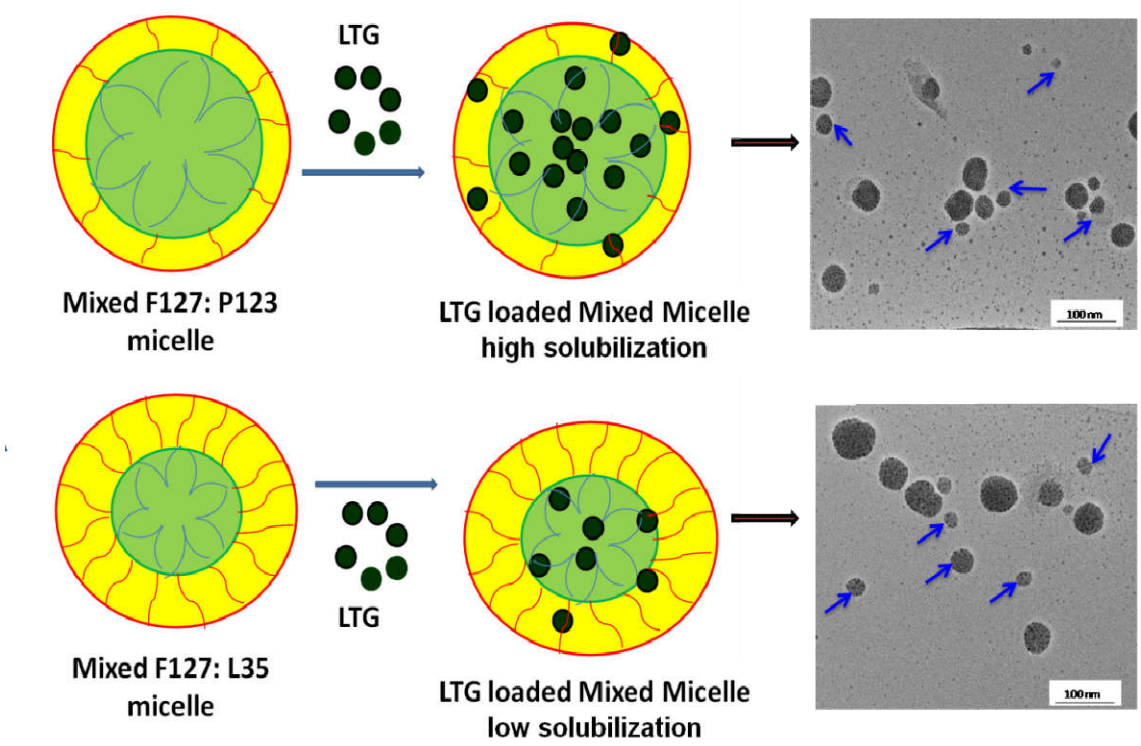
Self-assemblies of mixed PEO-PPO-PEO triblock copolymeric systems for Lamotrigine drugs at body temperature



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6.1: Introduction

Currently, the healthcare industry is confronted with the challenge to find formulations for a hydrophobic drug that has poor aqueous solubility which results in poor bioavailability. The bioavailability of the hydrophobic drugs is often enhanced through micellar solubilization using surfactants [1-3]. Surfactant micellar based solubilization is an important aspect due to the sufficiently high content in aqueous solution and transport through membranes etc. which has to facilitate in order to have efficiently working pharmaceutical formulations. However, this depends largely on the type of drug that will have specific and individual interactions and compatible with a given surfactant micelle [4]. Now a day, one of the major challenges in pharmaceutical formulations to design and deliver the effective surfactant micelle for better carriers [5]. Several surfactant micellar systems have been investigated for improved drug absorption and efficacy and in which polymeric micellar systems have emerged as one of the most promising approaches. The polymeric micelles are capable of enhancing the solubility and stability of hydrophobic drugs, enhancing cellular uptake and achieving in-vivo benefits [6,7]. Recently, the mixing of two or more amphiphilic polymers to obtain a mixed polymeric micelle has been an excellent approach to improve the micelle properties [8]. Such mixed micelles enhanced thermodynamic and kinetic stabilities, higher drug encapsulation efficiency, more control particle size and simple ways to modify their surface with different moieties [9].

The focus of current work is on triblock copolymers composed of a middle hydrophobic polypropylene oxide(PPO) block (at above 20°) that connects to hydrophilic polyethylene oxide (PEO) side blocks, structurally PEO_n-PPO_m-PEO_n Fig.6.1(a), are known as Pluronics in another tradename as Pluronic® block copolymers (BASF). Pluronic Poloxamers are found applications in diverse technological demands at cosmetics, food, coating, paints, petroleum and pharmaceutical industries [10-16]. High surface activity, temperature based micellization and reversible thermo-rheological behavior of Pluronics are considered them as the versatile materials [11,12,17,18]. Many Pluronic polymers are FDA approved and used as effective drug delivery nano carriers with thermo-responsive behavior [14-16]. The Pluronic polymer forms the core-shell micelles in aqueous media with the PPO core and shell of PEO at higher than critical micelle concentration (cmc) and critical micelle

temperature (cmt). The PPO core is hydrophobic at room temperature, leading to an aggregation against water and facilitating a local hydrophobic distinctive environment for the encapsulation of hydrophobic drug, while hydrophilic PEO shell keeps the dispersion stability. The encapsulation of hydrophobic drug into Pluronic micelles can enhance its solubility and stability that upgrade their pharmacokinetics and biodistribution. As encouraging nanomedicine technology, Pluronic micelles have shown notable applications in drug delivery systems and used as micellar nanocarriers for several lipophilic drugs [17-25].

For the treatment of tumors and neuro diseases, the blood-brain barrier (BBB) represents a formidable impediment limiting drug transport to the brain. Polymeric micelles have been explored for enhancement of CNS drugs delivery to the brain by two paths. The first path is based on a refinement of polymeric micelles with antibody molecules or ligand capable of transcytosis across brain microvessel endothelial cells, comprising the BBB. The second path, polymeric micelles were used to inhibit drug efflux systems, particularly P-gp, and selectively raise the permeability of BBB to P-gp substrates [19,20]. Two main mechanisms are involved in the P-gp modulating function of specifically Pluronic polymers. First, the Pluronic can influence mitochondria function and the energy conservation in cells expressing P-gp leads to inhibition of its function. Second, an interaction of Pluronic polymers with P-gp containing membrane also contributes to the inhibition of P-gp activity [26-33]. Pluronic is non-toxic and providing exciting opportunities to modulating P-gp and promoting the delivery of antiepileptic drugs (AEDs). Therefore, we aimed to study the solubilization of lamotrigine (LTG) in Pluronic micelles and specifically with mixed Pluronic micelles.

Lamotrigine (LTG) is a third generation anticonvulsant drug used in the treatment of epilepsy. It was widely used orally in adults with partial seizures and in the therapy of generalized seizures, either alone or mixed with other anticonvulsants [34,35]. It also works by inhibiting voltage-dependent sodium channels, resulting in decreased release of the excitatory neurotransmitters, glutamate, and aspartate [36].The LTG structure (shown in Fig.1(b) comprises four acidic amino hydrogen bond donors along with two basic hydrogen bond acceptors i.e. amino-pyridine nitrogen atoms giving rise to a variety of hydrogen bonding donor/acceptor sites making it a potential target. It has low aqueous solubility (approximately 0.17 mg/mL) which limits its absorption and dissolution rate and thus no

potent formulation is available [37]. Earlier some research work was done by our group on the encapsulation of LTG using Pluronic F127 micelle [38, 39]. The amount of LTG incorporated; with the micellar size of less than 20 nm, spherical shape and thermodynamic stability showed that Pluronic F127micelle is a potent nanocarrier for LTG. Synthesized LTG-incorporated Pluronic F127 micelles through the thin film hydration method showed the enhanced solubility and better compatibility of LTG. Liu et al [20] were also investigated the solubilization of LTG using highly hydrophobic Pluronic P123 micelles. They were observed the significantly high amount of LTG encapsulated and very rapid delivery into the brain.

Due to increased micelle stability and effective solubilization capacity in the previous reports, a mixed micelle composed of two or more kinds of Pluronic polymers is found better to those composed of an individual Pluronic [40,41]. The mixed Pluronic micellar systems based on Pluronic F127 and Pluronic P123 have been highly studied due to stabilization effect of long PEO moieties of Pluronic F127 blended with Pluronic P123 in micelles which might prevent the stacking of cylindrical aggregates formed by the long PPO moieties in therapeutic applications [41-45]. We have also reported our preliminary study on the mixed Pluronic micelle using the Pluronic F127, Pluronic P123 and Pluronic L35 for solubilization of LTG drug at room temperature [46]. Results clearly motivated that these Pluronic micelles are biocompatible and potent for oral administration of LTG.

Thus, taking this into consideration in the present work, we systematically reported the proper solubility studies of LTG in the variety of Pluronic polymers in an aqueous medium for better screening. Also, we have investigated the mixed Pluronic micellar systems composed with Pluronic F127, Pluronic P123, and Pluronic L35, as micellar nanocarriers, for LTG at body temperature (37°C) and properly compared using UV-Visible spectroscopy, dynamic light scattering, and small angle neutron scattering measurements. In extension, the in-vitro release profile and stability studies of LTG in the prepared mixed Pluronic micelle are also performed for further understanding of these mixed Pluronic micellar systems as potent nanocarriers for the futuristic formulation of LTG.

6.2: Experimental Section

6.2.1: Materials

Different PEO-PPO-PEO triblock copolymers (Pluronics) are supplied by Sigma-Aldrich (St.Luice, MO, USA) and used without further purification. They are listed in Table.1 which gives the trade name, composition, hydrophilic-lipophilic balance (HLB) numbers, and cloud points at 1 wt% concentrations. LTG is received as a gift sample from local suppliers and used with further purification. The D_2O for SANS analysis are of Sigma-Aldrich (St.Luice, MO, USA) and used as received. All the solvents and chemicals used are of HPLC grade.

Table.6.1: Molecular characteristics of Pluronic polymers studied in current work.

Pluronic	M.W.(g mol^{-1})	Composition	%PEO	CP of 1% Soln. (°C)	HLB
F127	12600	$E_{100}P_{65}E_{100}$	70	>100°	22
F88	11400	$E_{104}P_{48}E_{104}$	80	>100°	28
F68	8400	$E_{76}P_{29}E_{76}$	80	>100°	>24
P123	5750	$E_{20}P_{69}E_{20}$	30	90°	8
L121	4400	$E_5P_{68.2}E_5$	10	14°	1
L64	2900	$E_{13}P_{30}E_{13}$	40	58°	12-18
17R4	2650	$P_{14}E_{24}P_{14}$	40	46°	7-12
L35	1900	$E_{11}P_{16}E_{11}$	50	73°	19

6.2.2: Preparation of single and mixed Pluronic micellar solutions

The stock solutions of Pluronic (10 wt%) are prepared in HPLC grade water (pH 7.0) and left at 4°Cover night to produce unimers. The aqueous solution of accurate 1 wt% Pluronic was prepared using stock solutions of various Pluronic with proper dilution and stored at room temperature (30°C) for the experiments of solubilization screening. Mixed Pluronic F127:P123 and Pluronic F127:L35 micellar solutions with total 10 wt% concentrations (also weight ratio 70:30, 50:50 and 30:70) are prepared from stock solutions and transferred to 37°C and incubated for more than 2hrs before further experiments. The compositions of prepared mixed Pluronic micelle are represented in Table.6.2.

Table.6.2: The composition of mixed Pluronic systems studied in current work.

No.	System	Mixture composition X^{F127}: $X^{P123/L35}$	System code name
1	10 wt% F127	1.0 : 0.0	F127
2	7 wt% F127 + 3 wt% P123	0.7 : 0.3	MP73(7:3)
3	5 wt% F127 + 5 wt% P123	0.5 : 0.5	MP73(5:5)
4	3 wt% F127 + 7 wt% P123	0.3 : 0.7	MP73(3:7)
5	10 wt% P123	0.0 : 1.0	P123
6	7 wt% F127 + 3 wt% L35	0.7 : 0.3	MP75(7:3)
7	5 wt% F127 + 5 wt% L35	0.5 : 0.5	MP75(5:5)
8	3 wt% F127 + 7 wt% L35	0.3 : 0.7	MP75(3:7)
9	10 wt% L35	0.0 : 1.0	L35

6.2.3: Solubilization experiments

6.2.3.1: Solubility for a screening of Pluronic systems for LTG

The powder of LTG was added to the prepared 1 wt% aqueous solutions of various Pluronic and equilibrated by continuous stirring for 2days. The unsolubilized LTG is removed by filtration (Millipore PVDF syringe filter; 0.45µm pore size) and the concentration of the drug in the Pluronic micelle was determined after 10 to 100 times dilution with methanol. The absorbance of the obtained solutions was measured at the absorption maximum (i.e. 307 nm) using 1 cm cuvettes in a UV–Visible double beam spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan).The solubility of LTG is calculated using a calibration curve (supporting information S2) which established by measuring different concentrations of the LTG in methanol as solvent. All the measurements made in triplicate.

6.2.3.2: Solubility of LTG in mixed Pluronic micelles at body temperature

Powdered LTG added to the prepared mixed Pluronic micellar solutions of F127:P123 and F127:L35 with 10 wt% total concentrations and then stirred vigorously with the magnetic stirrer. All the samples were kept at 37°C for 48 hours to equilibrate the samples for the proper incorporation of LTG into mixed Pluronic solutions. By removing the excess LTG through filtration (Millipore PVDF syringe filter; 0.45µm pore size), the supernatant is suitably diluted using methanol for analysis. The absorbance maximum

(λmax=307 nm) of sample solutions are measured by UV–Visible spectrophotometer. The solubility of LTG is further calculated using the calibration curve mentioned before.

6.2.4: Characterization of Pluronic micellar systems

6.2.4.1: Dynamic light scattering (DLS)

The DLS measurements are performed at a fixed scattering angle of 90° on single and mixed Pluronic micelle solutions using a ZetasizerNano-SZ100 (Horiba) equipped with a He–Ne laser operating at 37°C. The average diffusion coefficient and hence the hydrodynamic micellar size in the form of average hydrodynamic diameter (D_h) is obtained by the method of cumulants. Each measurement is repeated at least four times.

6.2.4.2: Small-angle neutron scattering (SANS)

For a structural understanding, we performed a structural characterization of Pluronic F127, Pluronic P123, Pluronic L35, and mixed Pluronic micelle of F127:P123 and F127:L35 with and without LTG by means of SANS analysis. SANS analysis is performed on SANS diffractometer operating in GT laboratory at Dhruva Reactor, BARC, Mumbai, India [47]. The mean incident neutron beam wavelength (λ) was 5.2 Å with a resolution (Δλ/λ) of about 15%. The scattered neutrons are searched in an angular range of 0.5–15° through a linear position sensitive detector (PSD). The scattering vectors 'Q' in the range of 0.015-0.3 $Å^{-1}$ of scattered neutrons are measured. The measured SANS data are corrected for the background, the empty cell contribution, and the transmission and were presented on an absolute scale with standard protocols. All the data are recorded at 37°C. The theoretical explanations of SANS analysis are shown in the supporting information (S3).

6.2.4.3: Transmission electron microscopy(TEM)

The morphological examination of the selected mixed Pluronic micelle of F127:P123 and F127:L35 with LTG was performed by transmission electron microscopy (TEM) operating at 120kV(Model JEM-2100, Jeol, Tokyo, Japan). one drop of the nanocarrier dispersion solution was deposited on the surface of the carbon-coated copper grid (200 mesh), negatively stained with a drop of fresh uranyl acetate solution then allowed to dry at room temperature for 10 min for investigation.

6.2.5: In vitro release study of LTG drug

The amount of LTG release from micellar solutions of mixed F127:P123 (weight ratio 50:50) and F127:L35 (weight ratio 50:50) systems are carried out using dialysis bag diffusion techniques at 37°±0.1°C. LTG encapsulated mixed micellar solutions (MP73(5:5) and MP75(5:5) are placed into a dialysis bag (regenerated cellulose dialysis membranes; MW cut off of 2500 g/mol) sealed and placed in a Falcon® conical tube (15 mL) containing the release medium (deionized water, pH7.0). Then, each Falcon® conical tube is placed in an orbital gyratory device at 40 rpm and 37 °C. At every time intervals, the whole medium is withdrawn and replaced with an equal volume of fresh medium pre-heated at 37 °C. The released LTG amounts are quantified spectrophotometrically at λ_{max}=307 nm described as above with correction. The results are compared by different mathematical models to understand the mechanism and possible reason for LTG release from the mixed micelle. The equations used kinetic models are mentioned in Chapter 4.

6.2.6: Stability study of mixed Pluronic micelles for LTG

The stability of LTG in the representative mixed F127:P123 (MP73(5:5)) and F127:L35 (MP73(5:5)) micelle are determined at body temperature. Both the mixed micellar solutions are stored in the closed dark chamber maintaining the 37°±0.5°C temperature using thermostats and LTG retention is quantified at fixed time interval upto 10 days after the preparation. The amounts of LTG drug in the mixed micelles are evaluated using UV-Visible spectroscopy.

6.3: Results and Discussion

The applicability of any Pluronic is mainly guided by their self-associative nature to form micelles. The micelles of Pluronic consist of a desolvated PPO core and a solvated PEO shell. Hydrophobic Pluronic due to low cmc and cmt shows very condensed core which attracts the poorly soluble compounds more than the hydrophilic Pluronic polymer with high cmc and cmt. The values of cmc and cmt of Pluronic are of key factors for its application in drug solubilization and delivery.

Table.6.3 display the values of cmc and cmt which one reported in the earlier literature for Pluronic polymers used in the present work [17,30,49-54]. The stability of Pluronic micelles against possible dilution in body fluids was determined by their cmc value [13, 17]. It has been proven that the increase in the PPO block length enhances the overall hydrophobicity of the Pluronic polymer which effectively decreases cmc and helps to form condensed hydrophobic micellar core [55–57]. On the contrary, an increase in the length of the PEO blocks decrease the hydrophobicity which rises the cmc and finally reduces the stability of the micelle [17].

Table.6.3: Reported cmc and cmt values of studied Pluronic polymers in the water at RT/high temperature.

Pluronic	**cmc, (wt%)**		**cmt, (°C)**
	@ room temperature	@ high temperature	
F127	0.1[17]	0.02 [52]	24°[17]
F88	---	1.7[17]	38°[17]
F68	---	7.0[17]	50°[17]
P123	0.005[17]	0.001[17]	16°[17]
L121	---	0.00044[48]	15°[50]
L64	1.5[17]	0.4[17]	31.5°[17]
17R4	---	0.91[49]	33-34°[53]
L35	---	10.07[48]	60-70°[54]

Here, the cmc values (listed in Table.6.3) were shown that L35 micelles found with minimum stability while P123 micelles has better stability. It is evident from cmt values that

some Pluronic with higher %PEO and lower mol.wt. do not aggregate at room temperature, but undergoes micelle formation at higher temperatures. For the equivalent mol.wt. of PPO core, cmt increased with increase in the %PEO, whereas the increase in the mol.wt. of the core favored aggregation and hence formed micelles at comparatively lower temperatures [24].

6.3.1: Solubilization

6.3.1.1: Pluronic polymers for LTG solubilization for screening

We systematically varied the molecular architecture of the Pluronic with different PEO/PPO ratio and investigated its effect on the solubilization of LTG into aqueous media using UV-Visible spectroscopy. The aqueous solubility of LTG was 0.17 mg/mL at 30°C [38, 39] and the results for its solubilization in the aqueous solutions of different Pluronics (fixed 1.0 wt% concentration) are shown in Fig.6.2. These results show that the structure of the Pluronic polymers has a much more pronounced influence on the solubilization for LTG drug in the range of zero to 0.6 mg/mL. The order of the enhancement of solubilization for LTG is as follow:

L121>P123>F127>L64>17R4>F88>F68>L35.

Alexandridis et al [17] showed a large dependence of the cmt of Pluronic on PPO block length and size of the PEO head group. L121 and P123 showed good solubilization for LTG drug because of high hydrophobicity and low cmts. In contrast, F88, F68, and L35 did not show much solubilization due to high hydrophilicity in the molecule and high cmt as well. F127, L64, and 17R4 are also shown the moderately enhanced solubilization of LTG drug. According to the solubilization performance by various Pluronic polymers, the L121 may be the best choice in the formation of a mixed Pluronic micelle for LTG, but its aqueous solutions are very temperature sensitive. At below cmt, they dissolve as unimers, whereas at higher temperatures, above CPT (cloud point temperature), they very quickly form larger vesicles. The working window of the vesicles, i.e. between the cmt and the CPT, is rather small since the cmt is 15°C and the CPT is very low nearby at 14°C [50]. Such physical properties limit the potential use of L121 in pharma applications.

In this context, we selected the mixture of F127 and P123 which has low mol.wt. difference and the similar chain length of PPO blocks. This mixed F127:P123 micelle were also proved to overcome the problems of the low solubility of many pharmaceutical excipients with its benefits of high kinetic and thermodynamical stability [42-46].The choice of F127 here was to increase PEO block length at mixed micelle surface, which ultimately helps in long circulation time and improves the biocompatibility of the drug. To compare with mixed F127:P123 systems, other mixed systems were selected with F127 and the lowest performer Pluronic L35. As L35 shows the lowest solubilization for LTG among the all other studied Pluronic because of its unimeric form. It may be differently behaving in the mixed micelle [54].

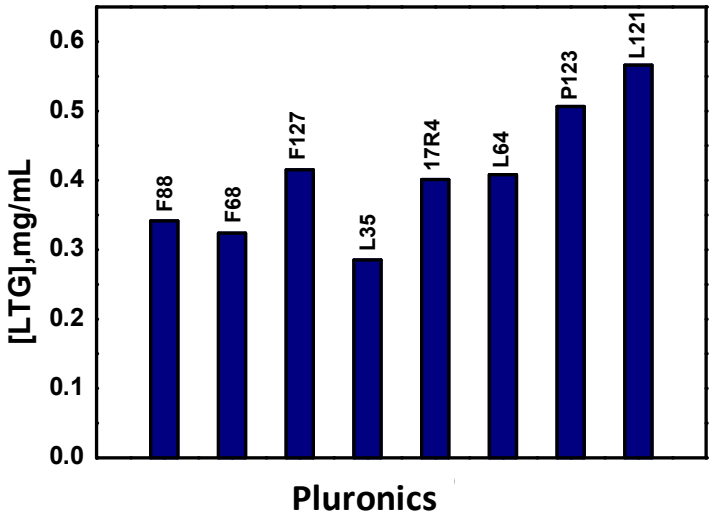

Fig.6.2: The solubility of LTG in 1.0 wt % aqueous solutions of Pluronic polymers at room temperature (30°C).

6.3.1.2: Solubilization of LTG in mixed Pluronic micelles

The solubility of LTG in micellar solutions of mixed Pluronic systems at 37°C is determined by UV-Visible spectroscopy. The solubility (mg/mL) of LTG in the aqueous solutions of mixed F127:P123 and F127:L35 micelles systems with the total polymer concentration of 10 wt% at 37°C are shown in Fig.6.3. The solubility of LTG in an aqueous solution of 10 wt% F127, P123, and L35 are 1.0631, 1.6738 and 0.5698 mg/mL, respectively. Pluronic polymers with high PO units (69 for P123 and 65 for F127) is better for solubilization of LTG drug as more hydrophobicity in the systems [58]. PluronicL35 with

lower PO units of 16 shows the low solubilization of LTG as less hydrophobic in compare to F127 and P123 with its unimeric nature in the aqueous medium [54].

Further analyzing our solubility values of LTG drug in the mixed F127:P123 micelles; MP73(3:7), MP73(5:5) and MP73(7:3), we have observed that increasing P123 composition in MP73 mixtures increases the solubilization of LTG almost above 1.5 mg/mL (Fig.6.3). Oh, et al [59] were also observed that mixed Pluronic micelles are good solubilizers of hydrophobic drugs, where the increase in the encapsulated efficiency by the mixed micelles of Pluronics for mixtures with a higher amount of hydrophobic Pluronic. Due to the high hydrophobicity of P123 in mixed F127:P123 micelles improved the solubility of LTG as cargo core of PPO blocks. On the contrary, the mixed F127:L35 micelles show the lower solubilization of LTG with an increase in the composition of L35. The presence of L35 in the increases the hydrophilicity which affect the overall solubilization of LTG. Such results also influenced due to the unimeric form of PluronicL35 at 37°C which is far below its cmt (60°-70°C) [54].

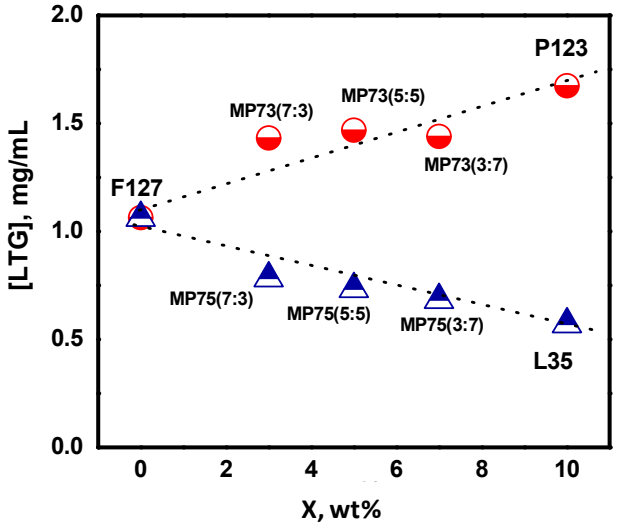

Fig.6.3: The solubility of LTG in aqueous solutions of mixed Pluronic micellar systems at body temperature (37°C).

6.3.1.3: Thermodynamic parameters of LTG solubilization in mixed Pluronic micellar systems

The thermodynamic parameters of solubilization of LTG drug in mixed F127:P123 and F127:L35 micellar systems are investigated at 37° in the form of the partition coefficient (P) and standard Gibbs free energy (ΔG°) changes.

Here, the partition coefficient(P) values are calculated using the equation;

$$\mathbf{P} = \frac{S - S_w}{S_w}$$

The P is expressed as the ratio of LTG solubility in the mixed micelles of Pluronic (S) to the LTG concentration in water (S_w).

The standard Gibbs free energy (ΔG°) of the system designates the spontaneity of the solubilization and determined using the relation;

$$\triangle \mathbf{G}^{\circ} = -RT\ ln\ \mathbf{P}$$

where R is the gas constant, T is the working temperature(in Kelvin), and P is the partition coefficient [38,58-60].

The calculated values of P and ΔG° for all the mixed F127:P123 and F127:L35 micelles are listed in Table.6.4. All the data shows that the standard Gibbs free energy of solubilization was negative for F127, P123, L35, including their mixed MP73 and MP75 micelles. This way the spontaneous LTG solubilization in the aqueous solutions of mixed Pluronic micelles systems was clearly observed. The ΔG° values are nearby - 4.0 KJ/mol for MP73 systems and almost approx. -1.5 KJ/mol for MP75 systems. The increase in the hydrophobic character of the mixed Pluronic micelles decreased the ΔG°, which favors its spontaneous solubilization as a more number of LTG drug molecules can be incorporated into Pluronic micelles i.e. mixed F127:P123 micelles. The low negative values of ΔG° in mixed F127:L35 systems for LTG are due to obvious hydrophilic L35 which not efficiently participated in a mixed condensed micellar core in compare to P123 in the micelles.

Table.6.4: Thermodynamic parameters of LTG drug solubilized mixed Pluronic micellar systems at 37°C.

Pluronic System	Partition coefficient, P	Standard Gibbs free energy, ΔG^0 (KJ/mol)
P123	5.5255	-4.40565
MP73(3:7)	4.6116	-3.93971
MP73(5:5)	4.7228	-4.00107
MP73(7:3)	4.5836	-3.92397
F127	3.1446	-2.95285
MP75(3:7)	1.6635	-1.31174
MP75(5:5)	1.8580	-1.59678
MP75(7:3)	2.0530	-1.85391
L35	1.2214	-5.15551

6.3.2: Micelle characterization

It is important to note that the small sizes of the drug-encapsulated micelles indicated that they achieve longevity during systemic circulation and enough to evade detection and destruction by the reticuloendothelial system (RES) [19,42].

6.3.2.1: Dynamic light scattering(DLS)

The average hydrodynamic sizes (D_h) of the micelles of F127, P123, and mixed F127:P123 micelles in absence and presence of LTG at 37°C are analyzed using DLS technique and presented in Fig.6.4. The mean diameter of micelles, as well as mixed micelles of both the Pluronic and mixed Pluronic (MP73), were in-between 15 and 22 nm, with an acceptable PDI of 0.2 and 0.3 ranges. The D_h of F127 is larger than that of P123 since its high MW of 12,600 $gm.mol^{-1}$ with long hydrophilic chain length (%PEO: 70)[44]. Here, the D_h of the mixed F127:P123 micelles, MP73(7:3), MP73(5:5) and MP73(3:7), is slightly increased than the blank Pluronic micelles. Results indicate that the weight fraction of P123 first increases then decreases the D_h in the mixed systems due to strong hydrophobic-hydrophobic interactions between the P123 polymers. The D_h go through a higher level as the weight fraction of P123 increases as the increase in aggregation number, which favors a large radius. But due to a decrease in length of the coronal chains favors a small radius. Such changes are shown because of a gradual change from a soft to a hard interaction potential in the mixed micelles [41,46]. It was also noticed that the LTG drug solubilization in the micelles had not much effect on the overall sizes of the micelles but slightly increases the D_h of the mixed micelle. DLS results clearly indicate growth in micelles when LTG are incorporated. The micelle size dramatically increases with the incorporation of LTG in polymeric micelles. The results are supported by the solubilization results and found a similar trend.

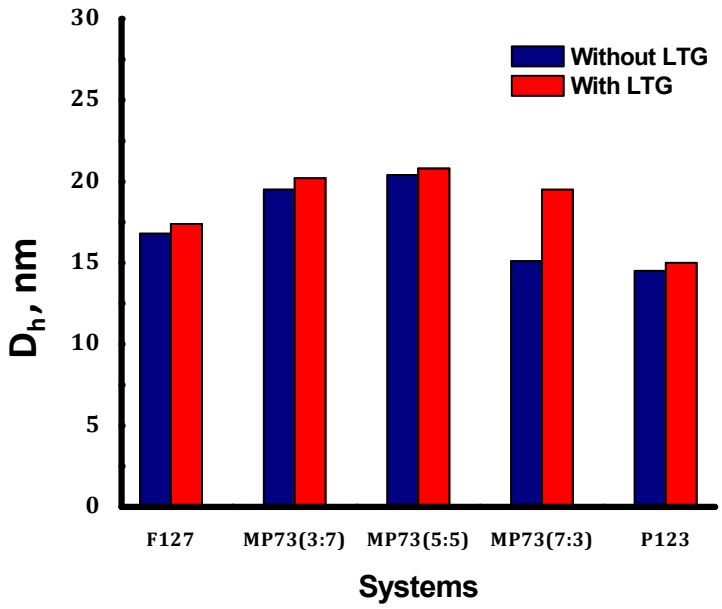

Fig.6.4: Average D_h values of F127, P123, and mixed F127:P123 micellar systems with and without LTG drug at 37°C.

Fig.6.5 shows the average hydrodynamic size (D_h) of the micelles of F127, L35, and its mixed F127:L35 micelles in absence and presence of LTG drug at 37°C. DLS studies have revealed that plain L35 demonstrated large particle sizes and was not found in the range. It is amazing that the Pluronic architecture mainly did not much effect at 10 wt% concentration of L35 as a unimeric form [61]. The D_h values of mixed F127:L35 micelle were higher in comparison to F127:P123 due to the added L35 has monomeric form expanded the PEO shells of micelles. In Fig.6.5, it was also shown that the solubilization of LTG drug into the PPO core of the micelles increased the D_h values of the mixed systems. The DLS analysis of mixed Pluronic micelles; F127:P123 and F127:L35 has indicated the particle sizes were smaller than 25 nm at applicable 37°, which is a great advantage for their further utilization in pharmaceutical formulations [40,42].

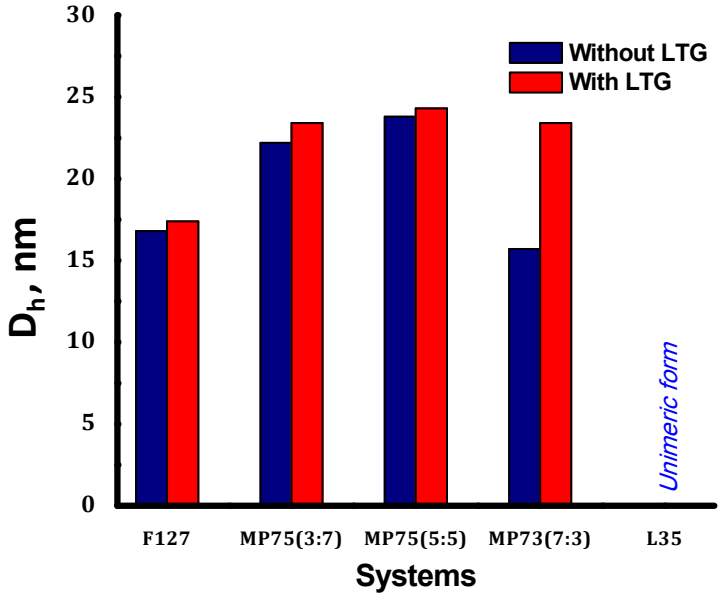

Fig.6.5: Average D_h values of F127, L35, and mixed F127:L35 micellar systems with and without LTG drug at 37°C.

6.3.2.2: Small-angle neutron scattering (SANS)

Small angle neutron scattering (SANS) studies are done here to understand the change in the micellar structure upon solubilization of LTG into the mixed Pluronic micelles. SANS distribution curves for 10 wt% of pure F127, P123 and L35 in D_2O with and without LTG drug at 37°C are represented in Fig.6.6. Different micellar parameters which characterized micelle-like mean core radius(R_c), hard sphere radius (R_{hs}), a volume fraction of micelles(ϕ) and polydispersity(δ) are determined as fitting parameters from the SANS analysis and tabulated in Table.6.5. The SANS data clearly confirms the information of Pluronic micelle i.e. morphological shape, size and aggregation behavior in the presence and absence of LTG. The SANS intensity profile of F127 and P123 shows the signatures of form as well as structural factors which govern by scattered intensities. Both the micelles are found polydispersed with sphere assemblies. The micellar core radius (R_c) of 5.31 nm for F127 and 5.75 nm for P123 was similar with reported values [62, 63]. The P123 has bigger R_c value than F127 as high hydrophobicity. But L35 has given very weak Q dependence and small intensities in the SANS profile. The reason for such behavior as we also found with DLS study that L35 does not micellized at 37°C with the studied concentration and fully dispersed in a monomeric form [48]. In the SANS distribution curve of 10 wt% of F127, P123 and

L35after the solubilization of LTG showed the higher scattering intensities than the pure micelles. The micellar R_c of F127 and P123 with LTG are increased from 0.5 nm to 1.3 nm (Table.6.5). However, such increment reflected the certain increased in the hydrophobic PPO micelle core size due to the incorporation of LTG in it [38].

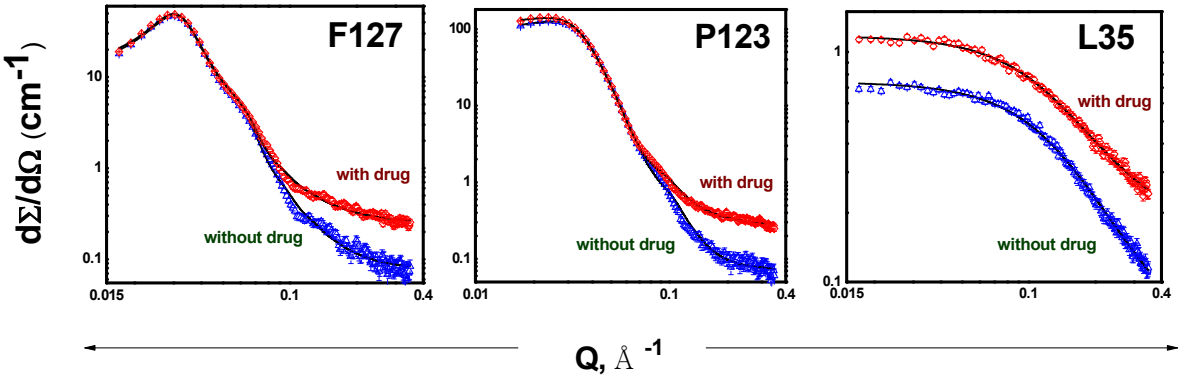

Fig.6.6: Neutron scattering curves for 10 wt% solutions of F127, P123, and l35 in the absence and presence of LTG in D_2O at 37°C.

The SANS profiles of blank and LTG solubilized mixed F127:P123 and F127:L35 micelles in D_2O at 37°C are shown in Fig.6.7 and Fig.6.8. Both the systems showed small PDI values, indicating a complete micellization. All the mixed Pluronic micelles show polydispersed with a spherical core and a Gaussian distribution of chains attached to them, interacting with hard sphere potential. The sizes of the micelles of mixed F127:P123 systems are found higher than the pure F127 and P123 respectively. The micellar R_c is almost increased up to 3 to 8 nm in the mixed F127:P123 systems than the pure Pluronicsystems (listed in Table.6.5).

The R_c of the mixed F127:P123 micelles also had shown the increase when LTG is incorporated. It was associated with an improvement of the hydrophobic interactions in the micellar core upon LTG solubilization, enhancing the self-aggregation tendency of the polymers. The sizes of the micelles of mixed F127:L35 micelles are found nearby the pure F127 micelle. The R_c of the mixed F127:L35 micelles also had shown a very negligible increase when LTG was encapsulated. SANS results are completely correlated with the observed DLS results.

Table.6.5: Various SANS parameters of mixed Pluronic micellar systems at 37°C.

Pluronic micellar systems	Core radius R_c (Å)		PDI		Hard sphere radius R_{hs}(Å)		Volume fraction ϕ	
	with LTG	without LTG	with LTG	without LTG	with LTG	without LTG	with LTG	without LTG
F127	53.1	53.6	0.30	0.30	95.8	96.0	0.27	0.27
MP73(7:3)	61.2	63.5	0.25	0.21	105.6	97.1	0.23	0.20
MP73(5:5)	61.3	62.9	0.22	0.21	103.7	102.7	0.22	0.22
MP73(3:7)	60.2	64.2	0.20	0.28	98.3	85.7	0.20	0.22
P123	57.5	58.8	0.22	0.22	86.7	88.5	0.15	0.14
MP75(7:3)	52.3	52.9	0.30	0.27	97.8	96.4	0.21	0.19
MP75(5:5)	51.1	51.9	0.29	0.20	99.6	89.5	0.16	0.07
MP75(3:7)	48.2	49.9	0.27	0.25	98.2	97.4	0.11	0.11
L35	*unimers at a studied concentration*							

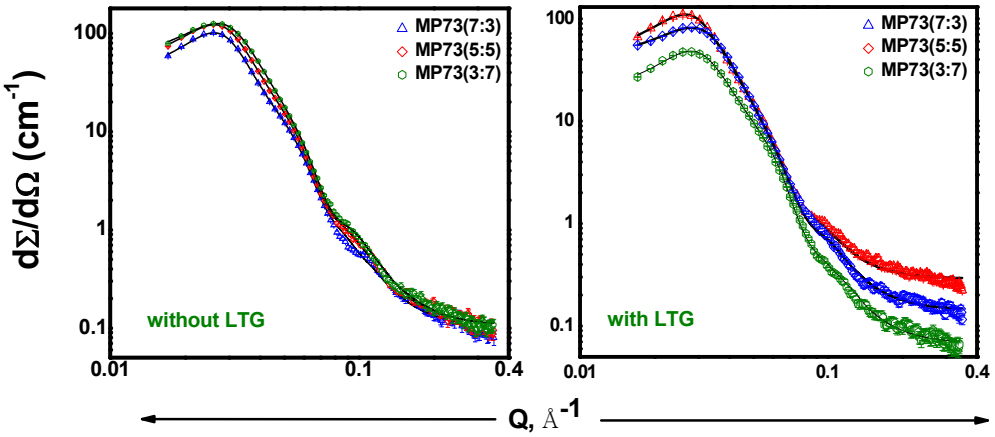

Fig.*6.7: Neutron scattering curves for 10 wt% solutions of mixed F127: P123 micellar systems with and without LTG at 37°C.*

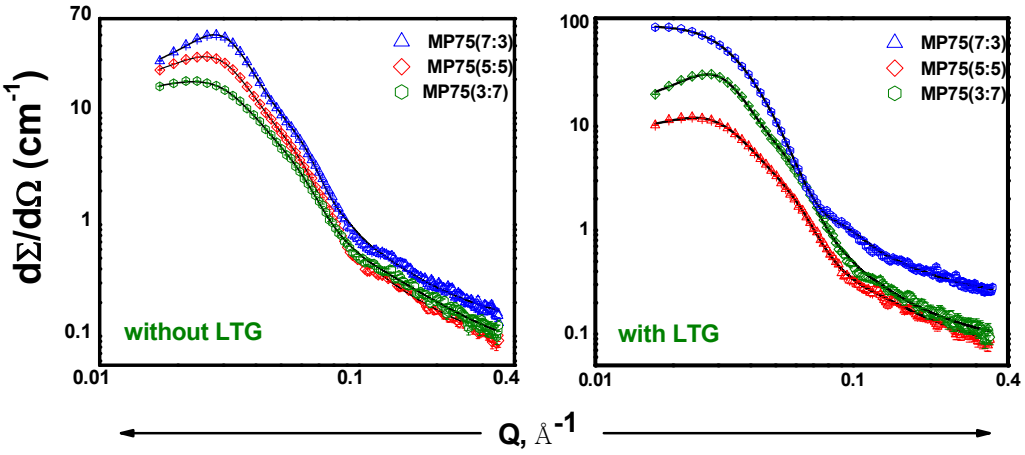

Fig.6.8: Neutron scattering curves for 10 wt% solutions of mixed F127:L35 micellar systems with and without LTG at 37°C.

6.3.2.3: Transmission electron microscopy(TEM) study

The TEM images of selected LTG encapsulated mixed F127:P123 micellar (MP73(5:5)) and mixed F127:L35 MP75(5:5)) systems were shown in Fig.6.9. The TEM micrographs of both the systems were indicated that micelles exhibited a spherical shape, smooth surface, and low polydispersity forming homogeneous nano micellar structures with a size in line with DLS and SANS results. From Fig.6.9, it was possible to note two distinct bright and dark regions in the mixed micelles. The dark region confirmed to the high-density core of the micelle (hydrophobic PPO region) and the bright region corresponds to the hydrophilic PEO corona shell. These core-shell structure of mixed micelles are highly important in the drug delivery system of the hydrophobic drug in the body.

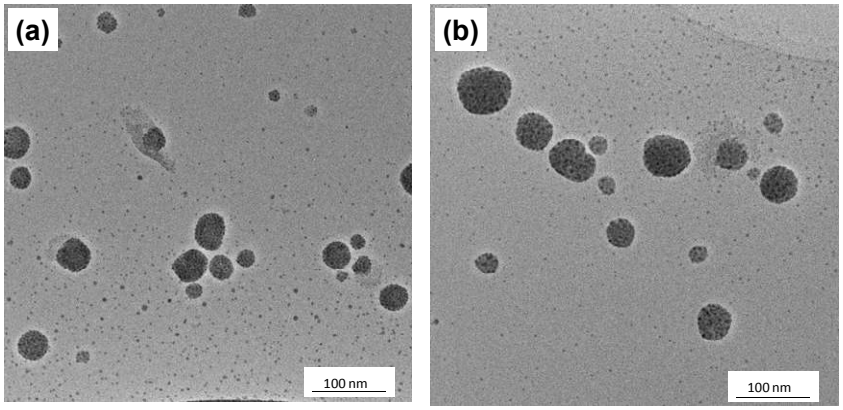

Fig.6.9: TEM images of drug loaded mixed F127:P123 and mixed F127:L35 micellar systems.

6.3.3: In-vitro release study

The in vitro release profiles of LTG from two mixed Pluronic micelles; MP73(5:5) and MP75(5:5) under the sink condition are investigated using the dialysis bag method. The release profile curves with time are shown in Fig.6.10. The drug release in both the mixed Pluronic micelles, MP73(5:5) and MP75(5:5), with an initial burst release for about 4 h followed by a sustained release for more than 80 h. This biphasic release may be attributed to LTG molecule entrapped nearby micellar surface causing initial burst release. After an initial burst release, the release rate decreased, reflecting the release of drug entrapped in the strong hydrophobic micellar core of the system. Such release profile of both the mixed Pluronic micelles was indicating a slow and sustained release behavior, which is similar to previous studies [42,64]. This release property was attributed to the encapsulated LTG as a separate phase inside the micellar PPO core, subsequently leading to a slower or even retarded drug release[19,42,45,61].

For release kinetics, results are analyzed using the four different kinetic models viz. zero-order, first-order, Higuchi and Korsmeyer-Peppas kinetics (Table.6.6).

Table.6.6: Release rate constants (K) and regression coefficients (R^2) for LTG solubilized mixed Pluronic micelles.

Release kinetic model	**MP73(5:5)**		**MP75(5:5)**	
	K	R^2	*K*	R^2
Zero-order	0.004767	0.60861	0.011201	0.48825
First order	0.037727	0.39319	0.036007	0.33153
Higuchi	**0.029745**	**0.84048**	**0.072118**	**0.77656**

Data were analyzed for the initial % drug release in 24 hrs only which is equivalent to 60% of total release amount. Based on the regression coefficient analysis, we found that the model of Higuchi is best fitted with LTG drug release kinetics for both the mixed Pluronic micelles. Such behavior of formulations allows the encapsulated LTG to reach and or interact with its target with minimal drug loss.

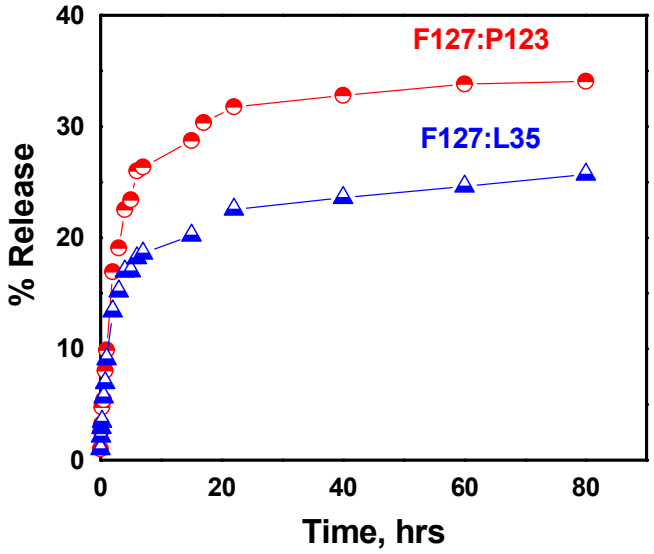

Fig.6.10: In vitro release profile of LTG drug from mixed F127:P123 and mixed F127:L35 micellar systems at 37°C.

6.3.4: Stability study

The stability of LTG drug in the two mixed Pluronic micelles; MP73(5:5) and MP75(5:5) are assessed for a period of 9 days at a controlled temperature of 37°C. Fig.6.11 reveals the solubility of LTG over the given period of time. It is clearly observed that no significant loss in drug retention in the mixed F127:P123 micelles systems within the time period. The decrease in the solubility of LTG of 0.15 mg/mL is shown in MP75(5:5) micelles. In line with earlier observations, better stability of LTG is noticed in MP73(5:5) as compared to MP75(5:5). The stability study is proved the better interaction of LTG molecules with mixed micelles of F127:P123 which considered better compatibility. As all other measurements and data, stability results also not encouraged the mixed F127:L35 micelles for LTG formulations.

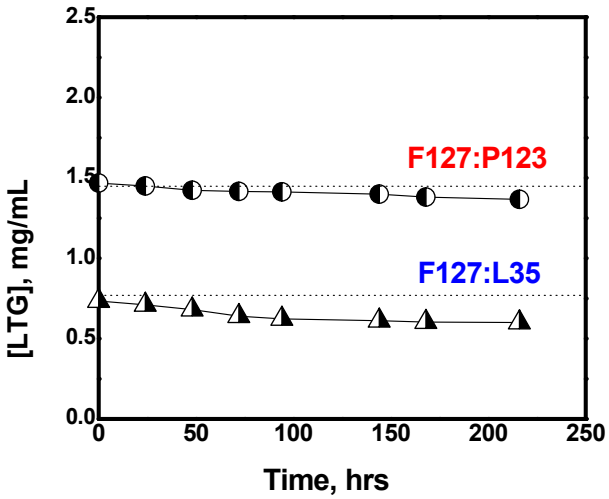

Fig.6.11: Stability study of LTG drug from mixed F127:P123 and mixed F127:L35 micellar systems at 37°C.

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