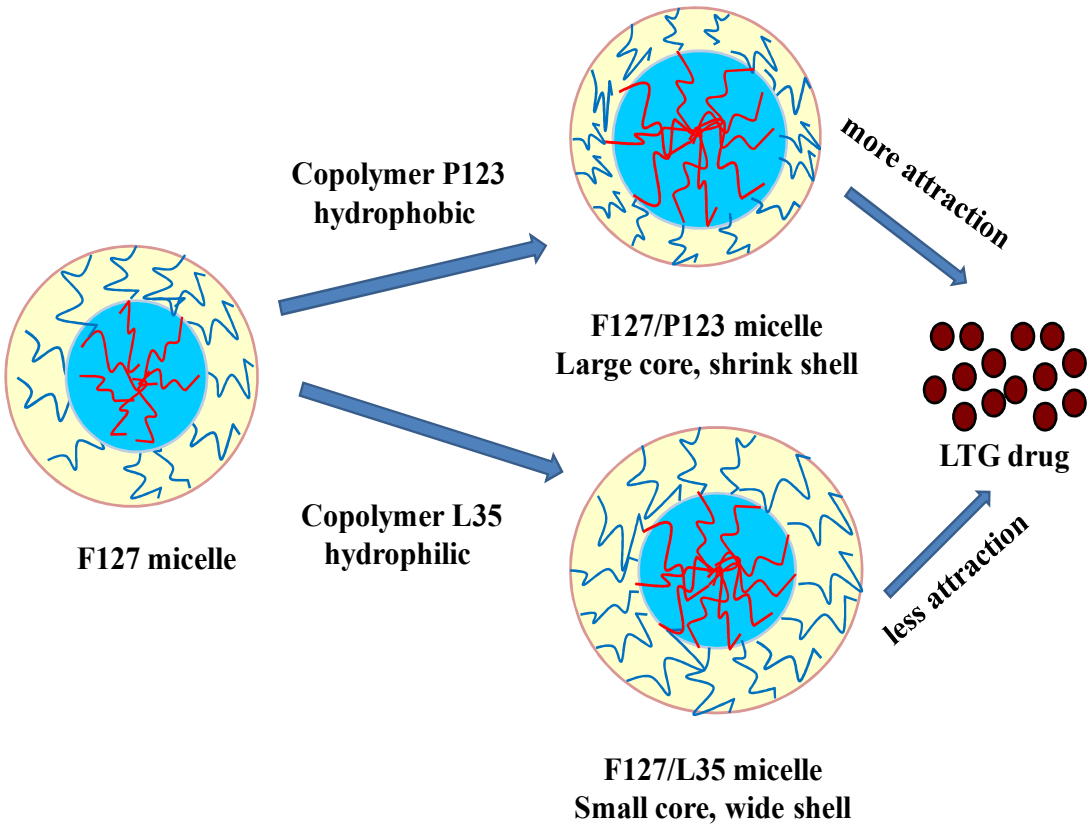
Self-assemblies of mixed PEO-PPO-PEO triblock copolymeric systems for Lamotrigine drugs



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5.1. Introduction

The amphiphilic block copolymers have been used in as pharmaceutical and biotechnology excipients for more than four decades. The associative behavior of amphiphilic block copolymer in the form of micelles as a solubilizer for hydrophobic drugs have been explored in high amount and considered as a fast-growing field of nanomedicine technology [1-6]. In an aqueous environment, the hydrophobic blocks of the copolymer form the core of the micelle while the hydrophilic blocks construct the corona/shell. The hydrophobic core serves as a microhabitat for the placement of lipophilic drugs, while the hydrophilic corona works as a stabilizing interface between the hydrophobic core and the outer environment.

One specific class of such amphiphilic block copolymers, polyethylene oxide (PEO)-polypropylene oxide (PPO)-polyethylene oxide (PEO) block copolymers are commercially available non-ionic surface active agents approved by FDA as pharma excipients and well known by their trade name Pluronic® (BASF) [7-9]. They self-assemble into nanosized micelles in aqueous media with a core consisting of a PPO block surrounded by a heavily hydrated corona of PEO block [10,11]. These Pluronic polymer form nanosized micelles at above the critical micelle concentration (cmc) and the critical micelle temperature (cmt), and it is fully dependent on their concentration and temperature in the system. At high concentrations, the closed packing of micelles results in the formation of a variety of gel type ordered structures. Thus, these Pluronic polymers are capable of displaying thermally reversible gelation behavior [12-14]. Pluronic micelles can show spherical, cylindrical or lamellar morphology. This depends on the relative length of both PEO and PPO blocks, which changes the hydrophilic-lipophilic balance (HLB) of the block copolymers [13-16]. Due to their small micelles/particle sizes (<100 nm), Pluronic systems show many good advantages like targeting ability, long circulation time and easy production on effective delivery of drugs. The encapsulation of low molecular weight hydrophobic drugs into Pluronic micelles can increase the solubility and stability of the drug, which improves their pharmacokinetics and

biodistribution. Therefore, Pluronics polymers have found notable applications in drug delivery systems [14-23].

Pluronic F127 ($PEO_{100}PPO_{65}PEO_{100)}$ versatile tri-block copolymer has generated much interest in the research of controlled drug solubilization and delivery, due to its structural ability to changes with temperature and concentration [24-26]. It was found that drugs like naproxen and indomethacin decrease the micellar sizes and aggregation numbers as well as lowering of the gelation temperature of Pluronic F127 solutions [27]. The micellization of Pluronic F127 micelle was systematically studied when different hydrophobicities of drug molecule were incorporated in the micellar PPO cores [28]. Scherlund et al [29] also confirmed that cmt and gelation temperature of Pluronic F127 was decreased with dilution and an increase in the pH of the medium with added local anesthetics. However, sometimes poor solubility and low stability of many lipophilic drugs in Pluronic F127 solutions, make it difficult to use alone as nanocarriers. Copolymer F127 also shows poor drug-loading capacity [30-33]. The Pluronic F127 self-assembled into nano-sized micelles in aqueous solutions due to the entropy-driven association of their PPO blocks. A major problem was encountered with F127 is the relatively high cmc compared to other hydrophobic c Pluronic like P123, which results in the dissociation of micelles upon dilution many times [27,33]. The dissociation of micelles favors releasing of drug well before it reaches the required site. To overcome this instability of nano-sized micelles, several stabilization approaches were investigated including the formation of mixed micelles with two or more kind of Pluronic polymers [16,22,23,34].

Binary Pluronic micellar systems may compensate for the disadvantages of a mono system and control the physicochemical properties for better drug delivery system. As per the earlier study, mixed micelles composed of Pluronic polymers show synergistic properties to those composed of the single Pluronic such as increased micelle stability and more effective solubilization capacity [14]. Here, we would like to develop the binary mixed micellar systems with PluronicF127 as one of the components because of its versatility in therapeutic applications. The binary mixture with Pluronic F127 and

Pluronic L121 was developed by Oh et al [23]. They show cooperative aggregation with extra energy input such as heating and ultrasonication to form stable nano-sized particles. Mixed Pluronic F127/L121 micelles exhibited 10-fold higher solubilization capacity compared to that of Pluronic F127 micelles. Dutra et al [34] have been reported the binary mixture of Pluronic F127/P123 as nanocarriers for griseofulvin drug. Pluronic P123 shows better solubility, whereas F127 shows good colloidal stability and form stables nano-sized mixed micelles in water. Lee et al [16] have been also developed the binary mixed system with Pluronic P123 and Pluronic L121 as a nano-sized carrier for model drug Sudan III. The lamellar micelle forming PluronicL121 was incorporated with P123 to produce nano-sized dispersions with greater stability due to P123 and high amount of solubility because of Pluronic L121.

In this context, we were attended to develop binary mixtures of Pluronic polymers with the similar length of the hydrophobic block, Pluronic F127/P123, and different length of both the blocks, PluronicF127/L35 for solubilization of an anticonvulsant drug, lamotrigine (LTG).

The oral antiepileptic drug (AED), lamotrigine (LTG), chemically 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine is widely used in the treatment of in pediatrics and adults [35,36]. It also works by inhibiting voltage-dependent sodium channels [37]. Here, the prepared binary mixed Pluronic micellar systems have been characterized in terms of cmc, particle size as micelle hydrodynamic diameter (D_h) and morphological observations using the techniques like UV-Visible spectroscopy, dynamic light scattering, and small angle neutron scattering. The solubilization capacity of the binary Pluronic systems was evaluated for the expectation, of this new modality with potentially enhance the bioavailability of LTG drug, thereby improving patient compliance.

5.2: Experimental Section

5.2.1: Materials

Three PEO-PPO-PEO triblock copolymers (Pluronics®) were procured from Sigma-Aldrich (St.Luice, MO, USA) and used without further purification. Their characteristic is represented in Table.5.1. Lamotrigine (LTG) drug was received as a gift sample from PAB Organics, Gujarat, India. The pyrene probe for cmc determinations and D_2O for SANS sample preparations were of Sigma-Aldrich (St.Luice, MO, USA) and used as received. All the solvents used were of HPLC grade. Triple distilled water for all the sample preparations and Pyrex ™ glass apparatus was always used.

Table .5.1: Molecular characteristics of the studied Pluronic polymers.

Pluronic polymer	MW, ($g.mol^{-1}$)	%EO	nEO	nPO	CP, (°C) @1.0wt%soln.	HLB
F127	12600	70	100	65	>100°	22
P123	5750	30	20	69	90°	8
L35	1900	50	11	16	73°	19

5.2.2: Preparation of binary mixtures of Pluronic polymers

To prepare the solutions of binary mixtures of Pluronic polymers, Pluronic F127/P123 and Pluronic F127/L35 (10wt% total concentration and 70:30, 50:50, 30:70 copolymer weight ratio), the required amount of each Pluronic was first dissolved in deionized water (pH 7.0) at 4°C and then equilibrated at room temperature (30 °C) and kept for more than 1 h. The composition and code of prepared binary Pluronic mixtures are shown in Table.5.2.

Table .5.2: Composition of Pluronic block copolymers and their binary mixtures.

No.	Systems	Pluronic polymer, (wt%)			% Composition	Code name
		F127	P123	L35	X_{F127}: $X_{P123/L35}$	
1.	F127	10	--	--	1.0 : 0.0	F127
2.	F127 + P123	7	3	--	0.7: 0.3	FP70
3.	F127 + P123	5	5	--	0.5 : 0.5	FP50
4.	F127 + P123	3	7	--	0.3: 0.7	FP30
5.	P123	--	10	--	0.0 : 1.0	P123
6.	L35	--	--	10	0.0 : 1.0	L35
7.	F127 + L35	7	--	3	0.7: 0.3	FL70
8.	F127 + L35	5	--	5	0.5 : 0.5	FL50
9.	F127 + L35	3	--	7	0.3: 0.7	FL30

5.2.3: Determination of critical micelle concentrations

The cmc of pure Pluronic (F127, P123, and L35) and their binary mixtures with the different ratio in water were determined at 30°C using pyrene as a UV probe as reported earlier [38]. The desired amount of pyrene was dissolved in methanol and added to the Pluronic solutions in the ratio of 1:100 (50µL: 5 mL). Thus, solutions of Pluronic polymers ranging from 0.0001 to 10 wt% concentrations and a fixed amount of $1x10^{-4}$M of pyrene were obtained. The absorbance measurements of the prepared samples were taken after around 24 h with proper filtration using UV–Visible double beam spectrophotometer (Shimadzu, Japan, UV-2450).

5.2.4: Solubilization experiments

Solubilization of LTG drug was determined with "shake flask" method as reported by Aliabadi et al [39]. Pluronic polymers and their binary mixtures of respective concentrations were prepared in deionized water (10 mL). Then, a portion of LTG drug (w ≈ 10 mg, excess than normal) was added to an aliquot of Pluronic solution. The system was slowly stirred at 30 °C (± 0.1 °C) for four days in a thermostatic bath. After that, 3 mL of supernatant was filtered (0.45 µm Millipore) to remove any non-solubilized LTG drug. Aliquots of the filtered samples were diluted with methanol and the LTG drug concentration was monitored through UV-Visible spectroscopy at the wavelength of 307

nm using the calibration curve (shown in chapter.3) based on Beer's law All measurements were made in triplicate.

5.2.5: Micelle characterization

5.2.5.1: Dynamic light scattering (DLS) analysis

Dynamic light scattering (DLS) technique is based on the Brownian motion, where the time-dependent fluctuations in the intensity of scattered light through a suspension of particles undergoing random. Analysis of such intensity fluctuations allows obtaining the diffusion coefficients which one used in Stokes-Einstein equation for determination of the particle size in the form of average hydrodynamic diameter (D_h).

$$D = k\ T/6\ \pi\ \eta\ R_h$$

Where, D = diffusion coefficient, k = boltzmann constant, T= temperature, η = dynamic viscosity and R= hydrodynamic radius

The D_h of Pluronic and their various binary mixtures in presence and absence of LTG drug was determined using the equipment Horiba-Zetasizer, SZ-100 with a fixed scattering angle of 90°at 30°C. The measurements were made using the filtered aliquots remaining from the solubilization procedure. All the measurements were made in triplicate.

5.2.5.2: Small angle neutron scattering (SANS) measurements

The micellar morphology and structural details of Pluronic F127 and their binary mixtures with Pluronic P123 and Pluronic L35 (F127/P123 and F127/L35) with and without LTG drug in D_2O were determined through SANS measurements. SANS measurements were carried out on SANS diffractometer operating at Dhruva Reactor, Bhabha Atomic Research Centre (BARC), Mumbai, India [40]. All the data were recorded at 30°±0.1°C.

5.3: Results and Discussion

Amphiphilic molecules tend to associate into micellar systems as a spontaneous process in water. The micelles of PEO-PPO-PEO triblock copolymers consist of a desolvated PPO core and a solvated PEO corona. Pluronic F127 is currently approved for pharma applications by the FDA [17]. Owing to high molecular weight with 70% of polyethylene oxide (PEO) units, Pluronic F127 is forming micelles at quite high concentration [27,33].

In the present study, the combination of Pluronic F127/P123 and PluronicF127/L35 have been chosen in order to combine the advantages of the two distinct block copolymers, the binary Pluronic mixture has low molecular weight difference and similar length of PPO blocks (F127/P123) and, other binary mixture has very high difference molecular weight with different length of PEO as well as PPO blocks (F127/L35).

5.3.1. cmc_s of binary Pluronic mixtures

The cmc of single Pluronic polymers and their binary mixtures (F127/P123 and F127/L35) in water was studied by UV-Visible spectroscopy using pyrene as a probe. Pyrene is hydrophobic with poor solubility in water but has increased solubility in micelles environment. When the concentration of Pluronic polymers is less than cmc, the absorbance changes slightly, and it differs dramatically at above cmc. The phenomenon is related to the reality that pyrene can move into the core of the micelles from the aqueous phase, which results in an alteration in the polarity around [38].

Fig.5.1 shows the plot of cmc values versus weight percentage of the copolymer in solution (X, wt%) at 30°C. The observed cmc values of Pluronic F127 and their binary mixtures were listed in Table.5.3 and compared with those reported in the literature [34]. The cmc of Pluronic F127 and Pluronic P123 were 0.0698 wt% and 0.0067 wt%, which is in accordance with reported one [41]. It was found that cmc is affected by the difference in length of the PEO block and PPO block. Although the effect of PEO on the

cmc is less pronounced than that of PPO, the CMC becomes dependent on the number of EO units when two Pluronic polymers have the same PPO block length. We can observe that the cmc of Pluronic F127 (PPO length: 65) is higher than that of Pluronic P123 (PPO length: 69), as the hydrophilic PEO block of Pluronic F127 is five times bigger than that of Pluronic P123 [42]. Another copolymer, L35 does not micellize up to studied 10 wt% concentration at 30°C and stood as the monomeric form. Kabanov et al [43] have been reported the cmc of Pluronic L35 greater than 10 wt% at body temperature (37°C). Lower cmc values of Pluronic polymers are of great interest for drug solubilization because they provide high stability of their micelles in solutions upon dilution in the blood [15,22,41,42]. Results show that the cmc values for binary Pluronic F127/P123 mixtures were found to be intermediate compared to those found for the pure copolymers. The cmc values for binary mixture FP70, FP50 and FP30 were 0.0220 wt%, 0.0176 wt% and 0.0150 wt%, respectively.

Table .5.3: CMC values of Pluronic block copolymers and their binary mixtures in water at 30°C.

Systems	CMC, %w/v	CMC, %w/v (reported)
F127	0.0698	0.06*[34]
FP70	0.0220	0.018*[34]
FP50	0.0176	0.0019#[57]
FP30	0.0150	0.027*[34]
P123	0.0067	0.0034*[34]
L35	---	10.07*[43]
FL70	0.0807	---
FL50	0.1160	---
FL30	0.1603	---

dye solubilization method at 30°C* at 37°C.

Data listed in Table 5.3 indicates that the Pluronic F127 ratio in binary the mixtures decreased, cmc also decreased, as the lower cmc of hydrophobic P123. The low cmc values of these binary Pluronic systems at room temperature bring to them promising pharmacological applications as it remains stable even upon dilution in the blood [14-16,39,41,42]. Other binary Pluronic system, F127/L35, the cmc values were

found increased in order to cmc of pure Pluronic F127. The measured cmc values for binary mixture FL70, FL50 and FL30 were 0.0807 wt%, 0.1160 wt%, and 0.1603 wt%, respectively. Results have been indicated that the ratio of hydrophilic PluronicL35 in the mixtures increases, cmc also increased. Pluronic L35 was monomeric form up to 10 wt% may be the factor for the increase in the cmc of the binary mixtures. Gaisford et al [44] also noticed that the Pluronic polymers with similar PPO moieties exhibited cooperative aggregation; in meanwhile those having different PPO moieties presented non-cooperative binding. All the cmc values were suggested that the binary mixture of Pluronic F127/P123 and Pluronic F127/L35 micellar solutions could be utilized in drug solubilization.

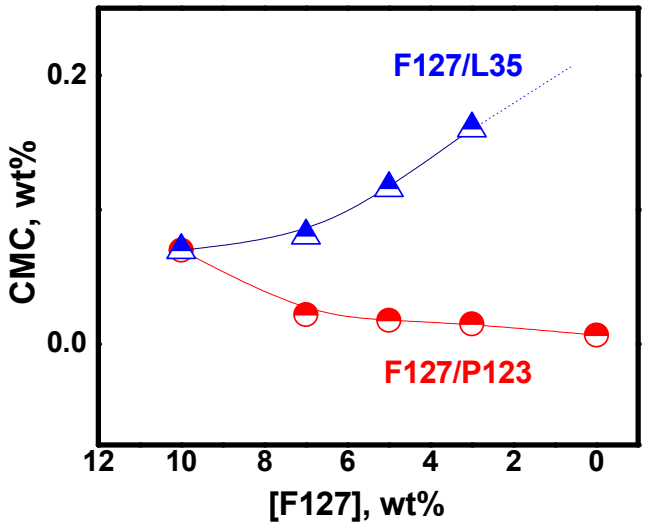

Fig.5.1: Plot of CMC versus weight percentage of mixed Pluronic polymers at 30°C. (No value of cmc up to 10wt% for L35)

5.3.2. Micellar characterization

In order to achieve longevity during systemic circulationof micelles in blood stream, the polymeric micelle must be small enough to evade detection and destruction by the reticuloendothelial system (RES) [22].

In our study, the average micelle sizes i.e. hydrodynamic diameter (D_h) of both empty and LTG loaded micelles of binary mixtures of Pluronic F127/P123 and Pluronic F127/L35 are illustrated in Fig.5.2. The D_h Values of empty micelles, as well as mixed micelles of both the binary systems, are in between the 15 to 25 nm, with an acceptably good PDI with the range of between 0.15 to 0.32. Normally polydispersity is used to describe the degree of "non-uniformity" of a distribution. In DLS and SANS technique, the native distribution is the intensity distribution which indicates how much light is scattered from the various sizes. The mean size and the standard deviation from that mean can be obtained directly from the statistics of the distribution. Here, the relative polydispersity can be obtained by PDI = standard deviation/mean. For a uniform sample, the PDI would be 0, whereas it is 0.1 to 0.4 for a polydispersed distribution. The D_h of copolymer F127 is larger than that of P123 (Figure.5.2) since PluronicF127 has a long hydrophilic chain length of PEO (%EO: 70) compare to Pluronic P123 (%EO: 30) [34]. The D_h of the micelles is copolymer specific. It was noticed that the D_h of the Pluronic L35 was not found in the range. It is amazing that the copolymer architecture basically does not affect at 10 wt% concentration of L35 investigated (Figure.5.3) [45].

Here, the particle size (D_h) of the FP70, FP50, and FP30 was slightly increased than the empty micelles. Data indicates that the amount of Pluronic P123 first increases then decreases the D_h in the binary systems due to strong hydrophobic-hydrophobic interactions between the Pluronic polymers.

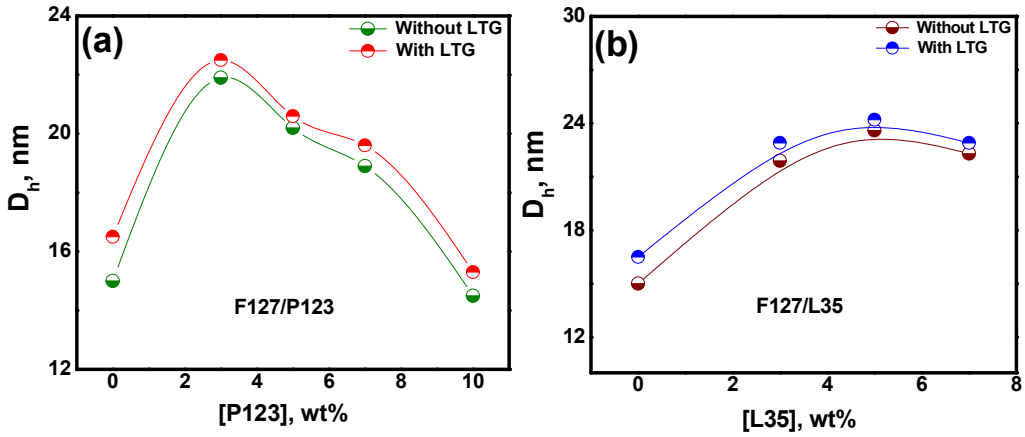

Fig.5.2: Hydrodynamic diameter(D_h) of binary Pluronic systems (a) F127/P123 and (b)F127/L35 with and without LTG drug at 30°C.

Here, the average hydrodynamic diameter goes through the higher level as the weight fraction of Pluronic P123 increases as an increase in aggregation number (N_{agg}), which favors a large radius. But due to the decrease in length of the coronal chains favors a small radius. Such changes are shown because of a gradual change in the mixed micelles from a soft to a hard interaction potential [14]. Similar observations have been also found in case of Pluronic F127/L35 binary systems. The D_h values of Pluronic F127/L35 systems were higher in comparison to Pluronic F127/P123 due to Pluronic L35 has 50% EO in its molecule, which was expanded the micellar sizes.

Table .5.4: Hydrodynamic diameter(D_h)values of Pluronic block copolymers and its binary mixtures in the absence and presence of LTG drug at 30°C.

System	Hydrodynamic diameter(Dh), nm	
	without LTG drug	*with LTG drug*
F127	15.0	16.5
P123	14.5	15.3
FP70	21.9	22.5
PF50	20.2	20.6
FP30	18.9	19.6
L35	---	---
FL70	22.3	22.9
FL30	21.9	22.9
FL50	23.6	24.2
FL30	21.9	22.9

It was also shown in Fig.4.2 that the incorporated LTG drug had virtually not much but slightly increased the D_h of mixed micelles of binary Pluronic F127/P123 and Pluronic F127/L35 systems up to 1 to 2 nm. However, the very slight and statistically insignificant average size was increased after LTG loading might nevertheless reflect a certain increase in the hydrophobic micelle core size also. It was clearly understood in Fig.5.3, where the hydrodynamic radius (R_h) was also simultaneously increased because of LTG solubilization [22]. The DLS analysis of binary mixtures of Pluronics® has indicated the particle sizes were smaller than 30 nm, which is a great advantage for their applications in pharmaceutical sciences [16,22].

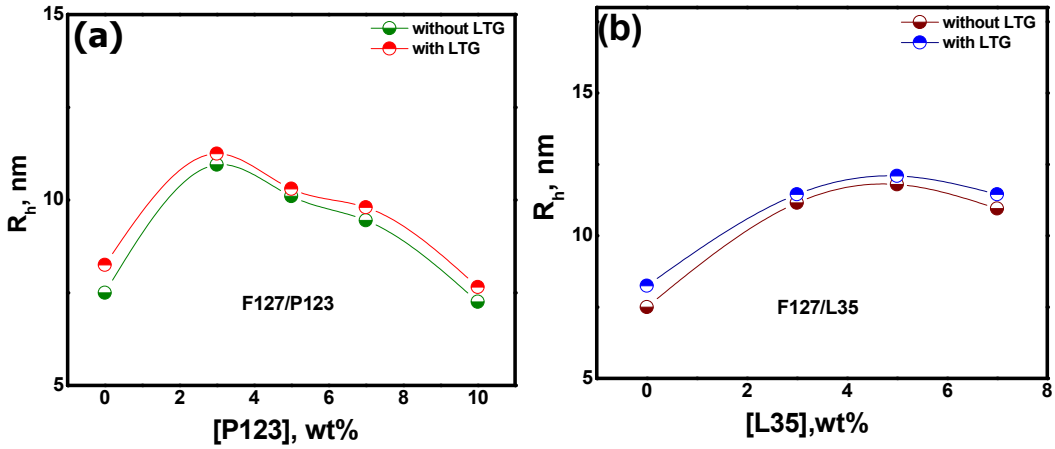

Fig.5.3: Average hydrodynamic radius (R_h) of a binary Pluronics systems (F127/P123 and F127/L35) with and without LTG drug at 30°C.

SANS is a unique tool for characterizing soft-condensed matter and has been widely used to examine micelles of Pluronic polymers and its associative interactions with drugs (pharma excipients). Fig.5.4(a) depicts the SANS distribution curve for 10 wt% of Pluronic polymers F127, P123 and L35 in D_2O at 30°C. The experimental data were fits using a structural hard-sphere core-shell model. The mean core radius (*Rc*), hard sphere radius (*Rhs*), the volume fraction of micelles (ϕ) and polydispersity (δ) were

determined as the fitting parameters from the analysis. The micellar parameters obtained by fitting of the data based on spherical core-shell micelles are reported in Table.5.5.

The SANS intensity profile of Pluronic F127 and Pluronic P123 shows the signatures of both form factor as well as structural factor governed scattering. The Pluronic micelles are found to be polydispersed micelles with a spherical shape and the obtained core radius(*Rc*), 5.06 nm for F127 and 5.47 nm for P123, respectively which are a good agreement with reported values [46,47].

Table .5.5: Various micellar parameters of Pluronic block copolymers and their binary mixtures obtained from SANS analysis at 30°C.

System	Core radius R_c(Å)	Polydispersity	Radius of gyration R_g(Å)	Hard sphere radius R_{hs}(Å)	Volume fraction ϕ
Without LTG drug					
F127	50.6	0.31	21.6	95.0	0.27
FP70	58.6	0.25	–	104.3	0.23
FP50	59.9	0.22	–	103.4	0.22
FP30	59.1	0.20	–	97.9	0.21
P123	54.7	0.22	12.0	85.2	0.15
L35	–	–	11.0	–	–
FL70	48.6	0.32	11.0	96.8	0.21
FL50	47.9	0.31	11.0	98.5	0.15
FL30	47.0	0.31	11.0	100.0	0.10
With LTG drug					
F127	51.1	0.30	21.6	95.0	0.27
FP70	60.1	0.21	–	96.9	0.21
FP50	62.6	0.21	–	102.5	0.22
FP30	62.5	0.28	–	85.5	0.22
P123	55.2	0.22	12.0	84.8	0.15
L35	–	–	11.0	–	–
FL70	50.1	0.29	11.0	97.4	0.19
FL50	61.0	0.18	11.0	86.8	0.08
FL30	47.8	0.28	11.0	96.5	0.10

In the SANS profile of Pluronics L35, the scattering function shows relatively weak Q dependence and small intensities. This behavior may be due to the fact that at the studied temperature (30°C or below) the copolymer does not micellize as hydrophilic in nature. The resulting radius of gyration, Rg = 11 Å and not specified core radius (*Rc*) of L35 seems, however, the copolymer was really fully dissolved in monomeric form.

The SANS distribution curve for LTG loaded micelles of 10 wt% of Pluronics F127, P123 and L35 in D_2O at 30°C is represented in Fig.5.4(b). It shows the signatures of both form factor and structural factor governed scattering in the LTG loaded Pluronic micelles like empty micelles. Pluronic L35 was also shown the similar distribution curve with LTG drug too. The analysis of the data (Table.5.5) reveals slightly increases in the micellar core radius of Pluronic F127 and Pluronic P123 with LTG drug. However, this increase might nevertheless reflect a certain increase in the hydrophobic PPO micelle core size because of LTG solubilization [48].

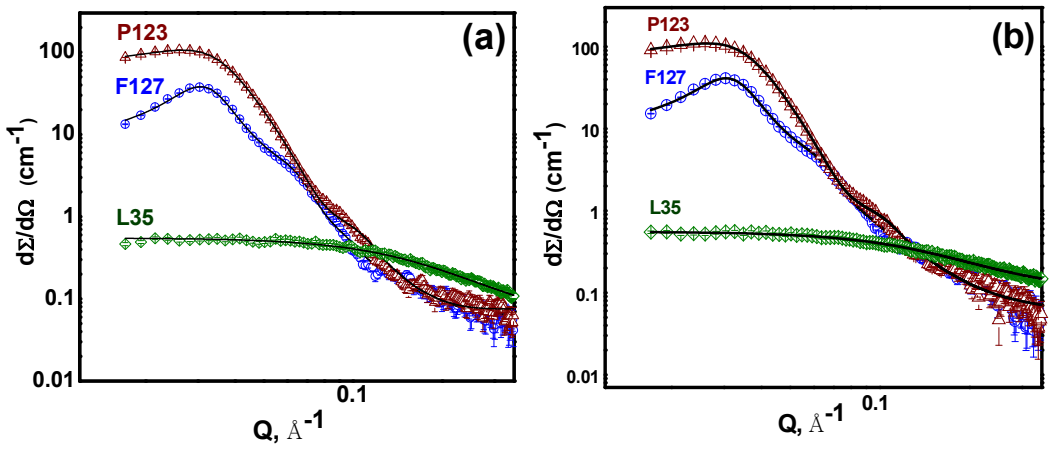

Fig.5.4: Scattering curves for 10 wt% Pluronic F127, P123 and L35 (a) without and (b) with LTG drug in D_2O at 30°C.

The SANS profile of both empty and LTG loaded micelles of binary mixtures of Pluronic F127/P123 and Pluronic F127/L35 in D_2O at 30°C is shown in Fig.5.5 and Fig.5.6. All the binary Pluronics mixtures were shown polydispersed with a spherical core

and a Gaussian distribution of chains attached to them, interacting with hard sphere potential. The analysis of the data (Table.5.5) reveals a slightly increased in the micellar core radius in the presence of LTG drug. The observed increase in micelle core radius upon solubilization of LTG resulted in in the incorporation of LTG in the PPO core. The micellar volume fractions, on the other hand, remain almost the same. This can be explained based on the fact that micellar solubilization of LTG is accompanied by simultaneous micellar dehydration. These results are very much correlated with the observed DLS analysis.

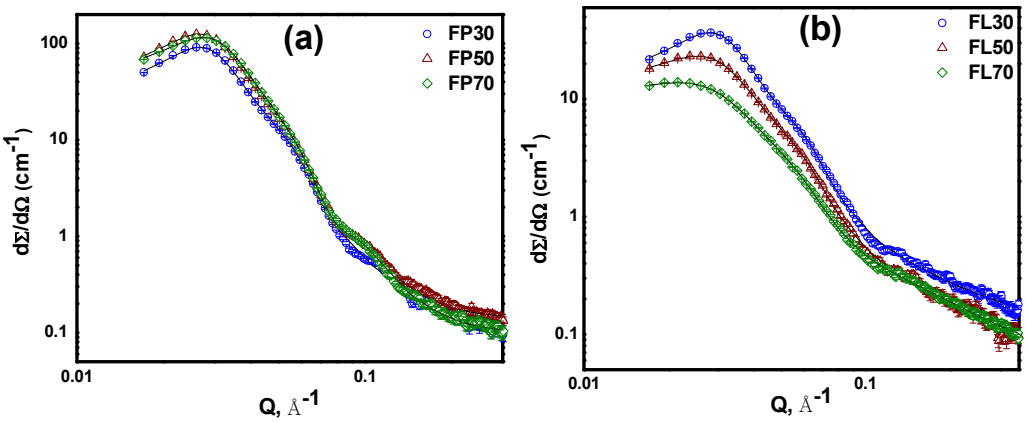

Fig.5.5: Scattering curves for binary Pluronic mixtures of (a) F127/P123 and (b) F127/L35 in D_2O at 30°C.

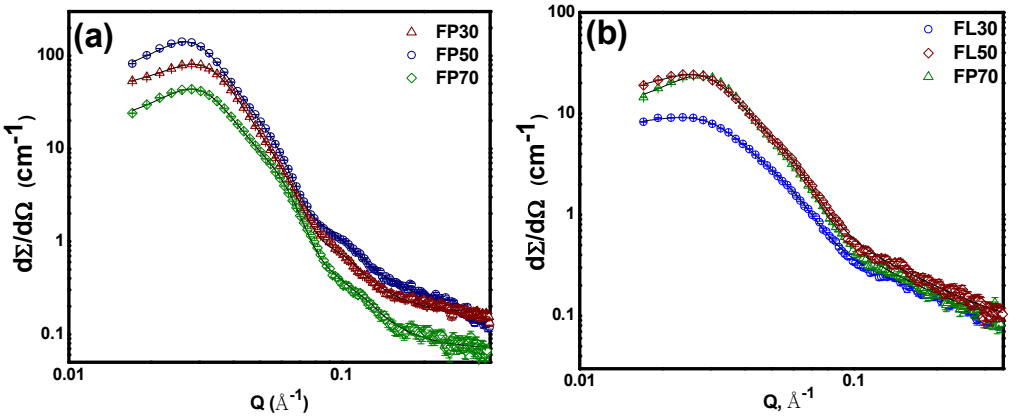

Fig.5.6: Scattering curves for binary Pluronics mixtures of (a) F127/P123 and (b) F127/L35 in the presence of LTG drug in D_2O at 30°C.

5.3.3: Solubilization of LTG in binary Pluronic mixtures

Polymeric micelles are effective approach in the drug delivery as they enhance drug solubility, stability and also can improve drug pharmacokinetics and biodistribution. These are nontoxic and protect drugs from degradation by many enzymes. Many PEO-PPO-PEO copolymers are FDA approved and used in ingestion and topical formulations. These copolymers have been used to solubilize many drugs such as griseofulvin [34], paclitaxel [15, 22], doxorubicin, epirubicin [49], apigenin [50], curcumin [51] and amphotericin B [52], etc. The formulation, with the name, SP1049C, was developed by Supratek Pharma is a successfully prepared doxorubicin loaded mixed micelle system of Pluronics. This is in phase II clinical trial for the treatment of metastatic esophageal carcinoma.

With this approach, we have investigated the micelles of Pluronic F127 with hydrophobic Pluronic P123 and hydrophilic Pluronic L35 for the solubilization of a poorly water-soluble drug, lamotrigine. Here, the solubilization capacities (S_{CP}) of the Pluronic solutions for LTG drug were determined by UV-Visible spectroscopy.

The solubilization capacity was calculated as [41,53-55] the relation; $S_{CP}= S - S_0$, where S is the solubilized LTG drug in copolymeric solutions and S_0 is the solubility of LTG in water [41,53-55]. The values of S_0 for LTG drug was 0.1754 mg/mL at 30°C, similar to those reported by Ribeiro et al [55].

The values of S_{CP} mg/mL) obtained for LTG loaded micelles of binary mixtures of Pluronic F127/P123 and Pluronic F127/L35 have been shown in Fig.5.7 Generally, the S_{CP} of diluted Pluronic solutions, with hydrophobic PPO blocks, showed low values when it compared with values of other types of copoly(oxyalkylenes) [41,55,56]. However, Lee et al [16] studied the S_{CP} and aqueous stability of Pluronic polymers analyzing their HLB (hydrophilic/lipophilic balance) and observed that the solubility of the drug in these Pluronic micellar systems do not just depend on hydrophobicity of the PPO block, but also on the length of the hydrophilic PEO block. The S_{CP} values of Pluronic F127, P123, and L35 were 0.6206, 1.3611 and 0.3061 mg/mL at 30°C, respectively (Figure.8). The HLB values for the Pluronic P123 and Pluronic F127 are 8

and 22. The higher the HLB value, the higher the hydrophilicity and these balance values were indicative that the loading efficiency of PluronicP123 for hydrophobic drugs would be better than that of F127 [57,58]. Here, the Pluronic L35 shows the low S_{CP} value due to the non aggregated form in the water at a studied temperature [43].

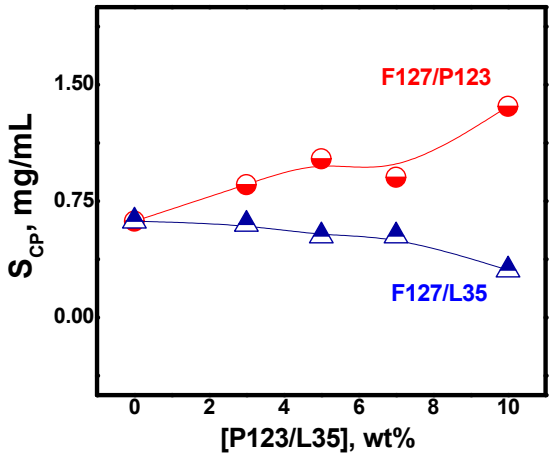

Fig.5.7: Solubilization capacity (S_{CP}) of LTG drug in 10 wt% Pluronic solutions and their binary mixtures in the water at 30°C.

The Pluronic P123 with low cmc has increased the thermodynamic stability due to tight hydrophobic interactions with PPO blocks, while the Pluronic F127 with long hydrophilic chain increased kinetic stability due to steric hindrance for micelle aggregation [23]. These results suggest that Pluronic polymers with relatively low HLB increases the thermodynamic stability, but do not affect the kinetic stability. Therefore, incorporating long PEO chains in micelles form the kinetically stable dispersion which prevents secondary micellar aggregation by steric hindrance.

Binary mixing of Pluronic F127 and Pluronic P123 is investigated in this study to overcome the limitations of low S_{CP} and combine advantages of high kinetic and thermodynamic stability. Further analyzing S_{CP} values of FP30, FP50, and FP70, we also observed that the increase of Pluronic P123 composition in FP mixtures increase the solubilization of LTG drug (shown in Figure.8). These results were understood, as observed by Oh et al [23] and Kulthe et al [59], that the binary Pluronic binary mixtures

are good solubilizers of hydrophobic drugs, where the increased in the loading efficiency by the mixed micelles for mixtures had a higher ratio of hydrophobic polymer in it. The weight fraction of Pluronic P123 increases in FP mixtures decreased in length of the coronal chains favors the interaction of PPO core to LTG drug. The improved S_{CP} in Pluronic F127/P123 mixtures is due to increase interactions between LTG drug and copolymers. Contrary to such data, in the binary mixtures of Pluronic F127/L35 show lower values of S_{CP} because of Pluronic L35 in the systems. It can be understood that the overall solubilization of LTG was affected due to the increase in the hydrophilicity of the solutions in the presence of Pluronic L35.

To understand the interaction between LTG drug and binary mixture of Pluronic from experimental data, micelle/water partition coefficient (P) can be calculated using the equation; $P=S_{CP}/S_0$. The P is defined as the ratio of LTG drug in the Pluronic micelle as solubilization capacity(S_{CP}) to the drug concentration in water. The Gibbs free energy change($\Delta G°$) was also calculated from the temperature dependence of P values. The $\Delta G°$ which indicates the spontaneity of the solubilization and calculated using the relation; $\Delta G° = -RTlnP$, where R is the gas constant, T is the temperature (in Kelvin), and P is the micelle/water partition coefficient [53]. The values of P and $\Delta G°$ for all the studied systems are listed in Table.5.6.

Table.5.6: Solubilization capacity(S_{CP}), Partition coefficient(P) and Gibbs free energy change(ΔG^0) for all the studied systems at room temperature.

System	S_{CP}, (mg/mL)	P	ΔG^0
F127	0.6206	3.5381	-3183.23
FP70	0.8556	4.8779	-3992.16
FP50	1.0224	5.8289	-4440.84
FP30	0.9040	5.1539	-4130.78
P123	1.3611	7.7599	-5161.67
L35	0.3061	1.7451	-1402.76
FL70	0.6004	3.4230	-3099.87
FL50	0.5252	2.9942	-2762.76
FL30	0.5250	2.9931	-2761.80

As reported by Kadam et al [53] in their previous studies using Pluronic polymers for solubilization of carbamazepine drug, our data also shows the free energy of solubilization is negative in all the Pluronic systems, including their binary mixtures, Pluronic F127/P123, and Pluronic F127/L35. This way the spontaneous LTG drug solubilization in the aqueous solutions of these copolymeric systems is manifested by the negative values of ΔG°.

Results clearly indicate that the increasing the hydrophobic character in the mixed micelles decreases ΔG°, what favors its spontaneous solubilization, since increases the drug/unimer ratio, i.e., the higher number of drug molecules can be accommodated into micelles i.e. binary Pluronic F127/P123 mixtures. As usual, the Pluronic L35 was not found efficient in comparison to Pluronic P123 in combination with F127 with low P and high ΔG° values.

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