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On the formation, physicochemical properties and antibacterial activity of colloidal systems containing tea tree (*Melaleuca alternifolia*) oil

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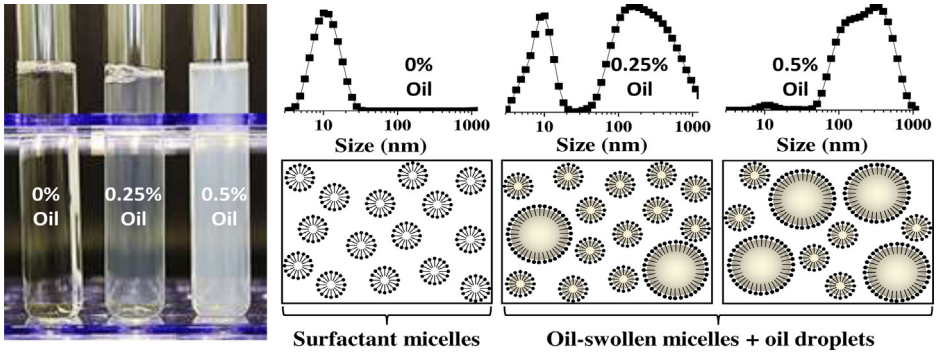
HIGHLIGHTS

- The formation of oil-swollen micelles and oil droplets depended on the tea tree oil concentration.
- Their physicochemical properties such as size, turbidity, zeta potential and fluidity were investigated.
- Oil droplets are physically unstable since they undergo a gradual transition to oil-swollen micelles under storage.
- The antimicrobial activity of tea tree oil in free and encapsulated forms was compared.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we investigated the influence of tea tree oil (TTO) on the formation and physicochemical properties of colloidal systems stabilized by the nonionic surfactant polysorbate 80. These systems were prepared by spontaneous emulsification with quantities of TTO ranging from 0% to 0.5% w/w, at a fixed surfactant concentration (2% w/w). The dispersed structures usually found in microemulsions (oil-swollen micelles) and emulsions (oil droplets) were produced under these experimental conditions. The relative contribution of the oil-swollen micelles (~11 nm) to the overall scattered intensity decreased as a function of TTO concentrations, while an opposite behavior was observed for the oil droplets (~275 nm). Such variations led to significant increases in the z-average particle size and turbidity of the TTO containing samples. Additional investigations also revealed that the surface charge and fluidity of oil-swollen micelles and oil-droplets are very similar. Although their sizes remained practically unchanged over the storage time, the oil droplets were considered to be physically unstable since they undergo a gradual transition to oil-swollen micelles. Finally, *in vitro* susceptibility studies with TTO containing colloidal systems showed that the encapsulation of this essential oil with polysorbate 80 did not improve its antimicrobial

Abbreviations: TTO, tea tree oil; DLS, dynamic light scattering; EPR, electron paramagnetic resonance; NTU, nephelometric turbidity unit; NLLS, nonlinear least-squares; DSA, doxyl stearic acid; R, rotational diffusion constant; S_0, order parameter.

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activity. This effect has been attributed to the electrostatic repulsion between the dispersed structures and the bacterial outer membrane as well as a greater tendency of essential oil molecules to remain dissolved in the hydrophobic core of colloidal systems.

1. Introduction

The essential oil of *Melaleuca alternifolia*, also known as tea tree oil (TTO), has been used in Australian traditional medicine and more recently worldwide mainly due to its antimicrobial and anti-inflammatory activities [1]. The action mechanism of TTO against fungus and bacteria has been partially elucidated on the basis of its chemical composition, which is comprised mostly of monoterpenes, sesquiterpenes, and their associated alcohols [2–4]. Because terpenes are considered potent membrane fluidizing agents, it has been proposed that TTO compromise the permeability barrier of microbial membrane structures, thereby leading to chemiosmotic control loss and cell death [5–8]. The variability of amounts and profiles of its components seems to contribute to reduce the occurrence of resistance to TTO, since numerous targets would have to adjust to overcome the antimicrobial actions of each terpene. In fact, clinical resistance to TTO has not yet been reported since the 1920s, when its medical use in Australia was approved. Moreover, resistance to conventional antibiotics has not presented to influence susceptibility to TTO, suggesting that cross-resistance does not occur either [1,9–11]. In the light of the potential benefits of TTO for the drug resistant infection treatment and microbial resistance control, its use in several biologically active formulations is gaining increasing acceptance throughout the world. Nowadays, several types of creams and gels containing TTO can be found in the market and compounding pharmacies for the treatment of superficial infections caused by bacteria and fungus. Evaluations on the efficacy and safety of this essential oil as a topical antimicrobial agent are presented in numerous studies [12–16]. In addition, this essential oil has been used more recently as an active ingredient in some commercial brands of mouthwashes such as AloeDent®, Jason® and Desert Essence® for the elimination of odor-causing bacteria and controlling gingivitis. The effectiveness of TTO in decreasing the gingival inflammation is attributed, among other factors, to its water-soluble components that are able to suppress the cytokine production by human monocytes [17–20]. Although the use of theses mouthwashes has been widespread, there is still very little information on the preparation and physicochemical properties of TTO containing aqueous systems.

Because essential oils are insoluble or sparingly soluble in water, its dispersion in aqueous medium has been often achieved through their encapsulation in colloidal systems such as emulsions, microemulsions, liposomes, nanoemulsions, and solid particles made from carbohydrates and crystalized lipids [21–28]. Besides contributing to a uniform distribution of essential oils in a hydrophilic matrix, these systems can also increase the physical stability of the active substances, decrease their volatility and enhance their bioactivity [21,22]. Of the above listed colloids, emulsions and microemulsions are amongst the commercially most frequently used systems. Emulsions are metastable colloids consisting of at least two immiscible liquids, such as oil and water, with one liquid being dispersed in the other in the form of droplets, stabilized by the presence of an emulsifying agent [29]. They are optically turbid dispersions and can only be obtained by mechanical mixing of the components because of their thermodynamic instability. On the other hand, microemulsions are thermodynamically stable systems, which imply that form spontaneously at certain concentrations of oil, water and amphiphile, and the formation is limited only by the diffusion of the molecules [30]. Another important advantage of microemulsions over emulsions is that they can be designed to be optically transparent by making the particle dimensions much smaller than the wavelength of light so that light scattering is relatively weak. Thus, they can be incorporated into products that need to be optically transparent such as mouthwashes. However, from an application standpoint, since microemulsions require a relatively large amount of surfactant than emulsions, the choice of chemically stable and less toxic surfactants is required. As there is a consensus that nonionic surfactants tend to be less toxic than ionic surfactants, they are more widely used not only in mouthwashes but also in a number of pharmaceutical formulations and as food additive [31,32]. Perhaps the most pharmaceutically acceptable nonionic surfactants are the polyoxyethylene derivatives of triglycerides and castor oil and polyoxyethylene sorbitan fatty acid esters (generally referred to as polysorbates or tweens). The first group of nonionic surfactants has been preferred for intravenous administration, while polysorbates have been often used in parenteral and oral formulations [33].

The purpose of this study was first to examine the influence of TTO concentration on the formation of colloidal systems stabilized by the nonionic surfactant polysorbate 80. In particular, the particle size distributions obtained from dynamic light scattering (DLS) measurements were carefully analyzed in order to identify the experimental conditions for which microemulsions and/or emulsion are produced. Other physicochemical properties such as surface charge and fluidity of dispersed structures were also investigated by zeta potential and electron paramagnetic resonance (EPR) measurements, as well as the relationship between the mean particle size (z-average) and the turbidity of samples. As the physical stability of colloidal systems is important for their applications in mouthwashes, this issue was also addressed in the second part of this paper. Finally, the *in vitro* susceptibility of a gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria to the colloidal systems was evaluated to explore the question of whether or not the TTO improves its antibacterial effect after having been formulated as oil-in-water microemulsions and emulsion. Overall, we believe this study provides useful information for the formation and rational design of TTO containing aqueous systems for pharmaceutical industry applications.

2. Materials and methods

2.1. Materials

Polysorbate 80 (CAS Number 9005-65-6), dimethyl sulfoxide (DMSO) resazurin, and brain-heart infusion (BHI) broth were acquired from Sigma Chemical Co. (St. Louis, MO, USA). The essential oil of *M. alternifolia* (tea tree oil) was supplied by Ferquima Indústria e Comércio LTDA (Vargem Grande Paulista, SP, Brazil). The main components of this essential oil are: terpinen-4-ol (42%), γ-terpinene (22%), α-terpinene (10%), and 1,8-cineole (1.5%). The spin probes 5- and 16-doxyl stearic acid (5- and 16-DSA, respectively) were purchased from Avanti Polar Lipids (Alabaster, Al, USA). 5-DSA has the magnetic fragment (doxyl moiety) attached to the 5th carbon of the acyl chain, whereas for 16-DSA this fragment