Summary and Conclusions

Due to versatile features, research on block copolymers (BCs) has been a popular demand in many fields, such as biomedicine, biomaterials, microelectronics, photoelectric materials, catalysts, etc. BCs are a fascinating class of polymers that consist of two or more covalently joined blocks forming a variety of structures like linear, star block, miktoarm, etc. Self-assembly of BCs has attracted much attention for many decades because it can yield ordered structures in bulk and in solutions. The yielded various morphologies include spheres, cylinders, bicontinuous structures, lamellae, vesicles, and many other complexes or hierarchical assemblies. Compared to low molecular weight surfactant micelles, BC micelles exhibit higher stability and durability due to their physical and mechanical properties.

Majority of researchers in the area of medical sciences have been tried for new treatments using the self-assemblies of BCs with different-different morphologies that are safer, faster, less invasive, show high % efficacy using lower doses and effective therapeutic agents to a target cell. In this context, the focus of present thesis work is on triblock copolymers composed of a middle hydrophobic polypropylene oxide (PPO) block (at above 20°C) that connects to hydrophilic polyethylene oxide (PEO) side blocks, structurally PEO_n-PPO_m-PEO_n, are known as Poloxamers in other tradename as Pluronic® (BASF). Pluronic polymers have been the subject of interest as its micelles considered as potent drug nanocarriers because of their benefits like small particle size(< 100 nm), good solubilization capacity and high colloidal stability. The Pluronics used in the present research work are listed in Table.8.1.

Table .8.1: Pluronics used in present research work.

Pluronic	Poloxamer	Mol.Wt. (g mol^{-1})	Composition	%PEO	HLB
F127	P407	12600	$EO_{100}PO_{65}EO_{100}$	70	22
F88	P238	11400	$EO_{104}PO_{48}EO_{104}$	80	28
F68	P188	8400	$EO_{76}PO_{29}EO_{76}$	80	>24
P123	P403	5750	$EO_{20}PO_{69}EO_{20}$	30	8
L121	P401	4400	$EO_5PO_{68.2}EO_5$	10	1
L64	P184	2900	$EO_{13}PO_{30}EO_{13}$	40	12-18
17R4	M174	2650	$EO_{24}PO_{14}EO_{24}$	40	7-12
L35	P105	1900	$EO_{11}PO_{16}EO_{11}$	50	19

This thesis is mainly divided into eight chapters covering the study on various self-assemblies/micelles of single and mixed Pluronic polymers and its applications in drug solubilization and delivery at target. The micellar behavior of various Pluronic polymers and their mixtures in aqueous media and their applications as nanocarriers for solubilization of some model hydrophobic drugs like curcumin, lamotrigine and quercetin have been examined. Several drug-loaded Pluronic micelles were synthesized using direct dissolution and thin-film hydration methods. These micelles were well characterized through modern techniques like DLS, SANS, TEM, DSC, TGA, XRD, UV-VIS spectroscopy. The structural parameters of these drug-loaded Pluronic micelles and its interaction with respective drug molecules have been properly addressed. The in-vitro release, storage stability, and the antioxidant studies of drug-loaded Pluronic micelle have been explored for its better pharma applications.

In order to meet all objectives, the contents of chapters are summarized as follows.

Chapter-1: Introduction

Chapter-1 covers the general introduction of block copolymers and its self-assemblies in bulk and solutions. The complete overview and importance of PEO-PPO-PEO triblock copolymers (Pluronics) and its micelles has been provided. The spherical, rod-like or lamellar micelles of Pluronic depending upon the length of PPO and PEO chains, concentration, the temperature has been discussed with appropriate theories. In the chapter, various preparation methods and characterization techniques of drug-loaded Pluronic micelles have been furnished. The applications of Pluronic micelles as nanovehicles in drug solubilization and delivery at target are systematically elaborated. The recent scenario of the use of Pluronic micelles in cancer treatment has been provided for better understanding and importance of the research on Pluronic polymers. The most important and relevant research articles related to self-assemblies of BCs and specifically for Pluronic micelles in drug delivery applications are reported in this chapter.

Chapter-2: Self-assembly of PEO-PPO-PEO triblock copolymeric system for Curcumin drug

In this chapter-2, micelles of Pluronic F127($EO_{100}PO_{65}EO_{100,}$ Mw.=12600 $g.mol^{-1}$) have been studied for the drug, Curcumin - the potent anticancer agents.

Curcumin (called *Indian solid gold*) was found to be very effective against many different types of cancer cells. The clinical applications of curcumin in the treatment of

cancer and other diseases have been limited due to its poor aqueous solubility, stability, and bioavailability. The solubility of curcumin drug in various concentrations of Pluronic F127 in aqueous media was determined by UV-Visible spectroscopy. Results of solubility motivated for the use of Pluronic F127 micelles for the curcumin drug. The curcumin-loaded Pluronic F127 micelles (PMsCur) has been prepared using the thin-film hydration technique. The characterization of prepared PMsCur was carried out using DLS, SANS, UV-VIS, FTIR, PXRD and DSC measurements. The incorporation efficiency, drug loading and solubility of the curcumin in optimized PMsCur were 48.07%, 41.511%, and 0.02117 mg/mL, respectively. All the measurements of characterization of PMsCur have confirmed the encapsulation of curcumin drug into Pluronic F127 micelles and interaction between them. DLS results showed that PMsCur has a particle size (D_h) of 26 nm with low PDI=0.195. SANS analysis reveals an increase in spherical micellar core radius of Pluronic F127 from 55.1 Å to 58.2 Å, while curcumin was encapsulated.

The in-vitro release study shows the slow and sustained release of curcumin at physiological pH. Stability study also showed the PMsCur is stable upto three months and better drug compatibility.

This study suggests that Pluronic F127 micelles improves the bioavailability of curcumin and serve as a promising delivery system to target cancer cells. *This work is the part of paper published in Cancer Reports(Wiley), 2(1), 2018, e1133.*

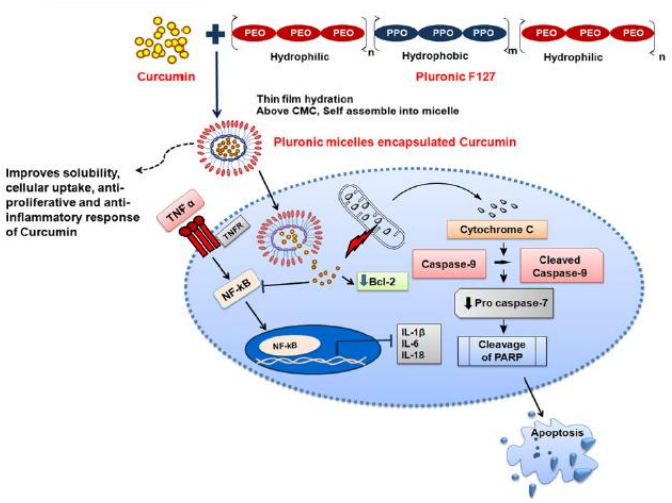

Fig.8.1: Schematic representation of Pluronic F127 micelles for solubilization and delivery of curcumin drug (Adapted from Cancer Reports, 2(1), 2018, e1133)

Chapter-3: Self-assembly of PEO-PPO-PEO triblock copolymeric system for Lamotrigine drug: effect of hydrophiles

The aim of this chapter-2 was to design micelles composed of PEO–PPO–PEO triblock copolymer, Pluronic F127 for the incorporation of a poorly soluble anticonvulsant drug- Lamotrigine.

LTG is a known oral antiepileptic drug (AED) and applied for the treatment of partial seizures and generalized seizures, either alone or in combination with other anticonvulsants in pediatrics and adults. This BCS class II drug having low aqueous solubility (approximately 0.17 mg/mL at RT) which limits its medicinal applications. The solubility of LTG in the aqueous micellar solutions of Pluronic F127 was investigated using UV-visible spectroscopy. The solubility of LTG increases markedly with increase in the concentration of Pluronic F127. Pluronic F127 micelles are able to solubilize a good amount of LTG because of the increased number of micelles at higher concentration and the interaction between LTG and Pluronic F127 molecules. The amount of LTG incorporated, with micellar sizes of less than 20 nm, spherical shapes, and thermodynamic stability were investigated using modern techniques like the UV–visible, DLS and SANS and proved that Pluronic F127 is a good nanocarrier for the incorporation of the LTG drug. The effect of hydrophilic polymers (PEG1500 and F68) on the LTG-incorporated Pluronic F127 micelles was also studied and found inefficient for enhancement of the solubility of LTG. The apparent micelle-water partition coefficient (P) and standard free energy change ($\Delta G°$) of solubilization of LTG in Pluronic F127 micellar solutions were also indicated the spontaneous process.

The powder forms of LTG-incorporated Pluronic F127 micelles with and without hydrophilic polymers were successfully prepared using thin-film hydration method and designated as LPMs. The FTIR, XRD, and TGA analysis have been ensured the compatibility of the LTG drug with Pluronic F127 micelles in prepared LPMs. Rapid release of almost 70% in the first stage followed by sustained and slow release of more than 85% over prolonged time of 60 h was found from in-vitro release studies of LPMs. No significant loss in drug retention within 3 months for LPMs were observed in stability studies.

The present chapter study concludes that Pluronic F127 micelles serve as potent nanocarriers for the formulation of LTG drug. *This work has been published in the Journal of Surfactants Detergents (Springer), 20(3), 2017, 695-706.*

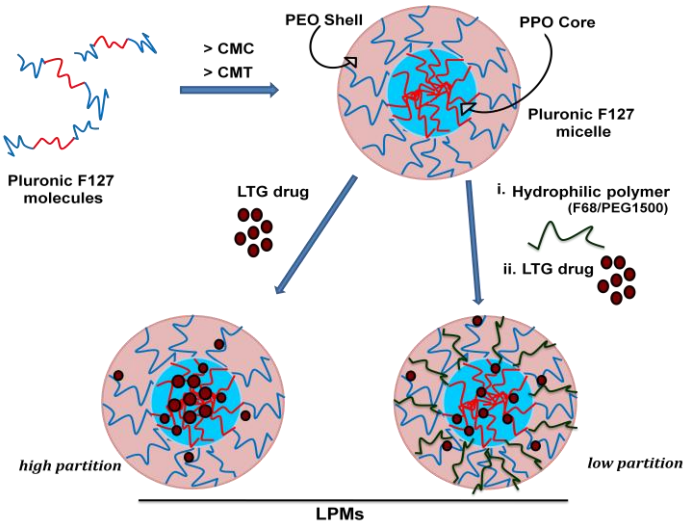

Fig.8.2: Schematic representation of Pluronic F127 micelles with and without hydrophiles for LTG drug (Adapted from JSD)

Chapter-4: Self-assemblies of PEO-PPO-PEO triblock copolymeric systems for hydrophobic drugs of different polarity

In this chapter-4, various Pluronic micelles have been examined for three hydrophobic drugs of different polarity such as Curcumin - anticancer agents as CUR, Quercetin- antioxidant agents as QCN and lamotrigine- antiepileptic agents as LTG.

In the aqueous medium, the LTG drug is considered as hydrophilic while CUR and QCN are hydrophobic in nature. The solubilization and solubilization capacities of these three drugs in the various aqueous solutions of Pluronic polymers of different molecular architecture have been investigated using UV-Visible spectroscopy. The Pluronic solutions (micellar or unimeric form) show enhanced solubility of all the studied drugs. More hydrophobic nature of drug dissolved more in the Pluronic solutions. The QCN solubilized by 1000-fold more than the LTG in an aqueous medium by using Pluronic micelles. Pluronics having high cmt values with more than 30°C (F88, F68, L35, and 17R4) clearly dissolve the fewer amounts of drugs in comparison to low cmt (< 30°C) contained Pluronic (L121, P123, F127, and L64). According to the evaluation of solubility performance, the Pluronic L121 and Pluronic P123 are the best choices for all the drugs. But the aqueous solutions of Pluronic L121 are very temperature sensitive and its cmt and CPT values are nearby 15°C. Therefore, PLuronic P123 was chosen for the further investigations. Using

Pluronic P123, three drug-loaded Pluronic P123 micelles (designated as PLC for CUR, PLQ for QCN, and PLL for LTG) were prepared using direct dissolution method and fully characterized using UV-VIS, DLS, SANS, CPT and TEM measurements. The sizes (as D_h) of the PLC, PLQ, and PLL were 21.3 nm, 22.5 nm, and 18.6 nm, respectively. The TEM and SANS measurements clearly show that all the drug-loaded Pluronic P123 micelles were spherical in shape. In the in-vitro release study, CUR and QCN showed the slow release following zero-ordered rate respectively while LTG showed fast release following the Higuchi model. The PLC and PLQ were also assessed for their antioxidant potential by DPPH assay method as CUR and QCN are well-known anti-oxidants. Both the drug-loaded Pluronics P123 micelles were confirmed the resistance to oxidation more significantly effective than the free drug.

Overall, this study concludes that Pluronic P123 micelles use as an efficient nanovehicles for the formulation of CUR, QCN, and LTG drugs as it possesses characteristics like smaller particle size, higher drug loading capacity, sustained drug release, and good anti-oxidant activity. *This work is communicated in Colloids and Surfaces A : Physiochemical Engineering Aspects (Elsevier).*

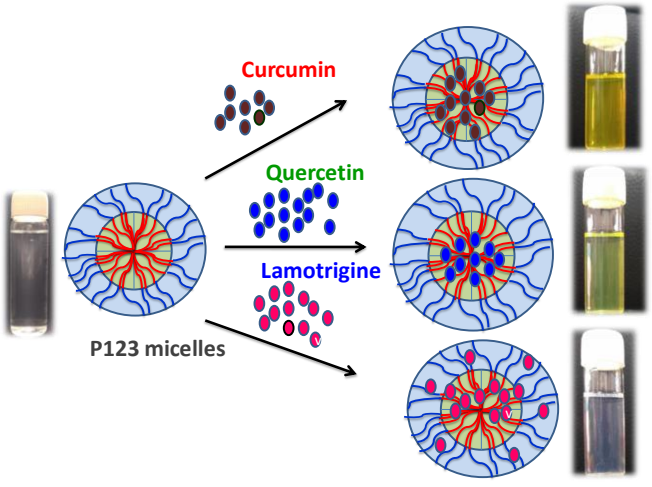

Fig.8.3: Schematic representation of Pluronic P123 micelles for CUR, QCN and LTG drugs

Chapter-5: Self-assemblies of mixed PEO-PPO-PEO triblock copolymeric systems for Lamotrigine drugs

Mixed Pluronic micellar systems may compensate for many disadvantages of a single Pluronic micellar system and control the physicochemical properties for better drug solubilization and delivery. Mixed micelles composed of Pluronic polymers show synergistic properties to those composed of the single Pluronic such as increased micelle stability and more effective solubilization capacity.

In this context, we have systematically reported the binary mixed micellar systems (Pluronic F127/P123 and Pluronic F127/L35) as nanosized drug carriers for lamotrigine(LTG) drug in the Chapter-5. First, the cmc of single Pluronic polymers and their binary mixtures (F127/P123 and F127/L35) in water was determined through UV-Visible spectroscopy using pyrene as a probe. All the binary Pluronic systems form stable mixed micelles with low cmc in water. The cmcs were located between cmc of the individual Pluronic in F127/P123 mixture and found increased in the F127/L35 mixtures. The low CMC values of both these binary systems at 30°C bring to these mixtures as promising pharma excipients as it remains stable even upon dilution in the blood.

The characterization of mixed micelles has been performed using UV-Visible spectroscopy, DLS and SANS measurements. The DLS and SANS measurements have been confirmed the spherical micelles with less than 30-nm diameter in size for both the mixed systems. Not much but mixed micelle became slightly larger after solubilizing of LTG drug. The solubilization capacity (SCP) of the binary systems was monitored using UV-Visible spectroscopy. The mixed F127/P123 micellar system shows relatively high solubilization capacity as more hydrophobicity and the mixed F127/L35 system had low solubilization capacity because of hydrophile nature of L35. Results of the micellar water partition coefficient (P) and Gibbs free energy changes ($\Delta G°$) of solubilized LTG drug were also confirmed the spontaneous process in the mixed micellar systems. Results clearly indicate that the increasing the hydrophobic character in the mixed micelles decreases $\Delta G°$, which favors its spontaneous solubilization, since increases the drug/unimer ratio, i.e., the higher number of drug molecules can be accommodated into micelles i.e. F127/P123 mixtures. The Pluronic L35 was not found efficient in comparison to Pluronic P123 in combination with Pluronic F127 micelles with low P and high $\Delta G°$ values.

Finally, this work of chapter-5 was evaluated that the F127/P123 mixed micellar systems are better than mixed F127/L35 systems as nanosized carriers for encapsulation of LTG drug for delivery in CNS. *This work is published in the Journal of Polymer Research (Springer) 25(3), 2018 ,73(1-10).*

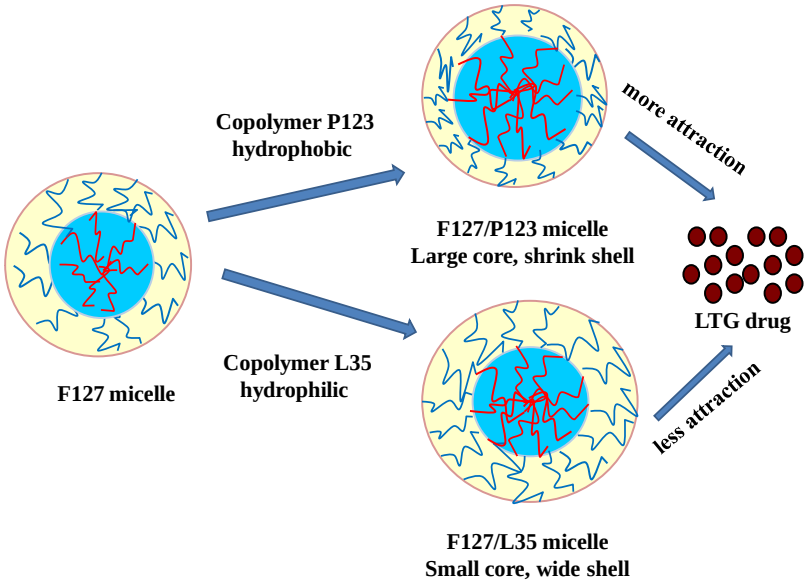

Fig.8.4: Schematic representation of Mixed Pluronic micelles for LTG drug

Chapter-6: Self-assemblies of mixed PEO-PPO-PEO triblock copolymeric systems for lamotrigine drugs at body temperature

With the understanding in Chapter-5, we motivated to investigate further the mixed Pluronic F127/P123 and mixed Pluronic F127/L35 micellar systems for encapsulation of LTG drug at body temperature.

In the present investigation in Chapter-6, we systematically performed the solubilization study of LTG drug in various Pluronic polymers solutions using UV-Visible spectroscopy. High solubilization of LTG in Pluronic micelles with low cmts is observed. We have successfully examined the mixed Pluronic systems (F127:P123 and F127:L35) as possible micellar nanocarriers for LTG drug at body temperature through the UV-Visible spectroscopy, DLS, SANS, TEM, in-vitro release and storage stability analysis. The solubility of LTG drug shows relatively high in mixed F127:P123 micellar systems as higher hydrophobicity and had low in mixed F127:L35 systems because of the hydrophilic nature of

L35. Thermodynamic parameters, P and ΔG° of solubilized LTG are also proved that the mixed F127:P123 micellar system shown better partition and spontaneity in comparison to mixed F127:L35 systems.

Results of DLS and SANS measurements confirmed the spherical assembly with average micelle sizes of < 25 nm of both the mixed Pluronic micellar systems with and without LTG drug. The clear spherical shape micelles was shown in TEM images. In vitro drug release profile of both the mixed Pluronic micellar systems demonstrated an initial burst release for about 4 h followed by a slower and sustained release for more than 80 hrs and results that release of LTG is driven by a Korsmeyer-Peppas process. The stability studies are confirmed the better biocompatibility of LTG drug in the mixed F127:P123 micellar systems.

The present work clearly proved that mixed F127: P123 micellar system could be efficiently nanosystems for better bioavailability of LTG drug. *This work is communicated in the Asian Journal of Pharmaceutical Sciences.*

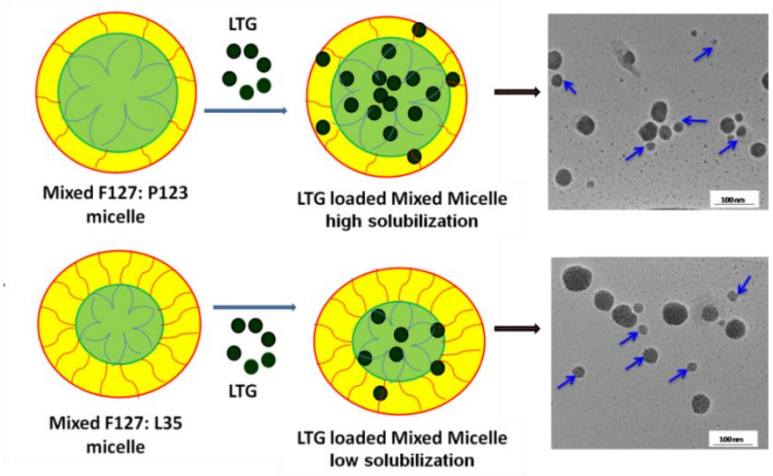

Fig.8.5: Schematic representation of Mixed Pluronic micelles for LTG drug at body temperature.

Chapter-7: Self-assemblies of mixed PEO-PPO-PEO triblock copolymer-Phosphatidylcholine(PC) systems for Curcumin drugs

In view of the advantages of mixed micelles of Pluronic F127/P123 for pharmaceutical applications, it is critically important to investigate these mixed Pluronic micelles with biocompatible useful compound like lecithin (L-α- phosphatidylcholine) for better medicinal applications.

Chapter-7 covers the aggregation behaviour of mixed Pluronic F127/P123 with phosphatidylcholine(PC) in aqueous media. Solubilization of curcumin drug into these mixed PC-Pluronic solutions has been provided for its possible pharma applications.

The aggregation behaviour of phosphatidylcholine(PC) is significantly modified with mixed micelles of Pluronic F127/P123 that has a strong impact on morphology. These mixed PC-Pluronic solutions have been characterized through DLS, SANS and TEM measurements. The vesicle of 1% PC is shifted to spherical micelles in the presence of mixed micelles of Pluronic F127/P123 of 2.5 wt% of each polymer in the system.

The addition of Pluronic might have interfered with PC molecules causing perturbations in the surface of the lipid bilayer, which is showed in the changes of the order and relaxation parameters of the bilayer. It decreases the bending rigidity and inducing a transition from unilamellar vesicle to the quite small mixed vesicle phase. At high Pluronics concentration might be due to the presence of mixed micelles, the spherical micelles were shown rather than vesicles morphology. The solubility of curcumin drug in micellar solutions of PCF, PCP, and PCPF at 30°C has been found using UV-Visible spectroscopy. About 28% of the curcumin was released from mixed micelles of PC-Pluronic F127/P123 solutions at pH 7.4 after 100 hrs has been observed in in-vitro release study. It indicates the slow release of curcumin which one favorable at the tumor site. The pure curcumin showed 30% inhibition upto 3.0 µg/mL, while mixed PFPC micelles inhibited almost 85% at the same concentration. It was clearly observed that mixed PFPC micelles showed higher antioxidant activity than the pure curcumin drug.

This study concludes that the mixed Pluronic micelles with PC could be a promising approach to improve oral and parenteral delivery of curcumin. *This work is in under preparation for possible publication in the journal of repute.*

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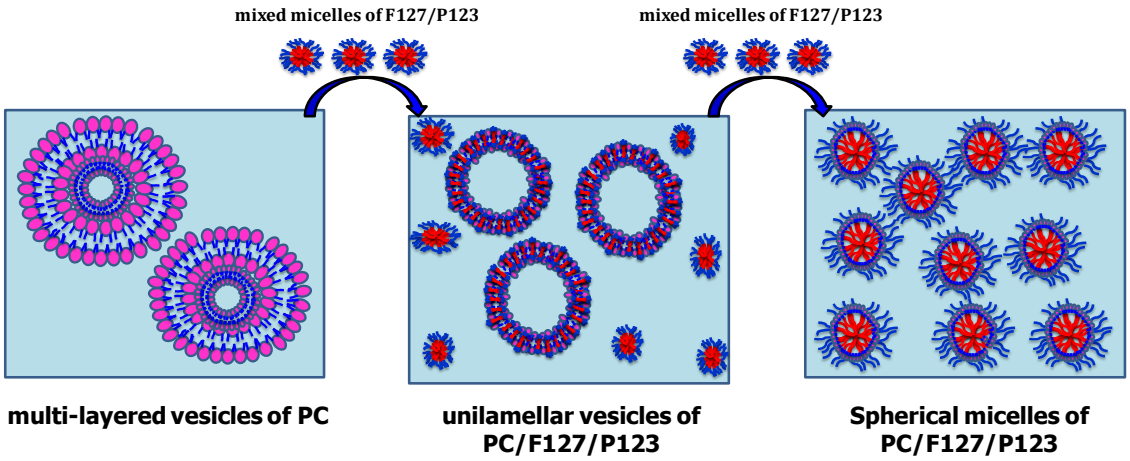

Fig.8.6: Schematic representation of Mixed Pluronic-PC assemblies at increasing concentration of Pluronics

In brief, this thesis presents the studies on Pluronic micelles and their applications for solubilization of hydrophobic drugs such as curcumin, quercetin, and lamotrigine for better pharma outcomes. The design, synthesis, and characterization of single and mixed Pluronic micelles have been highlighted as nanovehicles for drug delivery applications. The work also explored the uses of various advanced techniques, such as UV-Visible, DLS, SANS, TEM, DSC, TGA, FTIR, and XRD to confirm the structural insights of the drug-loaded Pluronic micelles. The biological significance of the Pluronic micelles has been provided with the in-vitro release, stability, and anti-oxidant studies.

Overall, this work concludes that self-assemblies of block copolymers and specifically Pluronic block copolymers play a vital role in the solubilization and delivery of drugs in pharma applications. With the versatility in the flexible structure of Pluronic polymers, there are lots of possibilities to investigate the appropriate micellar assemblies for numerous drugs for better and safer medicinal applications.