International Journal of Pharmaceutics 520 (2017) 267–274

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

# Hydrophobic ion pairing: Key to highly payloaded self-emulsifying peptide drug delivery systems

Janine Griesser[a], Gergely Hetényi[a], Michael Moser[a], Frédéric Demarne[c], Vincent Jannin[c], Andreas Bernkop-Schnürch[a,b,*]

[a] Thiomatrix Forschungs-und Beratungs GmbH, Trientlgasse 65, Innsbruck, Austria
[b] Center for Chemistry and Biomedicine, Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria
[c] Gattefossé SAS, 36 chemin de Genas, 69804 Saint-Priest Cedex, France

ARTICLE INFO

*Article history:*
Received 11 January 2017
Received in revised form 2 February 2017
Accepted 6 February 2017
Available online 7 February 2017

*Keywords:*
Leuprorelin
Insulin
Desmopressin
Self-emulsifying drug delivery systems
Hydrophobic ion paring
Peptide delivery

ABSTRACT

*Aim:* The aim of this study was the formation and characterization of various ion pairs of therapeutic peptides with different surfactants in order to reach a high payload in self-emulsifying drug delivering systems (SEDDS).
*Methods:* Hydrophobic ion pairs (HIP) were formed between the anionic surfactants sodium docusate, dodecylsulfate and oleate and the peptides leuprorelin (LEU), insulin (INS) and desmopressin (DES). The efficiency of HIP formation was evaluated by quantifying the amount of formed complexes, log P value determination in n-octanol/water via HPLC and zeta potential measurements. Solvents and surfactants were screened regarding their complex solubilizing properties. Subsequently, peptide complexes were incorporated into SEDDS followed by payload and stability determination.
*Results:* Independent from the type of peptide, docusate showed the most efficient HIP properties followed by dodecylsulfate and oleate. Ratios of 2:1 for LEU, 6:1 for INS and 1.5:1 for DES led to the highest quantity of formed complexes with docusate and log P increased at least by 3 units. The more docusate was added to each peptide, the more negative became the zeta potential of the resulting complex. Incorporating these optimized complexes into novel SEDDS containing Capryol 90, Labrafil M 2125 CS, Labrasol ALF, Peceol, propylene glycol, tetraglycol, Transcutol HP and Tween 20 allowed payloads of the LEU, DES and INS complexes above 10%. Moreover, SEDDS exhibited high stability and constant negative zeta potential over a 4 h incubation time.
*Conclusion:* Following the procedure described herein payloads >10% can be achieved for peptide drugs in SEDDS.

© 2017 Elsevier B.V. All rights reserved.

## 1. Introduction

Self-emulsifying drug delivery systems (SEDDS) representing isotropic mixtures of oils, solvents and emulsifiers are likely opening the door for the oral administration of as challenging drugs as therapeutic peptides and proteins (Leonaviciute and Bernkop-Schnürch, 2015). Incorporating peptide drugs in lipid droplets protects them towards thiol/disulfide exchange reactions with food and endogenous glutathione as well as towards an enzymatic degradation in the gastrointestinal tract (Dahm and Jones, 1994; Ijaz et al., 2016; Schmitz et al., 2006). Moreover, these lipid droplets can be designed in a way that they are able to permeate the mucus gel barrier in a comparatively efficient manner (Friedl et al., 2013). Once having reached the underlying absorption membrane, SEDDS were shown to exhibit even permeation enhancing properties for peptide drugs (Leonaviciute and Bernkop-Schnürch, 2015).

Up to date, however, this promising strategy has by far not reached its full potential, as various hurdles still need to be overcome. One of these hurdles is certainly the poor solubility of peptide drugs in the lipophilic phase of SEDDS. Because of their mainly hydrophilic nature peptides and proteins can likely only be incorporated in lipids via hydrophobic ion pairing (HIP). But even by utilizing this technique only comparatively low payloads were achieved. Hintzen et al. (2014), for instance, reached a payload of just 0.4% for leuprorelin, Karamanidou et al. achieved

* Corresponding author at: Center for Chemistry and Biomedicine, Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, 6020, Innsbruck, Austria.
*E-mail addresses:* andreas.bernkop@uibk.ac.at, a.bernkop@thiomatrix.com (A. Bernkop-Schnürch).

http://dx.doi.org/10.1016/j.ijpharm.2017.02.019
0378-5173/© 2017 Elsevier B.V. All rights reserved.

1.13% for insulin (Karamanidou et al., 2015) and Zupančič et al. (2016) obtained 0.25% for desmopressin in SEDDS. According to these results, the low peptide payload of SEDDS had to be identified as worrying bottleneck for this otherwise promising strategy.

In order to address this problem, it was the aim of this study to focus just on this issue and to provide a broader understanding on parameters influencing the efficiency of HIP and the subsequent incorporation of peptide complexes in SEDDS. For comparison reasons three peptides including cyclic or non-cyclic as well as high molecular weight and low molecular weight peptides were chosen. As leuprorelin (LEU), insulin (INS) and desmopressin (DES) are cationic peptides, the anionic surfactants sodium docusate, sodium dodecylsulfate and sodium oleate exhibiting different acidic groups, namely a sulfonate, sulfate and carboxylic moiety, were tested for HIP. In order to achieve comparatively high payloads, the solubility of the most lipophilic complexes was investigated in various solvents. Those solvents in which complexes could be dissolved most efficiently were in the following utilized to form SEDDS.

## 2. Materials and methods

### 2.1. Materials

Leuprorelin acetate and desmopressin acetate were purchased from Chemos GmbH, Germany. Insulin from porcine pancreas was bought from Prospec Protein, Israel. Lipids utilized within this study were provided by Gattefossé, France. Tween 20 was a gift from Croda, Germany. All other reagents were purchased from Sigma-Aldrich, Austria.

### 2.2. Methods

#### 2.2.1. HPLC analysis

Peptides were analyzed on a Hitachi Elite LaChrom HPLC-System equipped with L-2130 pump, L-2200 autosampler and L-2400 UV detector. As mobile phase a binary solvent system of solvent A – water with 0.1% (v/v) trifluoroacetic acid (TFA) and solvent B – acetonitrile with 0.1% (v/v) TFA was used. Leuprorelin was quantified utilizing XBridge BEH300 C18 3.5 μm 4.6 mm x 150 mm as stationary phase. An isocratic method (70% mobile phase A and 30% mobile phase B) over 12 min at 40 °C with a flow rate of 0.8 ml/min was applied. Moreover, all samples were stored at 10 °C within the auto sampler until injection of 20 μl and thereafter analyzed at a wavelength of 222 nm. Insulin was quantified with LiChrosorb RP-18 LiChroCART 5 μm, 100 Å, 125 × 4 as stationary phase. An isocratic method (65% mobile phase A and 35% mobile phase B) over 7 min at 40 °C with a flow rate of 1 ml/min was used. Aliquots of 50 μl were injected and analyzed at a wavelength of 214 nm. For desmopressin Nucleosil 100-5 C18 5 μm 4 mm x 250 mm as stationary phase was used. An isocratic method (75% mobile phase A and 25% mobile phase B) over 10 min at 40 °C with a flow rate of 1 ml/min was applied. The injection volume was 20 μl and samples were analyzed at a wavelength of 222 nm.

#### 2.2.2. Hydrophobic ion pairing (HIP)

First, 1 ml of each peptide solution was prepared in a concentration of 10 mg/ml utilizing 0.01 M HCl as solvent. As leuprorelin, desmopressin and insulin exhibit different net positive charges at this pH, several ratios of peptide to surfactant were analyzed. Thereafter, surfactants as listed in Table 1 were dissolved in 1 ml of demineralized water applying ratios as indicated in Table 1. The solution of each surfactant was added drop wisely to the peptide solution under vigorous stirring (400 rpm). The mixtures were stirred for two hours and subsequently centrifuged at 12,500 rpm for 10 min with High-Speed Mini Centrifuge (Fisher Scientific, Illinois, USA). During the reaction, an immediate white precipitation indicated the formation of the hydrophobic peptide complex. The supernatant was separated from the precipitate and washed with 0.01 M HCl. Thereafter, the water-soluble fraction of the peptide remaining in the supernatant was determined by measuring its concentration via HPLC as described above. Finally, the pellets were frozen, lyophilized and stored at −30 °C. As blank reference, the surfactant solutions were added to 0.01 M HCl without peptide. Precipitation efficiency was defined utilizing the following equation:

$$\text{Precipitation efficiency}[\%] = 100 - \left(\frac{\textit{Peptide concentration after HIP}}{\textit{Peptide concentration before HIP}} \times 100\right)$$

#### 2.2.3. Log P determination

To 1 ml of n-octanol/water (1:1), 1 mg of peptide or 1 mg of complex was added and incubated at 37 °C while shaking at 300 rpm for 24 h. Samples were centrifuged for 10 min at 10,000 rpm with High-Speed Mini Centrifuge (Fisher Scientific, Illinois, USA). Thereafter, 100 μl aliquots were withdrawn from both of the aqueous and the n-octanol phase and diluted with 300 μl of methanol containing 0.1% (v/v) TFA. The concentration of

**Table 1**
Complexes of leuprorelin, insulin and desmopressin with indicated surfactants.

| Peptide | Net positive charges | Basic amino acids (AA) | Tested surfactants | Molar ratios [surfactant: peptide] |
|---|---|---|---|---|
| Leuprorelin | 2 | Arginine<br>Histidine | Sodium docusate<br>Sodium dodecyl sulfate<br>Sodium oleate | 0.5:1<br>1:1<br>2:1<br>3:1<br>4:1 |
| Insulin | 6 | Arginine<br>Histidine<br>2 Lysine<br>2 N-terminal AA | Sodium docusate<br>Sodium dodecyl sulfate<br>Sodium oleate | 1:1<br>3:1<br>6:1<br>9:1<br>12:1 |
| Desmopressin | 1 | Arginine | Sodium docusate<br>Sodium dodecyl sulfate<br>Sodium oleate | 0.5:1<br>1:1<br>1.5:1<br>2:1<br>3:1 |

Download English Version:

https://daneshyari.com/en/article/5550421

Download Persian Version:

https://daneshyari.com/article/5550421

Daneshyari.com