Journal of the Taiwan Institute of Chemical Engineers 000 (2016) 1–9

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

Small-angle neutron scattering studies of microenvironmental and structural changes of Pluronic micelles upon encapsulation of paclitaxel

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ARTICLE INFO

Article history:
Received 27 April 2016
Revised 26 September 2016
Accepted 21 November 2016
Available online xxx

Keywords:
Pluronic prodrug
SANS
Paclitaxel
Nanocarrier
Drug delivery system

ABSTRACT

Pluronic micelles represent a novel type of nanocarriers that enhance the therapeutic efficacy of paclitaxel (PTX) by increasing its solubility and circulation time. However, encapsulation or incorporation of a drug influences the micellar structure and subsequently alters the pharmacokinetic behavior of the drug delivery system. In this study, we investigated the structural properties of micelles after micellar solubilization of PTX. Proton nuclear magnetic resonance (^{1}H NMR) and Fourier transform infrared spectroscopy (FTIR) studies showed that PTX was incorporated into the poly (propylene oxide) (PPO) blocks of the micelle cores. Small-angle neutron scattering (SANS) was used to probe changes in the core-shell structure of Pluronic P123 micelles as a function of increasing PTX and Pluronic concentration. Model-independent analyses and cryogenic- transmission electron microscopy (cryo-TEM) confirmed that both pure and PTX-incorporated micelles were spherical. However, an increase in PTX concentration led to a slight increase in the micellar dimension and was associated with the appearance of the larger Pluronic-drug aggregates as confirmed by evaluation of cryo-TEM images. At higher Pluronic concentration and a constant PTX concentration, the dimensions of micelles increased, most likely due to the altered hydrophobic environment promoting the interaction of PPO and poly (ethylene oxide) (PEO) blocks.

1. Introduction

Paclitaxel (PTX) is a well-known anti-cancer drug with proven efficacy against a variety of malignancies, including non-small cell lung, and metastatic breast, colon, and ovarian cancers [1–3]. However, PTX is highly hydrophobic with a very low water solubility (0.3 μg mL^{-1}) that leads to precipitation of the drug upon dilution [4]. One strategy to overcome low solubility is modification of the drug to a prodrug form through the introduction of hydrophilic moieties with little or no pharmacological activity [5]. Prodrugs prolong drug circulation and enhance pharmacokinetics until they reach the target organ and then undergo an enzymatic and/or chemical transformation to release the active parent drug [6,7]. Consequently, dosage frequency can be reduced. Among the reported macromolecular PTX prodrugs, the implementation of Pluronic micelles as non-toxic vehicles for PTX delivery has been the focus of extensive studies [1,2,8,9]. The main advantages of Pluronics are P-glycoprotein mediated efflux inhibition [10], overcoming multidrug resistance (MDR) [11], and improvement of the enhanced permeability and retention (EPR) effect [12,13]. However, the pharmacokinetic behavior and accumulation of micelles in the tumor site via the EPR effect rely on the morphology and dimensions of the PTX-loaded micelles.

Pluronic micelles can physically encapsulate and deliver drug nanocrystals to a tumor site, potentially preserving the original drug activity. However, this method suffers from low capacity and rapid release of the encapsulated drugs [14]. Indeed, dilution in bodily fluids is the main constraint for the physical encapsulation approach, which can lead to dissociation of micelles into monomer and drug precipitation. Alternatively, PTX molecules can be accommodated into the PPO hydrophobic core, while the tendency of PEO to dissolve in water enhances the solubility of the nanocarrier in the body fluid [1,15]. Nevertheless, incorporation of PTX into micelles also alters the balance of hydrophobic and hydrophilic domains in favor of PPO hydrophobicity, which in turn influences the structural properties of the micelles such as size, aggregation number, and dissolution [16]. Several studies suggested that PTX-pluronic conjugates results in increased drug solubility upon dilution [1,8]. However, conjugation may alter the

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http://dx.doi.org/10.1016/j.jtice.2016.11.027
1876-1070/

biological properties of the drug, causing further complication in the characterization and regulatory approval [14].

In Pluronics, the micellar formation is highly sensitive to solvent composition and the presence of hydrophilic or hydrophobic molecules either interrupts or enhances the Pluronic self-assembly in solution [17,18]. On this basis, small-angle neutron scattering (SANS) is a useful tool to measure changes in the core-shell structural parameters such as micelle core and corona sizes, polydispersity, and aggregation number. It is the technique of choice to probe bulk of the system, providing quantitative data and real statistical averages. One further advantage is that the distinction between hydrogen and deuterium scattering, which helps to extract the structural information of a sample having similar X-ray scattering length density as solvent. Likewise, SANS has been shown to be an excellent tool in identifying hydrophobic domains in micellar systems composed of PEO–PPO–PEO triblock copolymers.

Pluronic-drug interactions have been the focus of many recent SANS investigations [16,19,20]. Sharma et al. reported that the presence of PTX decreased the Pluronic CMC by 40 % while keeping the same core and corona size. The greatest changes were observed in the aggregation number and intermicellar interaction and inter-distance [16,19]. The same behavior was reported for the effect of ibuprofen on Pluronics P104 and P105, which each contain a similar number of PPO units but a different number of PEO units [20]. For both these Pluronics, larger PPO cores were observed as a result of drug incorporation. In another study, the encapsulation of flurbiprofen in solutions of Pluronics P123 and P103 was investigated by Alexander et al. [21] who demonstrated that incorporation of the drug into Pluronic building blocks reduced the CMC and led to an increased fraction of the micellized polymer as well as an increase in micelle dimension.

Therefore, the objective of the present work was to study the structural changes in Pluronic-PTX suspensions prepared by micellar solubilization and the encapsulation of PTX using P123 micelles. Firstly, the interactions of the drug with Pluronic blocks and micellar microenvironment were studied through proton nuclear magnetic resonance (^{1}H NMR) and Fourier transforms infrared (FTIR) spectroscopies. Furthermore, the effects of drug and Pluronic concentration on micellar structures were studied using SANS and cryogenic transmission electron microscopy (cryo-TEM) techniques.

2. Experimental section

2.1. Materials

All compounds including PTX, heavy water (D_2O), acetonitrile, and Pluronic P123 (P123) were supplied by Sigma-Aldrich and used as received.

2.2. Sample preparation

Pluronic solutions were prepared by dissolving the polymer to D_2O. The PTX-loaded P123 nanocarriers were prepared by an emulsion/solvent evaporation technique [22]. Briefly, the Pluronic and PTX in various feed volume ratios were dissolved in acetonitrile (5 mL), ultrasonicated for 20 min at room temperature and emulsified for 2 h in PBS (10 mL). The solution was then evaporated under a stream nitrogen. The resultant thin film was rehydrated at 70 °C with D_2O and ultrasonicated for 30 min. The concentrations of Pluronic and PTX within the samples are presented in Table 1. The physical mixture of P123 and PTX prepared by recrystallization of PTX using acetonitrile followed by dissolution of the crystals in P123 solution. The incorporation and interaction of PTX were assessed by ^{1}H NMR and FTIR studies.

The ^{1}H NMR studies were conducted on a Bruker instrument operating at 500 MHz. The spectra of Pluronic and Pluronic-PTX solutions in D_2O were measured at 25 °C. The samples were allowed to equilibrate at 25 °C for at least 15 min prior to measurement. Infrared spectra were recorded on a Digilab FTIR spectrometer (ITS-40) with fully computerized data storage and data handling capability. For all the reported spectra, 64-scan data accumulation was used at a resolution of 4 cm^{-1}. In order to observe small changes in band intensity and frequency, subtraction and baseline correction procedures were used. High-performance liquid chromatography (HPLC, Perkin Elmer Series 200) was used to measure the PTX concentrations. The drug loading efficiency was calculated from the following formulation: ((concentration of the feeding PTX − concentration of the free PTX) / concentration of the feeding PTX) × 100%). The hydrodynamic sizes and zeta potential of the Pluronic micelles were determined using dynamic light scattering (DLS) on a Beckman Coulter®N4 PLUS machine at a wavelength of 514 nm and detection angle of 90°.

2.3. Small angle neutron scattering experiments

SANS measurements were carried out on the NGB30 SANS instrument at the National Institute of Standards and Technology (NIST) Center for Neutron Research, Gaithersburg, MD. Three configurations were used: one, with the detector offset by 25 cm and a sample-detector distance of 1 m; two, with a sample-to-detector distance of 4 m and five inserted neutron guides to enhance flux on the sample and therefore improve statistics; three with the detector offset by 10 cm and a sample-detector distance of 13 m was applied to give a scattering vector range of 0.005–0.600 $Å^{-1}$. Cold neutrons with a wavelength of 6.0 Å were used, and all samples were measured in demountable titanium cells with 1 mm path length. Unless otherwise stated, samples were measured at 37 °C. The raw data were reduced using Igor Pro macros [23]. A standard data reduction method was used to correct for empty cell and blocked beam scattering and to rescale the radially averaged data to an absolute cross section scale. The reduced data were fitted using the core-shell model with constant poly core-shell ratio (PolyCoreShellRatio) of the NCNR Igor Pro data analysis package [24]. The value for solvent scattering length density (SLD) for D_2O was kept constant at 6.38 $\times 10^{-6}$ $Å^{-2}$ for all fittings.

The software program GNOM from ATSAS was also used to obtain information on the particle distance distribution function (PDDF), also known as the $P(r)$ function [25]. As an indirect transformational program for small-angle scattering data processing, GNOM reads in one-dimensional scattering curves and evaluates the PDDF. It provides a histogram of all distances between point pairs within particles weighted by the excess SLD at the point, which is useful to compute the radius of gyration (R_g), maximum particle dimension (D_{max}), and Porod volume [26,27].

2.4. Cryogenic-transmission electron microscopy

The micellar structure in solution was visualized by cryo-TEM images. Specimens for cryo-TEM were prepared with an FEI Vitrobot Mark IV vitrification system in a controlled environment. Samples were applied onto holey carbon films. After the blotting process, the grids were immediately plunged into liquid ethane and then stored in liquid nitrogen before imaging. Finally, TEM imaging was conducted on an FEI Tecnai T12 transmission electron microscope operating at 120 kV.

3. Results and discussion

3.1. Pluronic P123 micelles

The solutions of P123 in D_2O were studied as a function of both Pluronic and PTX concentrations. The structure of the P123 PEO_{20}–

Please cite this article as: K. Dehvari et al., Small-angle neutron scattering studies of microenvironmental and structural changes of Pluronic micelles upon encapsulation of paclitaxel, Journal of the Taiwan Institute of Chemical Engineers (2016), http://dx.doi.org/10.1016/j.jtice.2016.11.027