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Structural characteristics of oil-poor dilutable fish oil omega-3 microemulsions for ophthalmic applications

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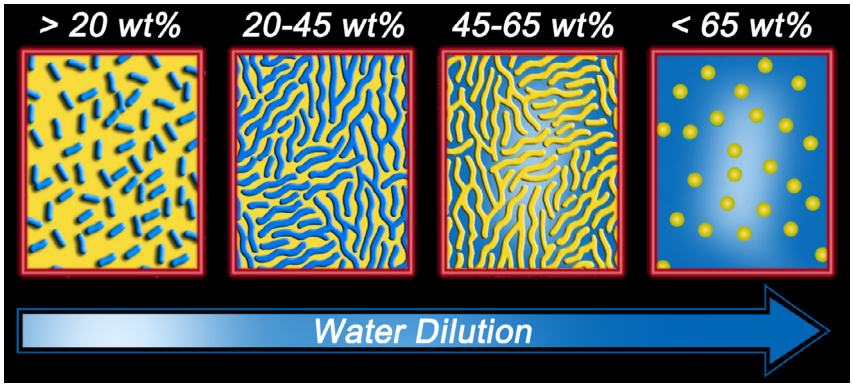
GRAPHICAL ABSTRACT



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ABSTRACT

Docosahexaenoic acid (DHA) promotes synthesis of anti-inflammatory prostaglandins and relief of dry eye symptoms. However, topical ophthalmic application of DHA is difficult because of its lipophilic property. Therefore, it is important to develop aqueous-based formulation with enhanced capabilities.

Novel, unique water-dilutable microemulsions (MEs) were constructed to allow loading of naturally occurring rigid long-chain triglyceride of DHA (TG-DHA). The TG-DHA serves as solubilizate and as the oil phase, therefore preparation is poor in oil. The structural transformations of MEs upon water dilution were studied by SAXS, viscosity, electrical conductivity, self-diffusion NMR, DSC, cryo-TEM, and DLS techniques.

At low water content a new type of water-in-oil (W/O) structure is formed. The glycerol/water phase hydrates the headgroups of surfactants, and the oil solvates their tails, forming "ill-defined bicontinuous domains". Upon further water dilution more structured bicontinuous domains of high viscosity are formed. After additional dilution, the mesophases invert to oil-in-water (O/W) droplets of ~8 nm.

In the structures composed of up to 25 wt% water, the TG-DHA spaces and de-entangles the surfactant tails. Once the bicontinuous structures are formed, the surfactants and TG-DHA content decrease and their interfacial layer shrinks, leading to entanglement and buildup of viscous non-Newtonian mesophase. Above 70 wt% water TG-DHA is embedded in the core of the O/W droplets, and its effect on the droplets' structure is minimal.

This new dilutable ill-defined microemulsion can be a potential delivery vehicle for ophthalmic TG-DHA transport.

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1. Introduction

Microemulsions (MEs) are isotropic transparent nanostructured liquids, composed of mixture of water, oil, and certain amphiphiles, spontaneously formed, thermodynamically stable, and Newtonian [1–4]. They are formed once the oil/water interfacial tension is reduced to ultra-low values by a mixture of surfactants, cosurfactants, and cosolvents. The existence of lipophilic and hydrophilic domains in MEs enables solubilization of water-insoluble and oil-insoluble guest molecules within their core, or at the interface of the nanosized droplets. The nanostructures can function as vehicles for transport of bioactives across human membranes [5–8]. These structures are known for their ability to enhance bioavailability and skin permeability of bioactive molecules [9–13]. MEs are widely studied in ophthalmology as delivery vehicles for drugs to the anterior and posterior departments of the eyes [14–16].

Docosahexaenoic acid (DHA) is an essential omega-3 polyunsaturated fatty acid (PUFA). It is important 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 [17–19]. DHA is located as a phospholipid derivative within the membranes of retinal photoreceptor outer segments and may affect membrane permeability, fluidity, and properties of the lipid phase [20,21]. Several studies suggest that oral uptake of omega-3 fatty acids may help to protect adult eyes from macular degeneration and dry eye syndrome [19,21–24]. Dry eye syndrome is a multifactorial disease, affecting tears and the ocular surface, accompanied by increased osmolarity of tear film, and inflammation of the ocular surface [25]. DHA promotes synthesis of anti-inflammatory prostaglandins and relieves the dry eye symptoms [24]. It also was shown that topical PUFA treatment reduces symptoms of dry eyes in mice [26]. In addition, the encapsulation of fish oil into what is called (by mistake) "microemulsion" structures (200–1200 nm) that are non-thermodynamically stable, not dilutable, and not real microemulsions enhances bioavailability of DHA in rats [27]. However, topical ophthalmic use of DHA is limited because of its lipophilic properties. Therefore, it is important to develop a convenient aqueous ophthalmic dosage formulation of DHA. MEs are promising liquid vehicles for pharmaceutical formulations [7,8,28]. We reported in our previous study that DHA can be solubilized into true fully dilutable MEs [29]. In addition, O/W fish oil MEs with food-permitted components have been developed [30]. However, these MEs were not aimed to be used for ocular applications.

In the present study, we specially designed MEs for ophthalmic use loaded with DHA in its triglyceride form (TG-DHA) rather than the ethyl esters of DHA. The effect of TG-DHA on the ME structure and its location was presently studied as a function of water dilution using small-angle X-ray scattering (SAXS), viscosity, electrical conductivity, self-diffusion NMR (SD-NMR), differential scanning calorimetry (DSC), cryogenic transmission electron microscopy (cryo-TEM), and dynamic light scattering (DLS) methods.

We formed unique structure of TG-DHA-loaded systems in the absence, or low content of water, forming ill-defined bicontinuous mesophases, transformed into O/W microemulsions. This is relevant and important delivery vehicle for future ophthalmic preparation for treatment of dry eye syndrome.

2. Materials and methods

2.1. Materials

Tween 80 (polyoxyethylene-(20)-sorbitan monooleate) and Cremophor EL (polyoxyethylene-(35)-castor oil) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Glycerol (99%) was obtained from Frutarom (Haifa, Israel). Docosahexaenoic acid in triglyceride form (TG-DHA) (Meganol-D (DHA 85 TG)) was purchased from AK BioTech Co., Ltd. (Sanggae, South Korea). Deuterium oxide (D_2O) was purchased 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

Mixtures of two permitted for ophthalmic use surfactants (Tween 80 and Cremophor EL) were prepared. The optimal composition containing the lowest surfactant and highest glycerol contents was selected. 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. The TG-DHA-loaded concentrate was prepared by solubilization of 6.5 wt% TG-DHA within the empty concentrate. The loaded ME was stirred at 50 ± 2 °C until a transparent concentrate was formed. Empty and TG-DHA-loaded concentrates are able to be diluted to any water content without phase separation.

2.3. Viscosity

Viscosity measurements were performed at 25 ± 1 °C on empty and TG-DHA-loaded MEs (Thermo Electron GmbH, Karlsruhe, Germany) using a cone (6.0 cm with 3.5 cm diameter, 1° angle), and glass plate. Shear rates were 0–100 s^{-1}.

2.4. Electrical conductivity measurements

Electrical conductivity measurements were performed at room temperature (22 ± 1 °C) using a conductivity meter, type CDM 730 (Mettler Toledo GmbH, Greifensee, Switzerland). Measurements were made on empty and TG-DHA-loaded samples upon dilution with water up to 90 wt%. Electrolyte was not added to the ME; therefore the conductivity is attributed to the ionic impurities of the non-ionic surfactants [31].

2.5. Small angle X-ray scattering (SAXS)

Scattering experiments were performed using Cu Kα radiation (λ = 0.154 nm) from a Rigaku RA-MicroMax 007 HF X-ray generator (Rigaku Inc. Tokyo, Japan) operated at a power rating up to 1.2 kW and generating a $70 \times 70\ \mu m^2$ focal spot. The Osmic CMF12-100CU8 unit produced a beam size at the sample position of $0.7 \times 0.7\ mm^2$. The scattered radiation passed through an He-filled flight path and was detected by a Mar345 imaging plate detector from MAR research (Norderstedt, Germany). Samples were inserted into 1.5 mm quartz capillaries and scanned for 15 min at T = 25 ± 1 °C. The sample to detector distance was calibrated using silver behenate [32,33].

2.6. Self-diffusion NMR (SD-NMR)

The self-diffusion coefficients were determined using pulse gradient spin echo NMR [29,33–35]. NMR measurements of empty and TG-DHA-loaded samples were performed at 25 ± 0.2 °C on a Bruker AVII 500 spectrometer (Frankfurt, Germany), with the BGU II gradient amplifier unit, and 5 mm BBI probe equipped with a z-gradient coil, providing a z-gradient strength of up to 54 G cm^{-1}.

2.7. Differential scanning calorimetry (DSC)

A Mettler Toledo DSC 822 (Greifensee, Switzerland) system was used for calorimetric measurements. The DSC measurements were