4.1 Introduction:

Block copolymeric micelles have been of interest for few decades because of their structural array and tuning compatibility to achieve the desired properties for specific applications. Ethylene oxide-propylene oxide block copolymers commercially available as linear (Pluronics®) and star shaped (Tetronics®) amphiphiles are well-known for their surface activity, strong temperature dependant micelle formation and reversible thermo-rheological behavior [1, 2, 3]. Pluronics® are FDA approved and have been proved to be highly effective drug delivery vehicles with thermo-responsive behavior [4]. However, the related X-shaped Tetronics® were practically ignored until their recent investigation as efficient pharmaceutical excipient [4-7]. These copolymers form pH sensitive and thermodynamically stable micellar systems and are synthesized by the sequential reaction of the acceptor ethylenediamine molecule, initially with propylene oxide (PO) and then later with ethylene oxide (EO) precursors, resulting in a four branched arms, each one individually consisting of two -EO and -PO blocks attached to the central ethylenediamine core as shown in Scheme-I [8, 9].

![Scheme-I: Structure formula of (a) Tetronic® (T904) and (b) Quercetin (QN).](image)

The centrally located two tertiary amine groups in T904 can be easily protonated [10-12]. Tetronic® block copolymers flaunt a great potential as “smart” polymers in drug delivery systems, in tissue engineering, as blended nanoparticle carriers for gene delivery due to the low toxicity and cost-effectiveness. Their micelles possess good ability to solubilize hydrophobic drugs [6, 13-18]. Despite such attractive prospective, the physico-chemical data on Tetronics®, in particular concerning micellization in aqueous solution which is influenced not only by temperature/ionic strength, but also by pH are limited and less explored [4, 9, 19, 20].

The special architecture of Tetronics® suggests that pH, temperature and ionic
Chapter 4  Temperature/pH/salt modulate........

strength of the medium strongly influence their solution behavior [21]. Thus by controlled variations in such solution conditions may remarkably amend the role of Tetronics® and could offer interesting features for developing their skill to host/release drugs [22-24].

Our work deals with the solubilization study of hydrophobic hypolipidemic drug Quercetin, (3, 5, 7, 3’, 4’-pentahydroxyflavone, hereafter referred to as QN) a common flavonoid abundantly present in nature and consists of two aromatic rings (A and B) linked by an oxygen containing heterocycle (ring C) as shown in Scheme-I. Sufficiently found in the human diet, this anionic polyphenol has potent antioxidant and metal ion chelating capacity. It also exhibits anti-inflammatory, anti-neoplastic, cardio-protective activities and is anticipated to be one of the next anticancer drugs with extremely high efficiency [25]. QN is very sensitive to the solution conditions, which would alter the solubility, hydrophobicity and electrochemical properties and eventually its antioxidant capability. Its solubility in water being very low (~0.015mg/ml or 0.05mM at 30°C) prevents its practical applications. Thus formulation of QN in T904 micellar system is an attractive approach to overcome its low solubility and limited oral bioavailability.

Our findings provide an insight into the self-associative process of Tetronic® T904 in aqueous media. In order to elucidate its potential as stimuli-responsive in drug delivery systems, the sensitiveness of the copolymeric micelles is evaluated as a function of pH, ionic strength and temperature in the physiological range. The solubility of QN in T904 micelles was examined by UV-Vis spectrophotometry; changes in the micellar size with drug solubilization were studied by dynamic light scattering (DLS) while the possible locus of the drug molecule in the micellar aggregates was estimated from two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY). The probable mechanism of QN release from the micelle is discussed considering various kinetic models. Thus study correlates to the optimization of this star shaped copolymer aiming to be employed in the controlled release drug delivery systems.
4.2 Experimental:

4.2.1 Materials:

Tetronic® T904 and Pluronic® P84 were received as a gift sample from BASF Corporation, NJ, USA. Quercetin (QN) from (Sigma-Aldrich) was used. NaCl of AR grade was used. Sample solutions and buffers of different pH were prepared in triply distilled water for different sets of experiments.

4.2.2 Methods:

4.2.2.1 Preparation of micelles:

T904 solutions between (1-5%) were prepared, refrigerated and then equilibrated for 24 h prior to use.

4.2.2.2 Drug solubilization:

Shimadzu (UV-2450) UV-Visible double beam spectrophotometer was used to examine the solubility of the QN at 371 nm, against a blank/reference copolymer solution without the drug. T904 solution was poured into sterilized glass vials containing QN in large excess. These vials were shaken in a temperature-controlled horizontal shaker with steady rate and at desired temperatures for at least 48 h. The solutions were then filtered through 0.45 μm cellulose acetate membranes to remove the unsolubilized drug. These filtered solutions were then diluted properly with methanol. Calibration with dilute drug solutions ranging from 0 - 30 mg/ml dissolved in methanol gave satisfactory Beer-Lambert plot (not shown) with $R^2 = 0.9991$ [26].

Several descriptors presented below were used to characterize the ability of T904 to solubilize QN [6, 16].

(i) Molar solubilization capacity ($\chi$) defined as the moles of QN solubilized per mole of T904 was evaluated using

$$\chi = \frac{S_{\text{tot}} - S_w}{C_{\text{T904}} - \text{CMC}}$$

Where $S_{\text{tot}}$ is the total QN solubility; $S_w$ is the solubility in water (in molar concentration), $C_{\text{T904}}$ is the molar concentration of the copolymer. As T904 unimer concentration above the CMC remains constant and is equal to the CMC, its concentration in the micellar form can be estimated as $C_{\text{T904}} - \text{CMC}$.

(ii) The ratio of QN in the micelle to that in water, for 5% T904 was obtained using micelle-water partition coefficient ($P$) as
(iii) Standard free energy of solubilization, was estimated from the molar micelle-water partition coefficient, $P$

$$\Delta G^0_s = -RT \ln \frac{\chi(1 - \text{CMC})}{S_w}$$

……... (3)

(iv) Solubilization capacity in (mg) per gram of hydrophobic block calculated as the amount of QN dissolved in the copolymer solution in excess of the amount dissolved in an equivalent volume of solvent medium referred to the mass fraction of hydrophobic blocks in each copolymer,

(v) Number of QN molecules solubilized per micelle, calculated as

$$n_s = \frac{(S_{tot} - S_w)N_A}{n_m}$$

……... (4)

In above equations, $S_{tot}$ is the total QN solubility; $S_w$ is the solubility in water (in molar concentration); and $n_m$ is the number of micelles per liter of solution, estimated as

$$n_m = \frac{(C_{T904} - \text{CMC})N_A}{N}$$

……... (5)

$N_A$ is Avogadro number, and $N$ is the aggregation number.

4.2.2.3 Viscosity:

Solution viscosity was measured using a regular Ubbelohde suspended level capillary viscometer at temperature stability of about ±0.1°C. After thorough cleaning and drying, the flow time for constant volume of solution was measured thrice using a calibrated stopwatch [27].

4.2.2.4 Measurement of the micellar size ($D_h$) by dynamic light scattering (DLS):

The DLS measurements on QN free and QN loaded micelles were done using a Malvern 4800 Autosizer employing 7132 digital correlator and a light source of argon ion laser operating at 514.5 nm. Filtered solutions were measured at least 3 times. The correlation functions at neutral pH were analyzed using the CONTIN method in order to obtain the hydrodynamic size of micellar aggregates in aqueous medium using the Stokes-Einstein equation.
4.2.2.5 $^1$H-NMR and Two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY):

The 2D NMR experiments ($^1$H, gradient NOESY with and without solvent suppression) were performed on a Bruker AVANCE-II 400 MHz spectrometer at StFX University. The mixing times and the delay times for the NOESY experiments were estimated from the spinlattice relaxation times ($T_1$ values) of the surfactant determined in separate experiments. In all cases, an acquisition delay of $\gg 3 \times T_1$ and a mixing time of $\gg 1 \times T_1$ were used to obtain the NOESY spectra. For all acquisitions, 256 transients of either 2 or 4 scans over 512 complex data points were acquired. All experiments were done in phase sensitive mode, with and without the saturation of the water resonance at $\approx 4.70$ ppm. The data were zero-filled twice in dimension 1 and multiplied by a squared sine function in both dimensions before 2DFT. (18). Solutions were prepared directly in small sample vials, and these were dispensed into the NMR tubes with a volumetric pipette [28, 29].

4.2.2.6. In vitro drug release:

Dissolution cell placed in a 500 mL Borosil beaker that served as the receptor cell consisted of Himedia dialysis membrane bag (cut-off molecular weight: 12000-14000 kDa) previously soaked in water and clamped at one end. 5 mL T904 solution containing drug was subjected to the release studies in phosphate buffer saline (PBS) (50 ml) having pH~7.4 in the dissolution cell. The contents of the dissolution cell were agitated with the help of a glass stirrer. 250 mL PBS was placed in the receptor cell. The receptor cell contained a magnetic bead and was rotated at a constant speed of about 100 rpm at 37°C. At predetermined time intervals, 2 mL of sample was withdrawn and replaced with an equal volume of fresh PBS. For comparison, QN release from bulk powder (dispersed in 5 mL of water by vortexing) placed in a dissolution cell was performed under the same conditions. The samples were analyzed for QN content at 371 nm using a UV-Visible spectrophotometer [30].

4.3 Results and discussion:

Tetronic® T904 having 40% PEO and intermediate polarity was used without further purification. The molecular characteristics and hydrophilic-lipophilic balance (HLB) presented in Table 4.1 characterize the hydrophobicity of PEO-PPO based amphiphile. $\beta$, can be used to characterize the hydrophobicity of copolymer,
calculated (~2.29) as the ratio between the Flory radius of the PPO blocks to that of the PEO blocks in the given polymer by using below equation [16].

\[
\beta = \frac{I_{PO}(N_{PO})^3}{I_{EO}(N_{EO})^3}
\]  

......... (6)

Where \(N_{PO}\) and \(N_{EO}\) are the numbers of -PO and -EO units while \(I_{PO}\) and \(I_{EO}\) are the corresponding lengths of the -PO and -EO units respectively.

**Table 4.1: Physical characteristics of Tetronic® T904 in aqueous medium.**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Mol. wt.</th>
<th>% EO</th>
<th>HLB</th>
<th>(pK_{a1})</th>
<th>(pK_{a2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>((EO_{15}PO_{17})<em>2 - NCH_2CH_2N - (PO</em>{17}EO_{15})_2)</td>
<td>6700</td>
<td>40</td>
<td>12-18</td>
<td>4.0</td>
<td>7.8</td>
</tr>
</tbody>
</table>

The proton dissociation (\(pK_a\)) makes the study of protonation degree, self aggregation and the surface-active properties of the polymer more feasible to understand. The titration profile of T904 presented in Fig. 4.1 shows two inflections that correspond to the proton dissociation from the nitrogen atoms on the ethylenediamine group. The \(pK_{a1}\) and \(pK_{a2}\) values obtained are in agreement to those previously found for T904 [16].

![Figure 4.1 Potentiometric titration curve for 5% T904 in aqueous medium without drug as a function of pH.](image)
Studies inferred the complication in the micellization process as the decrease in pH leads the CMC shift to higher order and hence decrease the micellar size. The high impact of the solvent conditions on the aggregation motivated us to explore the behavior of Tetronics® displaying a broad range of EO/PO ratios and molecular weights at pH values below pK_a, where only unimers exist with similar degree of protonation [9, 16, 28].

4.3.1 Shape transition in micelles:

Relative viscosities of 5% T904 at different pH and varying salt concentrations are shown in Fig. 4.2. A slight initial decrease in viscosity followed by a gradual increase at higher pH or salt concentration is observed for both the cases. The repulsive or weak attractive intermicellar interactions play a significant role, especially at ambient temperature and salt concentration for a given copolymer concentration [21].

![Relative viscosity vs pH](image)

**Figure 4.2** Relative viscosity of 5% T904 solution as a function of (a) pH and (b) [NaCl] at pH~7.5 recorded at 30°C.

The large increase in relative viscosity observed for the copolymer solution at pH>12 (Fig. 4.2a) is associated with the growth of the micelles which is suggestive of the occurrence of a micellar transition or an onset of attractive intermicellar interaction in the presence of alkali. Studies have revealed the dual role of NaOH in
enhancing the hydrophobicity and consequently altering the characteristics of micelles. At low concentrations, NaOH stabilizes the micelles by removing the protonated form of T904 molecule while at higher concentration, it promotes micellar growth due to the dehydrating effect of the OH\(^-\) ion on T904 molecules. The dehydrating effect of NaOH is likely to be determined by the OH\(^-\) ions with the Na\(^+\) playing a minor role [31-33]. In the presence of NaCl (Fig. 4.2b), a slight decrease in viscosity followed by an abrupt increase above 2.0 M NaCl is observed which mainly attributes two governing factors: firstly, due to the change in shape of the micelles and secondly due to the magnitude of the intermicellar interactions at higher salt concentrations (where the micelles are large).

4.3.2 Effect of copolymer/salt concentration on drug solubilization:

Fig 4.3a shows a linear increase in solubility of QN with the increase in T904 concentration which is attributed to the strong interaction between the drug and the copolymer molecules. Such an increase in T904 concentration may lead to an increase in the number of micelles rather than micellar growth [4, 6, 12, 34-36]. Theoretical studies have suggested that the micelle size increases with molecular weight. Also linear correlation between the surfactant micelle/water partition coefficients to solubilize various polyaromatic hydrocarbons is reported. These studies confirm the ability of amphiphilic molecules to significantly enhance the solubility of hydrophobic solutes in aqueous solution [35, 37, 38].
Since drugs employed for the medical formulations usually comprise salts for pH buffering and ionic strength balance, QN tends to critically influence the behavior of T904 in aqueous medium. The solubility of QN in water and in T904 in the presence of salt is shown in Fig 4.3b. The CMT of 5% T904 is ~27°C where the copolymer begins to aggregate at this temperature and induces micellization [19]. The solubility of QN at that temperature is apparently low. The addition of salt decreases the CMT, thus promotes aggregation and drug solubility. The increase in ionic strength shields the overall repulsion, causes a little salting-out effect that decreases the hydrophilicity of the PEO chains and weakens the hydrogen bonding interactions [36, 39]. Consequently, the entropy driven force for aggregation and the enthalpic compensation is enhanced. Greater demicellization enthalpies in high ionic strength medium, compared to water, have also been reported in case of poloxamers [40].
4.3.3 Effect of salt on micellar size:

The DLS results for 5% T904 in water and at different salt concentrations in presence of QN are shown in Fig. 4.4. Initially, a slight decrease in micellar size with increase in salt concentration is observed due to the tightening of PEO shell while further an increase in micellar size is attributed due to the quenching of water molecules from the core region causing a simultaneous intercalation of the drug molecule within the micelle leading to micellar growth/transition [25, 41].

Figure 4.4 Hydrodynamic diameter of 5% T904 as a function of NaCl in presence of QN at 37°C.

In quantitative terms, 5% T904 enhances QN solubility to about 59 times than in water. Results shown in Table 4.2 clearly indicate an increase in the solubilization capacity (\(\chi\)) and partition coefficient (P) of T904 as the copolymer/salt concentration is increased. The standard free energy of solubilization (\(\Delta G_s^\circ\)) appeared to be negative in magnitude indicating spontaneous migration process of drug molecules in the monomer state. However, more negative \(\Delta G_s^\circ\) value with increasing copolymer/salt concentration indicate more favored solubilization of QN due to strong hydrophobic interaction. The core of the copolymer, which is the most hydrophobic, is able to host the drug species.
Table 4.2 Solubilization parameters measured for QN as a function of T904/salt concentration at 37°C.

<table>
<thead>
<tr>
<th>[T904], %</th>
<th>x x 10^{-3}</th>
<th>P</th>
<th>ΔG_{s}^0, kJmol^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>141</td>
<td>4</td>
<td>-2.26</td>
</tr>
<tr>
<td>2</td>
<td>275</td>
<td>16</td>
<td>-3.98</td>
</tr>
<tr>
<td>3</td>
<td>335</td>
<td>29</td>
<td>-4.48</td>
</tr>
<tr>
<td>4</td>
<td>395</td>
<td>46</td>
<td>-4.91</td>
</tr>
<tr>
<td>5</td>
<td>397</td>
<td>58</td>
<td>-4.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>[NaCl], M in 5%T904</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>0.4</td>
</tr>
<tr>
<td>0.6</td>
</tr>
<tr>
<td>0.8</td>
</tr>
<tr>
<td>1.0</td>
</tr>
<tr>
<td>1.5</td>
</tr>
<tr>
<td>2.0</td>
</tr>
</tbody>
</table>

4.3.4 Effect of pH on QN solubilization in different copolymeric micelles:

The presence of a central diamine group in the molecular structure of Tetronics® renders the copolymer to be thermo- and pH- responsive. However limited work is known in this direction explaining the micellization behavior of Tetronics® at different pH and impact of basic/acid equilibrium upon aggregation [12]. The influence of pH on the micellar solutions of Pluronic® P84 having the weight fraction of the PEO block (40%) similar to that of T904 was also studied. The experimental data on QN solubilization as a function of pH in the aqueous solution of two different copolymers are presented in Fig. 4.5 and are quite interesting.
Fig 4.5 Solubility of QN in 5% T904, P84 and in water at 37°C as a function of pH.

Fig. 4.5 represents almost negligible solubility of QN in water which increases slightly in alkaline medium (after pH~6) due to its ionization. For T904, QN solubility is almost same as in water till pH <4, and above pH 4 a drastic increase in the solubility is observed. For comparison QN solubility in P84 was determined. Here the solubility is around 3mM in acidic pH and slightly increase at pH >6. The pH does not impose its effect on Pluronic® unimers as they do not possess pH-sensitive moieties in their structure and show solubility even in acidic pH where the solubility increases due to the dehydrating effect of OH⁻ ion as discussed in section 3.1.

The solubilization capacity (χ) and partition coefficient (P) presented in Table 4.3 remain almost constant up to pH <4 and above that it increase, indicating more QN solubilization in T904 micelles. Such enhanced solubility of QN in T904 above pH 4 is attributed to the hydrophobic nature as well as anionic state of QN. Initially, above pH 4, the hydrophobicity of QN is found responsible for aggregation of T904 and later at less acidic pH (5-6), QN molecules get ionized. Such anionic form facilitates QN to interact electrostatically with quaternary ammonium groups of T904 and generates a stable complex which makes possible for QN to solubilize in T904 micelles [9, 12, 28]. Similar trend is observed for Gibb’s energy (∆G_s). The solubilization capacity of hydrophobic block (i.e. n_s) using equation 4 was measured as 7.10 and 19.65 mg g⁻¹ for T904 and P84, respectively. However, the fraction of the
drug that is hosted by the micelles as well as the amount of drug incorporated per gram of PPO blocks clearly rises as the solubility increases with pH.

**Table 4.3** Solubilization parameters and Gibb’s energy ($\Delta G_s^o$) for QN dissolved in 5% copolymer solutions at different pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>$x \times 10^{-3}$</th>
<th>P</th>
<th>$\Delta G_s^o$, kJmol$^{-1}$</th>
<th>$x \times 10^{-3}$</th>
<th>P</th>
<th>$\Delta G_s^o$, kJmol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>0.6</td>
<td>6.86</td>
<td>259.7</td>
<td>60.0</td>
<td>-3.11</td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
<td>0.8</td>
<td>6.12</td>
<td>264.1</td>
<td>61.0</td>
<td>-3.15</td>
</tr>
<tr>
<td>3</td>
<td>5.5</td>
<td>0.8</td>
<td>6.12</td>
<td>272.7</td>
<td>63.0</td>
<td>-3.23</td>
</tr>
<tr>
<td>4</td>
<td>80.6</td>
<td>11.8</td>
<td>-0.81</td>
<td>265.8</td>
<td>61.0</td>
<td>-3.17</td>
</tr>
<tr>
<td>5</td>
<td>94.3</td>
<td>13.8</td>
<td>-1.22</td>
<td>268.4</td>
<td>62.0</td>
<td>-3.19</td>
</tr>
<tr>
<td>6</td>
<td>113.0</td>
<td>16.6</td>
<td>-1.70</td>
<td>264.1</td>
<td>61.0</td>
<td>-3.15</td>
</tr>
<tr>
<td>7</td>
<td>166.0</td>
<td>24.4</td>
<td>-2.69</td>
<td>290.1</td>
<td>67.0</td>
<td>-3.39</td>
</tr>
<tr>
<td>8</td>
<td>268.0</td>
<td>39.2</td>
<td>-3.91</td>
<td>324.7</td>
<td>75.0</td>
<td>-3.68</td>
</tr>
<tr>
<td>9</td>
<td>428.0</td>
<td>62.6</td>
<td>-5.11</td>
<td>350.7</td>
<td>81.0</td>
<td>-3.89</td>
</tr>
<tr>
<td>10</td>
<td>540.1</td>
<td>79.0</td>
<td>-5.72</td>
<td>385.3</td>
<td>89.0</td>
<td>-4.12</td>
</tr>
</tbody>
</table>

In general, at low pH T904 chains are di-protonated which leads to coulombic repulsion and curtail aggregation [26]. Due to this strong electrostatic barrier micellization can not be possible and hence QN can not be solubilized in acidic pH. But in this study, QN solubility in acidic medium (> pH4) can be well understood from spectral analysis (Fig. 4.6). Our findings relate to the influence of QN on the protonation and hydrophobicity of T904.

![Figure 4.6 Absorbance vs. wavelength for QN at different pH.](image-url)
As shown in Fig. 4.6, above pH 4 absorbance intensity of QN increases which indicates that solubilization capacity of T904 enhances as pH increases. The plausible reason for such a behavior is the ionization of QN and its coulombic interaction with T904. Below pH 4 (in acidic medium), QN molecule interacts with T904 unimers with $\lambda_{\text{max}}$ near 293 nm. While above pH 4, a red shift is observed at $\lambda_{\text{max}}$ 371 nm which is due to the interaction between QN and T904 micelles. Further in higher alkaline medium i.e. above pH 10, the most acidic phenolic -OH groups in QN on position 17 and 19 of the molecule dissociate and results in the mixture of neutral and anionic species with one or two charges as shown in scheme II [42]. As a consequence QN becomes more anionic, shows blue shift at 326 nm and its solubility in water enhances dramatically. Inset shown in Fig. 4.6 indicates the change in the wavelength (shifts) as a function of pH.

Scheme II: Charged QN in blue shift region above pH 10 (higher alkaline medium).

These findings correlate well from the potentiometric titration curve of T904 in the presence of QN (Fig. 4.7) which show only one inflection point quite different from the two inflection points as observed in Fig. 4.1. This corresponds to the proton dissociation of the nitrogen atoms on the central ethylenediamine group by QN molecule indicating hydrophobic/electrostatic interaction between QN and T904 thus increasing QN solubility.
Figure 4.7 Single inflection point depicted in potentiometric titration curve obtained for QN solubilized in 5% T904 as a function of pH.

4.3.5 Location of QN in Tetronic micellar solution:

2D-NOESY being an established non-invasive method is employed to gain significant information on the intra- and inter molecular proximity of proton in T904 micellar system with solubilized QN. It revealed valuable information on the distance between the pairs of coupled protons from the intensity of the cross-peaks and the strength of the interaction between T904 molecule and QN [43-45]. This study gave an insight about the location and the resulting microenvironment of QN in micellar solution of T904.
Fig. 4.8a: $^1$H NMR spectra of T904 in D$_2$O recorded at 20°C. Inset shows QN interaction with protons of PPO -CH$_2$ and PPO -CH$_3$.

Fig. 4.8 depicts three resolved signals of T904. Signals observed at 1.16ppm, broad peaks from 3.65 to 3.45ppm and the intense resonance at around 3.7ppm attribute to the protons of the -CH$_3$ PPO, -CH$_2$ PPO and -CH$_2$ PEO respectively. The additional signal for the very small peak at 3.20 to 2.20ppm is the proton resonance of -NCH$_2$-CH$_2$N- linkage. The 2D-NOESY contour plot for T904 micellar system in solubilized QN is presented in Figure 4.8b. For this system, the cross-peaks are observed between the protons of QN (δ ≈ 6.2 - 7.3ppm) with the -CH$_3$ PPO (δ≈1.16 ppm) / -CH$_2$ PPO (δ≈ 3.6 ppm) protons of the T904. This indicates interaction of QN molecule with PPO protons of T904 though difficult to interpret which part of QN interacts. QN molecule being almost planar indicates that there is no particular preference for QN protons to interact with PPO blocks of T904. Irrespective of the preference given to the protons of QN, a hypothetically predicted location of QN molecule will favor A ring (Scheme 1) towards micellar core in slightly acidic condition (~pH 6-6.5). This could be due to the electrostatic interaction of anionic form of QN with the positively charged amine group of T904 and may vary with pH.
Figure 4.8b: The 2D NOESY spectrum of QN solubilized in 5% T904.

4.3.6 In vitro release of QN from T904 micelles:

Figure 4.9 shows the cumulative release profile of QN versus time (hrs) from solutions containing different T904 concentrations. The time intervals used were based on the trial and error approach. On the basis of the preliminary studies, release media containing 0%, 1%, 5% and 10% T904 were selected for intended application and employed for in vitro release studies. Fig. 4.9 shows a low burst effect with about 19%, 8% and 6% drug released within the first 24 h for 1%, 5% and 10% T904 concentration respectively indicates the incorporation of QN in T904 micelles. About 68% of QN was released from bulk powder i.e. 0% T904 initially and became constant after 24 h. While the release rate of QN from T904 micelles in PBS was observed to be more rapid at lower polymer concentration giving initial burst release compared to the higher concentration. The amount of QN released from T904 micelles of varying concentration followed the trend: 1% > 5% > 10%. The amount of QN released from T904 micelles was found to be lower than its release in aqueous solution (0% T904) which infers the controlled rate of drug release. Upto the 9th day,
the QN release profiles displayed a sustained release phase but a very contrast behavior was noticed after 216 hours (9 days) for higher concentration of T904 where QN showed maximum release for 10% T904. Finally at about 288 hours (12 days), a total cumulative drug released from micelles followed the trend: 10% > 5% > 1%. Such increased QN release from T904 micelles can be attributed to the solubilizing ability exhibited by the polymeric micelles.

![Figure 4.9](image)

**Figure 4.9** The in vitro release profile of QN in PBS (pH~7.4) as a function of T904 concentration at 37°C.

The possible mechanism of QN release from the polymeric micelle is evaluated mathematically by analyzing the release data employing the zero-order kinetics, first-order kinetics and Higuchi equation models. The criterion for selecting the most appropriate model was based on best goodness-of-fit ($R^2$ values). To predict the release pattern of QN from T904 micelles, the rate constant and correlation coefficient were calculated for all the three models and reported in Table 4.4.

**Table 4.4** Different kinetic models depicting the release pattern of QN from T904 micelles.

<table>
<thead>
<tr>
<th>[T904], %</th>
<th>Zero order release model</th>
<th>First order release model</th>
<th>Higuchi release model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K(h^{-1})$</td>
<td>$R^2$</td>
<td>$K(h^{-1})$</td>
</tr>
<tr>
<td>1.0</td>
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The results showed that the release of QN from polymeric micelles followed the Higuchi equation for the 1% polymeric solution while it followed zero-order kinetics for the 5% and 10% concentration of polymer. It has been well known that Higuchi and Zero-order equations describe respectively the release processes under the controls of drug diffusion and interface movement. This indicates that at lower concentration of T904, the QN release is mainly due to the diffusion process while at higher concentration the interface movement is responsible for the release phenomenon.

4.4 Conclusion:

Our study evaluates the ability of thermo- and pH-responsive star-like Tetronics® T904 micelles to host a poorly water-soluble hypolipidemic drug QN and serve as efficient solubilizers and carriers for their pharmaceutical formulations. Solubilization furnishes trivial insight to the fraction of drug that is hosted by the micelles. This paper describes a notable micellar behavior of T904 micelles in presence of salt, with/without drug as a function of pH. Results capitalize the improved aqueous solubility of the model drug QN by its incorporation into T904 micelles. The solubilization study was monitored systematically using spectral techniques. Scattering data stipulated our findings and showed the change in the micellar size in presence of QN. 2D-NOESY evidenced the expected locus (site) of the QN to the copolymer and successfully correlates by significant and positive cross peaks. The controlled release study of QN from T904 micelles was examined using different order kinetic models. Our findings reveal hydrophobic and electrostatic force as the main driving factors responsible for the interaction between the drug and copolymer micelle. Increase in the apparent solubility of QN in aqueous T904 solutions was favored by varying T904 concentration, salt concentration and pH. The solubility data and the release pattern explored the important role of pH-responsive polymer concentration in drug loading and drug release profile creation and if conveniently modulated could make the Tetronic® micelles more promising in designing carriers in site-specific drug delivery systems.
Solubilize dissolution of QN (anionic drug) in T904 core-shell aggregates possessing nonpolar PPO interior and relatively polar PEO shells is well presented as a function of pH.

References

Chapter 4  

**Temperature/pH/salt modulate**


