Summary and Conclusions

In the advancement of polymer science, amphiphilic block copolymers (ABCs) have played a vital role, and this class of materials has been exploited in a variety of science fields. ABCs have attracted attention since their building blocks are non-compatible, which results in a microphase separation. But due to the covalent linkages between the different blocks, this restricts phase separation and produces the self-assembled structures with typical diameters of between 10 to 100 nm. The ABCs can produce a variety of morphologies, like spherical, cylindrical, lamellae, vesicles, and other typical assemblies. Therefore, ABCs are of specific interest in the field of nanomedicine and are used to encapsulate hydrophobic drugs for targeted drug delivery.

Triblock copolymers of PEO and PPO, also known as Poloxamers and sold commercially as Pluronics® (BASF), are known for their surface-active properties, which come from their non-compatible blocks (hydrophilic PEO and hydrophobic PPO). These Pluronics (*PEO-PPO-PEO block copolymers*) are self-assembled into a variety of morphological structures depending upon the block copolymer concentration, PPO/PEO composition, temperature, and the presence of additives. The versatile physico-chemical characteristics of Pluronics establish them as pharmaceutical ingredients. Some Pluronics are FDA and EPA approved. The Pluronics characteristics used in the present research work are shown in Table 7.1.

Table 7.1: Characteristics of the Pluronics studied in the present work.

Pluronic®	**F127**	**F88**	**F68**	**P123**
Composition	$EO_{100}PO_{65}EO_{100}$	$EO_{76}PO_{29}EO_{76}$	$EO_{76}PO_{29}EO_{76}$	$EO_{20}PO_{70}EO_{20}$
HLB	22	28	>24	8
Mol.Wt. ($g.mol^{-1}$)	12600	11400	8400	5800
CMC (%w/v)	0.02	1.7	7.0	0.001
CMT (°C)	24°	38°	50°	16°

In the present research work, the physicochemical and biological evaluation of three different classes of lipophilic drugs like quercetin(QCN), curcumin(CUR), and glipizide(GLN) has been investigated using the mixed Pluronic micellar systems as the nanovehicles for better solubilization and oral bioavailability. The micellar size, shape, stability of developed drug-loaded mixed Pluronic micelles and their interactions with these potent drugs were characterized using the modern techniques like UV-Vis, DLS, SANS, TEM, FTIR, XRD, and DSC. *In-vitro*, *ex-vivo*, and *in-vivo* studies of cumulative drug release, DPPH scavenging antioxidant activity, cell proliferation, and cell viability have been

done to test the biological activities of drug-loaded mixed Pluronic micelles. The full results of the work's research are written up in seven chapters. These chapters include an introduction, a description of the materials and how they were made, four new Pluronic formulations for lipophilic drugs, a summary of the research, and a conclusion.

Chapter-1: General Introduction

In this Chapter 1 of a general introduction, initial research aspects and the importance of ABCs have been discussed. The current scenario, general classification, and self-assemblies of ABCs have been shown with the latest literature citations. The packing parameters for various morphologies of ABC's micellar systems are discussed with appropriate theories. ABCs' micellization and solubilization behaviours have also been described in order to gain a better understanding of their applications in various fields.

The significance and advances in the research of Pluronic polymers are highlighted in the chapter. The details of the information regarding the structure, synthesis, commercial availability, and physicochemical properties of Pluronic polymers are elaborated. The micellization of Pluronic and its mixtures and their characterization methods have been furnished. Applications of Pluronic micelles and mixed micelles as nanovehicles for drug delivery have been demonstrated using chemistry, making them more promising future materials in the pharmaceutical industry.

This chapter gives an up-to-date introduction to the present research work, which focuses on Pluronic micelles and mixed micelles for biomedical applications.

Chapter-2: Materials, experimental design, and characterization methods

Firstly, the molecular structure, specifications, and procurement of materials used for the work, like Pluronics, phosphatidylcholine (PC), vitamin E conjugate (TPGS), and stearic acid (SA) have been furnished in Chapter 2.

The brief introduction and basic profiles of the lipophilic drugs QCN, CUR, and GLN have been given, along with their respective calibration curves for further use in drug loading and encapsulation efficiency measurements. The linear statistical parameters of the calibration curves of drugs are tabulated.

In the chapter, the experimental design (D-optimal design and CCD) used in the thesis work has been described in detail. This design was used to find the best drug formulations.

Various methods and techniques used in the present work, like CMC determination through pyrene-probe UV-Vis and fluorescence analysis, particle size and size distribution, morphologies, and viscosity using DLS, SANS, SEM, TEM, and rheometer measurements, and solid state characterization through FTIR, XRD, and DSC measurements with their parameters of data evaluation, have been discussed with sample executions. The locations of solubilized drugs in the micelles were determined through ^{1}H-NMR analysis.

Materials procurement for biological investigations is also covered in this chapter.

Chapter 3: Mixed Pluronic self-assemblies for quercetin drug for anticancer evaluations

In this Chapter-3, to enhance the bioavailability of the poorly water-soluble drug QCN, the QCN-incorporated mixed Pluronic P123/F88 micelles were developed and systematically examined in the present study. The mixed P123/F88 micelles have a low CMC (0.0092 %w/v), which signifies they can maintain a stable micelle structure even in a diluted environment.

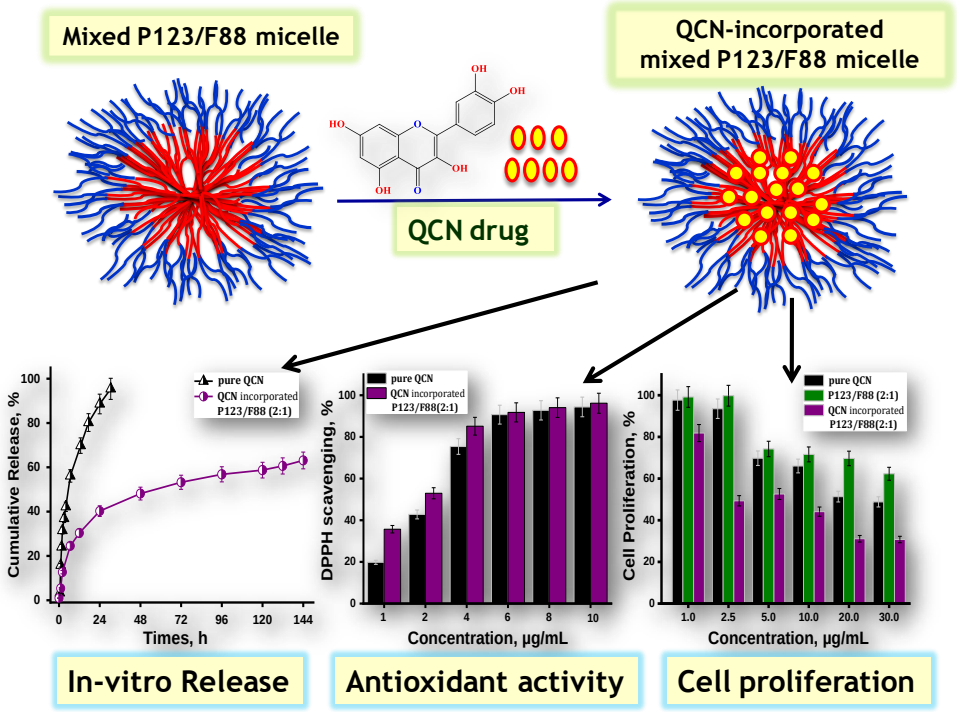

Figure 7.1: Graphical presentation of Pluronic mixed P123/F88 micelles with and without QCN encapsulation with their biological application.

With an increase in temperature, the QCN exhibited increased solubility in the mixed P123/F88 micellar system. The average particle size and the ζ potential of the QCN-incorporated mixed P123/F88 micelles were 21.8 nm and −16.2 mV, respectively. The shape of the QCN-incorporated mixed P123/F88 micelles was spherical and quite stable at RT. The *in-vitro* release behaviour of QCN from the mixed P123/F88 micelles showed the slower and sustained release. The resistance to oxidation of QCN-incorporating mixed P123/F88 micelles was found to be significantly greater than that of pure QCN. While the results from *in-vitro* cell proliferation studies on MCF-7 cells demonstrated that QCN-incorporated mixed micelles were effective in inhibiting the growth of tumour cells.

The current study demonstrated a novel method for delivering QCN through mixed Pluronic polymeric micelles. *In-vitro* experiments have revealed the outstanding advantage of QCN-incorporated mixed P123/F88 micelles. Thus, QCN-incorporated mixed P123/F88 micelles may represent an effective approach for enhancing QCN's oral bioavailability, antioxidant activity, and cell viability.

Chapter-4: Mixed Pluronics/phosphatidylcholine self-assemblies for curcumin drug for antimicrobial evaluations

In the Chapter-4, a sustainable mixed polymeric nanomicellar PFPC formulation was developed by incorporating biocompatible PC into the structure of Pluronic P123/F68 mixed micelles. These findings open the door for developing thermodynamically and kinetically stable CUR-PFPC mixed nanomicellar formulations for improved solubilization and absorption of lipophilic drugs such as CUR. The D-optimal design was used to optimize the CUR-PFPC mixed micellar formulation, which were fabricated using the fine-film hydration method. The obtained optimal formula, composed of 5 %w/v P123, 4.98 %w/v F68, 1 %w/v PC, and 2 mg CUR, had the highest desirability value and was chosen and evaluated.

The optimized CUR-PFPC nanoformulation had a particle size of 67.43 nm, a PDI of 0.528, and zeta potential of -15.1 mV with a spherical shape. Our results showed the CUR-PFPC achieves higher aqueous solubility (dispersibility) of CUR in water, better compatibility, and stability for 25 days, which ultimately enhances its use in pharmacological formulations. However, only over 60% of the incorporated CUR had been released 10 days from the CUR-PFPC nanoformulation, indicating a slow and sustained CUR release. The antioxidant investigations showed that the CUR-PFPC had better oxygen resistance than free

CUR. Our results also proved that the CUR-PFPC nanoformulation improved the antimicrobial activity of CUR toward bacterial strains and fungi. The CUR-PFPC nanoparticles were nano-sized, spherical shaped, and had improved aqueous dispersibility and stability. These findings suggest that the CUR-PFPC nanoformulation may be a promising nano-vehicle CUR option as an antibacterial and antifungal agent.

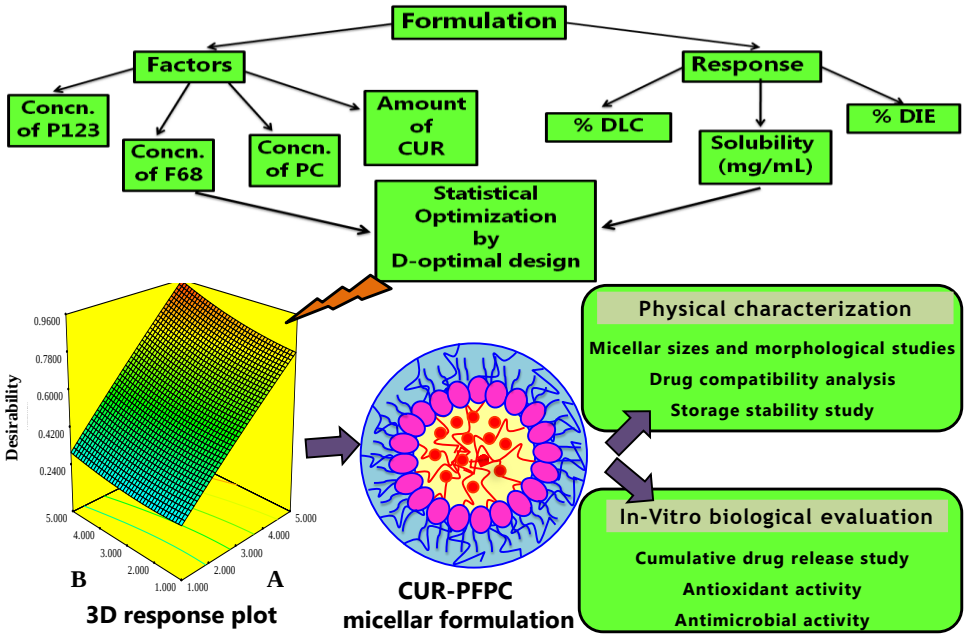

Figure 7.2: Graphical presentation of CUR encapsulated Pluronic mixed P123/F68/PC micelles with their application.

Chapter-5: Mixed Pluronics/phosphatidylcholine self-assemblies for curcumin drug for anticancer evaluations

In the Chapter-5, the micellar behaviour of PC is modified using the mixed F127/P123 micellar solutions, which has impact on micellar shape. These mixed PC/Pluronic micellar solutions have been characterized through DLS, SANS, rheology, and TEM measurements. With 5% w/v mixed F127/P123 micelles (2.5% w/v of each polymer), the vesicle of 1% PC is shifted to spherical micelles. The addition of Pluronic has interfered and caused the surface of the lipid bilayer to change, which is reflected in the parameter changes of the bilayer. It decreases the bending rigidity and creates a transition from the unilamellar vesicle to the smaller mixed micelle phase. The introduction of Pluronic into the PC domain layer was confirmed by steady shear rheological data. All the analysis on mixed PC/Pluronic solutions confirms incorporation of the PC in the Pluronic matrix. The location of curcumin in the inner core of the mixed PC/Pluronic micelles is confirmed by NMR analysis.

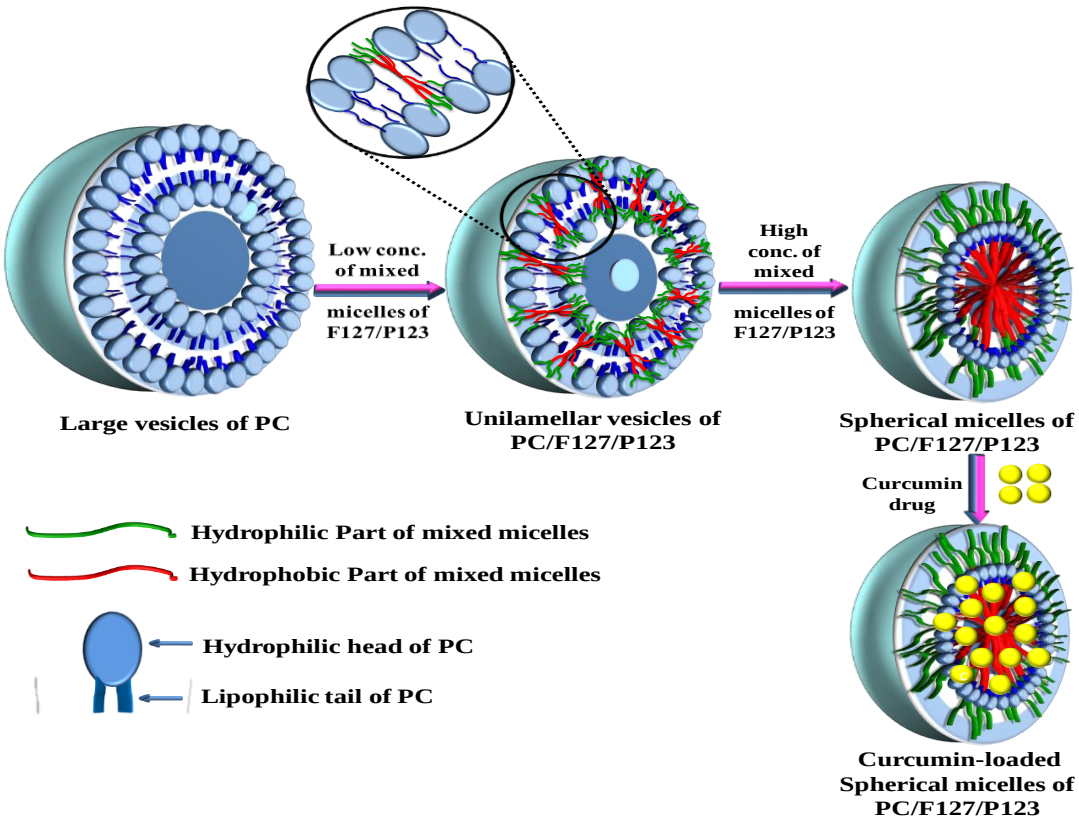

Figure 7.3*:* Graphical presentation of mixed PC/F127/P123 micellar system for CUR drug solubilization.

The pure CUR was released at 100% in 22 hrs. But only 28% of the CUR was released in the 100 hrs investigation from mixed PCFP micellar solutions, which clearly indicates the slower release. Pure CUR inhibited DPPH by 30% up to 3.0 g/mL, whereas mixed PCFP micellar solutions inhibited DPPH by 85% at the same concentration. This observation proves the higher antioxidant activity of mixed PCFP micelles compared to pure CUR.

In-vitro cell proliferation and cell viability investigation on MCF-7 cells revealed that a curcumin-loaded mixed PCFP micellar system significantly restricts cell proliferation and induces death in the cancer cell. Mixed PC/Pluronic micelles are the "smart nano-carriers" that offer a new stratagem for efficient drug delivery of anti-cancer agents with better efficacy, and could be utilized for therapeutic intervention for the development of safer new drug.

Chapter-6: Mixed Pluronic/Vitamin E conjugates self - assemblies for glipizide drug for antidiabetic evaluations

The SA-F127 was successfully synthesized in order to create a better mixed polymeric micellar carrier with the TPGS for oral bioavailability of the GLN drug. The GLN-PMM micellar system was optimized through CCD, fabricated by thin-film technique, and thoroughly characterized using the multi-technique approach. The optimized GLN-PMM had a particle size of 67.86±2.06 nm, a PDI of 0.582±0.06, and a zeta potential of -3.85±1.39 mV, with a spherical shape. Results revealed that the GLN-PMM achieved higher aqueous solubility of GLN, better compatibility, and good stability up to three months, which ultimately enhanced its pharmacological activities. The *in-vitro* release study indicated the sustained release of GLN from the mixed polymeric micelles in different acidic, alkaline, and neutral media. *Ex-vivo* analysis showed high drug permeation, which favours improved oral bioavailability. The GLN-PMM system showed better *in-vivo* anti-diabetic activity than marketed formulation in reducing the blood sugar level after oral administration in rats. All of the results showed that the GLN-PMM mixed polymeric micellar system developed in the present work is far better than the use of pure GLN.

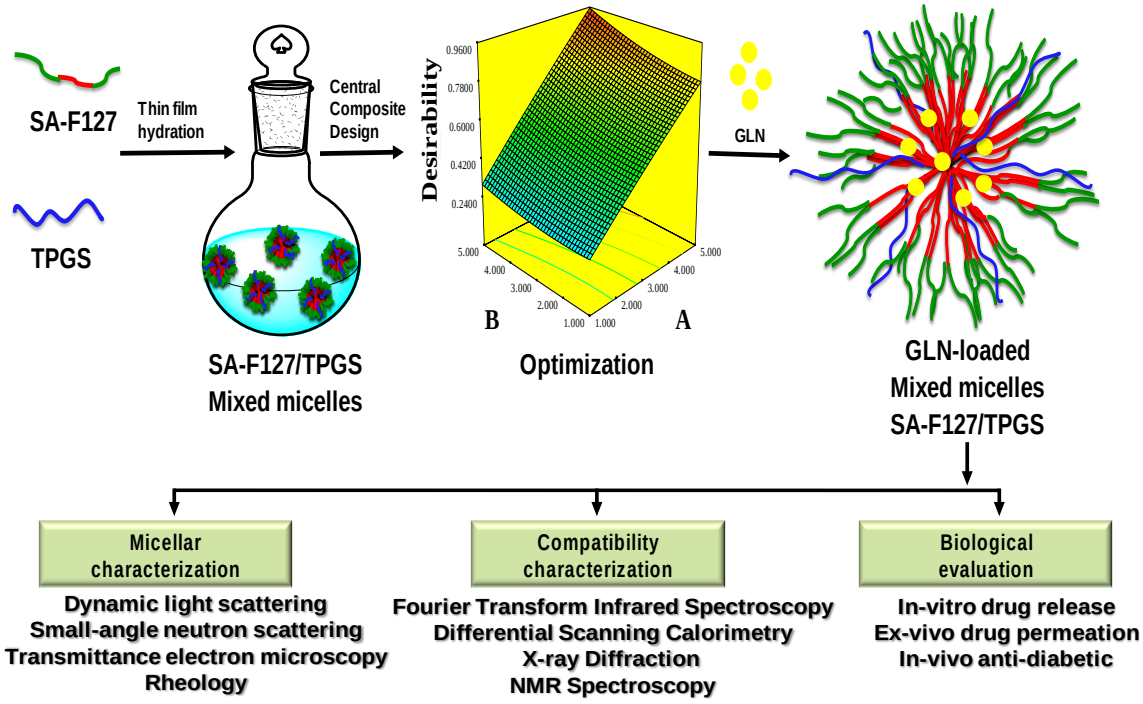

Figure 7.4: Schematic representation of mixed SA-F127: TPGS micelles for bioavailability of the GLN drug

Overall, the interest in applications of self-assemblies of Pluronics and its mixture with other Pluronic or biocompatible natural materials has emerged as a newer area of research due to its non-toxicity, availability, high solubilization and loading ability, and passive accumulation in tumor regions. Pluronic micellar applications have now shifted the focus of formulation research largely towards targeted nanomedicines, and in consideration of this, the present work thoroughly investigated these Pluronic mixed micellar assemblies for solubilization and oral bioavailability of important lipophilic drugs QCN, CUR, and GLN. The current study advances research on Pluronic systems for biological and pharmaceutical applications.