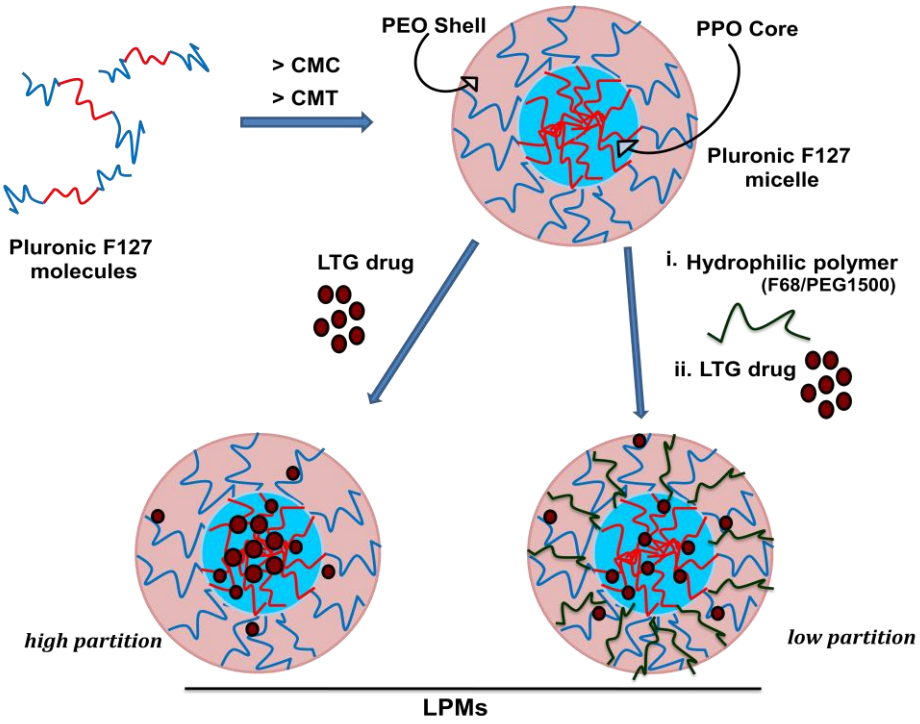
# Self-assembly Of Single PEO-PPO-PEO Triblock Copolymeric System For Lamotrigine Drug: Effect of Hydrophiles

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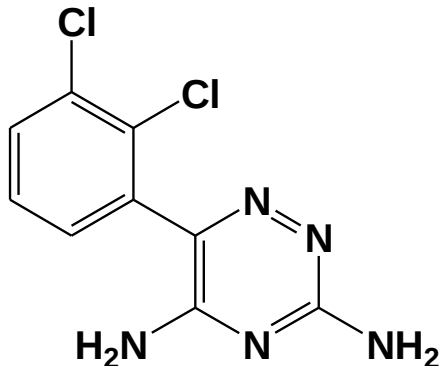
## 3.1: Introduction

Epilepsy is a very general chronic neurological disorder characterized by recurrent unprovoked seizures [1]. The risk avoidance of permanent brain damage requires quick management of seizures. Parenteral administration allows transport of drugs to the brain abstaining BBB, providing a unique feature and better choice to target drugs (for example Lamotrigine) to the brain with rapid onset of action in case of emergencies like epilepsy.

Lamotrigine (LTG), chemically 6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine (Scheme.1) is an anticonvulsant drug marketed under the brand name Lamictal by GlaxoSmithK line for oral administration as the chewable tablet. LTG has widely used the oral antiepileptic drug (AED) in adults with partial seizures and for the treatment of generalized seizures, either alone or in combination with other anticonvulsants in pediatrics and adults [2,3]. It also works by inhibiting voltage-dependent sodium channels, resulting in decreased release of the excitatory neurotransmitters, glutamate, and aspartate [4]. It is a BCS class II drug having low aqueous solubility (approximately 0.17 mg/mL at 25°C) which limits its absorption and dissolution rate and thus the bioavailability [5,6].




***Scheme.3.1: Chemical structure of LTG drug.***

Recently nanotechnology based on amphiphilic polymers has been recognized as one of the most attractive and rapidly-growing areas of nanomedicine technology [7-10]. Among these systems, Pluronic® block copolymers (also known as Poloxamers) that consist of hydrophilic polyethylene oxide (PEO) and lipophilic polypropylene oxide (PPO) blocks,

arranged in a basic $PEO_nPPO_mPEO_n$ structure are well-known [11-13]. Pluronic block copolymers self-assemble to form nano-sized aggregates (micelles) in aqueous solutions and exhibit a unique core-shell structure which having low polydispersity that is strongly dependent on temperature/concentration with spherical/rod-like morphology. Pluronic micelles have a micelle core consisting of PPO blocks surrounded by a heavily hydrated, shell of PEO [14,15]. Pluronic micelles have a slower rate of dissociation and allow retention of incorporated drugs for a longer time than other classical surfactant-based drug delivery systems; which may deliver a higher accumulation of the active species at the target site. Therefore, Pluronics have found noteworthy applications in drug delivery systems [16-20]. As the literature studied by Bandyopadhyay et al. [21] the formation of block copolymer micelles-drug complexes was initially proposed Dorn et al. [22]. When insoluble drug molecules are mixed with Pluronic molecules and the temperature is raised, the drug molecules assembled in the hydrophobic PPO cores. The hydrated PEO coronas are nontoxic and prevent the drug molecules from being removed from the PPO core. The solubility of the hydrophobic drugs, therefore, increases substantially in an aqueous medium, enhancing the bioavailability of the drugs [23]. It is reported that the passive accumulation of drugs incorporate in Pluronic micelle at solid tumor cells is more efficient than that of free drugs due to the long circulation time of the drug-incorporated micelles and the slow dissociation of drugs from these micelles in the blood circulation system [16,17]. Drug-incorporated Pluronic micelles can also enhance the transport of drugs throughout the blood-brain and intestinal barriers. Many more pharmaceutical advantages of Pluronic micelles make it a promising contender as a nanocarrier for the drug delivery.

Recently, Pluronic F127, $PEO_{100}$-$PPO_{65}$-$PEO_{100}$, has generated much interest in the research of controlled drug delivery due to its structural ability to changes in temperature and concentrations [24-26]. It has been reported that the micellar sizes and aggregation numbers, and gelation temperature of Pluronic F127 solutions decrease in the presence of naproxen and indomethacin drug [27]. The behavior of Pluronic F127 micelles has been systematically studied with drug molecules of different hydrophobicities incorporated in the micellar cores [28]. It was observed that most of the hydrophobic drugs increased the sizes of the micellar core and corona, and generally decreased the micellar aggregation numbers. Scherlund et al.

also observed the decrease in critical micellization temperature (cmt) and gelation temperature when local anesthetics to Pluronic F127 solutions [29]. They also confirmed that the cmt and gelation temperature of Pluronic F127 was decreased with dilution and increases in the pH of the mediums.

In the present investigations, we have focused on the solubilization of LTG in the Pluronic F127 micelles by the usual technique of UV– visible spectroscopy. Pluronic F127 micelles with and without LTG drug been characterized using DLS and SANS measurements. The effects of hydrophilic polymers, i.e., PEG1500 and F68 on LTG-incorporated Pluronic F127 micelles have also been studied. Thermodynamic parameters like the apparent micelle-water partition coefficient (P) and standard free energy change (ΔG°) of solubilization of LTG in Pluronic F127 micellar solutions were also calculated. The powdered form of LTG incorporated Pluronic F127 micelles (LPMs) were also prepared using the thin-film hydration method and characterized with modern techniques to understand the biocompatibility of LTG drug with Pluronic F127 micelles and its possible pharma applications

# 3.2: Experimental Section

## 3.2.1: Materials

Two triblock copolymers, Pluronic F127, and Pluronic F68 were procured from Sigma-Aldrich (St.Luice, MO, USA) and used as received. Polyethylene glycol (PEG1500) was obtained from Aldrich. Specifications of all the polymers are listed in Table.3.1.

***Table .3.1: Specifications of polymers used in the present work.***

| Polymer | Mol. Wt., g.mol$^{-1}$ | %(EO) / g mol$^{-1}$ | nEO | nPO | CP, °K | HLB |
|---|---|---|---|---|---|---|
| Pluronic F127 | 12600 | 70 | 100 | 65 | > 100°C | 22 |
| Pluronic F68 | 8400 | 80 | 76 | 29 | > 100°C | > 24 |
| PEG | 1500 | 100 | --- | --- | > 100°C | |

LTG was obtained as a complimentary gift sample from PAB Organics Limited (India) and used with further purification.

***Table.3.2: Specification of LTG drug.***

| Code Name | M / g mol$^{-1}$ | *pka* value | Partitioncoefficient (*P*) | Aqueous solubility (mg/mL) |
|---|---|---|---|---|
| **LTG** | 256.09 | 5.7 | 2.5 | 0.17$^{\#}$ |

**#solubility according to Ref. no.5**

Solvents like ethanol, methanol were analytic grade and utilized after appropriate distillation using a BUCHI Rota-vapor, R-210. LA390-10MT dialysis membrane was obtained from Merck, India was used. Triple distilled water was used for the sample preparation. All solutions were prepared in Pyrex $^{TM}$ glass containers.

## 3.2.2: Solubilization experiments

### *3.2.2.1: Calibration of Lamotrigine (LTG) drug*

Initially, the 1.0 mg/mL of LTG solution was prepared in methanol solvent and scanned in the UV-Vis range between 200 to 800 nm for determination of its λmax with required dilution. LTG in methanol showed an absorption maximum at 307 nm and this wavelength was chosen as the analytical wavelength. Suitable aliquots of the stock solution of LTG (1.0 mg/mL) were taken out into 10 mL standard measuring flasks and the volume was made up to 10 mL with methanol to give final concentration ranging from 0.02 to 0.12 mg/mL of the drug. All the solutions were mixed using vortex mixer and their absorbances measured at λmax = 307 nm using methanol as a reference on Shimadzu 2450 UV-Visible spectrophotometer (Shimadzu, Japan, UV-2450, double beam), and a calibration curve was plotted (shown in Fig.3.1).

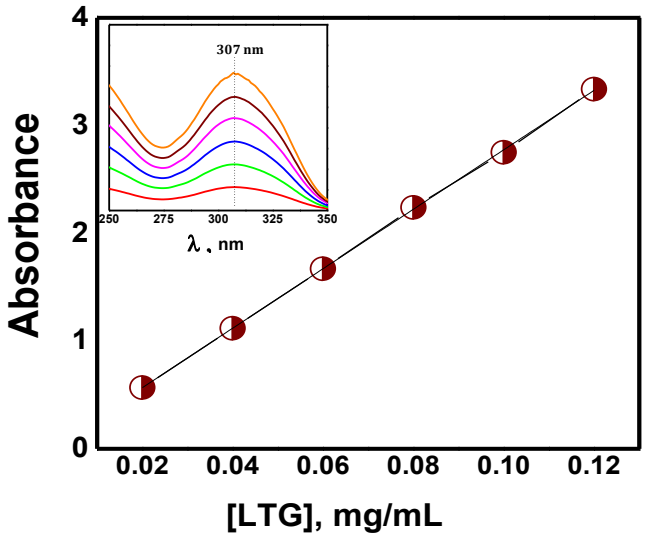



***Fig.3.1: Calibration curve of LTG drug at 307 nm at 37°C in methanol (Insert the absorbance spectra of LTG).***

Results clearly showed that Beer's law was obeyed in the 0.01 and 0.12mg/mL concentration range as the correlation coefficient was found to 0.9997. Parameters indicating linearity for the used UV spectrometric method of analysis for LTG are shown in Table 3.3.

***Table 3.3: Parameters for UV spectrometric method of analysis for LTG in Methanol***

| Parameters | Result |
|---|---|
| $\lambda_{max}$ | 307 nm |
| Linearity rang | 0.02-0.12 mg/mL |
| Regression equation | y=a+b*c |
| Correlation coefficient | 0.9997 |

#### *3.2.2.2: Phase solubility study of LTG in Pluronic F127 solutions with and without hydrophiles*

Solubility experiments were carried out on a Shimadzu (UV-2450) UV–visible double beam spectrophotometer with a matched pair of stoppered fused silica cells of 1 cm optical path length. Saturated LTG incorporated Pluronic solutions were prepared by mixing LTG into aqueous Pluronic solutions (with and without PEG1500 and F68) in glass vials with stirring at constant temperature (37°C) for 2 days. The solutions were filtered (Millipore, 0.45 lm) to remove the unsolubilized drug. The soluble amount of drug was determined by measuring absorbance at λ=307 nm. In the solubilization experiments, the filtered Pluronic solutions were diluted 60–120 times using methanol, the amount of water after dilution was negligible and applied for the calibration plot. Each solubility value was determined in triplicate, and the results are reported as the mean of the three.

## 3.2.3: Dynamic light scattering(DLS) measurements

The hydrodynamic diameter (Dh) of aqueous solutions of 5 wt% Pluronic F127 and LTG-incorporated Pluronic F127 micellar solution was determined by DLS measurements using Horiba-Zetasizer, SZ-100 instrument with a fixed scattering angle of $90^0$ at 37°C. The average diffusion coefficients and hence the hydrodynamic size was obtained by the method of cumulants. The freshly prepared sample was placed into a quartz cuvette without any treatment. Each measurement was repeated at least three times and expressed as a mean size ± 0.1 nm.

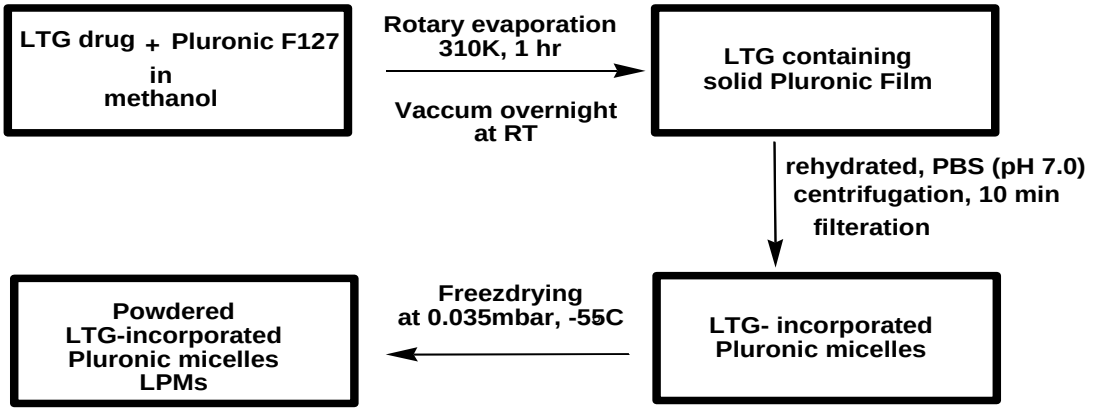
## 3.2.4: Small-angle neutron scattering(SANS) analysis

SANS analysis is used to determine the morphology and other structural details of empty Pluronic F127 micelles and LTG-incorporated Pluronic F127 micelles. SANS

experiments were carried out on samples prepared in D2O at the DHRUVA reactor, Bhabha Atomic Research Centre, Mumbai, India [30]. In SANS, one measures the coherent differential scattering cross-section ($d\Sigma/d\Omega$) per unit volume as a function of wave vector transfer Q ($q = 4p \sin(\theta/2)/\lambda$, where $\lambda$ is the wavelength of the incident neutrons and h is the scattering angle). The mean wavelength of the monochromatized beam was 5.26 A ° with $\Delta \lambda / \lambda$*15%. The angular distribution of neutrons scattered by a sample is recorded using a 1 m long one-dimensional He3 position sensitive detector. The instrument covers a Q-range of 0.017–0.35 $A^{\circ -1}$. The temperature of all the samples during the measurements was kept fixed at 37°C. The detail of measurements formulae is mentioned in section 2.2.6.2 of Chapter 2.

## 3.3.5: Preparation of LTG-incorporated Pluronic F127 micelles(LPMs)

LTG-incorporated Pluronic F127 micelles (LPMs) were prepared by thin film hydration method as shown in Scheme.3.2. A fixed amount of Pluronic F127 and LTG drug with and without hydrophilic polymers are dissolved in methanol with stirred for an hour for proper mixing and then poured into the glass plates. The methanol was completely evaporated in the vacuum oven at room temperature overnight to gave LTG-containing Pluronic film. The solid film was rehydrated with deionized water (pre-warmed at 37°C, pH 7.0) at the optimized volume to prepare LPMs. The LTG-incorporated Pluronic F127 micellar solutions were pre-frozen at -55 °C for 4 hr and then lyophilized under vacuum for 24 hrs.




***Scheme.3.2: Flowchart of preparation of LPMs.***

## 3.3.6: Incorporation efficiency and drug loading of LTG in LPMs

The percentage of LTG incorporated in the prepared LPMs were determined spectrophotometrically at λ=307 nm using the following formulas;

$$\text{Incorporation efficiency (\%)} = \frac{W_{loaded}}{W_{added}} \times 100$$

$$\text{Drug loading (\%)} = \frac{W_{loaded}}{W_{total}} \times 100$$

where $W_{loaded}$ is the LTG amount incorporated into Pluronic micelles, $W_{added}$ is the initially added LTG drug, and $W_{total}$ represents the amount of both LTG and Pluronic F127in the prepared LPMs.

## 3.3.7: Characterizations of Solid LPMs

### *3.3.7.1: UV-Visible spectroscopy (UV-VIS)*

The UV-Visible spectra of aqueous solutions of 1 wt% of LTG drug, Pluronic F127 and LPMs were observed using UV–visible double beam spectrophotometer (UV-2450, Shimadzu, Japan) with matched pair of stoppered fused silica cells of 1 cm optical path length.

### *3.3.7.2: Fourier transform infrared spectroscopy (FTIR)*

FTIR spectra of LTG drug, Pluronic F127, and three prepared LPMs were recorded in order to analyze the chemical structure and possible compatibility of systems. Samples were analyzed by spectrophotometer (FTIR-8400S, Shimadzu Co., Kyoto, Japan) using potassium bromide (KBr) pellet method using a frequency range of 4000-400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$**.**

### *3.3.7.3: X-ray diffraction spectroscopy (XRD)*

X-Ray diffraction (XRD) patterns were recorded for LTG drug, Pluronic F127 and prepared LPMs using an X-Ray diffractometer (Philips X' Pert MPD, USA) with a scanning rate of 0.5˚/s in the 2θ angle of 2-50˚.

#### *3.3.7.4: Thermogravimetric analysis (TGA)*

TGA for the degradation patterns of the pure LTG drug, Pluronic F127 and prepared LPMs were performed on Shimadzu, TGA-50 at a heating rate of 10ºC/min for the evaluation of thermal behavior of LTG drug and Pluronic molecules alone and in presence of each other.

## 3.3.8: In vitro release study of LTG from LPMs

Amount of LTG drug release from prepared LPMs was carried out using dialysis bag diffusion techniques in the phosphate buffer saline of pH 7.0 at 37°C. Released LTG drug was measured spectrophotometrically at λ=307 nm for every sample on predetermined time interval regularly at 37°C.

## 3.3.9: Stability Test of LPMs

To evaluate the physical stability of all the prepared LPMs, samples were stored at room temperature in the closed chamber and drug retention was monitored over three months and quantified using UV-Visible spectroscopy.

## 3.4: Results and discussion

LTG is a nitrogen-based drug and present in the positively charged protonated form. We observed the solubility of LTG in water is 0.17 mg/mL at 30°C, which is a close agreement with reported value [5]. Fig 3.2 shows the UV-Visible absorbance spectra of LTG drug in the water at 37°C. The solubility of LTG drug was found to 0.1754 mg/mL ,but still, not promising for its pharma applications.

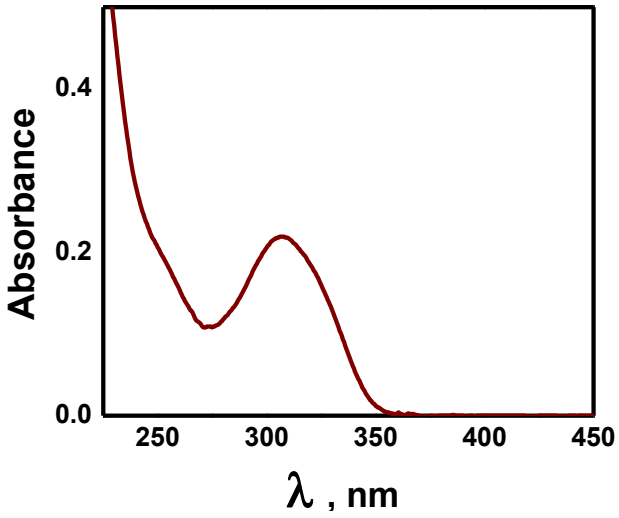



***Fig.3.2: UV-Visible spectra of LTG drug solubilized in water****(diluted with methanol)* ***at 37°C.***

### 3.4.1. Solubilization of LTG drug in Pluronic F127 solutions

The amount of LTG solubilized in different concentrations of Pluronic F127 was determined by UV-Visible spectroscopy. The phase solubility curve of LTG solubilized in aqueous micellar solutions of Pluronic F127 in a concentration range of 1.0 wt% to 10 wt% is shown in Fig.3.3. The solubility of the LTG increased markedly with increase in the concentration of Pluronic F127. It shows that Pluronic F127 micelles are able to solubilize a good amount of the LTG due to the interaction between LTG drug and Pluronic F127 molecules and increased number of micelles at a higher concentration which contributes to

increasing hydrophobic interaction [31]. Pluronic F127micelles have large PPO core which generated the hydrophobic environment for the drug to attract.

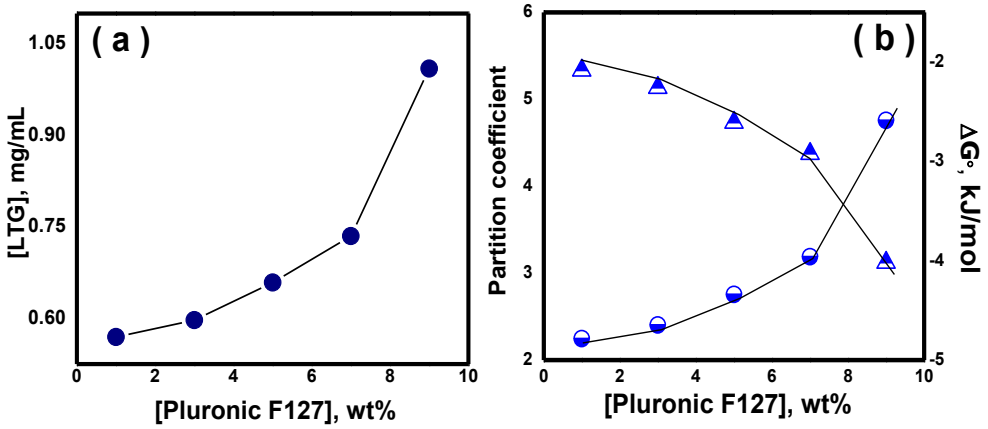



***Fig.3.3: (a) Solubility of LTG drug in aqueous solutions of Pluronic F127 at 37°C and (b) Partition coefficient and Gibbs free energy of solubilization of LTG in Pluronic F127 at 37°C.***

To understand the interaction between LTG drug and Pluronic F127 from experimental data, micelle/water partition coefficient (*P)* can be calculated from UV-Visible results. The *P* is defined as the ratio of LTG drug in the Pluronic micelle to the drug concentration in water and obtained using the following equation [32].

$$P = \frac{S_{total} - S_w}{S_w}$$

Where $S_{total}$ is the LTG solubility in Pluronic micelles and $S_w$ is the solubility of the drug in water. The partition coefficient was calculated and shown in Fig. 3.3(b).

The standard free energy change (ΔG°) for transfer of one mole of the drug in the micellar phase was also calculated from the temperature dependence of micelle/water partition coefficient (P) values. ΔG° indicates the spontaneity of the solubilization and was calculated using the following equation.

$$\Delta G° = -RT \ln P$$

where R is the gas constant, T is the temperature (in Kelvin), and P is the micelle/water partition coefficient. The ΔG° values were found to be negative, indicating that the solubilization of LTG is spontaneous. The partition coefficient values also increased with increasing concentration of Pluronic F127. Thermodynamic parameters clearly indicate that Pluronic F127 micelles are good carriers for LTG drug.

## 3.4.2. Solubilization of LTG drug in Pluronic F127 i.p.of hydrophilic polymers

The amount of LTG solubilized in 5 wt% Pluronic F127 in the presence of hydrophilic polymers (PEG1500 and F68) was determined, and the phase solubility curve is plotted in Fig.3.4(a). The solubility of the LTG was decreased in the presence of PEG1500 and F68. Results indicate that the Pluronic F127 micelle was not favored the solubilization of LTG drug after the added of the hydrophilic polymers. Such effects were occurred due to an increase in the hydrophilicity of Pluronic F127 micelles. F68 is slightly better compared to PEG1500 because of the presence of PPO part (nPO : 29 ) in its molecule.

The micelle/water partition coefficient(P) (Fig.3.4b) and standard free energy change(ΔG˚) (Fig.3.4c) values of LTG solubilized in Pluronic F127 (5 wt% fixed) micelles with PEG1500 and F68 in concentration range(0.01 to 1 wt%) were also not encouraged and indicate the hindrance into spontaneous partition of LTG drug into the Pluronic F127 micelles.

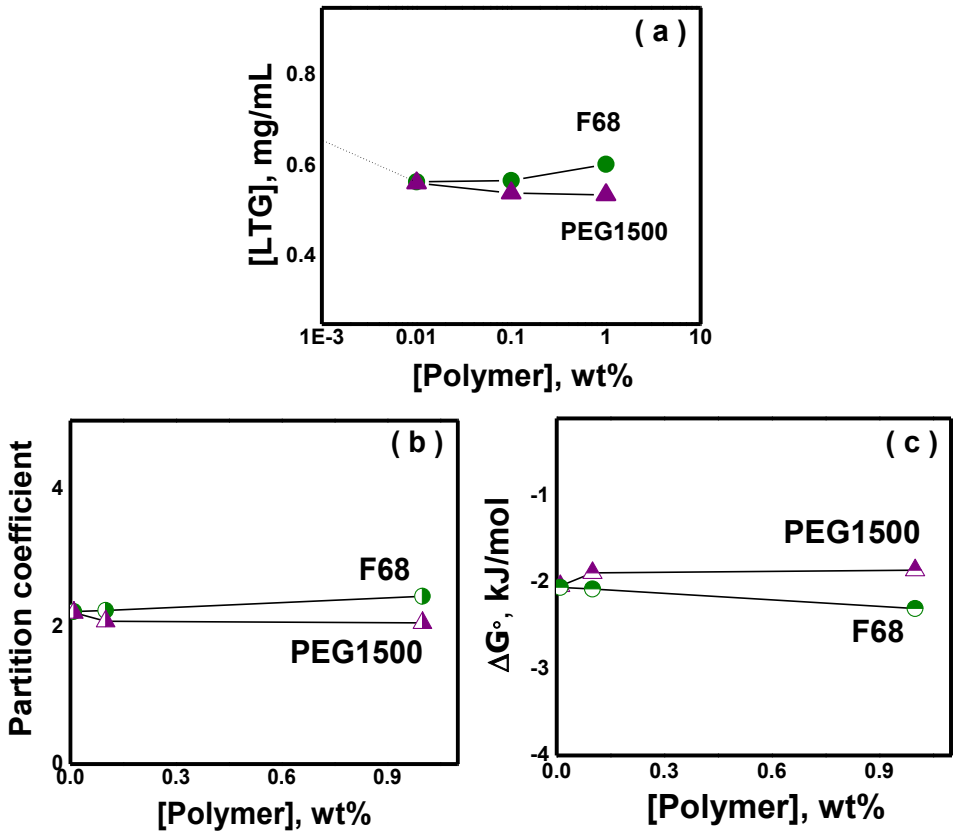



***Fig.3.4: (a) Solubility of LTG drug in Pluronic F127 (5.0 wt% fixed) solution i.p.of hydrophilic polymers at 37°C, (b) Micellar/water partition coefficient and (c) Gibbs free energy of solubilization of LTG drug in Pluronic F127 (5.0 wt% fixed) i.p.of hydrophilic polymers at 37°C.***

For the determination of micelle size and change in presence of LTG- incorporated Pluronic F127 micelles, DLS was used [33]. In the drug delivery, the micelle size of the drug carrier is the important as it plays a key role in drug accumulation and penetration in cells i.e. the micelle size should be large enough for circulation as well as small enough for their penetration [34]. All studies were performed at 37°C, which is well above the cmt (19.5°C) for 5 wt% Pluronic F127 [14]. The intensity weighted size distribution plot of the empty and LTG-incorporated Pluronic F127 micelles is presented in Fig.3.5. The hydrodynamic diameter of an empty Pluronic F127 micelle was found to be 18.9 ± 0.1 nm. After the solubilization of LTG in the Pluronic F127 micelle, the hydrodynamic diameter of LTG-incorporated Pluronic F127micelle was 19.3 ± 0.1 nm. Results clearly demonstrated the

increase in micellar size due to the encapsulation of LTG in the PPO coreas well as PEO shell of the F127 micelle.

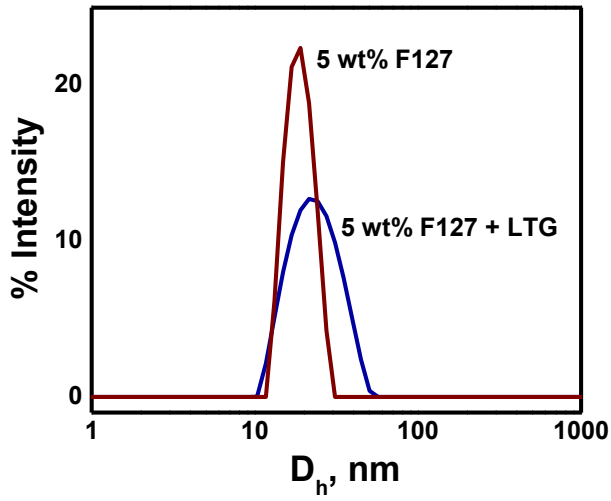



***Fig.3.5: %Intensity versus hydrodynamic diameter ($D_h$) plot for 5 wt%Pluronic F127 the solution before and after incorporated LTG drug at 37°C.***

SANS measurements are widely used to study the associative interactions between Pluronic polymers and drugs. The SANS profile of the 5 wt% Pluronic F127 and LTG incorporated in 5 wt% Pluronic F127 in $D_2O$ at 37°C is shown in Fig.3.6. SANS data on Pluronic F127 micelles is well studied [35], but LTG-incorporated Pluronic F127 micelles are reported here for the first time. The SANS intensity profile of 5 wt% Pluronic F127 show signatures of both form factor as well as the structural factor governing scattering. The Pluronic F127 micelles are found to be polydispersed micelles with spherical core and a Gaussian distribution chains attached to them, interacting with hard sphere potential [36]. The analysis of the data (Table.3.4) reveals an increase in the micellar core radius of Pluronic F127 with LTG drug from 55.1 Å to 56.9 Å. The hard sphere radius is 101.1 Å, which is much larger compared to the core radius because the interaction radius also involves shell of PEO blocks. The micellar volume fraction on the other hand, decreases in case of LTG incorporated Pluronic F127. This can be explained based on the fact that micellar solubilization of drug is accompanied by simultaneous micellar dehydration. The observed increase in micellar core upon solubilization of LTG results due to the incorporation of LTG in the PPO core and a simultaneous increase in micellar aggregation. The morphology of

LTG-incorporated Pluronic F127 micelles was determined from SANS measurements to be spherical, and the particle size was similar to that determined by DLS.

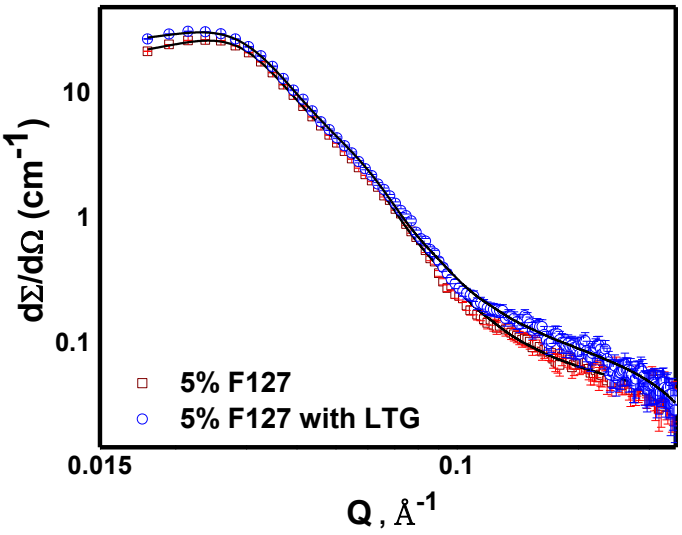



***Fig.3.6: SANS profile for 5 wt% Pluronic F127 with and without LTG drug.***

***Table .3.4: Various parameters of SANS analysis for 5 wt% Pluronic F127 solution with and without LTG drug at 37°C.***

| System | Core radius Rc, (Å) | PDI | Radius of gyration Rg, (Å) | Hard sphere radius Rhs, (Å) | Volume fraction Φ |
|---|---|---|---|---|---|
| 5wt% F127 | 55.1 | 0.36 | 21.6 | 101.1 | 0.17 |
| 5wt% F127 + LTG | 56.9 | 0.36 | 21.6 | 100.3 | 0.16 |

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## 3.4.3. Characterization of LPMs

To determine the efficiency of LTG incorporation, we also prepared solid LTG-incorporated Pluronic F127 micelles with and without hydrophilic polymers (PEG1500 and F68) using the thin-film hydration method. The composition of LTG drug, Pluronic F127 and hydrophilic polymers are shown in Table 3.5.

***Table .3.5: Composition of LPMs with and without hydrophilic polymers.***

| Drug | Pluronic | Hydrophile | $W_{drug}$ :$W_{Plu}$ : $W_{add}$ | Code name |
|---|---|---|---|---|
| LTG | F127 | --- | 0.1 : 2.0 : 0.00 | LPM7 |
| LTG | F127 | PEG1500 | 0.1 : 2.0 : 0.04 | LPM7P |
| LTG | F127 | F68 | 0.1 : 2.0 : 0.04 | LPM7F8 |

The incorporation efficiency and drug loading percentage of LTG incorporated into Pluronic F127 micelles with and without polymer additives are summarized in Table.3.6. Percentage loading (1.28%) and incorporation efficiency (65.87%) of LTG was highest in LPM7 micelles. The other two systems show the lower IE% (56.77% for LPM7F8, 54.01% for LPM7P) and DL% (1.11% for LPM7F8, 1.056% for LPM7P) with compared to LPM7. Results clearly indicated that Pluronic F127 micelle alone is a better carrier for the incorporation of LTG drug than with the added hydrophilic polymers. Better incorporation efficiency of LPM7 could enhance therapeutic efficacy by improving pharmacodynamic property [20]. LPM7 demonstrates the prospect for a practically useful drug delivery carrier for LTG with improved drug loading capacity.

***Table .3.6: Drug loading and Incorporation efficiency of LPMs at 37°C.***

| System | DL% | IE% |
|---|---|---|
| LPM7 | 1.28 | 65.87 |
| LPM7F8 | 1.11 | 56.77 |
| LPM7P | 1.056 | 54.01 |

The UV-Visible spectra of 1 wt% solutions of prepared LPMs in the water at 37°C is presented in Fig.3.7. The spectra clearly show that LTG drug has a very low peak intensity at 307 nm in water due to limited water solubility. All the LPMs gave intense peaks at 307 nm indicating that LTG molecules are transformed from the bulk phase to the micellar phase. The trend of the increase in the intensity of LTG is as follows;

LPM7 > LPM7F8 > LPM7P

The spectra were indicated the incorporation of LTG drug was better in LPM7 due to the hydrophobic F127 micelles [37].

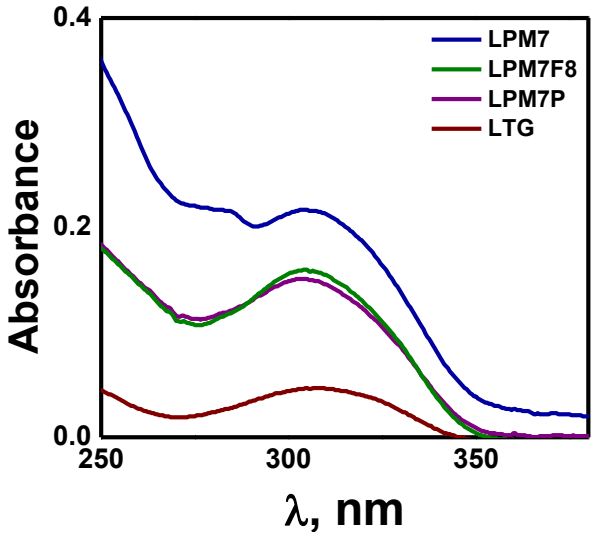


***Fig.3.7: UV-VIS Spectra of LTG drug and prepared LPMs (1 wt% fixed concentration) in the water at 37°C***

The FT-IR spectrums of LTG drug, Pluronic F127 and all the three LPMs are presented in Fig.3.8. The LTG shows principal absorbance peaks 3446 $cm^{-1}$ (-N-H aromatic stretching), 3208 $cm^{-1}$ (-C-H aromatic stretching), 1640 $cm^{-1}$ (-N=N stretching) and 794 $cm^{-1}$ (-C-Cl stretching) in the IR spectrum [45].The Pluronic F127 spectra show two prominent peaks at 2882 $cm^{-1}$ of (–C–H stretching) and 1112 $cm^{-1}$ of (–C–O stretching ). The IR spectrums of the LPMs shows two prominent peaks at 2878 $cm^{-1}$ of (-C-H stretching) and 1105 $cm^{-1}$ of (–C–O stretching), but not found any peak of aromatic nucleus present in the LTG drug. Also, the peak intensities of the LTG drug in the spectra of LPMs were smoothened indicating the compatibility of a drug with the micellar core of Pluronic F127.

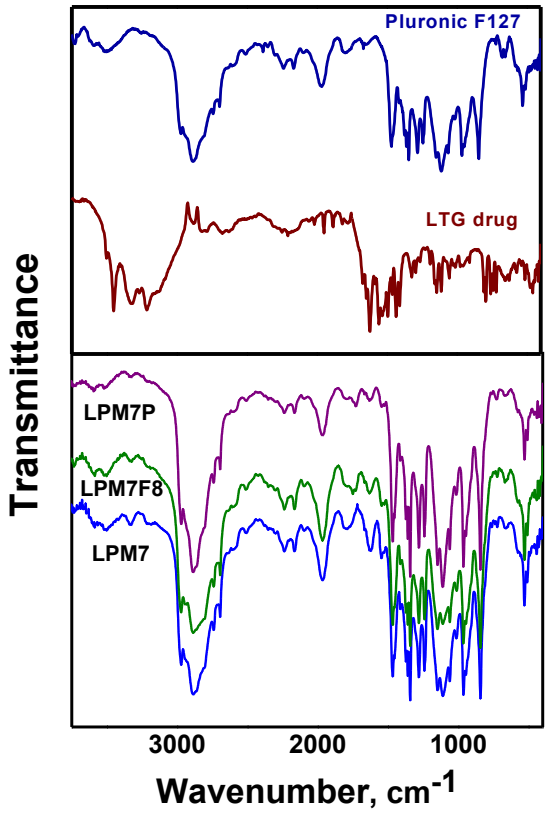


***Fig.3.8: (a) FTIR spectrums of LTG Drug and Pluronic F127 and (b)FTIR spectrums of prepared LPM7, LPM7F8, and LPM7P.***

X-Ray diffraction was carried out to analyze potential changes in the inner structure of LTG-incorporated Pluronic F127 micelles in the powder form. The extent of such changes depends on the chemical nature and physical hardness of the active ingredient. In Fig.3.7, the XRD pattern of pure LTG shows many characteristic peaks in the studied 10° to 35° range of 2θ, indicative of its crystalline nature. Meanwhile, the two sharp high intense peaks at 2θ of 19.0° and 23.2° were observed in an XRD pattern of Pluronic F127 due to the presence of PEO groups [37,38]. The XRD pattern of LPMs shows the absence, broadening and reduction of major LTG diffraction peaks, but two minor peaks of PEO group of Pluronic F127 were present in all the cases. This clearly indicates the masking of crystalline peaks of LTG drug after incorporated into Pluronic F127 micelles in LPMs.

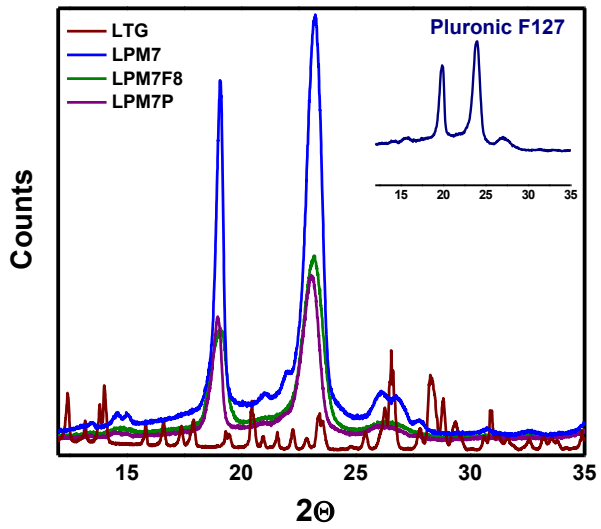


***Fig.3.9: XRD patterns of LTG drug, LPM7, LPM7F8 and LPM7P (Inserts the pattern of Pluronic F127)***

Fig.3.10 shows the TGA thermograms of the prepared LPMs. The TGA patterns of LTG drug and Pluronic F127 are presented in inserts of Fig.3.10. LTG showed multistage decomposition without any stable intermediates with weight loss from 145 to 640°C temperature. The first depression in the curves was a measure of moisture content. The Pluronic F127 shows the percentage of weight loss from 145 to 300°C with single stage decomposition. The fast processes of weight loss were shown in all the LPMs between 150 to 325°C. The LTG-incorporated Pluronic F127 micelles decomposed between that of LTG and Pluronic F127. Results indicated thermal stability for LPMs relatively lower to pure LTG but higher than the Pluronic F127. Lack of crystallinity in LPMs degradation suggests better drug dispersion and increased drug-Pluronic interactions, which favor slow-release kinetic [39].

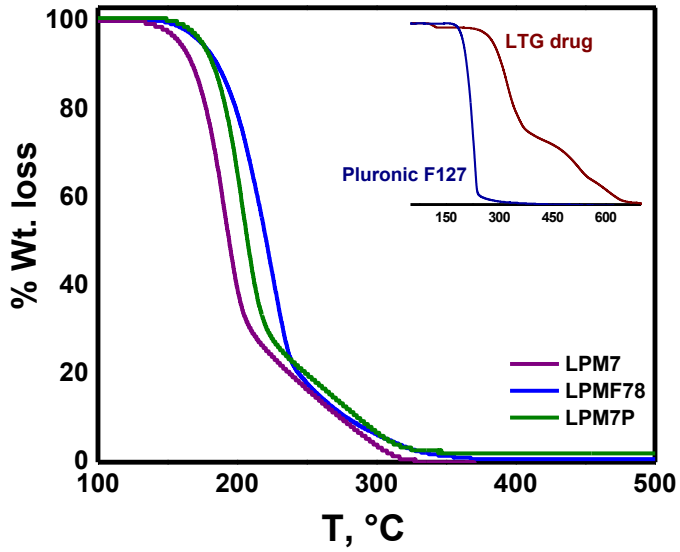


***Fig.3.10: TGA curves of LTG drug, Pluronic F127 and prepared LPMs.***

## 3.4.4. In-vitro release study of LPMs

The in vitro release of LTG from micellar formulation under sink condition was investigated by the dialysis method. Fig.3.11 shows the release profile of LTG drug from LPMs in phosphate buffer saline of pH=7.0 up to 60 hours. The release of a lipophilic compound from core-shell micelle nanocarriers is mainly depended on hydrophobic properties of the micelle inner core and hydrophobic interaction between the drug and micellar core. The stronger the interaction between the drug and the core-forming block, the slower the release of drug from micelle. Two-phase release profile was observed in the case of all the prepared LPMs. There was a rapid release of almost 70% in the first stage followed by a sustained and slow release of more than 85% over the prolonged time of 60 hrs. The order of dissolution enhancements was LPM7>LPM7F8>LPM7P was attributed to compatibility of the micelles of Pluronic F127.

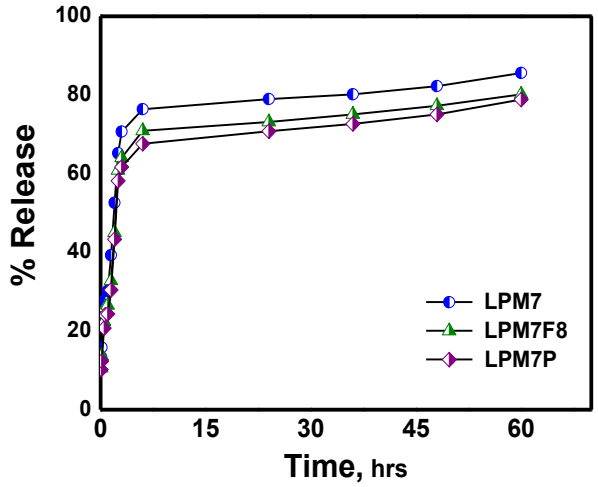


***Fig.3.11: In vitro release profile of LTG drug from LPM7, LPM7F8, and LPM7P in PBS of pH 7.0 at 37°C.***

## 3.4.5. Stability of LPMs

The storage stability of prepared LPMs was studied for 3 months at room temperature. From Fig.3.12, no significant loss in drug retention within the time period was observed. It means that the powder forms of LTG-incorporated Pluronic F127 micelles are stable up to 3 months. Sahu et al. also reported good stability using Pluronic F127 [37]. Results also proved the compatibility of the LTG drug with Pluronic F127 with and without hydrophilic polymers PEG1500 and F68.

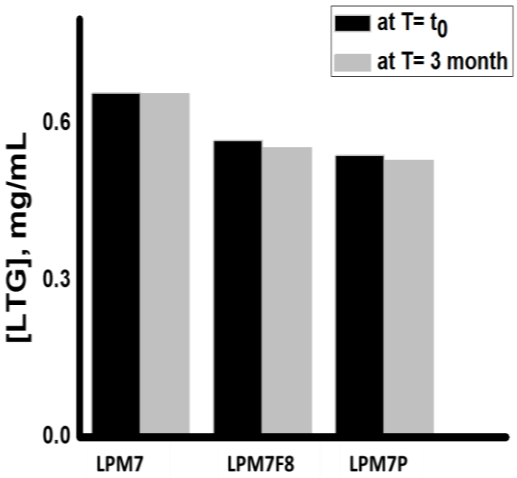


***Fig.3.12: Storage stability of prepared LPM7, LPM7F8, and LPM7P at RT.***

## 3.5: References


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