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Development and *in vitro* evaluation of zeta potential changing self-emulsifying drug delivery systems for enhanced mucus permeation

Wongsakorn Suchaoin[a], Irene Pereira de Sousa[a], Kesinee Netsomboon[a,b], Hung Thanh Lam[a,c], Flavia Laffleur[a], Andreas Bernkop-Schnürch[a,*]

[a] Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, A-6020 Innsbruck, Austria
[b] Faculty of Pharmacy, Thammasat University, Rungsit Campus, Phahonyothin Road, Khlong Luang, Pathumthani, Thailand
[c] Department of Pharmaceutical Technology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Can Tho City, Viet Nam

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ABSTRACT

The aim of this study was the development of zeta potential changing self-emulsifying drug delivery systems (SEDDS). Various cationic surfactants were incorporated into a formulation consisting of 30% Cremophor EL, 30% Capmul MCM, 30% Captex 355 and 10% propylene glycol (w/w). A substrate of intestinal alkaline phosphatase (IAP), 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic acid sodium (PA), was thereafter incorporated into SEDDS. Size, zeta potential and polydispersity index were determined. Phosphate release studies were performed using three different models, namely, isolated IAP, Caco-2 cell monolayer and rat intestinal mucosa and the amount of released phosphate was quantified by malachite green assay. Interaction of SEDDS and mucus was investigated regarding surface charges and mucus diffusion studies were performed using rotating tube technique. SEDDS were diluted 1:100 in 100 mM HEPES buffer and a negative zeta potential was obtained. By addition of isolated IAP, 15% to 20% phosphate was liberated from SEDDS within 3 h and a shift of zeta potential from negative to positive was observed. On Caco-2 cell monolayer and rat intestinal mucosa, 12% and 23% phosphate were released, respectively, from SEDDS diluted 1:1000 in glucose-HEPES buffer. Positively charged droplets were bound to negatively charged mucus resulting in a decrease of zeta potential, whereas negatively charged SEDDS showed no interaction. Furthermore, negatively charged SEDDS diffused faster through mucus layer as higher extent of incorporated Lumogen was present in deeper mucus segments in comparison to positively charged ones. Accordingly, zeta potential changing SEDDS provide an effective mucus permeation combined with higher cellular uptake when droplets reach absorptive epithelium membrane.

1. Introduction

Mucus gel layer covering epithelial surfaces along the entire gastrointestinal tract serves as a protective barrier against xenobiotics and pathogens. Accordingly, the adhesive three-dimensional network formed by mucus is also an effective hindrance for drugs and in particular for drug carrier systems such as micro- and nanoparticles (NPs) (Netsomboon and Bernkop-Schnürch, 2016). To design drug carriers that can overcome this barrier, one of the crucial factors that must be taken into consideration is their surface charge. In fact, mucus contains anionic substructures, namely, sialic acid and sulfonic acid that have a negative net charge (Bernkop-Schnürch and Greimel, 2012). It was shown that negatively charged and uncharged NPs were capable of moving across mucus while positively charged ones were immobilized and could not permeate through mucus layer because of ionic interactions (Pereira de Sousa et al., 2015). However, a major drawback of using negatively charged NPs is that when they access to the intestinal epithelium, cell uptake via endocytosis is less pronounced in comparison to positively charged carriers (Miller et al., 1998).

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

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Recently, drug carrier systems that can change zeta potential from negative to positive were developed to overcome this obstacle (Bonengel et al., 2015a; Bonengel et al., 2015b; Perera et al., 2015). The benefits of this strategy are that negatively charged NPs can diffuse faster across mucus network and surface charges are shifted to positive once they have reached the intestinal epithelium allowing enhanced cellular uptake. To achieve this, phosphorylated NPs could be designed loosing anionic phosphate groups via being cleaved off by intestinal alkaline phosphatase (IAP) expressed on intestinal epithelium brush border. The proof of this concept was demonstrated using NPs produced by polyelectrolyte complexation between phosphotyrosine-modified chitosan and carboxymethyl cellulose (CMC). Phosphate was cleaved from NPs by IAP resulting in conversion of zeta potential from −5 mV to +8 mV (Perera et al., 2015). Likewise, the zeta potential of the NPs containing 6-phosphogluconic acid-coupled polyethylene imine was shifted from −6 mV to +3 mV after incubation with IAP (Bonengel et al., 2015b). Proof-of-concept was additionally provided by transfection efficiency studies on Caco-2 and HEK-293 cells using phosphorylated nanocomplexes for pDNA delivery. Transfection efficiencies were lower in presence of phosphatase inhibitor in both cell lines as zeta potential of the NPs remained negative (Bonengel et al., 2015a).

Among various candidates of drug carrier, self-emulsifying drug delivery systems (SEDDS) appear to represent a promising approach. SEDDS composed of oil, surfactants and co-surfactants that are spontaneously emulsified in gastric fluid are not only used to incorporate poor water-soluble drugs but their protection against enzymatic degradation is also shown (Friedl et al., 2013; Rohrer et al., 2016; Zupančič et al., 2016). In addition, SEDDS can rapidly diffuse through mucus due to their small average droplet size and their shape deformation ability and exhibit high affinity to phospholipid bilayer of intestinal cells. For these reasons, mucus permeation and drug absorption might be improved by combination of the zeta potential changing strategy and SEDDS.

It was therefore the aim of this study to design novel zeta potential changing SEDDS which might be a promising tool for oral drug delivery. Several cationic surfactants and a phosphate ester bearing compound, 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidic acid sodium (PA), were incorporated into SEDDS, respectively. As a substrate of IAP, PA generates a shift of zeta potential via enzymatic cleavage of phosphate substructure. The *in vitro* release studies were performed in three different models using isolated IAP, Caco-2 cell monolayer and isolated rat intestine. Furthermore, the interaction of SEDDS and mucus as well as mucus penetration of SEDDS were investigated regarding surface charges of droplets within this study.

2. Materials and methods

2.1. Materials

PA was supplied by Carbosynth (Berkshire, UK). Capmul MCM (mono/diglycerides of caprylic acid, HLB = 5–6) and Captex 355 (caprylic/capric triglyceride) were obtained from Abitec Corporation, USA. Propylene glycol was purchased from Gatt-Koller (Absam, Austria). Lumogen red was obtained from Kremer pigmente GmbH & Co., KG (Aichstetten, Germany). Alkaline phosphatase from bovine intestinal mucosa (IAP; ≥6500 DEA units/mg protein, ≥10.0 mg protein/mL), ammonium molybdate tetrahydrate, benzalkonium chloride (BAC), cetrimonium bromide (CTAB), cetylpyridinium chloride monohydrate (CPC), Cremophor EL, dimethylsulfoxide (DMSO), ethyl oleate, HEPES, malachite green oxalate salt, octylamine (OA), potassium phosphate monobasic (KH_2PO_4), sulfuric acid (H_2SO_4), phosphatase inhibitor cocktail 2, triacetin and Triton-X 100 were purchased from Sigma-Aldrich (Vienna, Austria). All other chemicals were of analytical grade and received from commercial sources.

2.2. Methods

2.2.1. SEDDS preparation

Each formulation was prepared by weighing the excipients and homogenous mixing them at 50 °C. The compositions of each formulation and their nomenclatures are given in Table 1. Afterwards, several cationic surfactants, namely, octylamine (OA), benzalkonium chloride (BAC), cetrimonium bromide (CTAB) and cetylpyridinium chloride (CPC) were pre-incorporated into the formulations (Fig. 1). They were finally spiked with 0.4% (w/w) of PA. During each step, SEDDS formulations were diluted in 100 mM HEPES buffer (pH 7.0) in a dilution of 1:100 before characterization.

2.2.2. SEDDS characterization

The droplet size and polydispersity index of SEDDS were determined by dynamic light scattering using a particle sizer (Nicomp PSS 380 DLS/ZLS, Particle Sizing Systems, Inc., Port Richey, Florida) equipped with a He-Ne laser source at 632.8 nm. The measurement was performed at 23 °C under an angle of 90 and analyzed by intensity-weighted particle size from Gaussian Analysis. The sample was considered as monodisperse when polydispersity index <0.2. Zeta potential was measured using electrophoretic light scattering. The conversion from electrophoretic mobility to zeta potential was based on Debye-Hückel approximation as low ionic strength buffer was used and the droplet size of SEDDS was in nanorange (Nørby and Esmann, 1997;

Table 1
SEDDS formulations. Data are means ± SD (n = 3).

Nomenclature	Composition	Particle size (nm)	Zeta potential (mV)	Polydispersity index
F_1	30% Cremophor EL 30% Capmul MCM 30% Captex 355 10% Propylene glycol	37.9 ± 4.1	−1.70 ± 0.13	0.10
F_2	50% Tween 80 20% Capmul MCM 20% Captex 355 10% Glycerol 85	57.0 ± 8.2	−2.59 ± 0.20	0.15
F_3	50% Tween 80 30% Ethyl oleate 10% Glycerol 85 10% Triacetin	37.5 ± 6.8	−0.27 ± 0.10	0.25
F_4	50% Tween 80 40% Olive oil 10% Glycerol 85	342.1 ± 96.5	0.32 ± 0.05	0.18