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SEDDS: A game changing approach for the oral administration of hydrophilic macromolecular drugs☆

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ABSTRACT

Since the development of self-emulsifying drug delivery systems (SEDDS) in 1980's, they attract the attention of researchers in order to confront the challenge of poor water-solubility of orally given drugs. Within recent years, SEDDS were also discovered for oral administration of hydrophilic macromolecular drugs such as peptides, proteins, polysaccharides and pDNA. Due to hydrophobic ion pairing (HIP) with oppositely charged lipophilic auxiliary agents the resulting complexes can be incorporated in the lipophilic phase of SEDDS. Depending on the solubility of the complex in the SEDDS pre-concentrate and in the release medium drug release can be adjusted on purpose by choosing more or less lipophilic auxiliary agents in appropriate quantities for HIP. Within the oily droplets formed in the GI-tract drugs are protected towards degradation by proteases and nucleases and thiol-disulfide exchange reactions with dietary proteins. The oily droplets can be made mucoadhesive or highly mucus permeating depending on their target site. Furthermore, even their cellular uptake properties can be tuned by adjusting their zeta potential or decorating them with cell penetrating peptides. The potential of SEDDS for oral administration of hydrophilic macromolecular drugs could meanwhile be demonstrated via various in vivo studies showing a bioavailability at least in the single digit percentage range. Owing to these properties advanced SEDDS turned out to be a game changing approach for the oral administration of hydrophilic macromolecular drugs.

Contents

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1. Introduction

The oral route of administration has always been of great interest, as it offers a comparatively high patient convenience, capacity for self-administration and flexibility in regimen. From manufacturing perspective, oral dosage forms do not require sterile environment and therefore production costs are extremely low [1]. However, hydrophilic macromolecular drugs including herein mainly therapeutic peptides, proteins, polysaccharides and DNA-based drugs exhibit poor oral bioavailability due to numerous barriers associated with GI tract [2,3]. In order to address this issue, a variety of strategies has been employed such as structural drug modifications [4], co-administration of auxiliary agents [5] and development of nanocarriers [6].

Among lipophilic nanocarriers [7], self-emulsifying drug delivery systems (SEDDS) used in this review as broad terminology for both self-microemulsifying and self-nanoemulsifying drug delivery systems (SMEDDS/SNEDDS) [8] appear a promising approach for oral itinerary of therapeutics [9,10]. In comparison to other nanocarriers such as polymer-based nanoparticles, liposomes, niosomes, micelles or carbon nanotubes, scale-up and manufacturing of SEDDS on industrial scale is more simply and cost-efficient as it is almost just the preparation of a solution. Furthermore, nanocarrier related problems such as their tendency to aggregate and limited batch uniformity do not count for SEDDS as nanodroplets are formed in the GI-tract and not before. The first oral SEDDS product (Sandimmune Neoral) made its way to the market already over twenty years ago. Since then, however, pharmaceutical industry on the one hand has considered SEDDS only for delivery of lipophilic drugs and academia, on the other hand, mostly dedicated its research towards solid nanocarriers. The transfer of the enormous knowledge gathered over decades for solid nanocarriers to SEDDS [11–14], however, brought them back in the lime light within recent years. Inclusion of novel multifunctional auxiliary agents such as muco-inert compounds and cell penetrating peptides and the choice of appropriate excipients help in eradicating the limitations associated with oral SEDDS technology for hydrophilic macromolecular delivery. As a result, advanced SEDDS provide novel nanoemulsions with drastically improved functional properties such as increased stability in GI fluids, prolonged GI residence time, improved mucus permeating properties, enhanced permeation and improved cellular uptake leading consequently to a drastically raised bioavailability of the incorporated hydrophilic macromolecular drug [15]. This review describes in detail the broader role of this new generation of SEDDS as well as their future potential.

2. Oral dosage forms for SEDDS

In order to sufficiently dissolve hydrophilic macromolecular drugs in the oily phase of SEDDS hydrophobic ion pairing (HIP) turned out to be the likely most efficient technique. Utilizing the most appropriate hydrophobic counter ion as well as drug to counter ion ratio payloads even above 10% can be achieved [16]. The potential of HIP has already been shown for peptides [17,18], proteins [19], polysaccharides [20,21] and DNA-based drugs [22]. The association of oppositely charged lipophilic molecules to hydrophilic macromolecular drugs leads to improvement in lipophilicity [23]. For example, insulin representing the likely most described hydrophilic macromolecular drug in literature can be converted into a lipophilic complex by various methods [24–26]. Li et al. described the complexation of surfactants such as distearyldimethylammonium bromide and soybean phosphatidylcholine with insulin and its loading into SEDDS [24]. In another study, Karamanidou et al. established a hydrophobic insulin/dimyristoyl phosphatidyl glycerol complex and incorporated it into SEDDS for oral delivery. The insulin payload was 1.2% [27]. Rao and Shao formed HIPs of lactamase with phosphatidylcholine [19]. Griesser et al. investigated various parameters in detail with the aim to achieve a high payload of ion-paired therapeutic peptides in SEDDS. The critical parameters included assortment of appropriate surfactants, number of surfactant molecules attached to the peptide and selection of suitable solvents in order to dissolve the complex in SEDDS. Taking all these parameters into account payloads above 10% can be achieved for therapeutic peptides [16]. Zupančič et al. reported HIP of daptomycin, an anionic peptide antibiotic, with the cationic surfactant dodecylamine hydrochloride in order to increase its hydrophobicity and to incorporate it into SEDDS [28]. The log *P* value shifted from −5.0 to +4.8 corresponding to an almost 10^{10}-fold increase in lipophilicity. In another study, Zupančič et al. described the complexation of desmopressin with sodium docusate leading to alteration of log *P* value of drug from initial −6.13 to 0.33 [29]. Meanwhile the potential of HIP was also demonstrated for polysaccharides. The low molecular weight heparin enoxaparin, for instance, was successfully ion paired with dodecylamine. In another study HIP was formed between heparin and a lipophilic cationic polymer of β-cyclodextrin [21]. Furthermore, HIPs of pDNA are well-established since the 1980s [30–32]. Hauptstein et al. were likely the first to form HIPs of a pDNA and to incorporate the most lipophilic complex in SEDDS [22]. Composition and characterization of SEDDS containing HIPs is summarized in Table 1.

As on the one hand the diameter of oily droplets formed in the GI-tract should be in the nanometer range and on the other hand the diffusion coefficient of macromolecular drugs is below 10^{-8} cm^2/s, HIPs are released within a second from the lipophilic phase until equilibrium between the lipophilic and the aqueous phase is reached according to their distribution coefficient. The distribution coefficient between the lipophilic phase (SEDDS) and the hydrophilic phase (release medium = RM) – the designated log $D_{SEDDS/RM}$ – can therefore be regarded as the key parameter for drug release [36]. Taking the volume of the administered SEDDS preconcentrate and that of the GI-fluid into consideration, a log $D_{SEDDS/RM} > 2.5$ seems advantageous in order to provide a sustained release being controlled just by the drug absorption process and not at all by the delivery system. Assuming the oral administration of 1 ml SEDDS preconcentrate and an available intestinal fluid of 30 ml, for instance, just around 10% of a drug with Log D of 2.5 is immediately released. From a formulators' perspective, however, beneficial properties of SEDDS like a protective or mucus permeation enhancing effect can never become effective for these immediately released 10%. In case of log $D_{SEDDS/RM} > 4$ drug release is likely insufficient but can in this case be accelerated and controlled by lipase degradability of SEDDS [37].

SEDDS containing HIPs hold fundamental features including small droplet size (10 to 500 nm) and kinetic stability. The so far only marketed SEDDS containing a peptide (Sandimmune Neoral) is administered as soft gelatin capsule. However, complications regarding process control, leakage of the enclosed formulation and limited storage stability are shortcomings of this dosage form [38–40]. Moreover, unless sealed in aluminum blister pack volatile components of SEDDS tend to evaporate through capsule shells resulting in precipitation of the incorporated drug. To overcome these shortcomings solid SEDDS were

Table 1
Composition and characterization of SEDDS containing HIPs.

Hydrophilic macromolecular drug	Hydrophobic surfactant	SEDDS composition	Payload	Shift in log *P* value	Ref.
Daptomycin	Dodecylamine complex	35% Dermofeel MCT, 30% Capmul MCM and 35% Cremophor RH40	5.5%	−5.0 to +4.8	[28]
Desmopressin	Sodium docusate	5% Transcutol HP, 20% Peceol, 10% Capryol 90, 35% Labrasol ALF, 30% Tween 20	10.7%	−2.7 to +0.3	[16]
Enoxaparin	Dodecylamine	31.5% Labrafil 1944, 22.5% Capmul PG-8, 9% propylene glycol, 27% Cremophor EL and 10% DMSO	2%	–	[20]
Exenatide	Sodium docusate	35% Cremophor EL, 25% Labrafil 1944, 30% Capmul-PG 8 and 10% propylene glycol	1%	−1.1 to 2.1	[33]
Heparin	Cationic lipophilic polymer of β-cyclodextrin	Liquid paraffin, Tween 80, propylene glycol and ethanol	–	–	[21]
Insulin	Sodium docusate	30% Tetraglycol, 30% Peceol, Labrasol ALF 40%	10.7%	−1.0 to +2.0	[16]
Insulin	Dimyristoyl phosphatidyl glycerol	Lauroglycol/Cremophor EL/Labrafil (3/5/1.5)	1.1%	–	[27]
Lactamase	Phosphatidylcholine	Lauroglycol FCC, Cremophor EL, Transcutol; (5:4:3)	2400 mU/g	–	[19]
Lanreotide	Sodium deoxycholate	25% Capmul MCM, 30% Kolliphor EL, 45% Miglyol 840	6.4%	−10 to +2–6	[34]
Leuprorelin	Sodium docusate	5% Transcutol HP, 20% Peceol, 10% Capryol 90, 35% Labrasol ALF, 30% Tween 20	10.7%	−1.9 to +2.8	[16]
Leuprorelin	Sodium oleate	30% Cremophor EL, 30% Capmul MCM, 10% propylene glycol, 30% Captex 355	0.4%	–	[17]
pDNA	Hexylamine, dodecyltrimethylammonium, cetylpyridinium chloride monohydrate, stearalkonium chloride and cetrimide	30% Cremophor EL, 30% Capmul MCM, 10% propylene glycol, 30% Captex 355	0.5%	–	[22]
pDNA	Cetrimide	30% Cremophor EL, 30% Capmul MCM 30% Crodamol, 9% propylene glycol, 1% cell penetrating peptide (TAT)-oleoyl conjugate	0.5%		[35]

developed offering the benefits of solid dosage forms and at the same time retain most properties of liquid SEDDS. By addition of adsorbents or porous carriers such as cross-linked porous silicon dioxide, magnesium aluminum silicate and microporous calcium silicate the liquid SEDDS preconcentrate is transferred to solid [41, 42]. SEDDS containing low molecular weight heparin, for instance, was solidified with microporous calcium silicate [43]. In another study Sander and Holm developed liquid SEDDS loaded tablets containing cyclosporine. They could show exactly the same pharmacokinetic of cyclosporine in dogs being administered via these tablets in comparison to soft gelatin capsules containing a liquid SEDDS preconcentrate (Neoral®) [44]. As cyclosporine is a very special molecule - in fact a peptide behaving like a lipophilic small molecule for that HIP is not necessary, these data do not really proof the potential of solid SEDDS for oral peptide delivery. Drug release from these solidified systems is most of the time not complete and not stable over time due to the diffusion of the drug/formulation in deep pore pockets [45, 46]. Furthermore, the stability of HIPs might be lowered due to the adsorptive binding on inorganic surfaces. Alternatively, solid SEDDS can be prepared by using mixtures of solid or semi-solid excipients and processed by techniques like melt granulation, spray drying or hot melt extrusion [47]. Hydrophilic macromolecular drugs, however, have to exhibit a sufficiently thermal stability when being incorporated in melted lipids.

3. Barriers for oral delivery of hydrophilic macromolecular drugs

The GI tract exhibits an environment that provides protection against external invasion. The efficiency of oral SEDDS whether for local or systemic treatment is highly dependent on their capability to overcome barriers of the GI tract. The following section describes the barrier functions comprising wide ranging pH, enzymatic degradation, sulfhydryl barrier, mucus gel and intestinal epithelial cell layer.

3.1. pH barrier

Absorption of most orally administered drugs takes place in the small intestine (pH 5.5–8.0). However, hydrophilic macromolecular drugs must resist the extremely low pH (pH 1.0–2.0) of the stomach before reaching the small intestine. Unless being administered as enteric coated dosage form, SEDDS should therefore not only be resistant towards harsh acidic gastric fluids per se but should also provide protection for encapsulated acid labile drugs. Mercuri et al. investigated the stability of SEDDS towards the gastric fluid showing high stability of all tested formulations towards the acidic environment [48]. Nevertheless, the efficiency of SEDDS becomes considerably variable when solubility of the encapsulated HIP is highly pH dependent. In fact, the stability of HIPs can be pH dependent. When the complex falls apart in the aqueous medium at low pH, for instance, log $D_{SEDDS/RM}$ drops tremendously, as drug solubility in the GI-fluid increases. This can even cause the immediate entire release of the drug going hand in hand with the loss of all beneficial properties of the delivery system described in detail in the following chapter. According to this, stability of HIPs over a pH range of 1.0–8.0 needs to be investigated in order to be able to more precisely characterize their release behavior from SEDDS. Furthermore, other parameters such as the impact of multivalent ions, especially that of Ca^{2+} and Mg^{2+} competitively interacting with HIPs in a pH dependent manner needs to be addressed in this connection.

3.2. Enzymatic barrier

In the GI-tract hydrophilic macromolecular drugs are subject to degradation by various types of enzymes. Therapeutic peptides and proteins are rapidly cleaved by luminally secreted enzymes such as trypsin, chymotrypsin, elastase and carboxypeptidase A and B as well as by brush border membrane bound enzymes such as aminopeptidase N or endopeptidase 24.11 [3]. In contrast, most therapeutic polysaccharides such as heparins or chondroitinsulfate are very stable in the GI-tract, as they mainly bear stabilizing sulfate substructures that mask the recognition site for polysaccharidases. Generally, our knowledge about peptidases, proteases and polysaccharidases is broad and enzymatic cleavage sites are well-described allowing very accurate in-silico calculations about the degradation pathway of hydrophilic macromolecular drugs in the GI-tract. In contrast very little is known about the degradation of DNA-based drugs in the GI-tract. Neither all types of nucleases are known nor do we know their recognition sites. At least it was shown that DNA-based drugs are facing a rapid enzymatic degradation in the GI-tract. Loretz et al., for instance, showed an almost entire degradation of pDNA in porcine gastric and intestinal fluid as illustrated in Fig. 1 [49].

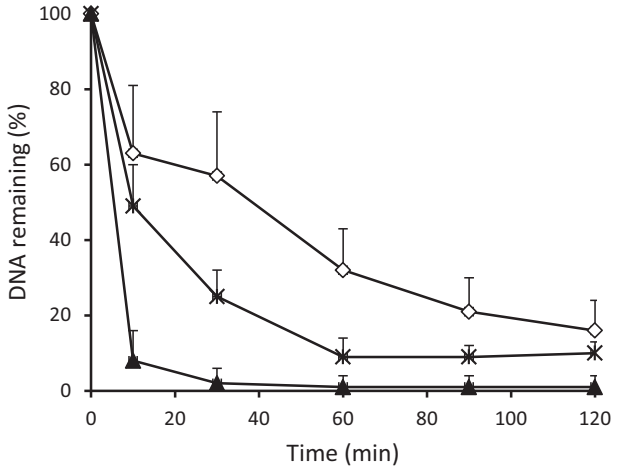

Fig. 1. Degradation of pDNA in porcine gastric juice diluted 1:2 (◇), in porcine small intestinal juice diluted 1:2 (x) and with 8.33 unit/ml DNAse I (▲). Adopted from Loretz et al. [49].

The overall activity was thereby determined to correspond to 0.02 Kunitz units of DNAse I per ml [49]. When it comes to lipases even SEDDS are vulnerable to degradation themselves and this enzymatic degradation is a major physiological hurdle that limits their full potential in oral drug delivery of hydrophilic macromolecular drugs. Gastric and pancreatic lipases are the most vibrant digestive enzymes concerning lipid based formulations [50]. Furthermore, once destabilized the incorporated drugs are released becoming a substrate for peptidases, proteases and nucleases as well. SEDDS comprising triglycerides are highly susceptible for degradation by gastric lipases. Degradation by lipases is further enhanced by the catalytic effects of pancreatic enzymes. The outcome of SEDDS degradation is often a too fast drug release and loss of the protective effect of the formulation against the hostile GI environment.

3.3. Sulfhydryl barrier

The sulfhydryl barrier is comprised of reduced thiols like glutathione, homocysteine and/or cysteine either intracellular or from dietary meal. The GI thiol content as part of food fluctuate is in the range between ≤350 nM/g for vegetables and ≤135 nM/g for fruits [51,52]. Orally administered SEDDS, comprising peptides and proteins, should therefore provide protection against extemporaneous thiol-disulfide exchange reactions with sulfhydryl moieties that can result in up to entire inactivation of these drugs [53].

3.4. Mucus barrier

Mucus covers most surfaces of the body, where epithelia bear living cells on their surfaces. The three dimensional mucus gel layer not only traps pathogens and xenobiotics but it is also a major barrier that orally administered drug delivery systems have to face [54,55]. Mucus layer is interwoven creating a mesh size of ~10–200 nm within its microstructure that blocks all particles too large to permeate it. Generally, the mean size of oily droplets formed by SEDDS falls in this size range. Though, semipermeable nature of the mucus gel allows passage of water, ions, nutrients, some proteins and particles/droplets of <50 nm to pass freely without altering it structurally [56].

3.5. Intestinal epithelial barrier

The epithelium lining of the GI tract portrays the largest and most important barrier to systemic drug delivery via SEDDS. Permeability and diffusion through this cellular lining is mainly dependent on the physicochemical properties. The selective permeability of epithelial barrier is arbitrated either by transcellular pathway or paracellular pathway [57] and is maintained by a protein network that seals the adjacent cells via adhesive complexes such as desmosomes, adherens junctions and/or tight junctions [58,59]. Most oral delivery systems for therapeutic peptides and polysaccharides that have been developed so far are aiming to open tight junctions and to channel in entire nanocarriers or at least the drug itself in the systemic circulation via this route. Over the last decades, however, this absorption route moved more and more out of focus of research as nanocarriers indeed can reach the systemic circulation via this route but just to a minor extent [60]. For hydrophilic macromolecular drugs per se the uptake is also poor even when junctions are opened reaching its limit at least from the industrial point of view already at a size of 6 kDa for peptides and in case of polysaccharides even earlier on. In contrast, SEDDS for cyclosporine being even a substrate for efflux pumps are teaching us that via the transcellular route an oral bioavailability dependent on the patient population as great as almost 90% can be reached [61]. The key for this route of uptake seems to be lipophilicity of the drug or HIP rather than its size. Because of the poor solubility of HIPs in aqueous media, however, it is very challenging to set up valid in vitro permeation experiments to analyze their uptake behavior in more detail.

4. Teaching old formulations new tricks

During past few years, massive knowledge attained for solid nanocarriers has been transferred to SEDDS. The application of novel functional excipients and selection of suitable lipids/surfactant combinations has enabled this new generation of SEDDS to overcome barriers having been described above. The composition of SEDDS is primarily dictated by lipids providing sufficient solubility of HIPs. Once, appropriate solvents for HIPs have been identified they are integrated in well-established SEDDS compositions. For all these well-established formulations, PEGylated surfactants with a HLB value between 12 and 18 such as Cremophor EL or Tween 20 seem to be essential to provide sufficient self-emulsifying properties and to guarantee a droplet size below a diameter of 500 nm. Advanced SEDDS are smart enough to provide resistance towards enzymatic degradation, to prolong residence time via mucoadhesion, to enhance mucus permeation and to boost cellular uptake. These properties of advanced SEDDS are discussed in detail in the following section.

4.1. SEDDS providing protection against enzymatic degradation

In order to provide a protective effect for drugs being incorporated in SEDDS towards a presystemic metabolism in the GI-tract, SEDDS need to be per se stable towards enzymatic degradation. GI enzymes are mostly hydrophilic in nature and unable to penetrate the hydrophobic oily droplets, unless SEDDS themselves are liable to degradation. The protective ability of SEDDS is highly demarcated by the nature and composition of the constituents employed. Leonaviciute et al. illustrated that SEDDS being stable against hydrolysis are capable of providing protective effect for oral drug delivery. They developed leuprolide oleate loaded SEDDS differing in amounts of ester linkages and evaluated their stability against pancreatic lipases [37]. SEDDS based on excipients without ester linkages showed resistance towards lipase hydrolysis. This observation opened the door for the protection of incorporated drugs towards other GI-enzymes such as peptidases and nucleases. Recently, Hetényi et al. provided evidence, that the serine proteases trypsin, chymotrypsin and elastase are too hydrophilic to penetrate into SEDDS and that these proteases do not show any enzymatic activity in SEDDS [62]. Hauptstein et al., for instance, loaded SEDDS with ion pairs formed between pDNA and various cationic surfactants. Incubating these SEDDS with artificial intestinal fluid containing DNAse showed a pronounced protective effect of the delivery system towards the enzyme [22].

4.2. SEDDS providing protection against sulfhydryl barrier

Protection against thiol-disulfide exchange reactions is also provided by SEDDS. Experimental evidence was provided by Ijaz et al. [34] investigating oral SEDDS loaded with the peptide drug lanreotide bearing a disulfide bond. The hydrophilic peptide lanreotide acetate was first complexed with the anionic surfactant sodium deoxycholate and loaded into different SEDDS. As illustrated in Fig. 2 SEDDS provided significant protection towards thiol-disulfide exchange after incubation with glutathione and enriched casein peptones at 37 °C for 3 h. Furthermore, an oral SEDDS formulation for desmopressin developed by Zupančič et al. was found protective against pre-systemic inactivation by thiol-disulfide exchange reactions in an in-vitro experiment with glutathione [29].

4.3. Mucoadhesive SEDDS

Mucoadhesive drug delivery systems are designed to achieve a prolonged residence time at mucosal surfaces. Generally, SEDDS are not capable of adhering to mucosal surfaces. However, like many nanoparticulate drug delivery systems, SEDDS can be made mucoadhesive via the incorporation of hydrophobic mucoadhesive polymers. In case of SEDDS, selection of an appropriate mucoadhesive polymer in terms of lipophilic properties and compatibility is primarily important. Apart from the traditional polymers that adhere by hydrogen bonding or weak non-specific electrostatic interactions, the choice is there for next-generation mucoadhesive polymers such as thiomers having capability of enhanced attachment via covalent bonding [63]. In this regards, Bonengel et al. developed thiolated alkyl-modified carbomers [64] and provided evidence for their emulsification and mucoadhesive properties. It was recognized that rate and extent of cross-linking in thiolated alkyl-modified polymer had a significant effect upon emulsifying properties, emulsion stability and mucoadhesive properties [65].

The combination of thiolated polymers with SEDDS was introduced by Sakloetsakun et al. for oral insulin delivery [66]. By incorporating thiolated chitosan to SEDDS they revealed an improved in-vitro release profile and significantly increased in-vivo serum insulin levels as compared to control. However, no evidence for mucoadhesive properties was specifically provided in this study. Recently, Leonaviciute et al. explicitly reported about the mucoadhesive properties of SEDDS after incorporation of a hydrophobic thiolated polymer [67]. In their study, thiolated Eudragit® S100 with greatly enhanced mucoadhesive properties was synthesized. The inclusion of 1.5% (w/w) of hydrophobic thiomer to SEDDS led to a strong improvement in the mucoadhesive properties observed on porcine intestinal mucosa. The outcome of the mucoadhesive studies are shown in Fig. 3A. Moving further in the line of mucoadhesive SEDDS, Hetényi et al. incorporated preactivated thiomer, the so called second generation of thiomers, in order to address the oxidation issues of free thiol groups [68]. SEDDS containing 0.3% (w/v) of amphiphilic hydrophobically modified preactivated Pemulen TR-2 demonstrated significant improvement in mucoadhesive properties. With the successful introduction of hydrophobic ion pairing techniques for loading of hydrophilic drugs, the same could be employed to overcome solubility issues of thiolated and preactivated polymers in SEDDS. Recently, Elbahwy et al. developed mucoadhesive SEDDS for ocular delivery of econazole utilizing preactivated thiolated Eudragit® L100–55. The newly developed SEDDS did not only demonstrate high mucoadhesive properties on the ocular mucosa but also a sustained drug release [69].

Apart from covalent attachment by thiolated polymers, mucoadhesive properties of SEDDS can also be enhanced via ionic interactions. In a recent study, Efiana et al. described the design of mucoadhesive SEDDS by incorporation of acyl chitosan [70]. The extent of mucoadhesion in terms of fluorescence labelled SEDDS remaining on the mucosal surface is illustrated in Fig. 3B. First they synthesized acylated chitosan using different fatty acid chlorides (palmitoyl chloride, lauroyl chloride, octanoyl chloride) to make it lipophilic. Afterwards, SEDDS containing acylated chitosan were evaluated for mucoadhesive properties on porcine mucosa. All SEDDS formulations containing 2% of acylated polymer exhibited >75% of model drug adhering to the mucosa. Various attributes and composition of mucoadhesive SEDDS are listed in Table 2.

In particular for therapeutic enzymes such as lactase for treatment of lactose intolerance [74] or antibodies such as anti-TNF-α for treatment of intestinal inflammations [75] a prolonged residence time of SEDDS in the mucus gel layer seems advantageous.

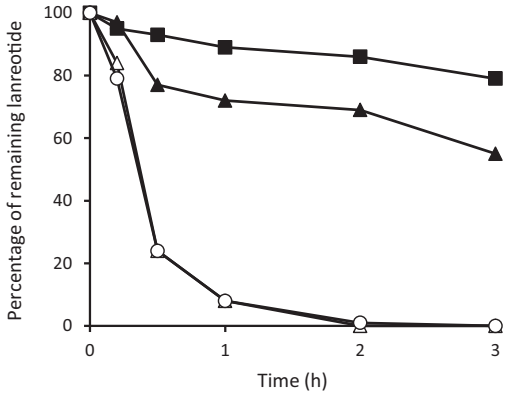

Fig. 2. Degradation profile of lanreotide acetate (○) and Lan/Deo-SN* (■) in GSH in 50 mM acetate buffer pH 6; and of lanreotide acetate (△) and Lan/Deo-SN (▲) in thiol enriched casein peptones in 50 mM phosphate buffer pH 6.8 at 37 °C. Adopted from Ijaz et al. [34] * Lan/Deo-SN = Lanreotide-deoxycholate loaded SEDDS.

4.4. Mucus permeating SEDDS

In case of systemic delivery the ability of drug delivery system to permeate the mucosal barrier and to reach the epithelial membrane is essential [76]. In comparison to other nanocarrier systems such as liposomes and solid nanoparticles SEDDS tend to permeate mucus to higher extent [77]. Recently, their mucus permeating properties were further improved by the addition of functional excipients. Friedl et al. developed a simple mucus diffusion test system by coating the filter of Transwells with a thin layer of freshly isolated porcine mucus and quantifying the amount of SEDDS permeating this layer as a function of time. They demonstrated that droplet size and composition of SEDDS significantly influence the mucus permeation behavior [78]. It was shown that a formulation with droplet size of 12 nm exhibits 8.4-fold higher diffusion compared to another formulation with a mean droplet size of 456 nm. This size dependency was recently confirmed by Griesser et al. for SEDDS displaying a mean droplet size of 25, 50, 124, 226 and 503 nm [77]. Apart from droplet size factors influencing the permeation of SEDDS include lipophilicity, surface charge, mucolytic properties and the chemical substructures of the carrier system. Moreover, composition of SEDDS is also very important factor as change in concentration of one excipient can have huge impact on the diffusion behavior of the nanoemulsion through intestinal mucus [78].

4.4.1. Mucus permeating PEG-coated SEDDS

With a very few exceptions [79], SEDDS are containing PEG-ylated surfactants being essential for their self-emulsifying properties. Because of their hydrophilic character the PEG-substructures are assembling on the surface of the oily droplets providing a muco-inert coating and consequently high mucus permeating properties [80]. In contrast to solid nanocarriers exhibiting also strongly improved mucus permeating properties due to PEG-substructures on their surface [81], the preparation of PEG-coated SEDDS is much easier and can be reduced to the

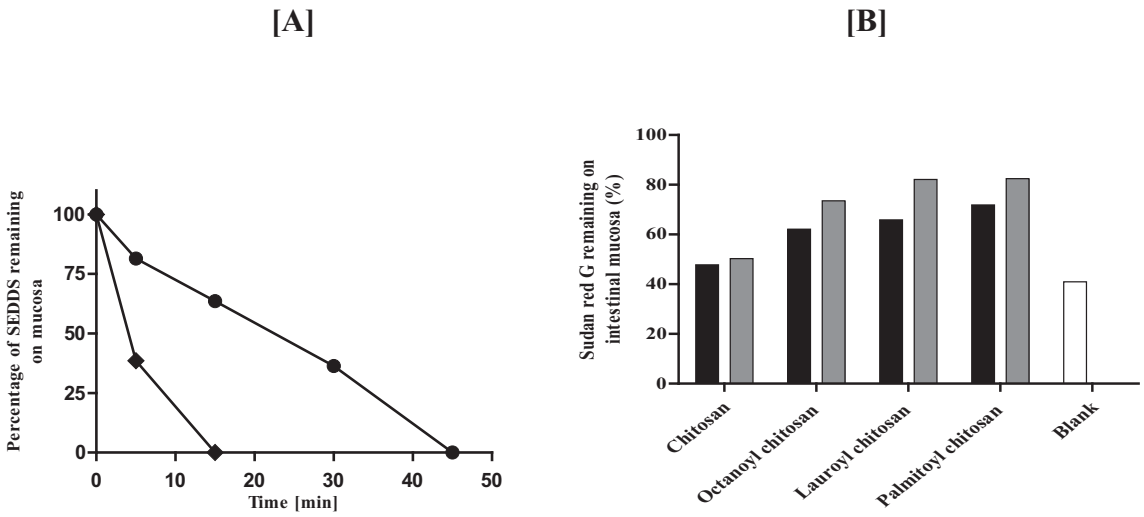

Fig. 3. Mucoadhesive properties of SEDDS on porcine intestinal mucosa; [A] SEDDS comprising thiomers; with (●) and without (♦) thiolated Eudragit S100, adopted from Leonaviciute et al. [67] and [B] SEDDS comprising mucoadhesive polymers; 1% (black bars) and 2% (grey bars) of chitosan, octanoyl chitosan, lauroyl chitosan and palmitoyl chitosan. SEDDS without mucoadhesive polymer (white bar) served as control. Adopted from Efiana et al. [70].

preparation of a simple solution as the PEG-substructures of surfactants assemble themselves on the surface of the oily droplets in the GI-fluid.

4.4.2. Mucus permeating mucolytic SEDDS

Further in this direction, Rohrer et al. introduced SEDDS with mucolytic properties as mucus permeating drug delivery systems [71]. For this purpose, thiobutylamidinedodecylamine and thioglycolicacid-octylamine were synthesized and introduced into SEDDS. The study was based on the fact that inclusion of thiol bearing moieties to the nano-emulsion breaks disulfide bonds of the mucus network. The thiolated SEDDS exhibited a mucus diffusion coefficient of up to $0.871 \pm 0.122 \times 10^{-9}$ cm^2/s that was 66-fold higher than that of the same SEDDS without the thiolated excipients and even higher than that of a virus used for comparison reasons. Furthermore, Leichner et al. developed papain functionalized mucus permeating SEDDS. Incorporation of a mucolytic enzyme into SEDDS was feasible via hydrophobic ion pairing with sodium deoxycholate. Papain functionalized SEDDS exhibited a decrease in viscosity of porcine intestinal mucus, an enhanced permeation through mucus gel layer in Transwell diffusion system as described above and a prolonged mucosal residence time as compared to control [72]. As papain can also cleave therapeutic peptides and proteins, however, the system seems to be primarily useful for oral administration of polysaccharides and DNA-based drugs. Recently, Efiana et al. have effectively incorporated these proteolytic enzymes into SEDDS via lipidization with palmitoyl chloride. Apparently, with this lipidization method a payload of enzymes in SEDDS of up to 4.5% was achieved [73]. An overview about mucoadhesive and mucus permeating SEDDS is provided in Table 2.

4.4.3. Mucus permeating zeta potential changing SEDDS

In the line of development of drug delivery systems that can permeate mucus, a critical consideration is the surface charge of the droplets [82]. Mucus itself has a net negative charge because of anionic substructures particularly sialic and sulfonic acid. Droplets possessing a positive charge are restrained in the mucus because of ionic interactions. Their mucus permeation behavior is therefore comparatively poor. In contrast, neutral and negatively charged droplets can move across the mucus layer [83,84]. Having reached the intestinal epithelium the fundamental issue with negatively charged droplets is their poor interaction with epithelial cells representing the initial step for fusion with the cell membrane or endocytosis. As the cell surface has a negative charge due to heparan sulfate substructures, a positive charge of the droplets would now be advantageous [85]. Accordingly, zeta potential changing droplets showing reduced ionic interactions within the mucus changing their charge to positive once having reached the

Table 2
Characterization of mucoadhesive and mucus permeating SEDDS.

SEDDS category	SEDDS composition	Key component	Droplet size (nm)	Zeta potential (mV)	Ref.
Mucoadhesive	20% Miglyol 840, 30% Tween 20, 30% Chremophor EL, 10% Polyethylene glycol, 10% DMSO	Thiolated Eudragit® S100	114	−10.2	[67]
Mucoadhesive	40% tetraglycol, 25% Captex 355, 25% Tween 20 and 10% Croduret 50	Pemulen-Cysteine methyl ester Pemulen-Cysteine methyl ester-2-MNA	178 to 196	−14 to −18	[68]
Mucoadhesive	40% DMSO:*N*-methylpyrrolidone:propylene glycol (1:1:1) 20% Labrafil M 1944 CS 40% Chremophor EL	Preactivated thiolated Eudragit® L100-55-Benzalkonium chloride	62	3.7	[69]
Mucoadhesive	Capryol 90, Imwitor 742, Captex 300, Labrafil M 1944 CS, Caprol 3GO, Tween 20, Kollipor EL, PEG 400 and propylenglycol	Palmitoyl chitosan Lauroyl chitosan Octanoyl chitosan	80 to 300	0.1 to 0.8	[70]
Mucus permeating	Cremophor EL 30%, Capmul MCM 30%, Captex 355 30% and propylene glycol 10%	3% Thio butyl amidine dodecylamine (TBA-D) 3% Thio glycolic acid-octylamine (TGA-O)	44 53.39	−2.1 −2.2	[71]
Mucus permeating	20% Glycerol 85/Ethanol, 40% Cremophor EL, 40% Miglyol 840	Papain-deoxycholate	130.6	–	[72]
Mucus permeating	Captex 355, Kollipor EL and propylenglycol (2:3:5)	Bromelain-palmitate Papain-palmitate Trypsin-palmitate	~180 to 312	−3 to −5	[73]

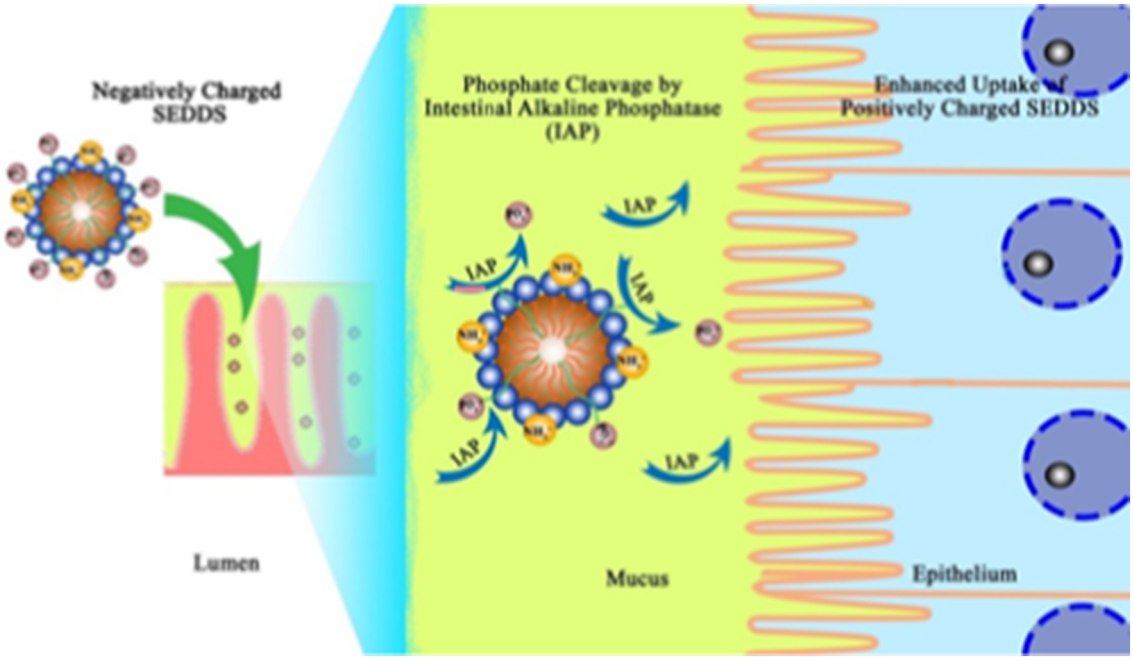

Fig. 4. Diagrammatic description of the steps involved in mucus permeation of zeta potential changing SEDDS.

absorption membrane are likely key to success [86,87]. The concept of how zeta potential changing systems work is depicted in Fig. 4.

Suchaoin et al. optimized SEDDS of negative zeta potential shifting to a positive charge required for cellular uptake once having reached the epithelium [88]. A cationic surfactant and 1,2-dipalmitoyl-sn-glycero-3-phosphatidic acid sodium (PA), a phosphate ester, were thereby combined in SEDDS. Enzymatic cleavage of phosphate substructure of PA by intestinal alkaline phosphatase led to an alteration of the surface charge. Mucus diffusion experiments were performed via the rotating silicon tube method. Freshly excised porcine mucus is filled in a silicone tube and the SEDDS formulation is applied on one end of this tube. In the following the tube is rotated at 37 °C for several days to allow the oily droplets to diffuse in the mucus gel layer. In the following the tube containing the mucus is frozen, cut into slices and the amount of diffused SEDDS quantified in each slice. Utilizing this technique the interaction of SEDDS formulation of different surface charge with mucus was explored. Furthermore, in-vitro phosphate release was investigated via three different models i.e. isolated intestinal alkaline phosphatase, Caco-2 cell monolayer and ex-vivo rat intestine. Recently, Griesser et al. have developed zeta potential changing SEDDS based on phosphorylated polysaccharides. Maize starch and hydroxypropyl starch after being phosphorylated with phosphorus pentoxide were incorporated into SEDDS. It was shown that SEDDS containing hydroxypropyl starch phosphate demonstrated higher mucus diffusion properties, accentuated by a substantial change in zeta potential [89]. The composition and in-vitro characterization of zeta potential changing SEDDS are shown in Table 3.

4.5. Cell penetrating SEDDS

As compared to conventional therapy, DNA-based drugs are highly specific and efficient towards the target and consequently exhibit less adverse effects [90]. Proficient delivery of these drugs at their target site, however, is a great challenge [91]. The likely two most important concerns regarding successful gene delivery are stability towards enzymatic degradation and sufficient cellular uptake.

Hauptstein et al. were likely the first to discover the potential of SEDDS for effective oral gene delivery. Hydrophobic ion pairing of pDNA encoding for green fluorescent protein was accomplished using Lipofectin and numerous other lipids such as hexylamine, dodecyltrimethylammonium, cetylpyridinium chloride monohydrate, stearalkonium chloride and cetrimide [22]. HIP was formed between the cationic amino group of lipids and anionic backbone of pDNA. The pDNA- hydrophobic lipid complex was dissolved in the SEDDS preconcentrate. Utilizing these SEDDS a comparatively high transfection rate was achieved. In a follow up study the efficacy of SEDDS as gene delivery systems was further improved by the addition of the cell penetrating peptide HIV-1 Tat-protein 49-57 as illustrated in Fig. 5 [35]. Before incorporating into SEDDS, the peptide was attached to oleic acid providing a lipophilic anchor in the oily droplets. The cell penetrating peptide decorated SEDDS exhibited 1.7-fold higher transfection efficiency for pDNA as compared to Lipofectin. The results of transfection studies are demonstrated in Fig. 6. The cellular uptake was confirmed through confocal microscopy and later quantified via a fluorescent marker. In-vitro analysis described clathrin mediated and caveolae

Table 3
Composition and characterization of zeta potential changing SEDDS; data are means ($n = 3$).

SEDDS composition	Phosphorylated complex	Droplet size (nm)	Zeta potential with PA (mV)	Zeta potential after treatment with IAP[a] (mV)	Ref
30% Cremophor EL, 30% Capmul MCM, 30% Captex 355 and 10% propylene glycol and benzalkonium chloride	1,2-Dipalmitoyl-sn-glycero-3-phosphatidic acid sodium (PA)	57.0	−1.14 ± 0.77	+0.84 ± 0.27	[88]
30% Cremophor EL, 30% Capmul MCM, 30% Captex 355 and 10% glycerol	Hydroxypropyl starch phosphate	38.7	−6.3 ± 0.1	+1.0 ± 0.1	[89]

[a] IAP = Intestinal alkaline phosphatase.

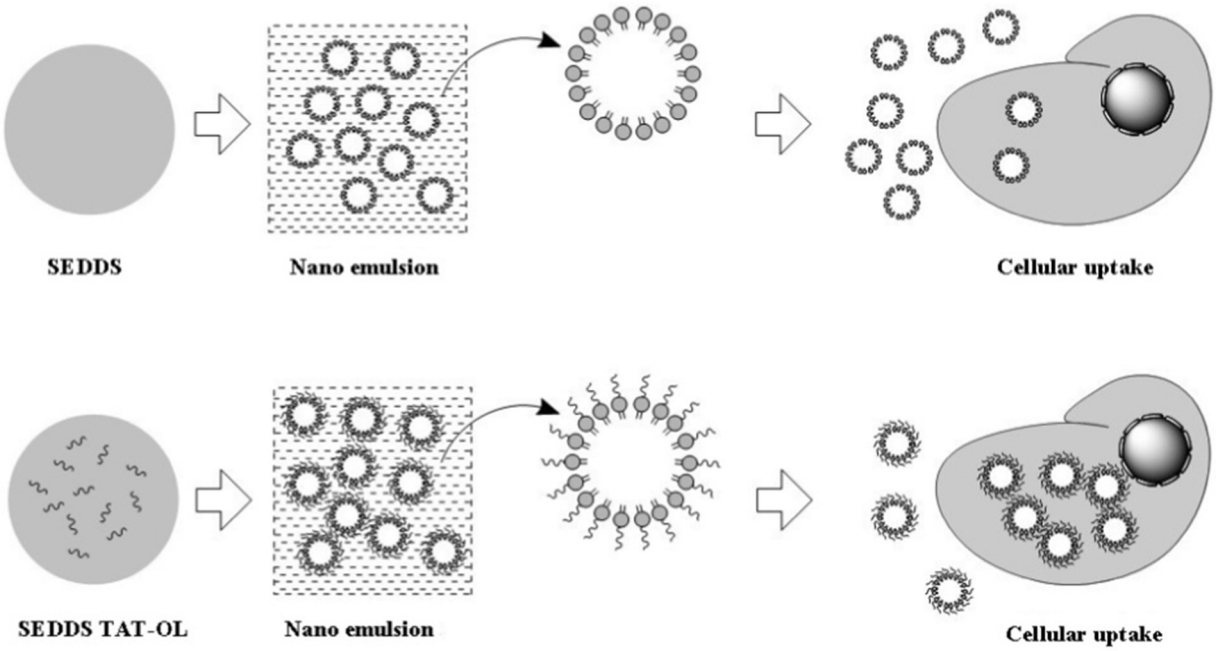

Fig. 5. Schematic diagram illustrating the enhanced cellular uptake of SEDDS containing a DNA-based drug by a cell penetrating peptide (HIV-1 Tat protein 49-57; TAT) -oleoyl (OL) conjugate (TAT-OL).

mediated endocytosis as the major pathways for cellular internalization. Moreover, the inclusion of peptide not only enhanced the cellular uptake but mucus diffusion was also improved.

5. In-vivo studies

Generally, the in vitro – in vivo correlation of SEDDS is comparatively poor. Main reasons for this are on the one hand the high complexity of these systems resulting out of the huge impact of the GI environment on them such as interactions with bile salts and fatty acids with lipases or with the mucus gel layer and absorption membrane. Furthermore, they can be taken up via various routes such as transcellular, paracellular or via the lymphatic system. Although, on the one hand, an impressive progress was made in recent years improving the validity of in vitro methodology such as the establishment of an accurate method to evaluate the impact of lipases [92] and an improved understanding about drug release from SEDDS [36], on the other hand, various valid in vitro methods allowing a more precise prediction of their in vivo performance are still missing. Good examples are in vitro permeation studies on cell monolayers or freshly excised tissues. As drugs that can

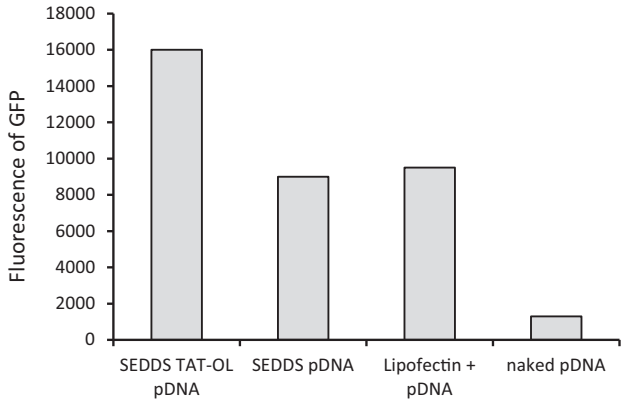

Fig. 6. Transfection efficiencies of $_{p}$DNA loaded SEDDS with and without cell penetrating peptides upon HEK-293 cells. Naked pDNA and complexation with Lipofection® served as controls. Adopted from Mahmood et al. [35] * GFP = Green fluorescence protein.

be incorporated in SEDDS are lipophilic in nature, they exhibit insufficient solubility in aqueous permeation media. The addition of organic solvents and/or surfactants to these media improves drug solubility but has in turn an unpredictable impact on membrane permeability.

With regard to SEDDS for oral administration of hydrophilic macromolecular drugs only a few in-vivo data are available. Hintzen et al. described SEDDS containing lipophilic complex of the peptide drug leuprorelin with oleate [17]. They documented results from an in-vivo study using rats that displayed a 17.2-fold higher oral bioavailability of the peptide drug in complex form loaded into SEDDS formulation as compared to control (leuprorelin solution). Furthermore, Bonengel et al. could demonstrate the high impact of the type of HIPs on oral bioavailability of octreotide in pigs. In maximum an oral bioavailability of even 5% was achieved by ion pairing the peptide drug with deoxycholate, whereas systemic uptake of HIPs with docusate and decanoate was comparatively lower [18]. Results of this study are illustrated in Fig. 7.

Apart from small peptides exhibiting a molecular mass of approximately 1 kDa, in vivo studies with the GLP-1 analogue exenatide with a molecular mass of 4.2 kDa were performed. In this study a relative oral bioavailability vs. sc. of even 14% was achieved in rats showing also a pronounced decrease in blood glucose level [33], whereas the intraduodenal bioavailability of unformulated exenatide is negligible [93,94]. The plasma-concentration curve of exenatide is demonstrated in Fig. 8. Furthermore, Sakloetsakun et al. could improve the oral bioavailability of insulin exhibiting a molecular mass of 5.8 kDa 3-fold by incorporating the peptide in mucoadhesive SEDDS [66]. This improvement was even topped by Zhang et al. achieving a 6-fold higher oral bioavailability for insulin with their SEDDS formulation [95]. Although an oral SEDDS formulation containing insulin was already in phase I and II clinical trials, unfortunately no details about these Chinese studies are available [96]. The fact that SEDDS for oral insulin delivery were already subject of clinical trials and taking also the marketed oral cyclosporine formulation into account, however, underline the potential of the technology in particular for peptide drug delivery.

The potential of SEDDS for oral hydrophilic macromolecular drug delivery was apart from therapeutic peptides also shown for therapeutic polysaccharides. Ito et al. were able to reach with a solid SEDDS containing low molecular weight heparin an oral bioavailability of 18.8% in rats

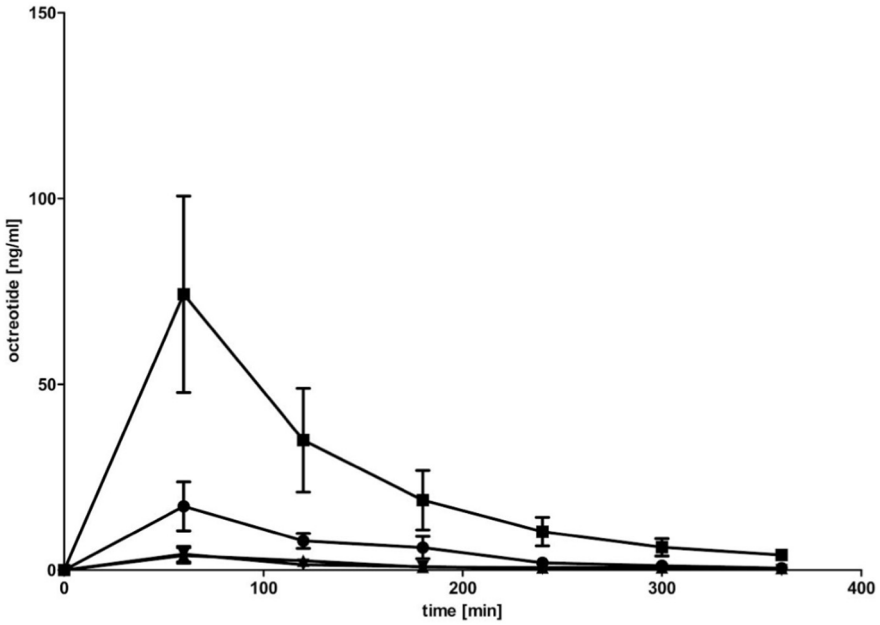

Fig. 7. Plasma concentration curves of octreotide after administration of an octreotide acetate solution (▲), octreotide-decanoate SEDDS (♦), octreotide-docusate SEDDS (●) and octreotide-deoxycholate SEDDS (■) to pigs (dose = 50 mg). Indicated values are means from 4 applications ± SD. Adopted from Bonengel et al. [18].

and comparatively high anti-Xa activity in dogs [43]. Recently, Zupančič et al. developed SEDDS formulation for oral delivery of another low molecular weight heparin via ion pairing of enoxaparin with dodecylamine hydrochloride. They demonstrated through an in-vivo analysis on male Sprague-Dawley rats that the model polysaccharide drug was successfully delivered to systemic circulation utilizing this delivery system [20].

6. Future perspectives

Generally, the technology seems to be already sufficiently advanced to overcome the enzymatic barrier, sulfhydryl barrier and mucus barrier in a very efficient manner providing convincing advantages over likely all other delivery technologies in this regard. In contrast, the full potential of SEDDS to overcome the intestinal epithelial barrier has by far not been reached. A prerequisite for such developments is certainly an improved knowledge and understanding about the fate of HIPs and SEDDS on the intestinal epithelium. First orientating confocal/STED-laser microscopic analyses of the cell uptake behavior of peptides, surfactants, HIPs and SEDDS being labelled with differently colored fluorescence dyes showed a great variety of possible interactions with epithelial cells including the accumulation of droplets on the cell membrane, the fusion of SEDDS with the cell membrane and even the uptake of intact HIPs and droplets into cells. Most parameters being responsible for the fate of HIPs and SEDDS on the intestinal epithelium are still unknown, although key to more potent SEDDS for oral macromolecular drug delivery. One of them, for instance, seems to be the stability of HIPs in the intestinal fluid and on the cell membrane. Other substantial parameters are likely the zeta potential and the type of surfactants used

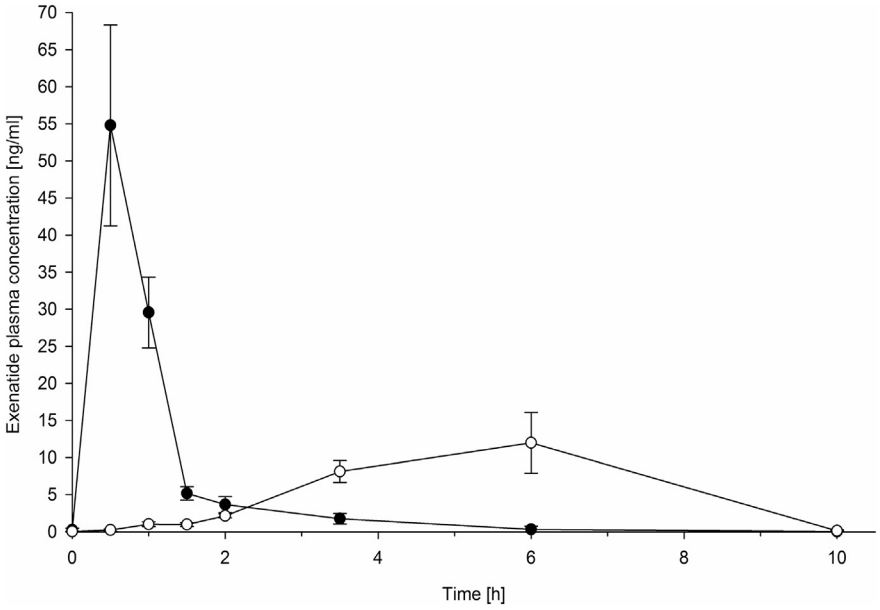

Fig. 8. Exenatide plasma concentration-time curve for orally administered exenatide/DOC SEDDS (○) and s.c. exenatide solution (●). Illustrated values are the means of at least three experiments ± standard deviation, Adapted from Menzel et al. [33].

in SEDDS exhibiting also permeation enhancing properties. Labrasol, for instance, was shown to exhibit comparatively high permeation enhancing properties for hydrophilic macromolecular drugs and can be easily incorporated in SEDDS [97]. An improved understanding of these parameters and the overall cell uptake mechanisms of HIPs and SEDDS will perhaps even pave the way for the oral administration of comparatively much bigger hydrophilic macromolecular drugs such as therapeutic proteins and antibodies.

7. Conclusion

These are still very early days in the development of advanced SEDDS for oral delivery of macromolecular drugs. Progress made so far, however, promises a great potential of this technology. In particular the protective effect of SEDDS towards GI-enzymes (I), the comparatively high mucus permeating properties (II) and their capability of serving as vehicle for improved membrane permeability (III) make them to a powerful tool. In comparison to solid nanocarriers the manufacturing process is simple and apart from newer excipients such as thiolated compounds, chitosan esters or cell penetrating peptides just excipients that are generally regarded as safe (GRAS) are needed. Although a proof-of-concept has already been provided via various in vivo studies in rodent and non-rodent animal models, the full potential of the technology has by far not been reached. Challenges in front are overall an improved understanding about processes taking place directly at the absorption membrane and the optimization of SEDDS based on this knowledge. Addressing these points, SEDDS can be regarded as game changing approach in oral administration of hydrophilic macromolecular drugs.

Conflict of interest

The authors have no conflict of interest.

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