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PEO–PPO based star-block copolymer T904 as pH responsive nanocarriers for quercetin: Solubilization and release study

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ABSTRACT

Solubilization of quercetin (QN), a *hypolipidemic drug* in aqueous micellar solution of a star-like octablock Tetronic® T904 covering different salt concentration, pH and temperature is investigated. The change in pH modulates the charge of the copolymer which alters the dibasic nature of the centrally located ethylenediamine moiety and makes T904 undergo deprotonation favoring self assembly. At low pH, the columbic repulsion among the positively charged amine groups of Tetronic® hinders micellization while presence of salt facilitates it. The drug solubility data for micelles in aqueous/salt solutions determined by UV–Visible spectroscopy and micellar size with loaded drug from dynamic light scattering (DLS) are reported. Hydrophobic/anionic QN, deprotonates T904 and induces the micellization in acidic pH thus assisting solubilization. The expected locus (site) of the QN in T904 micelles was successfully correlated by the significant and positive cross peaks obtained from two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY). The evaluated *in vitro* release profile employing different kinetic models explains the controlled release of drug from T904 micelles.

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 [1–3]. Some 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 1 [8,9].

The centrally located two tertiary amine groups in T904 can be easily protonated [10–12]. Tetronic® block copolymers flaunt great potential as "*smart*" polymers in drug delivery systems and 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

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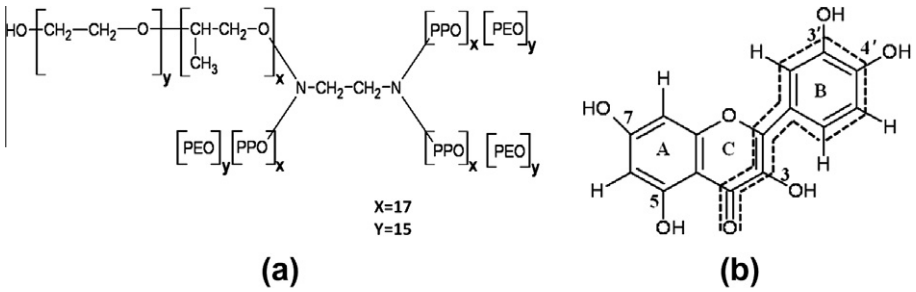

Scheme 1. Structural formula of (a) Tetronic® (T904) and (b) quercetin (QN).

that pH, temperature and ionic strength of the medium strongly influence their solution behavior [21]. Thus 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 1. Sufficiently found in the human diet, this anionic polyphenol has potent antioxidant and metal ion chelating capacity. It also exhibits anti-inflammatory, anti-neoplastic, cardioprotective 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 (~0.015 mg/ml or 0.05 mM 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 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 micelles is discussed considering various kinetic models. Thus, present study correlates to the optimization of this star shaped copolymer aiming to be employed in the controlled release drug delivery systems.

2. Experimental

2.1. Materials

Tetronic® T904 and Pluronic® P84 were received as a gift samples 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.

2.2. Methods

2.2.1. Preparation of micelles

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

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 to 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 (χ) defined as the moles of QN solubilized per mole of T904 was evaluated using

$$\chi = \frac{S_{tot} - S_w}{C_{T904} - CMC} \quad (1)$$

where Stot is the total QN solubility; Sw is the solubility in water (in molar concentration), CT904 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 CT904 – 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

$$P = \frac{S_{tot} - S_w}{S_w} \quad (2)$$

(iii) Standard free energy of solubilization, was estimated from the molar micelle–water partition coefficient, *P*