Mixed Pluronic self-assemblies for quercetin drug for anticancer evaluations

3.1: Introduction

Phytochemicals are naturally occurring components found in plants. The applications of phytochemicals derived from dietary ingredients have been a great scientific interest in pharmaceutical drug development as very useful to treat various ailments, especially cardiovascular diseases and cancer [1-3]. Specifically, the focus has been on multiple polyphenol dietary compounds.

QCN is a bioactive flavonoid that is in the glands located on the surface of leaves, flowers, or fruits of plants [4,5]. Chemically, QCN (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4H-chromen-4-one) consists of three aromatic rings with five hydroxyl groups, where the two rings I and II, are linked by oxygen-containing six-membered heterocyclic ring III. QCN has a wide spectrum of pharmacological and biological properties, including anti-carcinogenic, anti-diabetic, antiulcer, anti-inflammatory, antiviral, and anti-allergic effects [6,7]. As a potent antioxidant scavenges free radicals directly inhibits xanthine oxidase, alters antioxidant defense, and inhibits lipid peroxidation [8-11]. However, the application of QCN has been limited as a therapeutic molecule due to poor oral bioavailability because of low aqueous solubility. With low solubility, QCN shows the shortcomings of poor permeability, instability in physiological medium, short biological half-life, extensive first past metabolism before reaching systemic circulation [2,12].

Nowadays, many researchers have attempted to overcome these problems using polymer based carriers for the bioavailability of QCN [2,12]. Recent studies show that the incorporation of hydrophobic drugs in polymeric micelles is one of the most attractive approaches [13]. Polymeric micelles having many beneficial properties, like effectively solubilizing hydrophobic drugs, superior stability, longevity, biocompatibility, and changing the release profile of incorporated drug molecules [14]. Block copolymeric micelles with an amphiphilic polymer are core-shell structures with nanoscopic particle sizes (10-100 nm). The micellar inner core forms through hydrophobic part which incorporate water insoluble drugs, whereas the hydrophilic part serves as corona which maintains the stability of the block copolymeric micelles [15]. Block copolymeric micelles show greater thermodynamic and kinetic equilibrium even at low CMC and hence do not easily disassemble even upon

extreme dilution in parenteral administration. Its hydrophilic surface also prevents nonspecific uptake by the RES [16-18].

The most widely studied block copolymers are Pluronics made of PEO and PPO with a molecular arrangement of PEO-PPO-PEO. Depending on the molecular properties achieved by changing the PPO/PEO ratio and/or the various HLB values, they are commercially available as Poloxamers (Pluronic, BASF) [19,20]. Because of their minimal immunological response, many Pluronics have been approved as safe pharmaceutical excipients by the US-FDA and EMA. Although Pluronics are not biodegradable, pharmacokinetic data reveals their clearance from the kidney. Slight accumulation in the liver has been related to saturation in its ability to convert micelles into unimers [21-24]. Pluronics self-assemble in an aqueous medium to form dynamic yet thermodynamically stable core-shell micelles with size around 5 to 100 nm [25]. Pluronic micelles have shown high loading and solubilization capacity for hydrophobic drugs [26]. Besides, the morphology of micelles can be tuned to achieve desirable structural features. Numerous reports are published in literature dealing with the solubilization of hydrophobic drugs in the Pluronic micellar systems [27,28]. With low immunogenicity and a unique core-shell structure, Pluronic micelles are an attractive drug delivery carrier for topical and systemic administration. Bahadur et al. [29] has systematically reported the physicochemical and design aspects of Pluronic micelles relevant to targeted drug delivery to cancer cells. Micelles can be injected without any risks of embolism [30,31]. The hydrophobic core facilitates the incorporation of multiple hydrophobic drugs while the hydrophilic corona protects against aggregation, protein adsorption, and opsonization in the physiological milieu. Moreover, polarity changes along the core to shell enable the straight forward incorporation of solubilizates with a range of molecular characteristics [32]. The loading capacity can be improved by combining two or more Pluronics to obtain mixed Pluronic micellar systems. The former relies on the participation of Pluronic, hydrophobic blocks, in particular, to enable superior engagement of drug molecules. Similarly, filamentous micelles (filomicelles) offer higher drug loading and are much less susceptible to phagocytic clearance [33,34].

The present work was undertaken to study a mixed Pluronic micellar systems comprised of Pluronic P123 and F88 to increase QCN drug bioavailability. The CMCs of prepared mixed P123/F88 micelles were determined by UV-Vis method as pyrene probe. The

QCN-incorporated mixed P123/F88 micelles were prepared by thin film hydration technique and characterized using UV-Vis, DLS, TEM, SEM, SANS, FTIR, and DSC techniques. This work is also focused on *in-vitro* analysis of drug release profile, storage stability, antioxidant activity and cell proliferation of the mixed P123/F88 micellar systems for the QCN bioavailability.

3.2: Experimental Section

3.2.1: Preparation of the Mixed P123/F88 Micelles

The P123 (10% w/v) and F88 (10% w/v) stock solutions were made by dissolving the polymers in triple distilled water at cool conditions and keeping them overnight. The mixed P123/F88 micelles (1% w/v total concentration) were prepared by mixing the P123 stock solution with the F88 stock solution followed by dilution with water and equilibrating the prepared solutions at room temperature for at least 24 h before use. The mixed P123/F88 micellar systems with a polymer weight ratio of 1:2, 1:1, and 2:1 (shown in Table 3.1) were prepared for the investigations.

3.2.2: Characterization methods

CMCs of blank mixed P123/F88 micelles were determined using pyrene as a UV probe method. The physicochemical properties of the blank mixed P123/F88 micelles and QCN-incorporated mixed P123/F88 micelles were characterized using CPT, UV-Vis, DLS, TEM, SANS, FTIR, and DSC techniques. The detailed procedure of the all-mentioned techniques is shown in Chapter 2.

3.2.3: *In-vitro* release study of QCN–incorporated mixed P123/F88 micelles

The dialysis membrane bag diffusion method was used to determine the drug release profile of QCN drug and QCN-incorporated mixed P123/F88(2:1) micelles. The 3mL solution of QCN-incorporated P123/F88(2:1) micelles was introduced into the dialysis membrane and put into 100 mL of the mixture of PBS solution (pH=7.4) and methanol to a final ratio of 2:1. The drug release system was maintained at 30º±1ºC with constant stirring at 200±5 rpm. A fixed amount (5 mL) of the sample was taken from the release medium at regular specific time intervals, and an equal amount of freshly prepared media was replenished. The collected samples were filtered through a 0.45 µm syringe filter before quantifying the QCN release amount using a UV-Vis spectrophotometer at 369 nm (λ_{max} for QCN). The pattern of QCN drug release was determined using a solution of propylene glycol acting as a release medium under same conditions of drug incorporated micelles. All samples were performed in triplicate.

3.2.4: *In-vitro* antioxidant activity of QCN-incorporated mixed P123/F88 micelles

In this experiment, the fixed amount of DPPH (0.1 mmol/L, 2.0 mL) was added as an influential free radical to test the antioxidant activity of QCN drug and QCN-incorporated mixed P123/F88(2:1) micelles with a variety of concentrations (1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 µg/mL) [38]. The sample solutions were vigorously mixed before measurement, and the mixtures were placed for 15 min in a dark condition at RT. At 516 nm, the samples were then analyzed through UV-Vis spectroscopy. The experiments were performed in triplicates. The scavenging rate was calculated using the following equation;

$$\text{Scavenging rate} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\% \ldots..(1)$$

3.2.5: *In-vitro* cell proliferation activity studies

The stock concentration of 130 µg/mL of mixed P123/F88(2:1) micelles and QCN-incorporated mixed P123/F88(2:1) micelles was freshly prepared in the DPBS solutions. The QCN was freshly prepared in DMSO with a stock concentration of 5 mg/mL. The MCF-7 cells were cultured in DMEM medium supplemented with 10% FBS and 1% PSN. The cells were cultured in a humidified condition of 5% CO_2 at 30°C. Exponentially growing MCF-7 were treated with QCN, mixed P123/F88(2:1) micelles, and QCN-incorporated mixed P123/F88(2:1) micelles with different-different concentrations (1, 2.5, 5, 10, 20, and 30 µg/mL) for 24 h. The control was taken as untreated cells in 0.6 %w/v DMSO.

The half-maximal inhibitory concentration (IC_{50}) was determined by the MTT assay method [39]. Briefly, $1.0x10^4$ MCF-7 cells were treated with prepared1.0, 2.5, 5.0, 10.0, 20.0, and 30.0 µg/mL of QCN, mixed P123/F88(2:1) micelles, and QCN-incorporated mixed P123/F88(2:1) micelles for 24 h. After that, the cells were washed with DPBS and incubated with MTT (5.0 mg/mL) for 4 h at 30˚C in a dark environment. After incubation, the MTT was removed, and DMSO was added to each well. The absorbance was recorded at 570 nm with a reference wavelength of 650 nm using a Multimode microplate reader (SpectraMax $M2^e$, Molecular Devices, USA). The results were represented as a percentage of cell proliferation, and the IC_{50} value was evaluated.

The represented data was analyzed by student t-test using Sigma Stat 2.0 statistical analysis software. The Shapiro-Wilk test tested the normality of data before the student t-test. p values **$p \leq 0.01$, ***$p \leq 0.001$ were considered statistically significant.

3.3: Results and discussion

3.3.1: Self-aggregation of mixed P123/F88 micelles

Mixed Pluronic micellar system has demonstrated great potential as novel drug delivery carrier for parental administration to overcome the obstacles of inadequate aqueous solubility and oral bioavailability.

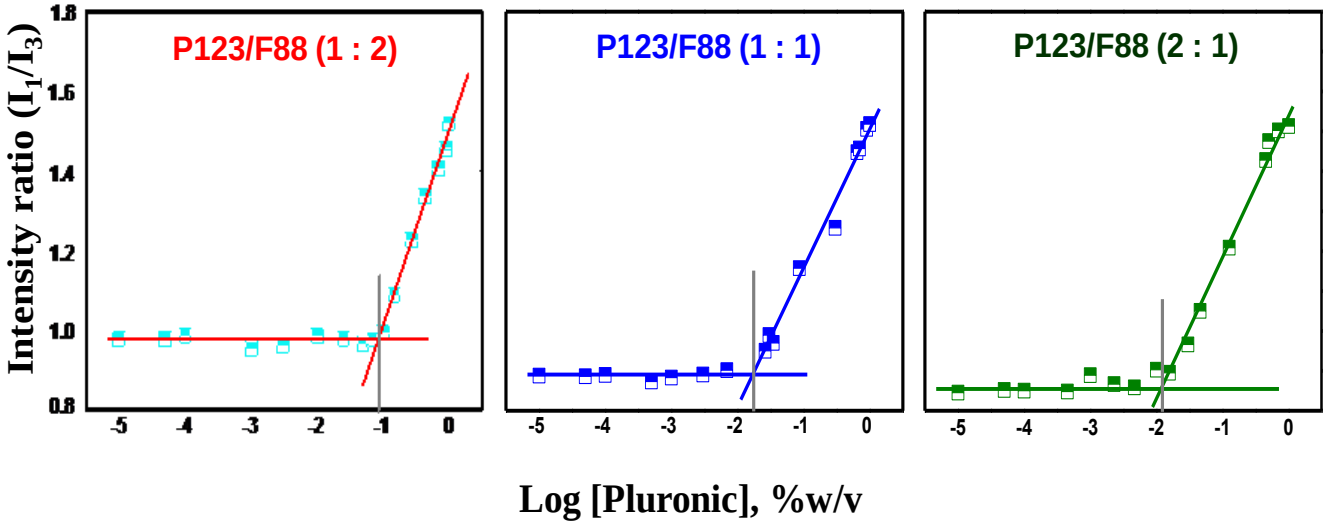

Figure 3.1: Plot of Intensity ratio (I_1/I_3) against Log concentration of mixed Pluronic polymers in water at 30°C

In the current study, Pluronic P123 was used to produce mixed micelles with Pluronic F88 as potential nanocarriers for QCN drug. The linear thermo-responsive copolymer P123 is highly hydrophobic, while F88 is highly hydrophilic in nature. The self-aggregation in terms of CMC for prepared mixed P123/F88 micellar systems with the weight ratio of 1:2, 1:1, and 2:1 were investigated using UV-Vis spectroscopy with pyrene as a probe [35]. Figure 3.2 presents the plot of intensity ratio (I_1/I_3) against Log concentration of the mixed P123/F88 solutions, and the inflection point was considered as the CMC. The CMC values were presented in Table1. The CMC values of mixed P123/F88 micellar systems with a polymer weight ratio of 1:2, 1:1, and 2:1 were found to be 0.1723%w/v, 0.0838%w/v, and 0.0091%w/v, respectively. These low CMC values of mixed micelles show high stability and anti-dilution properties, favoring drug delivery applications [36].

3.3.2: CPT of mixed P123/F88 micelles

CPT is the temperature at which a homogenous dispersion of amphiphilic compounds in water separates into a surfactant-rich and a surfactant-poor phase [37]. At higher temperatures, the CPT is obtained by dehydrating the hydrophilic moieties of nonionic surfactants..Therefore, it is to be expected that the CPT of mixed micellar systems considerably differs from that of single micelles. Table 3.1 summarizes the CPT values examined for single and mixed P123/F88 micelles. P123 showed a low value of CPT (90.5°C) due to the high hydrophobicity and oppositely, the high CPT value (>100°C) displayed by F88 due to the high content of the hydrophilic part [19]. The CPT values were found >100°C for P123/F88(1:2), 98°C for P123/F88(1:1), and 95.5°C for P123/F88(2:1) micellar solutions. The CPT values of mixed micelles were higher than the single P123 micelles, indicating the hydrophilic nature of the mixture. It should be noted that all micellar systems showed one single CPT, indicating the formation of a mixed micellar system [38].

Table: 3.1. Composition of Pluronics, CMC and CPT values of the studied systems.

Pluronic®	**Mixture composition**	**% Composition P123:F88**	**Code name**	**CMC, %w/v**	**CPT, °C**
P123	--	1.0	P123	0.001*	90.5
P123:F88	1:2	0.3:0.7	P123/F88(1:2)	0.0838	>100
P123:F88	1:1	0.5:0.5	P123/F88(1:1)	0.01723	98
P123:F88	2:1	0.7:0.3	P123/F88(2:1)	0.009192	95.5
F88	--	1.0	F88	1.7*	>100

** from Ref.25*

3.3.3: Size and zeta potential of QCN drug-free mixed P123/F88 micelles

The particle size and surface properties of nano-vehicles may influence the rate of absorption in the GI tract when administered orally [39]. The mean hydrodynamic diameter (D_h) and size distributions of prepared mixed P123/F88 micellar systems with the weight ratio of 1:2, 1:1, and 2:1 without QCN drug were characterized through the DLS technique (Figure 3.2 and Table 3.2). All the mixed micelles showed a unimodal distribution with D_h values of 21.54 nm for P123/F88(1:2), 20.90 nm for P123/F88(1:1), and 20.53 nm for P123/F88(2:1), respectively. All the micelle systems showed a small PDI value in the range

of 0.1 to 0.25, which considered the complete micellization. Results confirm that the increase in P123 weight fraction decreases the D_h of mixed P123/F88 micellar systems because of strong interactions between the hydrophobic and hydrophobic PPO core of P123. These results were in line with the CMC and CPT values observed (listed in Table 3.1).

Table: 3.2. Hydrodynamic diameter (D_h), zeta (ζ) potential, PDI, core radius (R_c), and radius of gyration (R_g) values of mixed P123/F88 micelles in absence and presence of QCN drug in water at 30°C.

System	D_h, nm*	ζ potential, mV*	PDI*	R_c, nm#	R_g nm#	Shape#$
P123/F88(1:2)	21.54	-5.08	0.235	-	-	-
QCN-incorporated P123/F88(1:2)	56.70	-7.30	0.376	-	-	-
P123/F88(1:1)	20.90	-10.60	0.208	-	-	-
QCN-incorporated P123/F88(1 :1)	24.85	-15.1	0.283	-	-	-
P123/F88(2:1)	20.53	-12.1	0.171	6.56	1.50	Spherical
QCN-incorporated P123/F88(2:1)	21.85	-16.2	0.233	6.70	1.50	Spherical

*Data obtained from *DLS, #SANS, and $TEM*

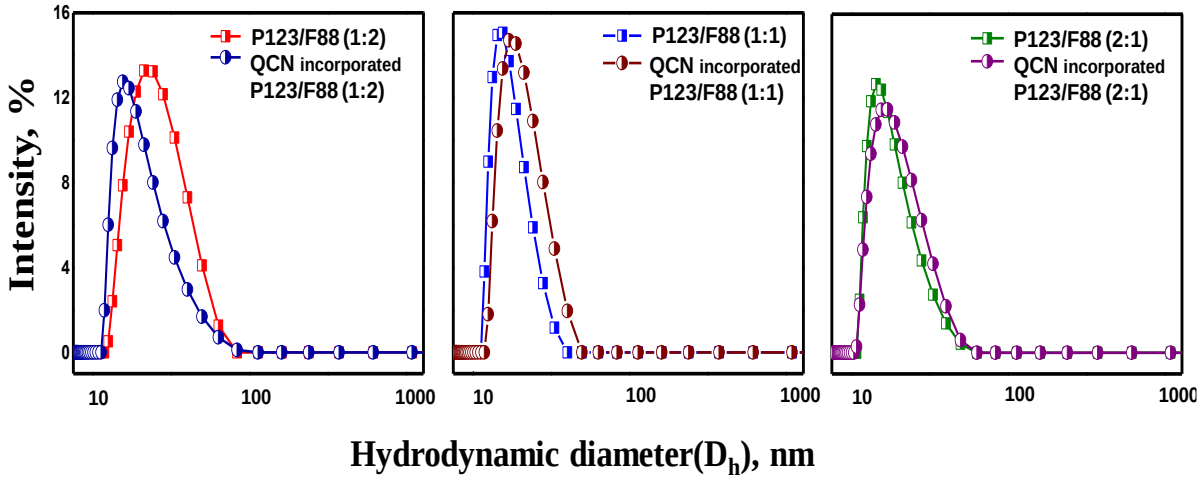

Figure 3.2: Micellar size and size distribution graphs of mixed P123/F88 micelles in absence and presence of QCN drug at 30°±0.5° C. Results are expressed as mean± S.D (n=3).

The storage stability of colloid dispersion systems is greatly influenced by the ζ potential of particles. Particle aggregation is quite probable if the ζ potential of the particles is very low to create enough electric repulsion or steric barriers. [40]. The ζ potential values of all the micelles were negative as generally found for uncharged PEO amphiphilic

copolymers. The ζ potential analysis of blank mixedP123/F88 micelles showed the negatively charged surfaces with values of -5.08 mV for P123/F88(1:2), -10.6 mV for P123/F88(1:1), and -12.1 mV for P123/F88(2:1), respectively. The negative value of the ζ potential indicates the better stability of the micelles (Figure 3.2 and Table 3.2)**.** According to the ζ potential values of mixed micelles, the mixed P123/F88(2:1) micelle was the most stable.

3.3.4: Solubilization of QCN drug in the mixed P123/F88 micelles

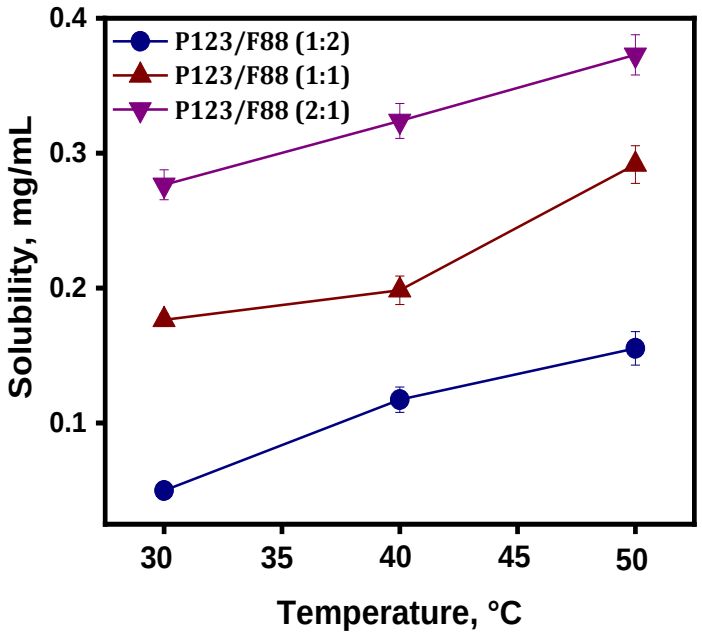

Figure 3.4: Solubility of QCN drug in the aqueous mixed P123/F88 micellar solutions at different temperatures.

Polymer micelles' ability to increase the water solubility of hydrophobic molecules is related to their hydrophobic core, which provides an appropriate microenvironment for a hydrophobic solute. Generally, temperature plays a vital role in the micellization and solubilization capacity of Pluronics [41]. Solubility of QCN in the mixed P123/F88 micellar systems with the weight ratio of 1:2, 1:1, and 2:1 was investigated using UV-Vis spectroscopy. Figure 5 shows the solubility of QCN in aqueous mixed P123/F88 micellar solutions at various temperatures. The solubilization of drugs in a micellar system increases as the temperature rises due to micellar expansion. The effects of mixed polymeric micelles

produced with P123 on increasing QCN solubility have previously been reported [42]. The data clearly showed that increasing the temperature increases the thermal vibration of the Pluronic molecules incorporated in the mixed micelle, thus increasing the space available for drug solubilization. The results show that the QCN-incorporated mixed P123/F88(2:1) micelles perform much better for QCN drug solubilization.

3.3.5: Characterization of QCN-incorporated mixed P123/F88 micelles

The mean hydrodynamic diameter (D_h) of the QCN-incorporated mixed P123/F88 micelles (weight ratio of 1:1, 1:2, and 2:1) at 30°C was determined using the DLS technique and presented in Figure 3.2. All of the QCN-incorporated mixed P123/F88 micelles had a unimodal distribution, with D_h values of 56.70 nm for P123/F88(1:2), 24.85 nm for P123/F88(1:1), and 21.85 nm for P123/F88(2:1), respectively. Here, the D_h values of QCN-incorporated mixed P123/F88 micelles were somewhat higher than those of drug-free mixed P123/F88 micelles. In mixed systems, the weight fraction of P123 first makes the Dh go up and then makes it go down. This is because the PPO core of P123 has strong hydrophobic-hydrophobic interactions. It was also observed that QCN solubility in the mixed micelles might have a little impact on the total micelle sizes. These results demonstrated that the increment of QCN in the micelles improves the self-aggregation tendency of the mixed micelles. The ζ potential values for every mixed P123/F88 micelle were negative, in a similar manner to the drug-free mixed micelles (Table 3.2)**.** The ζ potential values for QCN-incorporated mixed P123/F88(2:1) micelles found the highest negative value indicates good stability of the drug in this nanomicellar system.

Taking into account the QCN solubilization, particle size, size distribution, and ζ potential characterizations of the mixed P123/F88 micelles, we have chosen the QCN-incorporated mixed P123/F88(2:1) micelles as the representative system for further investigation of morphological, compatibility and *in-vitro* biological evaluation.

TEM and SEM are useful methods to visualize the morphology of micelles. The QCN-incorporated mixed P123/F88(2:1) micelle produced uniform nanometric sphere, as shown in the TEM image (Figure 3.5(a))**.** The QCN-incorporated mixed P123/F88(2:1) micelles showed the size of the nanosphere is about 19.8 nm. In general, the outer shell was formed by the PEO moieties of a spherical mixed Pluronic micelle, and the inner core

consisted of hydrophobic PPO moieties of mixed micelle and drug. In addition, the micelles showed good formability and uniform dispersion without much aggregation. SEM analysis also confirmed the spherical morphology of the QCN-incorporated mixed P123/F88(2:1) micelles (Figure 3.5 (b))**.** As appeared in the TEM & SEM micrograph, the mean size of the micelles was in good agreement with the size obtained from DLS.

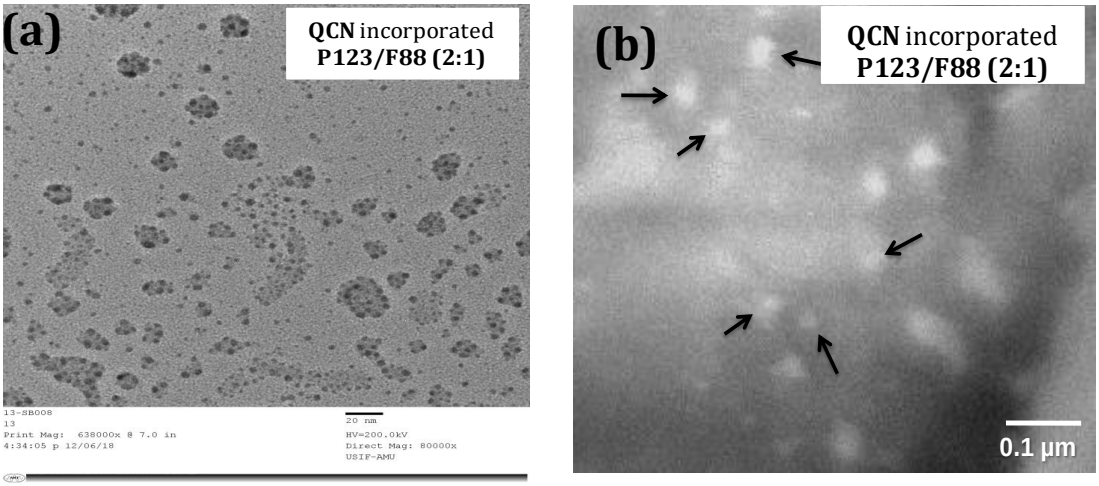

Figure 3.5: (a)TEM image of QCN-incorporated P123/F88(2:1)micellar system, and**(b)** SEM image of QCN-incorporated P123/F88(2:1)micellar system

The SANS profile of the mixed Pluronic P123/F88(2:1) micelles and QCN-incorporated mixed P123/F88(2:1) micelles in D_2O at 30°C were examined and shown in Figure 3.6. The mean core radius(R_c), polydispersity(δ), and radius of gyration(R_g) were determined as the fitting parameters from the analysis. The R_c of mixed P123/F88(2:1) micelles and QCN-incorporated mixed P123/F88(2:1) micelles were 6.57 nm and 6.7 nm, respectively. The mixed P123/F88 micelles with and without QCN drug showed almost similar scattering intensities in the SANS distribution curve. Here, even though the incorporation of QCN molecules in the mixed P123/F88 micelles, the number density of the micelles had not much changed as the number of drug molecules in the micelles was significantly lower than the mixed P123/F88 micellar core. The morphology of mixed P123/F88(2:1) micelles and QCN-incorporated mixed P123/F88(2:1) micelles were found to be spherical in shape. The SANS results were matched with the DLS and TEM.

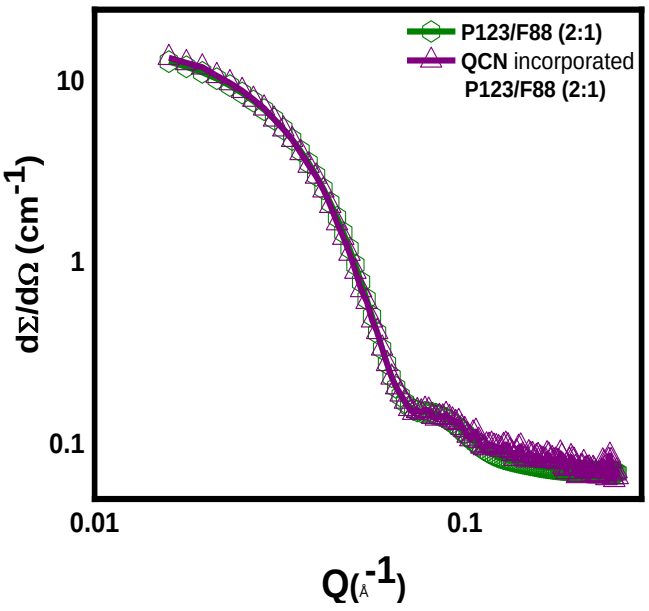

Figure 3.6: SANS profile of mixed P123/F88(2:1) and QCN-incorporated mixed P123/F88(2:1) micellar system at 30°C.

3.3.6: Compatibility of QCN drug with mixed P123/F88 micelles

To confirm the encapsulation of QCN in mixed P123/F88 micelles, FTIR spectroscopy analyses were performed. The IR spectra of pure QCN, mixed P123/F88 micelles, and QCN-incorporated mixed P123/F88(2:1) micelles are shown in Figure 3.7. In all three samples, a major peak is found around 3400-3500 cm^{-1} with a minor difference in wave number due to intermolecular interactions. The spectrum of QCN had a wide O-H stretching vibration in the region 3200-3400 cm^{-1}, a carbonyl C=O stretching vibration at 1660 cm^{-1}, and a C-O-C ether vibration at 1110 cm^{-1} [43]. The characteristic peaks of -C=C- double bonds stretching of an aromatic moiety at 1511 cm^{-1} were present in the IR spectrum of QCN. The spectra of mixed P123/F88 micelles showed the C-C(alkane) peaks at 2890 cm^{-1}, the aliphatic CH_2 and CH_3 peaks at 1343 cm^{-1} and 1373 cm^{-1}, respectively, and the C-O-C ether peaks at 1100 cm^{-1} [44]. The spectrum of QCN-incorporated mixed P123/F88(2:1) micelles had no new peaks except the shifting of wavenumber ranges incomparison to the blank P123/F88 micelles. Results also clearly showed that all the peaks of QCN drug also vanished in the IR spectrum of QCN-incorporated mixed P123/F88(2:1) micelles. Such observations in FTIR analyses indicate the interactions between mixed P123/F88 micelles

and QCN drug. It was found that the QCN drug was compatible with the micellar core of the mixed P123/F88 micelles.

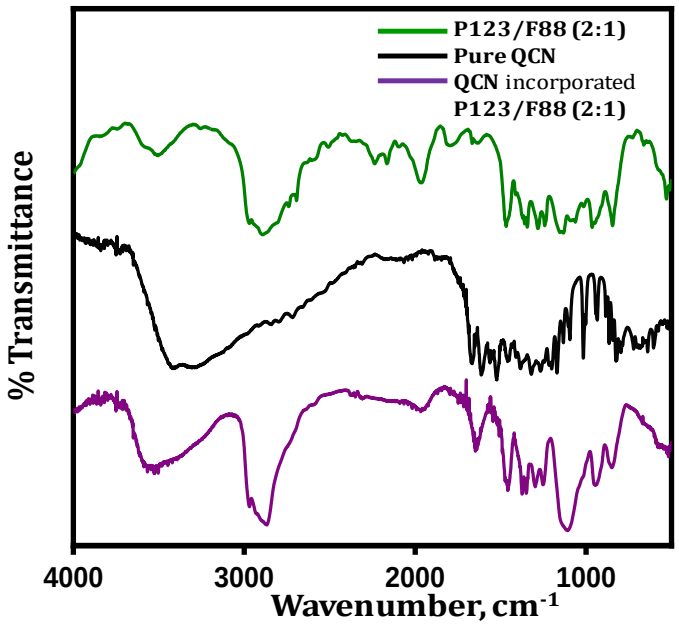

Figure 3.7: FTIR spectrums pure QCN, mixedP123/F88(2:1) micelles,and QCN-incorporated mixed P123/F88(2:1) micelles.

DSC was used to determine the physicochemical state of QCN in the mixed P123/F88 micelles. The DSC thermograms (shown in Figure 3.8) revealed that the QCN thermogram had two endothermic peaks at 177.77°C and 317.57°C [47]. According to the DSC findings, the phase state of QCN in mixed P123/F88 micelles had changed, and the drug was extensively dispersed throughout the micelles system. The alteration in QCN structure in mixed micelles revealed that QCN was in a non-crystalline form, such as an amorphous or disordered crystalline phase of molecular dispersion [45], which was beneficial for improving the solubility of poorly water-soluble drugs. The DSC results also supported the FTIR analysis.

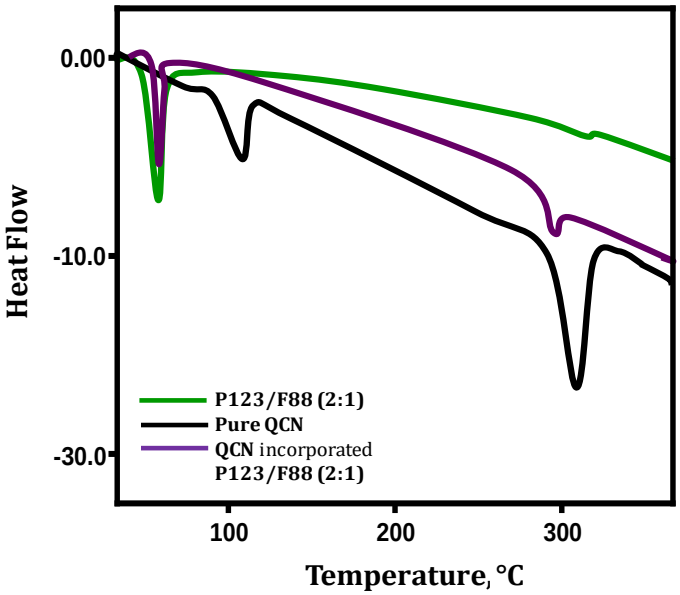

Figure 3.8: DSC thermograms of pure QCN, mixedP123/F88(2:1) micelles, and QCN-incorporated mixed P123/F88(2:1) micelles.

3.3.7: Storage stability study of QCN-incorporated mixed P123/F88 micelles

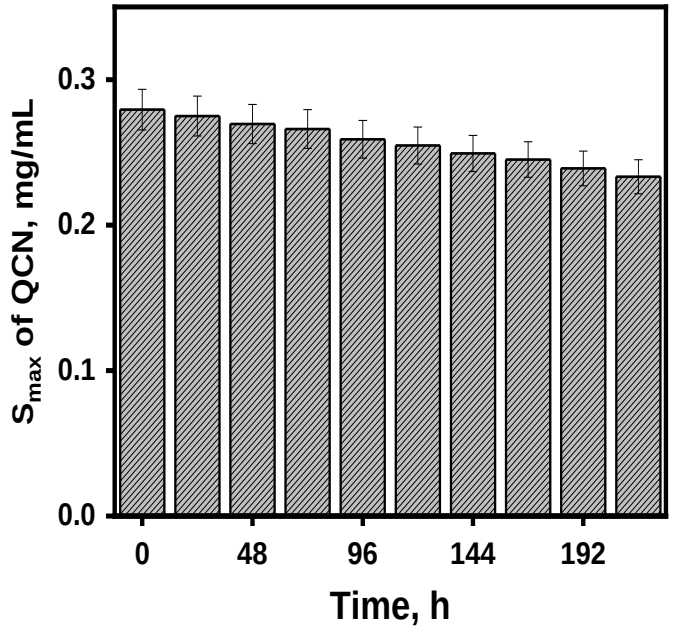

Figure 3.9: Storage stability of prepared QCN-incorporated mixed P123/F88(2:1) micelles at 30°C.

Stability studies on nanodelivery systems represent an important issue for the applicability of the formulation in daily practice and thinking about industrial production.

The stability of QCN-incorporated mixed P123/F88(2:1) micelles was monitored for 10 days at room temperature (Figure 3.9). The physical parameters remained practically unchanged during the storage period. The solubility values of QCN remain almost the same even after 10 days, ranging from 0.2874 mg/mL to 0.2318 mg/mL, respectively. No significant changes like turbidity and layer separations were observed and also not much retention of the drug was found during the period of 10 days.

3.3.8: *In-vitro* drug release profile

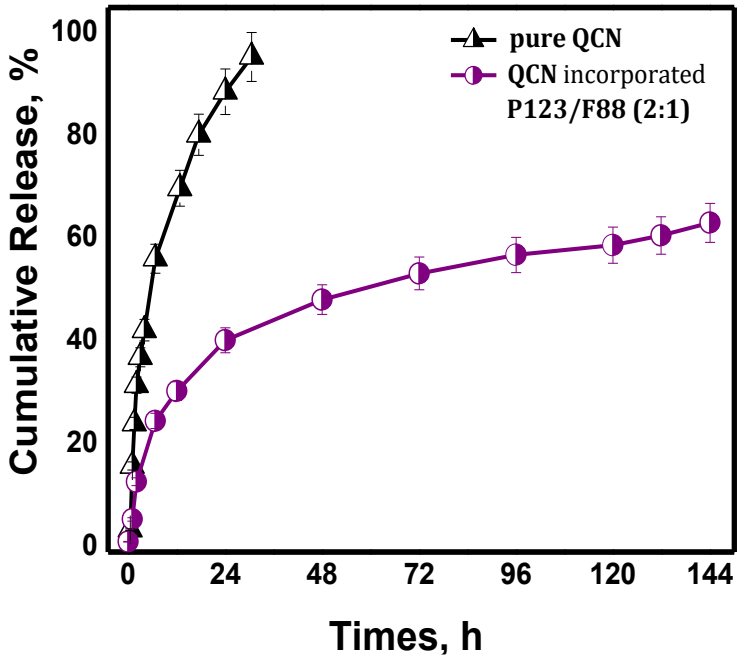

Figure 3.10: *In-vitro* release profile of QCN and QCN-incorporated mixed P123/F88(2:1) micelles at 30°C

The QCN *in-vitro* release studies from mixed P123/F88(2:1) micelles were performed at pH 7.4 (Physiological pH) using the dialysis bag method, in comparison to the QCN drug. As shown in Figure 3.10, only 30% of QCN was released from QCN-incorporated mixed P123/F88(2:1) micelles in the initial 12 h, while 60% of QCN was released from the QCN in propylene glycol solutions [46]. At the end of the study, ~60% of QCN incorporated in the mixed P123/F88(2:1) micelles had been released. Therefore, the QCN-incorporated mixed P123/F88(2:1) micelles showed a controlled and sustained release profile. The incorporation of QCN in the core of mixed micelles could explain the slower release of QCN from QCN-

incorporated mixed P123/F88(2:1) micelles compared to QCN in QCN-propylene glycol solution. Micelles are expected to expand as a result of water absorption, resulting in drug diffusion and polymer erosion [47]. However, because of the hydrophobic PPO core structure, water diffusion into micelles may be slowed, contributing to their prolonged release. This characteristic helped to prevent fast leakage and precipitation inside the GI lumen during drug delivery through oral administration.

3.3.9: *In-vitro* antioxidant activity

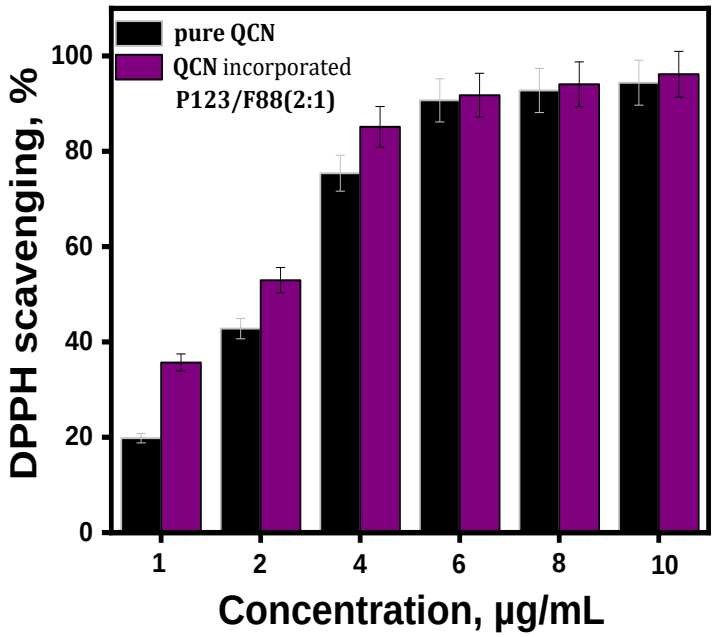

Figure 3.11: % inhibition of DPPH at different concentrations of pure QCN and QCN-incorporated mixed P123/F88(2:1) micelles.

The capacity of free QCN and QCN-incorporated mixed P123/F88(2:1) micelles to resist the oxidation process was checked by comparing the difference in DPPH scavenging behavior. The QCN behaves as a pro-oxidant in many situations, despite its antioxidant properties. During the scavenging of free radicals, QCN undergoes chemical conversion into oxidation compounds such as orthoquinone/quinonemethide intermediates, which are mutagenic. These compounds have a high reactivity for thiols, which have been shown to impair the function of certain proteins. According to many reports, QCN pre-treatment may also protect DNA from oxidative damage [48]. As presented in Figure 3.11, the maximum

scavenging rate of pure QCN reached almost 89% upto 10.0 µg/mL while QCN-incorporated mixedP123/F88(2:1) micelles inhibited almost 96% at the same concentration. The difference in scavenging activity could be ascribed to the potential enhancement of the antioxidant ability of QCN by the mixed Pluronic micelle formulation. This is because the mixed micelle formulation of QCN resulted in smaller particle size and improved solubility, which in turn enhanced the neutralization of the DPPH radicals as well as the antioxidant effect of the drug. Studies of QCN and QCN Incorporated mixed P123/F88(2:1) micelles confirmed oxidation resistance more significantly effective in mixed micelle than the free drug.

3.3.10: *In-vitro* cell proliferation activity

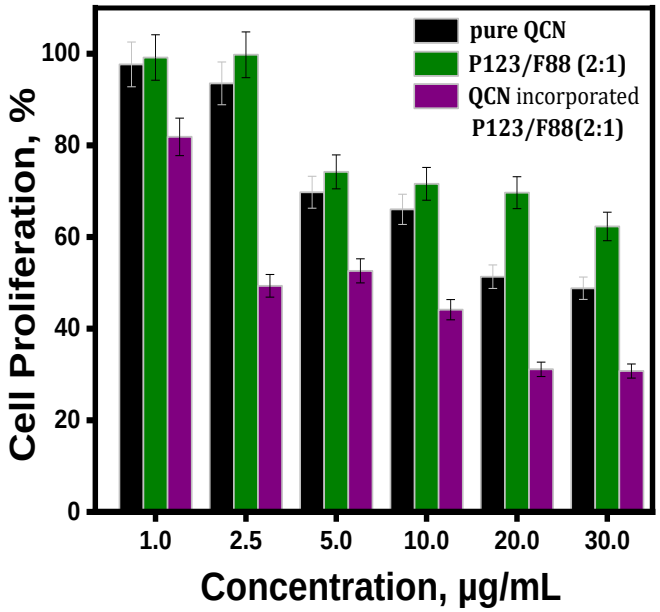

Figure 3.12: Cell Proliferation activity of pure QCN, mixed P123/F88(2:1) micelles and QCN-incorporated mixed P123/F88(2:1) micelles.

Cell proliferation activity of pure QCN, mixed P123/F88(2:1) micelles, and QCN-incorporated mixed P 123/F88(2:1) micelles was evaluated by MTT assay. Figure 3.12 exhibits the results percentage inhibition of cell proliferation obtained using MCF-7 cells after exposure for 24 h. Nonetheless, MCF-7 breast cancer cells showed no apparent toxicity to combine with mixed P123/F88(2:1) micelles, suggesting that the micellar system was safe, biocompatible, and nontoxic. The mixed P123/F88 micelles may solubilize a broad variety of

poorly water-soluble compounds and serve as a safe nanocarrier for delivering a drug without the use of highly unsafe excipients. The IC_{50} (concentration of drug needed to achieve 50% cell proliferation) value of free QCN, mixed P123/F88(2:1) micelles, and QCN-incorporated mixed P123/F88(2:1) micelles, was calculated. The IC_{50} values of free QCN, mixed P123/F88(2:1) micelles and QCN-incorporated mixed P123/F88(2:1) micelles were 29.9µg/ml, >30 µg/ml and 6.60 µg/ml, respectively. Our results indicate that aqueous soluble QCN-incorporated mixed P123/F88(2:1) micelles, exhibited increased cell proliferation activity in MCF-7 cells as compared to pure QCN.

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