European Journal of Pharmaceutics and Biopharmaceutics 116 (2017) 38–50

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Hydrogel-thickened nanoemulsions based on essential oils for topical delivery of psoralen: Permeation and stability studies

Thaís Nogueira Barradas [a,*], Juliana Perdiz Senna [a], Stephani Araujo Cardoso [a], Sara Nicoli [c], Cristina Padula [c], Patrizia Santi [c], Francesca Rossi [d], K. Gyselle de Holanda e Silva [b], Claudia R. Elias Mansur [a]

[a] Instituto de Macromoléculas Professora Eloisa Mano, Universidade Federal do Rio de Janeiro (IMA/UFRJ), Centro de Tecnologia, Bl. J, Avenida Horácio Macedo, 2030, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, Brazil
[b] Departamento de Fármacos e Medicamentos, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro (UFRJ), Avenida Carlos Chagas Filho, 373, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, Brazil
[c] Dipartimento Farmaceutico, Università degli Studi di Parma, Parco Area delle Scienze 27/A, 43100 Parma, Italy
[d] Instituto IMEM-CNR, Parco Area delle Scienze 37/a, 43100 Parma, Italy

ARTICLE INFO

Article history:
Received 13 May 2016
Revised 10 October 2016
Accepted in revised form 16 November 2016
Available online 17 November 2016

Keywords:
Hydrogel-thickened nanoemulsions
Permeation studies
Essential oils
Clove essential oil
Sweet fennel essential oil
Eugenol
Trans-anethole
Psoralens
Terpenes
8-MOP
Chitosan
Transdermal delivery

ABSTRACT

Nanoemulsions (NE) have attracted much attention due to their as dermal delivery systems for lipophilic drugs such as psoralens. However, NE feature low viscosity which might be unsuitable for topical application. In this work, we produced hydrogel-thickened nanoemulsions (HTN) using chitosan as thickening polymer to overcome the low viscosity attributed to NE. The aim of this study is to develop and characterize oil-in-water (o/w) HTN based on sweet fennel and clove essential oil to transdermal delivery of 8-methoxsalen (8-MOP). NE components (oil, surfactant) were selected on the basis of solubility and droplet size and processed in a high-pressure homogenizer (HPH). Drug loaded NE and HTN were characterized for particle size, stability under storage and centrifugation, rheological behavior, transdermal permeation and skin accumulation. Transdermal permeation of 8-MOP from HTN was determined by using Franz diffusion cell. Transdermal permeation from HTN using clove essential oil showed strong dependency chitosan molecular weight. On the other hand, HTN using sweet fennel oil showed an unexpected pH-dependent behavior not fully understood at the moment. These results need further investigation, nevertheless HTN revealed to be interesting and complex dermal delivery systems for poorly soluble drugs.

1. Introduction

Psoralens are naturally occurring tricyclic furocoumarins used, in association with ultraviolet light, in photochemotherapy (PUVA), a well-known treatment for psoriasis and vitiligo, which involves systemic or topical administration of psoralens and exposure of UVA light. PUVA can induce repigmentation by stimulation of melanogenesis, immunomodulation and activation of growth factors, though the exact mechanism is still unknown [1]. Among psoralens (8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 4,5′,8-trimethylpsoralen (TMP)) 8-methoxsalen (8-MOP) is the most widely used in PUVA therapy [2].

Psoralens are lipophilic molecules with logP between 2 and 3, and are therefore, in principle, suitable for oral and topical administration. However, systemic treatment presents many drawbacks, such as liver carcinogenicity, tachycardia, herpes simplex, depression and insomnia, among others. The topical treatment, despite being a safer approach, has limitations related to intense local phototoxicity and slow therapeutic effect, due to insufficient drug penetration into the skin layers [3–5].

When activated by ultraviolet radiation (200–400 nm), psoralens cause phototoxic reactions in the epidermis with an intensity which can vary according to the concentration and exposure time [6–8]. The oral therapy with psoralens is related to the high frequency of toxicity, such as liver carcinogenicity. The most frequent side effect is acute phototoxicity, similar to severe sunburn and may persist for days, followed by systemic effects such as

* Corresponding author at: Instituto de Macromoléculas Professora Eloisa Mano, Universidade Federal do Rio de Janeiro (IMA/UFRJ), Centro de Tecnologia, Bl. J, Avenida Horácio Macedo, 2030, Cidade Universitária, Ilha do Fundão, Rio de Janeiro CEP: 21941-598, Brazil.
E-mail address: thaisbarradas@ima.ufrj.br (T.N. Barradas).

http://dx.doi.org/10.1016/j.ejpb.2016.11.018
0939-6411/

tachycardia, herpes simplex, fever, nausea, headache, dizziness, depression and insomnia, unusual rashes with pain and deep distress in the skin. Psoralens can also reach the eyes and interact with proteins of retina, causing opacity, conjunctival changes and early cataracts, which makes this treatment unsuitable for children under 12 years. For this reason, the eye photoprotection must be emphasized during the treatment [5,9,10].

In the long term, phototherapy can cause photoaging, hepatotoxicity and liver damage, renal toxicity, hypertension, hyperlipidemia and immunosuppression. The risk of carcinoma and melanoma is by an average of 2.6 times higher than the rest of the population. Furthermore, the psoralens have the ability to inhibit enzymes of the P450 2A6 cytochrome family, responsible for the metabolism of numerous substances and drug [9–12].

In the intention to reduce the systemic effects of oral therapy, topical PUVA has become an alternative. Topical PUVA does not produce systemic effects, enhances the therapeutic effects of the treatment of various dermatoses and does not require prolonged ocular photoprotection [13,14].

However, the local phototoxicity is intense and fast, being a dose-dependent phenomenon. Adverse effects are usually immediate such as erythema, edema and vesicles that occur due to uncontrolled psoralen photoreaction in the epidermis with radiation because the drug is freely available in the dosage form to react with the skin surface after topical application [15,16].

Besides the cases of toxicity, topical treatment with psoralen imposes complications since these drugs have low penetration capacity into the deeper skin layers, which requires the administration of higher doses and increased exposure time to UVA radiation, increasing the risk of serious adverse effects such as carcinogenesis [5,17,18].

Is well known that the vehicle in which a certain drug is included has a significant effect on its dermal or transdermal penetration. In general, it is possible through a careful selection of vehicle components to optimize skin penetration of an active molecule. Over recent years, several nanosystems have been proposed in order to increase the penetration of psoralens across the skin [19–22]. Among them, nanoemulsions are the most commonly used, for their ability to favor dermal drug penetration. In addition, several authors have demonstrated that it is possible to control drug release and skin permeation by adjusting the formulations, enabling dose reduction and, therefore, reducing adverse effects [5,23,24]. Another strategy to increase drug permeation through the skin is the addition of permeation enhancers, including terpenes, especially those of vegetable origin, which are described as a class of products with low toxicity, low irritation potential and listed in insurance excipients FDA [25,26].

One of the disadvantages of the use of nanoemulsions for topical application is the low viscosity, which limits their clinical application and makes their topical administration inconvenient. In this context, the development of a hydrogel-thickened nanoemulsion, by the introduction of a gel-forming polymer to the outer phase of the formulation, can solve this disadvantage and make the nanoemulsion suitable for topical application [27–30]. Such system can combine the characteristics of stability and skin penetration of nanoemulsions with the properties of sustained drug release of hydrogels thus optimizing the therapeutic effects and allowing a reduction of the dose [31,32].

One of the polymers more suitable for topical application is chitosan, a semisynthetic polymer capable of forming hydrogels by entanglement of polymer chains which entrap a large amount of water molecules, swelling in the presence of this solvent. In particular, chitosan hydrogels show mucus and bioadhesion properties, capable of increasing drug residence time on the skin and promote drug skin penetration. This is due to the cationic nature of chitosan, which allows its polymeric chains to establish ionic interactions with the surface of the *stratum corneum*. Such characteristics make hydrogels based on chitosan good candidates for the development of dermal drug delivery systems [33–35].

This paper proposes the development of chitosan-thickened nanoemulsions containing essential oils rich in terpenes, for the topical application of 8-MOP. The effect of different surfactants (Pluronic and Cremophor) as well as of the presence and viscosity of chitosan was evaluated as well.

2. Experimental part

2.1. Materials

The materials used in this study were: the psoralen 8-methoxysalen (1, MW = 216.2 g/mol, m.p. = 142–148 °C, Xanthotoxin, 99% purity) was used as received Sigma-Aldrich, São Paulo, Brazil; Sweet Fennel, Peppermint, Orange, Lime, Buriti, Avocado, Clove and Copaiba essential oils obtained from Ferquima, São Paulo, Brazil; cellulose acetate membrane (pore size of 0.2 μm), acquired from Sigma-Aldrich, Missouri, USA; the poly(ethylene oxide)-poly(propylene oxide) block copolymer, Pluronic F68, obtained from Sigma-Aldrich, Missouri, USA; Cremophor RH 40 (PEG-40 hydrogenated castor oil), obtained from Oxiteno, São Paulo, Brazil and Chitosan of low (50,000–190,000 Da), medium (190,000–310,000 Da) and high (310,000–375,000 Da) molecular weight obtained from Sigma-Aldrich, Missouri, USA. All Chitosan grades were at least 75% deacetylated according to the supplier.

2.2. Methods

2.2.1. Screening of oil phase

Due to its low water solubility, 8-MOP was subjected to preliminary solubility tests in various oil concentrations of 1–20% w/v. Therefore, we employed canola, almond, grape seed, avocado and buriti, copaíba, lemongrass, lime, sweet fennel, lemon balm, mint, clove (stem and button) and orange vegetable essential oils. For this purpose, we performed a preliminar selection of essential oils by visual observation of solubilization of 8-MOP using a qualitative criterion based on the United States Pharmacopeia (USP) in the chapter entitled "Description and Relative solubility" [36].

1 mg of 8-MOP was added to 1 mL of each essential oil mentioned above. Then, the systems were subjected to ultrasonic cavitation to accelerate solubilization for 15 min, and solubility was assessed visually after 24 h. To the oils that promoted solubilization, the above procedure was repeated consecutively until the observation of precipitation for each system.

The essential oils in which 8-MOP had higher solubility classification were chosen to be evaluated quantitatively by Ultraviolet (UV) spectrophotometry. The determination of 8-MOP concentration was performed in sweet fennel and clove essential oils. The samples for determining the solubility were prepared by adding an excess quantity of 8-MOP to a defined volume of each essential oil. These suspensions were kept under stirring for 24 h at room temperature (25 ± 1 °C), after which the suspensions were centrifuged at 3000 rpm for 5 min. Then the supernatant was removed with the aid of a Pasteur pipette and each sample was filtered through cellulose filter element. An aliquot of each solution was diluted with ethanol and subsequent dilutions were prepared in ethanol. The absorbance of these solutions was determined using quartz cuvette (1 cm) and wavelength 300 nm, a calibration curve was prepared in ethanol.

2.2.2. Development of NE containing 8-MOP

The formulations were produced by employing a high energy was performed by means of a high-pressure homogenizer (HPH).