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Elucidating chlorin e6–sucrose ester interaction using coarse-grain modeling and fluorescence spectroscopic technique

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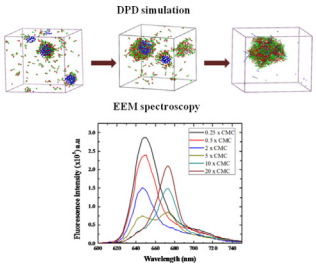
HIGHLIGHTS

- Disaggregation of chlorin e6 by sucrose ester surfactants was studied.
- Extent of disaggregation was quantitatively estimated by EEM fluorescence.
- The interaction was qualitatively visualized by DPD simulation.
- Sucrose ester with longer alkyl chain length showed better disaggregation.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aimed at visualization and elucidation of aggregate-to-monomer conversion of chlorin e6 (Ce6) by non-ionic surfactant sucrose esters. Excitation–emission matrix (EEM) fluorescence spectra of Ce6 were recorded in the presence of different concentrations of the surfactants with alkyl substituents of various chain lengths [lauryl (SEL)/myristyl (SEM)/palmityl (SEP)]. The EEM spectra were deconvoluted and parallel factor (PAFARAC) algorithm was applied to determine the concentrations of the underlying constituents (aggregates and monomers) for each Ce6–sucrose ester system. The experimental results were further correlated by dissipative particle dynamics (DPD), a coarse grain simulation technique. The results showed that disruption of Ce6 aggregates was primarily initiated at sub-micellar concentration of the sucrose ester and completed at concentrations higher than their corresponding critical micelle concentrations. Thermodynamic study revealed that Ce6 monomers were encased in the hydrophobic micellar cores of the sucrose esters. PARAFAC analysis and DPD response parameters substantiated the overall disaggregation rank order as SEP > SEM > SEL. This study successfully demonstrated Ce6–surfactant interaction using a novel multivariate technique while DPD simulation served as a visualization tool for illustrating underlying changes in the Ce6–sucrose ester systems at different surfactant concentrations.

1. Introduction

Computer-aided simulations have emerged as a powerful tool in elucidating the nature of interaction of systems comprising drug–polymer, polymer–surfactant and drug–surfactant, respectively [1]. Atomistic simulations can provide detailed information on the behavior of a system with much less time required for simulation but possible only for limited number of molecules and is inefficient for specific complex systems [2]. In this respect, mesoscopic simulation technique has gained increased attention in recent years owing to the availability of larger time frame for simulation with larger number of molecules incorporated into the system. In reality, the simulation of many complex systems such as emulsion, polymer mixture and drug formulations performed

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effectively in mesoscale owing to afore-mentioned advantages [3]. Dissipative particle dynamics (DPD) is a mesoscale simulation method, which has been successfully used to simulate complex systems such as emulsions and microscale drug formulations. In DPD, cluster of atoms are designated as fluid particles, which interact with similar or dissimilar particles according to Newton's law of motion [4–6].

Aggregation is typically exhibited by hydrophobic dyes in aqueous media, where the dye molecules form intermolecular association through hydrogen bonding or hydrophobic force of interaction. On the basis of spectral shift of the absorption maxima of aggregates relative to that of the monomers, the aggregates are classified as J- and H-aggregates. J-aggregates exhibit bathochromic shift in the absorption band and form a head-to-tail structure with high fluorescence intensity. On the other hand, H-aggregates are formed by face-to-face stacked structure where fluorescence is strongly quenched, resulting in reduced quantum yield [7]. In recent years, chlorin e6 (Ce6) has been used as a photosensitizer in photodynamic therapy (PDT) for the treatment of superficial carcinomas [8]. PDT provides the advantages of being non-invasive and inexpensive therapy, using the strong emission in the red light region and causing less side effects. The PDT efficacy of Ce6 is largely limited by its tendency to form aggregates at the tumour's physiological pH. Paul et al. has recently shown the pH-dependent reduction in quantum yield of H-aggregates of Ce6 in aqueous media. The fluorescence life-time and subsequent singlet oxygen generation were also markedly compromised in aqueous media, implicating its reduced efficiency as a potential PDT drug [9]. Conversion of Ce6 aggregates to monomers using disaggregating agents such as surfactants and macromolecules have been explored by many researchers. Surfactants have often been reported to facilitate monomerization of dye aggregates when they are employed well above their critical micelle concentration [10]. For biopharmaceutical consideration, the use of non-toxic and biocompatible disaggregating agents is of potential choice as these can be easily exploited as a drug carrier for product development. Sucrose esters are examples of biocompatible non-ionic surfactants, which are currently used in various food industries as taste masking agent. Additionally, they still have not been explored as a disaggregating agent for photosensitizers.

It has been observed that the aggregate to monomer conversion is preceded by three stages, namely pure aggregates, a mixture of aggregates and monomers and pure monomers, at very low, intermediate and maximum concentrations of the disaggregating agent, respectively [10]. These events lead to the variation in emission maxima, which can be regarded as the qualitative estimate of aggregate to monomer conversion. The disaggregation of dye molecules has been demonstrated using such indicative spectral shifts [10,11]. The present study compared the disaggregation efficiency of the various sucrose esters by determining the amounts of Ce6 aggregates and monomers at different concentrations of the surfactant. Determination of aggregated or monomeric Ce6 concentrations, corresponding to specific emission maxima, is possible with the use of excitation–emission-matrix (EEM) spectroscopy. EEM fluorescence spectroscopy has gained interest in recent years owing to their suitability to accurately quantify and determine the individual components in a mixture of fluorophores. The process involves exciting a sample over a range of wavelengths and recording the fluorescence emission over another range of wavelengths [12,13]. Combining the data produces a contour map, often referred to as a "fingerprint", displaying fluorescent peak locations and intensities. The peak locations indicate the type of fluorescent substance and the intensity represents the concentration. Therefore, the present study aimed to elucidate the disaggregation efficacy of different sucrose esters on the Ce6 aggregates using novel techniques, such as EEM spectroscopy and DPD simulation. It was hypothesized that the relative proportion of pure aggregates and monomers could be determined using EEM fluorescence spectroscopy, while DPD simulation could be used as an effective tool to visualize the monomerization of Ce6 aggregates at various surfactant concentrations. Furthermore, the specific DPD response parameter such as diffusion coefficient and end-to-end distance of surfactants would provide information of the physical changes of drug and surfactants during different simulation steps.

1.1. DPD simulation

1.1.1. DPD theory

DPD is a coarse-grained simulation technique often used to simulate complex fluid dynamical behavior. A DPD bead represents a group of atoms or a volume of fluid that is large on the atomistic scale but still macroscopically small. In DPD simulations, a set of beads move according to Newton's equation of motion [14]

$$\frac{d\mathbf{r}_i}{dt} = \mathbf{V}_i, \quad m_i \frac{d\mathbf{V}_i}{dt} = \mathbf{f}_i, \tag{1}$$

where $\mathbf{r}_i$, $\mathbf{V}_i$, m_i, $\mathbf{f}_i$ are the position, velocity, mass and force of bead i, respectively. All bead masses are assumed to be the same and set to unity for simplicity. The force acting on each bead is the sum of conservative force ($\mathbf{F}_{ij}^{C}$), dissipative force ($\mathbf{F}_{ij}^{D}$), and random force ($\mathbf{F}_{ij}^{R}$).

$$\mathbf{f}_i = \sum_{j \neq i} (\mathbf{F}_{ij}^{C} + \mathbf{F}_{ij}^{D} + \mathbf{F}_{ij}^{R}), \tag{2}$$

The conservative force for non-bonded particles is defined by soft repulsion acting along the line of centers and given by:

$$\mathbf{F}_{ij}^{C} = \begin{cases} a_{ij}(1 - r_{ij})\hat{\mathbf{r}}_{ij}, & (r_{ij} < 1) \\ 0, & (r_{ij} \geq 1) \end{cases} \tag{3}$$

where a_{ij} is a maximum repulsion between bead i and bead j with the following relation;

$$\mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j, \mathbf{r}_{ij} = \left|\mathbf{r}_{ij}\right|, \hat{\mathbf{r}}_{ij} = \frac{\mathbf{r}_{ij}}{\left|\mathbf{r}_{ij}\right|} \tag{4}$$

The dissipative force is regarded as a frictional force, which depends on the position and relative velocities of the beads, and given by:

$$\mathbf{F}_{ij}^{D} = -\frac{\sigma^2(\omega(\mathbf{r}_{ij}))^2}{2kT}(\mathbf{V}_{ij}.\hat{\mathbf{r}}_{ij})\hat{\mathbf{r}}_{ij}, \tag{5}$$

The random force is the random interaction between two adjacent beads i and j and given by:

$$\mathbf{F}_{ij}^{R} = \frac{\sigma\omega(r_{ij})\hat{\mathbf{r}}_{ij}\zeta}{\sqrt{\delta_t}}, \tag{6}$$

where $\mathbf{V}_{ij} = \mathbf{V}_i - \mathbf{V}_j$, ζ is a random variable with zero mean and variance 1, δ_t is the time step used, the r-dependent weight function $\omega(r) = 1 - r$ for $r < 1$ and $\omega(r) = 0$ for $r > 1$ [14].

1.1.2. Spring force

In the DPD model, atoms of each molecule are grouped together into beads. According to Groot and Warren, the molecules are designated by beads, which are joined together by simple harmonic spring force according to the following relation [14]:

$$\mathbf{F}_i^{S} = \sum\nolimits_j C\mathrm{r}_{ij} \tag{7}$$

where C is the spring constant, and the mean distance between two consecutive chain beads is governed by the spring force and repulsive interaction. Simple harmonic potential was used for simulating