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Small angle neutron scattering study of doxorubicin–surfactant complexes encapsulated in block copolymer micelles

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Abstract. Self-assembling behaviour of block copolymers and their ability to evade the immune system through polyethylene oxide stealth makes it an attractive candidate for drug encapsulation. Micelles formed by polyethylene oxide–polypropylene oxide–polyethylene oxide triblock copolymers (PEO–PPO–PEO), pluronic P123, have been employed for encapsulating the anti-cancer drug doxorubicin hydrochloride. The binding affinity of doxorubicin within the micelle carrier is enhanced through complex formation of drug and anionic surfactant, aerosol OT (AOT). Electrostatic binding of doxorubicin with negatively charged surfactants leads to the formation of hydrophobic drug–surfactant complexes. Surfactant-induced partitioning of the anti-cancer drug into nonpolar solvents such as chloroform is investigated. SANS measurements were performed on pluronic P123 micelles in the presence of drug–surfactant complex. No significant changes in the structure of the micelles are observed upon drug encapsulation. This demonstrates that surfactant–drug complexes can be encapsulated in block copolymer micelles without disrupting the structure of aggregates.

Keywords. Micelles; polymer solutions; neutron scattering.

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1. Introduction

Doxorubicin hydrochloride is a widely used anti-cancer drug, but its cytotoxicity to normal tissues and inherent multidrug resistance remain a major problem [1]. To minimize toxicity and improve its therapeutic efficiency, several strategies have been explored such as encapsulation in liposomes and drug-conjugated nanoparticles. Recently amphiphilic block copolymers are gaining much attention in drug research due to their unique characteristics [2]. Block copolymers self-associate in aqueous solution forming a micellar structure having a diameter less than 100 nm. Small size of the micelle enables easier tissue and cell penetration [3]. These self-assemblies have a hydrophobic core surrounded by a shell of hydrophilic blocks consisting of hydrophobic blocks. The core serves as a non-aqueous reservoir for

hydrophobic drug and the shell interacts with the biological environment, preventing the capture of these species by the reticuloendothelial system (RES). This increases the circulation time of the drug in the body. In this study, attempts have been made to develop a drug delivery system consisting of a drug–surfactant complex encapsulated in a PEO-based polymeric micelle-like pluronic P123. The electrostatic interaction of the drug with a negatively charged species is expected to make the drug hydrophobic enough to partition into the hydrophobic core of the micelle [4].

2. Materials and methods

DOX in the form of hydrochloride salt was obtained as a gift from Sun Pharmaceuticals, Baroda. AOT (dioctyl sulfosuccinate, sodium salt) and pluronic P123 [(ethylene oxide)$_{20}$-(propylene oxide)$_{70}$-(ethylene oxide)$_{20}$] were obtained from Aldrich. DOX–AOT complex solutions were prepared at different concentrations ranging from 10 to 100 μM. Encapsulation of the complex into micelle was achieved by adding 1% pluronic P123 solution to each DOX–AOT solution. The complexation of drug with surfactant and its hydrophobicity were confirmed using a Chemito Spectrascan UV-2600 double beam spectrophotometer. SANS experiments were carried out on samples prepared in D_2O using the SANS diffractometer built at Dhruva reactor, BARC, Trombay, India. The mean wavelength of incident radiation was 5.2 Å. The magnitude of scattering vector was varied from 0.02 to 0.3 Å^{-1}.

3. Results and discussions

3.1 *Complexation of DOX with AOT and loading into P123 micelle*

Doxorubicin hydrochloride molecule contains a protonated amino group which makes it hydrophilic in nature (figure 1). The water soluble nature of DOX makes it difficult to physically load within the micelles in sufficient amount. Several strategies have been reported in literature to encapsulate ionic salts of cytotoxic drugs in lipophollic compartments. One method is to add organic counterions to form ion-pairs with charged drug molecules [5]. Complexation of drug with ionic polymers to form hydrophobic drug–polymer complexes or nanoparticles has also been reported [6]. We investigated the effect of an anionic surfactant, AOT for electrostatic complexation with cationic drug. The hydrophobicity of the drug was established by observing its partitioning between chloroform and water. The effect of varying AOT concentration on the drug extraction into the organic phase was examined spectrophotometrically. The absorbance of DOX decreased with increasing AOT concentration as shown in figures 2a and 2b. The decrease in the absorbance of DOX in aqueous phase is attributed to the formation of DOX–AOT complex that is being extracted to the chloroform phase. Moreover, the absorbance in the aqueous phase almost vanished at equimolar concentration of AOT, indicating the possibility of 1 : 1 complex formation.

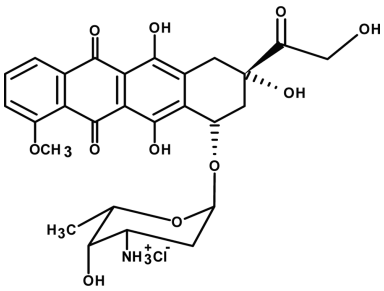

Figure 1. Chemical structure of doxorubicin hydrochloride.

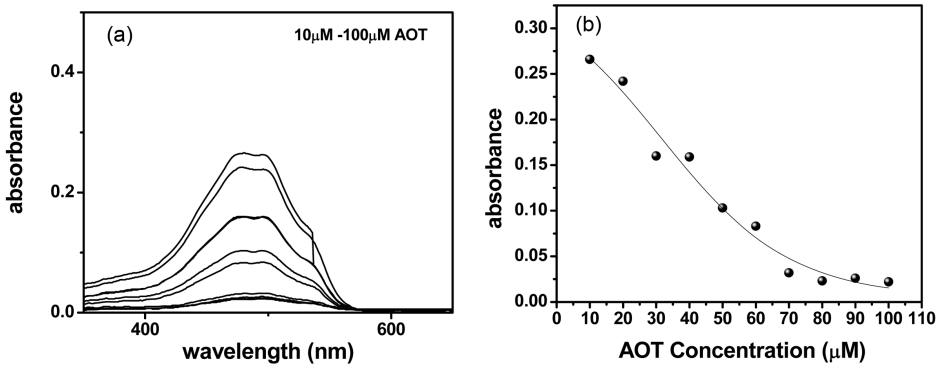

Figure 2. (**a**) Effect of AOT on the partition of DOX between aqueous and chloroform phase at different wavelengths. (**b**) Variation of absorbance of DOX with AOT concentration.

In the presence of P123 micelles (1% P123), the optical absorption of DOX–AOT complex is similar to that of pure DOX–HCl. The increased absorbance of DOX–AOT complex in water in the presence of P123 micelles indicates the solubilization of the drug–surfactant complex within the micelles. A slight change in the spectral features is also observed upon addition of AOT. For DOX–HCl in water, $\lambda_{\max}$ is $\sim$480 nm with a shoulder at 498 nm (figure 3), while for the complex the intensity of the shoulder peak is increased marginally. This change probably indicates the effect of complexation of drug with surfactant.

3.2 *Small angle neutron scattering*

The structure of P123 micelles in the presence of DOX–AOT complex has been confirmed from SANS measurements. The evolution of the SANS spectra for P123–DOX and P123–DOX–AOT (P123 – 1%, DOX – 100 μM, AOT – 100 μM) at room temperature is shown in figure 4. The differential scattering cross-section per unit volume ($\mathrm{d}\Sigma/\mathrm{d}\Omega$) of monodisperse micelles can be written as

$$\mathrm{d}\Sigma/\mathrm{d}\Omega = NF_{\mathrm{mic}}(q)S(q) + B. \tag{1}$$

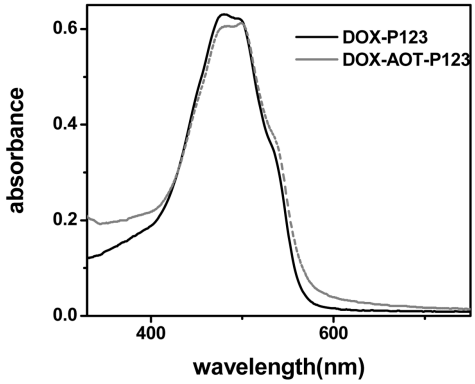

Figure 3. Absorbance spectra of DOX–P123 and DOX–AOT–P123.

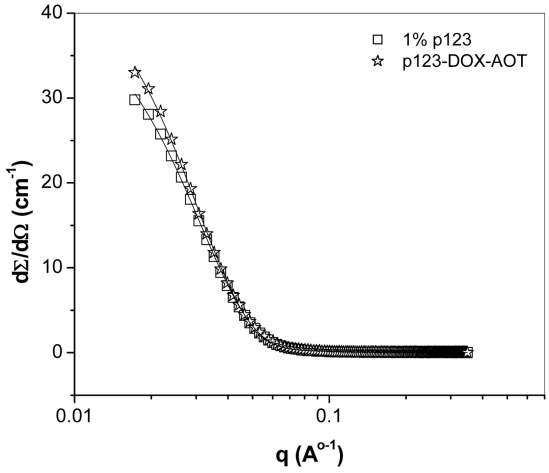

Figure 4. SANS spectra of 1% P123 micelles with and without DOX.

N is the number density of the micelles and B is a constant term that represents the incoherent background scattering mainly from the hydrogen atoms present in the sample. $F_{\mathrm{mic}}(q)$ is the form factor characteristic of specific size and shape of the scatterers and $S(q)$ is the structure factor that accounts for the interparticle interaction. The structure of P123 block copolymer micelles has been described using a model consisting of PEO chains attached to the surface of the PPO core. The form factor for such a micelle structure was formulated by Pedersen [7]. In this model, the core is described as a sphere and the shell as consisting of non-interacting Gaussian polymer chains. Non-penetration of the chains into the core region is mimicked by moving the center-of-mass of the chains by a distance R_{g} away from the surface of the core, where R_{g} is the radius of gyration of the chains. The interparticle structure factor $S(q)$ for block copolymer micelles is usually captured from the analytical solution of the Ornstein–Zernike equation in the Percus–Yevick approximation, employing hard sphere potential. Since our analyses were carried

out on dilute (1%) solutions, we have considered $S(q)$ as unity. A polydispersity in the micellar core size has been accounted for by a Schultz distribution. Analysis of the SANS data of similar block copolymer indicates that micelles are polydisperse in nature with significant water of hydration [8]. Accordingly the R_g of the polymer ($R_g = 9$ Å), volume fraction of the hydrated micelles ($\phi = 0.02$) and polydispersity parameter ($z = 35$) are used to restrict the number of parameters in the fit. The core radius of the micelle is used as the only variable in the fit. Data analysis based on the above parameters show that the micelles are composed of a hydrophobic core of radius 57 Å and no significant change in the size of the micelles is observed upon drug solubilization.

4. Conclusions

This report investigates the solubilization of the anti-cancer drug, doxorubicin in block copolymer micelles through electrostatic complexation. Complexation of doxorubicin with anionic surfactant aerosol OT was confirmed from optical absorption measurements. Electrostatic binding between the surfactant and the drug makes the complex hydrophobic and gets partitioned into chloroform. Addition of pluronic block copolymer (P123) in an aqueous suspension of drug–AOT complex solubilizes hydrophobic complex in micelles. The structure of block copolymer micelles are retained even after drug solubilization.

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