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Water-dilutable microemulsions for transepithelial ocular delivery of riboflavin phosphate

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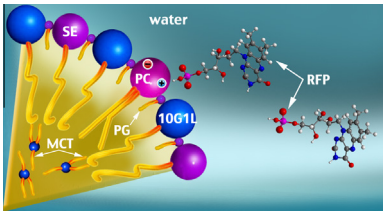
GRAPHICAL ABSTRACT



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ABSTRACT

Riboflavin phosphate (RFP) is an essential compound in the treatment of keratoconus – a degenerative, non-inflammatory disease of the cornea. Currently, the quantitative and efficient transport of riboflavin to the cornea is possible after mechanical removal of the epithelium. To avoid surgical intervention, it is therefore important to develop a method for quantitatively transporting riboflavin across the intact epithelium.

In the present study, an RFP-loaded microemulsion was prepared, which could potentially function as an ocular drug delivery system crossing the eye epithelium. The specially designed water-dilutable microemulsion was based on a mixture of nonionic surfactants. Propylene glycol and glycerol acted as cosurfactant and cosolvent assisting in the solubilization of the RFP. The glycerol-rich water-free concentrate consisted of *direct micelles* for which glycerol served as the hydrophilic phase. In formulations with up to 40 wt% water, the hydrophilic surfactant headgroups and glycerol strongly bind water molecules (DSC and SD-NMR). Above 60 wt% water, globular, O/W nanodroplets, ~14 nm in diameter, are formed (SAXS, cryo-TEM, and SD-NMR). The structure of microemulsions loaded with 0.14–4.25 wt% RFP (0.29–8.89 mmol per 100 g formulation) is not significantly influenced by the presence of the RFP. However, in the microemulsions containing 10–80 wt% water, the mobility of RFP in the microemulsion is constrained by strong interactions with the surfactants and cosurfactant, and therefore free transport of the molecule can be achieved only upon higher (>80 wt%) water dilutions.

1. Introduction

Microemulsions (MEs) are isotropic and thermodynamically stable nano-sized mixtures of water, oil, and amphiphiles [1–4]. Often they require a cosolvent or cosurfactant in order to achieve the lowest interfacial tension and the proper surfactant packing parameter. They are capable of solubilizing pharmaceutically active components within or at the interface of the nano-sized

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droplets, and function as excellent vehicles for transport of these molecules inside the human body [5,6]. They are known for their ability to enhance skin permeability to bioactive molecules [7–10] and are also widely studied as delivery vehicles for ophthalmic drugs, primarily to the front of the eye [11,12].

Riboflavin (vitamin B_2) is a naturally occurring micronutrient found in relatively high levels in, or added to, various foods and beverages. It plays an important role in biochemical redox reactions in humans and animals [13]. It also acts as an antioxidant and is essential for the health of skin, hair, eyes, and liver [14]. Riboflavin phosphate, a derivative of riboflavin, is included in recently approved procedures for treating progressive keratoconus, a common degenerative disorder characterized by corneal ectasia that results in significant visual distortion [15,16]. In the procedure called corneal collagen cross-linking (CXL), riboflavin phosphate is activated *via* UVA illumination and acts as a photosensitizer inducing cross-linking of collagen in the stromal layer. This mediates the recovery of the cornea's mechanical strength [15,17,18]. However, prior to treatment, the epithelial layer must be removed in order to ensure adequate and uniform saturation of RFP in the stroma. The damage that can be caused to deeper layers of the cornea by the debridement of the epithelium may be significant and irreversible. Therefore, efforts are currently being made to develop alternative methods for quantitatively targeting the drug to the stroma. These methods include the use of topical drugs and synthetic peptides to loosen tight junctions; applying limited, full-thickness, epithelial debridement in a grid-like pattern, with islands of intact epithelium to facilitate more rapid postoperative healing; introducing intrastromal channels with a femtosecond laser; using mucoadhesive films loaded with riboflavin; iontophoresis; and phonophoresis [19–27]. Recently it was shown that nanoemulsions can improve the penetration of riboflavin into the stroma across the intact epithelium [28]. Penetration of riboflavin and riboflavin phosphate was evaluated by measurement of corneal absorption and transmittance. However, these nanoemulsions (droplet diameter $\sim$ 70 nm) are prepared by ultrasound irradiation, they are not thermodynamically stable, and are not fully water-dilutable. We also noted that the efficiency of the CXL treatment has still not been demonstrated [28].

In the present study, we suggest the use of a novel ethanol-free and preservative-free, thermodynamically stable, and self-assembled ME system based on a mixture of non-toxic, non-ethoxylated surfactants as a possible vehicle for transport of RFP across the eye epithelium [29]. The specially selected ME components self-assemble, forming transparent, thermodynamically stable, non-viscous, and fully water-dilutable structured systems. RFP was loaded into the ME at 0.14–4.25 wt%, i.e., 0.29–8.89 mmol per 100 g formulation, which is high enough to induce CXL in the cornea and to prevent damage to the deeper ocular structure due to UVA absorption [30,31].

In order to evaluate the potential of this system as a drug delivery vehicle, it is necessary to characterize and determine physical and structural properties of the empty and RFP-loaded systems. We used several analytical and structural methods, including electrical conductivity and viscosity measurements, self-diffusion NMR (SD-NMR) spectroscopy, differential scanning calorimetry (DSC), small angle X-ray scattering (SAXS), and cryogenic transmission electron microscopy (cryo-TEM) [32,33].

2. Materials and methods

2.1. Materials

Decaglycerol monolaurate (10G1L), SY-Glyster ML-750, was obtained from Sakamoto Yakuhin Kogyo Co., Ltd. (Osaka, Japan). Sucrose ester monolaurate (SE), L-1695, was purchased from Ryoto Sugar Esters Division, Mitsubishi-Kagaku Foods Corporation (Tokyo, Japan). Soybean lecithin (Emulpure IP), consisting of a mixture of polar phospho- and glycolipids was purchased from Degussa BioActives (Hamburg, Germany). Medium chain triglycerides, MCT (Neobee M-5), containing 66% caprylic and 32% capric fatty acids, were obtained from Stepan (Northfield, IL, USA). 1,2-Propanediol (PG) (99.5%) was purchased from Merck KGaA (Darmstadt, Germany). Glycerol (99%) was obtained from Frutarom (Haifa, Israel). Riboflavin 5′-monophosphate sodium salt (RFP) (Ph Eur) was purchased from Fluka (St. Gallen, Switzerland). Deuterium oxide (D_2O) was from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). All components were used without further purification. The water was double distilled.

2.2. Preparation of the microemulsions

The empty ME was prepared by combining a mixture of 10G1L/glycerol with mixtures of SE/PG and lecithin/MCT. The transparent concentrate (henceforth, "the concentrate") is fully dilutable with water. For preparation of the RFP-loaded MEs, an aqueous RFP solution (4.5 wt%) was used as the dilution medium instead of water, meaning that as the dilution progresses, the content of RFP as a weight fraction of the total mixture increases.

2.3. Viscosity

Viscosity measurements were performed at 25 ± 1 °C on empty and RFP-loaded MEs (Thermo Electron GmbH, Karlsruhe, Germany) using a cone (6.0 cm diameter, 1° angle), and glass plate. Shear rates were 0–100 s^{-1}.

2.4. Differential scanning calorimetry (DSC)

A Mettler Toledo DSC 822 (Greifensee, Switzerland) system was used for calorimetric measurements. The instrument was calibrated every two weeks with indium, lauric acid, water, and ethyl acetate to ensure the accuracy of the calorimetric data. The calibration heating rate was 5 °C min^{-1}. The DSC measurements were carried out as follows: 7–11 mg ME samples were weighed, using a Mettler M3 microbalance, into standard 40 μL aluminum pans and immediately sealed by a mechanical press. The samples were cooled in liquid nitrogen from +25 to −100 °C at 5 °C min^{-1}. Each sample remained at this temperature for 20 min and was then heated at a rate of 5 °C min^{-1} to +25 °C. An empty pan was used as a reference. The enthalpy changes associated with the thermal transitions of water were obtained by integrating the area of the water peak [34–36]. DSC temperatures reported here were reproducible within ±0.5 °C.

2.5. Electrical conductivity measurements

Electrical conductivity measurements were performed at 22 ± 1 °C using a conductivity meter, type CDM 730 (Mettler Toledo GmbH, Greifensee, Switzerland). Measurements were made on empty and RFP-loaded samples upon dilution with water up to 95 wt%. Aside from the RFP present in the loaded ME, no other electrolyte was added to the samples.

2.6. Self-diffusion NMR (SD-NMR)

The self-diffusion coefficients were determined using pulse gradient spin echo NMR [37–40]. NMR measurements of empty and RFP-loaded samples were performed at 25 ± 0.01 °C on a Bruker AVII 500 spectrometer, with the BGU II gradient amplifier unit,