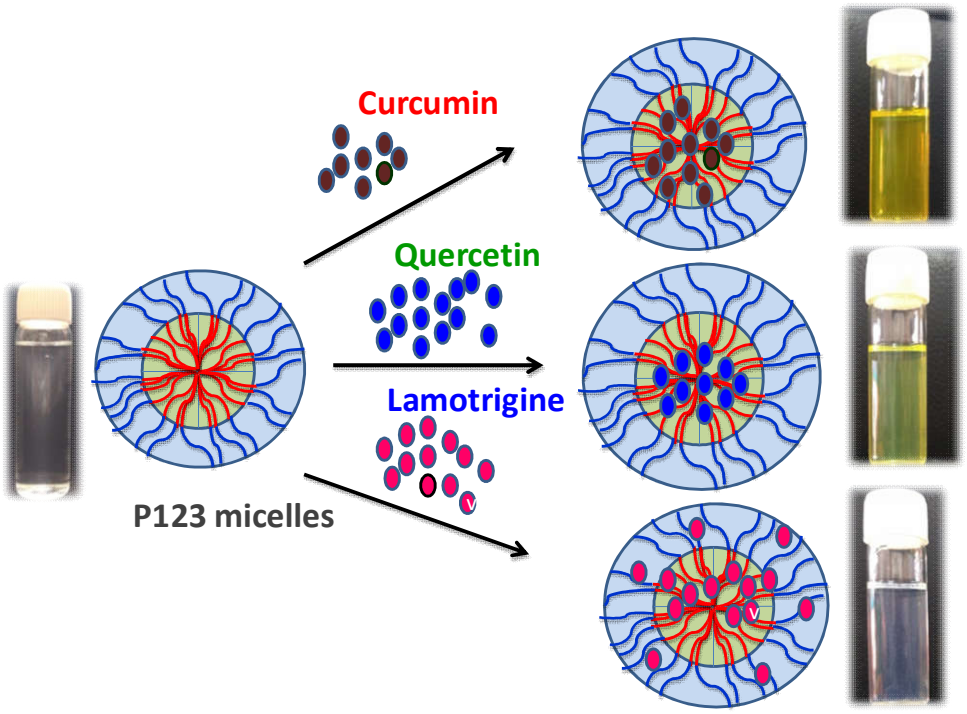
Self-assemblies of PEO-PPO-PEO triblock copolymeric systems for hydrophobic drugs of different polarity



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4.1: Introduction

More than the past twenty years, triblock copolymers of polyoxyethylene (POE) and polyoxypropylene (POP) with the structure of POE-POP-POE (generally known as Poloxamers of Pluronic or Synperonic) have received great attention in the field of nano-biotechnology [1-3]. These linear triblock copolymers, Pluronics®, are not much expensive and available commercially, few of which are FDA approved [4,5]. When Pluronic dissolved in water, it self-assembles into nano-sized spherical micelle, which is constructed with POP as hydrophobic inner core and POE as hydrophilic outer shell [6-8]. The main role of these polymers is to solubilize the hydrophobic drugs within the POP core of micelles as drug delivery nanovehicles. Another advantage of using POE as the hydrophilic shell of micelles shields and protects the drug present in the core from the outside medium [9-12]. The applicability of any Pluronic is mainly driven by their various micelle structures [13]. The micellar behavior of the Pluronics in water is dependent on the molecular constitution like the lengths of their POE parts and POP parts as well as their EO-PO ratio [14, 15]. Extensive studies were reported on Pluronic structures at different temperatures, concentrations and pH environments showing a large influence of molecular architecture on their phase behavior [16-19]. Recently, Pluronics are widely utilized to prepare thermodynamically stable micellar systems in aqueous media that are capable to solubilize insoluble pharmaceutically active ingredients [5,8] and lipophilic oils [20]. These solubilized water-insoluble compounds can also influence the structure of Pluronic through increasing the aggregation number, the micellar size and the volume fraction of micellized polymer [21-23].

Pluronics are applied to increase the bioavailability of various hydrophobic drugs and provide metabolic stability with increasing circulation time in the delivery at target [4,12,24]. This versatile behavior of Pluronics enables them to work as a scavenger for cardio-toxic drugs [25]. The solubilization of hydrophobic compounds in Pluronic micelles also found the tendency to form the micelles at a much lower critical micelle concentration (cmc) or critical micelle temperature (cmt) [26-28]. Hence the study on bioavailability of various hydrophobic drugs using Pluronic micelles are considering their basic and applied interests

We have focused here on three well-known pharmaceutical active ingredients, curcumin (CUR), quercetin (QCN) and lamotrigine (LTG). The molecular structure of the drugs is shown in Fig.4.1.

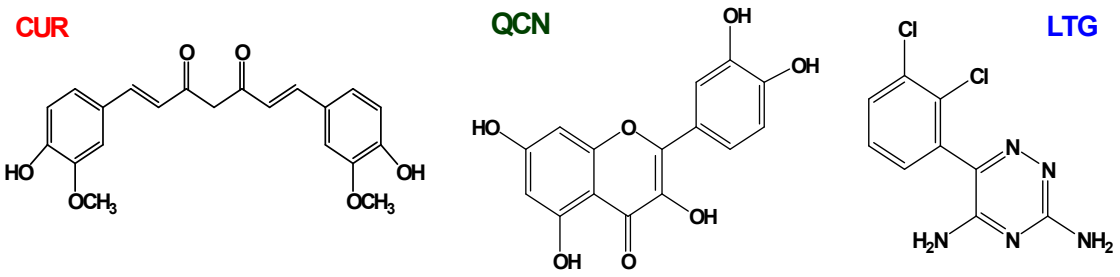

Fig.4.1: Molecular structures of CUR, QCN and LTG drug.

Despite its pharmacological effects, the therapeutic applications of these drugs (CUR, QCN, and LTG) are limited greatly due to its low solubility as 0.00034 mg/mL, 0.0004 mg/mL and 0.17 mg/mL, respectively in aqueous media [29-31]. Various Pluronics which contains different units of propylene oxide and ethylene oxide has been applied as a solubilizer for these three important pharmaceutical active ingredients. Amongst these Pluronics, the Pluronic P123, composed of $POE_{20}POP_{68}POE_{20}$, we found the best candidate to study in detail for formulations of these hydrophobic drugs. Pluronic P123 represents a novel type of nanocarrier that can increase solubility, improve circulation time and proper release drugs at the target sites. Two important features like high solubilization capacity and time-dependent micellar growth of Pluronic P123 micelles has attracted immense attention of researchers [32-37].

The encapsulation of hydrophobic drugs using Pluronic P123 micelles has prevented aggregation and improved the solubility of formulations like genistein [31], docetaxel[38], SCR7 [39], paclitaxel [31,40,41], hypericin [42], lamotrigine [43], ibuprofen [44], and quercetin [31,45].

Thus, taking this into consideration, we report here a systematic study showing the micellar characteristics and efficiency of Pluronic P123 micelles for CUR, QCN and LTG

drug. For the structural understanding, we systematic performed a structural characterization of these micelles by means of UV-Visible spectroscopy, dynamic light scattering (DLS), small angle neutron scattering (SANS), transmission electron microscopy (TEM) and cloud point temperature techniques. The in-vitro release profile, stability studies and antioxidant study of this drug-loaded Pluronic P123 micelles were also performed for better understanding of the systems. The results of such analytical characterization and biological evaluation then shall give the basis for a thorough understanding of these Pluronic micelles that can be applied to develop the most efficient drug delivery system in the future.

4.2: Experimental Section

4.2.1: Materials

4.2.1.1: Triblock copolymers (Pluronics)

Various POE-POP-POE triblock copolymers (Pluronics) with different ethylene oxide and propylene oxide composition were selected from Pluronic grid [3] and obtained from Sigma-Aldrich (St. Luice, MO, USA). They are listed in Table.1 which shows the trade name, molecular mass, hydrophilic-lipophilic balance (HLB), cloud point temperature and reported values of cmc, and cmt [46-52].

Table.4.1: Molecular characteristics of Pluronic polymers studied in present work.

Pluronic	M.W. (g mol^{-1})	nEO	nPO	%EO	CP(°C) @ 1wt%	HLB	CMC (wt%)	CMT (°C)
F127	12600	100	65	70	>100°	22	*0.02[47]	24°[46]
F88	11400	104	48	80	>100°	28	#1.7[46]	38°[46]
F68	8400	76	29	80	>100°	>24	#7.0[46]#	50°[46]
P123	5750	20	69	30	90°	8	0.001[46]#	16°[46]
L121	4400	5	68.2	10	14°	1	0.00044[48]*	15°[50]
L64	2900	13	30	40	58°	12-18	0.4[46]#	31.5°[46]
17R4	2650	24	14	40	46°	7-12	0.91[49]*	33-34°[51]
L35	1900	11	16	50	73°	19	10.07[48]*	60-70°[52]

#@room temperature,*@high temperature

4.2.1.2: Hydrophobic drugs

Two pharmaceutical compounds, curcumin (CUR) and quercetin (QCN) (both with the purity of ≥ 99%) were purchased from Sigma-Aldrich. Lamotrigine(LTG) drug was obtained as a gift sample from local suppliers and used with further purification.

Triple distilled water and all pyrex® glass apparatus were used in the study. The D_2O for SANS analysis are of Sigma-Aldrich (St. Luice, MO, USA) used as received. All the solvents and chemicals used are of HPLC grade.

4.2.2: Methods

4.2.2.1: Drug solubilization experiments for the ratiocination of Pluronics

The powdered drug was added in the aqueous solutions of various Pluronics with different-different concentration and equilibrated by continuous stirring for 24 h at room temperature (30°C). The unsolubilized drug was removed by filtration (Millipore PVDF syringe filter; 0.45µm pore size) and the amount of solubilized drug in the Pluronic micelles solutions was determined after 10 to 100 times dilution with methanol/ethanol solvent using UV–Visible spectroscopy (Shimadzu, UV-2450, Japan, double beam mode 200-800 nm). The solubility of the drug is evaluated by measuring its absorbance at the wavelength of 425 nm for CUR, 370 nm for QCN and 307 nm for LTG, respectively. To observe the solubility,the calibration curve had been prepared by measuring different concentrations of each drug in alcohol solvent in (Fig.4.2). All the measurements were made in triplicate.

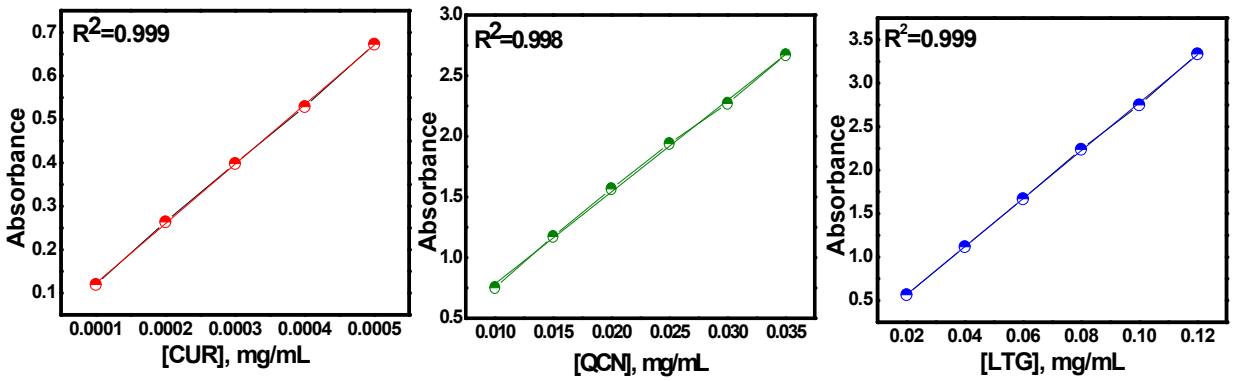

Fig.4.2: Calibration curve of studied hydrophobic drugs (diluted with methanol/ethanol) at 30° C.

4.2.2.2: Preparation of drug-loaded Pluronic P123 micelles

An appropriate amount of Pluronic P123 to obtain a fixed concentration of 5 wt% was dissolved in HPLC grade water. The desired drug was then added into the micellar solution of Pluronic P123 and stirred vigorously with a magnetic stirrer for 2 hrs at room temperature (30°C). The resulting drug-loaded Pluronic P123 micellar solutions were then sonicated for an hour and filtered through a 0.22 µm nylon filter to remove undissolved drug or

contaminants. The dispersion of drug-loaded Pluronic P123 micelles was further used for measurements. The scheme of the procedure is shown in Figure.4.3. The prepared drug-loaded Pluronic P123 micelles were coded as PLL, PLQ, and PLL for CUR, QCN and LTG, respectively.

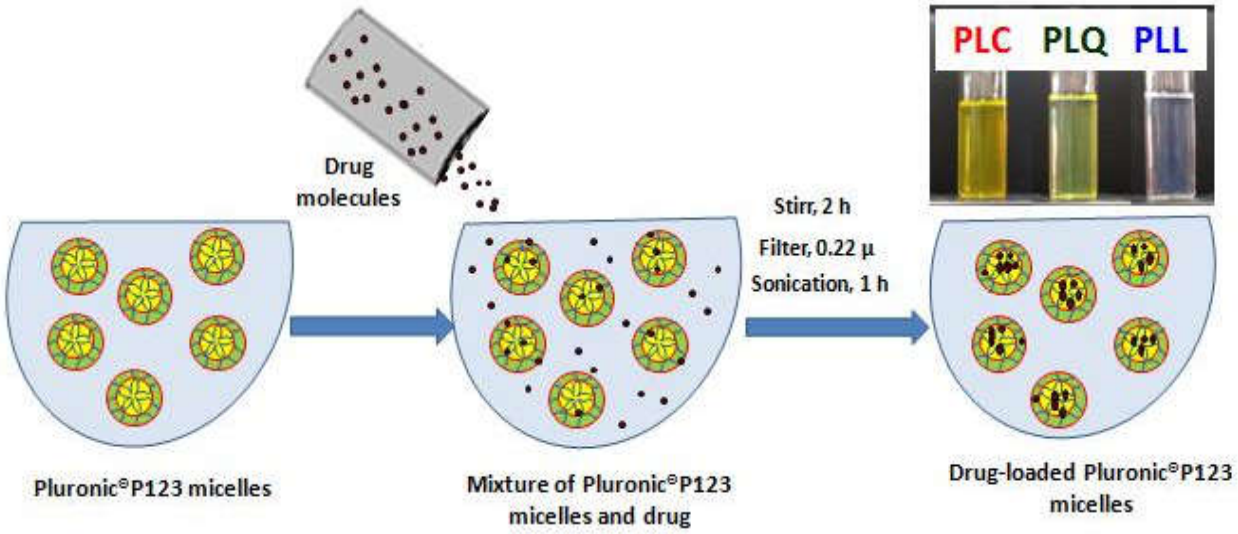

Fig.4.3: Schematic representation of the preparation of drug-loaded Pluronic P123 micelles.

4.2.3: Characterization of drug-loaded Pluronic P123 micelles

4.2.3.1: UV–VIS Spectroscopy

The UV–visible spectra of Pluronic P123 micelles, PLC, PLQ, and PLL micelles were measured using UV–Visible double beam spectrophotometer (Shimadzu, Japan, UV-2450) with a matched pair of stoppered fused silica cells of 1 cm optical path length.

4.2.3.2: Dynamic light scattering (DLS)

The average hydrodynamic sizes of the blank Pluronic P123 micelle and PLC, PLQ, and PLL were obtained through DLS measurements using a Zetasizer Nano-SZ 100 (Horiba) instrument. The scattering angle was kept at fixed 90°. Before the measurements, samples were properly filtered through Millipore PVDF syringe filter; 0.45µm pore size at room

temperature (30°C). Results are reported as the mean of multiple measurements up to ± 0.5 nm.

4.2.3.3: Small-angle neutron scattering (SANS)

SANS was performed for evaluation of sizes and shapes of PluronicP123 and drug-loaded Pluronic P123 micelles (PLC, PLQ, and PLL). These experiments were carried out at SANS diffractometer operating in Guard Tube laboratory at Dhruva Reactor, BARC, Mumbai, India. The mean incident neutron beam wavelength (λ) was 5.2 Å with a resolution (Δλ/λ) of about 10%. The scattered neutrons were searched in an angular range of 0.5–15° through a linear position sensitive detector (PSD). The scattering vectors 'Q' in the range of 0.015-0.3 $Å^{-1}$ of scattered neutrons were measured. The measured SANS data were corrected for the background, the empty cell contribution, and the transmission and were presented on an absolute scale with standard protocols. All the data were recorded at room temperature (30°C).

4.2.3.4: Cloud point temperature (CPT)

The cloud point temperatures were determined by gradually heating the blank Pluronic P123, PLC, PLQ, and PLL solutions in 20 mL thin glass vials dipped in a water bath. The solution temperature was increased steadily at the rate of 1°C min^{-1} with constant stirring using magnetic stirrer equipped with a heater. The average of appearance and disappearance of turbidity was taken as the cloud point temperature for the Pluronic micellar solution used. The process was repeated several times and the average temperature was taken as the CPT. The results were reproducible within ± 0.5°C.

4.2.3.5: Transmission electron microscopy (TEM)

The micelles morphology of Pluronic P123 and other three formulations PLC, PLQ, and PLL were evaluated by transmission electron microscopy using the transmission electron microscope (TEM, JEOL JEM-2100, Jeol Ltd., Japan) accelerating at a working voltage of 120kV. The sample solution drop was put on the carbon-coated copper grid (200 mesh) followed by drying for a few minutes (at RT). After that, fresh uranyl acetate solution drop was placed on the grid having the dried sample. At the same temperature, the grid was again dried.

4.2.4: In vitro release study of drug-loaded Pluronic P123 micelles

The amount of drugs released from drug-loaded Pluronic P123 micellar solutions was carried out using a dialysis bag method at body temperature (37°±0.1°C). Drug-loaded micellar solutions were placed into a pre-swelled dialysis bag (HIMEDIA®LA395 dialysis Membrane -110) with two ends fixed with a string. Each tube was then placed in 500 ml of release medium (20:80 methanol: water, pH 7.4) in the dissolution apparatus. A constant stirring (100 rpm) was maintained to prevent the formation of the stagnant layer at the membrane and outer solution interface. Care was taken to protect the drug against light during the experiments. The 4 mL aliquots were taken from the medium at fixed time intervals and replaced with an equal volume of fresh medium. This replenishing assured the maintenance of good sink conditions. The kinetics for release of all the drug from Pluronic P123 micelles was fitted by different mathematical models to understand the release mechanism and possible reason for drug release from the micellar systems. The equations used kinetic models are presented below.

Mathematical models reported by Thakkar et al[53] are used to understand drug release kinetics for the lamotrigine drug:

- *Zero-order model*: As per the pharmacokinetics, the release of drug from the dosage form can be presented by the equation:

$$C_0 - Ct = K_0 t$$

$$Ct = C_0 + K_0 t$$

where Ct is the amount of drug released at time t, C_0 is the initial concentration of drug at time t=0, K_0 is the zero-order rate constant. The process of constant drug release from a drug delivery system and drug level in the blood remains constant throughout the delivery is considered as zero order kinetics.

- *First order model*: The release of drug follows first-order kinetics are represented by the equation:

$$DC/dt = -K_1 C$$

Where, K_1 is the first order rate constant, expressed in time^{-1}. The first order process is the one whose rate is proportional to the concentration of drug. It follows linear kinetics. After modifing the above equation,

$$\log C = \log C_0 - K_1 t/2.303$$

where K_1 is the first order rate constant, C_0 is the initial concentration of the drug, C is the % drug remaining at time t. Hence to study the drug release kinetics data obtained from in-vitro dissolution study is plotted against time i.e., log % of drug remaining vs. time and the slope of the plot gives the first order rate constant.

- *Higuchi model*: The drug release involves both dissolution and diffusion. The basic Higuchi equation is represented by

$$Q = \sqrt{D (2 C_0 - Cs) Cs t}$$

where Q is the cumulative amount of drug released in time t per unit area, C_0 is the initial drug concentration, C_S is the drug solubility in the matrix and D is the diffusion coefficient of the drug molecule in the matrix. Higuchi equation can also be represented in the simplified form:

$$Q = K_H \times t^{1/2}$$

where K_H is the Higuchi constant.

The data obtained are plotted as % drug release versus square root of time. Therefore, the simple Higuchi model will result in a linear Q versus $t^{1/2}$ plot having a gradient, or slope, equal to K_H and considered as the matrix follows $t^{1/2}$ kinetics.

- *Korsmeyer-Peppas model*: Korsmeyer and Peppas gave a simple relationship which described the drug release from a polymeric system. $Mt/M_\infty = K_{kp} t^n$

where, Mt/M_∞ is a fraction of drug released at time t,

$$\log(Mt/M_\infty) = \log K_{kp} + n \log t,$$

Mt is the amount of drug released in time t, M_∞ is the amount of drug released after a time ∞, n is the drug release exponent, K_{kp} is the Korsmeyer release rate constant. To study release kinetics a graph is plotted between log(Mt /M∞) vs. log t [53].

4.2.5: Stability study of drug-loaded Pluronic P123 micelles

To assess the stability of each CUR, QCN, and LTG in the Pluronic P123 micelles was performed at room temperature. The drug-loaded Pluronic P123 micelles were stored in the closed dark chamber maintaining the temperature (30°±0.5°C) using thermostat. At

predetermined time of intervals up to 15 days, the amount of each drug in the PluronicP123 micellar solutions was determined by UV-Visible spectroscopy method.

4.2.6: Antioxidant activity of drug-loaded Pluronic P123 micelles

The antioxidant activities of two selected systems of curcumin and quercetin-loaded Pluronic P123 micelles were checked by measuring their ability to scavenge 2,2′-diphenyl-1-picrylhydrazyl (DPPH) stable radicals. Here, the solution of DPPH having a concentration of 0.1 mM DPPH in methanol was prepared and mixed with the tested samples with different concentrations (1, 2, 4, 6, 8 and 10 µg/ml) solution one by one. After that, this mixture was vigorously mixed and left over for 30 min in the dark, at room temperature for incubation. The color intensity of the mixture changed by the scavenging of free radicals from DPPH was measured by a spectrophotometer at 517 nm. The scavenging capacity of the tested samples was calculated by comparison of the color of the sample with the control. The experiments were carried out in triplicates. The % inhibition was calculated by using the following formula:

$$\% \text{ inhibition} = (A_0 - A_1)/A_0 \times 100$$

Where A_0= control and A_1= sample present

The IC_{50} value is calculated by plotting % inhibition and concentration curve and finding out the concentration of analyte at 50% inhibition of DPPH.

4.3: Results and discussion

4.3.1: Evaluation of solubility of CUR, QCN, and LTG drug

In our investigations, we systematically varied the molecular architecture of the Pluronics and studied its effect on the solubilization of curcumin, quercetin, and lamotrigine drug into the aqueous solution. Here, we also measured the solubility of these hydrophobic drugs at varied concentration with the range of 1 to 5 wt% of Pluronics and described those systems comprehensively.

The results of the solubility of the three different drugs (CUR, QCN, and LTG) in the aqueous solution of various Pluronics are presented in Fig. 4.4(a). The solubilization capacities of various Pluronics relative to the aqueous solubility of the respective drugs are also presented in Fig. 4.4(b). The solubility studies were carried out at fixed 5 wt% concentration of Pluronics in water. The different aggregation behavior of Pluronics can remarkably impact their performance. As the process of solubilization is a result of hosting the drug to the micelles, the changes in the cmc and the structure of micelles could significantly affect the capability of the polymers [54, 55]. Enhanced solubility of all the drugs was observed in the Pluronics L121, P123, F127, and L64 by virtue of their moderate hydrophobicity and the presence of micelles which have POP core with sufficient space to accommodate the drugs. The almost negligible or slight increase in the solubility was found with the Pluronics i.e. F88, F68, L35, and 17R4 for all the drugs, due to insufficient hydrophobicity and unmicellized form of the Pluronic molecules in the aqueous media. The solubilization of CUR, QCN and LTG drug follows the order: L121>P123>F127>L64>17R4>F68>F88>L35. The differences in the solubilizing ability of Pluronics are interpreted by their various structures rely on cmc and cmt. High cmt values with more than 30°C of Pluronics (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) [46].

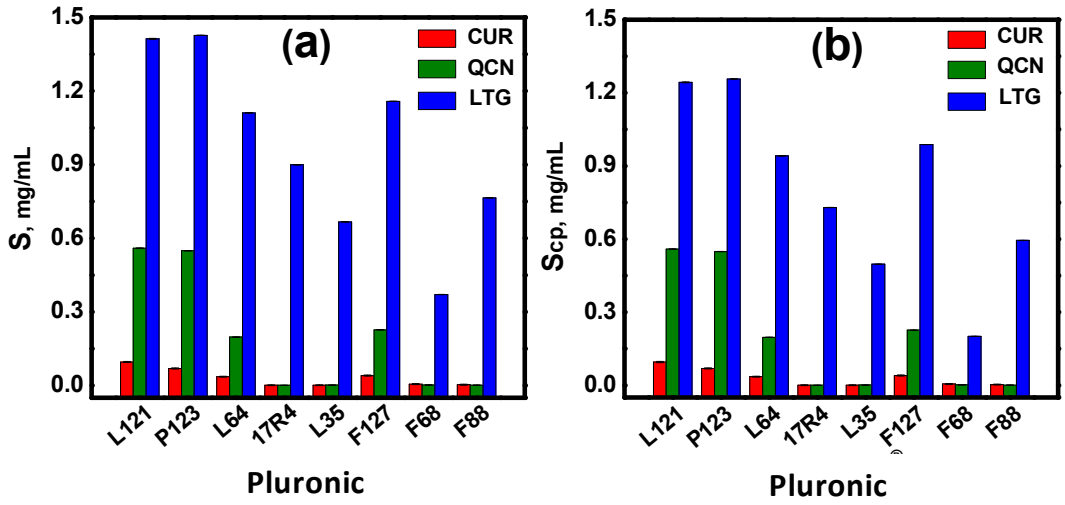

Fig.4.4: (a) solubility and (b)solubilization capacity of CUR, QCN, and LTG drug in various aqueous Pluronic solutions (5 wt% fixed concentration) at 30°C.

Fig.4.5 shows the solubilization of all the three drugs (CUR, QCN, and LTG) in the aqueous solution of various Pluronics at the different concentration from 1 wt% to 5 wt%. The solubility of all the drugs increases as the concentration of Pluronic increases, especially a remarkable increase for the two Pluronics, L121 and P123 was found. This can be attributed to the fact that solubilization requires the presence of micellar aggregates, which is only the case of high hydrophobic nature of the respective Pluronic. These Pluronics have the number of propylene oxide unit is of 68 for L121 and 69 for P123, respectively. These numbers show the high hydrophobicity of the Pluronics compared to all other studied Pluronics. Both the Pluronics has high surface activity as well as low cmc which make them better solubilizer of the hydrophobic drugs. In quantitative terms, 5 wt% Pluronic P123 solutions reveal a sharp solubility increase from the aqueous solubility of 0.00034 to 0.06954 mg/mL for CUR, 0.0004 to 0.5494 mg/mL for QCN and 0.1754 to 1.427 mg/mL for LTG. The results were representing the approximately 174-fold, 1375-fold and 8.4-fold increase, respectively. In case of 5 wt% Pluronic L121 solutions, solubility increase from the aqueous solubility of 0.0004 to 0.9597 mg/mL for CUR, 0.0004 to 0.5595 mg/mL for QCN and

0.1701 to1.413 mg/mL for LTG. It also represents the approximately 283-fold, 1400-fold and 8.4-fold increase, respectively.

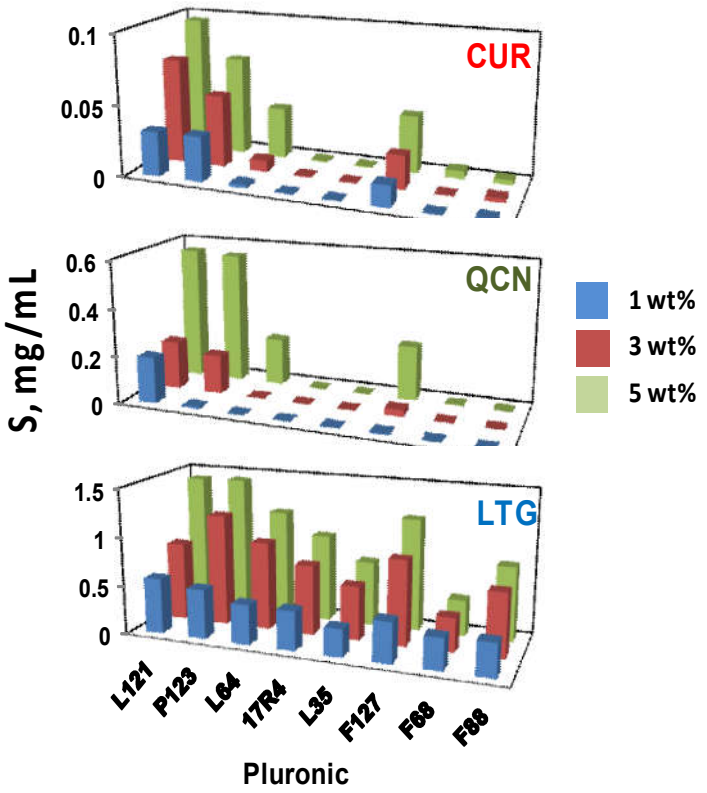

Fig.4.5: The solubility of CUR, QCN and LTG drug in different concentrations of Pluronic polymer solutions at 30°C.

According to the evaluation of solubility performance, the micelles Pluronic L121 and Pluronics P123 are the best choices for all the drugs, CUR, QCN and LTG. But the aqueous solutions of Pluronic L121 are very temperature sensitive. At below cmt, they dissolve as unimers, whereas at higher temperatures, above CPT (cloud point temperature), they very quickly form larger aggregates than vesicles. Fig.4.6 shows the clear images of Pluronics L121 at much-closed temperature range. The working window of the vesicles, i.e. between the cmt and the CPT, is rather small since the cmt is approximately 15°C and the CPT is almost very low nearby at 14°C [56]. This limits the potential use of Pluronics L121. Hence, further studies were limited to Pluronic P123 micelles only.

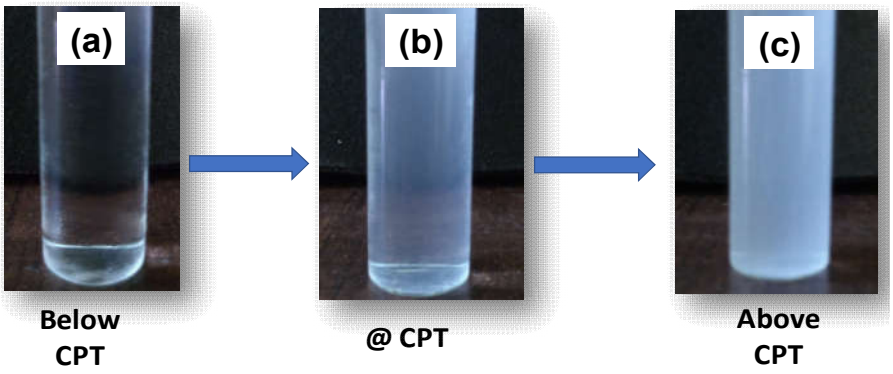

Fig.4.6: Images of Pluronic L121 (a) in the water at 10 °C; below its CPT and b) @ CPT,(C) above its CPT

4.3.2: Thermodynamics of solubility of CUR, QCN, and LTG in Pluronic P123 micelles

The results of solubility of drugs in Pluronic P123 micelles clearly indicate that the trend of solubility increase fold was better for QCN than CUR and low in case of LTG. As Pluronic P123 micelles are nonionic, but due to dissociation of the acidic phenolic group of QCT during its solubilisation in micelles the surface charge was developed. The anionic charge on Pluronic P123 micelles might have resulted in the increased solubility of QCN [57]. The aqueous solubility of LTG in water also reflected the hydrophilic nature of drug which ultimately found low fold increase in solubility for a micellar solution.

Table .4.2: Percentage enhancement and thermodynamic parameters of solubilized drugs in Pluronic P123 micellar systems at 30°C.

Drug	Conc.,(wt%)	% enhancement	P	ΔG^0 (KJ/mol)
CUR	wt%	79.00	91.764	-11.3
	3.0	127.0	148.02	-12.8
	5.0	174.0	203.52	-13.3
QCN	1.0	20.00	18.955	-7.4
	3.0	406.0	404.50	-15.5
	5.0	1374	1372.5	-18.2
LTG	1.0	3.000	1.9788	-1.70
	3.0	6.700	5.7352	-4.5
	5.0	8.400	7.3941	-5.0

The thermodynamic parameters of solubilization for all the drugs, CUR, QCN, and LTG in Pluronic P123 micelles have been investigated at room temperature and the partition coefficient(P) and standard Gibbs free energy(ΔG°) changes were calculated. Here, the partition coefficient(P) values are calculated using the equation;

$$\mathbf{P} = \frac{S - S_w}{S_w}$$

The P is determined as the ratio of drug solubility in the Pluronic P123 micelles (*S*) to the drug concentration in water (S_w).

The standard Gibbs free energy (ΔG°) of the system designates the spontaneity of the solubilization and determined using thee quation;

$$\triangle \mathbf{G}^{\circ} = -RT\,ln\,\mathbf{P}$$

where R is the gas constant, T is the working temperature (in Kelvin), and P is the partition coefficient [58-60]. The calculated values of P and ΔG° for all the micellar solutions of Pluronic P123 are listed in Table.4.2.

The partition of QCN, CUR, and LTG shows the increasing trend in Pluronics P123 micellar solutions. At 5 wt% concentrations, the partition of QCN is best amongst the CUR and LTG drugs. Data indicated that the less polar QCN drug attracted more towards the POP core of Pluronic P123 micelles. The values of ΔG° of solubilization of all the three drugs are found negative, which confirmed the spontaneous drug solubilization in the aqueous Pluronic micellar system. The increase in the hydrophobic character of the Pluronic P123 micellar solutions with concentration decreased the ΔG°, which favors its spontaneous solubilization as a more number of drug molecules can be incorporated into Pluronic P123 micelles.

4.3.3: Characterization of drug-loaded Pluronic P123 micelles

The drug-loaded Pluronic P123 micelles coded as PLC for CUR, PLQ for QCN and PLL for LTG drug was prepared through direct dissolution method. The PLC, PLQ, and PLL physically appeared as well drug dispersive solutions (shown inside of Fig.4.3). The structures of the PLC, PLQ, and PLL were characterized and successfully confirmed by multiple techniques like UV-VIS spectroscopy, DLS, SANS, CPT, and TEM.

The UV-Visible absorbance spectra of all the prepared drug-loaded Pluronic P123 micelles (PLC, PLQ, and PLL) is shown in Fig. 4.7(a). All the drug-loaded Pluronic P123 micelles gave intense peaks indicating that drug molecules are transformed from the bulk phase to the micellar phase. The trend for the intensity of loaded drug is as follows; PLQ > PLC > PLL. The drugs CUR, QCN, and LTG in water did not give any visible peaks due to its limited water solubility. The spectra again indicate the incorporation of the drug was better with the hydrophobic nature of the Pluronic P123 micellar solutions. The hydrophobicity of the PluronicP123 micellar solutions is increased with QCN molecules. Therefore, it gave a better intense peak in comparison to CUR and LTG.

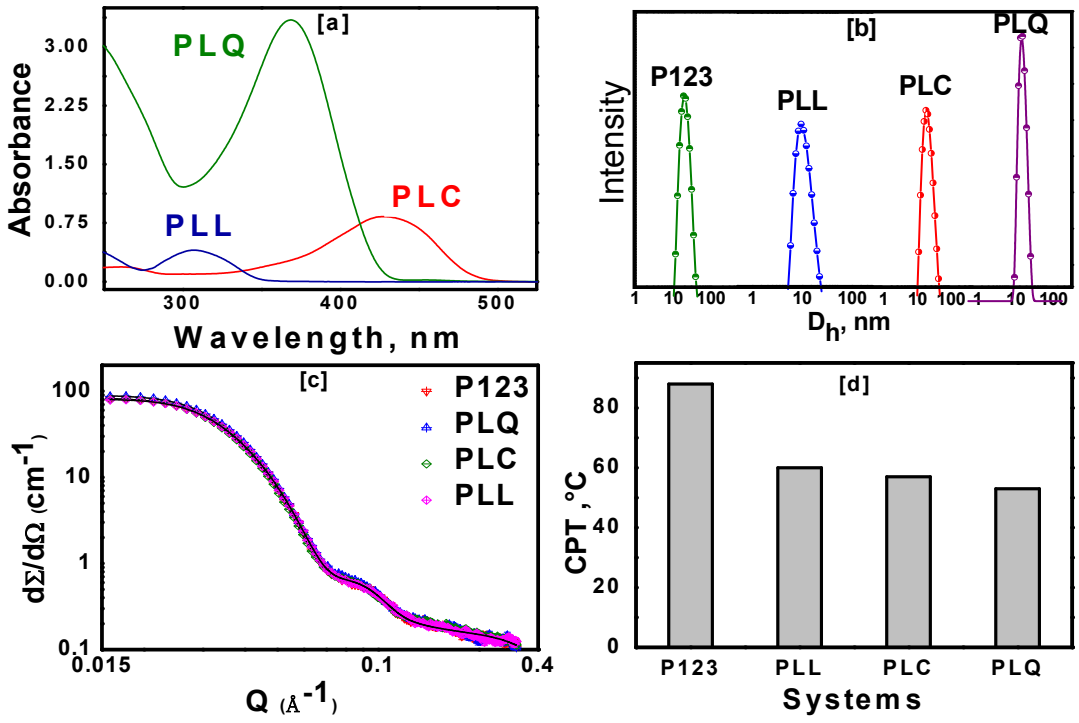

Fig.4.7: (a)UV-Visible absorbance spectra of PLC, PLQ and PLL in the water at room temperature (*The spectra were taken with 100 times dilution of PLC and PLL and 10 times of PLQ in respective solvents. The aliphatic PluronicP123 and drugs not shown any peak in the spectrum).***
(b) DLS study of drug-loaded Pluronic P123 micelles at 30°C.
(c) SANS study of drug-loaded Pluronic P123 micelles at 30°C.
(d) CPT of 5 % Pluronic P123 in absence and presence of drugs.

The hydrodynamic diameter (D_h) of the Pluronic P123 micelles with and without drugs at 30°C was determined using DLS technique and presented in Fig.4.7(b). The D_h of the Pluronic P123 micelle was 18 nm. The Dh of PLC, PLQ and PLL were 21.3, 22.5 and 18.6 nm, respectively. The overall results indicated that the D_h of the micelles is increased with the increase in the hydrophobicity of the systems. It has been noticed that the encapsulation of LTG drug in the PLL had not much effect on the overall sizes of the micelles but significant increment showed in the D_h of PLC and PLQ. It indicates the encapsulation of CUR and QCN was in the POP core of the PluronicP123 micelle. The DLS analysis of the drug-loaded Pluronics P123 micelles was indicated the small particle sizes of <23 nm, which is a great advantage for their further utilization in pharmaceutical formulations [39,40].

Table.4.3: SANS,DLS and CPT parameters PluronicP123 micellar systems at 30°C.

Pluronic micellar systems	CP (°C)	*Dh (nm)	R_c(Å)	PDI	R_{hs} (Å)	Volume fraction ϕ
P123	88°	18.0	65.2	0.17	93.7	0.06
PLL	60°	18.6	65.3	0.17	93.8	0.06
PLC	57°	21.3	66.0	0.17	95.1	0.07
PLQ	53°	22.5	65.5	0.17	93.8	0.06

* data obtained from DLS

Small angle neutron scattering (SANS) studies are carried out here to understand the change in the micellar structure upon solubilization of drug into the Pluronic micellar systems. SANS distribution curves for 5wt% of Pluronic P123 and drug-loaded Pluronics P123 micelles (PLC, PLQ, and PLL) in D_2O at 30°C were represented in Figure.4.7(c). The important micellar parameters mainly core radius (R_c), hard sphere radius (R_{hs}), volume fraction (ϕ) and polydispersity(δ) are determined from the SANS analysis and tabulated in Table.4.3. The SANS data clearly elaborates the information of Pluronic P123 micelle i.e. morphological shape, size and aggregation behavior in the presence and absence of drugs. The SANS intensity profile of Pluronic P123 and drug-loaded Pluronic P123 micelles (PLC,

PLQ, and PLL) shows the signatures of both form factor as well as structural factor governed scattered intensities. The Pluronic P123 micelles are found polydispersed with a spherical shape. The micellar core radius (R_c) of 65.2 Å for PluronicP123 was similar with reported values [49]. The Pluronic P123 has bigger R_c value due to as high hydrophobicity. In the SANS distribution curves of PLC, PLQ and PLL showed the higher scattering intensities than the pure Pluronic P123 micelles. The R_c values of drug-loaded Pluronic P123 micelles are increased from 0.1 nm to 0.8 nm, respectively. Here, such increment reflected the certain increased in the hydrophobic POP core size due to the incorporation of QCN and CUR drug in it. Very hydrophobic drugs like QCN and CUR have been found to be soluble in the POP cores of Pluronic micelles, which increase the diameter of the hydrophobic core. In contrast, low hydrophobic compounds like LTG drug have been shown to interact with the hydrophilic corona without a change in micellar diameters [61–63]. Therefore, our results imply that QCN and CUR may be located in the hydrophobic cores, while LTG may be mainly embedded in the hydrophilic coronas of the Pluronic P123 micelles. SANS results were completely correlated with the DLS results.

The CPTs of Pluronic P123, PLC, PLQ and PLL are shown in Fig.4.7(d). All the drug molecules showed a significant lowering of the CPT of the Pluronics P123. It has been observed that the QCN was decreased CPT more than the CUR and LTG drugs. It was found that the more hydrophobic drug increased the micellar core volume also seen from micellar size (Fig.4.7(b)) and ultimately decreases the CPT. Similar kinds of results were obtained by several other researchers [64-66]. The very low decrease in CPT of Pluronic P123 in case of PLL was due to the hydrophilic nature of the LTG drug.

Fig.4.8 shows the TEM images of Pluronic P123 micelles and drug-loaded Pluronic P123 micelles (PLC, PLQ, and PLL). TEM images clearly demonstrated that all the Pluronic P123 micelles were spherical in shape with quite a smooth surface and mono-dispersed. Earlier studies also reported that Pluronic micelles solubilized hydrophobic drugs like CUR, QCN, and LTG has spherical morphologies [44,45,67].

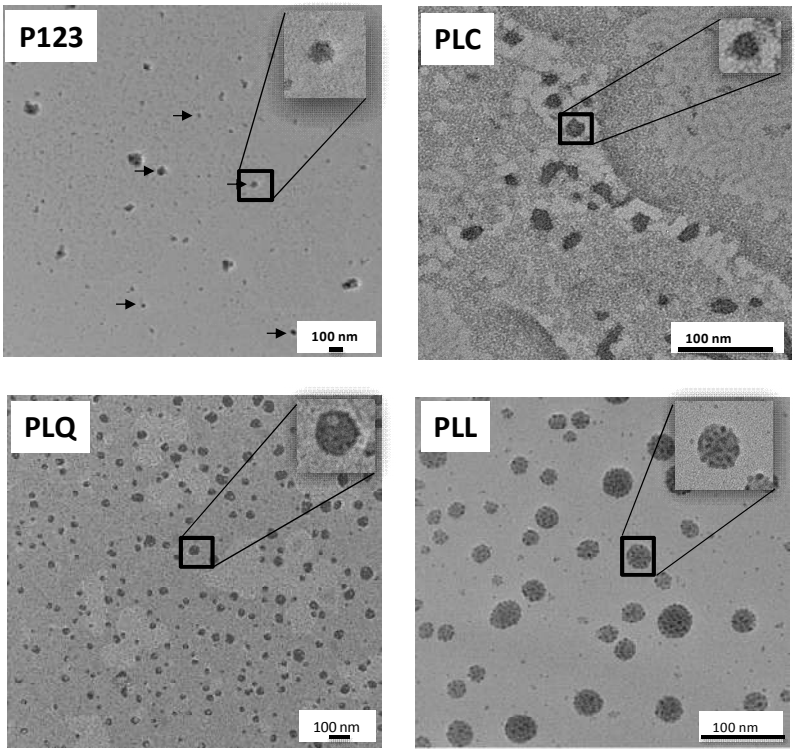

Fig.4.8: TEM images of drug-loaded Pluronic P123 micelles at 30°C.

4.3.4: In-vitro release study of drug-loaded Pluronic P123 micelles

The in vitro release behaviors drugs from drug-loaded Pluronics P123 micelles were investigated under physiological conditions (37°C). Fig.4.9 shows the In-Vitro release profile for drugs in PLC, PLQ, and PLL micelles. A typical two-phase release profile was observed (Fig.8). A rapid release in the initial stage followed by sustained and slow release over a prolonged time up to 15 days was observed. On comparing the release profiles, it can be estimated that LTG from PLL initial rate of release is quite high which later becomes slow and stable and release of CUR from PLC was slower initially which later increased gradually with time. Conversely, the release of QCN from PLQ remains slow throughout the time studied. In the case of LTG and CUR around 60% of drug released during the studied time period whereas for QCN around 30% of drugs release was observed. Overall, the release of these drugs was slow and sustained from Pluronics P123 micelles [57].

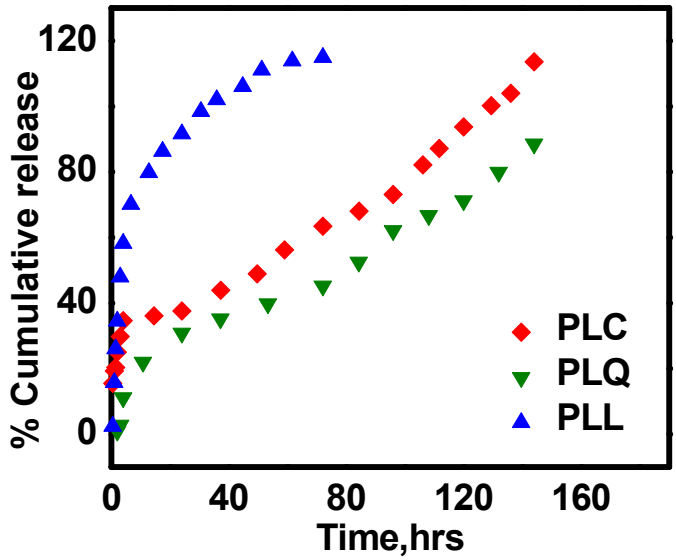

Fig.4.9: In vitro profile of drug-loaded Pluronic P123 micelles at 30°C.

The drug release data were analyzed using various mathematical models. The criterion for selecting the most appropriate model was based on the best goodness-of-fit (R^2 values). To predict the release pattern of the drug from the PluronicP123 micelles, the correlation coefficient and rate constants were calculated for all the models and reported in Tables 4.4. From the R^2 values, it was observed that the CUR and QCN drug release profiles best fit in the zero order equation. The LTG drug release profile was followed by the Higuchi equation. Highest drug release rate and maximum % drug release were observed for PLL.

Table.4.4: Release rate constants (K) and regression coefficients (R^2) for the PLC, PLQ and, PLL.

Release kinetic model	**PLC**		**PLQ**		**PLL**	
	K	***R^2***	***K***	***R^2***	***K***	***R^2***
Zero order	**0.59690**	**0.97471**	**0.61187**	**0.97301**	1.3128	0.72818
First order	0.00506	0.89599	0.00939	0.71529	0.0120	0.31436
Higuchi	7.20815	0.94403	8.37801	0.94004	**13.317**	**0.87162**

4.3.5: Stability study of PLC, PLQ, and PLL

Stability of PLC, PLQ and PLL were evaluated at room temperature over a period of 15 days which reveals that 85% to 90% of the drug was retained in the formulations. The drug-loaded Pluronics P123 micelles are stable up to 15 days which also proved the compatibility of these drugs with PluronicsP123 micelles. Hence, It can be anticipated that drug incorporated in more hydrophobic Pluronic micelles like PluronicsP123 micelles exhibit enormous potential as drug carriers in the area of nanomedicine.

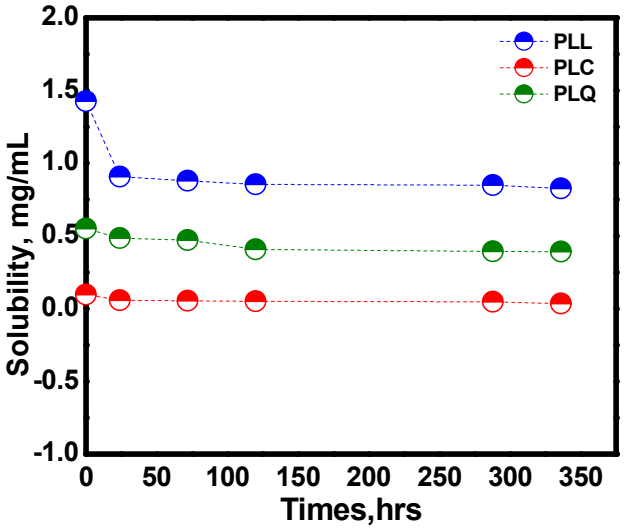

Fig.4.10: Storage stability of drug-loaded Pluronic P123 micelles at 30°C.

4.3.6: Anti-oxidant study of drug-loaded Pluronics P123 micelles

Oxidative stress is responsible for various types of diseases in our body because of the formation of free radicals. Antioxidant substances are always benefit in preventing the free radical hazards by scavenging and stabilizing the free radical [68,69]. Mostly, DPPH has been applied to determine the potential of antioxidant substances for scavenging the free radical. The deep purple color of DPPH radical is due to an odd electron present on the nitrogen atom of DPPH, shown at 517 nm. DPPH is a stable free radical that has the ability to abstract hydrogen from an electron-donating compound having a capacity to donate an atom by the antioxidant compound. When DPPH accepts the electron it's color changes from

purple to yellow as formed the nonradical DPPH in the reaction. A greater change in purple color of DPPH toward yellow showed the higher antioxidant potential of that compound. The antioxidant activities of two representatives drug-loaded Pluronics P123 micelles, PLC and PLQ, was performed by the DPPH method and presented in Fig.4.11(a&b). The PLC shows better antioxidant activity compared to pure CUR drug.

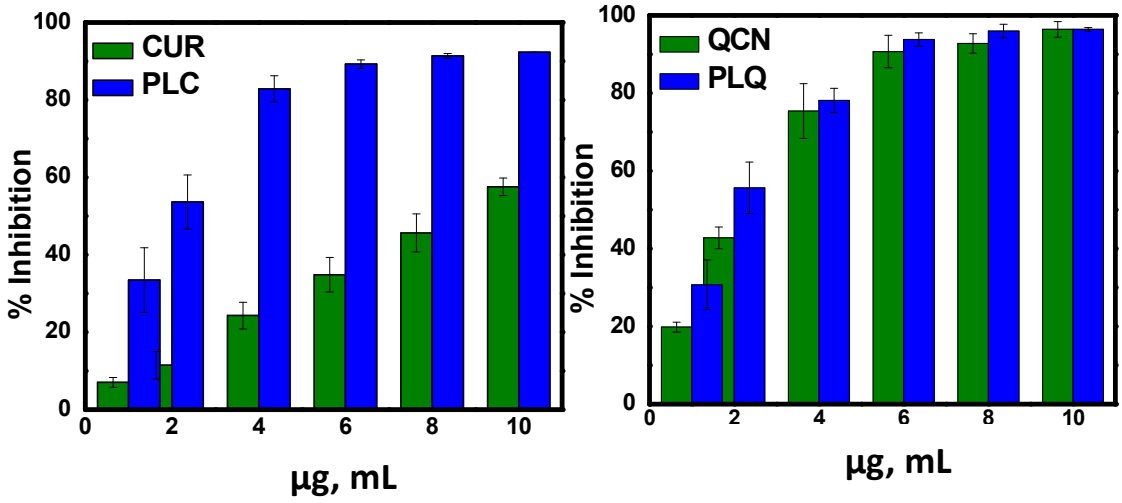

Fig.4.11: Antioxidant activity of CUR & QCN drug-loaded Pluronic P123 micelles at 30°C.

CUR protects against free radical damage because it is a strong antioxidant [69]. Results were in good agreement with the work related to antioxidant potential or DPPH scavenging of free curcumin [70]. As shown in Fig.4.11(b), other formulations PLQ was also observed the higher of antioxidant activity compared to pure QCN drug. Boots et al [71] explained that quercetin in many circumstances acts as a pro-oxidant known as "quercetin paradox". When quercetin scavenges free radicals, it is chemically converted into oxidation substances such as ortho-quinone/quinone methide intermediates. These substances display a high reactivity towards thiols which decrease some protein functions. The quercetin pre-treatment can protect DNA from oxidative damage also [71]. The studies of both the drug-loaded Pluronics P123 micelles, PLC and PLQ, were confirmed resistance to oxidation more significantly effective than the free drug. Such results motivate the further use of Pluronic polymers in the formulation of CUR and QCN drugs.

4.4: References

1) Almeida, M., Magalhaes, M., Veiga, F., Figueiras, A., Poloxamers, poloxamines and polymeric micelles: definition, structure and therapeutic applications in cancer. *J. Poly. Res.*, 25(1), **2018**, 31.
2) Batrakova, E.V., Bronich, T.K., Vetro, J.A., Kabanov, A.V., Polymer micelles as drug carriers. *Nanopart. Drug Carr.*,1, **2006**, 57-93.
3) Pitto-Barry, A., Barry, N.P., Pluronic block-copolymers in medicine: from chemical and biological versatility to rationalisation and clinical advances. *Poly. Chem.*, 5(10), **2014**, 3291-3297.
4) Batrakova, E.V., Kabanov, A.V., Pluronic block copolymers: evolution of drug delivery concept from inert nanocarriers to biological response modifiers. *J. Cont. Rel.,* 130(2), **2008**, 98-106.
5) Chiappetta, D.A., Sosnik, A., Poly (ethylene oxide)–poly (propylene oxide) block copolymer micelles as drug delivery agents: improved hydrosolubility, stability and bioavailability of drugs. *Eur. J. Pharma. Biopharma.*, 66(3), **2007**, 303-317.
6) Brown, W., Schillen, K., Almgren, M., Hvidt, S., Bahadur, P., Micelle and gel formation in a poly (ethylene oxide)/poly (propylene oxide)/poly (ethylene oxide) triblock copolymer in water solution: dynamic and static light scattering and oscillatory shear measurements. *J. Phys. Chem.,* 95(4), **1991**, 1850-1858.
7) Nakashima, K., Bahadur, P., Aggregation of water-soluble block copolymers in aqueous solutions: recent trends. *Adv. Colloid Int. Sci.*, 123, **2006**, 75-96.
8) Alexandridis, P., Poly (ethylene oxide)/poly (propylene oxide) block copolymer surfactants. *Cur. Opin. Colloid Int. Sci.*, 2(5), **1997**, 478-489.
9) Rösler, A., Vandermeulen, G.W., Klok, H.A., Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. *Adv. Drug Del. Rev.*, 64, **2012**, 270-279.
10) Allen, C., Maysinger, D., Eisenberg, A., Nano-engineering block copolymer aggregates for drug delivery. *Colloids Surf. B. Bio:*, 16(1-4), **1999**, 3-27.
11) Kazunori, K., Masayuki, Y., Teruo, O., Yasuhisa, S., Block copolymer micelles as vehicles for drug delivery. *J. Cont. Rel.*, 24(1-3),**1993**, 119-132.

12) Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y., Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. *J. Cont. Rel.*, 82(2-3), **2002**, 189-212.

13) Sezgin, Z., Yüksel, N., Baykara, T., Preparation and characterization of polymeric micelles for solubilization of poorly soluble anticancer drugs. *Eur. J. Pharma. Biopharma.*, 64(3), **2006**, 261-268.

14) Mortensen, K., Brown, W.Y.N., Poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) triblock copolymers in aqueous solution. The influence of relative block size. *Macromolecules*, 26(16), **1993**, 4128-4135.

15) Almgren, M., Brown, W., Hvidt, S., Self-aggregation and phase behavior of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) block copolymers in aqueous solution. *Colloid Poly. Sci.*, 273(1), **1995**, 2-15.

16) Mortensen, K., Pedersen, J.S., Structural study on the micelle formation of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) triblock copolymer in aqueous solution. *Macromolecules*, 26(4), **1993**, 805-812.

17) Linse, P., Malmsten, M., Temperature-dependent micellization in aqueous block copolymer solutions. *Macromolecules*, 25(20),**1992**, 5434-5439.

18) Basak, R., Bandyopadhyay, R., Encapsulation of hydrophobic drugs in Pluronic F127 micelles: effects of drug hydrophobicity, solution temperature, and pH. *Langmuir*, 29(13), **2013**, 4350-4356.

19) Wanka, G., Hoffmann, H., Ulbricht, W., Phase diagrams and aggregation behavior of poly (oxyethylene)-poly (oxypropylene)-poly (oxyethylene) triblock copolymers in aqueous solutions. *Macromolecules*, 27(15), **1994**, 4145-4159.

20) Liu, H.Y., Prévost, S., Gradzielski, M., Solubilisation of oils of different polarity in aqueous solutions of Pluronic triblock copolymers. *Zeit. Phy.Chemie*, 226(7-8), **2012**, 675-694.

21) Foster, B., Cosgrove, T., Hammouda, B., Pluronic triblock copolymer systems and their interactions with ibuprofen. *Langmuir*, 25(12), **2009**, 6760-6766.

22) Alexander, S., Cosgrove, T., Prescott, S.W., Castle, T.C., Flurbiprofen encapsulation using Pluronic triblock copolymers. *Langmuir*, 27(13), **2011**, 8054-8060.

23) Holmqvist, P., Alexandridis, P., Lindman, B., Modification of the microstructure in poloxamer block copolymer-water-"oil" systems by varying the "oil" type. *Macromolecules*, 30(22),**1997**, 6788-6797.

24) Alakhov, V.Y., Moskaleva, E.Y., Batrakova, E.V., Kabanov, A.V., Hypersensitization of multidrug resistant human ovarian carcinoma cells by pluronic P85 block copolymer. *Bioconjugate chem.*, 7(2),**1996**, 209-216.

25) Varshney, M., Morey, T.E., Shah, D.O., Flint, J.A., Moudgil, B.M., Seubert, C.N., Dennis, D.M., Pluronic microemulsions as nanoreservoirs for extraction of bupivacaine from normal saline. *J. Am. Chem. Soc.*, 126(16), **2004,** 5108-5112.

26) Patel, V., Dey, J., Ganguly, R., Kumar, S., Nath, S., Aswal, V.K., Bahadur, P., Solubilization of hydrophobic alcohols in aqueous Pluronic solutions: investigating the role of dehydration of the micellar core in tuning the restructuring and growth of Pluronic micelles. *Soft Matt.*, 9(31), **2013**, 7583-7591.

27) Sharp, M.A., Washington, C., Cosgrove, T., Solubilisation of model adjuvants by Pluronic block copolymers. *J. Colloid Int. Sci.*, 344(2), **2010**, 438-446.

28) Causse, J., Oberdisse, J., Jestin, J. and Lagerge, S., Small-Angle Neutron Scattering study of solubilization of tributyl phosphate in aqueous solutions of L64 Pluronic triblock copolymers. *Langmuir*, 26(20), **2010**, 15745-15753.

29) Singh, J., Garg, R., Gupta, G.D., Enhancement of solubility of lamotrigine by solid dispersion and development of orally disintegrating tablets using 32 full factorial design. *J. Pharma.*, ***2015***.

30) Yallapu, M.M., Jaggi, M., Chauhan, S.C., Curcumin nanoformulations: a future nanomedicine for cancer. *Drug Dis. Today*, 17(1-2), **2012**, 71-80.

31) Raval, A., Pillai, S.A., Bahadur, A., Bahadur, P., Systematic characterization of Pluronic micelles and their application for solubilization and in vitro release of some hydrophobic anticancer drugs. *J. Mol. Liq.*, 230, **2017**, 473-481.

32) Kadam, Y., Ganguly, R., Kumbhakar, M., Aswal, V.K., Hassan, P.A., Bahadur, P., Time dependent sphere-to-rod growth of the pluronic micelles: investigating the role of core and corona solvation in determining the micellar growth rate. *J. Phy. Chem. B*, 113(51), **2009**, 16296-16302.

33) Ganguly, R., Kumbhakar, M., Aswal, V.K., Time dependent growth of the block copolymer P123 micelles near cloud point: employing heat cycling as a tool to form kinetically stable wormlike micelles. *J. Phys. Chem. B.*, 113(28), **2009**, 9441-9446.

34) Patel, V., Dey, J., Ganguly, R., Kumar, S., Nath, S., Aswal, V.K., Bahadur, P., Solubilization of hydrophobic alcohols in aqueous Pluronic solutions: investigating the role of dehydration of the micellar core in tuning the restructuring and growth of Pluronic micelles. *Soft Matt.*, 9(31), **2013**, 7583-7591.

35) Bhattacharjee, J., Verma, G., Aswal, V.K., Patravale, V., Hassan, P.A., Microstructure, drug binding and cytotoxicity of Pluronic P123–aerosol OT mixed micelles. *RSC Adv.*, 3(45), 2013, 23080-23089.

36) Kadam, Y., Yerramilli, U., Bahadur, A., Solubilization of poorly water-soluble drug carbamezapine in Pluronic micelles: Effect of molecular characteristics, temperature and added salt on the solubilizing capacity. *Colloids Surf. B. Bio.*, 72(1),**2009**, 141-147.

37) Zhao, L.Y., Zhang, W., Recent progress in drug delivery of Pluronic P123: pharmaceutical perspective. *J. Drug Targ.*, 25(6), **2017**, 471-484.

38) Liu, Z., Liu, D., Wang, L., Zhang, J., Zhang, N., Docetaxel-loaded Pluronic P123 polymeric micelles: in vitro and in vivo evaluation. *Int. J. Mol. Sci.*, 12(3), **2011**, 1684-1696.

39) John, F., George, J., Vartak, S.V., Srivastava, M., Hassan, P.A., Aswal, V.K., Karki, S.S., Raghavan, S.C., Enhanced efficacy of pluronic copolymer micelle encapsulated SCR7 against cancer cell proliferation. *Macromol. Bio. Sci.*, 15(4), **2015**, 521-534.

40) Han, L.M., Guo, J., Zhang, L.J., Wang, Q.S., Fang, X.L., Pharmacokinetics and biodistribution of polymeric micelles of paclitaxel with Pluronic P123. *Acta Pharma. Sinica*, 27(6), **2006**, 747.

41) Wang, Y., Li, Y., Zhang, L., Fang, X., Pharmacokinetics and biodistribution of paclitaxel-loaded Pluronic P105 polymeric micelles. *Arch. Pharma. Res.*, 31(4), **2008**, 530-538.

42) Montanha, M.C., Silva, L.L., Pangoni, F.B.B., Cesar, G.B., Gonçalves, R.S., Caetano, W., Hioka, N., Tominaga, T.T., Consolaro, M.E.L., Diniz, A., Kimura, E., Response

surface method optimization of a novel Hypericin formulation in P123 micelles for colorectal cancer and antimicrobial photodynamic therapy. *J. Photochem. Photobio. B: Bio.*, 170, **2017**, 247-255.

43) Liu, J.S., Wang, J.H., Zhou, J., Tang, X.H., Xu, L., Shen, T., Wu, X.Y., Hong, Z., Enhanced brain delivery of lamotrigine with Pluronic P123-based nanocarrier. *Int. J. Nanomed.*, 9, **2014**, 3923.

44) Cao, S.W., Zhu, Y.J., Wu, J., Wang, K.W., Tang, Q.L., Preparation and sustained-release property of triblock copolymer/calcium phosphate nanocomposite as nanocarrier for hydrophobic drug. *Nano. Res. Lett.*, 5(4), **2010**, 781.

45) Zhao, L., Shi, Y., Zou, S., Sun, M., Li, L., Zhai, G., Formulation and in vitro evaluation of quercetin loaded polymeric micelles composed of Pluronic P123 and Da-tocopheryl polyethylene glycol succinate. *J. Biomed. Nanotech.*, 7(3), **2011**, 358-365.

46) Alexandridis, P., Holzwarth, J.F. and Hatton, T.A., Micellization of poly (ethylene oxide)-poly (propylene oxide)-poly (ethylene oxide) triblock copolymers in aqueous solutions: thermodynamics of copolymer association. *Macromolecules*, 27(9), **1994**, 2414-2425.

47) Perry, C.C., Sabir, T.S., Livingston, W.J., Milligan, J.R., Chen, Q., Maskiewicz, V., Boskovic, D.S., Fluorescence of commercial Pluronic F127 samples: temperature-dependent micellization. *J. Colloid Int. Sci.*, 354(2), **2011**, 662-669.

48) Kabanov, A.V., Batrakova, E.V. and Alakhov, V.Y., Pluronic block copolymers as novel polymer therapeutics for drug and gene delivery. *J. Cont. Rel.*, 82(2-3), **2002**, 189-212.

49) Chu, B., Structure and dynamics of block copolymer colloids. *Langmuir*, 11(2), **1995**, 414-421.

50) Li, F., de Haan, L.H., Marcelis, A.T., Leermakers, F.A., Stuart, M.A.C. and Sudhölter, E.J., Pluronic polymersomes stabilized by core cross-linked polymer micelles. *Soft Matt.*, 5(20), **2009**, 4042-4046.

51) Mustafina, A., Elistratova, J., Zakharova, L., Kudryashova, Y., Bochkova, O., Burilov, V., Konovalov, A., Soloveva, S., Diverse effect of POE–POP–POE and POP–POE–POP triblock copolymers on temperature responsive behavior of

luminescent hard–soft colloids. *Colloids Surf. A: Phys. Engg. Asp.*, 392(1), **2011**, 343-349.

52) De Neve, L., York, M., Dickens, J., Leys, J., Meurs, G., Sinnaeve, D., Van der Meeren, P., Molecular structure and ionic strength both affect the micellization and solubilization behavior of POE-POP-POE surfactants. *Colloids Surf. A: Phys. Engg. Asp.*, 536, **2018,** 172-179.

53) Thakkar, V.T., Shah, P.A., Soni, T.G., Parmar, M.Y., Gohel, M.C., Gandhi, T.R., Goodness-of-fit model-dependent approach for release kinetics of levofloxacin hemihydrates floating tablet. *Diss. Tech.*, 16(1), **2009**, 35-39.

54) Chiappetta, D.A., Facorro, G., de Celis, E.R., Sosnik, A., Synergistic encapsulation of the anti-HIV agent efavirenz within mixed poloxamine/poloxamer polymeric micelles. *Nanomed. Nanotech., Bio. Med.*, 7(5), **2011**, 624-637.

55) Chiappetta, D.A., Hocht, C., Taira, C., Sosnik, A., Efavirenz-loaded polymeric micelles for pediatric anti-HIV pharmacotherapy with significantly higher oral bioavailability. *Nanomedicine*, 5(1), **2010**, 11-23.

56) Li, F., de Haan, L.H., Marcelis, A.T., Leermakers, F.A., Stuart, M.A.C. and Sudhölter, E.J., Pluronic polymersomes stabilized by core cross-linked polymer micelles. *Soft Matt.*, *5*(20), **2009,** 4042-4046.

57) Patra, A., Satpathy, S., Shenoy, A.K., Bush, J.A., Kazi, M., Hussain, M.D., Formulation and evaluation of mixed polymeric micelles of quercetin for treatment of breast, ovarian, and multidrug resistant cancers. *Int. J. Nanomed.*, 13, **2018,** 2869.

58) Shaikh, S., Ray, D., Aswal, V.K., Sharma, R.K., Incorporation of Lamotrigine drug in the POE–POP–POE triblock copolymer (Pluronic F127) micelles: effect of hydrophilic polymers. *J. Surf. Det.*, 20(3), **2017**, 695-706.

59) Oh, K.T., Bronich, T.K., Kabanov, A.V., Micellar formulations for drug delivery based on mixtures of hydrophobic and hydrophilic Pluronic block copolymers. *J. Cont. Rel.*, 94(2-3), **2004**, 411-422.

60) Kadam, Y., Yerramilli, U., Bahadur, A., Bahadur, P., Micelles from POE–POP–POE block copolymers as nanocontainers for solubilization of a poorly water soluble drug hydrochlorothiazide. *Colloids Surf. B: Bio.*, 83(1), **2011**, 49-57.

61) Nagarajan, R., Solubilization of "guest" molecules into polymeric aggregates. *Poly. Adv. Tech.*, 12(1-2), **2001**, 23-43.

62) Ganguly, R., Kuperkar, K., Parekh, P., Aswal, V.K. Bahadur, P., Phenol solubilization in aqueous Pluronic solutions: Investigating the micellar growth and interaction as a function of Pluronic composition. *J. Colloid Int. Sci.*, 378(1), **2012**, 118-124.

63) Parekh, P., Singh, K., Marangoni, D.G., Aswal, V.K., Bahadur, P., Solubilization and location of phenol and benzene in a nonlinear amphiphilic EO–PO block copolymer micelles: 1H NMR and SANS studies. *Colloids Surf. A: Phys. Engg. Asp.*, 400, **2012**, 1-9.

64) Pandit, N., Trygstad, T., Croy, S., Bohorquez, M., Koch, C., Effect of salts on the micellization, clouding, and solubilization behavior of Pluronic F127 solutions. *J. Colloid Int. Sci.*, 222(2),**2000**, 213-220.

65) Ohashi, K., Hashizaki, K., Taguchi, H., Saito, Y., Effects of inorganic salts on micellization and solubilization in an aqueous solution of poly (ethylene oxide)/poly (propylene oxide)/poly (ethylene oxide) triblock copolymer. *J. Disp. Sci. Tech.*, 30(5), **2009**, 720-724.

66) Saito, Y., Sato, T., Effects of inorganic salts on solubilization of estriol in an aqueous solution of poly (ethylene oxide)/poly (propylene oxide)/poly (ethylene oxide) triblock copolymer. *Drug Dev. Ind. Pharma.*, 24(4),**1998**, 385-388.

67) Mohanty, C., Sahoo, S.K., The in vitro stability and in vivo pharmacokinetics of curcumin prepared as an aqueous nanoparticulate formulation. *Biomaterials*, 31(25), **2010**,.6597-6611.

68) Pandey, S., Sah, S.P., Sah, M.L. and Mishra, D., An antioxidant potential of hydromethanolic extract of urticaparvifloraroxb. *J. Basic Clin. Pharma.*, 1(3), **2010**, 191.

69) Parveen, A., Akash, M.S.H., Rehman, K., Kyunn, W.W., Recent investigations for discovery of natural antioxidants: a comprehensive review. *Critical Reviews™ in Eukaryotic Gene Expression*, 26(2),**2016,** 143-160.

70) Tan, C., Xie, J., Zhang, X., Cai, J., Xia, S., Polysaccharide-based nanoparticles by chitosan and gum arabic polyelectrolyte complexation as carriers for curcumin. *Food Hydrocolloids*, 57, **2016**, 236-245.

71) Boots, A.W., Li, H., Schins, R.P., Duffin, R., Heemskerk, J.W., Bast, A., Haenen, G.R., The quercetin paradox. *Tox. App. Pharma.*, 222(1), **2007**,89-96.