Colloids and Surfaces B: Biointerfaces 173 (2019) 226–232

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Potentiality of microemulsion systems in treatment of ophthalmic disorders: Keratoconus and dry eye syndrome – *In vivo* study

Nina Lidich[a,1], Sharon Garti-Levy[b], Abraham Aserin[a], Nissim Garti[a,*]

[a] The Casali Center for Applied Chemistry, The Institute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Jerusalem 9190401, Israel
[b] Lyotropic Delivery Systems, High Tech Village, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Jerusalem 9190401, Israel

ARTICLE INFO

Keywords:
Microemulsions
Riboflavin
Omega fatty acids
Solubilization
Drug delivery system
Transepithelial permeation
Keratoconus
Dry eye syndrome
Collagen cross-linking

ABSTRACT

Microemulsions are widely studied as potential ocular drug delivery vehicles. In the present study we show the versatility of possible use microemulsions as ocular delivery vehicle. The ME is loaded with a hydrophilic drug, riboflavin phosphate (RFP) and a lipophilic, docosahexaenoic acid in triglyceride form (TG-DHA), each separately. These drugs treat keratoconus and dry eye syndrome, respectively. The advantage of using ME loaded with RFP is in overcoming eye epithelium debridement during collagen cross-linking therapy for treatment of keratoconus. ME loaded with lipophilic TG-DHA provides convenient dosage in liquid aqueous form of administration of highly lipophilic TG-DHA, which is known as a protective molecule in dry eye syndrome. The capability of RFP-loaded MEs was demonstrated in terms of improvement of biomechanical strength of the rabbit cornea, as a result of successful penetration of RFP through the intact epithelium. TG-DHA-loaded microemulsion applied topically onto an eye with induced dry eye syndrome showed the significant relief of the dry eye condition.

1. Introduction

One of the major challenges in drug delivery is ophthalmic delivery, due to the complex structure of the human eye, which constitutes a number of static (corneal epithelium, stroma, retina) and dynamic barriers (lacrimal fluid secretion, choroidal and conjunctival blood flow). [1] Therefore only ~5% of the topically applied dose approaches the target site [2–4]. This has led to much effort in the ophthalmic drug delivery field for developing improved topical ophthalmic formulations.

Colloidal drug carriers such as microemulsions (MEs) are of high interest in the area of pharmaceuticals, food, and cosmetic sciences, [5–12] and also are widely studied as potential ocular drug delivery vehicles [13–18]. MEs are isotropic, transparent, and thermodynamically stable nano-sized mixtures of water, oil, and amphiphiles [19–21]. The advantages of using MEs include improvement in solubility and stability of the applied drug, enhancement of membrane permeability, and ability of active molecules to overcome the epithelial barrier, control release of the drug at the target site, increase retention time of the drug and as a result less frequent application, and ability to solubilize hydrophilic and lipophilic active molecules [13–15,17,22–25].

Riboflavin (RF, vitamin B_2) is 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. [26] It also acts as an antioxidant and is essential for the health of skin, hair, eyes, and liver [27]. Riboflavin is sparingly soluble in water. Riboflavin phosphate (RFP), a water-soluble derivative of riboflavin, is included in recently approved procedures for treating progressive keratoconus, a common degenerative disorder of the cornea characterized by stromal thinning and ectasia, which results in significant visual distortion [28–30]. In the procedure called corneal collagen crosslinking (CXL), RFP is activated *via* UVA illumination and acts as a photosensitizer inducing photo-oxidative reduction of corneal collagen cross-links in the stromal layer. [28,31] Riboflavin also prevents UV damage to the endothelial cells [32].

This mediates the recovery of the cornea's mechanical strength. [28,33,34] However, currently, the quantitative and efficient transport of RFP to the cornea is possible only after complex mechanical removal of the epithelium prior to the treatment in order to ensure adequate and uniform saturation of RFP in the stroma. The damage that can be caused to the 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

* Corresponding author.
E-mail address: garti@mail.huji.ac.il (N. Garti).
[1] The results presented in this paper are part of Nina Lidich's Ph.D. dissertation in Applied Chemistry, The Hebrew University of Jerusalem, Israel.

https://doi.org/10.1016/j.colsurfb.2018.09.063
Received 7 June 2018; Received in revised form 4 September 2018; Accepted 24 September 2018
Available online 27 September 2018
0927-7765/

targeting the drug to the stroma. These methods include the use of topical drugs and 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; iontophoresis; and phonophoresis [35–40].

Another important nutraceutical for ocular applications is docosahexaenoic acid (DHA) in its triglyceride form (TG-DHA). DHA is an essential omega-3 polyunsaturated fatty acid that is an important compound for the development of cognitive functions, reducing the risk factor for cardiovascular diseases, decreasing acute and chronic inflammation, inducing anti-microbial resistance, and enhancing appropriate pre- and post-natal development of the central and peripheral nervous systems. [41–43] Oral uptake of omega-3 fatty acids may help to protect adult eyes from macular degeneration and dry eye syndrome [44–47]. However, topical ophthalmic use of DHA is limited because of its lipophilic properties.

The present research is aimed to demonstrate the ability of MEs and its versatility to serve as an efficient drug delivery system to the anterior chamber of the eye. For these reason two well characterized ME formulations were selected: one loaded with RFP [48] at its interface and the second loaded with triglyceride of DHA (TG-DHA) [49,50], serving as an oil phase. These formulations were prepared with a high amount of water in order to prevent as much as possible the side effects, which can be related to high surfactant content in MEs containing low water amount. In addition, the formulations are fully water dilutable and will not separate to two phases after application on the eye and exposure to the tears.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

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 phospholipids and glycolipids was purchased from Degussa BioActives (Hamburg, Germany), Epikuron 200 (92% phosphatidylcholine), Degussa BioActives (Hamburg, Germany). Tween 80 (polyoxyethylene-(20)-sorbitan monooleate) and Cremophor EL (polyoxyethylene-(35)-castor oil) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Medium chain triglycerides, MCT (Neobee M-5), containing 66% caprylic and 32% capric fatty acids, were obtained from Stepan (Northfield, IL, USA). Docosahexaenoic acid in triglyceride form (TG-DHA, omega-3) (Meganol-D (DHA 85 TG)) was purchased from AK BioTech Co., Ltd. (Sanggae, South Korea). 1,2-Propanediol (PG) (99.5%) was purchased from Merck KGaA (Darmstadt, Germany). Glycerol (99%) was obtained from Frutarom (Haifa, Israel). Riboflavin 50-monophosphate sodium salt (RFP) (Ph. Eur.) was purchased from Fluka (St. Gallen, Switzerland). Sodium chloride (min 99%) was purchased from J.T. Baker (Phillipsburg, NJ, USA). All components were used without further purification. The water was triple distilled.

2.2. Preparation of the microemulsions

2.2.1. Preparation of the microemulsions containing RFP

For keratoconus treatment three different formulations were constructed and studied. One of these formulations, termed 1-RFP, was selected for a detailed structural characterization. [45] This ME exhibited the highest loading capacity and is rich in glycerol. It is composed of direct nanodroplets of oil phase dispersed in glycerol/water mixture rather than in water. The system can be fully diluted with water (full dilution line). RFP (Fig. 1) was found to be located at the interface of the ME nanometric droplet.

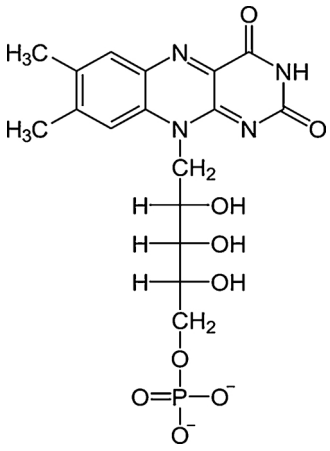

Fig. 1. Structure of riboflavin 5′-phosphate – a water-soluble derivative of vitamin B_2 used as a photosensitizer in treatment of keratoconus.

2.2.1.1. Formulation 1-RFP. The empty 1-RFP formulation was prepared by combining a mixture of 10G1L/glycerol with mixtures of SE/PG and lecithin/MCT. The transparent concentrate (no water) is fully dilutable with water. For preparation of the RFP-loaded MEs, the concentrate was diluted up to 97 wt% water content with saline and appropriate amount of RFP (Fig. 1) was weighted and added, resulting in final 0.24 wt% RFP.

2.2.1.2. Formulation 2-RFP. A mixture of Tween 80/Cremophor EL/glycerol (6.4/3/1) was stirred at 50 ± 2 °C. Then 1.2 wt% of IPM was added, resulting in formation of a transparent concentrate. This concentrate is fully dilutable with water. For preparation of the RFP-loaded MEs, the concentrate was diluted up to 97 wt% water content with saline and appropriate amount of RFP was weighted and added, resulting in final 0.24 wt% RFP.

2.2.1.3. Formulation 3-RFP. A mixture of Epikuron 200/PG/Tween 80 (1/9.7/22.7) was stirred at 50 ± 2 °C resulting in transparent mixture. This concentrate is fully dilutable with water. For the preparation of the RFP-loaded formulation, the mixture was diluted up to 97 wt% water content with saline and appropriate amount of RFP was weighted and added, resulting in final 0.24 wt% RFP.

2.2.2. Preparation of the microemulsions containing TG-DHA

For the treatment of dry eye syndrome formulation based on 2-RFP formulation was selected. At this ME TG-DHA serves as oil phase [46] and is located between the surfactants' tails. This ME is also fully water-dilutable. At low water content (up to 25 wt% water) the system is composed of "ill-defined bicontinuous structures". At 25–65 wt% water, a more defined bicontinuous structures are formed, and above 70 wt% water, the oil is dispersed in water forming O/W nanodroplets. The pseudo-ternary phase diagram of this ME is presented in Fig. 2.

A mixture of Tween 80/Cremophor EL/glycerol (2.1/1/0.2) was stirred at 50 ± 2 °C; this mixture is called the empty concentrate. Since the concentration of TG-DHA for topical treatment of dry eye syndrome was unknown three TG-DHA-loaded concentrates were prepared by solubilization of 8.0 wt%, 8.5 wt%, and 9.0 wt% TG-DHA (Fig. 3) within the empty concentrate. The loaded concentrates were stirred at 50 ± 2 °C until transparent mixtures were formed. TG-DHA-loaded concentrates were diluted with 85 wt%, 90 wt%, and 95 wt% saline, correspondingly, resulting in MEs containing 1.2 wt%, 0.85 wt% and 0.45 wt% TG-DHA respectively.