International Journal of Pharmaceutics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Comparative percutaneous permeation study using caffeine-loaded microemulsion showing low reliability of the frozen/thawed skin models

Amnon C. Sintov *, Igor Greenberg

Department of Biomedical Engineering, Faculty of Engineering Sciences, Ben Gurion University of the Negev, Beer Sheva 84105, Israel

ARTICLE INFO

Article history:
Received 12 April 2014
Received in revised form 20 May 2014
Accepted 22 May 2014
Available online xxx

Keywords:
Microemulsion
Transdermal drug delivery
Percutaneous permeation
Skin models
Porcine skin
Caffeine

ABSTRACT

The aim of this study was to explore the transdermal delivery potential of a new caffeine-containing microemulsion system. The skin permeability of caffeine (CAF) was measured in vitro using skin excised from three different animal species: rat, rabbit and pig. As shown, microemulsion containing 20% aqueous phase enhanced CAF permeation across fresh rat skin by one order of magnitude ($P_{app} = 8.2 \times 10^{-3}$ vs. 0.86×10^{-3} cm/h; enhancement ratio = 9.6). The permeability coefficient value, the cumulative permeation amount, and the percent of dose permeated after 24 h, decreased with the increase of water content from 60% to 80% in microemulsions due to the apparent increase in the droplet size. Importantly, differences were noted between caffeine transport rates across fresh and frozen/thawed pig skin whereas microemulsions delivered caffeine at similar rates across rat and rabbit skin, either fresh or frozen/thawed. It has been shown that the permeability of caffeine through frozen/thawed pig skin was abnormally high and was independent of its vehicle properties, i.e., its hydrophilic or lipophilic nature. It has been hypothesized that the reason for this abnormality is that porcine stratum corneum has a higher ceramide-to-cholesterol ratio compared to rat and rabbit skin. This unusual phenomenon observed in a non-freshly used porcine skin places a question mark on its suitability to in vitro evaluation of transdermal drug delivery systems.

1. Introduction

The unique class of optically transparent, thermodynamically stable, and isotropic dispersion, known as microemulsion, has been widely and extensively utilized for a variety of pharmaceutical purposes including transdermal and dermal drug delivery (Kreilgaard et al., 2000, 2001; Alvarez-Figueroa and Blanco-Mendez, 2001; Kreilgaard, 2001; Rhee et al., 2001; Lee et al., 2003; Sintov and Shapiro, 2004; Hua et al., 2004; Sintov and Botner, 2006; Sintov and Brandys-Sitton, 2006; Liu et al., 2007; Shevachman et al., 2008; Heuschkel et al., 2008; Gannu et al., 2010; Zhao et al., 2011; Zhang et al., 2011; Zhang and Michniak-Kohn, 2011). Microemulsions, which are versatile systems (oil-in-water, water-in-oil, or bi-continuous systems as well as various structures and components), possess a high solubilizing capacity of diverse compounds, hydrophilic and lipophilic in nature. The increase in solute concentration and the tendency of the vehicle to favor partitioning into the stratum corneum, make microemulsions a useful vehicle to enhance transdermal drug permeability.

The present report describes a new microemulsion system for transdermal drug delivery and deals with its contribution to percutaneous delivery of caffeine. Caffeine (1,3,7-trimethylxanthine; CAF) is most commonly consumed by humans from tea leaves, coffee, and cocoa beans. In cosmetics, CAF has demonstrated an anti-cellulite activity (Bertin et al., 2001), an inhibitory effect on UV-induced skin carcinogenesis by functioning as a sunscreen, and a stimulatory effect on apoptosis in the epidermis of UVB-treated mice (Lu et al., 2002, 2004, 2005, 2008; Conney et al., 2013). In addition, a stimulating effect on hair growth has been indicated after CAF application (Fischer et al., 2007). In therapy, CAF has a stimulatory activity suitable for the treatment of neonatal apnea via percutaneous delivery (Amato et al., 1991). Numerous transdermal studies have used CAF as an agent driven by a hydrophilic character, aiming to evaluate (Otberg et al., 2008; Trauer et al., 2009, 2010; Tfaili et al., 2013) and to enhance (Boucaud et al., 2001; Nicoli et al., 2004, 2005; Thakur et al., 2007; Duracher et al., 2009) its skin permeability by various means. Several nano-particulate systems have also been studied for

* Corresponding author at: Laboratory for Biopharmaceutics, E.D. Bergmann Campus, Ben-Gurion University of the Negev, P.O. Box 653, Beer Sheva 84105, Israel. Tel.: +972 8 647 2709.
E-mail address: asintov@bgu.ac.il (A.C. Sintov).

http://dx.doi.org/10.1016/j.ijpharm.2014.05.040
0378-5173/

transdermal CAF delivery. Nanoparticles made from starch derivatives were tested for permeation through human heat-separated epidermis (Santander-Ortega et al., 2010), revealing low apparent permeability coefficient (P_{app}) values of particle-bound and free caffeine with no statistically significant differences. However, the skin permeability of caffeine through excised pig skin increased by using microemulsion formulations, in particular oil-in-water (O/W) type formulations (Zhang and Michniak-Kohn, 2011; Naoui et al., 2011).

In this report, we present (i) a significant enhancement of caffeine permeability by using W/O microemulsions containing nonionic surfactants, PEG-8 caprylic/capric glycerides (Labrasol®) and glyceryl oleate, (ii) an advantage of a water-in-oil (W/O) type microemulsion over the inverse O/W type, and (iii) a comparative penetration data obtained from three skin models: rat abdominal skin, rabbit ear skin and pig ear skin at two usage conditions, i.e., being immediately tested as fresh or being used after skin had been frozen and thawed. It has been shown previously (Sintov and Botner, 2006) that frozen/thawed pig skin had a higher permeability compared to fresh skin. The present results have further demonstrated that the permeability in certain cases through frozen pig skin is abnormally high and independent of the effect of different formulations, placing a question mark on the validity of the not freshly-used porcine skin for in vitro penetration studies.

2. Materials and methods

2.1. Materials

Caffeine was obtained from Sigma, Rehovot, Israel. Labrasol® (PEG-8 caprylic/capric glycerides) was purchased from Gattefosse (Saint-Priest Cedex, France), and glyceryl oleate was obtained from Croda Europe Ltd. (East Yorkshire, England). Isopropyl palmitate and propylene carbonate were purchased from Sigma, Rehovot, Israel. High-performance liquid chromatography (HPLC) grade water and solvents were obtained from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ).

2.2. Preparation of microemulsions

The formulation was prepared according to Sintov and Botner (2006) and Sintov et al., 2010. Shortly, water-in-oil (W/O) liquid microemulsions were prepared by mixing Labrasol® and glyceryl oleate (surfactants), propylene carbonate (co-surfactant) and isopropyl palmitate (oil), followed by gently titration of distilled water. The co-surfactant/surfactants (CoS/S) weight ratio was 1:5, while the surfactants ratio was 1:3 glyceryl oleate/Labrasol®. The microemulsion formed spontaneously at room temperature as a clear monophasic liquid. The final concentration of CAF in the microemulsions was 1.0 wt%. As a convenient method to represent an increase of water content while decreasing CoS/S levels, water dilution lines were drawn from the water apex to the opposite oil side of the phase diagram (Fig. 1). The line was arbitrarily denoted as the value of the line intersection with the oil scale (e.g., DL11 means line representing a surfactant-to-oil ratio of 89:11). Formulations along a dilution line DL11 was prepared and analyzed for droplet size by light scattering.

Microemulsion gel was prepared by incorporating 10% amorphous silica (CAB-O-SIL TS-530, Cabot Corp., USA) into W/O microemulsion containing 20% water phase (DL11).

2.3. Light scattering

The hydrodynamic diameter spectrum of microemulsion nanodroplets was collected using CGS-3 Compact Goniometer System (ALV GmbH, Langen, Germany). The laser power was 20 mW at the He–Ne laser line (633 nm). Correlograms were calculated by ALV/LSE 5003 correlator, which were collected at 90°, during 10 s for 20 times, at 25 °C. Measurements were performed at permanent angle of 90°. The droplet size was calculated using the Stokes–Einstein relationship, and the analysis was based on regularization method as described by Provencher (1982).

2.4. Electrical conductivity (EC) tests

Formulations were measured for electrical conductivity by a CyberScan Con 510 Meter (Eutech Instruments Europe B.V., Nijkerk, Netherlands). For EC tests, an aqueous phase consisting of 0.9% (w/v) sodium chloride solution replaced purified water. The EC values were recorded for microemulsion samples along DL11.

2.5. Differential scanning calorimetry (DSC)

Microemulsion microstructures were evaluated using a Mettler-Toledo DSC 1 STAR System (module DSC823e/700/484LN2, Mettler-Toledo AG, analytical, CH-8603 Schwerzenbach, Switzerland). Microemulsion sample (7–10 mg) was weighed accurately in an aluminum hermetic sample pan and sealed with the lid quickly to prevent the sample evaporation. DSC tests were run at the following conditions: equilibration at 25 °C, staying isothermal for 2 min, cooling the sample at the ramp rate of 5 °C/min to −80 °C, staying isothermal for 0.5 min, and heating the sample at the ramp rate of 5 °C/min–30 °C (Zhang and Michniak-Kohn, 2011).

2.6. In vitro skin penetration study

The permeability of CAF through animal skin was determined in vitro with a Franz diffusion cell system (Crown Bioscientific, Inc., Clinton, NJ). The diffusion area was 1.767 cm^2 (15 mm diameter orifice), and the receptor compartment volumes varied from 11 to 12 ml. The solutions in the water-jacketed cells were thermostated at 37 °C and stirred by externally driven, Teflon-coated magnetic bars. Each set of experiments was performed with at least four

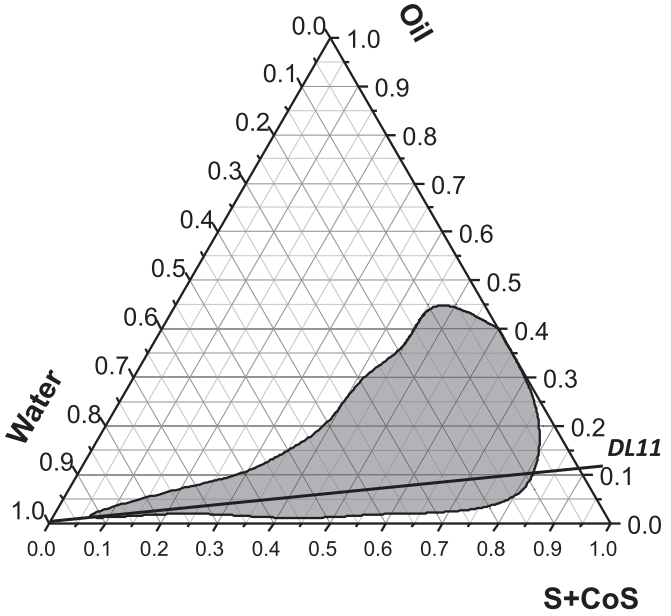

Fig. 1. Pseudo-ternary phase diagram of the microemulsion system. The co-surfactant/surfactants (CoS/S) ratio was 1:5, and the surfactants ratio was 1:3 glyceryl oleate/Labrasol®. Note that the drawn dilution line DL11 is representing a surfactant-to-oil ratio of 89:11.

Please cite this article in press as: Sintov, A.C., Greenberg, I., Comparative percutaneous permeation study using caffeine-loaded microemulsion showing low reliability of the frozen/thawed skin models, Int J Pharmaceut (2014), http://dx.doi.org/10.1016/j.ijpharm.2014.05.040