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Preparation and characterization of a lutein loading nanoemulsion system for ophthalmic eye drops

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ABSTRACT

Nanoemulsions (NE) are advantageous nanosized delivery agents for ophthalmic medications because of their ability to penetrate into the ocular structure, as well as their sustained effects. We prepared a NE system composed of isopropyl myristate, triacetin, Tween 80, and ethyl alcohol to increase the solubility and permeability of lutein, an effective medication in macular degeneration. The pseudo-ternary phase diagram was constructed to identify the self-emulsifying region. Eight formulations were selected to characterize each formulation. We examined physical characteristics including particle size, drug solubility, formulation stability, and turbidity. We selected the optimized formulations NE 5 (NE-5) and NE-8, both of which are transparent. The particle size of NE was *ca.* 10–12 nm with a narrow size distribution. Neither separation nor change in the particle size was observed for 7 days. The lutein loading NEs demonstrated a significant increase in lutein release and sustained release. In contrast, lutein prepared with oil and starch had limited drug release profiles under 5%. The prepared lutein NE formulation is a potential alternative for lutein delivery systems.

1. Introduction

Age-related macular degeneration (AMD) is a leading cause of blindness in elderly people [1,2]. In most cases, AMD slowly progresses from 'dry (atrophic)' to 'wet (neovascular or exudative)' by 20%. There is no known treatment for dry AMD and wet AMD is responsible for almost 90% of the severe cases of blindness [3]. Over the past decade, the number of people living with AMD has increased significantly due to an exponentially aging population. There have been significant advances in anti-angiogenesis therapy for age-related macular degeneration. Fortunately, we can now effectively prevent blindness and restore vision with new technologies [4]. However, these therapies are expensive and not readily available in many countries.

Recently, xanthophylls carotenoids lutein has been intensively studied in the pathogenesis of AMD [5,6]. As a main constituent of the macular pigment in the retina, lutein was initially thought to prevent or limit retinal damage by filtering out phototoxic short wavelength visible light and by quenching the reactive oxygen species stress, thereby acting as an antioxidant [7]. In addition, clinical and epidemiological studies have suggested that low lutein concentrations in human plasma may be a risk factor of AMD; lutein supplementation for 140 days resulted in significant risk improvement [5,8]. Therefore, lutein has been recognized as an important supplement in the prevention of ocular diseases [3].

Lutein is a poorly soluble (solubility:0.732 μg/ml) [9] and lipophilic drug in water (logP 7.8) [10]. In order to enhance its bioavailability, previous groups studied lutein oral intake with vegetable oil or oil-in-water (o/w) emulsions [11–13]. However, there are few studies that address ophthalmic drug delivery systems for lutein. Ophthalmic drug delivery is one of the most attractive and promising research areas facing pharmaceutical scientists. Most ocular diseases are treated with topical solutions that are applied as eye drops. These common dosage forms account for approximately 90% of the currently available marketed formulations because of their cost advantage, easy formulation development, production, and patient acceptance [14]. However, ocular

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drug delivery with eye drops is disadvantageous for several reasons. For instance, there is very low bioavailability of drugs via this route because of restricted corneal permeation and rapid drug removal from the absorption site by eye blinking [15]. From various delivery platforms, especially ocular use, the nanoemulsion (NE) has been known for excellent wetting and spreading properties thereby increasing membrane permeability and drug uptake [16]. It also able to penetrate to deep layers of the ocular structure [17,18]. Sustained release of the drug from NE in the cornea allows the drug to penetrate into the deeper layers of the ocular structure by its relatively high viscosity and extremely small particle size [11,16]. The industrial production and sterilization of NE are relatively simple and inexpensive because of the thermodynamic stability and solubilizing capacity of lipophilic drugs [19]. However, even though many advantages of NE, it has been still debated whether topical formulations could treat the posterior eye diseases but some studies showed that topical application of nanoformulations may overcome the barriers such as cornea and conjunctiva [16,18,20]. In this study, we studied o/w NE for topical ocular application containing lutein for AMD. The preparation, physicochemical characterization, and *in vitro* evaluation were investigated to determine their utility.

2. Materials and methods

2.1. Materials

Lutein (80% food grade) was purchased from Xhengsheng Kangyuang Biomedical Co. (Xianyang, China). Lutein analytical standard and croton oil were purchased from Sigma-Aldrich (St. Louis, USA). Isopropyl myristate (IPM), triacetin, Tween 80 (polysorbate 80), ethyl alcohol 100%, olive oil, and soybean oil were purchased from Samchun Co. (Seoul, Korea). The samples of peceol, laurogylcol 90, labrasol, lauroglycol FCC, and labrafil M 1944 CS were kindly donated by Gattefosse (Montesquieu, France). All other chemicals used were of analytical grade.

2.2. High-performance liquid chromatography (HPLC)

Quantitative determination of lutein was performed using an HPLC (Agilent 1200 series, Agilent Tech., USA) equipped with an auto-sampler, high pressure gradient pump, and UV–Vis detector. A reverse phase Spherisorb ODS 2 column (250 × 4.6 mm pore size 5 μm, Waters Corp., USA) was used. The mobile phase, consisting of a mixture of acetonitrile and methanol (70:30 v/v %), was delivered isocratically. The retention time of lutein was 6.7 min when the flow rate was 1.0 ml/min. The column oven temperature was maintained at 30 °C. The column effluent was detected at 446 nm, and the concentration of lutein was calculated using a linear calibration curve of standard lutein solutions.

2.3. Solubility of lutein in oils and surfactants

The solubility of lutein in various oils, surfactants, and co-surfactants was determined by adding an excess amount of lutein in 1 ml of each excipient, followed by 30 min of stirring and 15 min of ultra-sonication. After 6 h of incubation at 25 °C, the lutein solutions were filtered through a 0.45 μm PVDF membrane filter to remove insoluble lutein. The concentration of lutein in each excipient was measured by HPLC (Agilent Tech. Co., USA).

2.4. Construction of pseudo-ternary phase diagram

A pseudo-ternary phase diagram was constructed using the water titration method to determine the self-emulsifying area [21]. First, the oil phase (composed of 1:4 v/v % of IPM and EtOH) and the surfactant phase (with 1:3 v/v % of triacetin and Tween 80) were mixed thoroughly at all combinations (1:0, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 0:1) in glass vials. Each mixture was titrated with PBS buffer as the water phase was stirred under visual observation. This was performed to build the pseudo-ternary phase diagram. The total water consumed was noted in terms of v/v %. During titration of the oil-surfactant ratio, observations were made for phase clarity. The concentrations of water where turbidity-to-transparency and transparency-to-turbidity transitions occur were derived from the volume measurements. These values were used to determine the boundaries of the NE region that corresponded to the selected value of oil and surfactant mixture ratio [22].

2.5. Preparation of NE formulation

Composition of NE was determined based on the solubility of lutein in various components (i.e., oil, surfactant and co-surfactant) and the pseudo-ternary phase diagram study. An excess amount of lutein (10 mg/ml) was added to the mixture of oil and surfactant, followed by stirring and sonication (Vivra Cell®, Sonics & Material, USA). The water phase was added to the lutein dissolved oil-surfactant mixture with mild stirring and sonication to make a homogeneous NE. After equilibration, all of the formulations were filtered through a 0.45 μm PVDF membrane filter to remove insoluble lutein. The NE was stored at ambient laboratory conditions before use.

2.6. Lutein solubility and transmittance of NE formulation

The clear solutions were analyzed for lutein content with HPLC at 446 nm. The percentage transmittance of NE formulations at $\lambda = 580$ nm were measured using a UV-1200 Spectrophotometer (Labentech, Incheon, Republic of Korea). All experiments were repeated three times in order to produce reliable data.

2.7. Determination of particle size and size distribution

The effective hydrodynamic diameters (D_{eff}) and size distribution of the NE were measured by photon correlation spectroscopy using a "Zetasizer Nano-ZS" (Malvern Instruments, UK) equipped with the multi-angle sizing option (BI-MAS). The software provided by the manufacturer was used to calculate the D_{eff}. Each sample was diluted approximately 40 fold with distilled water. Then, the average D_{eff} values were calculated from three measurements performed on each sample. For the NE stability study, each formulation was stored at room temperature and observed by measuring the particle size using a Zetasizer Nano-ZS' (Malvern Instruments, UK) for 7 days.

2.8. Morphology of the aqueous dispersion of NE using hyperspectral imagery

A Nikon light microscope with Cytoviva high-resolution adapter (CytoViva, Auburn, AL) was used to visualize the NE. Illumination was controlled with a DC-stabilized 150-W halogen light source with a constant voltage of 11 V. An internal neutral density filter was used to decrease the light intensity. Hyper Visual Software from the Institute for Technology Development (ITD) (Stennis, MS) was used to obtain images. The hyperspectral imagery microscope system can quantify particle abundance and cluster size using a high-intensity, dark-field illuminator to view nanoscale structures, particularly liquid samples. The hyperspectral imaging system does not require additional chemical manipulation of the sample and allows for visualization of small objects, including nano-sized