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Development and characterization of micellar systems for application as insect repellents

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ABSTRACT

N,N-diethyl-meta-toluamide (DEET) is a widely used insect repellent due to its high efficacy. In this work, micellar systems based on poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer were developed and studied for the purpose of controlling the release and cutaneous permeation of DEET, using concentrated solutions of the copolymer Pluronic F127 to form thermoreversible gels. The formulations presented thermoreversible gelation above 5 °C and altered rheological behavior at 15 and 25 °C. The presence of the drug drastically changed the sol–gel transition temperatures. The micrographs suggest that DEET induced the formation of anisotropic structures, and Maltese Crosses were observed. The formulation containing 10 wt% DEET and 15 wt% Pluronic F127 presented sustained drug release for up to 7 h. DEET release profile followed the Higuchi kinetics model. There was a reduction of approximately 35% in the amount of DEET absorbed through the skin after 6 h. About 62% of DEET from the formulation consisting of Pluronic F127 and DEET remain retained on the skin. The anisotropic structure may constitute a barrier to diffusion and thereby controlling the drug release effectively. These tests suggest that the tested samples exhibit safety profile greater than some commercially available products.

1. Introduction

In the world, millions of people suffer each year from diseases transmitted by mosquitoes, which are considered to be the main cause of contagious diseases (Tolle, 2009). Besides these diseases, insect bites and stings cause local discomfort and irritation. Although more common in tropical and subtropical regions, outbreaks of insect-borne disease can occur any place in the world. Public strategies which aim vector control and reduce disease incidence can lower the risk of epidemics. However, individual protection is still one of the main forms of prevention and is particularly important because of the absence of vaccines and curative treatments for the great majority of these diseases (Bissinger and Roe, 2010; Impoinvil et al., 2007).

Among the commercial repellents, products containing N,N-diethyl-meta-toluamide (DEET) (Fig. 1) have been used for decades due to its good repellent activity (Sudakin and Osimitz, 2010). However, there are reports, although rare, of severe adverse reactions to products containing DEET (Antwi et al., 2008; Masson, 2011). These events are usually related to the presence of the molecule in the bloodstream, since DEET is capable of passing through the cutaneous barrier, reaching deeper skin layers by diffusion and entering the blood vessels quickly (Winter, 2005).

Polymeric micelles can be used in drug carrier systems as well as for guided release due to their high encapsulation capacity. Pluronic F127 or Poloxamer 407 is a commercial block copolymer with A–B–A architecture, a symmetrical structure composed of a central segment of poly(propylene oxide) (PPO) and two peripheral segments of poly(ethylene oxide) (PEO). Therefore, this block copolymer presents surfactant activity and it is widely used in formulating pharmaceutical products because of its thermoreversible gelation characteristic, allowing the drug to remain at the administration site for longer periods and enhancing its therapeutic efficacy (Alexandridis et al., 1996; Pepic et al., 2004; Ruel-Gariepy and Leroux, 2004; Sharma and Bhatia, 2004; Sharma et al., 2008). The gelation phenomenon of concentrated solutions of Pluronic F1227 have been attributed to the formation of ordered structures such as liquid crystals with lamellar, cubic or hexagonal arrangements (Ivanova et al., 2000; Liu and Chu, 2000). Recently, gels based on Pluronic F127 have been receiving special attention for the

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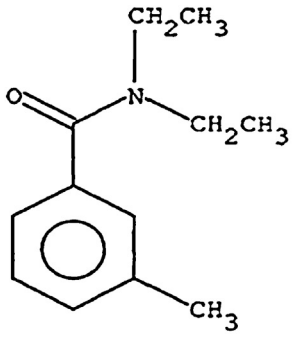

$\log k_{oct}$	2,02
MW	191,27 Da
ρ(30°C)	0,984g/cm³
P_{vp} (30°C)	0,00267 torr
bp_{760}	288-292°C

Fig. 1. Structure and physical properties of DEET (Santhanam et al., 2005).

development of dermal and transdermal release systems, to hasten or retard the permeation of drugs through the skin (Antunes et al., 2011). The thermoreversible characteristic and release pattern make Pluronic F127 a good candidate to be a carrier of drugs through various administration routes (Bivas-Benita et al., 2004; Kojarunchitt et al., 2011).

The attempt to reduce the permeation rate of synthetic repellents through the skin is a challenge that must be overcome during the development of new repellent formulations, so as to minimize the risk of cutaneous absorption of molecules such as DEET. In this context, the application of polymers to modify the drug diffusion trough the formulation's vehicle, or for microencapsulation of active substances to control their release, is of fundamental importance for the development of new formulations.

2. Experimental

2.1. Materials

The synthetic insect repellent DEET (N,N-diethyl-meta-toluamide) (Clariant S/A, São Paulo, Brazil) cellulose acetate membrane (pore size of 0.2 μm and thickness of 43 mm) (Sigma, MO, USA); and the poly(ethylene oxide)–poly(propylene oxide) block copolymer, Pluronic F127 (Sigma, MO, USA) were used in this study.

2.2. Methods

2.2.1. Preparation of the formulations

The formulations were prepared by adding a mass of the solid triblock copolymer to an appropriate amount of ultrapure water. The polymer/water mixture was then cooled to 2 °C until all the solids were dissolved for 24 h. Appropriated amounts of DEET were added to the systems and then the samples were processed in an Ultra-Turrax® (IKA, model T10) homogenizer at 8000 rpm for 2 min. All the samples were kept in a refrigerated bath at 4 ± 2 °C. The concentrations of polymer and repellent are expressed as percentage by weight (wt%) (Table 1).

2.2.2. Rheological characterization of the micellar systems

The dynamic and steady-state analyses were carried out in a rotational rheometer Rheostress 600 (Haake, Karlnuhe, Germany). Measurements were performed in a stainless steel cone-plate geometry, with a cone diameter of 35 cm and angle of 1° and a gap of 0.052 mm. The temperature was controlled by a Phoenix II cooling and heating system (precision of 0.1 °C). In order to minimize water evaporation, a solvent trap has been used during rheological experiments. All samples were analyzed at three temperatures (5, 15 and 25 °C). The reliability of the measurements was assured by obtaining the rheograms in triplicate at all temperatures studied.

Table 1
Formulations prepared and the ratio between G'/G'' at three temperatures studied.

Formulation	Composition		Temperature	G'/G''
I	Pluronic F127	12%	5 °C	5.9
	DEET	5%	15 °C	6.4
	H_2O qsp 20 mL		25 °C	3.6
II	Pluronic F127	12%	5 °C	4.3
	DEET	7%	15 °C	4.3
	H_2O qsp 20 mL		25 °C	4.2
III	Pluronic F127	12%	5 °C	1.7
	DEET	10%	15 °C	4.3
	H_2O qsp 20 mL		25 °C	4.2
IV	Pluronic F127	15%	5 °C	5.4
	DEET	10%	15 °C	4.5
	H_2O qsp 20 mL		25 °C	5.6

The rheological characterization was performed in four steps:

(a) The flow experiments were carried out in a shear rate range between 0.01 and 100 s^{-1}. The flow curves were determined with two consecutive continuous shear rate ramps of 0.01–100 s^{-1} with ascending and descending cycle.
(b) The sol-gel transition was determined by observing the viscosity variation under heating at a rate of 3 °C min^{-1}, in the temperature interval of 5–40 °C. The experiments were conducted at a constant shear rate of 100 s^{-1}.
(c) Oscillatory tests were conducted to determine the region of linear viscoelasticity with variation of deformation from 0.1 to 600% at a frequency of 1.0 Hz.
(d) The viscous modulus (G'') and elastic modulus (G') were determined as a function of the variation of frequency from 0.1 to 90 Hz, under constant deformation of 0.3%.

2.2.3. Polarized light microscopy

An Olympus BX50F-53 microscope was used in this study. A droplet of each sample was pressed between the slide glass and the coverslip so as to have thickness of only a few microns. Then, the slide system was placed in a sample holder and analyzed under polarized light, at room temperature (25 ± 2 °C).

The micrographs were captured by a Nikon Coolpix 5400 camera coupled to the ocular lens and were digitized using a video capture card, which transmitted the data directly to a personal computer.

2.2.4. Quantification of DEET by high performance liquid chromatography (HPLC)

We initially constructed a calibration curve using a Jasco MDS-2010 Plus chromatograph, with an NST C18 column (25 cm) and a PDA detector, using the method developed and validated by Kasichayanula et al. (2005). The mobile phase was composed of methanol and water (70:30), with flow of 1 mL min^{-1}, loop of 20 μL,