Synopsis

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Self-assembly of Block Copolymeric Systems: Design and Drug Delivery Perspectives

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Self-assembly of Block Copolymeric Systems: Design and Drug Delivery Perspectives

Block copolymers (BCs) have been the focus of much interest during the last 30 years because their constituent blocks are generally immiscible, leading to a microphase separation. Since the different blocks are linked together, the microphase separation is normally limited and results in self-assembled structures whose characteristic sizes are of 10 to 100 nm. Self-assembly of BCs has attracted considerable attention for many decades because it can yield ordered structures in a wide range of morphologies, including spheres, cylinders, bicontinuous structures, lamellae, vesicles, and many other complex or hierarchical assemblies[1,2]. Fig.1 shows the number of publications with the topic "Block copolymer" and "Pluronic" over the past three decades.

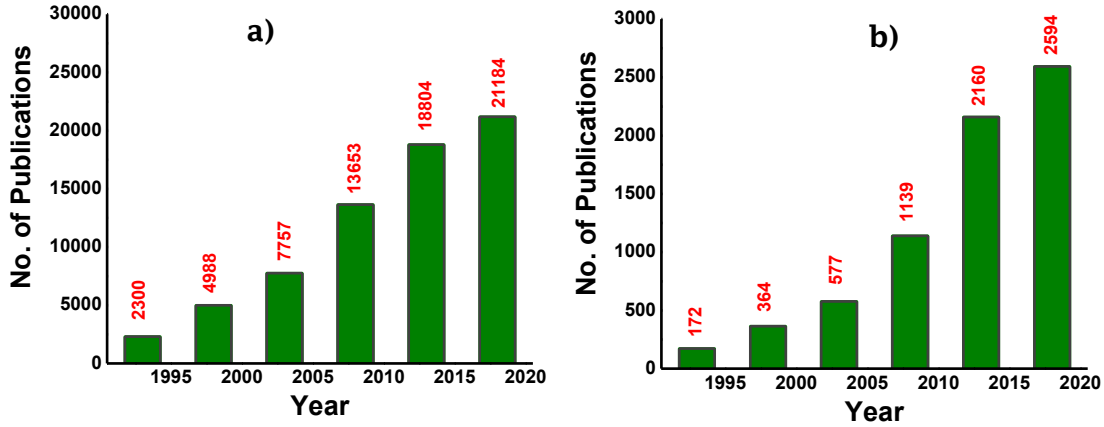

Fig.1 : The number of publications with a) Block copolymer and b) Pluronic as topic against year. The datawere obtained from Web of Science (2017 Clarivate Analytics).

The focus of present research 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, are known as Poloxamers in other tradename as Pluronic® block copolymers (BASF). Pluronic® polymers have been the subject of interest as its self-assemblies (micelles) considered as potent drug nanocarriers due to their benefits of high collloidal stability, small particle size and good solubilization capacity[3-7]. The Pluronics used in the present project work are listed in Table.1.

As our aim is to design and characterize the various self-assembly of single and mixed block copolymers for possible applications in drug solubilization and delivery at target, we have been studied the aggregation behavior of various PEO–PPO–PEO triblock copolymers (Pluronics®) and their mixtures in aqueous media and designed as nanocarriers for solubilization of some model hydrophobic drugs like curcumin, lamotrigine and quercetin.

Table.1 : General characteristics of the used Pluronics® in present work.

Pluronics®	M.W. (g mol^{-1})	Composition	% PEO	CP of 1% (°C)	HLB
F127	12600	$EO_{100}PO_{65}EO_{100}$	70	>100°	22
F88	11400	$EO_{104}PO_{48}EO_{104}$	80	>100°	28
F68	8400	$EO_{76}PO_{29}EO_{76}$	80	>100°	>24
P123	5750	$EO_{20}PO_{69}EO_{20}$	30	90°	8
L121	4400	$EO_5PO_{68.2}EO_5$	10	14°	1
L64	2900	$EO_{13}PO_{30}EO_{13}$	40	58°	12-18
17R4	2650	$EO_{24}PO_{14}EO_{24}$	40	46°	7-12
10R5	1950	$EO_{22}PO_8EO_{22}$	50	69°	12-18
L35	1900	$EO_{11}PO_{16}EO_{11}$	50	73°	19

Various modern techniques like small angle neutron scattering(SANS), dynamic light scattering(DLS), transmission electron microscopy (TEM) as well as conventional UV-VIS spectroscopy, cloud point(CP), tensiometry, and necessary biological analysis have been used to evaluate the self-assemblies of Pluronic® polymers for better drug delivery applications.

In order to meet all the objectives, the contents of thesis are summarized mainly into seven chapters.

The brief introduction regarding the self-assembly of block copolymers and PEO-PPO-PEO triblock copolymers (Pluronics®) has been provided in the **Chapter-1**. It covers the micellar and solubilization behaviour of Pluronic® block copolymers. The most important and relevant research articles to the work are reported systematically in this chapter.

Curcumin was found to be very effective against many different types of cancer cells. However, its clinical applications in cancer and other diseases have been limited due to poor aqueous solubility, stability, and bioavailability. The self-assembly of PEO-PPO-PEO triblock copolymer (Pluronic®F127) have been examined as nanocarriers for solubilization of curcumin and synstematically reported in **Chapter-2.** The curcumin-loaded Pluronic® micelles (PMsCur) have been prepared using thin film hydration method. The characterization PMsCur has carried out using UV-VIS, DLS, SANS, FTIR, PXRD and DSC measurements. All the techniques of characterization of PMsCur were proved the incorporation of curcumin into Pluronic®F127 micelles and interaction between them. The PMsCur has particle size (Dh) of ~ 24 nm with low PDI=0.195 and found spherical in shape. Result showed that PMsCur decreases cell viability and exhibits low toxicity response in a dose and time dependent manner in MCF-7 cells. The study of this chapter suggests that Pluronic®F127 micelles improves the bioavailability of curcumin and serve as promising delivery system to target cancer cells[8].

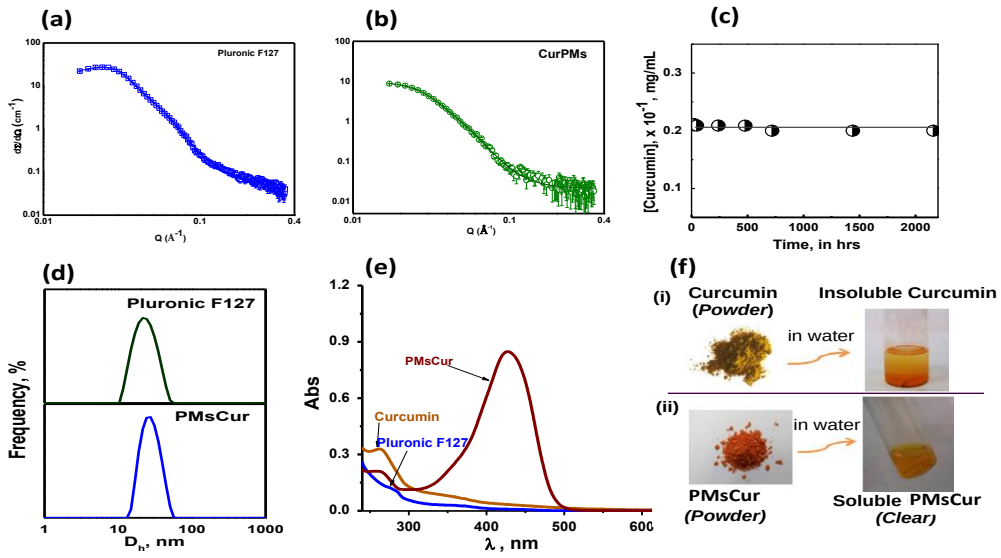

Fig.1 :(a) SANS curveof F127 in D_2O,(b)SANS curve ofPMsCur in D_2O, (c)Stability study of F127 micelles and PMsCur at RT, (d)Intensity weighted size distribution plot for empty F127 and PMsCur in water,(e) Absorbance spectrums of curcumin, F127 and PMsCur in water, and(f) Images of solubilization ofcurcumin in F127 micellar solutions.

Lamotrigine (LTG) is widely used in partial seizures as well as in treatment of generalized seizures, either alone or in combination with other anticonvulsants in pediatrics and adults. Micelles of Pluronic®F127 for solubilization and incorporation of LTG drug were investigated using UV-VIS, DLS, SANS, FTIR, TGA and PXRD measurements in **Chapter-3**. The LTG-incorporated Pluronic®F127 micelles (LTGPMs) showed fairly high entrapment efficiency, loading capacity and sustained release profile of drug. The effects of hydrophiles i.e. PEG1500 and Pluronic®F68 on aggregation behaviour these LTGPMs were also investigated. Results indicated the interaction of the F127 micelles with PEG and the formation of micelles clusters, but no such interaction occurs with F68. SANS and DLS were confirmed that the F127 micelles (Dh=19.3 nm) was spherical in absence and presence of hydrophiles. The study of this chapter indicated that LTGPMs improves the bioavailability of LTG drug and serve as promising delivery system, but there are no benefits of addition of polymer additives (PEG1500 & F68) in the formulations[9].

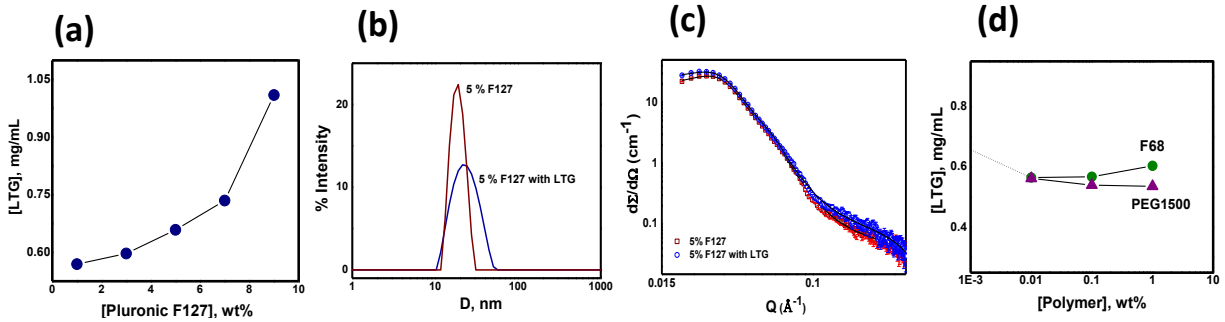

Fig.2 : (a)Solubility of LTG in F127 micelles (b) Intensity v/sDh plot for empty & LTGPMs (c) SANS profile for empty &LTGPMs at 37°C, and (d) Solubility of LTGin F127 micelles i.p.ohydrophiles.

As Pluronic polymer micellar approach could be considered as an efficient drug nanocarriers, the solubilization of three drugs (Lamotrigine(LTG), curcumin(CUR) and quercetin(QCN)) in the aqueous solutions of various Pluronic® polymers have been studied using UV-VIS spectroscopy in **Chapter-4**. The LTG shows enhanced solubility by Pluronics® that form micelles and have good water structure breakers unimers. The CUR and QCN drugs showed better solubilization in Pluronic® polymers which form micelles and have low CMTs. Among all, Pluronic®P123 found to be the best candidate for all the three drugs. Drug-encapsulated Pluronic®P123 micelles were fully characterized through DLS, SANS and TEM measurements. The drug-encapsulated Pluronic®P123 micelles are stable upto 15 days. The in-vitro release study of all the drugs from P123 micelles showed the slow and sustainable release. Formulation P123+LTG, P123+CUR, and P123+QCN has been assessed for their antioxidant potential by in vitro DPPH assay. Antimicrobial properties are also checked by using E.coli zone of inhibition method. The study of this chapter clarified the Pluronic®P123 micelles are good option for the bioavailibility of all these pharmaceutical active ingradients.

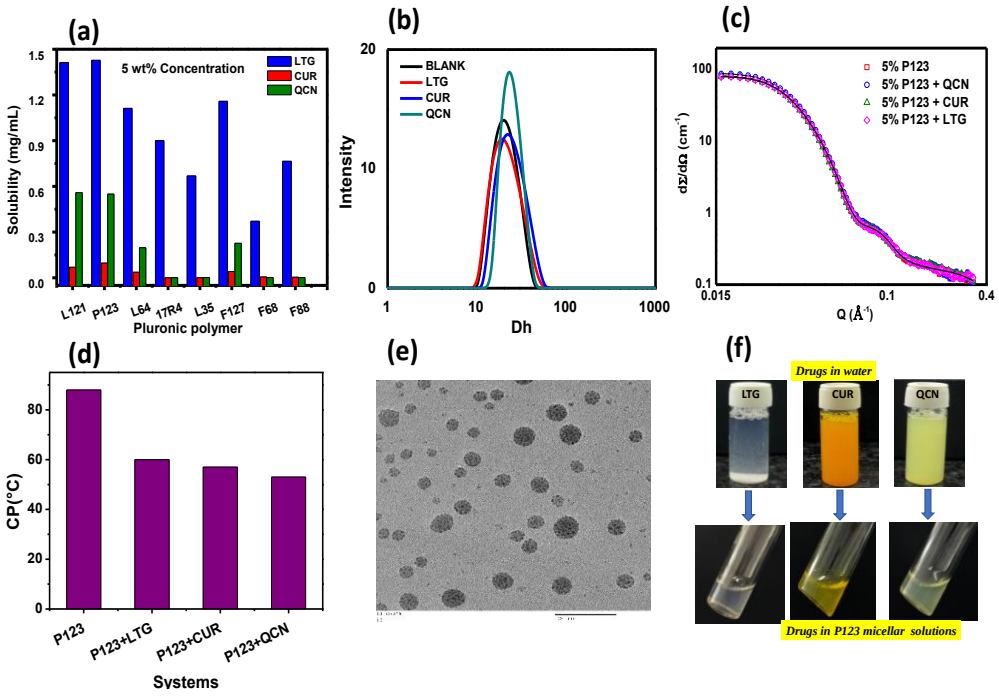

Fig.3 : (a) Solubilization of drugs in various Pluronic polymers, (b) Intesityweightsized distribution curve ofdrugs in P123 micelles, (c) SANS profiles of 5 wt% P123 with and without drug loading, (d) CPs of drugs in P123 micelles, (e) TEM image of LTG encapsulated P123 micelles, and (f) Pictures of drugs in water and P123 micellar solutions.

Chapter-5 consist study of binary mixed micellar systems, Pluronic®F127/P123 and Pluronic®F127/L35, as nanosized carriers for solubilization of an anticonvulsant drug, lamotrigine (LTG) at room temperature. The micellar characterization has been performed using UV-VIS spectroscopy, DLS and SANS techniques. All the binary systems formed stable mixed micelles

with low CMC in water. The CMCs were located between CMC of the individual copolymer in F127/P123 mixtures and found increased in the F127/L35 mixtures. The DLS and SANS measurements had been confirmed the spherical micelles with less than 30-nm diameter in size for both the binary systems. The F127/P123 system shows relatively high S_{CP} as more hydrophobicity and the F127/L35 system had low S_{CP} because of hydrophile nature of L35. This work of chapter has been evaluated that the F127/P123 mixed micellar systems are better than F127/L35 systems as nanosized carriers for encapsulation of LTG for delivery in CNS at room temperature (30°C)[10].

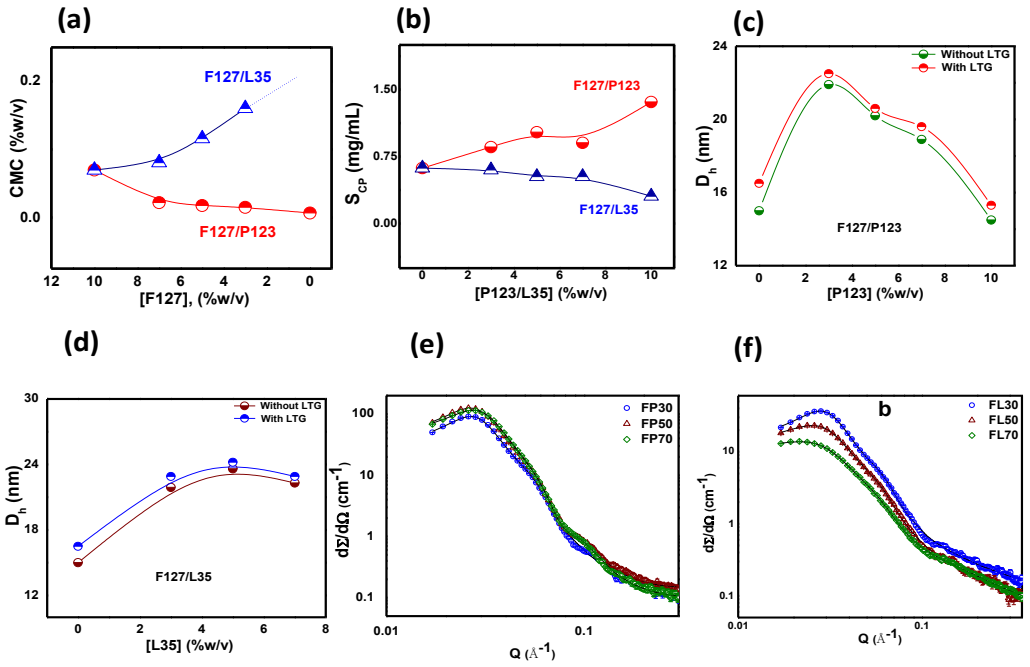

Fig.4 : (a) CMCs of binary F127/P123 andF127/L35 systems at 30°C, (b) Scp of mixed Pluronic F127/P123 and F127/L35 micellar systems, (c) Hydrodynamic diameter(Dh) plot for empty and LTG loaded Pluronic F127/P123 micelles, (d) Hydrodynamic diameter(Dh) plot for empty and LTG loaded Pluronic F127/L35 micelles, (e) SANS profile for LTG loaded Pluronic F127/P123 micelles at 30°C,and (f) SANS profile for LTG loaded Pluronic F127/L35 micelles at 30°C.

Similaraly, mixed Pluronic® micellar systems (F127/P123 and F127/L35) as micellar nanocarriers for LTG drug were also investigated at body temperature in **Chapter-6**. The solubilization of LTG in binary mixed micellar systems, F127/P123 and F127/L35 are determined using UV-VIS spectroscopy. 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. Thermodyanmic 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. The scattering techniques, DLS and SANS, confirmed the spherical assembly with average sizes of < 25 nm of both the mixed Pluronic® micellar systems with and without LTG drug. In vitro drug release profile of both the mixed Pluronic® micellar

systems demonstrate an initial burst release for about 4 h followed by slower and sustained release for more than 80 h and go behind Korsmeyer-Peppas model of kinetics. The stability study has confirmed the better biocompatibility of LTG in the mixed F127:P123 micellar system. The study of this chapter confirms that LTG-solubilized mixed F127:P123 micellar system could be an efficient carriers for bioavailibility of LTG drug**.**

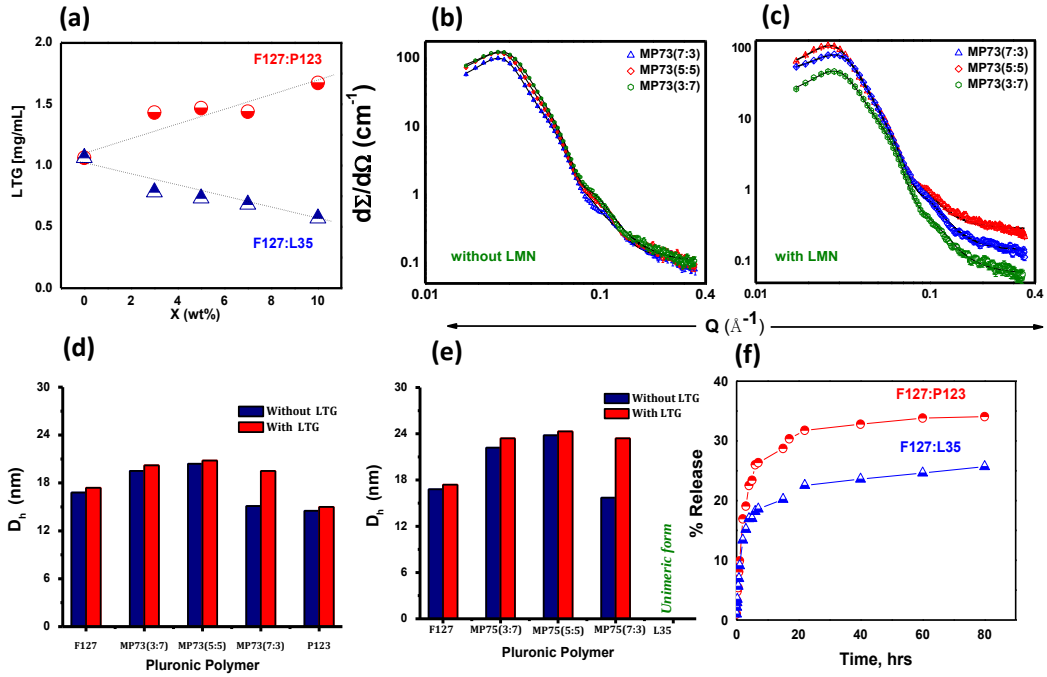

Fig.5 (a) Solubilizationof LTG in binary mixtures at 37°C, (b) SANS profiles of binary F127/P123 mixtures without LTG loading, (c) SANS profiles of binary F127/P123 mixtures with LTG loading, (d) Hydrodynamic diameters(Dh) of binary F127/P123 with and without LTG drug, (e) Hydrodynamic diameters(Dh) of binary F127/L35 with and without LTG drug, and(f)In-vitro release behaviour of Pluronic binary mixtures (F127/P123 and F127/L35) at 37°C.

As we found that the mixed micelles of Pluronic®F127 and Pluronic®P123 would be the best choice for the drug delivery aspects, these mixed Pluronic®F127/P123 micelles are combined with phosphatydyl choline(PC) and examined for the bioavailibilty of curcumin drug in **Chapter-7**. The PC has the ability to solubilize a variety of both hydrophilic and hydrophobic drugs and has been approved FDA as a safe pharmaceutical adjuvant[11]. Self-assembly of these mixed F127/P123/PC systems have been investigated using DLS, SANS, TEM and Optical microscopic techniques. Results show that 1% PC has vesicle structure in the medium and addition of spherical mixed micelles of F127/P123 shown transition of vesicles-to-unilamellar vesicles micelles-to-spherical micelles morphology. Solubilization of curcumin drug into these mixed systems is examined using UV-Visible spectroscopy. The study of this chapter highlighted that the unilamellar vesicle micellar systems of F127/P123/PC could be a promising approach to improve oral and parenteral delivery of curcumin.

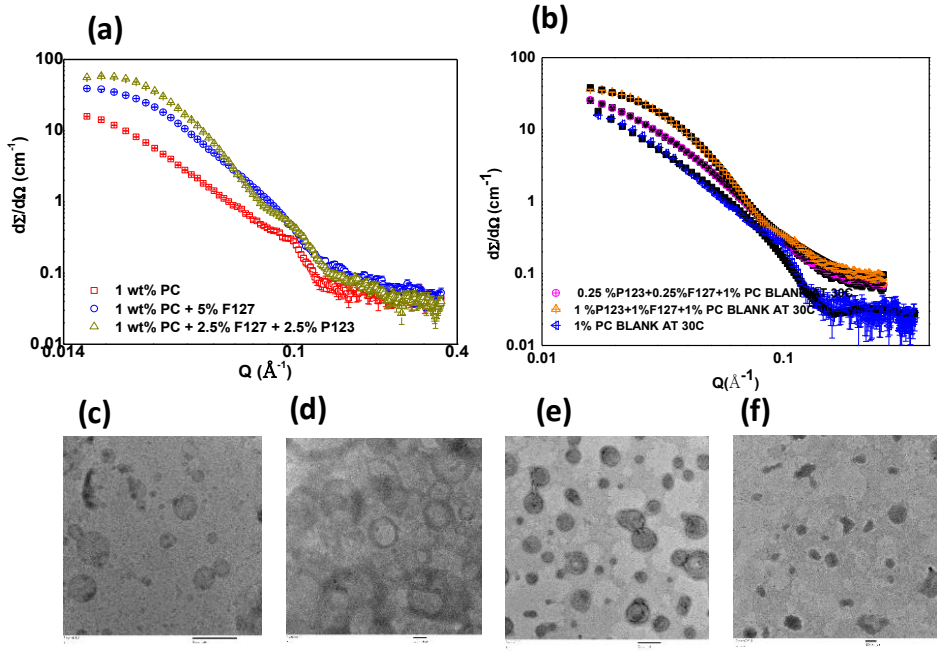

Fig.6: (a)SANS profiles of 1% PC, 1%PC with 5 % F127 and mixtures of 1%PC with 2.5%F127+2.5% P123in D_2O, (b)SANS profiles of 1% PC, 1%PC with 1% F127+1%P123 and mixtures of 1%PC with 0.25%F127+0.25%P123in D_2O, (c)TEM image of 1% PC, (d)TEM image of 1%PC with 0.25%F127+0.25% P123, (e)TEM image of 1%PC with 1%F127+1% P123, and (f)TEM image of 1%PC with 2.5%F127+2.5% P123.

The thesis ends with a summary of all the reported work and general conclusions drawn from the investigations.

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Signature of candidate

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Endorsement of Supervisor;
Synopsis is approved by me

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Research Papers published under the category of peer reviewed Journals

1) Incorporation of lamotrigine drug in the PEO–PPO–PEO triblock copolymer (Pluronic F127) micelles: effect of hydrophilic polymers
Shaikh, S., Ray, D., Aswal,V.K., Sharma, R.K., Journal of Surfactants Detergents, 20, 695–706 (2017).

2) Binary mixed micellar systems of PEO-PPO-PEO block copolymers for lamotrigine solubilization: A comparative study with hydrophobic and hydrophilic copolymer
Shaikh, S., Ray, D., Aswal,V.K., Sharma, R.K., Journal of Polymer Research, 25, 73 (2018).

3) Pluronic micelles encapsulated curcumin manifests apoptotic cell death and inhibits pro-inflammatory cytokines in human breast adenocarcinoma cells
Vaidya, F., Sharma, R.K., **Shaikh, S.**, Ray, D., Aswal,V.K., Pathak, C., Cancer Reports, 2018;e1133.

❖ **Papers presented at conferences/workshops**

1) Binary mixtures of polyethyelene oxide-polypropylene oxide-polyethelene oxide block copolymers for improved solubility of LTG drug
Shaikh, S., Ray, D., Aswal,V.K., Sharma, R.K., at National Conference on Frontiers in Chemical Sciences, Department of PG Studies and Department of Chemistry, PAHER University,Udaipur, Rajasthan, (6th October 2016) **(Poster)**

2) Encapsulation of lamotrigine drug in PEO-PPO-PEO triblock copolymeric micelles: effect of hydrophilic polymers
Shaikh, S., Ray, D., Aswal, V.K., Sharma, R.K., at 6th Conference on Neutron Scattering (CNS-2016), BARC, Mumbai, Maharashtra (21st-23rd November, 2016) (**Poster**)

3) Mixed micelles of PEO-PPO-PEO block copolymers as nanocarrier for lamotrigine drug
Shaikh, S., Ray, D., Aswal, V.K., Sharma, R.K., at International Conference on Expanding Horizons of Nanotechnology: Next Gen Challenges in Biomedical Sciences (NanoSciTech2017), Punjab University, Chandigarh (8th - 10th November, 2017) (**Poster**)

4) Solubilization of pharmaceutical active ingradients of different polarity using poloxamer polymers
Shaikh, S., Ray, D., Aswal, V.K., Sharma, R.K., at National Conference on Advances in Science & Technology – an Interdisciplinary approach (ASTIA-2018), Sofia College, Ajmer, Rajsthan (15th-16th October, 2018)(**Oral**)