Synopsis

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Design, Characterization and Biological Significance of Amphiphilic Block Copolymer Self-assemblies

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Design, Characterization and Biological Significance of Amphiphilic Block Copolymer Self-assemblies

In the advancement of polymer chemistry, amphiphilic block copolymers have played a vital role and this class of materials has been exploited in a variety of ways in the fields of chemistry, physics, material sciences, and biological sciences [1]. Amphiphilic block copolymers 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, the microphase separation is limited and produces the self-assembled structures with typical diameters of between 10 to 100 nm [2]. The amphiphilic block copolymers can produce ordered structures in a variety of morphologies, such as spheres, cylinders, bicontinuous structures, lamellae, vesicles, and many other complex or hierarchical assemblies. Therefore, amphiphilic block copolymers are of specific interest in the field of nanomedicine and are used to encapsulate hydrophobic drugs for targeted drug delivery [3,4]. Figure 1 shows the number of publications with the topic "*amphiphilic block copolymer*" over the past ten years, which reflects the importance of this research. Of course, the effect of COVID-19 is reflected in the increase in publications.

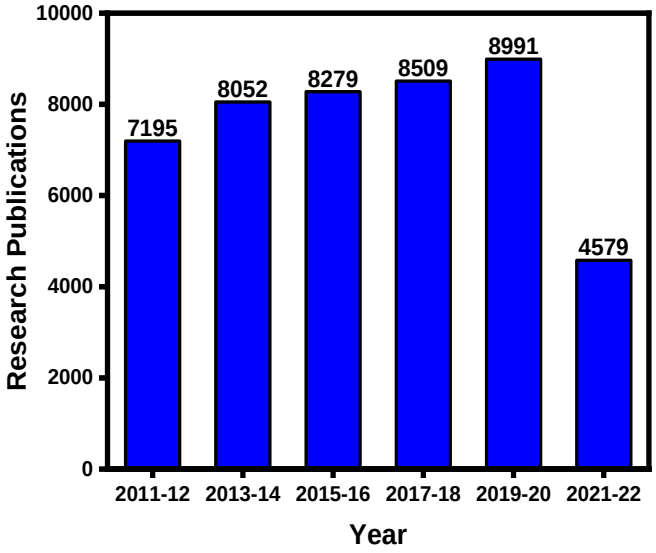

Figure 1: *The number of publications with "amphiphilic block copolymer" as the topic against year. The data were collected from* ***Web of Science (Clarivate Analytics)****.*

Triblock copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (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) [5]. These Pluronic polymers (*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 [6]. Pluronics' high surface activity, temperature-based micellization, and reversible thermo-rheological behaviour make them versatile materials with applications in the cosmetics, food,

coatings, paints, petroleum, and pharmaceutical industries [7]. Some of the Pluronics are FDA approved and used as effective drug delivery carriers with thermo-responsive behaviour [8]. Figure 2 shows the number of publications with the topic "*Pluronic*" over the past ten years, along with the molecular structure of the Pluronic polymer.

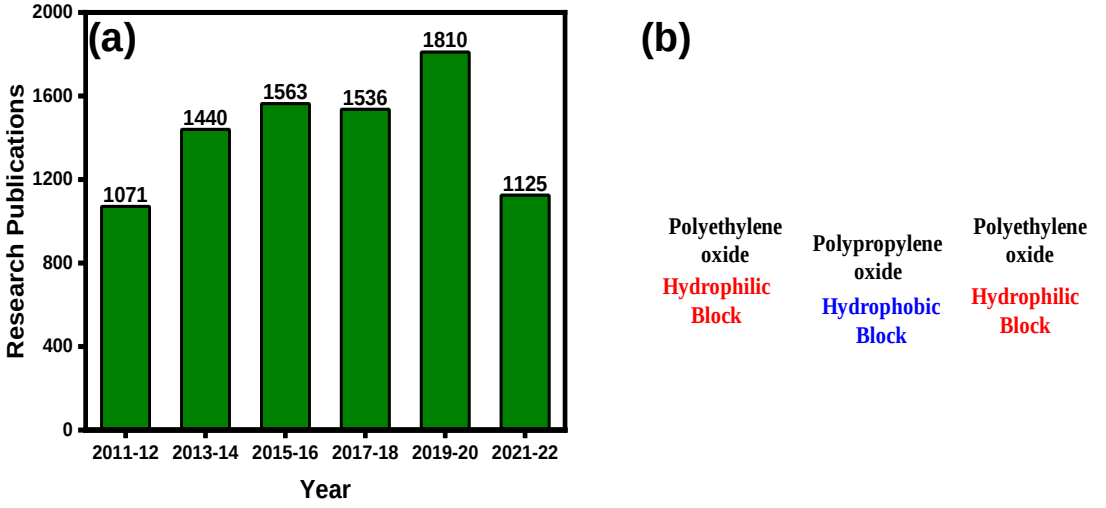

Figure 2: ***(a)*** *The number of publications with "Pluronic" as the topic against year. The data were collected from* ***Web of Science (Clarivate Analytics)*** ***(b)*** *molecular structure of Pluronic polymers.*

The Pluronic forms the core-shell micelles in water with the PPO core and shell of PEO at an above critical micelle concentration (CMC) and critical micelle temperature (CMT). The PPO core is hydrophobic and enables the local hydrophobic environment for the encapsulation of lipophilic drugs, while the hydrophilic PEO shell keeps the dispersion stability. The encapsulation of hydrophobic drugs into Pluronic micelles can enhance their solubility and stability, which upgrades their pharmacokinetics and biodistribution. Pluronics micelles and mixed micelles have been demonstrated to have significant applications in drug delivery systems and used as micellar nanovehicles for a variety of lipophilic drugs [9]. Pluronic polymers are suitable for systems requiring surface modification because they assemble on hydrophilic and hydrophobic surfaces, including biological media like platelets [10].

In this context, present thesis research work various mixed micellar systems of Pluronic polymers are designed, characterized, and potentially evaluated as the nanovehicles for drug delivery applications for poorly water soluble drugs.

The Pluronic polymers used in the present research work are shown in Table 1 with their characteristics.

Table 1: *Molecular properties of studied Pluronics.*

Pluronics®	Mol.Wt. (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

The physicochemical and biological evaluation of three different classes of lipophilic drugs like quercetin (antioxidant, BCS Class I), curcumin (anticarcinogenic/antimicrobial, BCS Class II), and glipizide (antidiabetic, BCS class I) have been investigated using the mixed Pluronic micellar systems for better potency. The molecular structures of the studied drugs are shown in Figure 3.

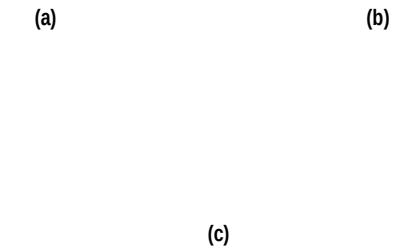

Figure 3: *Molecular structures of (a) Quercetin b) Curcumin and (c) Glipizide drug*

To investigate the drug-loaded mixed Pluronic micelles and their interactions with drugs, advanced characterization methods such as UV-Visible spectroscopy (UV-Vis), dynamic light scattering (DLS), small angle neutron scattering (SANS), transmission electron microscopy (TEM), fourier transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD), and differential scanning calorimetry (DSC) were used. 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.

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

Chapter 1: General Introduction

The introduction and self-assembly of block copolymers and Pluronic polymers have been discussed briefly using the most recent research relevance. The chapter focuses on Pluronic micelles and mixed micelles as the nanovehicles for lipophilic drug solubilizer and delivery applications. This chapter highlights the most important and relevant research outputs in the area of work in a systematic manner.

Chapter 2: Materials, experimental design, and characterization methods

The introduction and molecular properties of the materials involved like Pluronics, phosphatidylcholine, and Vitamin E conjugate in the present thesis have been shown in this chapter. The basic profiles and calibration curves of investigated hydrophobic drugs are presented. The experimental design used in the present thesis work has been presented here along with the applied theory. Various methods and techniques used in the present work will be shown in this chapter with their parameters of data evaluation.

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

In this chapter, the improvements in the aqueous solubility, oral bioavailability, and in-vitro cell proliferation activity of quercetin (QCN) drug, the mixed micelles of Pluronic P123 and F88 have been investigated. Mixed P123/F88 micelles and QCN incorporated mixed P123/F88 micelles are prepared using a direct dissolution method and well characterised through UV-VIS, DLS, TEM, SANS, and FTIR techniques. *In-vitro* antioxidant and *in-vitro* cell proliferation of QCN-incorporated mixed P123/F88 micelles are also evaluated. A low critical micelle concentration (CMC) of mixed micelles indicates the stability. The *in-vitro* drug release profile showed a sustained release pattern. The *in vitro* cell proliferation of the QCN-incorporated mixed P123/F88 micelles is investigated on MCF-7 breast cancer cells, which showed higher cell proliferation activity than the pure QCN drug. Figure 4 shows some of the results of the study of the mixed P123/F88 micelles for QCN bioavailability. Based on these results, the mixed micelles developed in this study might be a potential nano-drug delivery system for cancer treatment.

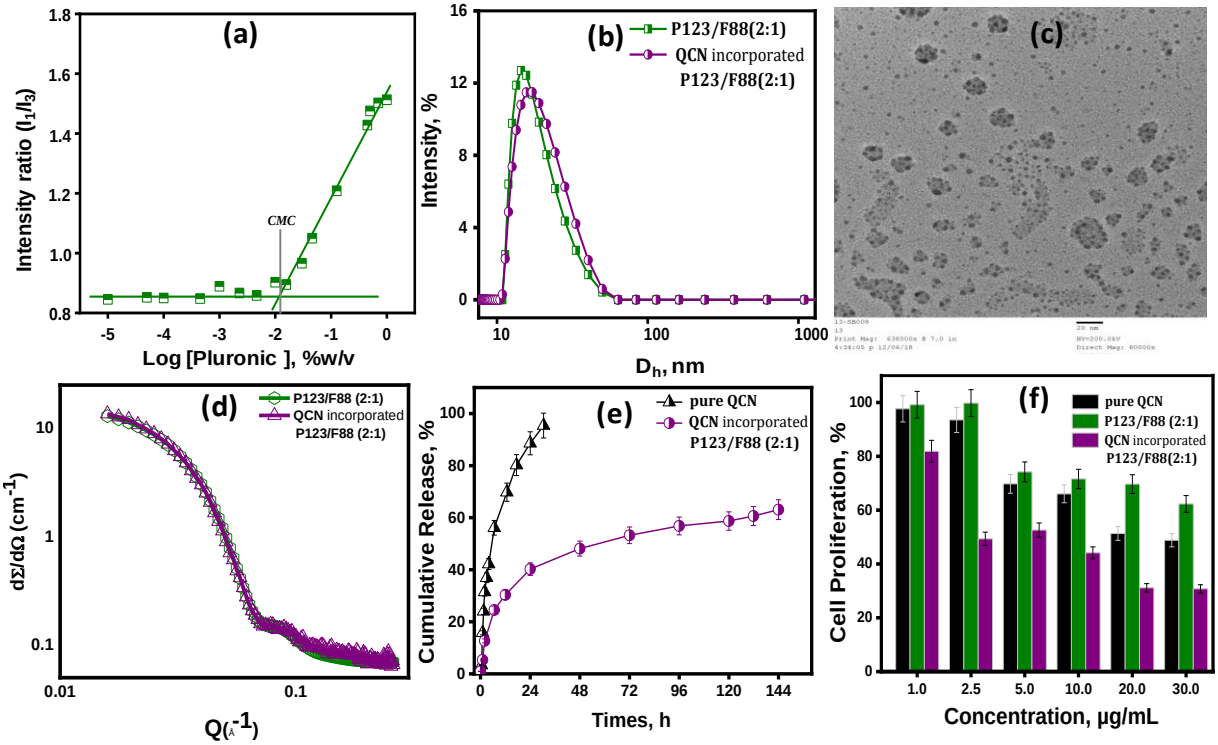

Figure 4: ***(a)****Plot of Intensity ratio (I_1/I_3) versus logarithmic concentration of mixed Pluronic polymers* ***(b)*** *Micellar size and size distribution graphs of mixed P123/F88 (2:1) micelles in absence and presence of QCN drug* ***(c)*** *TEM image of QCN-incorporated P123/F88(2:1) micellar system, and* ***(d)*** *SANS profile of mixed P123/F88(2:1) and QCN-incorporated mixed P123/F88(2:1)micellar system* ***(e)*** *In-vitro release profile of QCN and QCN-incorporated mixed P123/F88(2:1) micelles* ***(f)*** *Cell Proliferation activity of pure QCN, mixed P123/F88(2:1) micelles and QCN-incorporated mixed P123/F88(2:1) micelles.*

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

In this chapter, a mixed polymeric nanomicellar system based on Pluronic P123, F68, and phosphatidylcholine(PC) has been designed and examined as the nanovehicles for overcoming the major barriers of poor bioavailability related to curcumin (CUR). The CUR-

incorporated P123/F68/PC mixed nanomicellar formulation (CUR-PFPC) is fabricated by the thin film technique and investigated *in vitro*. The fabrication of CUR-PFPC is optimised through D-optimal design. CUR-PFPC morphology, size distribution, zeta potential, drug encapsulating and incorporation efficiency, compatibility, and crystallinity were characterised using DLS, TEM, FTIR, XRD, and DSC analysis. Moreover, the *cumulative* drug release, antioxidant assays, and antimicrobial properties of formulations are also examined. Figure 5 reports some of the results of investigations into the studied polymeric micellar formulations. The CUR-PFPC formulation exhibited a micellar size of 67.43 nm, a zeta potential of-15.1 mV, a PDI of 0.528, and a spherical shape. The mixed micellar formulation showed excellent compatibility and stability. The *in vitro* release profile of the CUR-PFPC reached over 60% in comparison to the 95% release of CUR, indicating a slow and sustained release. The DPPH assay showed that the CUR-PFPC had 96% antioxidant activity. Results show that the CUR-PFPC has powerful antibacterial and antifungal properties, which separates it from the free CUR. These findings suggest that the fabricated CUR-PFPC mixed polymeric nanomicellar formulation is thermodynamically and kinetically stable and may be considered a novel nanovehicle for hydrophobic antimicrobial drugs like CUR.

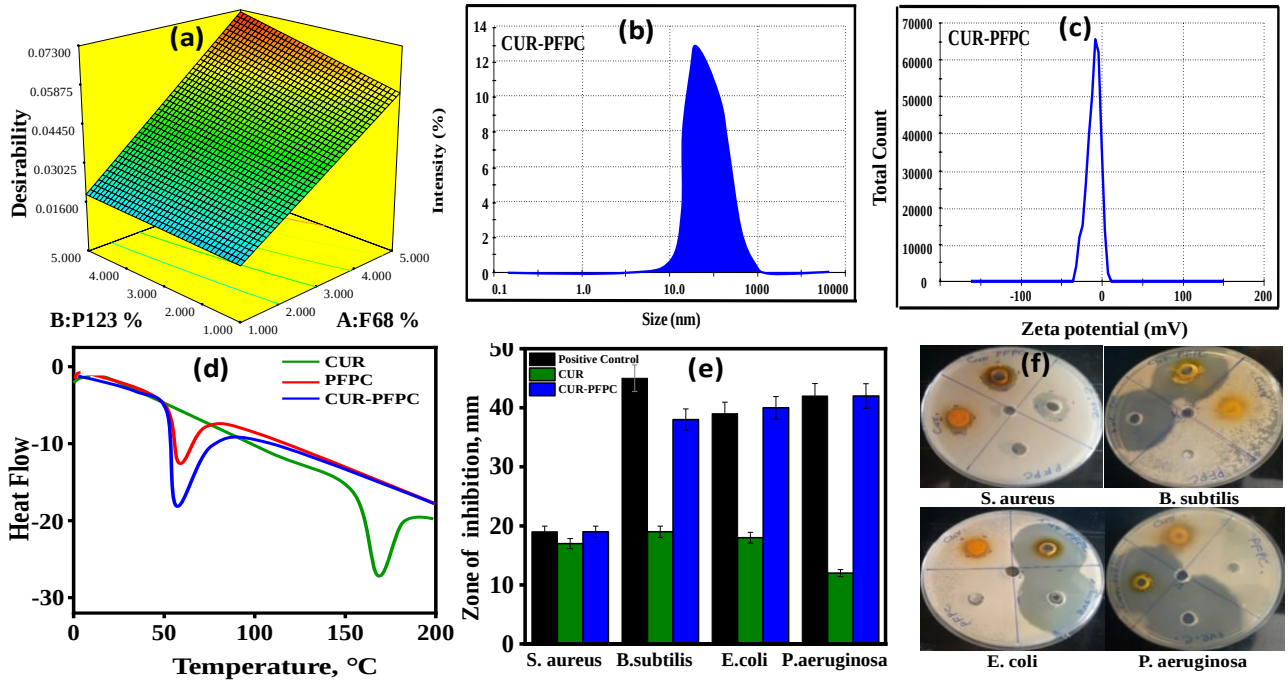

Figure 5: ***(a)*** *3D response surface plot,* ***(b)*** *Micellar size distribution of CUR-PFPC formulations* ***(c)*** *Zeta potential graph of CUR-PFPC formulations* ***(d)*** *DSC thermograms of pure CUR, PFPC and CUR-PFPC formulations* ***(e)*** *Antibacterial activities Graph of zone of inhibition against S. aureus, B. subtilis, E. coli and P. aeruginosa* ***(f)*** *The photographs of antibacterial activity against S. aureus, B. subtilis, E. coli and P. aeruginosa*

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

The self-assemblies of phosphatidylcholine (PC) liposomes and Pluronic polymers with varying compositions have been studied in this chapter. The micellar transition formed in the mixed PC and Pluronics (F127, P123, and mixed F127/P123) systems is investigated through

DLS, SANS, rheology, and TEM measurements. Results indicated that the PC appeared to be perfectly large bilayer vesicles. With an increasing concentration of Pluronics, the PC vesicle is also transformed into spherical micelles. The transitions from large lamellar vesicles to spherical micelles have been found with all the mixed systems, in which mixed F127/P123 performed better. The mixed PC/Pluronic micellar systems (PCFP) are being investigated for the problems associated with curcumin delivery, such as poor solubility and stability. The solubilization of curcumin in the PCFP systems has been examined and found to be better. The curcumin-loaded PCFP micellar system is synthesised through the thin-film method and evaluated in-vitro. Nuclear magnetic resonance (NMR) analysis indicated the location of curcumin has been found in the core of the PCFP micelles. The curcumin-loaded PCFP showed a slower and more sustained drug release under physiological conditions. The resistance to the oxidation of curcumin-loaded PCFP micelles is considerably higher than that of pure curcumin. Results also revealed that the curcumin-loaded PCFP effectively inhibits the cell proliferation of human breast adenocarcinoma cells (MCF-7) and induces cell death. Figure 6 shows some of the results of the study of the mixed PC/Pluronic micelles. This study suggests that the curcumin-loaded mixed PC/Pluronic micellar system enhances the bioavailability of curcumin.

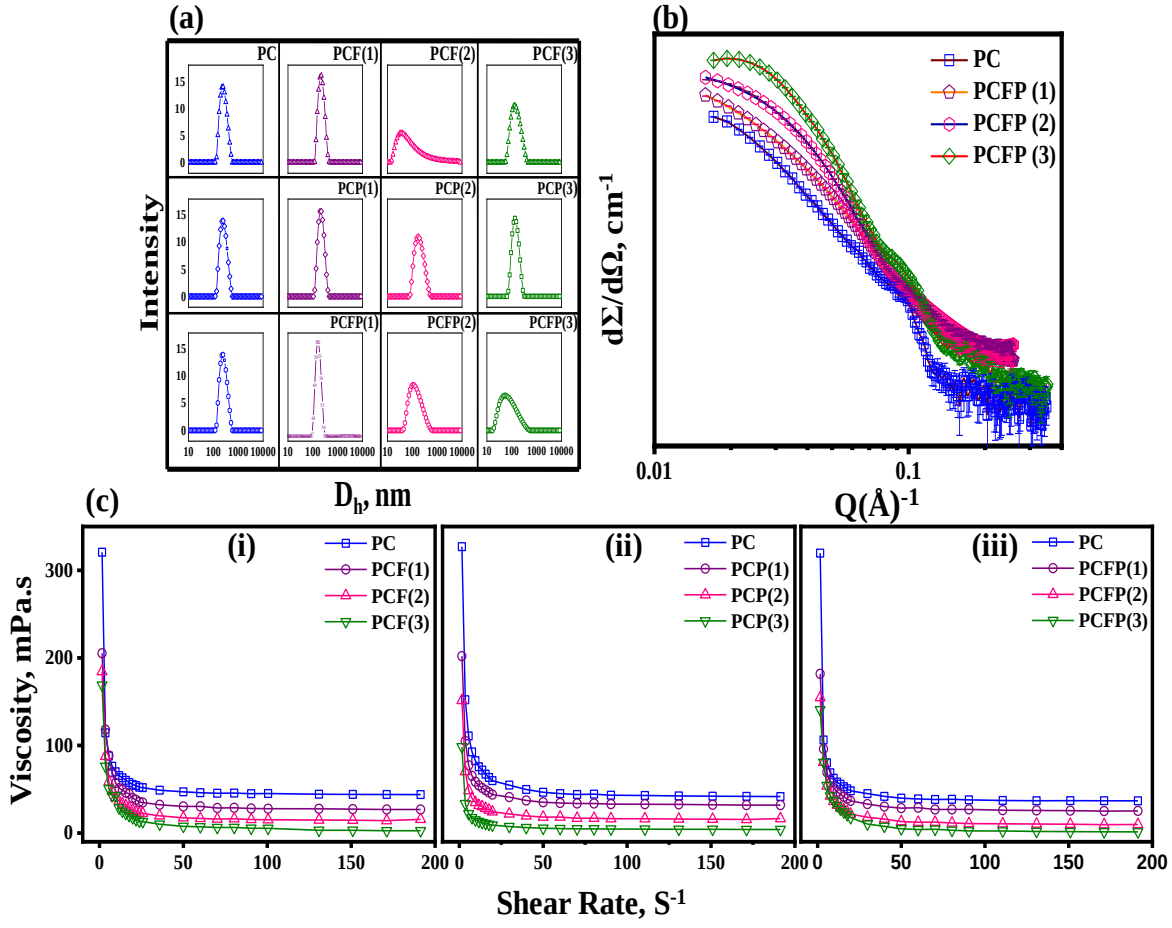

Figure 6: ***(a)*** *DLS stacks graph of PCF, PCP, and PCFP mixed micellar solutions* ***(b)*** *Experimental scattering curves for 1 wt% PC and PCFPs solutions in D_2O* ***(c)*** *Variation of the shear viscosity as a function of shear rate for mixed (i) PCFs, (ii) PCPs, and (iii) PCFPs micellar solutions.*

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

Glipizide (GLN), an antidiabetic agent, has potent biological advantages, but poor aqueous solubility restricts its better pharmaceutical applications. Recently, Pluronic polymers and their conjugation with biocompatible compounds have been widely applied as the nanocarriers for the bioavailability of poorly water-soluble drugs. The objective of the work in this chapter is to design and formulate the mixed polymeric nanomicellar system composed of stearic acid-conjugated Pluronic F127 (SA-F127) and tocopherol polyethylene glycol succinate (TPGS) as the nanocarriers for overcoming the major obstacles of poor bioavailability of GLN. Herein, we synthesized SA-F127 having a significantly lower critical micelle concentration, which helps in dissociation upon dilution during the delivery (Figure 7a). The GLN-encapsulated SA-F127/TPGS mixed micellar formulation (GLN-PMM) is fabricated by the thin film technique. The fabrication of GLN-PMM is optimized through central composite design (CCD).

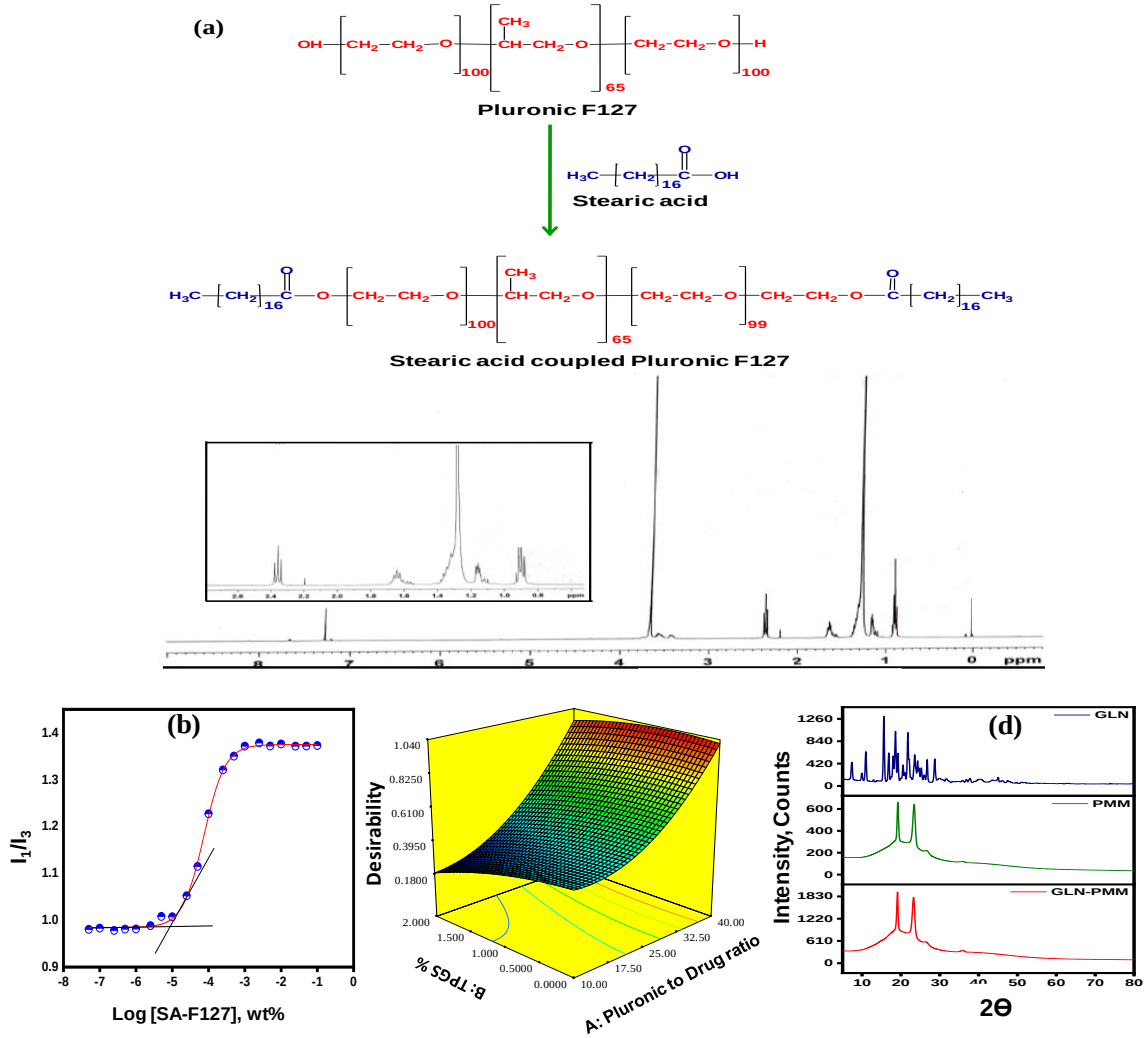

Figure 7: ***(a)*** *Synthesis scheme and* ^{1}*H NMR of SA-F127* ***(b)*** *Plot of Intensity ratio (I_1/I_3) versus logarithmic concentration of SA-F127 and* ***(c)*** *3D response surface plot* ***(d)*** *XRD pattern of pure GLN, PMM and GLN-PMM formulations*

GLN-PMM morphology, size distribution, zeta potential, drug loading and encapsulating efficiency, compatibility, and crystallinity are characterized using DLS, Zeta, TEM, SANS, FTIR, XRD, and DSC analysis. Figure 7b-d shows the CMC determination, optimization design, and solid state characterization of the micellar systems.

Also, the formulations are tested *in-vitro* for their cumulative drug release, *in-vitro* anti-diabetic study, and *in-vivo* pharmacokinetics study. The GLN-PMM formulation exhibited a micellar size of 67.86 nm, a zeta potential of -15.6 mV, a PDI of 0.481, and a spherical shape. The formulation showed excellent compatibility between drug and mixed micelles and was quite stable, amorphous in nature. The GLN-PMM *in vitro* release profile reached over 90% in the physiological pH environment after 24 hours, whereas free GLN was completely released after 5 hours, indicating sustained release. Results show that the GLN-PMM has powerful anti-diabetic properties and demonstrated that blood glucose levels are effectively controlled in the formulation compared with pure GLN. In conclusion, our findings suggest the potential use of mixed micelles of SA-F127 and TPGS as the nanocarriers for delivery of the hydrophobic drug GLN.

Chapter 7: Summary and Conclusions

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

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Signature of Candidate

(Patel Hemilkumar S.)

Endorsement of Supervisor;
Synopsis is approved by me

Dr. Rakesh K. Sharma
Guide

Head
Applied Chemistry Department

Dean
Faculty of Technology and Engineering

❖ List of the Research Publications

Under the Thesis work

1) **Patel H.S.**, Shaikh S.J., Ray D., Aswal V.K., Vaidya F., Pathak C., and Sharma R.K., Formulation, Solubilization, and *In Vitro* Characterization of Quercetin-incorporated Mixed Micelles of PEO-PPO-PEO Block Copolymers. Applied Biochemistry and Biotechnology. **2022**; 194:445-63. *doi.org/10.1007/s12010-021-03691-w*

2) **Patel H.S.**, Shaikh S.J., Ray D., Aswal V.K., Vaidya F., Pathak C., Varade D., Rahdar A., and Sharma R.K., Structural Transitions in Mixed Phosphatidylcholine/Pluronic Micellar Systems and Their *In Vitro* Therapeutic Evaluation for Poorly Water-soluble Drug. Journal of Molecular liquid. **2022**; 364: 120003. *doi.org/10.1016/j.molliq.2022.120003*

3) **Patel H.S.**, Kunjadiya A., Rahdar A., and Sharma R.K., Pluronic-Phosphatidylcholine Mixed Polymeric Nanomicellar Formulation for Curcumin Drug Bioavailability: Design, Fabrication, Characterization and *In-Vitro* Bioinvestigations. Journal of Bioactive and Compatible Polymers **(***Communicated***)**

Other than the Thesis work

1) Shaikh S.J., **Patel H.S.**, Ray D., Aswal V.K., and Sharma R.K., Mixed Poloxamer Nanomicelles for the Anticonvulsant Lamotrigine Drug: Solubility, Micellar Characterization, and *In-Vitro* Release Studies. Journal of Nanoscience and Nanotechnology. **2021**; 21:5723-35. *doi.org/10.1166/jnn.2021.19490*

2) Shaikh S.J., **Patel H.S.**, Ray D., Aswal V.K., Singh S., Vijayvargia R., Sheth U., and Sharma R.K., Enhanced Solubility and Oral Bioavailability of Hydrophobic Drugs Using Pluronic Nanomicelles: An *In-Vitro* Evaluation. Chemistry Select. **2021**; 6:7040-7048. *doi.org/10.1002/slct.202102123*

3) Patel S.S, **Patel H.S.**, Kunjadiya A., Rao V., and Sharma R.K., Synthesis, Characterization and Antimicrobial Activity of Poloxamer-Assisted Copper Nanoparticles: Investigating the Effacts of Different Concentrations of Ploxamer 407. Chemistry Select. **2022**; e20221477: 1-9. *doi.org/10.1002/slct.202201477*

❖ List of the Chapter in the Book

1) Shaikh S.J., **Patel H.S.**, and Sharma R.K., Self-assembly of PEO-PPO-PEO Block Copolymers: Advanced Nanosystems for Drug Delivery Applications**.** Advances in Nanotehcnology, NOVA Science Publishers, USA. Ch. No. 5 (**2020**) pp. 175-204. *ISBN: 978-1-53618-460-0*

❖ List of the Papers presented in the Conferences/Seminars/Workshops

1) Curcummin-loaded Mixed Pluronic P123/F68/Phosphatidylcholine Micelles: Formulation, Optimization and *In Vitro* Characterization.
   Patel H.S., and Sharma R.K., at National Conference on Current Trends and Advances in Chemical Science-2020 (NCBKM 2020), Department of Chemistry, B.K.M Science College, VALSAD, Gujarat, (12th January, 2020) **(Poster).**
2) Formulation, Optimization and In-vitro Characterization of Curcumin-Encapsulated Mixed Pluronic P123/F68/Phosphatidylcholine Micelles.
   Patel H.S., Sharma R.K., at National Seminar On Advances in Chemistry of Bioactive Molecules-2020 (ACBAM-2020), Department of Chemistry, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, (15th-16th October) (**Poster**).
3) Mixed Pluronics/Phosphatidylcholine Micelles for Bioavailability of Curcumin**.**
   Patel H.S., and Sharma R.K., at National Conference on Scientific World Around You and in Cosmos-2020 (NCSWAY-2020), Govind Guru Tribal University, Banswara Rajasthan, (27th-28th January, 2020) (**Oral-First Prize**).
4) Formulation, Solubilization, and *In Vitro* Evaluation of Pluronic P123/F88 Mixed Micelles as Efficient Drug Carriers for Hydrophobic Quercetin Drug.
   Patel H.S., and Sharma R.K., at World Chemistry Conference-2021 (WCC-2021), Department of Chemistry, Wilson College, Mumbai, Maharashtra (3rd-5th May, 2021) (**Oral**).
5) Formulation, Solubilization, and *In Vitro* Characterization of Quercetin-incorporated Mixed Micelles of PEO-PPO-PEO Block Copolymers.
   Patel H.S., Shaikh S., Ray D., Aswal V.K., Vaidya F., Pathak C., and Sharma R.K., at 2nd Virtual International Conference on Naturopathy, Nanotechnology, Nutraceuticals, and Immunotherapy in Cancer Research - 2021 (ICN3IC-2021), School of Life Sciences, B.S Abdur Rahman Crescent Institute of Technology, Chennai, India In Association with Purdue University, USA (11th-12th October) (**Poster-First Prize**).
6) Mixed Micellar PEO-PPO-PEO Triblock Copolymer-Phosphatidylcholine (PC) Systems for Curcumin Drugs: Formulation and Optimization through D-optimal Design Approach
   Patel H.S., Kunjadiya A., and Sharma R.K., at Prof. Ambikanandan Mishra Memorial International Conference on Recent Advances & Trends in Novel Drug Delivery Systems – 2021**,** Faculty of Pharmacy, The Maharaja Sayajirao University of Baroda, Vadodara (23rd September, 2021) (**E-Poster**).
7) Structural Transitions in Mixed Phosphatidylcholine/Pluronic Micellar Systems and Their *In Vitro* Bio-evaluation for Curcumin Drug.
   Patel H.S., Shaikh S., Ray D., Aswal V.K., and Sharma R.K., at International Seminar on Advanced Materials and Applications-2022 (ISAMA-2022), Faculty of Technology and Engineering, The Maharaja Sayajirao University of Baroda, Vadodara (18th July, 2022) (**Poster**).