

Study of block copolymer micelles as vehicles for hydrophobic drug Lamotrigine

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ABSTRACT

Objective: The objective of the study was to investigate the solubilization of poorly water-soluble drug Lamotrigine in pure & mixed Pluronic polymeric micelles. **Method:** Two different Pluronic (Pluronic F68, Pluronic L81) were chosen and micelle formulations were prepared by using various drug:polymer ratios and model drug Lamotrigine. Formulations were characterized by critical micellization concentration (CMC) values, cloud point of copolymers, micelle size and size distribution, zeta potential, loading efficiency, drug release and stability. **Result:** Mixed micelles (hydrophilic and hydrophobic) also helped to overcome the limitations of monosystem of Pluronic L81 and Pluronic F68. The solubilized drug and salt decreased the cloud point of copolymers. Results show that the solubilization of Lamotrigine enhances with the rise in concentration of block copolymers, negative Gs0 and temperature, but no significant increase was observed with added salt and at a lower pH the drug shows highest solubility. **Conclusion:** Mixed micelles showed fairly high entrapment efficiency, loading capacity and sustained release profile for Lamotrigine, a model hydrophobe than that of plain Pluronic micelles.

Key words: Pluronic F68, Pluronic L81, Micelles, Solubilization.

INTRODUCTION

In the pharmaceutical technology, attracted incredible attention for development of efficient drug delivery systems during the last two decades. The principal reason for the implausible growth of drug delivery technology is the best prospect for achieving large improvements over current therapies will occur through improved delivery of existing drugs. This necessity arises because of a drug molecule must overcome the enormous barriers before it reaches its target site within the body, where it can perform its biological role.¹

There are some serious problems associated with the therapeutic application of hydrophobic, poorly water-soluble agents, since low water solubility results in poor absorption and low bioavailability.² In addition, drug aggregation upon intravenous administration of poorly soluble drugs may lead to such complications as embolism³ and local toxicity.⁴ To overcome systemic toxicity a

very promising approach is the application of drug-loaded nanosized drug carriers, such as micelles, polymeric nanoparticles, dendrimers and liposomes.^{4,5}

Polymeric micelles are nano sized assemblies of amphiphilic block copolymers exhibiting an inimitable core-corona structure. The outer hydrophilic shell is important to stabilize the micelles in an aqueous environment and after systemic administration minimize clearance by the mononuclear phagocytic system (MPS) whereas the inner hydrophobic core functions as a drug reservoir.⁶ The assembled polymers are in dynamic equilibrium with free unimers, in contrast to polymeric nanoparticles and the particles are usually smaller (10 – 100 nm) displaying monodisperse size distributions.⁷

Block copolymers are commercially available as linear triblocks of the type ABA and BAB (Pluronics®) made of polyethylene oxide (PEO) and polypropylene oxide

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(PPO) with varying hydrophilic-lipophilic balance (HLB). These self-assemble polymeric amphiphiles to form nanosize aggregates (micelles) in aqueous solutions and exhibit a unique core-shell structure which having a fairly low polydispersity that is strongly dependent on temperature/concentration with spherical/rod-like morphology.^{8,9} Pluronic® aggregates have a micelle core consisting of a hydrophobic poly(propylene oxide) or PPO block surrounded by a heavily hydrated, hydrophilic poly(ethylene oxide) or PEO block.⁹ The Pluronic micelles have a slower rate of dissociation and allow retention of loaded drugs for a longer period than other conventional surfactant based drug delivery systems; hence, these surfactants may allow a higher accumulation of the active species at the target site and so Pluronic® have found significant applications in drug delivery systems.^{10,11} The size, morphology and solubilizing capacity of micelles depends on different factors, such as chemical structure of the amphiphile and the drug molecule, pH, temperature, and the ionic strength.¹² The solubilizing power of polymeric surfactants are generally considered to be better than conventional surfactants due to their low critical micelle concentrations (CMC) and the thermal stability of the micelles.¹³

A variety of block copolymers may be used to form polymeric micelles.¹⁴ In this study we used block copolymer such as Pluronic F68 and Pluronic L81 are used which are difunctional block copolymer surfactant terminating in primary hydroxyl groups from BASF.

Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures.¹⁵ In order to avoid the risk of permanent brain damage which requires quick management of seizures. Parenteral administration allows transport of drugs to the brain abstaining BBB, thus providing a unique feature and better option to target drugs (for example Lamotrigine) to the brain with rapid onset of action in case of emergencies such as epilepsy.

Lamotrigine is an anticonvulsant drug used in the treatment of epilepsy and bipolar disorder and widely used as mono or adjunct therapy in adults and children. The Lamotrigine a (biopharmaceutical classification system) BCS class II drug is currently available as a tablet which is administered 2–3 times per day as divided doses of 25–600 mg.¹⁶

Mixed micelles reveal synergistic properties, such as increased micelle stability and drug loading efficiency, superior to those of the individual components.¹⁷ In this study, mixed micelles made of hydrophobic Pluronic L81 and hydrophilic Pluronic F68 and pure micelles of individual component with Lamotrigine were prepared and also studied the solubilization efficiency of

Lamotrigine for binary mixture of Pluronic F68 and Pluronic L81. The mixed micelles of triblock copolymer surfactant Pluronic F68, Pluronic L81 with Lamotrigine has been studied as a function of temperature, presence of electrolyte (NaCl) and different pH

MATERIAL AND METHOD

Materials

Pluronic (Pluronic f68 and Pluronic L81) were purchased from Sigma-Aldrich. Lamotrigine was gift sample from Kopran Pharmaceuticals, Mumbai. All other chemicals and components for buffer solutions were of analytical grade.

Study of Block Polymers for Solubilization (CMC)

Surface tension method was used for CMC determination of Pluronic F68 and Pluronic L81 polymers. The CMC of the block copolymer at 25 °C was determined in pure water. The CMC determinations for Pluronic F68, Pluronic L81 were based on the change in surface tension with surfactant concentration. Stalagnometer was used to measure the surface tension. Each surface tension measurement was repeated three times, and the typical error in the CMC determination was less than 5%.

Cloud point (CP)

Cloud points of optimized formulation with and without drug were determined in the presence and absence of varying amounts of added sodium chloride by gradually heating the solution in thin 20 mL glass tubes immersed in a temperature controlled water bath. The solutions were stirred with a magnetic stirring bar while being heated. The heating rate of the samples was adjusted to 1 °C/min. The first appearance of turbidity was taken as the cloud point and cloud point values were found to be reproducible within 0.5 °C.

Preparation of drug-loaded polymeric micelles

Polymeric micelles containing Lamotrigine were prepared by direct dissolution technique using block copolymer (Pluronic L81, Pluronic F68) alone and in combination (1:1) ratio. Block copolymer was dissolved in water, Lamotrigine was then added to the polymer solution. The solution was stirred continuously on stirrer at room temperature at different rpm for period of 6 h to optimize the RPM for preparation of micelles. Nonincorporated drug remained insoluble and was separated by filtration of micelle suspension through a 0.2 µm filter. The filtered solution was diluted 10 times with polymeric solution. The Lamotrigine concentration in filtered solution was estimated by measuring UV absorbance at λ_{max} 306 nm.¹⁷

Table 1: Factors and Levels for the 33 factorial design

Independent Variables	Parameters	Levels		
		-1	0	+1
X1	Polymer:Drug ratio	1:1	1:5	1:10
X2	Time of stirring	12	24	48
X3	Type of block copolymer	Pluronic F68	Pluronic L81	Pluronic F68:L81(1:1)
Dependent Variables				
Y1	% drug loading			

Table 2: Factorial formulation according to factorial design

Batch code	Variables levels in coded form		
	X1	X2	X3
F			
F1	1	1	1
F2	-1	0	1
F3	0	0	-1
F4	-1	-1	1
F5	1	0	-1
F6	-1	1	0
F7	1	-1	1
F8	1	-1	-1
F9	0	0	0
F10	-1	0	-1
F11	-1	-1	0
F12	-1	0	0
F13	0	1	0
F14	1	-1	0
F15	0	1	-1
F16	0	-1	0
F17	1	1	0
F18	1	1	-1
F19	-1	1	1
F20	0	-1	-1
F21	1	0	1,
F22	1	0	0
F23	0	0	1
F24	-1	1	-1
F25	-1	-1	-1
F26	0	-1	0
F27	0	1	0

Optimization of polymeric micelles

Twenty seven Lamotrigine polymeric micelles formulation were prepared according to 3³ factorial design employing the factors and levels shown in (Table1and2).

Study the effect of operating variables on polymeric micelles

Polymeric micelles were prepared at different temperature (30,35, 45°C), pH(2,6,8), and salt (NaCl) concen-

trations (1.45%,0.9%,0.45%) keeping the stirring time constant i.e.,6 h. The effect of temperature, pH, and salt on the percentage of drug entrapment in polymeric micelles was calculated.

Drug entrapment

The filtered solution of drug containing polymeric micelles was diluted 10 times with methanol. Blank experiments, without Lamotrigine, were done to determine the solubility of the drug in water.The Lamotrig-

ine concentration in polymeric solution was estimated by measuring UV absorbance at λ_{\max} 306 nm.

After estimating the drug contents by UV, incorporated drug (%) and drug weight (%) in micelles were calculated using Equations (1) and (2):

$$\text{Drug incorporated (\%)} = (a/b) * 100$$

$$\text{Drug weight in micelle} = (a/(b+c)) * 100$$

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

Determination of micelles size and polydispersity index

The micelles size determination as performed using photon correlation spectroscopy within built zetasizer (model: nano zs, malvern instrument, USA). Aliquot pre-concentrate (0.5 mL) was diluted to 50 mL with distilled water; stir slowly to form a dispersion. Diluted samples were directly placed in to the module for measurements. All determination is made in triplicate.

Zeta potential determination

Sample (0.5 mL) was diluted to 50 mL with distilled water in glass beaker with constant stirring. Zeta potential of the resultant solution was determined using the zeta-sizer (model: nano zs, malvern instrument, USA). All determination is made in triplicate.

In vitro release of lamotrigine from polymeric micelles across the cellophane membrane

The release of Lamotrigine from polymeric micelles was determined using the membrane diffusion technique, 1 ml of polymeric micelles suspension was placed in a diffusion cell (glass tube) of diameter 2.5 cm, the lower open end of the glass tube was covered with soaked cellulose membrane. This cell then filled with phosphate buffer solution pH 6 and 8 (25 ml). This was constantly stirred at speed 50 rpm at 37 ± 10^0 on a magnetic stirrer with a thermostat. Aliquots were withdrawn at 15, 30, 60, 90, 120, min (up to 6 hours) intervals and replaced

simultaneously with equal volume of fresh phosphate buffer solution. The Lamotrigine concentration in the samples was analyzed spectrophotometrically, at wavelength 306 nm to determine the amount of drug release.

Stability studies

Stability test of micelles consisted of visual control and analytical measurement of drug content. For this purpose, lyophilized and aqueous solution forms of Lamotrigine loaded micelles were placed in a stability chamber at 25 ± 2^0 , 60% relative humidity for 3 months. Lyophilized forms were placed in 2 ml Eppendorf tubes and solution forms were placed in 5 ml bottle with a lid. Samples were taken to determine the drug content at the beginning and at the end of 3 months. Drug content was measured spectrophotometrically after extracting drug into methanol and UV spectrums were also checked.¹⁰

RESULT AND DISCUSSION

Determination of critical micelle concentration

The critical micelle concentration can be determined by carrying out surface tension measurement using stalagmometer by drop count method on a series of different Pluronic concentration, such as for F68 1 mg/ml to 8 mg/ml and for L81 from 10 $\mu\text{g/ml}$ to 80 $\mu\text{g/ml}$. Surfactant exhibit a specific surface tension curve as a function of the concentration. Initially the surfactant molecules increasingly enrich themselves at the water surface. During this phase the surface tension decreases from 59.79 N/m to 50.15 N/m for F68 and 41.28N/m to 37.65N/m for L81 linearly with the logarithm of the surfactant concentration. when the critical micelle concentration is reached, a further increase in surfactant concentration no longer has any considerable influence on the surface tension. The obtained critical micelle concentration values of polymers in aqueous solution were 4 mg/ml and 0.06 mg/ml for Pluronic F68 and Pluronic L81.

Table 3: Cloud points of Pluronic with and without drug (Lamotrigine) in salt solution

Type of Block polymer	Cloud point ($^{\circ}\text{C}$)							
	Distilled Water		0.45% w/v NaCl solution		0.9%w/v NaCl solution		1.45%w/v NaCl solution	
	Without drug	Drug saturated	Without drug	Drug saturated	Without drug	Drug saturated	Without drug	Drug saturated
Pluronic L81(1.25%)	21	20	20	18	19	18	18	17
Pluronic F68 (2.5%)	100 <							
Combination of Pluronic L81,F68(1:1) (2.25%)	23	21	22	21	21	20	20	18

Cloud point

The cloud point of nonionic surfactant depend on its molecular structures i.e, the lipophilic nonpolar and the hydrophilic parts (PEO). Increasing hydrophobicity decreases the cloud point whereas increasing the hydrophilicity increases the cloud point.¹⁸ The data presented in (Table 3) for cloud point (CP) of 1.25% of pure Pluronic L81, 2.25% of mixed Pluronic L81and Pluronic F68 with and without drug (Lamotrigine) in water and salt solutions with this behavior. An almost linear decrease in the cloud point of two copolymers and their combination with increasing salt concentration and. The effect was even more when copolymers are saturated with the drug (Lamotrigine) for same salt concentration .The solutions remain simply micellar without any anisotropy below the cloud point but transform into two phase system above it. At temperature below about 20^o, even micelles are absent and the copolymer remains dissolved. At the molecular level because of the hydrophilic nature of PEO in water at lower temperature. The micelle formation of EO–PO block copolymers is mainly dependent on concentration, temperature and hydrophobic interactions. Micelles grew large, particular at temperature near the

cloud point. Salt are known to change the cloud point of water soluble polymers such as PEO, PPO based non-ionic surfactant. The effect has been attributed due to their salting out action by dehydrating the PEO shell, similar to the effect of temperature and increases the hydrophobicity in the PPO block of the copolymer.¹⁸

Preparation of polymeric micelles

Optimization of RPM on the basis of highest drug loading for 6hrs was done ,since the optimized RPM for plain pluronic L81and combination of (pluronic L81, pluronic F68) was 600 RPM and 900 RPM for plain pluronic F68 micelles respectively, hence these were selected for the further study.

Loading of drugs in polymeric micelles

% drug loading was performed using 3³ factorial design for which the results are as shown in (Table 4) and (Figure 1 and 2)

Statistical analysis: The polynomial equation for 2F1model as given below:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3 + A_{11}X_1^2 + A_{22}X_2^2 + A_{33}X_3^2$$

Table 4: Summary of result of regression analysis for response Y.

Model	R2	Adjusted R2	Predicted R2	S.D.	Remarks
2F1	0.9838	0.9472	0.8150	1.72	Suggested

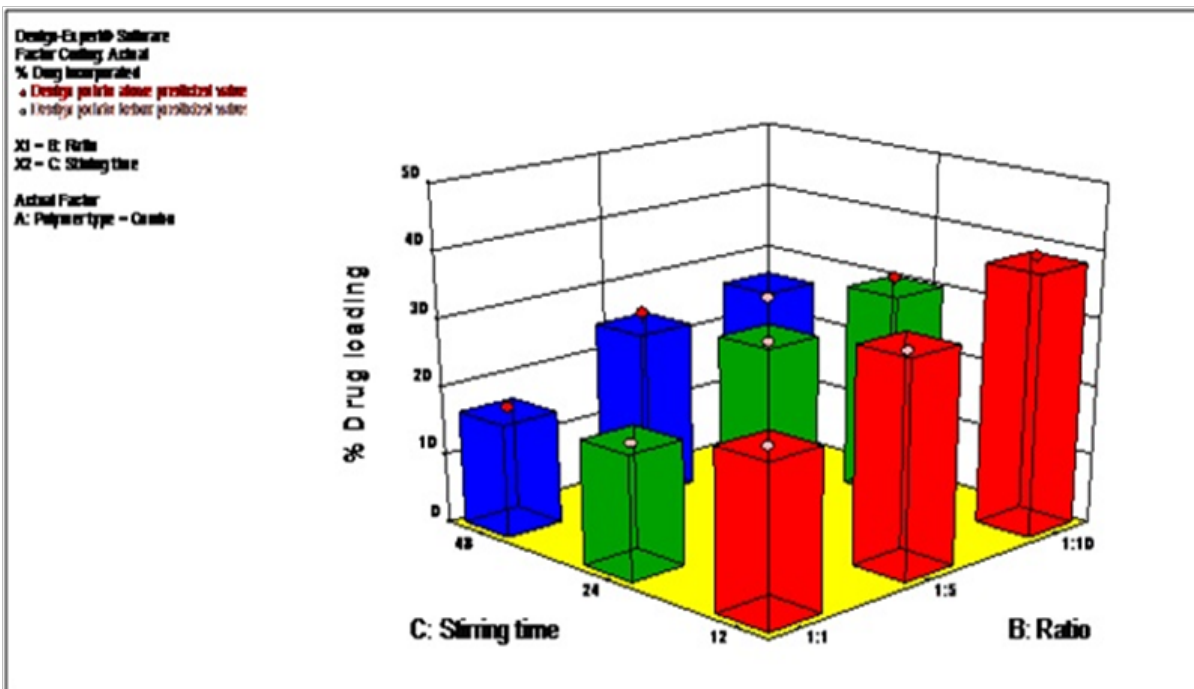


Figure 1: 3D graph of factor X1 and X2 for % drug loading

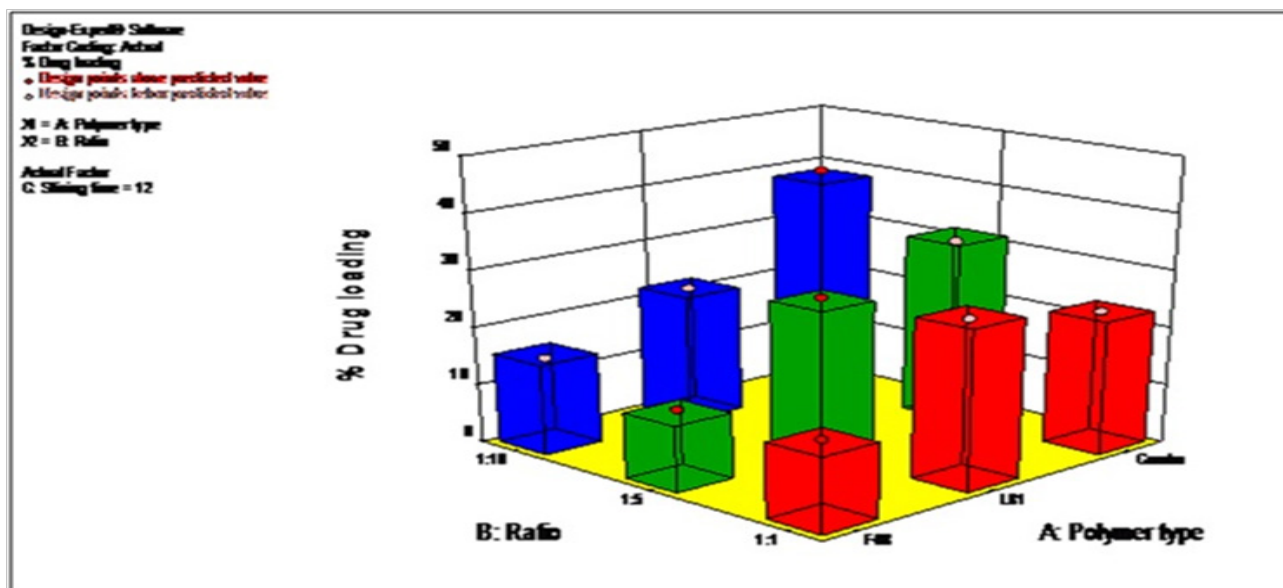


Figure 2: 3D graph of factor X1 and X3 for % drug loading

$$Y = 23.80 + (-6.26)X1a + 3.34X1b + (-1.15)X2a + 0.69X2b + (-0.39)X3a + (-2.39)X3b + 1.46X1aX2a + 4.47X1bX2a + (-1.54)X1aX2b + 0.55X1bX2b + (-3.67)X1aX3a + (-1.45)X1bX3a + 1.24X1aX3b + (-2.92)X1bX3b + (-0.82)X2aX3a + (-1.12)X2bX3a + (-0.24)X2aX3b + 0.64X2bX3b$$

Hydrophobic micelle core works as a cargo to improve solubility and stability of lipophilic drugs. Drugs loaded into micelle core by hydrophobic interactions and covalent/non-covalent bond formation. Consequently, depending on both hydrophobic interactions and steric properties it is not possible to load all kind of drugs into polymeric micelles. Several of the major factors which influence the loading capacity and loading efficiency of block copolymer micelles are nature of the solute, nature of the core forming block, core block length, total copolymer molecular weight, solute concentration and to a lesser extent, the nature and block length of the outer micelle shell.¹⁴ For reasons, we studied these two different polymer and their combinations. In both polymer types, no loading efficiency was obtained. Changing the drug:polymer ratios contribute to loading. Lamotrigine was loaded into micelles by direct dissolution. The maximum loading efficiency of 31.5% was obtained by loading Lamotrigine into Pluronic L81. Loading percent into Pluronic F68 micelles was less than Pluronic L81 micelles (Figure 2). With further increase in polymer:drug ratio from 1:1 to 10:1, there was an increase in loading efficiency of drug with both polymers. This might show that amount of added Lamotrigine was not exceeding the drug-carrying capacity of the core in these micelles. Contrary to Pluronic F68, in Pluronic L81 no regular increase on loading efficiency was observed with increasing polymer ratio. The interpenetrating network formed stabilized the micelles

at concentrations below the critical micellar concentration of free polymer, though the increased micellar stability was not permanent and disappeared over a time period of days to weeks. In order to overcome lack of stability we introduced the concept of binary mixing of hydrophobic (L81) and hydrophilic (F68) Pluronic block copolymers. Even though, large aggregates were primarily formed and phase separation apparent during the first 24–48 h, sonication (1–2 min) stabilized the dispersions. A combination of pluronic L81/ F68 (1:1% weight ratio) formed stable dispersions with a small particle size and displayed higher solubilization capacity of a hydrophobic drug compared to that of pure Pluronic F68 and Pluronic L81 micelles. Pluronic L81 contains 10% hydrophilic and 90% hydrophobic residue^{19,20} aggregated with a lamella structure. The hydrophobic block, PPO of Pluronic L81, aggregated due to its hydrophobic interaction in aqueous solution, forming a supramacromolecular structure. However, the hydrophilic block PEO of Pluronic L81 only has 10% of the whole molecular weight, not providing sufficient steric hindrance to form a stable dispersion. In this study, Pluronic F68 (EO76–PO30–EO76),^{20,21} was admixed into Pluronic L81 to prepare a stable micelle. The Pluronic F68 with long chains of PEO kinetically provided high stability and spherical structure for Pluronic L81. However, the stability pattern for formulation to those prepared with and without sonication was similar

Effect of variables on optimized formulation (combination 1:10)

Lamotrigine solubilized in block copolymer. The amount of drug solubilized was determined at different temper-

Table 5: Effect of variables on optimized formulation

Parameters	Temperature (°C)			pH			Salt concentration (%)		
	25	35	45	2	6	10	0.46	0.9	1.46
Drug incorporated(%)±SE ^a	25.16	32.2	39.3	34.42	26.47	24.9	34.93	34.3	29.35
Drug weight in micelles (%)	12.58	16.1	19.75	17.21	13.23	12.45	17.46	17.25	16.675

ature, pH and salt concentration. Lamotrigine solubilization monitored using UV spectroscopy and the results reveal a strong interaction between drug and copolymer. The solubility of drug increases with copolymer concentration, temperature and was also influenced by changing salt concentration and pH of the solution and data presented in (Table 5).

Effect of temperature

With an increase in temperature the amount of drug solubilized in micellar system increases due to micellar growth. The apparent concentration of Lamotrigine in optimized formulation as a function of temperature is shown in fig.(3). The solubility of Lamotrigine in water at 25 °C is 0.17 mg/ml.²² The solubility of Lamotrigine in Pluronic solution significantly increases with an increase in temperature. In general, with an increase in temperature, the dehydration of the PEO shell occurs and PPO chains become more hydrophobic, the drug might be expected to be solubilized in micellar core which is confirmed by UV spectroscopy due to the micellar environment is different than that of water.

Thermodynamic of solubilisation

Solubilization properties of the polymeric micelles are usually expressed in terms of micelle-water partition coefficient. Thermodynamic parameters are particularly useful in understanding the solubilization of hydrophobic species like Lamotrigine in block copolymer. The ratio of the drug concentration in the micelle to that in water for a particular copolymer concentration, as shown below;

$$P = \frac{S_{tot} - S_w}{S_w} \quad (1)$$

Where S_{tot} and S_w are concentrations of drugs in the micelles and in the water, respectively. The colloidal surfactants usually do not change the chemical poten-

tial of the solute, therefore the solubility of Lamotrigine in the extracellular phase was assumed to be equal to water (0.0533 mg/ml). Higher the partition coefficient the better hydrophobic microenvironment formed by the combination block co-polymers. The standard Gibbs energy of solubilization (ΔG_s^0) represents the energy change for the transfer of one mole of lamotrigine drug from a hydrophilic environment to the hydrophobic environment is given by

$$\Delta G_s^0 = -RT \ln P \quad (2)$$

Where R is the gas constant, P is the partition coefficient of the drug between the micelle and aqueous phase and solubilization can be considered as normal partitioning of the drug between the micelle and bulk phases, T is the temperature on the Kelvin scale. The standard Enthalpy and entropy of solubilization process were determined by equations 3 and 4 respectively, as shown below:

$$\Delta H_s^0 = -R \left[\frac{d \ln P}{d(1/T)} \right] \quad (3)$$

$$\Delta S_s^0 = \frac{\Delta H_s^0 - \Delta G_s^0}{T} \quad (4)$$

The thermodynamic parameters and micelle-water partition coefficient (P) for solubilization of Lamotrigine in block copolymer solutions as a function of concentration are shown in (Table 6). The values of Gibbs energy of solubilization (ΔG_s^0) are negative, indicating that more and more water molecules leach out from the micelle core and generating a more hydrophobic environment and with increasing block copolymer and the temperature the drug molecule spontaneously partitions into the micelles. The positive value of ΔS_s^0 might be due to liberation of water molecules surrounding the drug when it is partitioned from the aqueous phase to micelles. It also indicates that partitioning of the drug is enthalpically disfavoured process. Thus the solubilization was found to be endothermic in nature.

Table 6: Apparent partition coefficient and thermodynamic parameters for Lamotrigine by binary mixture of Pluronic L81, F68

Temperature, k	Partition coefficient	ΔG_s^0 , KJ mol ⁻¹	ΔH_s^0 , KJ mol ⁻¹	ΔS_s^0 , J mol ⁻¹ k ⁻¹
303	2.698529	-2.50091	66.64921	0.228218
308	3.735294	-3.37477		0.227351
318	4.883824	-4.1932		0.222775

Table 7: Micelles size, zeta potential and polydispersity index of polymeric micelles

Formulation	Micelles size (nm)	Zeta potential (mv)	Polydispersity index
F68(1:1)	221.5	-25.34	0.614
L81(1:5)	187.9	-7.35	0.557
Combination1:10	384.7	-4.37	0.491
Effect of salt0.46%	246.5	-19.45	0.576
Effect of salt0.9%	346.5	-18.87	0.576
Effect of temp25	> 1 μ m	-7.34	0.531
Effect of temp 45	439.4	-23.24	0.289
Effect of pH2	>1 μ m	-21.34	0.661
Effect of pH6	1082	-13.34	0.465
Effect of pH10	>1 μ m	-34.14	0.542

Effect of pH

The percent entrapment of Lamotrigine increases with the decrease in pH from 10 to 2 for mixed pluronic micelles. At lower pH the entrapment of Lamotrigine is significantly increased from 24.9% at pH 10 to 34.42% at pH 2. This can be explained by the fact that Lamotrigine is present as an ionized molecule at lower pH.

Effect of salt

The percent of the solubilized drug Lamotrigine was decreased in the presence of salt. On the addition of salt the Pluronics become more hydrophobic in nature i.e it contains fewer EO groups than PO groups. Since, the PPO core loses most of the associated water molecules. The PEO core would shrink due to dehydration and fall on the PPO core and it creating problem for the drug to incorporate in to the hydrophobic core of the aggregates.²³ Hence, we would expect that the partitioning of the drug inside the micelles would not be favoured, and thus, noticeable solubility of Lamotrigine is not observed.

Every proton containing chemical has a unique set of proton NMR signal. These signals may be affected by (eg.temperature, pH,hydrophobicity etc) nature of environment surrounding them transfer of protons from a polar region to a nonpolar region would be characterized by low frequency shift.

Effect of different concentration on the chemical shifts (δ) of the PO-CH₃ and EO-CH₂- signals are presented in fig.5. Addition of salt in different concentration extract water molecule from Pluronic core so at room temperature these protons become dehydrated and exhibit low frequency shifts in the peak, a remarkable decrease in chemical shifts of PO segments display that is greater the addition of salt, the larger the low frequency chemical shift of the block copolymer, which is similar to the behavior observed for the change in EO segments.

Micelles size and polydispersity index

(Table 7) shows the hydrodynamic size of plain micelles of Pluronic F68, Pluronic L81 and their mixed micelles and also depicts the effect of salt, pH and temperature on hydrodynamic size of optimized mixed micelles system, the size increases on addition of salt, the solubilization capacity was found to decrease. The micelles size as well as polydispersity index decreases on increasing temperature. Whereas with increases or decreases in pH the micelles size as well as polydispersity index increases.

Zeta potential

Zeta potential of lamotrigine-loaded micelles was measured as in (Table 7). It was shown that formulations have negative surface charges. Zeta potential of Pluronics and their combination micelles was within the ranges from -4.37 to -34.14 MV. The absolute value of zeta potential decreases. The zeta potential of mixed micelles showed that they are more stable against aggregation than pure Pluronic f68 and Pluronic L81 micelles.

Drug release

In vitro drug release experiments were carried out on Pluronic L81 and Pluronic F68 micelles and their mixed polymeric micelles. The release profiles of Lamotrigine from pure and mixed Pluronic micelles were evaluated at pH 8 and 6 over 6 hours by a simple diffusion method & were shown in (Figure.3&4). The percentages of Lamotrigine released from pure Pluronic F68 and Pluronic L81 Lamotrigine micelles after 6 hours at pH 6 and 8 were 92.9%, 65.75% and 80.03%, 61% and their combination were 59.36%, 36.47%. The release data showed that Lamotrigine release is statistically significantly higher at pH 6 than pH 8 (Figure.5 and 6). The Lamotrigine release behavior indicated the Lamotrigine incorporation stability and could be explained through the Lamotrigine location within the micelles. In case of plain Pluronic micelles Lamotrigine release rate more quick than that of mixed micelles. Hence the release

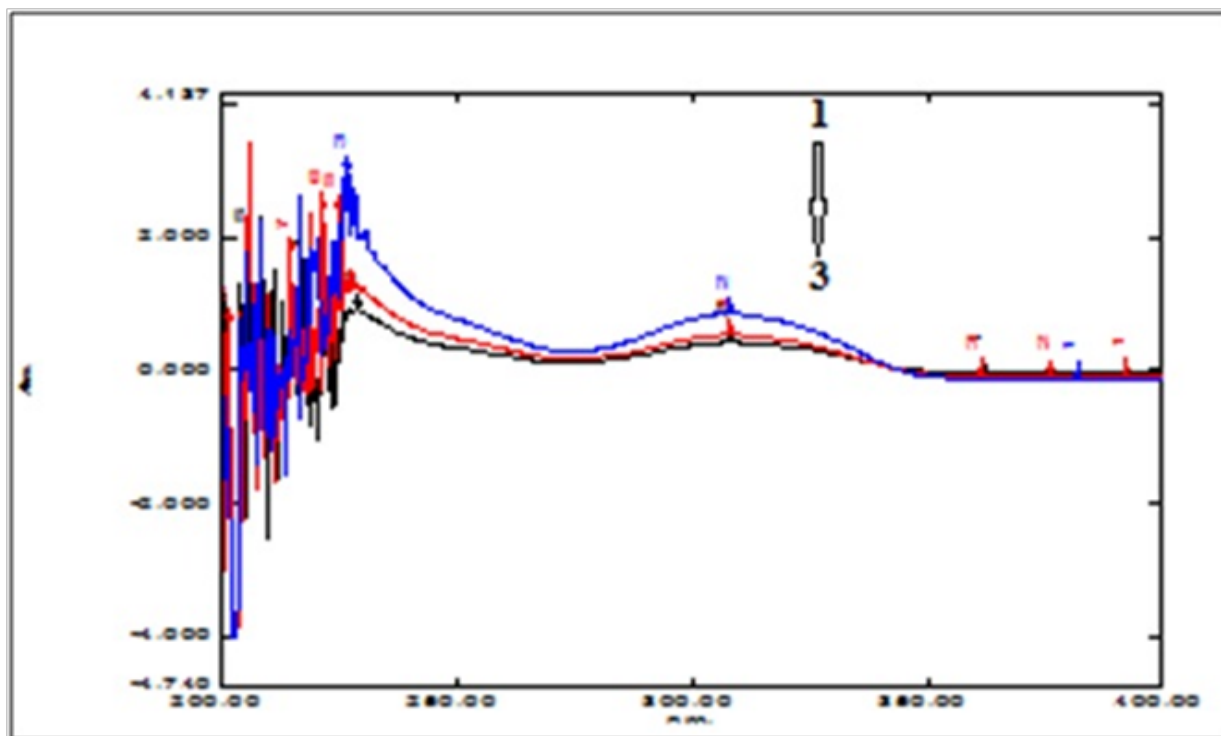


Figure 3: UV vis spectra of solubilized drug Lamotrigine (λ_{max} =306 nm) in mixed micelles at different temperature :1) 45 ° 2) 35 ° 3) 25 °C

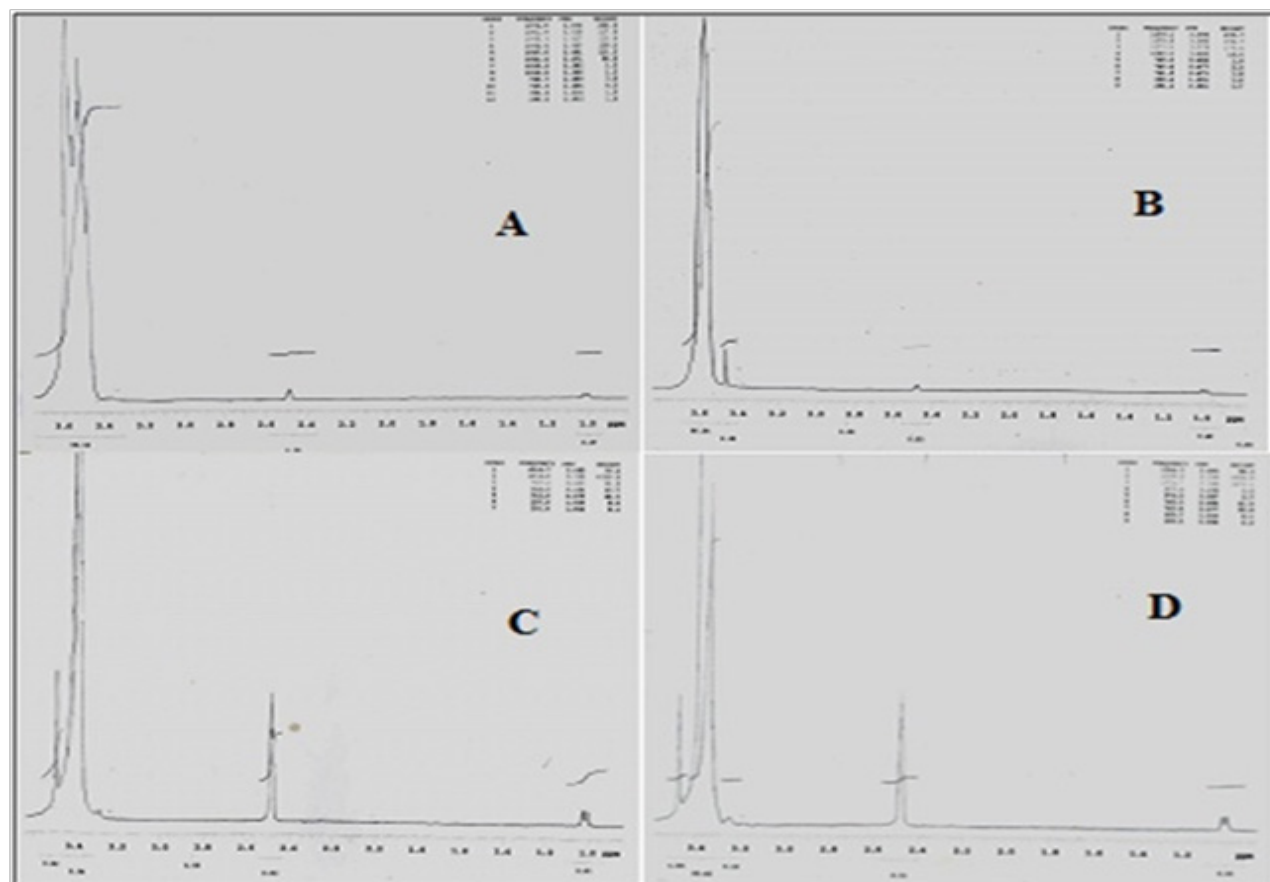


Figure 4: 1H NMR spectra of mixed pluronic micelles in DMSO solution showing A) 0%NaCl B)0.46% C)0.9% D)1.46%

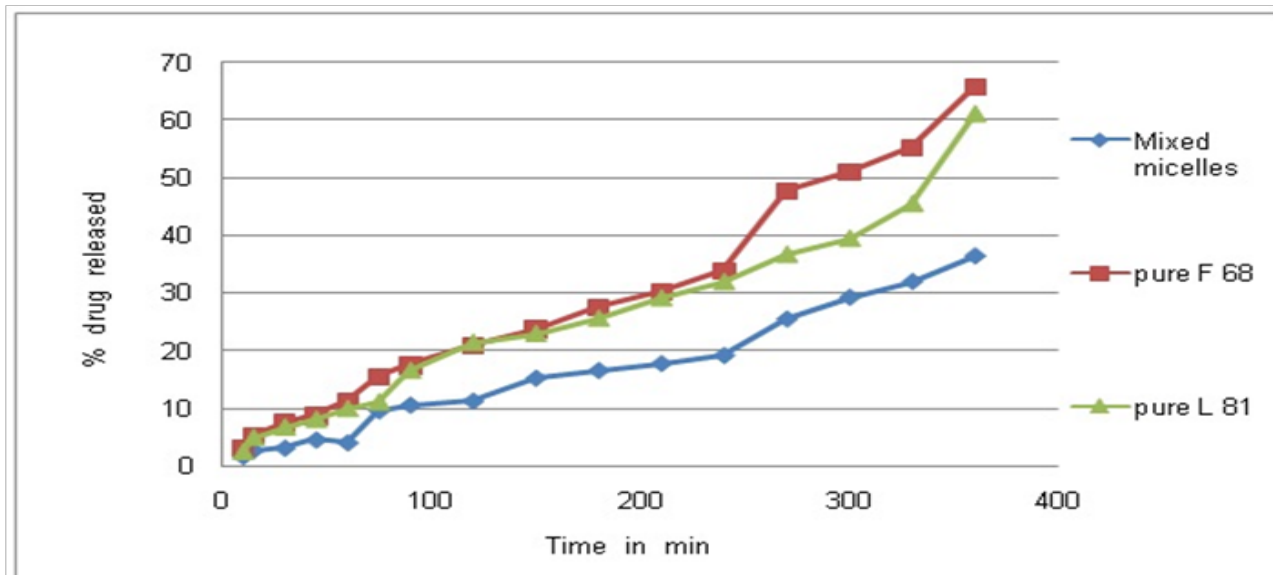


Figure 5: Comparison of drug release profiles of mono Pluronic F 68,Pluronic L81 micelles and their mixed micelles in pH 8 Buffer

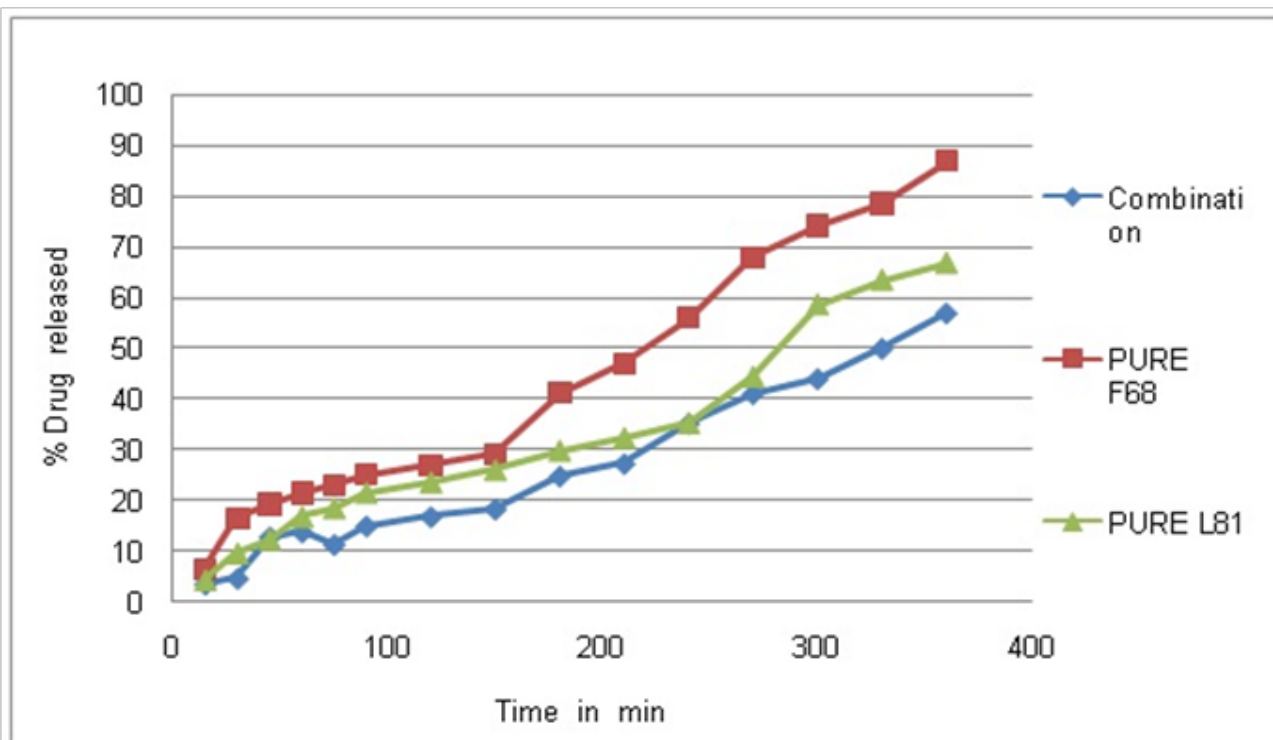


Figure 6: Comparison of drug release profiles of mono Pluronic F 68,Pluronic L81 micelles and their mixed micelles in pH 6 Buffer

behavior of Lamotrigine indicated the stability of Lamotrigine incorporation into the mixed micelles. The differences in the drug release rate reflect the advantages offered by the developed mixed micellar system over mono micellar system. A slow release even under the in vitro sink condition shows the Lamotrigine incorporated into the inner hydrophobic compartment stayed securely by the micelles.

Micelle storage stability

Lamotrigine-loaded plain and mixed micelles prepared from two different polymer types with the highest loading efficiency were used in stability study. Micellar structure is strongly depend on temperature. The temperature at which micelles are formed is known as critical micellization temperature. The micelles were lose their intact structure and drug would precipitate, if micelles were kept in refrigerator, temperature would fall lower than critical micellization temperature.²⁴ For this rea-

son, stability test was performed at 25 °. In the aqueous form of Lamotrigine-loaded pure Pluronic F68 & L81, 7.1%, 5.625% and for mixed micelles 3.2% decrease in Lamotrigine content was observed which indicate that stability of mixed micelles were more stable than that of plain micelles. This may be due to degradation of Pluronic micelle structure. Drug content of lyophilized form of mixed Pluronic micelles did not change showing high storage stability than that of aqueous form.

CONCLUSION

In this study plain and mixed Pluronic micelles were prepared. The high drug loading and entrapment efficiency developed by mixed micelles promise a high solubilization potential of the binary system was higher than mono system of Pluronic F68 and Pluronic L81 for hydrophobic drugs. Solubility of an anticonvulsant drug Lamotrigine in the mixed Pluronic micelle investigated under the influence of different stimuli; such as changes in pH or temperature and with varying salt/copolymer concentration. The solubility of the Lamotrigine could be significantly enhanced by increasing copolymer concentration, temperature or with lowering pH. The solubilization of Pluronic® micelle is not favored in the presence of NaCl. The sustained release behavior of drug from the mixed micellar system than plain micellar system since expects its role in controlled drug delivery.

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