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Characterization of chlorophyll derivatives in micelles of polymeric surfactants aiming photodynamic applications

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ABSTRACT

The spectrophotometric properties of chlorophylls' derivatives (Chls) formulated in the Pluronics® F-127 and P-123 were evaluated and the results have shown that the Chls were efficiently solubilized in these drug delivery systems as monomers. The relative location of the Chls in the Pluronics® was estimated from the Stokes shift and micropolarity of the micellar environment. Chls with phytyl chain were located in the micellar core, where the micropolarity is similar to ethanol, while phorbides' derivatives (without phytyl chain) were located in the outer shell of the micelle, *i.e.*, more polar environment. In addition, the thermal stability of the micellar formulations was evaluated through electronic absorption, fluorescence emission and resonance light scattering with lowering the temperature. The Chls promote the stability of the micelles at temperatures below the Critical Micellar Temperature (CMT) of these surfactants. For F-127 formulations, the water molecules drive through inside the nano-structure at temperatures below the CMT, which increased the polarity of this microenvironment and directly affected the spectrophotometric properties of the Chls with phytyl chain. The properties of the micellar microenvironment of P-123, with more hydrophobic core due to the small PEO/PPO fraction, were less affected by lowering the temperature than for F-127. These results enable us to better understand the Chls behavior in micellar copolymers and allowed us to design new drug delivery system that maintains the photosensitizer's properties for photodynamic applications.

1. Introduction

Chlorophylls (Chls) (Fig. 1A) are widespread in nature and have interesting photophysical characteristics as photosensitizers for photodynamic therapy (PDT) [1,2], such as high light absorption in the therapeutic window (600–800 nm), high lifetime of the excited state and high quantum yield of the singlet oxygen [3–6]. Also they exhibit high hydrophobicity [7], which increases the interaction with biological membranes, one of the most important target site in PDT [8]. However the high hydrophobicity of chlorophylls restricts its use because it leads to insolubility and/or self-aggregation in aqueous medium [9–12], which diminishes the bioavailability, light absorption capacity [13] and generation of singlet oxygen and, consequently, reduces the efficiency of photodynamic action [14]. Therefore, the use of nanostructured colloid as drug delivery system may improve the solubilization and stabilization of Chls in monomeric states for photodynamic applications [15–20].

The polymeric surfactant molecules, the Pluronics® type, are interesting drug delivery systems consisting of hydrophilic blocks of ethylene oxide (EO) and hydrophobic blocks of propylene oxide (PO) forming the poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), known as PEO-PPO-PEO structure [21–24].

The micelles from Pluronic® P-123 and F-127 were under investigation in our study, which both copolymers have similar PPO portion while the PEO section in F-127 is bigger than in P-123 (Fig. 1B). Some advantages of these polymeric micelles include their high structural stability - static and dynamic - *in vivo* and *in vitro*, high solubility in water, non-ionic characteristics with low toxicity compared to ionic micelles. Other advantages are attributed to the incorporation and release of different drugs in these micelles, and the ability to protect the encapsulated drug from degradation and other metabolism-related damage [25].

In water, these polymeric surfactants form micelles composed of an inner layer, the micelle core, consisting mainly of the hydrophobic block of the copolymers [25], stabilized by the outer layer of hydrated hydrophilic blocks, the corona region surrounded by water [26]. The surfactant concentration and the temperature are factors that affect the micellization and their drug solubilization capacity [27].

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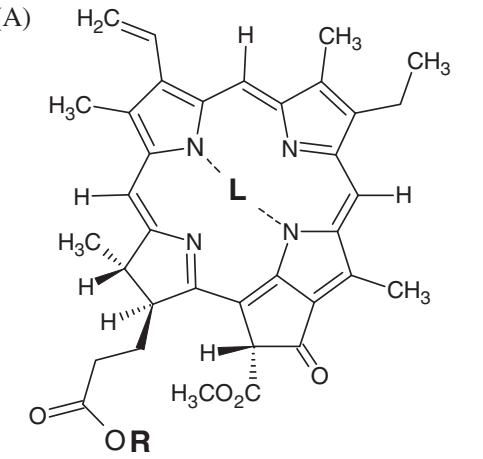
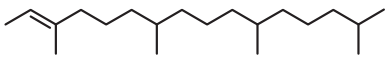
(A)



Phytyl=



Chls	Ligand (L)	Radical (R)
Mg-Chl	Mg	Phytyl
Zn-Chl	Zn	Phytyl
Pheo	2H	Phytyl
Pheid	2H	H
Zn-Chld	Zn	H

(B)

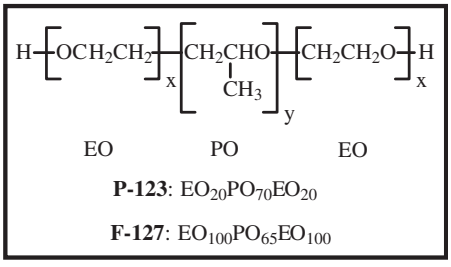


Fig. 1. Molecular structure of: (A) type *a* chlorophyll and their derivatives; (B) polymeric tri-block surfactants P-123 and F-127.

The temperature above the micellization is called Critical Micellar Temperature (CMT); at temperature below the CMT, PPO and PEO blocks are hydrated and dissolved in water solution, where the copolymer molecules exist as unimers [28]. At temperatures higher than the CMT, the dehydration of PO units occurs and, consequently, decreases the water solubility and leads to aggregation of the PPO block, while EO groups remain hydrated favoring the micelles' formation. Thus, the core of the micelles is composed of dehydrated PPO block whereas the corona is constituted by PEO hydrated blocks [29].

Therefore, in this work, the solubilization and monomerization of chlorophyll derivatives in polymeric micelles of P-123 and F-127 for possible application in PDT were investigated. The Chls studied were: Pheo, Mg-Chl and Zn-Chl, which presents the phytyl chain, and the phorbides, Pheid and Zn-Chld, without the phytyl group (Fig. 1). Thus, the thermal stability of formulations and the relative location of Chls in these nanocarrier systems were studied based on their micellar microenvironments properties at different temperatures through electronic absorption, fluorescence emission, stocks shift and resonance light scattering.

2. Materials and Methods

2.1. Materials

The investigated Chl compounds were Mg-Chl and their derivatives Pheo, Pheid, Zn-Chl and Zn-Chld (Fig. 1A), which were obtained and purified according to [14]. The Chls were characterized through electronic absorption (UV–vis Varian Cary 50) and ^{1}H NMR (Varian, Gemini 300 MHz) [30–34]. The polymeric surfactants of Pluronic classes P-123 (MM = 5800 g mol^{-1}) and F-127 (MM = 12,600 g mol^{-1}) were purchased from Sigma-Aldrich. All materials (solvents, surfactants and reagents) were of analytical grade (PA). The physicochemical analyzes were carried out using electronic absorption, fluorescence and Resonance Light Scattering RLS (Cary - Eclipse spectrofluorometer).

2.2. Formulation of Chlorophylls in Pluronic Micelles

The Chl (4.0 μmol L^{-1}) formulations in F-127 (2%, w/v; 1.59×10^{-3} mol L^{-1}) and P-123 (2%, w/v; 3.45×10^{-3} mol L^{-1}) were prepared through the dispersion solid method [35]. This method consists of the co-solubilization of the chosen Chl and surfactant in chloroform, followed by solvent's evaporation using a rotary evaporator, forming a thin-film of Chl/surfactant. The film was left to dry completely in a desiccator under reduced pressure for 24 h. After that, the film was hydrated with pH 7.4 PBS buffer at 60 °C, under constant stirring until complete solubilization, and the solution was transferred to a volumetric flask of 25.0 mL and filled up with distilled water. After that they were characterized.

In addition some fresh prepared formulated aqueous samples were submitted to lyophilization (freeze-drying methodology) and rehydration procedures. Aqueous samples ([Chls] = 4.0 μmol L^{-1}) prepared by solid dispersion method of Chls in F-127 and P-123 (2%, w/v) were characterized and the water was eliminated using a freeze-dryer apparatus (MicroModulyo). In sequence, the lyophilized "cake" was rehydrated, solubilized with water, and submitted to characterization procedures.

2.2.1. Characterization and Stability of Formulated Chlorophylls in Pluronic Micelles

The formulations Chls/pluronic micelles obtained through solid dispersion method were characterized by electronic absorption and fluorescence spectrophotometry, at 30.0 °C and pH 7.4. The excitation wavelength (λ_{exc}) was 411 nm for all Chls. The molar absorptivity (ε) of Chl/P-123 and Chl/F-127 were determined through dilution using those aqueous surfactants (2%, w/v). After each dilution, the electronic absorption spectrum was registered at 30.0 °C. The molar absorptivity value was calculated through Lambert-Beer's law.

The Stokes shift ($\Delta\nu$) was calculated for Chls in ethanol and in micellar aqueous formulations prepared by the solid dispersion method using the Eq. (1):

$$\Delta\nu(\mathrm{cm}^{-1}) = 10^7\left(\frac{1}{\lambda_{abs,\,max}} - \frac{1}{\lambda_{emi,\,max}}\right) \quad (1)$$

where $\lambda_{abs,max}$ and $\lambda_{emi,max}$ are the wavelength of maximum absorption and emission, respectively. The properties permitted to discuss the Chls position in the micelle.

In parallel, the samples were also characterized by resonance light scattering (RLS) in synchronous mode with $\Delta\lambda = 0$ using spectrofluorometer, which allowed estimating the size variation of the small aggregates in solution.

2.2.2. Influence of the Temperature on the Stability of Chls/Pluronic Micelles

The effect of temperature on the stability of the formulations in micelles of polymeric pluronics was investigated through electronic absorption spectrum and fluorescence emission. In parallel, the samples