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Development and characterization of microemulsions containing hyaluronic acid

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ABSTRACT

Tween80 and Span20 were used as surfactant mixture for developing non-ionic microemulsions (MEs) containing hyaluronic acid 22 kDa (HA). The effect of Tween80:Span20 ratio (T:S ratio) on microemulsion (ME) water intake and stability was studied. Moreover, the effect of HA on the consumed surfactant amount which is for stabilizing the MEs, for reducing water intake was investigated. Two W/O MEs containing HA were optimized. The first ME was composed of 2% HA, 13.8% Tween:80:Span20 (2:3), 4.2% water and 79.9% isopropylpalmitate (IPP). The second was composed of 2% HA, 16% Span20, 9.6% water:dimethyl sulfoxide (W:DMSO) (6:3.6) and 72.4% medium chain triglycerides (MCTG). The droplet sizes of MEs were determined using dynamic light scattering (DLS). The multilayer membrane system (MLMS) was used for testing the release of HA from both MEs and the released amount of HA was quantified using capillary zone electrophoresis (CZE). Furthermore, three phase diagrams and relevant rheological characteristics were generated. The droplet size of the ME without HA decreased and increased with increasing the temperature. Furthermore, the droplet size of the IPP-ME and MCTG-ME without HA and of the MCTG-ME with HA decreased with increasing temperature. In contrast to this results, the droplet size of the IPP-ME with HA increased with increased temperature. This ME belongs to the Newtonian fluids.

Compared to the first ME, the second ME shows droplet sizes at 25 °C of 6.5 nm without and 37 nm with HA. The droplet size in the second ME decreased proportionally with an increase of the temperature with and without HA. The release of HA was faster from the IPP ME compared to the MCTG-ME. The two developed MEs were stable, isotropic and their properties comply with ME properties concerning the droplet size and viscosity.

1. Introduction

Hyaluronic acid (HA) is a linear polysaccharide consisting of disaccharide units containing N-acetylglcosamine and glucuronic acid. Its molecular weight is usually in the order of 10^6–10^7 Da (Laurent, 1987). HA is used in ophthalmological and otological operations and in skin care (Rote Liste®, 2009). ^{1}H NMR-,^{13}C NMR-, Raman-, IR-, and UV–Vis-spectroscopy indicated the presence of a double bond in the pyrancarboxylic acid ring after the enzymatic digestion of HA. The double bond in pyrancarboxylic acid ring is necessary for reducing the toxicity of radicals during the UV irradiation of the human skin (Alkrad et al., 2003a).

The stratum corneum in the skin, which has lipophilic characteristic, is the main barrier for hydrophilic molecules such as HA (Bouwstra et al., 2003). Microemulsions (MEs) are ideal carrier systems for HA compared to semisolid formulations because of their rheological properties and their penetration enhancing capacity (Neubert, 2011). Furthermore, MEs can increase the local or systemic bioavailability of drugs by different mechanisms such as increase the incorporated dose, modification of diffusion barrier of the skin and increase the thermodynamic activity of the drug (Heuschkel et al., 2009; Kawakami et al., 2002; Langevin, 1991). MEs are thermodynamically stable colloidal systems consisting of oil in water (having a colloidal oily phase) or water in oil (having a colloidal aqueous phase) which are stabilized by surfactants and cosurfactants (Heuschkel et al., 2008; David, 1994). Dimethylsulfoxide (DMSO) is used usually as a penetration enhancer in semisolid formulations as well as due to its properties as a radical scavenger. Therefore, DMSO is a good candidate to be used in preparation for skin care (Reynolds et al., 2011; Kennedy and Symons, 1987).

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The aim of this study was the development and the characterization of W/O MEs containing HA as a radical scavenger using non-ionic surfactants for dermal application. The MEs were characterized by relevant methods.

2. Materials and methods

2.1. Materials

Chemicals and reagents are obtained from the following commercial companies: Hyaluronic acid (HA) was obtained from Hans-Knöll-Institut (Jena, Germany). Dimethyl sulfoxide (DMSO), sorbitan monolaurate (Span®20), polyoxyethylene sorbitan monooleate (Tween®80), isopropyl palmitate (IPP) and medium chain triglycerides (MCTG) were purchased from Fluka (Buchs, Switzerland). Collodium and glycerol which were used to prepare the hydrophilic membranes as an acceptor system for the release studies of HA were purchased from Caesar & Loretz GmbH (Hilden, Germany). Absolute ethanol and ether were obtained from Merck (Darmstadt, Germany). Water was used in bi-distilled quality.

2.2. Methods

2.2.1. Dynamic light scattering (DLS)

The light scattering hardware setup consists of commercially available equipment for simultaneous static and dynamic experiments made by ALV-Laser Vertriebsgesellschaft m.b.H. (Langen, Germany). A green Nd:YAG DPSS-200 laser (532 nm) from Coherent (Auburn, USA) with an output of 200 mW was used. The thermostated sample cell is placed on a motor-driven precision goniometer (±0.01°) which enables the photomultiplier detector to be moved from 20° to 150° scattering angle. The intensity time-correlation functions (ITCF) $g_2(t)$ are recorded with an ALV-5000 multiple tau digital correlator with fast option. The minimal sampling time of this correlator is 12.5 ns. The cylindrical sample cells, which have a diameter of 10 mm are made of Suprasil® quartz glass made by Hellma (Mühlheim, Germany).

2.2.2. Capillary zone electrophoresis (CZE)

The quantitative analysis using CZE for HA was performed on a Hewlett Packard Model G1600A, ^{3D}CE system (Waldbronn, Germany). Bubble capillaries (fused-silica) with 150 μm optical light path, 56 cm length to detector and 50 μm internal diameters were obtained from Hewlett Packard (Waldbronn, Germany).

2.2.3. Multilayer membrane system (MLMS)

The MLMS (see Fig. 1) described in the literature (Alkrad et al., 2003b; Knorst et al., 1997; Huth et al., 1996; Neubert and Wohlrab, 1990) was used for the release of HA from MEs described in Section 2.2.5. One release cell is composed of base disk and top disk, between which, depending on the experimental requirement multiple membranes can be arranged. The top disk is provided with a square hole of 4 cm^2, which allows the application of the formulation on the surface of the membranes. The cells were fitted together and placed in a chamber maintained at 32 ± 0.2 °C during the experimental period. Each acceptor system contained four glycerol membranes.

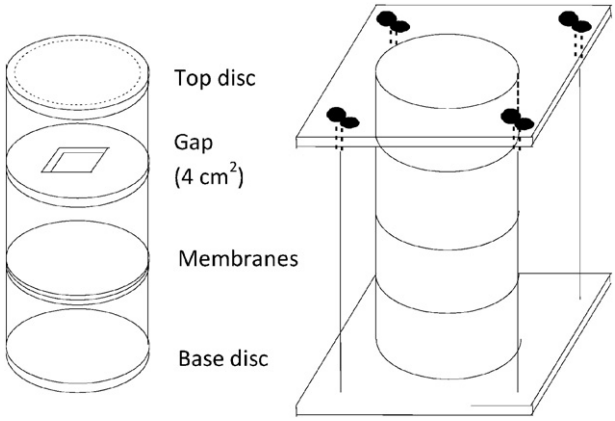

Fig. 1. Multilayer membrane system (MLMS).

2.2.4. Preparing of the hydrophilic membranes

100 g of ether and ethanol (85:15) mixture which contained 4 g of glycerol was mixed with 100 g 4% of collodium solution. Then the resulting mixture was placed on a glass surface of a film-forming device, Martin Luther University (Halle, Germany). The membrane was dried for 4 h at room temperature and cut into disks of 4 cm diameter. The content of glycerol in one membrane was 3.92 mg/cm^2 ± 0.08.

2.2.5. Viscosimetry

Rheograms were established for the MEs with increasing and decreasing shear force at 20 and 25 °C on the cup and bob viscometers. The apparatus consists of electric Rheometer: RHEOLAB MC1, Universal tool and Universal software: US200 (Physica, Stuttgart, Germany).

2.2.6. Polarization microscope

Polarization microscope (Carl Zeiss, Oberkochem, Germany) was used for testing isotropy of the MEs.

2.2.7. ME preparation

2.2.7.1. Non-ionic MEs for testing uptake capacity of water. Nine MEs which are composed of 4 ml IPP and 1 ml of Tween80:Span20 with different ratios (9:1, 8:2, 7:3, 6:4, 5:5, 6:4, 7:3, 8:2, 9:1) were prepared. Water was added drop wise to these MEs with continuous stirring using a magnetic stirrer until the ME begins to be slightly turbid and then turns over clear with continuous rotation. The added water volumes for each ME were recorded.

2.2.7.2. Preparing of MEs for testing the effect of incorporated amount of HA on water uptake and required surfactant mixture (Tween80:Span20) for forming a clear ME. Five series of MEs (series1, series2) were prepared with increased amount of HA (50, 100, 200 and 250 mg). The volume of water was increased gradually 0.2, 0.3, 0.4 and 0.5 ml in each series (in M1, M2, M3, M4 and M5, respectively). A fixed volume of IPP (4 ml) was added. Then Tween80:Span20 (2:3 ratio) was added drop wise under stirring using a magnetic stirrer until a clear ME is formed. The consumed quantities of the surfactant for forming clear MEs were recorded (Table 1).

2.2.7.3. HA-IPP-ME (2% HA-IPP-ME). 188 mg of HA (22 kDa) is dissolved in 0.4 ml water and then added to 7.5 ml IPP. A mixture of Tween80:Span20 (ratio 2:3) is added to dissolve HA in water and IPP till a clear ME is formed. The volume of surfactant mixture was 1.3 ml.

2.2.7.4. HA-MCTG-ME (2% HA-MCTG-ME). 200 mg of HA (22 kDa) was dissolved in 0.96 ml water:DMSO (6:3.6). This DMSO mixture was added to 7.24 ml MCTG. Span20 was dropped into dissolved HA in water till a clear ME was obtained.

2.2.8. Release studies

An accurately weighed quantity of the topical formulation (10 mg) was applied to the acceptor system which was fixed in a release cell with an exposed application area of 4 cm^2. The release cells were fixed in the model construction and placed in a chamber maintained at 32 ± 0.2 °C during the experiment. The model apparatus was removed from thermostated chamber at selected time intervals. After removal, the release cells were separated and the remaining amount of applied formulation on the first acceptor membrane was removed. The membranes were extracted with 3 ml distilled water, the lipid phase was