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In-vitro evaluation of cytotoxic and antioxidant properties of drugs solubilized in EO-PO star block copolymer micelles

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ABSTRACT

Self-assembly of a biocompatible, nontoxic, commercially available star shaped polyethylene oxide-polypropylene oxide block copolymer, Tetronics®1107 (mol. wt. = 15000, % ethylene oxide = 70, and HLB 18–23) in aqueous solution was examined using surface tension, fluorescence, viscosity, cloud point and dynamic light scattering. The copolymer being very hydrophilic shows poor surface activity and forms thermo-, pH and salt responsive nanosize core-shell micelles above critical micellization temperatures. These micelles showed markedly enhanced solubilization of hydrophobic drugs curcumin and quercetin. *In vitro* antioxidant activities (by free radical scavenging, reducing capacity and superoxide anion radical scavenging methods) and cytotoxicity (against CHO-K1 cell line) showed desirable properties in micellar drugs as compared to free one and similar cytotoxic effects were observed for free as well as micellar drugs. This suggests T1107 as promising tool for drug delivery.

1. Introduction

Nanocarriers help to improve solubility and stability of hydrophobic drugs [1] and micellar solubilization has been considered quite useful [2]. Core-shell micelles from amphiphilic polymers show huge potential for cancer treatment due to their small size, biocompatibility, biodegradability, prolonged circulation time in blood stream, enhanced drug loading capacity and easy chemical modification or surface functionalization [3]. These micelles are of interest because of their structure and polymorphic nature, and hence have received increased attention due to drug solubilization capacity [4–6], with improved pharmacokinetics and reduced off-target cytotoxicity [7].

Amphiphilic block copolymers of polyethylene oxide (PEO) and polypropylene oxide (PPO) are commercially available as linear triblocks of the type ABA (Pluronics®) and star-shaped (Tetronic®) with varying hydrophilic-lipophilic balance (HLB). These polymers self-assemble to form nanosized core-shell micelles in aqueous solution at critical micellar concentration (CMC) and critical micellar temperature (CMT). Micelles comprise of a hydrophobic core containing the PPO blocks surrounded by heavily hydrated corona of PEO blocks [8,9]. Tetronics® have four arms of amphiphilic terminal (PEO and PPO in different ratio) attached to central ethylenediamine moiety as shown in Scheme1. These copolymers are synthesized by the sequential reaction of the acceptor ethylenediamine molecule, initially with propylene oxide and later with ethylene oxide precursors, resulting in four branched arms [10]. The X-shaped Tetronics® impart strong pH dependence in their aggregation characteristics due to the presence of the central ethylene diamine unit. Micellization under a variety of conditions makes Tetronics® attractive for drug delivery applications due to a possibility of modulating them at physiological conditions [11,12]. Reports are available in literature on using Tetronics® as efficient pharmaceutical excipients vehicle, their micelles possess good ability to solubilize hydrophobic drugs [12–19]. Tetronics® are now becoming “smart” polymers for drug delivery systems due to low toxicity and cost. Two hydrophobic polyphenolic antioxidants, curcumin and quercetin are commonly used due to their wide pharmacological activities [2,20]. Curcumin derived from turmeric (*Curcuma longa*) possesses anti-inflammatory and anticancer activity, boosts immune system, prevents damage to biological membranes *etc*. [20]. Quercetin, a flavanoid present in onion, shows cytotoxic effects on cancer chemoprevention through cell cycle arrest, induction of apoptosis, and inhibition of angiogenesis [2]. However, both curcumin and quercetin are poorly soluble in water with low bioavailability and thus their use is restricted [2,21].

The aim of this study is to characterize micelles, monitor solubilization of curcumin and quercetin and study cytotoxic activity of free as well as micelle encapsulated drugs against CHO-K1 cell line. The present study also directs to develop micellar drug formulations capable of

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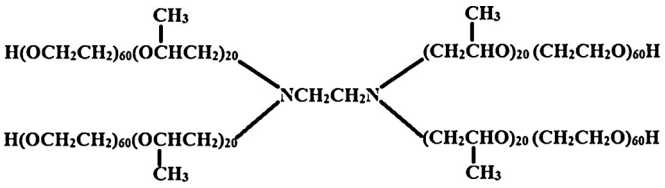
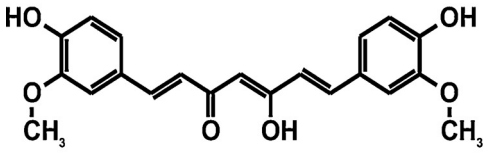


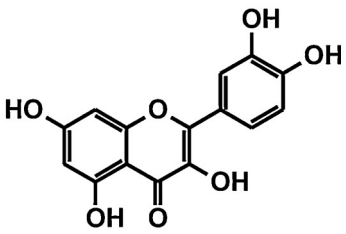
Tetronics®1107

(mol.wt. = 15000, %PEO = 70, HLB = 18-23, CP = >100°C, pK_a = 5.6, 7.9)



Curcumin

(Aqueous solubility = <0.1 mg/l, pK_a = 7.8, 8.5, 9.0)



Quercetin

(Aqueous solubility = <0.1 mg/l, pK_a = 6.4,-4)

Scheme 1. Structures of T1107, curcumin and quercetin.

preserving antioxidant activity. Free radical scavenging activity and reducing power potency of free and micellar curcumin and quercetin were investigated using three methods *i.e.*, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) assay, superoxide anion radical scavenging activity and reducing power activity (RPA) and compared with common antioxidants *viz.* ascorbic acid and gallic acid.

2. Experimental

2.1. Materials

Tetronic® 1107 was received as a gift sample from BASF Corp. Parsippany, NJ, USA, and used as received. Curcumin and quercetin were from Sigma Aldrich. Solutions for surface tension, fluorescence, viscosity, cloud point, dynamic light scattering and drug solubilization were prepared in Millipore Milli-Q purified water. CHO-K1 cell line was procured from ATCC, USA. Cell culture reagents were purchased from Gibco Invitrogen and Sigma Aldrich. All other reagents used were of AR grade purchased from Himedia.

2.2. Methods

2.2.1. Phase behavior and micelle characterization of T1107

Surface tension was determined by the Platinum ring method using a Lauda Tensiometer TDI (Lauda-Konigshofen, Germany) at 25 ± 0.5 °C.

CMC was determined by recording steady state fluorescence of pyrene using a SPEX fluorolog 1680 spectrofluorometer in the range 350–500 nm using an excitation wavelength of 330 nm. Pyrene at concentration of 6×10^{-7} M was added and acetone was evaporated. 10 ml of T1107 solutions at different concentrations were added to different tubes and heated for 3 h at 65 °C. Equilibration of the pyrene and polymeric micelles was achieved by keeping solutions overnight at RT.

The relative viscosities of the solutions were measured using calibrated Cannon Ubbelohde viscometers. The absolute viscosities of the solutions obtained were multiplied by viscometer constant to get kinematic viscosity in centistokes and density of solvent (water) to attain the solution viscosities in centipoise. These viscosities of solutions were divided by viscosity of water to obtain the relative viscosity [22–24].

Cloud point (CP) and gelation temperature were determined by gradual heating of the solution at a rate of 1 °C/min, with continuous stirring using a magnetic bar. The first manifestation of turbidity was taken as the CP. CPs of T1107 were also measured at different NaCl concentrations.

DLS measurements were carried out at 90° scattering angle on solutions using zetasizer 4800 (Malvern Instruments, UK) equipped with 192 channel digital correlator (7132) and coherent (Innova) Arion laser (light source) at a wavelength of 514.5 nm [25].

2.2.2. Preparation of drug loaded micelle and drug solubilization

Sonication was done to prepare loaded and non-loaded polymer micelles. For loaded micelles, the drugs were added till saturation using T1107 (prepared in Milli-Q water) followed by sonication at 37 °C for 1 h. Suspensions were then incubated at 37 °Candthen centrifuged for 10 min at 10,000 rpm. The obtained supernatant was filtered using 0.22 μ syringe filter. The absorbance of the clear solution was determined at262 nm for curcumin and 255 nm for quercetin after suitable dilution with methanol and the amount of drug encapsulated was quantified using linear calibration plot. Solubility was determined in triplicate and results reported are the mean of three trials [17].

2.2.3. In vitro cell viability assay

The effect of free and micellar drugs on the viability of CHO-K1 cell lines was determined by the MTT assay [26]. The exponentially grown CHO-K1 cells were seeded in 96-well plates at a density of 2×10^4 cells/well in 0.1 ml of MEM medium and incubated at 5% CO_2, 95% air at 37 °C for 24 h. The cells were then exposed to 12.5, 25, 50 and 100 μg/ml concentrations of free drugs and equivalent doses of drug-loaded T1107 micelles for 48 h. Untreated cell culture wells served as negative control. After the exposure, samples were removed and cells were incubated with 100 μl of MTT solution (1 mg/ml in media) for 4 h at 37 °C. Intracellular formazan crystals were extracted into 100 μl of DMSO and quantified by measuring the absorbance of the cell lysate at 570 nm. The relative growth inhibition compared to control cells was measured. The percent cytotoxicity was calculated as: