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Micellar structural transitions and therapeutic properties in tea tree oil solubilized pluronic P123 solution

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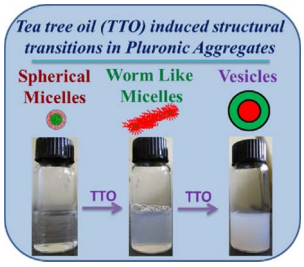
GRAPHICAL ABSTRACT



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ABSTRACT

Pluronics are used in industry as solubilizing agents for a variety of lipophilic compounds. The influence of different lipophiles on aggregation characteristics of Pluronics quite expectedly remained a subject of fundamental interest for the last few decades. In this manuscript, we show that solubilization of tea tree essential oil (TTO) brings about spherical-to-worm like micelles-to-vesicular structural transitions in aqueous solutions of Pluronic P123. TTO exhibits broad-spectrum antimicrobial properties and have therapeutic potential for wide range of diseases ranging from common wounds to different forms of cancers. Its solubilization in Pluronic solutions was carried out by subjecting the aqueous TTO-Pluronic systems into heat cycling through their phase separation temperature. We suggest that accelerated dynamics of micellar restructuring process in Pluronic P123 solutions at high temperature is responsible for solubilization of TTO upon heat cycling. Such instances of oil solubilization upon heat treatment and subsequent systematic micellar structural transitions are first of its kind in aqueous systems of nonionic surfactants. TTO solubilized Pluronic solutions exhibit antimicrobial property and cytotoxicity to breast (MCF7) and lung (A549) cancer cells, which suggest that therapeutic activity of TTO is not destroyed upon heating induced micellar solubilization.

1. Introduction

Pluronics are one of the most prominent classes of nonionic surfactants because of their widespread applications as solubilizing or dispersing agents and the rich structural polymorphism they exhibit in binary and ternary systems [1–11]. These polyethylene oxide-polypropylene oxide-polyethylene oxide based triblock copolymers are available with wide range of hydrophilic lipophilic balance (HLB). The copolymers with high HLB values like Pluronics F88 and F127, are approved by FDA as pharmaceutical excipients for different

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administration routes. The hydrophobic copolymers with low HLB values like P123, P85 etc. on the other hand, exhibit superior solubilizing capacity for lipophilic substances and have ability to facilitate delivery of drugs to multi drug resistant (MDR) cancer cells and across blood-brain barriers (BBB) [12–17]. The micro structural changes in the self-assembled structures of Pluronics associated with solubilization of lipophilic substances have always been a subject of great interest. In this manuscript, we have reported such structural changes occurring upon solubilization of tea tree essential oil (TTO) in aqueous solution of Pluronic P123. Recent reports showed potential application of Pluronic P123 as solubilization agents of oils [18–20] and drugs [21–28]. Aqueous solutions of this Pluronic exhibit time dependent sphere-to-rod micellar growth below their cloud points because of slow dynamics of micellar restructuring processes in them [4,29,30]. TTO on the other hand, has been subjected to extensive studies in recent times because of its broad spectrum antimicrobial properties and probable cure for diseases ranging from simple wounds [31–44] to critical ones like cancers [45–54]. It is sparingly soluble in water, so in the aqueous medium its therapeutic efficacies are studied using nonionic surfactants as dispersing agent [50–56]. A systematic study on the solubilization characteristics of TTO in aqueous solutions of non-ionic surfactants and subsequent changes induced in structure of surfactant aggregates has however, not been reported so far. Our studies showed that TTO solubilization in Pluronic P123 solution brings about a systematic structural change in P123 aggregates from spherical-to-worm like micelles-to-vesicles. The TTO containing Pluronic solution exhibit antimicrobial activity against *E. coli* and cytotoxicity to MCF7 (breast) and A549 (lung) cancer cells, which suggests that therapeutic activity of TTO remains unaffected upon micellar solubilization.

2. Experimental

2.1. Materials and sample preparation

TTO was procured from Allin Exporters, India and Pluronic P123 ($EO_{20}PO_{69}EO_{20}$) was purchased from Sigma–Aldrich. They were used as received. The copolymer solutions were prepared by weighing required amounts of water and copolymer, and keeping them in refrigerator overnight in tightly closed glass vials. TTO was solubilized by heating with the copolymer solution in boiling water for 2 min. Samples for cytotoxicity and antimicrobial studies were prepared under aseptic conditions using autoclaved phosphate buffer solution.

2.2. Methods

2.2.1. Dynamic light scattering (DLS)

DLS measurements on the TTO solubilized P123 solutions were carried out by using a Malvern 4800 Autosizer employing 7132 digital correlator. The light source was He-Ne laser operated at 632.8 nm with a maximum power output of 15 mW. Analyses of the electric field autocorrelation function vs. time data were carried out by method of CONTIN.

2.2.2. Small angle neutron scattering (SANS)

Experiments:

Small angle neutron scattering (SANS) experiments were performed using SANS facility operating at Dhruva Reactor, Bhabha Atomic Research Centre (BARC), Mumbai, India [57]. The mean wavelength (λ) of the incident neutron beam was 5.2 Å with a wavelength resolution ($\Delta\lambda/\lambda$) of approximately 15%. The scattered neutrons were detected using a one dimensional ^{3}He position sensitive detector (PSD) in an angular range of 0.5°–15° corresponding to wave vector transfer ($Q = 4\pi\sin(\theta/2)/\lambda$, where θ is scattering angle) range of 0.015-0.3 $Å^{-1}$. The corrections in the measured SANS data were made for the background, the empty cell contribution and the transmission. The corrected data were presented on an absolute scale using the standard protocols. The samples were prepared in D_2O to minimize the incoherent scattering and to increase contrast.

SANS Analysis:

In a SANS experiment, the differential scattering cross section ($d\Sigma/d\Omega$) per unit volume as a function of wave vector transfer (Q) is measured. For mono-dispersed particles in a medium, it can be given as [58–62]:

$$\frac{d\Sigma}{d\Omega} = n\,P(Q)S(Q) + B \quad (1)$$

where n is the particle number density. P(Q) is the intra-particle structure factor (square of the form factor) and S(Q) is the inter-particle structure factor. P(Q) characterizes the specific size and shape of the scattering objects, whereas S(Q) decides the spatial arrangement of the particles and thereby gives the information about the inter particle interaction. B is a constant term representing the incoherent background arising mainly from the hydrogen atoms present in the sample. The expressions for P(Q) for scattering objects of different shapes (spherical and long bi-layer) of particles used in the data analysis may be found in supplementary material file. For sufficiently dilute systems where inter particle interactions may be neglected, the inter-particle structure factor S(Q) may be approximated to unity. For concentrated systems on the other hand, S(q) is usually captured by the analytical solution of the Ornstein-Zernike equation with Percus-Yevick approximation, employing hard sphere interaction.

Throughout the data analysis corrections were made for instrumental smearing. The calculated scattering profiles were smeared by the appropriate resolution function to compare with the measured data. The parameters in the analysis were optimized by means of a nonlinear least-square fitting program [61].

2.2.3. Cytotoxicity study using MTT assay protocol

Human lung (A549) and breast (MCF7) adenocarcinoma epithelial cell lines were grown in DMEM medium supplemented with 10% FBS, 100 µg/ml streptomycin and 100 U/ml penicillin at 37 °C under 5% CO_2 and humidified air. For MTT assay, A549 cells (2×10^3 per well) and MCF7 cells (5×10^3per well) were seeded in 170 µl culture medium in a 96 well plate. 5–30 µl of 0.3% TTO solution in 1% P123 were added to it and the final volume of the medium was adjusted to 200 µl by further addition of necessary amount of pure 1% P123. This will give concentrations of TTO in range of 0.0075–0.045% (v/v) and a fixed P123 concentration of 0.15% in the treatment groups. The vehicle control consists of a group treated with P123 solution (0.15%) without TTO. The cells were then cultured for 48 h and processed for colorimetric MTT assay as reported previously [63]. The percentage (%) cytotoxicity was calculated from the decrease in absorbance at 570 nm of treated groups as compared to that of vehicle control group. The vehicle, P123 too was evaluated for cytotoxicity in the concentration range of 0.025–0.4% following the same method.

2.2.4. Antibacterial activity studies

Antibacterial activity of tea tree oil was checked by spot diffusion method against wild type *Escherichia coli (E. Coli.)* W1103 (gram negative) bacteria. Our laboratory doesn't have any class ii and class iii facilities to handle the infectious organisms so we have chosen *E. Coli* as a representative for showing antibacterial property of our system. Overnight grown *E. coli* culture was diluted and plated on LB agar. Spots of 5 µl solutions of 10% P123 solutions with varying concentrations of TTO and that of pure TTO were placed on the agar. These agar plates were incubated at 37 °C and were observed for inhibition area after 18 h [64,65].

2.2.5. Rheological measurements

Rheological studies of the samples were carried out by using an Anton Paar Physica MCR101 rheometer in double gap concentric cylinder geometry. The rates of oscillatory shear were varied from 0.001