European Journal of Pharmaceutical Sciences 81 (2016) 129–136

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

# Development and in vitro characterisation of an oral self-emulsifying delivery system for daptomycin

Ožbej Zupančič, Alexandra Partenhauser, Hung Thanh Lam, Julia Rohrer, Andreas Bernkop-Schnürch *

*Department of Pharmaceutical Technology, Institute of Pharmacy, Leopold-Franzens-University Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria*

ARTICLE INFO

*Article history:*
Received 17 August 2015
Received in revised form 28 September 2015
Accepted 8 October 2015
Available online 17 October 2015

*Keywords:*
Oral peptide delivery
Self-emulsifying drug delivery systems
Payload
Mucus permeation
Pancreatic lipase
α-Chymotrypsin

ABSTRACT

It was the aim of this study to develop an oral self-emulsifying drug delivery system (SEDDS) for the peptide drug daptomycin exhibiting an anionic net charge. Drug lipophilicity was increased by hydrophobic ion pairing with cationic surfactant dodecylamine hydrochloride in molar ratio of surfactant to peptide 5:1. Log P (octanol/water) of −5.0 was even raised to +4.8 due to complexation with dodecylamine hydrochloride. Various SEDDS formulations were developed and characterised regarding emulsification properties, droplet size, polydispersity index and zeta potential. When the daptomycin dodecylamine complex (DAP/DOA) was dissolved in a formulation containing 35% Dermofeel MCT, 30% Capmul MCM and 35% Cremophor RH40, a maximum payload of even 8.0% (*w*/*w*) corresponding to 5.5% pure daptomycin was achieved. The formulation was degraded by lipase within 90 min. Release studies of daptomycin from this formulation emulsified in 50 mM phosphate buffer pH 6.8 demonstrated a sustained drug release for at least six hours. Moreover, SEDDS exhibited also mucus permeating properties as well as a protective effect towards drug degradation by α-chymotrypsin. According to these results, SEDDS containing 8% DAP/DOA complex may be considered as a new potential oral delivery system for daptomycin.



## 1. Introduction

Oral drug delivery remains the preferred non-invasive route of administration for therapeutic peptide and protein drugs due to its low cost, ease of administration and high patient compliance (Zhang et al., 2012). However, drugs administrated by this route face physiological and biochemical barriers, which need to be taken into account in order to achieve a sufficiently high bioavailability (Karamanidou et al., 2015). Peptide drugs are often hydrophilic macromolecules, which are rapidly degraded in the deleterious gastrointestinal environment by luminal secreted endo- and exopeptidases (Morishita & Peppas, 2006; Woodley, 1993; Shen, 2003). In addition, the mucus layer represents a molecular filter for molecules with molecular mass cut off up to 800 Da, above which permeation is relatively low (Fricker & Drewe, 1996; Cone, 2009). In order to protect the peptide drug against enzymatic degradation and improve its permeation through mucus layer, various lipophilic and polymeric nanocarriers have been developed (Kammona & Kiparissides, 2012; Li et al., 2012; Fan et al., 2014). Among them self-emulsifying drug delivery systems (SEDDS) representing an isotropic mixture of oils, surfactants, co-surfactants and co-solvents are of particular interest (Pouton & Porter, 2008; Bahloul et al., 2015). These systems spontaneously form transparent and kinetically stable emulsions in gastric fluid under gentle agitation (Hintzen et al., 2014). Some SEDDS formulations were shown to exhibit mucus permeating (Karamanidou et al., 2015; Friedl et al., 2013) and absorption enhancing properties (Hintzen et al., 2013) as well as protection of the incorporated peptides against enzymatic degradation (Karamanidou et al., 2015; Hintzen et al., 2014; Borkar et al., 2015). Cyclosporine was the first successfully marketed peptide drug formulated to oral SEDDS (Neoral®, Sandimmune®). The lipophilic nature of cyclosporine ensures that it remains in the oily phase after emulsification with gastrointestinal fluid. In contrast, incorporation of hydrophilic peptides in SEDDS still remains very challenging (Date et al., 2010). Up to date the maximum reported payloads of hydrophilic peptide drugs in SEDDS range from 0.17 to 1.13% (*w*/*w*), which is indeed still a major limiting factor for this technology. A high payload in SEDDS is, however, key to success in order to reach therapeutic drug levels after oral administration.

Daptomycin is an anionic peptide antibiotic derived from the fermentation of *Streptomyces roseosporus*. It is used for the treatment of complicated skin infections, bacteremia, and right-side endocarditis caused by multiresistant Gram-positive bacteria (Qiu et al., 2011; D'Costa et al., 2012). To the best of our knowledge no oral delivery systems for daptomycin has been developed so far. The duration of treatment can last from one to up to six weeks (Chakraborty et al., 2009; Dvorchik et al., 2003). Incorporating the antibiotic into SEDDS would reduce the costs of treatment and increase patient's compliance. So far the

* Corresponding author.
*E-mail address:* andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

http://dx.doi.org/10.1016/j.ejps.2015.10.005
0928-0987/

development of an oral daptomycin SEDDS might have failed because of insufficient high payload. It was therefore the aim of this study to develop an oral SEDDS for daptomycin exhibiting a payload of at least 5% (*w*/*w*).

## 2. Materials and methods

### 2.1. Materials

Daptomycin was purchased from Eastbank Pharmaceuticals (Shanghai, China), Dermofeel MCT was a gift from Dr. Streatmans (Hamburg, Germany), Capmul MCM EP was a gift from Abitec (USA), Cremophor EL, Cremophor RH40, dodecylamine hydrochloride, cetrimonium bromide, benzalkonium chloride, bile salts, lipase from porcine pancreas (Type II, 54 units/mg using triacetin) and α-chymotrypsin (from bovine pancreas, Type II, 96 units/mg protein) were purchased from Sigma Aldrich (Steinheim, Germany), Float-a-lyser® (MWCO = 100 kDa) was purchased from Spectrumlabs (Netherlands). All other chemicals, reagents and solvents were of analytical grade and received from commercial sources.

### 2.2. HPLC analysis

A Hitachi EliteLaChrom HPLC-system equipped with an L-2130 pump, L-2200 autosampler and L-2450 photodiode array UV detector was used. A Nucleosil 100-5 C18 column (250 × 4 mm, 5 μm) was used as stationary phase. A binary solvent system was used at room temperature and flow rate of 1 mL/min. Solvent A was acetonitrile and solvent B was 0.1% (*v*/*v*) trifluoroacetic acid in water. Daptomycin was quantified by an isocratic method using 45% solvent A and 55% solvent B as mobile phase. The column was washed with a gradient method; 0–30 min: 25–90% A, 30–50 min: 90% A, 50–100 min: 90–60% A and 100–120 min: 60% A to remove any retained oils.

### 2.3. Hydrophobic ion pairing

Surfactant water solution (50 μL) was slowly added in a dropwise manner to daptomycin water solution (50 μL, 2 mg/mL, pH was adjusted to 6.8 with NaOH) at room temperature. Molar ratios of surfactant to daptomycin ranged from 1:1 to 10:1. The precipitated daptomycin-surfactant complex was isolated by centrifugation (5 min, 10,000 rpm). The amount of precipitated daptomycin-surfactant complex was determined by measuring the remaining amount of peptide in the supernatant by HPLC as described above. The precipitate was washed with purified water, lyophilised (Christ Gamma 1-16 LSC Freeze dryer) and stored at −20 °C for further use.

### 2.4. Determination of log P (octanol/water) of DAP/DOA complex

Dodecylamine hydrochloride solution (50 μL, 0.82–2.18 mg/mL) was slowly added in a dropwise manner to daptomycin water solution (50 μL, 2 mg/mL, pH was adjusted to 6.8 with NaOH) at room temperature. Molar ratios of dodecylamine hydrochloride to daptomycin ranged from 3:1 to 7:1. After five minutes, octanol (100 μL) was added to the mixture and samples were placed on a thermomixer (900 rpm, 25 °C) for 30 min. The complete phase separation was achieved by centrifugation (5 min, 14,000 rpm). The amount of daptomycin in octanol and water phase was determined by HPLC as described above. Octanol samples (10 μL) were diluted prior to injection in HPLC with isopropanol (80 μL) and water (110 μL) to yield a final volume of 200 μL.

### 2.5. SEDDS development, characterization and DAP/DOA complex incorporation

Dermofeel MCT and Capmul MCM EP were used as oils and Cremophor EL and Cremophor RH40 as surfactants for the preparation of SEDDS. Mixtures of oils and surfactants (1 g) were weighted in 2 mL reaction tubes and homogenized by a vortex mixer to form a single phase. SEDDS preconcentrates (100 mg) were emulsified in 5 mL of 50 mM phosphate buffer pH 6.8. Mean droplet size, polydispersity index and zeta potential of emulsions were determined by dynamic light scattering using a PSS Nicomp 380 DLS (Santa Barbara, CA, USA). Measurements were performed at 25 °C and 37 °C. Solubility of DAP/DOA in SEDDS was determined as follows: an exact amount of DAP/DOA (1 mg to 10 mg) was accurately weighted in 2 mL reaction tubes in 1 mg aliquots, then 100 mg of the SEDDS preconcentrate was added. The dispersions were left to stir on a thermomixer (40 °C, 400 rpm). After 48 h the tubes were centrifuged (13,000 rpm, 10 min) to separate the undissolved DAP/DOA complex form the oil. No sediment and a clear oily solution indicated the complete dissolution of the DAP/DOA complex. To determine the amount of pure daptomycin dissolved in SEDDS 10 mg of clear oil was emulsified in 2 mL of deionized water. The amount of pure daptomycin in SEDDS was determined by HPLC as described above.

### 2.6. In vitro release

Release of daptomycin from SEDDS was evaluated in 50 mM phosphate buffer pH 6.8 at 37 °C by using a dialysis tube (Float-a-lyser®, MWCO = 100 kDa). Daptomycin stock solution (3 mg/mL) was prepared in 50 mM phosphate buffer pH 6.8. Dialysis tube was filled with 1 mL of stock solution, 1 mL of daptomycin SEDDS emulsion (DAP-SEDDS, prepared by emulsifying 100 mg of SEDDS in 1 mL of stock solution) and 1 mL of SEDDS emulsion containing DAP/DOA complex (DAP/DOA-SEDDS, prepared by emulsifying 100 mg of SEDDS containing a DAP/DOA payload of 3% *w*/*w*, corresponding to 2.1% pure daptomycin in 1 mL of phosphate buffer). All three samples were dialysed against 25 mL of phosphate buffer at 3737 °C under stirring on a shaking board (Vibramax 100; Heidolph Instruments, Schwabach, Bavaria, Germany) at 300 rpm. At predetermined time points aliquots of 100 μL were withdrawn from the medium and replaced with phosphate buffer. The amount of released daptomycin in these aliquots was determined by HPLC as described above.

### 2.7. Permeability studies

For SEDDS mucus permeability studies a slightly modified method as described previously by Friedl et al. was used (Friedl et al., 2013). In brief, the intestine of freshly slaughtered pigs was collected from a local abattoir and stored on ice during the transport to the laboratory. Intestinal sections containing chime were removed and discarded. The mucosa of the remaining small intestine was incised longitudinally and scraped with a microscope slide to collect the mucus. Particulate debris was removed from the mucus via gentle agitation (stirring <40 rpm) followed by centrifugation (14,000 rpm). To 1 g of mucus, 5 mL of 0.1 M sodium chloride was added and agitated for 1 h after which the suspension was centrifuged for two hours and only the clean portion of the pellet was retained. The process was repeated once and the clean mucus was stored at −20 °C until required. Then 24-well plates (Greiner-BioOne) with transwell inserts (Greiner-BioOne, 3 μm pore size) covered with 50 mg of porcine mucus were prepared. Before the addition of samples the mucus was incubated on a shaking board (Vibramax 100; Heidolph Instruments, Schwabach, Bavaria, Germany) at 37 °C and 300 rpm for 15 min. The acceptor chamber was filled with 1250 μL of preheated 50 mM phosphate buffer pH 6.8 and the donor chamber with 250 μL of 0.08% daptomycin phosphate buffer solution or 250 μL of DAP/DOA-SEDDS (prepared by emulsifying 100 mg of SEDDS containing DAP/DOA payload of 8% *w*/*w*, corresponding to 5.5% pure daptomycin in 1 mL of phosphate buffer). The plate was covered with a plate lid and incubated at 37 °C on a shaking board at 300 rpm. At predetermined time points aliquots of 100 μL were removed from the acceptor compartment and replaced with the same