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Development of multiple W/O/W emulsions as dermal carrier system for oligonucleotides: Effect of additives on emulsion stability

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ABSTRACT

Multiple water-in-oil-in-water (W/O/W) emulsions are of major interest as potential skin delivery systems for water-soluble drugs like oligonucleotides due to their distinct encapsulation properties. However, multiple emulsions are highly sensitive in terms of variations of the individual components. The presence of osmotic active ingredients in the inner water phase is crucial for the generation of stable multiple emulsions. In order to stabilize the emulsions the influence of NaCl, $MgSO_4$, glucose and glycine and two cellulose derivatives was investigated. Briefly, multiple W/O/W emulsions using Span 80 as a lipophilic emulsifier and different hydrophilic emulsifiers (PEG-40/50 stearate, steareth-20 and polysorbate 80) were prepared. Stability of the emulsions was analyzed over a period of time using rheological measurements, droplet size observations and conductivity analysis.

In this study we show that additives strongly influence the properties stability of multiple emulsions. By increasing the concentration of the osmotic active ingredients, smaller multiple droplets are formed and the viscosity is significantly increased. The thickening agents resulted in a slightly improved stability.

The most promising emulsions were chosen and further evaluated for their suitability and compatibility to incorporate a DNAzyme oligonucleotide as active pharmaceutical ingredient.

1. Introduction

Multiple water-in-oil-in-water (W/O/W) emulsions represent complex systems consisting of both, water-in-oil (W/O) as well as oil-in-water (O/W) emulsions. For the generation of such a system, a primary W/O emulsion is dispersed into a continuous water phase and stabilized using a hydrophilic emulsifier. Due to their distinct structure and properties, multiple emulsions are of special interest for several drug delivery approaches, including carriers for the dermal application of pharmaceutical drugs in pharmaceutical products (Ferreira et al., 1995; Fukushima et al., 1987; Khopade and Jain, 1999; Lindenstruth and Müller, 2004), cosmetics (Tadros, 1992; Vasudevan and Naser, 2002) and the encapsulation of flavours in food (Garti and Benichou, 2004). However, multiple emulsions are thermodynamically unstable due to the excess of free energy associated with the two present interfaces. Compared to simple emulsions, both interfaces have to be stabilized. Therefore, two different emulsifiers are required: one with a low HLB to stabilize the inner W/O interface combined with a second one exhibiting a high HLB to stabilize the outer O/W interface. Both, the concentration and the chemical structure of the emulsifier strongly influence the properties of the formulation (Geiger et al., 1998; Jager-Lezer et al., 1997; Jiao and Burgess, 2003; Schmidts et al., 2009; Tirnaksiz and Ozlem, 2005). Water migration across the oil membrane is just one among several factors contributing to changes in the emulsion properties and leading to a destabilization process over the time. It has been demonstrated that the addition of electrolytes to the water phase in W/O emulsions results in a stabilizing effect, which can be explained by a counteraction of Laplace pressure (Aronson and Petko, 1993; Koroleva and Yurtov, 2003). Other additives in the aqueous phase, such as proteins, sugars and drugs, can also exert an osmotic effect (Hino et al., 2000; Ueda and Matsumoto, 1991). Stabilization hereby depends on the chosen concentration of the osmotic active ingredient, previously added to the inner water phase. There are several factors which can influence the migration of osmotic active ingredients, e.g. partition coefficient, ionisation, charge density, molecular weight and molecular mobility of the molecule (Sela et al., 1995). Water molecules passing from the outer into the inner water phase due to osmotic disequilibrium can also lead to swelling and potential bursting of inner water droplets (Jager-Lezer et al., 1997). In order to obtain a stable formulation, the concentration of electrolytes has to be high enough to allow for the regulation of Laplace pressure but at the same time suffi-

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ciently low to avoid osmotic effects. Another alternative to improve the stability of multiple emulsions can be achieved by increasing the viscosity of the inner water phase (Omotosho, 1990). Hence, the diffusion of electrolytes and water molecules between both water phases is hampered and modifications of the emulsion occur significantly slower.

DNAzymes of the 10–23 family are single-stranded DNA antisense oligonucleotides that exhibit a direct catalytic activity following binding of a corresponding RNA molecule. By sequence-specific cleavage of mRNAs they can suppress the expression of proteins that are involved in therapeutically relevant pathways, such as inflammatory processes (Breaker and Joyce, 1994) as well as active pharmaceutical ingredients (API) for the therapy of inflammatory diseases of the skin (Sel and Renz, 2008; Wraight and White, 2001). Their potential application in the therapy of dermal diseases is challenging since these molecules are highly sensitive to DNAses which are naturally present in and on the human skin (Santoianni and Rothman, 1961).

In this study, the influence of several modifications of the inner water phase on the properties and stability of multiple emulsions was investigated. Two groups of additives were studied: osmotic active ingredients as glucose, glycine, NaCl and $MgSO_4$, as well as thickening agents derived from cellulose derivatives.

Based on published data on delivery systems for sensitive drugs (Silverman, 2005; Singh et al., 1997) in the inner water phase of multiple emulsions, promising candidates were chosen and evaluated for their potential applicability for DNAzyme oligonucleotides.

2. Materials and methods

2.1. Materials

The oil heavy paraffin was supplied by Fagron (Fagron GmbH Co. KG, Barsbüttel, Germany). The lipophilic surfactant sorbitan monooleate 80 (Span^{TM}80) was kindly provided by Croda GmbH (Kaldenkirchen, Germany). The hydrophilic surfactants polysorbate 80 (Caelo GmbH, Hilden, Germany) as well as PEG 40/50 stearate and steareth-20, both from Croda GmbH, were used. NaCl (Merck, Germany), $MgSO_4 \cdot 7H_2O$, glycine and glucose (Caelo, Germany) were applied as osmolytes in the inner water phase. The thickeners hydroxyethylcellulose and sodium carboxymethyl cellulose were purchased from Caelo GmbH (Hilden, Germany). A 10–23 DNAzyme was generated and kindly provided by sterna biologicals GmbH & Co. KG, Germany, representing the Na-salt of single-stranded DNA molecule composed of 34 deoxynucleotide bases with a molecular weight of 10.6 kDa.

2.2. Preparation of emulsions

Multiple emulsions were prepared using a two-step procedure (Ueda and Matsumoto, 1991). Briefly, the primary W/O emulsion was prepared and then gently dispersed (40 wt.%) in the external water phase containing one of the following hydrophilic emulsifiers: polysorbate 80, PEG 40/50 stearate or steareth-20.

Regular W/O emulsions were prepared by adding an aqueous phase containing different concentrations of osmotic active ingredients to the oil phase. In detail, both phases were heated to approx. 70–75 °C before the water phase was added to the oil phase. The mixture was subsequently homogenized using a rotor/stator homogenizer (Diax 600, Heidolph Germany) at 9500 rpm for 2 min. The obtained primary emulsion was cooled down to room temperature and then slowly added to the outer water phase while the system was stirred at 1200 rpm using a EUROSTAR digital stirrer (IKA® Werke GmbH 6 Co. KG, Staufen, Germany), until a homogeneous emulsion was obtained. The compositions of the multiple emulsions are shown in Table 1.

2.3. Conductometric analysis

Conductivity measurements were carried out using a WTW Microprocessor Conductivity Meter LF 96 (WTW, Germany) at room temperature. Measurements were performed directly in the undiluted emulsion (mean ± S.D., $n = 3$). The standard deviation was negligible and therefore not plotted in the graphs.

2.4. Microscopic observation

The W/O/W multiple emulsions were analyzed using an optical immersion microscope TR 300 connected to a DV 2B (VWR, Germany) camera at ×1000 magnifying power (oil immersion). This method was used to allow a standardized quality control as well as a verification of the multiple emulsions.

2.5. Droplet size measurement

Mean water droplet size (*z*-average) in the primary W/O emulsion was determined by dynamic lights scattering (High Performance Particle Sizer (HPPS), Malvern Instruments, UK). Samples were diluted 1:1000 using light paraffin oil (viscosity: 33 mPa s) prior to measurement.

Oil droplet size and distribution in multiple W/O/W emulsions were determined using a laser diffraction particle size analyser (Mastersizer S, Malvern Instruments, England). The fundamental size distribution obtained with this technique is based on a volume distribution. The particle size distribution was calculated according to the Mie theory. Measurements were performed directly after samples have been diluted in distilled water (mean ± S.D., $n = 3$). The standard deviation was negligible and therefore not plotted in the graph. Microscopic observations revealed that the bimodal particle size distribution obtained by static light scattering measurements can be attributed to the occurrence of simple oil droplets without an encapsulated water phase and oil droplets with multiplicity (containing inner water droplets). Thus, peak maximum of the larger multiple droplets were chosen for characterisation of the W/O/W multiple emulsions.

2.6. Rheological measurement

Rheological analysis was performed at 25 °C using a RheoStress 300 Rheometer (Thermo Haake, France) with cone and plate geometry, diameter of two centimetres and an angle of 2°. The apparent viscosity was measured over a shear rate of 0.1–100 s^{-1}. The results are presented as mean values (mean ± S.D., $n = 3$). The standard deviation was negligible and therefore not plotted in the graphs.

Table 1
Compositions of multiple emulsions containing different osmolytes in the inner water phase.

Osmolyte solution	20.0%	Inner water phase
Heavy paraffin oil	15.8%	Oil phase
Span 80	4.0%	
Lecithin	0.2%	
Distilled water	ad. 100%	Outer water phase
Hydrophilic emulsifier	1%[a]/1.2%[b]	

[a] Polysorbate 80, steareth-20.
[b] PEG-40/50 stearate.