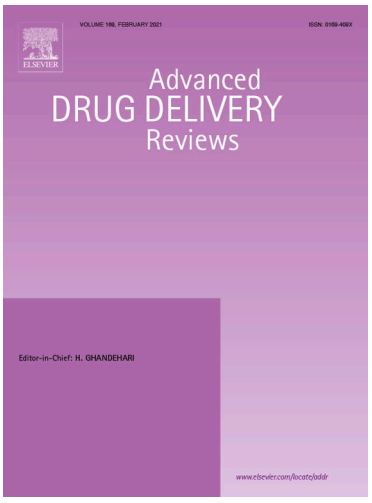
Journal Pre-proofs



Oral delivery of therapeutic peptides and proteins: Technology landscape of lipid-based nanocarriers

Soheil Haddadzadegan, Farid Dorkoosh, Andreas Bernkop-Schnurch

PII: S0169-409X(21)00490-7
DOI: https://doi.org/10.1016/j.addr.2021.114097
Reference: ADR 114097

To appear in: *Advanced Drug Delivery Reviews*

Received Date: 11 October 2021
Revised Date: 4 December 2021
Accepted Date: 21 December 2021

Please cite this article as: S. Haddadzadegan, F. Dorkoosh, A. Bernkop-Schnurch, Oral delivery of therapeutic peptides and proteins: Technology landscape of lipid-based nanocarriers, *Advanced Drug Delivery Reviews* (2021), doi: https://doi.org/10.1016/j.addr.2021.114097

Oral delivery of therapeutic peptides and proteins: Technology landscape of lipid-based nanocarriers

Soheil Haddadzadegan[1], Farid Dorkoosh[2,3] and Andreas Bernkop-Schnürch[1]

[1] Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria

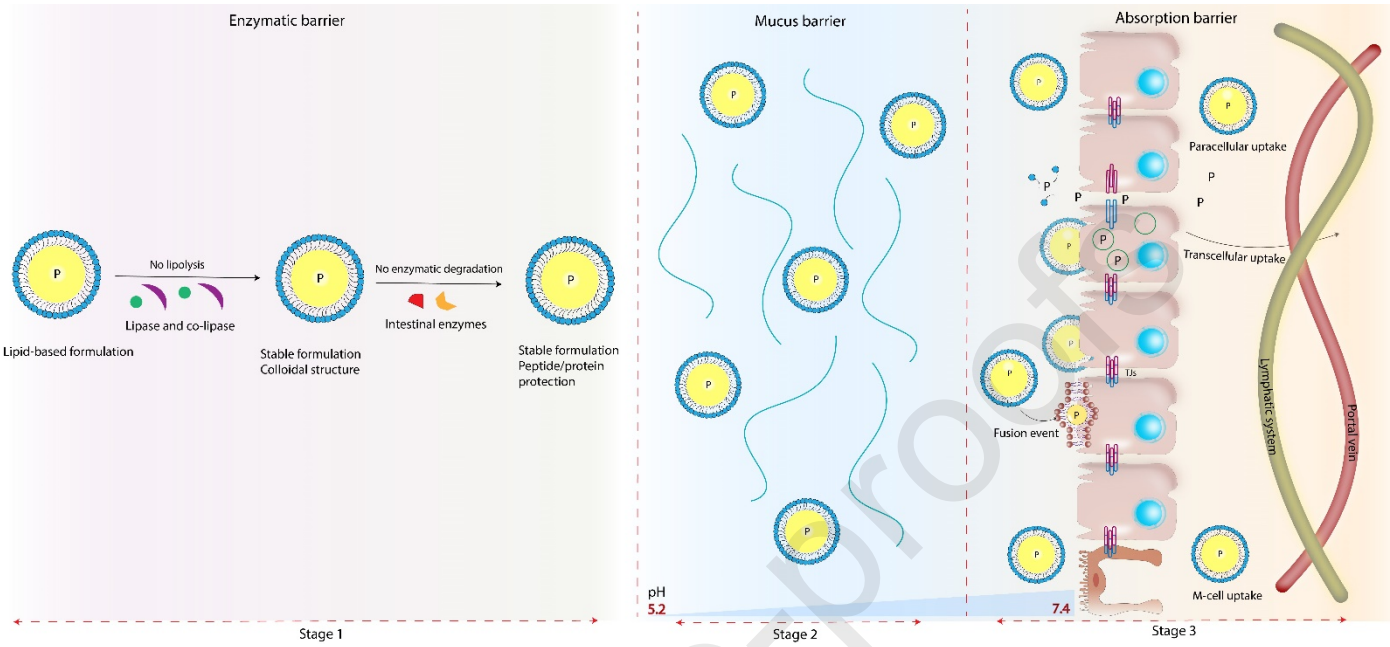
[2] Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

[3] Medical Biomaterial Research Center (MBRC), Tehran University of Medical Sciences, Tehran, Iran

Corresponding author: Andreas Bernkop-Schnürch, Center for Chemistry and Biomedicine, Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria. E-mail address: andreas.bernkop@uibk.ac.at

Contents

Graphical abstract



Abstract

The oral administration of therapeutic peptides and proteins is favoured from a patient and commercial point of view. In order to reach the systemic circulation after oral administration, these drugs have to overcome numerous barriers including the enzymatic, sulfhydryl, mucus and epithelial barrier. The development of oral formulations for therapeutic peptides and proteins is therefore challenging. Among the most promising formulation approaches are lipid-based nanocarriers such as oil-in-water nanoemulsions, self-emulsifying drug delivery systems (SEDDS), solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), liposomes and micelles. As the lipophilic character of therapeutic peptides and proteins can be tremendously increased such as by the formation of hydrophobic ion pairs (HIP) with hydrophobic counter ions, they can be incorporated in the lipophilic phase of these carriers. Since gastrointestinal (GI) peptidases as well as sulfhydryl compounds such as glutathione and dietary proteins are too hydrophilic to enter the lipophilic phase of these carriers, the incorporated therapeutic peptide or protein is protected towards enzymatic degradation as well as unintended thiol/disulfide exchange reactions. Stability of lipid-based nanocarriers towards lipases can be provided by the use to excipients that are not or just poorly degraded by these enzymes. Nanocarriers with a size < 200 nm and a mucoinert surface such as PEG or zwitterionic surfaces exhibit high mucus permeating properties. Having reached the underlying absorption membrane, lipid-based nanocarriers enable paracellular and lymphatic drug uptake, induce endocytosis and transcytosis or simply fuse with the cell membrane releasing their payload into the systemic circulation. Numerous *in vivo* studies provide evidence for the potential of these delivery systems. Within this review we provide an overview about the different barriers for oral peptide and protein delivery, highlight the progress made on lipid-based nanocarriers in order to overcome them and discuss strengths and weaknesses of these delivery systems in comparison to other technologies.

Key words: oral drug delivery; therapeutic peptides; lipid-based nanocarriers; self-emulsifying drug delivery systems; SEDDS; SLN; NLC; liposomes; micelles;

1. Introduction

Since the discovery of insulin in 1921 [1] and its approval for clinical use in 1982 [2], therapeutic peptides and proteins have experienced an enduring success story. As a building block of life, they offer numerous advantages such as high selectivity and highly specific interactions with their target based on a well-defined mechanism of action [3]. Peptides and proteins have a high safety profile and cause minor side effects in comparison to conventional small molecules [4]. Moreover, synthetic engineering and recombinant strategies have strongly advanced from those used to generate the first approved molecules such as insulin. However, therapeutic peptides and proteins have several significant limitations as therapeutics. Peptides and small proteins are rapidly cleared from systemic circulation by renal clearance, thus their efficacy is limited by a short circulating half-life. Furthermore, they are rapidly degraded by peptidases and poorly absorbed from mucosal membranes such as the small intestinal mucosa [5]. Consequently, peptides and proteins are mostly administrated via the parenteral route, which is inconvenient and sometimes even painful and risky. Formulation scientist favor therefore the development of non-invasive delivery systems. Among the various non-invasive routes of administration, oral drug delivery is the by far preferred one owing to the ease of administration and high patient compliance. As a result of the harsh GI environment, however, orally administered peptide and protein drugs face numerous challenges such as inactivation by harsh pH values, enzymatic degradation by GI peptidases, thiol/disulfide exchange reactions with endogenous thiols and poor membrane permeability [6]. Nonetheless, the number of oral peptide and protein formulations that are in clinical trials or already launched is continuously increasing. In Tab. 1 an overview about these oral peptide and protein delivery systems is provided. Among them, lipid-based nanocarriers are currently not the most favored approach. However, this situation will likely change in future as substantial progress was made on these formulations for oral peptide and protein delivery within recent years. Since, numerous surfactants and lipids that are listed in the pharmacopeia can be utilized for lipid-based nanocarriers and many of them can be found in the inactive ingredients list of approved oral drug products provided by the FDA [7], a great

flexibility for their design is provided resulting in various types of formulations. In particular oil-in-water nanoemulsions, self-emulsifying drug delivery systems (SEDDS), solid lipid nanoparticles (SLN), nanostructure lipid carriers (NLC), liposomes and micelles as illustrated in Fig. 1 are of relevance for oral peptide and protein delivery. Due to the formation of hydrophobic ion pairs (HIP) the lipophilic character of peptides and proteins can be tremendously raised. Consequently, they can be dissolved in the lipophilic phase of these delivery systems [8, 9], where they are protected towards GI peptidases and thiol/disulfide exchange reactions with endogenous thiols. In particular muco-inert lipid-based nanocarriers permeate the mucus gel layer reaching the absorption membrane in high quantities where their payload can enter the systemic circulation. As lipid-based nanocarriers can interact with cells in numerous ways including endocytosis, transcytosis and even fusion with the cellular membrane, these delivery systems have the potential to overcome even the epithelial barrier for therapeutic peptides and proteins very efficiently. Moreover, lipid-based formulations can be easily combined with permeation enhancers such as bile salts [10, 11] and fatty acids [12] which are currently the most favored approach. This review provides an overview about the different barriers for oral peptide and protein delivery and highlights the progress made on lipid-based nanocarriers as well as their key advances in order to overcome these barriers. Furthermore, we compare the strengths and weaknesses of lipid-based nanocarriers with other technologies for oral peptide and protein delivery.

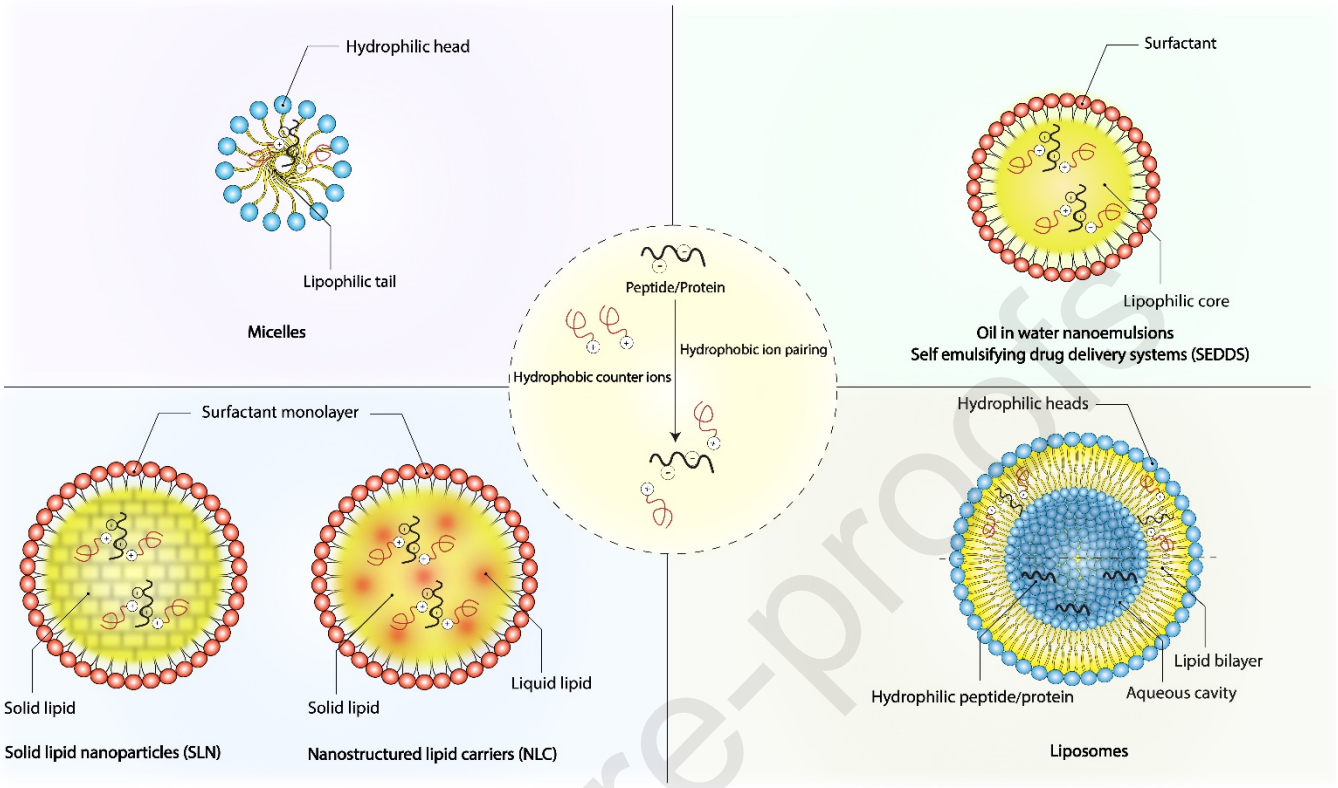


Figure. 1. Lipid-based nanocarriers containing hydrophobic ion pairs of therapeutic peptides and proteins and hydrophobic counter ions

Tab. 1. Oral peptide and protein delivery systems that are either in clinical trials or commercialized

Approach	Peptide/Protein	Dosage form	Comments	Company	Stage	Ref
Permeation enhancer	Insulin	Enteric-coated capsule	Formulation contains a mixture of solubilizer and permeation enhancer (aromatic alcohols)	Diabetology	Phase II	[13, 14]
	Insulin	Capsule	Bile salts were used as permeation enhancer and aprotinin as protease inhibitor	Oramed Pharmaceutics	Phase II	[15-17]
	Leuprolide	Tablet	Lyophilized leuprolide with maltodextrin, citric acid and acyl carnitine	Enteris BioPharma	Phase II	[18, 19]
	Parathyroid hormone (PTH)	Tablet	5-CNAC (8-(N-2-hydroxy-5-chloro-benzoyl)-amino-caprylic acid is used as permeation enhancer	Novartis	Phase II	[20]
	Salmon calcitonin	Tablet	5-CNAC is used as permeation enhancer. The combination of 5-CNAC forms with salmon calcitonin insoluble carriers at low pH, avoiding enzymatic degradation in the GI-tract.	Nordic Biosciences	Phase III	[21, 22]
	Salmon calcitonin	Enteric-coated capsule	*	Proxima Concepts Ltd	Phase III	[23, 24]
	Salmon calcitonin	Tablet	*	Tarsa Therapeutics	Phase III	[24-26]
	Semaglutide	Tablet	SNAC (sodium N- [8-(2-hydroxybenzoyl) amino caprylate) is used as permeation enhancer)	Novo Nordisk	approved	[27, 28]
Lipid-based formulation	Cyclosporine	Capsule Solution	Self-emulsifying drug delivery system forming in contact with GI fluid a homogeneous microemulsion	Novartis	approved	[29, 30]
	Exetanide	Capsule	Formulation consists of omega-3 fatty acid as a carrier and soy bean trypsin inhibitor as a protease inhibitor and sodium EDTA as a permeation enhancer	Oramed Pharmaceutics	Phase I	[31]
	Insulin	Gel capsules	Liposomes with a size <150 nm diameter loaded with insulin	Diasome	Phase II	[14, 32]
	Insulin	Enteric-coated tablet	Micelles based on medium-chain fatty acid glycerides	Novo Nordisk	Phase II	[14, 19]
	Insulin	Solution (Buccal spray)	Micelles >7 nm in size delivering insulin to the oral mucosa.	Generex	Phase III	[33]
	Octreotide	Capsule	Oily suspension based on transient permeation enhancer	Chiasma	approved	[34, 35]
Chemical modification	Dolcanatide	Tablet	*	Synergy Pharmaceuticals	Phase II	[36, 37]
	Desmopressin	Tablet	Certain L-amino acids are substituted by D-amino acids	Ferring Pharmaceuticals	approved	[19, 38]
	Insulin	Tablet	PEGylated alkylated insulin prodrug	Biocon Ltd	Phase I	[17, 39]
	Linaclotide	Capsule	Inhibitor cystine-knots (ICKs), also called knottins containing macrocycles formed by disulfide bonds between cysteines I and IV and II and V	Ironwood Pharmaceuticals	approved	[40]
Nanoparticles with hydrophobic surface	Insulin	Capsule	Silica nanoparticles with hydrophobic surface and branched polysaccharide	Oshadi Drug Administration	Phase II	[41]
pH sensitive formulation	Plecanatide	Tablet	Microcrystalline cellulose based formulations	Synergy Pharmaceuticals	approved	[42]

* Further details are not provided

2. Barriers for oral peptide and protein delivery

2.1. Enzymatic barrier

Most peptide and protein drugs are rapidly degraded by peptidases in GI tract. In stomach, pepsins are responsible for enzymatic cleavage of peptides and proteins under acidic conditions. Although peptides are absorbed from gastric mucosa, having first been demonstrated for salmon calcitonin [43] and in the following for semaglutide [44], the systemic uptake form this GI segment is comparatively lower than that from intestine. Only if peptide and protein drugs are stable at low pH and do not contain pepsin cleavage sites, their gastric absorption is relevant. In case of all other peptide and protein drugs an enzymatic degradation by pepsins can be avoided by a simple enteric coating. The endopeptidases trypsin, chymotrypsin and elastase and the exopeptidases carboxypeptidases A and B being secreted from the pancreas, however, cause a rapid degradation of most peptide and protein drugs. Under physiological conditions, for instance, insulin was shown to be almost entirely degraded by trypsin, α-chymotrypsin and elastase within an hour [45]. Moreover, in particular peptide drugs are degraded by membrane-bound enzymes on the brush border membrane including endopeptidases such as endopeptidase 24.11, exopeptidases such as aminopeptidase N and dipeptidases. Even cytosolic enzymes of enterocytes such as lysozymes are involved in this enzymatic degradation process. A more detailed overview about the enzymatic barrier for orally administered peptide and protein drugs is provided by various reviews [46, 47].

2.2. Sulfhydryl barrier

Therapeutic peptides and proteins bearing thiol and/or disulfide substructures are prone to thiol/disulfide exchange reactions in the GI-tract resulting in the formation of inactive conjugates. Apart from endogenous thiols such as glutathione and mucus glycoproteins exhibiting cysteine-rich subdomains, dietary proteins are involved in this process as well. The thiol content in vegetables, for instance, can vary from 3 to 349 nM per gram wet weight [48]. Desmopressin was shown to form three different disulfide conjugates with glutathione under physiological conditions [49]. In another

study entire degradation of lanreotide was shown in the presence of casein peptones serving as model for dietary proteins or glutathione within two hours [50].

2.3. Mucus barrier

In order to reach the absorption membrane peptide drugs have to overcome an up to 100 µm thick mucus gel layer covering GI epithelia. This mucus gel layer consists of mucus glycoproteins forming a three-dimensional network that hinders macromolecules to permeate it. Although its mesh size in the range of 100 – 200 nm would be wide enough for peptides to penetrate, interactions such as ionic interactions, hydrogen bonding or hydrophobic interactions are limiting the diffusion of peptides in mucus. It was demonstrated that peptides > 6.5 kDa can only to a minor extent permeate the mucus gel layer and for polypeptides with a molecular mass > 12.4 kDa permeation is almost negligible [51].

2.4. Epithelial barrier

Although the intestinal mucosa is highly vascularized and drugs have to overcome 'just' a monolayer of epithelial cells in order to reach first blood vessels and the systemic circulation, this epithelial barrier is the most challenging barrier of all. Generally, peptide and protein drugs can enter the systemic circulation from the intestinal lumen via the transcellular route (through cells), the paracellular route (between cells) and by endocytosis/transcytosis or by carrier-mediated transport. As membrane permeability has been found to be size dependent and decreases rapidly when molecular mass is greater than 1 kDa [52] even the absorption of small peptides is very limited. Furthermore, because of their hydrophilic nature, peptides and proteins do not partition in lipid bilayers of epithelial cells and are effectively excluded from entry. The paracellular route may be more compatible with peptide and protein drugs but the paracellular spaces contribute just less than 1% of the total mucosal surface. In addition, tight junctions between cells have to be transiently-opened to allow a systemic uptake. Most therapeutic peptides and proteins cannot overcome this barrier without auxiliary agents and smart formulations. The epithelial barrier and the different

routes via which peptides and proteins can overcome it is described in detail in numerous reviews [53, 54].

3. Hydrophobic ion pairing

In order to incorporate peptide and protein drugs in lipid-based nanocarriers their lipophilic character has to be strongly improved. Apart from chemical modifications such as the covalent attachment of fatty acids to peptides via ester or amide bond formation, reversible aqueous lipidization (REAL) and cyclization [55], hydrophobic ion pairing is a useful strategy. As chemical modifications of peptide drugs result in new active pharmaceutical ingredients (APIs) that have to pass the entire regulatory approval process, hydrophobic ion pairing is the preferred industrial approach. HIP of approved peptide and protein drugs fall under the 505(b)(2) regulatory approval pathway of the FDA allowing for fewer clinical and toxicity studies in order to obtain approval [56, 57]. Moreover, HIP of approved peptides and proteins that are formed in the oily phase of the final formulation likely do not need an additional approval at all. A good overview about HIP fromed between therapeutic peptides and hydrophobic counter ions has recently been provided by Ristroph and Prud'homme [58]. Since the majority of therapeutic peptides and proteins contain carboxylate anions because of the C-terminal carbonic acid and the amino acids glutamic acid and aspartic acid as well as cationic ammonium substructures because of the N-terminal primary amine and the amino acids lysine and arginine, lipophilic counterions can be ionically attached. By adjusting the pH of the medium in that the ion pair is formed at least two pH steps above or below the isoelectric point of the peptide of interest either a pronounced anionic or cationic net charge can be achieved, respectively. At this pH the hydrophobic counter ion has to be sufficiently charged as well. Strongly anionic surfactants such as sulfates or sulfonates with a pKa < 1 or quarternary ammonium surfactants exhibiting a permanent cationic charge are always ionized. In contrast, carboxylic acid and amino surfactants are only ionized at a sufficiently high or low pH, respectively. According to Coulomb's law (eq. 1)

$$F = k\, q_1\, q_2 / r^2\, \varepsilon \quad (eq.\ 1)$$

F = electrostatic force , K = constant (8.98755 x 109 Nm2C-2)

q_1, q_2 = point charges, r = distance between point charges, ε = dielectric constant

counter ions are more tightly bound to peptide and protein drugs when their point charge is strong. In case of lipophilic anionic counter-ions the following rank order of point charges can be established: sulfate > sulfonate > phosphate > carboxylate. Adjei et al., for instance, could show that leuprolide forms stable HIP with C6-C10 alkylsulfonates, whereas with carbonic acids such as dehydrocholate or salicylate improvement in lipophilicity was marginal [59]. As in case of cationic lipophilic counter ions just amines of similar point charge are of relevance, such a rank order cannot be established. HIP of daptomycin with dodecylamine resulted in an even 7 billion-fold increase in lipophilicity [60]. Nonetheless, quaternary ammonium compounds are advantageous over primary, secondary and tertiary amines, as they exhibit a permanent pH-independent cationic charge. Anionic lipophilic counter ions, however, are from the toxicological point of view prefered over cationic lipophilic counter ions. There are already various biodegradable and less toxic cationic surfactants such as arginine-, lysine- and betaine-fatty alcohol conjugates available [61-63]. These new auxiliary agents, however, are not registered yet. Another alternative might be divalent metal ions such as Ca^{2+} that allow the formation of HIP even between anionic charges on the therapeutic agent and anionic surfactants. In case of DNA-based drugs it has, for instance, already been demonstrated that lipophilic complexes between pDNA and anionic surfactants can be formed under the aid of Ca^{2+} forming simultanously ionic bonds between the different anion species [64]. In theory, di- or multivalent lipophilic counter ions would provide comparatively higher stability of HIP, as when one ion pair temporarily dissociates, association to the peptide or protein is still maintained by the other ion pair(s) formed to the same molecule. The likelihood of a synchronized dissociation of ion pairs on the same molecule at the same time decreases with the number of interacting charges per molecule. Complexes of polyanionic polymers with polycationic polymers such as polyacrylic acid/chitosan complexes are for example highly stable. Nevertheless, HIP having been formed with di- and multivalent counter ions such as pamoic and agaric acid did not show higher stability than

monovalent counter ions. Di- and multivalent lipophilic counter ions with exactly the same distance between their point charges as found for the counter point charges on the peptide or protein of choice are obviously difficult to find. As illustrated in Fig. 2, when the distance is too short or too long just one ionic substructure can form an ion pair and the additional ionic substructure(s) of the lipophilic counter ion remain dissociated. In contrast to DNA- and RNA- based drugs where the distance between their anionic phosphate substructures is always the same allowing the design of perfectly matching polycationic lipids for complexation, it is certainly not an easy task to find the perfect match for each therapeutic peptides and proteins as the distance between point charges can vary tremendously.

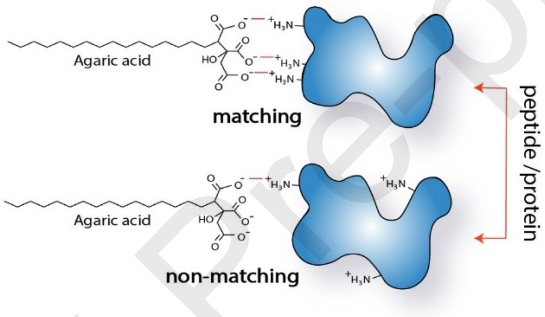


Fig. 2. Hydrophobic ion pairing of a peptide/protein with three cationic substructures with a multivalent counter ion (agaric acid); in case of a 'perfect match' all ionic substructures on the peptide/protein. are masked; moreover, if one ion pair dissociates the attachment of the hydrophobic counter ion is still provided by the two other ion pairs; in case of no match, however, after HIP formation there are more ionic charges on the peptide (two cationic and two anionic) than before;

Regarding the lipophilic substructure, branched lipophilic tails seem to be advantageous over linear ones. An explanation for this observation might be provided by the fact that branched tails like an umbrella can more efficiently shield the hydrophilic surface of peptides than linear ones [8, 65]. HIP formation of desmopressin was at least 5-fold more efficient with the branched counter ion docusate than with the linear counter ions oleate and dodecyl sulfate [8]. In another study docusate with just two hydrophobic arms was outperformed by tetraheptylammonium bromide exhibiting four hydrophobic arms, as HIP of exenatide with tetraheptylammonium showed an almost 2-fold higher oral bioavailability than those with docusate [66]. Furthermore, hydrophobic counter ions that are known for a permeation enhancing effect as listed in Tab. 2 are likely advantageous.

Tab. 2. Hydrophobic counter ions used for HIP formation that were shown to exhibit a permeation enhancing effect

Permeation enhancer	Log P	Mode of permeation enhancement	Experimental set up	Improvement	Model peptide/ protein/marker	Ref	Model peptide/protein used for HIP formation	Ref
Sodium oleate	6.78	transcellular	In-situ: Perfusion	-	Oxalate	[67]	Leuprorelin	[69]
			In-situ: Rectal	4-fold	Insulin	[68]	Insulin	[8]
							Desmopressin	[8]
							Salmon calcitonin	[70]
							Polymyxin B	[71, 72]
Sodium docusate	5.2	transcellular	In-vitro: Caco-2	-	Leuprolide	[73]	Exenatide	[66]
							Octreotide	[74]
							Desmopressin	[75]
								[76]
Sodium cholate	2.2	multimodal	Ex-vivo: Buccal	-	Insulin	[77]	Insulin	[78]
Sodium deoxycholate	3.8	multimodal	In-vitro: TR146	5.5-fold	Salmon calcitonin	[79]	Salmon calcitonin	[70]
			Ex-vivo: Buccal	93.7-fold	Salmon calcitonin	[79]	Octreotide	[74]
			In-vitro: Caco-2	2.8-fold	Octreotide	[80]		
			In-vitro: TR146	2.4-fold	Octreotide	[80]		
			Ex-vivo: Colon	7.4-fold	Insulin	[81]		
			In-vivo: Gavage	-	Heparin	[82]		
Sodium glycodeoxycholate	2.6	paracellular	In-vitro: Caco-2	6.4-fold	Insulin	[83]	Insulin	[83]
			Ex-vivo: Colon	2-fold	Insulin	[81]		
Sodium dodecyl sulfate	1.6	transcellular	In-vivo: Iv	8-fold	Enalaprilat	[84]	Dalargin	[85]
			In-vivo: Iv	-	Hexarelin	[84]	Insulin	[8]
							Insulin	[86]
							Gelargin insulin	[87]
							Octreotide	[88]
							Desmopressin	[8]
							Leuprorelin	[8]
Sodium taurocholate	0.79	multimodal	In-vitro: TR146	1.66-fold	Insulin	[89]	Salmon calcitonin	[91]
			In-vitro: Caco-2	2-fold	Insulin	[90]		
Linoleic acid	4.77	transcellular	Ex-vivo: Colon	3-fold	Adenylyl cyclase	[92]	Vancomycin	[93]

As these permeation enhancers reach the absorption membrane in form of HIP together with the therapeutic peptide or protein, their efficacy can be tremendously improved. Phan et al., for instance, showed that a concentration of just 0.0003% docusate is needed to enhance the permeation of horseradish peroxidase on a Caco-2 monolayer 4-fold, when the permeation enhancer is bound in form of a HIP to this model protein [94]. In another study permeation of toluidine blue O on Caco-2 monolayer and rat intestinal mucosa was 2-fold improved when HIP were formed with deoxycholate instead of applying the same concentration of this permeation enhancer without HIP formation [95]. In a recent study, Bashyal et al. showed that when insulin is ion-paired with sodium glycol deoxycholate (SGDC), permeation across TR146 buccal cell layer is 2.2-fold improved [96].

Apart from the counter ion the method for the preparation of HIP has also an essential impact on the hydrophobic character of the formed ion pairs. Recently Wibel et al. compared different preparation methods for the formation of HIP with peptide drugs. They showed that the Bligh-Dyer method and organic solvent-free method are advantageous over the biphasic metathesis reaction. In Table 3 the advantages and disadvantages of these and other methods are compared with each other [97].

Tab. 3. Direct comparison of different preparation methods for the formation of HIP with peptide and protein drugs.

Method	Brief description of the method	Advantages	Disadvantages	Reference
Organic solvent-free method	HIP are formed in aqueous media between the peptide and water–soluble hydrophobic counter ions; lipophilic HIP precipitate and can be removed by centrifugation or filtration;	• No toxic solvents • Ease of preparation • High product yield • Hydrophobic product obtained	• Restricted to water-soluble compounds • Complex formation efficiency is hampered when an excess of hydrophobic counter ions is used	[8, 97-99]
Bligh-Dyer method	HIP are formed in a mixture of water, methanol and chloroform; the resulting biphasic system allows the separation of HIP from unbound peptide and hydrophobic counter ion;	• Enables use of water-insoluble compounds • High product yield • Hydrophobic product obtained • Robust towards high hydrophobic counter ions concentrations • Direct incorporation of hydrophobic complexes into lipid-based nanocarriers via evaporation of organic solvents	• Toxic solvents • Organic solvents might have a negative influence on the activity of peptide and protein drugs	[97, 100]
Biphasic metathesis reaction	HIP are formed in biphasic systems consisting of water and a water-immiscible organic solvent	• Allows shift to non-toxic organic solvents • Direct incorporation of hydrophobic complexes into lipid-based nanocarriers based on evaporation of organic solvents possible	• Low product yield • Organic solvents might have a negative impact on the activity of peptide and protein drugs	[97, 101, 102]
Reverse micelle method	HIP are formed by dissolving peptide and protein drugs in an oily phase containing reverse micelles of the counter ion	• HIP are formed directly in the lipophilic phase		[103, 104]

In addition to HIP H-bond pairs can be formed between therapeutic peptides and non-ionic surfactants. As ion-pairs differ from the corresponding H-bond pairs just in the location of the proton, hydrophobic ion pairs and H-bond pairs are in close relation to each other. In fact, the conversion of an ion pair to an H-bond pair requires just the motion of the proton along the H-bond.

The formation of base pairs via H-bonding in DNA teaches us that these bonds are highly stable. Although the binding energy of dipole attractions between molecules and H-bonds with a range of binding energies between 10 and 400 kJ mol^{-1} is comparatively lower than that of ionic bonds with 200-1500 kJ mol^{-1}, these interactions are nonetheless of high relevance for the lipidization of peptide drugs as most peptide drugs bear at least 10-fold more H-bond donor and acceptor substructures than ionic substructures. The great potential of this approach has first been shown for polysaccharides. Hydrophobic complexes of dextran with a phospholipid, for instance, led to lipophilic complexes that were even soluble in C8/C10 mono- and diglycerides [105]. Transferring this concept to peptide drugs leuprolide was recently lipidized via hydrophobic H-bond pairing with sucrose esters showing that log P of this peptide can be up to 250-fold increased. Ex vivo permeation studies revealed 2-fold improved membrane permeation of hydrophobic H-bond pairing of leuprolide compared to free leuprolide [106]. In another study, the lipophilic character of exenatide was improved by incorporating this therapeutic peptide in reverse micelles formed by sorbitan oleate in a lipophilic phase that were subsequently incorporated into lipid-based nanocarriers. Although not mentioned by the authors exenatide was certainly anchored in the core of these reverse micelles via H-bond pairing. In diabetic mice an oral bioavailability of 4% was reached with this formulation [103]. Wang et al. used an oily phase containing reverse micelles to extract amino acids from aqueous media explaining the efficacy of this method by hydrophobic interactions and H-bond pairing [107]. As the binding energy of ionic bonds with 200-1500 kJ mol^{-1} is in the same range of single covalent bonds with 200 – 500 kJ mol^{-1} (C-C bond: 345 kJ mol^{-1}; C-N bond: 290 kJ mol^{-1}; C-O bond:350 kJ mol^{-1}), ionic bonds are comparatively strong. Nonetheless, their Achilles heel are ionic exchange reactions. In the GI environment hydrophobic counter ions are rapidly substituted by other counter ions such as electrolytes, bile salts, fatty acids and endogenous (poly)peptides. Nazir et al. investigated the stability of a HIP between bovine serum albumin and pamoic acid in aqueous media showing high stability in demineralized water but an almost entire dissociation of the complex in the presence of 154 mM sodium chloride within a few hours [108]. The high instability of HIP in the GI environment is

also the reason why the concept of HIP formation did not work for small therapeutic molecules so far, although there are numerous permeation enhancement data on different membranes including Caco-2 monolayer available [109-111]. Having been pioneered already in the 1960s and 1970s as a method to improve membrane permeability of biopharmaceutical classification systems (BCS) class 3 drugs, HIP showed over all these decades only in a very few in vivo studies potential and were never tested in clinical trials.

4. Key properties of lipid-based nanocarriers

4.1. Protective properties

Protective effect of lipid-based nanocarriers for peptide and protein drugs

As the lipophilic character of peptide and protein drugs is tremendously increased by HIP formation, they can be dissolved in the lipophilic phase of lipid-based nanocarriers. In this lipophilic phase, HIP are protected towards competing counter ions, as illustrated in Fig. 3 since they are too hydrophilic to enter it. Even bile salts and fatty acids can just assemble on the surface of lipid-based nanocarriers with their lipophilic tail heading into the lipophilic phase. Furthermore, the dielectric constant (ε) in the lipophilic phase is approximately 50-fold higher than in the surrounding aqueous phase. As according to Coulomb's law (eq. 1), the dielectric constant (ε) is indirectly proportional to the electrostatic force keeping ion pairs together. Their stability is consequently much higher. In case of H-bond pairs polar, aprotic excipients such as paraffin and squalene are advantageous to improve the stability of complexes in the lipophilic phase. Because of their hydrophilic character, peptidases and proteases cannot enter the lipophilic phase either. Therapeutic peptides are therefore protected in lipid-based nanocarriers towards degradation by these enzymes [112].

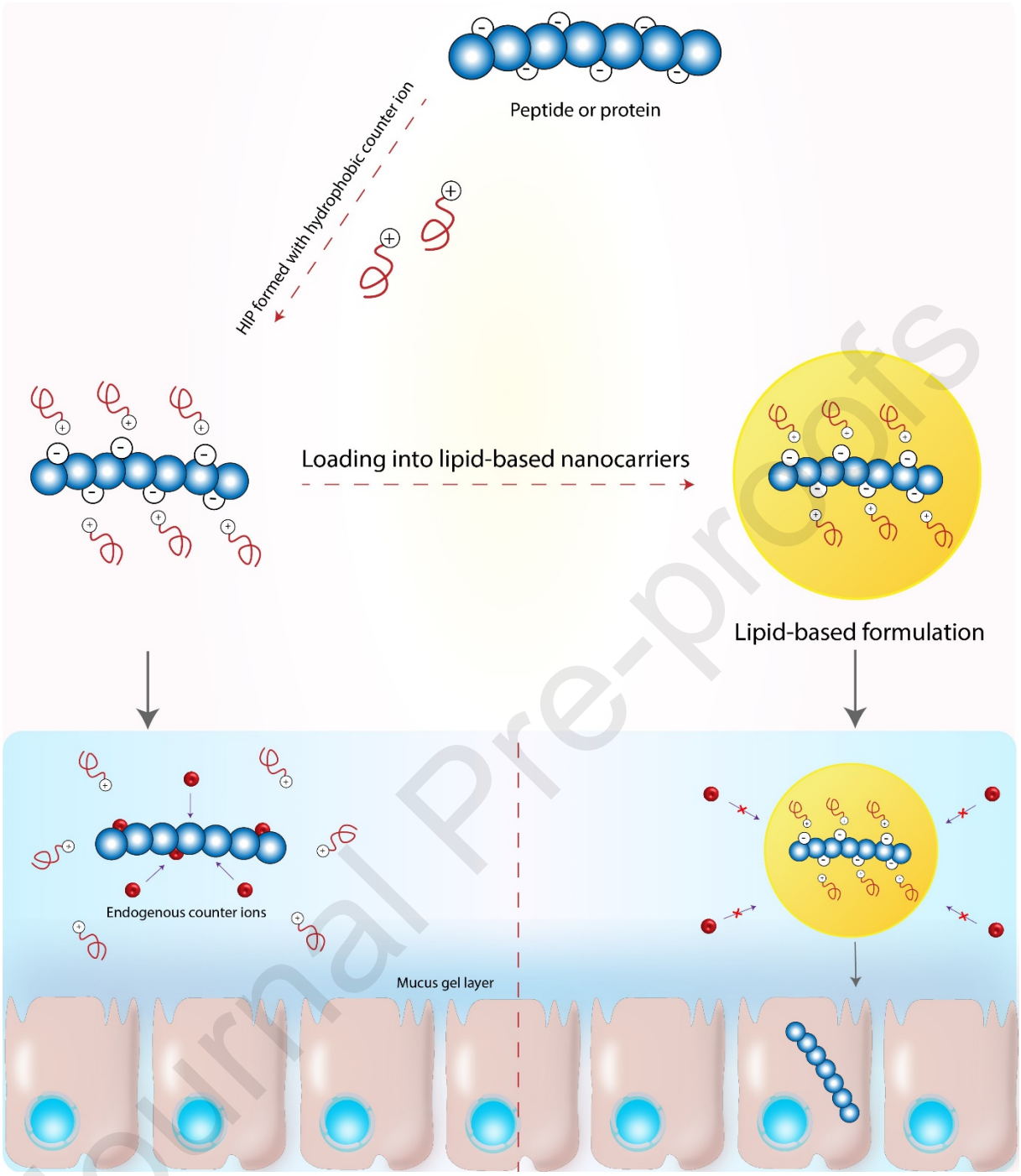


Fig. 3. HIP competing with intestinal counter ions such as bile salts and fatty acid

For instance, Leuprorelin being ion paired with oleate was entirely degraded by trypsin within 30 minutes, whereas at least 80% of the peptide remained intact when being incorporated in SEDDS. [69]. The protective effect of SEDDS was also evaluated for insulin that is rapidly degraded by pepsin and pancreatic proteases [45, 113]. The protective effect of SEDDS containing a hydrophobic ion pair of insulin and dimyristoyl phosphatidylglycerol was evaluated in the presence of trypsin and α-chymotrypsin, as illustrated in Fig. 4 [113].

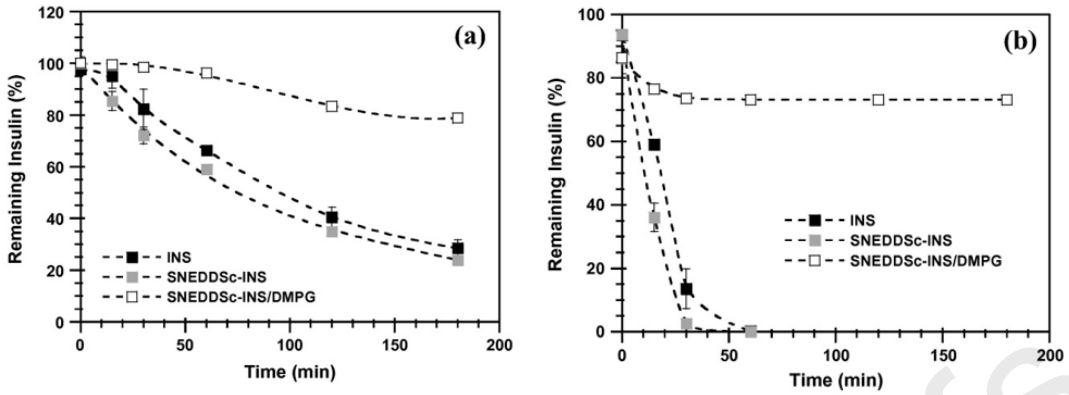


Fig. 4. Degradation profiles of insulin by trypsin (a) and α-chymotrypsin (b) in the form of insulin aqueous solution (INS), insulin mixed with SEDDS (SNEDDSc-INS) and insulin-dimyristoyl phosphatidylglycerol (INS/DMPG) HIP encapsulated in SEDDS (SNEDDSc-INS/DMPG). Results are presented as mean ± SD (n =3); adapted from Karamanidou et al. [113].

Liu et al. evaluated the protective effect of different SEDDS for incorporated insulin-phosphatidylcholine HIP towards degradation with α-chymotrypsin. They showed that insulin is more protected in SEDDS containing medium-chain glycerides than SEDDS containing long-chain glycerides. Moreover, SEDDS containing PEG-8 caprylic/capric glycerides showed a higher protective effect than SEDDS containing PEG-40 hydrogenated castor oil [114]. In another study, the protective effect of SEDDS towards chymotrypsin and elastase was shown for the opioid peptide dalargin, which was ion paired with sodium dodecyl sulfate [85]. Ansari et al. prepared insulin-loaded SLN by w\o\w solvent emulsification evaporation method with an entrapment efficiency of 51.9%. These nanocarriers provided a protective effect towards the GI enzymes pepsin and trypsin [115]. In another study, insulin was shown to be efficiently protected towards degradation by pepsin, trypsin and chymotrypsin by incorporation in bile salt containing liposomes [116]. Although none of these lipid-based nanocarriers could provide entire protection for the incorporated peptide or protein, these studies provide nonetheless evidence for the potential of this approach. The protective effect of different types of lipid-based nanocarriers can be mainly explained by the hydrophilic nature of proteases being effectively excluded from entry of the lipophilic phase of these carriers. In addition, the surface decoration of lipid-based nanocarriers seems to have an impact on their protective properties.

Thiol/disulfide exchange reactions with endogenous thiol-bearing peptides and proteins such as glutathione and dietary proteins can also be suppressed by incorporation in lipid-based nanocarriers. As illustrated in Fig. 5, Ijaz et al. demonstrated that lanreotide is entirely degraded by thiol/disulfide exchange reactions with glutathione within two hours, whereas it is degraded by just 10% being incorporated in SEDDS [50].

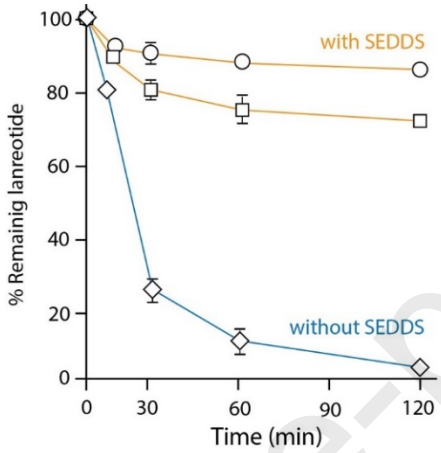


Fig. 5. Stability of lanreotide loaded SEDDS and lanreotide alone against glutathione within two hours; adapted from Ijaz et al. [50].

Stability of lipid-based nanocarriers in the GI environment

A prerequisite for the protective effect of lipid-based nanocarriers is that they are, per se, sufficiently protected towards destabilization and enzymatic degradation in the GI environment. In particular intestinal surfactants such as bile salts and fatty acids can destabilize lipid-based nanocarriers. This effect seems to be more pronounced in case of liquid formulations such as SEDDS [117] or liposomes [118] but is also known for solid formulations such as SLN [119] [120]. Liposomes containing phospholipids whose phase transition temperature is below body temperature – so-called 'fluid' liposomes – were totally disrupted in the presence of 10 mM bile salts releasing their entire payload. In contrast, 'solid' liposomes whose phase transition temperature is above body temperature remained almost stable [121]. The destabilization of lipid-based nanocarriers, however, can be addressed by a proper formulation design. Friedl et al., for instance, showed that the interaction of bile salts with SEDDS is much lower when they contain polyglycerol surfactants instead of PEG-

surfactants [122]. The oral bioavailability enhancing effect of lipid-based nanocarriers that are stable towards intestinal surfactants is in the presence of bile salts even enhanced. Zhang et al. showed a 1.8-fold improved oral bioavailability of a model drug loaded in lipid nanocarriers due to the addition of sodium cholate [123]. The addition of bile salts to the lipid bilayers of liposomes was believed to make the vehicles resistant to the detrimental effects of physiological bile salts in the GI tract and thus protect incorporated peptides and proteins from enzymatic degradation [118].

Overall, enzymatic degradation of lipid-based nanocarriers seems to be more important for their *in vivo* fate than destabilization by bile salts and fatty acids. Most lipid-based nanocarriers are rapidly degraded, especially by lipases in the intestinal fluid. Hu et al. investigated the lipolysis of lipid-based nanocarriers in mice, demonstrating that these formulations are degraded within the first 2 h after administration [124]. This degradation process of lipid-based nanocarriers by lipases goes hand in hand with the release of incorporated HIP [125]. Even when having been endocytosed, lipid-based nanocarriers are degraded by lysosomal acid lipase [126]. Maier et al. showed that lipid-based nanocarriers accumulate in late endosomes and lysosomes after endocytosis, where they are rapidly degraded [127]. Generally, not just liquid but also solid lipid-based nanocarriers are subject to lipolysis [128]. Triglycerides commonly used as excipients in lipid-based nanocarriers are broken down into diglycerides, then into monoglycerides and finally into glycerol and fatty acids [129]. Within this process, triglycerides are more rapidly degraded than monoglycerides [130]. Arnold et al. analyzed the degradation behavior of different excipients ranging from mixtures of tri-, di- and monoglycerides to non-ionic PEGylated and polyglycerol surfactants towards lipases [131]. As illustrated in Tab. 4, excipients with medium-chain length fatty acid esters were generally most rapidly and completely lipolysed, whereas PEGylated and polyglycerol surfactants showed comparatively slow and incomplete digestion kinetic. In a follow-up study, the digestibility of the various further surfactants was analyzed *in vitro* via a lipolysis test [132].

Tab. 4. Degree of degradability of different lipophilic excipients with ester substructures; adapted from Arnold et al. [131]

Excipient	Lipolysis degree after 3 h (%)
Lauroyl PEG-32 glycerides	100.0
Mono- and diglycerides of medium chain fatty acids	99.0
Mono-, di- and triglycerides, manly of caprylic and capric acid	93.6
Medium chain triglycerides	72.6
Propylene glycol monocaprylate	72.2
Polysorbate 80	53.3
PEG-40 Hydrogenated castor oil	34.7
Propylene glycol monolaurate	25.0
Linoleoyl PEG-6 glycerides	19.9

In order to provide high stability towards enzymatic degradation, lipid-based nanocarriers should therefore contain a low content of excipients with ester substructures that are readily cleaved by lipases and also proteases such as trypsin and chymotrypsin unable to discriminate between amide and ester bonds. Examples for ester-free excipients that can be used in lipid-based nanocarriers are given in Tab. 5.

Tab. 5. Examples for ester free excipients that are useful for the preparation of lipase-stable lipid-based nanocarriers.

Liquid excipients	Reference	**Solid excipients**	Reference
Liquid paraffins	[133]	Solid paraffins	[134]
Squalane	[135]	Cetyl stearyl alcohol	[136]
Benzyl alcohol	[137]	PEG-9 lauryl ether	[138]
Butyl alcohol	[139]	PEG-20 oleyl ether	[138]
Phenylethyl alcohol	[140]	PEG-100 stearyl ether	[141]
Oleyl alcohol	[142]	Stearic acid	[143]
Oleic acid	[144]	Poloxamer 188	[145]

Leonaviciute et al. showed that the amount and type of esters can accurately adjust the enzymatic degradation of SEDDS in the lipophilic phase. As illustrated in Fig. 6, the degradation profile of lipid-

based nanocarriers can be adjusted from a rapid degradation within almost one hour to no degradation at all.

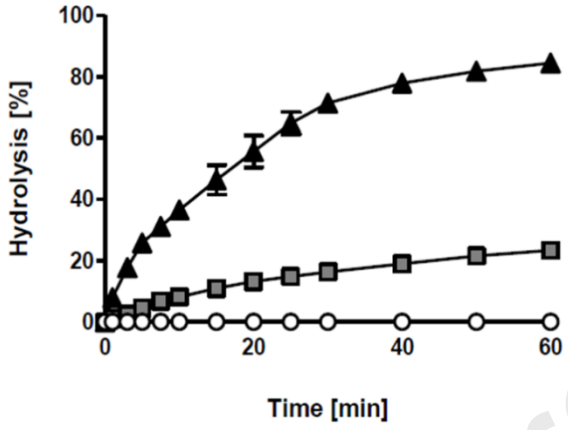


Fig. 6. Effect of pancreatic lipase on 1% SEDDS dispersions. SEDDS based on triglycerides (▲), SEDDS containing mono- and diglycerides (□) and SEDDS omitting ester linkages (○); adapted from Leonaviciute et al. [130]

Feeney et al. showed that the surface decoration of lipid-based nanocarriers has also an impact on their biodegradability. Coating of lipid-based nanocarriers containing a medium chain triglyceride with PEGs lowered the degree of digestion by lipases that resulted from steric hindrance of enzyme access to the oil–water interface. This shielding effect was shown to increase with an increase in PEG chain length. These observations were also confirmed by *in vivo* studies, where the relative bioavailability of a model drug after oral administration in two PEG-coated lipid-based nanocarriers increased to 120% and 182% in comparison to analogous digestible formulations [146]. Results are also in agreement with those of Shahzadi et al. showing that a PEG-coating of NLCs containing insulin provides a higher protective effect towards lipases and proteases than a polyglycerol coating. Furthermore, PEG-coatings based on PEG-ether surfactants provided higher stability than PEG-ester surfactants that can also be cleaved by lipases and proteases [147]. Christiansen et al. observed also for other non-ionic surfactatnts an inhibitory activity on lipolysis of triglycerides following the rank order: Cremophor RH40 > D-alpha-tocopheryl polyethylene glycol succinate (TPGS) > polysorbate 80 > Cremophor EL > sucrose laurate. They showed furthermore that the PEG-ester surfactants polysorbate 80, Cremophor EL and Cremophor RH 40 are digested by pancreatic enzymes and that

polysorbate 80 seems to inhibit lipases via a competitive mechanism [148]. Speranza et al. evaluated the influence of several non-ionic surfactants including polysorbate 20, polysorbate 40, polysorbate 60, Span-20, Span-60, and Span-80 on lipase activity and lipid bioaccessibility of o/w nanoemulsions concluding that the bioaccessibility is positively correlated with the hydrophilic/lipophilic balance (HLB) of the surfactant and inversely correlates to the surfactant aliphatic chain length [149]. Moreover, at least for analytical reasons potent lipase inhibitors such as orlistat can be added to lipid-based nanocarriers in order to avoid their enzymatic degradation [150].

Impact of drug release on the protective effect

As a protective effect for therapeutic peptides and proteins can only be achieved when they are entirely incorporated in the lipophilic phase of nanocarriers, their release behaviour from these delivery systems is also crucial. In order to escape a presystemic metabolism in the GI tract, a peptide or protein drug release before the lipid-based nanocarrier has reached the absorption membrane has to be avoided. Especially in case of liquid lipid-based nanocarriers this can only be achieved when the lipophilic characater of HIP is high enough so that these complexes remain in the oily phase. Taylor dispersion analysis experiments allow determining the partitioning coefficient (log D) of HIP between the SEDDS and continuous aqueous phase [151]. Alternatively, the maximum solubility of HIP in the SEDDS preconcentrate and the continuous aqueous phase can be determined. As the distribution coefficient between an organic phase and an aqueous phase directly correlates with the maximum solubility in both phases [152], log $D_{\text{SEDDS/release medium}}$ can be determined [153]. Generally a log $D \geq 3$ can be recommended as benchmark. In Table 6 log P of HIP between dalargin and different hydrophobic counter ions in various organic solvents that can be used for the formulation of SEDDS are listed, highlighting the challenge to reach this benchmark. Hydrophobic organic solvents such as ethanol, isopropyl alcohol, propylene glycol, DMSO and glycerol can enormously improve the solubility of HIP in the oily phase. However, as these solvents, are also rapidly released from the oily phase in aqueous media, they are likely not helpful, giving just the illusion of a proper embedment of HIP in the oily phase [154].

Tab. 6: Distribution coefficients (log D) of peptide/surfactant ion pair; adapted from Zupančič et al. [85]

Peptide	Dalargin	Dalargin palmitate	DAL-SDS	DAL-AOT	pDAL-SDS	pDAL-AOT	pDAL-DEO	pDAL-SOL
Log P (octanol in water)	NI	2.37	0.22	0.15	3.57	3.15	NI	NI
Log P (heptane in water)	NI	0.4	NI	NI	0.64	0.31	NI	NI
Log P (Campul 907 in water)	NI	3.38	3.35	2.95	3.1	3.44	2.84	2.71

NI: not investigated, could not detect any peptide thus the measurement of Log p was not possible, pDAL: dalargin palmitate, SDS: sodium dodecyl sulfate, AOT: sodium docusate, DEO: sodium deoxycholate, SOL: Sodium oleate

HIP that exhibit a pronounced hydrophilic and lipophilic partial structure like surfactants are likely also disadvantageous, as we assume that hydrophilic peptide-derived substructures assemble on the surface of lipid-based nanocarriers so that they can be cleaved by peptidases.

4.2. Mucus permeating properties

As the enzymatic activity of peptidases and proteases is comparatively much lower within the mucus gel layer than within the intestinal fluid, therapeutic peptides are to a minor extent – if at all – degraded in mucus. High mucus permeating properties of nanocarriers are therefore advantageous in order to avoid a pre-systemic metabolism of peptide drugs [155-157]. Furthermore, these carriers should reach the absorption membrane in order to provide a high systemic uptake of their payload. The mucus permeating properties of lipid-based nanocarriers depend on their size, surface charge and surface decoration as illustrated in Figure 7. In order to avoid ionic interactions with the negatively charged mucus gel layer a neutral or negative charge of lipid-based nanocarriers is advantageous to achieve high mucus permeating properties [158]. Griesser et al. showed for SEDDS that the smaller the size of these lipid-based nanocarriers is, the higher is their diffusion coefficient in mucus [75].

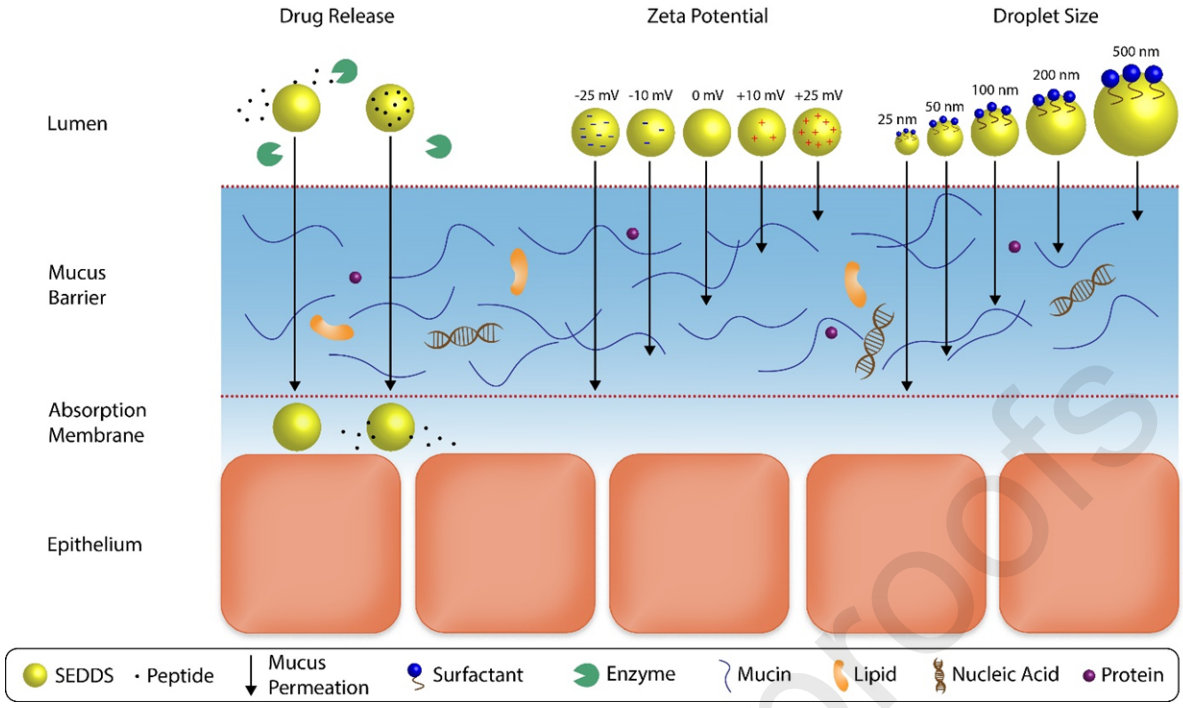


Fig. 7. Mucus permeation behavior of self-emulsifying drug delivery systems (SEDDS); in order to deliver peptide and protein drugs in intact form to the absorption membrane, lipid-based nanocarriers have to permeate the mucus gel layer with their payload; as the mucus exhibits a negative net charge SEDDS with a negative zeta potential can to a higher extent permeate the mucus gel layer than positively charged ones; in addition, the smaller lipid-based nanocarriers are, the higher is their diffusivity; surface decoration such as PEG or zwitterionic substructures that can be introduced via the polar head group of surfactants can further contribute to high mucus permeating properties; adapted from Griesser et al. [75].

As the mesh size of the mucus microstructure is in the range of 100-200 nm [159], lipid-based nanocarriers with a size below this cut off are of particular interest. Nonetheless, as the entire mucus layer is quite heterogeneous with numerous gaps in between regions of this well-defined microstructure showing a high dynamic, even much bigger lipid-based carriers can reach the underlying absorption membrane. Regarding surface decoration especially PEG- and zwitterionic surfaces were shown to exhibit high muco-inert properties [160, 161]. On the one hand, PEGylation reduces interactions of lipid-based nanocarriers with mucus glycoproteins. The reduced surface interactions of PEG-coated nanocarriers with mucus correlate to enhanced mucus permeating properties [162]. Such PEG-coatings can be simply obtained by the use of PEGylated surfactants that are anchored with their lipophilic tail in the lipophilic core of nanocarriers while heading with their hydrophilic PEG substructure into the aqueous phase [163]. The decoration of SLN with PEG2000-stearate, for instance, resulted in improved mucus permeating properties and a consequently 2-fold higher oral bioavailability of these nanocarriers in comparison to SLN without this PEG-coating [164].

By increasing the concentration of PEGylated surfactants in lipid-based nanocarriers, the density of PEG-coatings can be increased. The denser the PEG-coating is, the higher is the mucus diffusion behaviour of lipid-based nanocarriers until a plateau phase is reached [165]. On the other hand, many viruses exhibit zwitterionic surfaces allowing them to efficiently permeate the mucus gel layer. Both, the Norwalk and human papilloma virus were shown to diffuse in mucus and saline at the same rate [158]. Zwitterionic surfaces being also referred to as virus-mimicking surfaces provide special hydration behaviour across their surface. They bind water by Coulomb forces such as ion-dipole interactions whereas most other surfaces bind water via non-ionic interactions between water molecules and H-bond donor or acceptor groups. A single zwitterionic subunit was shown to bind eight moles of water, whereas a single PEG subunit can bind just one mole of water [166]. These super-hydrophilic properties prevent ion-ion interactions with mucus generating comparatively high muco-inert properties. Due to the addition of zwitterionic lipids such as phospholipids a zwitterionic surface decoration of lipid-based nanocarriers can be obtained. As most liposomes contain phospholipids such zwitterionic surfaces have unconsciously already been used for many decades [167]. More recently, Shan et al. compared the intestinal mucus interaction of nanocarriers with a zwitterionic surface based on dilauroyl phosphatidylcholine with PEG-coated nanocarriers. Both nanocarriers exhibited minimal interaction with purified mucus going hand in hand with high diffusivities and intestinal surface coverage [168, 169]. Apart from zwitterionic surfactants, the same effect can be achieved by the combination of anionic and cationic surfactants as long as they do not form HIP with each other. Yu et al. formed zwitterionic SLN by combining sodium dodecyl sulfate and benzalkonium chloride in these particles. In comparison to anionic and cationic SLN these zwitterionic SLN showed the fastest and highest systemic uptake after oral administration to mice likely because of this muco-inert surface decoration [170].

4.3. Uptake enhancing properties

The transport of peptides and proteins across the intestinal mucosa is a complex process involving several mechanisms. In case of lipid-based nanocarriers in particular endocytosis and transcytosis (i),

fusion events of lipid-based nanocarriers with the cell membrane (ii), the paracellular route of uptake (iii), and uptake M cells (iv) have been reported as illustrated in Fig. 8. In most cases several of these uptake mechanisms are involved. As the entire process is very complex and numerous parameters are involved that trigger the uptake of a given lipid-based nanocarrier by a particular mechanism rather than another, the design of nanocarriers being take up by just one of these mechanisms is so far not feasible. Generally, direct interactions of lipid-based nanocarriers with intestinal epithelial cells such as in case of endocytosis and fusion events seem to be much more important than their systemic uptake. Hu et al., for instance, investigated the fate of SLN and simulated mixed micelles after gastric gavage administration to mice observing not any systemic uptake of these lipid-based nanocarriers in intact form but their strong interaction with intestinal epithelial cells [171]. In Tab. 7 an overview about lipid-based nanocarriers providing an enhanced absorption of peptide and protein drugs is provided.

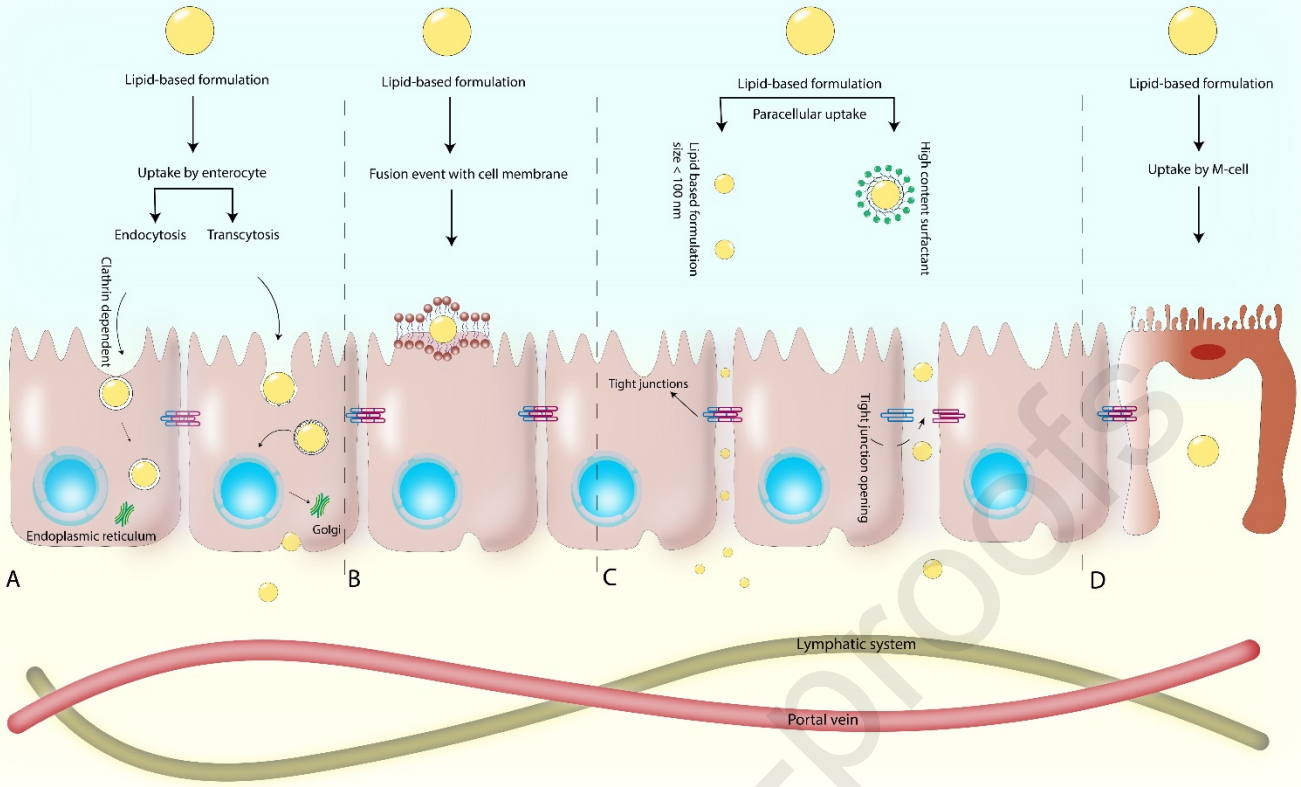


Fig. 8. Illustration of different modes of uptake of lipid-based nanocarriers from the absorption membrane. A: endocytosis and transcytosis, B: fusion event of lipid-based nanocarriers with cell membrane, C: Paracellular uptake, D: uptake via M cells.

Lipid-based nanocarriers are internalized by **endocytosis and transcytosis**. SEDDS and SLN were shown to be internalized via a clathrin-dependent endocytic pathway [172-174]. After endocytosis, lipid-based nanocarriers seem to assemble in the endoplasmic reticulum into prechylomicron transport vesicles and Golgi complexes [175]. Mature chylomicrons are then exocytosed at the basolateral membrane through another vesicular pathway [174]. Beloqui et al. showed for the peptidomimetic HIV protease inhibitor saquinavir a 3.5-fold improved permeation of Caco-2 monolayer using NLC. This improved permeation was based on the transcytosis of NLC. Size and amount of surfactant in these NLC influenced the transcytosis pathway. An NLC of size 247 nm and 1.5% surfactant content used both caveolae- and clathrin-mediated transcytosis, in contrast to other NLC formulations, which used only caveolae-mediated transcytosis [176]. In the following lipid-based nanocarriers are taken up by blood capillaries or the intestinal lymphatic system [177]. Similarly, already released peptide drugs can be transported to the systemic circulation by either the portal

vein or the lymphatic system [178-180]. *In vivo* studies investigating the contribution of lymphatic transport on oral absorption showed a minor up to 50% contribution of this route on systemic uptake [181-183].

Tab. 7. Improvement in the permeation of peptides by lipid-based formulations

Peptide/protein	Improvement in drug permeation	Type of formulation	Cell type	Likely involved uptake mechanism	Ref
Exendin-4	1.5-fold	SEDDS	Caco-2	Opening of tight junctions	[184]
Exendin-4	2.9-fold	Micelle	Caco-2	Endocytosis	[185]
Insulin	9.3-fold	SEDDS	Caco-2	Paracellular	[186]
Insulin	4.3-fold	SLN	Caco-2	Endocytosis	[187]
Insulin	2.5-fold	Micelle	Caco-2	Endocytosis	[188]
Insulin	2.5-fold	SLN	Caco-2	Opening of tight junctions	[189]
Insulin	4-fold	SLN	Caco-2	-	[190]
Insulin	22.3-fold	Micelle	Caco-2	Opening of tight junctions	[191]
Salmon calcitonin	10.8-fold	Liposome	Caco-2	-	[91]
Salmon calcitonin	2.3-fold	Micelle	Caco-2	Transcellular	[192]
β-Lactamase	24.9-fold	SEDDS	MDCK	-	[193]
Exenatide	2.92- fold	NLC	Caco-2	-	[194]
Cyclosporine A	1.8- fold	Micelle	Caco-2	Transcelluar	[195]

Fusion events of in particular liquid lipid-based nanocarriers with the cellular membrane seem to be involved in the uptake process as well. Analyses of these fusion events of lipid-based nanocarriers with bilayers showed a “contact-facilitated” mechanism. The initially occurring hemi fusion complexes are quickly expand radially to allow the massive flow of the content of the payload in the intermonolayer space resulting in a complete uptake into the cell [196]. Charge and shape of lipid-based nanocarriers seem to have a major impact on this fusion event. For example, lipids with small

polar head groups and broad hydrophobic tails facilitate the fusion process [197]. Yang et al. decorated liposomes with lipopeptides resulting in targeted membrane fusion with concomitant release of liposome encapsulated fluorescent dyes. Using a wide spectrum of endocytosis inhibitors and endosome trackers, they demonstrated that the major site of cargo release is at the plasma membrane [198].

Nanocarriers smaller than 100 nm can reach the systemic circulation via the **paracellular route**. Bunchongprasert et al. showed the permeation of oily droplets with a mean size of 30 nm formed by SEDDS across MDCK monolayer via the paracellular route of uptake [199]. In another study, the systemic uptake of a lipophilic model drug was 6-fold enhanced via the paracellular and transcellular pathway utilizing a SEDDS formulation [200]. Moreover, SEDDS increased the transepithelial permeability of insulin via the paracellular route [186]. The use of a high surfactant content employed in SEDDS formulations can cause an additional tight junction opening facilitating paracellular uptake [74].

Another entry port for lipid-based nanocarriers is the gut-associated lymphoid tissue (GALT). In particular, **M cells** of the Peyer's patches can take up lipid-based nanocarriers directly to the lymphatic system [201, 202]. Aramaki et al., for instance, studied the uptake of liposomes via Peyer's patches in rats showing that the uptake of these lipid-based nanocarriers was highest in the lower ileum which was rich in Peyer's patches compared to other segments [203]. Various other studies provide also evidence for the involvement of this rout of uptake for various types of lipid-based nanocarriers [204-209]. An uptake by the intestinal lymphatic pathway might help to avoid a first-pass metabolism of peptide drugs [210].

5. Types of lipid-based nanocarriers

5.1. Self-emulsifying drug delivery systems

Within this section the characteristics and properties of different types of lipid-based nanocarriers are summarized. In Tab. 8 a comparison of various fabrication methods is provided.

Tab. 8. Comparison of various fabrication methods for lipid-based nanocarriers

Method	Advantages	Disadvantages	Lipid-based nanocarriers	Ref
High-pressure homogenization (Hot/cold HPH)	Can be used for various types of nanocarriers; easy to scale-up; small particle size;	Droplet size is affected by intensity and duration of energy input; not suitable for heat sensitive drugs in case of hot HPH; potential for drug leaching into the aqueous phase; metallic contamination of the product may occur;	O/W nanoemulsions SLN/NLC Liposomes	[211-217]
Ultrasonication	Requires less energy in comparison to high-pressure homogenization; small particle size;	Less entrapment efficiency; metallic contamination of the product may occur;	SLN/NLC Liposomes	[211, 218, 219]
Spontaneous emulsification	Small particle size; energy-efficient;	Limited in the types of oils and emulsifiers that can be used; higher concentrations of surfactants are required;	O/W nanoemulsions	[220, 221]
Phase inversion temperature (PIT)	Small particle size; energy-efficient;	Limited in the types of oils and emulsifiers that can be used; higher concentrations of surfactants are required; not suitable for heat sensitive drugs;	O/W nanoemulsions SLN/NLC	[220, 222]
Coacervation	HIP formation of hydrophilic drugs; easy to scale-up; organic solvent free;	Not suitable for pH-sensitive drugs	SLN/NLC	[223-225]
Supercritical fluid method	Solvent-free; products obtained as dry powders;	Costly	SLN/NLC liposomes	[215, 226]
Solvent emulsification/evaporation	Mild conditions like ambient temperature; small particle size;	Use of organic solvents	SLN/NLC liposomes Micelles	[211, 227]
Film hydration	Easy to scale-up; solubility enhancement;	Use of organic solvents; nanocarriers are heterogeneous both in size and shape;	liposomes Micelles	[228-230]
Solvent injection	Easy and fast production process; narrow distribution of small liposomes (ethanol); solvent removal from product (ether);	Use of organic solvents	SLN/NLC Liposomes	[231, 232]
Adsorption to solid carrier	Easy to scale-up and cost-effective; high content uniformity; high drug loading;	Liquid components may be exuded during compression of solid carriers	Solid SEDDS	[233]
Spray drying	Single step process; cost effectiveness; high size uniformity; good dissolution profile;	Not suitable for heat sensitive drugs	Solid SEDDS	[234]
Hot melt extrusion	High drug loading; solvent free; high content uniformity;	Not suitable for heat sensitive drugs	Solid SEDDS	[235]
Freeze drying	Suitable for heat sensitive drugs; high stability/flowability;	Costly; slow process;	Solid SEDDS	[236]

Self-emulsifying drug delivery systems (SEDDS) are defined as isotropic mixtures of oils, surfactants, solvents and co-solvents/surfactants that have the ability of forming fine oil-in-water (o/w) emulsions when getting into contact with aqueous media [237]. As their upscale and production is comparatively simple, they are the preferred lipid-based nanocarrier for pharmaceutical industry [238]. The development of such formulations starts with the screening of organic solvents that can be used in SEDDS and that provide a sufficient high solubility of HIP [97]. Once the most appropriate organic solvent (/co-solvent combination) has been identified, the emulsifier and co-emulsifier are added to the HIP solution and the most suitable ratios of these components are identified via the establishment of pseudo-ternary phase diagrams [113, 239, 240]. In order to improve storage stability of such formulations SEDDS can be reformulated to solid SEDDS that are according to our definition solid at 25°C but liquid at body temperature. As temperature does not have to exceed 40°C during the preparation process, the thermal stress for the therapeutic peptide is limited [241]. To investigate the stability of proteins in SEDDS and solid SEDDS enzymes such as horseradish peroxidase and papain being inactivated by chemical modifications and conformational changes were utilized [242, 243]. Papain, for instance, was ion paired with deoxycholate and incorporated in liquid and solid SEDDS. The enzyme lost almost its entire activity in liquid SEDDS within two days, whereas it did not lose any activity in solid SEDDS within a month [243]. The *in vitro* characterization of SEDDS includes the determination of self-emulsification time, droplet size distribution, zeta potential, log D, stability towards components of the intestinal fluid including lipases, mucus and membrane permeation studies. Furthermore, storage stability studies are recommended [244]. As listed in Table 9, HIP of various therapeutic peptides and even proteins were incorporated in SEDDS and the potential of some of these formulations was demonstrated by *in vivo* studies.

Tab. 9: Oral delivery of therapeutic peptides and proteins utilizing self-emulsifying drug delivery method

Peptide/ protein	Outcome	Animal model	Hydrophobic counter ion	Mean size (nm)	Reference
β-Lactamase	Oral delivery of β-Lactamase SEDDS formulation resulted in a relative bioavailability of 6.34%.	Rat	-	<50	[193]
Dalargin	Formulation exhibited mucus permeating properties and a protective effect against enzymatic degradation by trypsin, α-chymotrypsin and elastase.	-	Sodium dodecyl sulfate	33-45	[85, 245]
Desmopressin	Negatively charged SEDDS formulations of smaller size showed higher mucus permeating properties.	-	Sodium docusate Sodium dodecyl sulfate Sodium oleate	25-500	[75, 151]
Exenatide	2.7-fold permeation enhancement was observed by utilizing exenatide docusate SEDDS. Oral administration of SEDDS led to a 20.6% decrease in AUC of blood glucose levels.	Rat	Sodium dodecyl sulfate Sodium deoxy cholate Sodium docusate Sodium oleate Sodium taurocholate	46	[246]
Exenatide	Relative bioavailability of 28% and 19.6% was achieved by oral administration of SEDDS containing exenatide-tetraheptylammonium and exenatide-docusate, respectively.	Rat	Tetraheptylammonium bromide Sodium docusate	20-25	[66]
Exenatide	Ex-vivo permeation experiment revealed 3.5-fold and 6.4-fold improvement in membrane permeability of SEDDS loaded with exenatide-docusate and exenatide- n-octadecyl sulfate HIP vs exenatide solution. Orally administrated SEDDS loaded with exenatide-docusate and exenatide-n-octadecyl sulfate HIP resulted in relative bioavailability of 19.6% and 15.2% compared to subcutaneous injection, respectively.	Rat	Sodium n-octadecyl sulfate Sodium docusate	30	[247]
Exendin-4	The permeability coefficient was improved by 1.5-fold with exendin-4 loaded self-emulsifying formulation compared to exendin-4 solution.	-	-	25-75	[184]
Insulin	9.3-fold improved transport across Caco-2 cells. Oral administration of SEDDS led to a reduction in glucose level.	Dog	Soy-bean phospholipids	-	[186, 248]
Insulin	SEDDS containing mono-acyl phosphatidylcholine (MCT) and Kolliphor RH40 (RH40) significantly decreased rat blood glucose level by 23.6% and 17.0% after 30 minutes, respectively	Rat	Soy-bean phospholipids	MCT 200-470 RH40 50-150	[69]
Leuprorelin	Protective effect against enzymatic degradation by trypsin.	-	Sodium oleate	300	[69]
Lanreotide	Protective effect against thiol-disulfide exchange reaction.	-	Sodium deoxycholate Sodium docusate Sodium taurocholate Sodium n-octadecyl sulphate	37-45	[50]

Octerotide	Reduced enzymatic degradation of the peptide caused by a longer residence time within protective lipid colloids. Results from ester free SEDDS formulations provide evidence for the stability of octreotide SEDDS formulations towards lipases. 17-fold improvement in oral bioavailability of SEDDS containing octreotide-docusate HIP compared to octreotide acetate.	Rat	Sodium docusate Sodium deoxycholate Sodium dodecyl sulphate Sodium oleate	232-235	[102, 125]
Octreotide	SEDDS formulation containing octreotide-decanoate, octreotide-docusate and octreotide-deoxycholate showed high stability towards degradation by lipase. 4.2-fold and 17.9-fold improvement in oral bioavailability of SEDDS containing octreotide-docusate and octreotide-deoxycholate HIP, respectively.	Pig	Sodium decanoate Sodium docusate Sodium deoxycholate	112-191	[74]

Octreotide, for instance, was ion paired with the anionic surfactants deoxycholate, decanoate and docusate. HIP were incorporated in SEDDS containing polyoxyethylen-10-oleylether, octyldodecanol, propylene glycol and ethanol in a concentration of 0.5% (w/v) and orally administered to pigs. Octreotide-decanoate containing formulations showed no improvement in the oral bioavailability of this peptide drug in comparison to a control formulation, whereas octreotide-docusate and octreotide-deoxycholate SEDDS resulted in a 4.2-fold and 17.9-fold higher bioavailability, respectively. With the octreotide-deoxycholate SEDDS formulation an oral bioavailability of approximately 5% was reached. Results of this study are illustrated in Fig. 9 [74]. In a similar study, octreotide was ion paired with docusate and incorporated in SEDDS comprising long-chain triglycerides. Oral bioavailability in rats was 17-fold improved when SEDDS containing octreotide-docusate HIP were used instead of SEDDS containing octreotide acetate. Within this study, the authors could provide evidence that this improvement resulted from reduced enzymatic degradation of the peptide caused by the protective effect of the oily droplets and not a result of permeation enhancement [102].

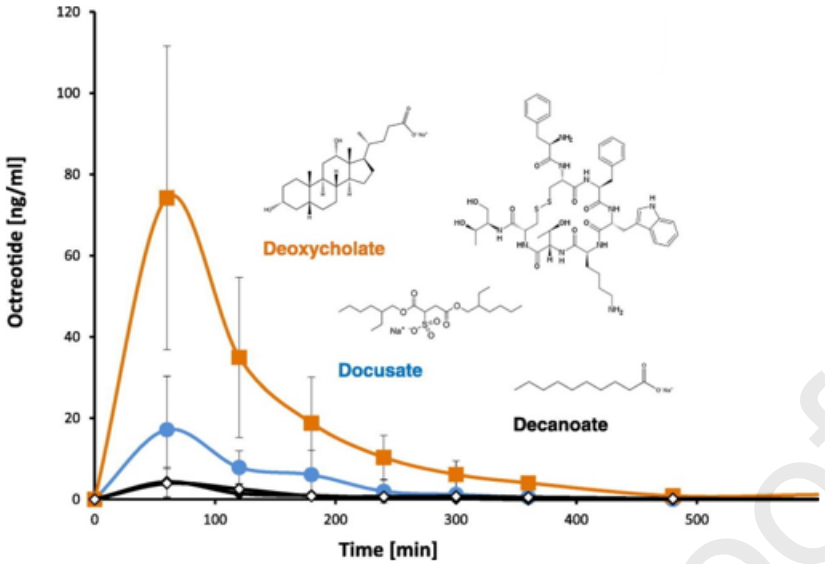


Fig. 9. Bioavailability of indicated octreotide ion pairs having been incorporated in SEDDS and orally administered to pigs. Adapted from Bonengel et al. [74].

In another study, the potent glucagon-like peptide-1 (GLP-1) analogue exenatide was ion paired with both anionic and cationic hydrophobic counter ions. N-octadecyl sulfate and docusate were used as anionic counterions, while tetraheptylammonium bromide was used as cationic counterion. HIP were incorporated in SEDDS and given orally to rats. With SEDDS containing the exenatide-tetraheptylammonium HIP, a relative oral bioavailability vs subcutaneous administration of even 28.0% was achieved, whereas, in the case of exenatide-n-octadecyl sulfate and -docusate HIP, the relative oral bioavailability was 19.6% and 15.2%, respectively [66, 246, 247]. Furthermore, SEDDS comprising medium-chain triglycerides and either monoacyl phosphatidylcholine (MAPC) or Kolliphor RH40 (RH40) were loaded with insulin-phosphatidylcholine HIP and administered to rats via intra-jejunal instillation. SEDDS containing MAPC and RH40 significantly decreased rat blood glucose level by 23.6% and 17.0% after 30 minutes, respectively. The addition of the lipase inhibitor orlistat to SEDDS showed no effect on insulin absorption [249]. In another study the oral bioavailability of leuprolide was 17.2-fold improved by ion pairing with oleate and incorporating this HIP into SEDDS [69].

5.2. SLN and NLC

Solid lipid nanoparticles (SLN) usually consist of solid lipids, surfactants, and/or co-surfactants, whereas nanostructured lipid carriers (NLC) are composed of both solid and liquid lipids as a core matrix [211]. Peptide and protein loaded SLN and NLC can be prepared by employing various techniques such as high-pressure homogenization techniques, solvent emulsification-evaporation, solvent emulsification-diffusion and microemulsion techniques [250]. It was shown that even proteins are able to endure the harsh procedures of SLN formulation by high pressure homogenization [251]. HIP are dissolved in an appropriate liquid or melted lipid phase that is added during the preparation process [73]. Leuprolide-docusate HIP, for instance, were encapsulated in SLN and NLC by high pressure homogenization leading to nanoparticles exhibiting a mean size of 120 nm and a platelet structure. The encapsulation efficacy was ≥75% [252]. Hu et al. developed a solvent diffusion method in an aqueous system. The model peptide gonadorelin was ion paired with stearic acid in acetone and ethanol at 50°C and the resultant organic solution was poured into an aqueous containing 1% polyvinyl alcohol (PVA) under mechanical agitation. HIP-loaded SLN were quickly formed and separated by centrifugation [253]. Yuan et al. developed a non-aqueous oil-in-oil (O/O) emulsion-evaporation technique in order to incorporate leuprolide-stearate HIP in SLN. Compared with the conventional method of solvent diffusion in an aqueous system, the efficiency of leuprolide entrapment with SLN increased from 28.0% to 74.6% by the combined technique of HIP formation and O/O emulsion-evaporation [120]. The method of choice depends on the thermal stability of the therapeutic peptide or protein. The characterization includes particle size distribution determinations, zeta potential measurements, entrapment efficacy determinations, drug release, lipolysis, mucus and membrane permeation and storage stability studies [254]. In Tab. 10 an overview about therapeutic peptides and proteins that have already been incorporated in SLN and NLC is provided.

Tab. 10: Peptides and proteins incorporated in SLN and NLC and properties of these formulations

Peptide/protein	Outcome	Animal model	Type of formulation	Hydrophobic counter ion	Mean size (nm)	References
Cyclosporine A	Oral administration of SLN formulation showed sustained release pattern and peak plasma concentration was less than 1000 ng/ml, which is beneficial in comparison to the commercial Sandimmune Neoral formulation that resulted in peak concentrations higher than 1000 ng/ml.	Pig	SLN	-	157	[255, 256]
Desmopressin, Leuprolide	Peptides lipophilicity was enhanced by formation of hydrophobic ion pairs (HIP) with sodium docusate.	-	NLC	Sodium docusate	<200	[252]
Exenatide, Liraglutide	NLC formulation showed 2.9-fold improvement in the permeability of exenatide across intestinal cell monolayer. Glucose-lowering effect was not observed. NLC formulation showed site-specific release of peptide under intestinal conditions.	-	NLC	-	161-225	[194]
Glargine insulin	Glucose-lowering effect was observed by oral administration of NLC.	Rat	NLC	Sodium dodecyl sulfate	302.4-499.9	[87]
Glargine insulin	Oral administration of NLC loaded with insulin-dodecyl sulfate HIP resulted in 6% oral bioavailability.	Rat	NLC	Sodium dodecyl sulfate	330-360	[9]
Insulin	SLN loaded with insulin showed a release pattern following of Weibull and Higuchi equation. Stability studies showed a relatively long-term stability after storage at 4◦C for 6 months.	-	SLN	Sodium cholate	114.7±4.68	[78]
Insulin	Cationic solid lipid nanoparticles (CSLN) loaded with insulin showed a biphasic release pattern, initial burst release in the first 30 min, followed by a sustained release of insulin. Transport across Caco-2 cell monolayer was 2.5-fold improved. CSLN provided a protective effect for insulin towards degradation by pepsin and trypsin.	-	SLN	-	-	[189]
Insulin	2.19-fold enhanced transepithelial permeation by utilizing SLN containing an endosomal escape agent.	-	SLN	-	148-171	[187]
Insulin	Chitosan-coated SLN loaded with insulin enhanced permeation of insulin across Caco-2 4-fold. Oral administration of chitosan-coated insulin SLN to diabetic rats resulted in a significant hypoglycemic effect.	Rat	SLN	-	225-395	[190]
Insulin	Modification of Insulin loaded SLN with wheat germ agglutinin-N-	Rat	SLN	-	57.7-68	[257]

	glutaryl-phosphatidylethanolamine (WGA-N-glut-PE) resulted in 7.11% oral bioavailability.					
Leuprolide	*In vitro* release of leuprolide from SLN showed a sustained release pattern.	-	SLN	sodium dodecyl sulfate	320-522	[258]
Salmon calcitonin (sCT)	Salmon calcitonin phospholipid loaded SLN showed bioavailability twice as high as that of aqueous sCT solution.	Rat	SLN	-	160.4 ± 1.81	[259]
Salmon calcitonin	SLN loaded with sCT exhibited reduction (17.44 ± 3.68%) in plasma Ca^{2+} level and bioavailability of 13.01 ± 3.24%.	Rat	SLN	-	-	[260]
Thymopentin	*In vitro* release of thymopentin from SLN showed a sustained release pattern.	-	SLN	Hexa decyl phosphate	-	[261]

One of the few studies providing evidence for the potential of SLN and NLC for oral peptide and protein delivery has recently been published by Muntoni et al.. In their study glargine insulin was ion paired with sodium dodecyl sulfate and incorporated in SLN and NLC. With the NLC formulation an oral bioavailability of 6% was reached in rats [9]. Results of the *in vivo* pharmacokinetic (PK) study are illustrated in Fig. 10.

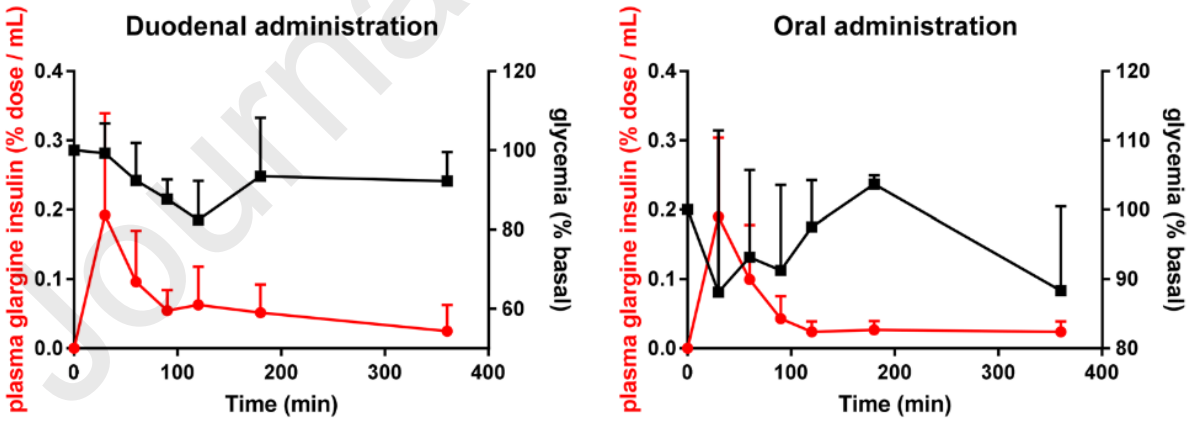


Fig. 10. *In vivo* pharmacokinetics in healthy rats of labelled glargine insulin-loaded NLC. Nanoparticles loaded with fluorescently labelled peptides were administrated (30 IU/Kg) to healthy male Wistar rats; plasma glargine insulin (●); glycemia (■); adapted from Muntoni et al. [9].

In comparison to most other lipid-based nanocarriers, SLN and NLC remain in the solid state after administration. As a consequence they are more stable in the GI environment providing a comparatively higher protective effect for incorporated peptide and protein drugs towards

enzymatic degradation. Furthermore, an unintended rapid release of rather hydrophilic HIP (log D < 2) into GI fluids can be avoided, as their diffusion out of the oily phase is limited by the solid nature of these nanocarriers. As soon as these nanocarriers have reached the absorption membrane, however, their solid nature is likely more of a hindrance than an advantage as these nanocarriers are not at all taken up into the systemic circulation [171]. The limited capability of SLN and NLC to overcome the epithelial barrier is likely the reason why an improved oral bioavailability of therapeutic peptides and proteins could so far be shown just in a very few studies as illustrated in Tab. 9. Whether these nanocarriers are able to release their payload on or in the intestinal epithelium deserves detailed further analyses. Hu et al. could at least already demonstrate that SLN strongly interact with intestinal epithelial cells [171].

5.3. Liposomes

Liposomes are spherical vesicles consisting of one or more lipid bilayers. The lipid bilayers are generated by the self-gathering of phospholipids. Hydrophilic peptides are trapped in the hydrophilic interior aqueous core, whereas lipophilic HIP are assumed to assemble within the hydrophobic lipid bilayers. In addition, peptides and proteins as well as HIP can be bound to the surface of these vesicles. In order to provide stability towards lipolysis and consequently to protect the incorporated peptide or protein towards proteases phospholipids can be substituted by tetraether lipids (TELs). Parmentier et al. improved the oral bioavailability of octreotide 4.1-fold with liposomes containing TELs [262]. Following another strategy, Niu et al. prepared liposomes containing sodium glycocholate, sodium taurocholate and sodium deoxycholate and evaluated their efficacy against degradation by various enzymes such as pepsin, trypsin, and α-chymotrypsin. Results showed the highest protective effect against enzymatic degradation of liposomes containing sodium glycocholate [116]. To increase the stability of liposomes in GI tract and to improve the permeation through the mucosa, Werle et al. introduced surface modified liposomes, which were coated with the mucoadhesive polymer chitosan. In order to provide also a protective effect towards proteases the protease inhibitor aprotinin was added. With these liposomes an increase in bioavailability of orally

administrated calcitonin was achieved [263]. Another surface modification was investigated by Yamazoe et al., who decorated liposomes with polyethylene glycol (PEG_{2000}). Results of this study revealed that oral absorption can be increased by surface PEGylation of liposomes. However, excessive PEGylation decreased oral bioavailability of a model peptide [264]. A mechanistic study focusing on the fate of liposomes on the intestinal epithelium provided evidence for enhanced transcellular permeation. Cellular uptake of liposomes was size independent for liposomes exhibiting a diameter between 80 nm and 400 nm but was tremendously lower at a size of 2000 nm. Furthermore, by the incorporation of bile salts in liposomes their cellular uptake was significantly improved [265]. In Tab. 11 an overview about peptide and protein loaded liposomes for oral administration is provided.

Tab. 11: Peptides and proteins employed in liposomes for oral administration

Peptide/protein	Outcome	Animal model	Hydrophobic counter ion	Mean size (nm)	Ref
Bovine serum albumin (BSA)	Chitosan coated liposomes provided a protective effect towards proteases. BSA was released from uncoated liposomes in a sustained manner, which was further enhanced by chitosan coating.	-	-	144-246	[266]
Calcitonin	Thiomer coated liposomes showed 3.8-fold enhanced permeation through rat intestine. Oral bioavailability of sCT was 8.2-fold improved compared to free sCT solution.	Rat	-	-	[267]
Calcitonin	Chitosan-aprotinin coated liposomes provided a protective effect towards trypsin. In comparison to calcitonin solution, the area above the blood calcium concentration–time curve (AAC) after oral administration of calcitonin loaded chitosan–aprotinin coated liposome increased around 15-fold.	Rat	-	4460	[263]
Calcitonin	10.8-fold improved transport across Caco-2 cells. 7.1-fold increase in oral bioavailability.	Rat	Sodium taurodeoxycholate	56.2	[91]
Exendin-4	Oral delivery of Exendin-4 loaded liposomes resulted in a relative oral bioavailability of 19.5%.	Rat	-	229	[268]
Gluthathion	Liposomal formulation of glutathione (GSH) exhibited 1.11-fold higher bioavailability than pure GSH.	Rat	-	167-175	[269]
Human growth hormone (h-GH)	Liposome containing tetraether lipids (TELs) and cetyl pyridinium chloride led to a bioavailability of around 3.4%, while free h-GH administered orally showed a bioavailability of just 0.01%.	Rat	-	200-250	[270]

Insulin	Insulin-glycocholate HIP loaded liposomes provided a high protective effect against enzymatic degradation by pepsin, trypsin, and α-chymotrypsin than liposomes containing the bile salts sodium taurocholate and sodium deoxycholate.		sodium taurocholate sodium glycocholate sodium deoxycholate	154	[116]
Insulin	Liposomes containing bile salts (sodium glycocholate) showed size dependent hypoglycemic effect and a sustained release pattern over a period of 20 hour with peak time around 8-12 hour.	Rat	-	150-400	[271]
Insulin	A remarkable hypoglycemic effect and improved absorption were observed after treating diabetic rats with biotinylated liposomes (BLPs).	Rat	-	150	[272]
Insulin	Biomimetic (thiamine and niacin) decorated liposomes showed comparable and sustained mild hypoglycemic effect.	Rat	-	125-150	[273]
Insulin	*In vitro* release of insulin was limited to 18.9±0.35% in simulated gastric fluid, whereas in simulated intestinal fluid, 73.3±0.7% was released after 48 h from chitosan (CS)-coated liposomes. Chitosan (CS)-coated insulin-loaded cationic liposoms showed outstanding glucose lowering effect 1 hour after oral administration that maintained up to 8 hours.	Mice	-	-	[274]
Lactoferrin	Study was based on modification of liposomes with two different polymers (pectin and chitosan). In-vivo pharmacodynamic studies of optimized formulations in the rat model revealed that polymer-modified liposomes particularly chitosan-modified liposomes, are promising carriers for transporting the drug to the intestinal lymphatic region, which resulted in increased oral bioavailability of lactoferrin. Recent observational study was performed in 75 patients with typical symptoms of COVID-19 who tested positive to IgM/IgG rapid test, results from this study revealed that liposomal formulation of lactoferrin possess antiviral, immunomodulatory and anti-inflammatory effects which might be important for the treatment of COVID-19 infection.	Rat	-	-	[275, 276]
Octreotide	4.1-fold improvement in oral bioavailability was observed by liposomes containing tetraether lipids (TELs).	Rat	-	130-207	[262]

Further evidence for the potential of liposomes for oral peptide and protein delivery was provided by Song et al.. They formed HIP between salmon calcitonin and taurodeoxycholate and incorporated the complex in pro-liposomes with an entrapment efficiency of 55%. A 10.8-fold increased permeability of Caco-2 cells and a 7.1-fold increased oral bioavailability in rats was shown for this formulation. The authors explain these effects primarily by the HIP formation increasing the permeability of the otherwise cation peptide across the biological membrane [91]. In comparison to other lipid-based

nanocarriers the protective effect of liposomes is poor. Only when liposomes are coated with polymers such as chitosan or other appropriate auxiliary agents such as bile salts are incorporated in these nanocarriers, a sufficiently high protective effect can be achieved. Furthermore, at least in comparison to SEDDS their manufacturing process and standardization is more complex.

5.4. Micelles

Micelles are considered as nanostructure colloidal drug delivery systems which are formed by self - gathered amphiphilic molecules. Generally, micelles consist of a central hydrophobic matrix which is covered by an exterior hydrophilic corona [277]. Their impressive ability to encapsulate macro-molecules while guaranteeing the physical and chemical stability of these molecules, makes them potential carriers for oral peptide delivery [278]. Aggregation of many amphiphilic peptides can be prevented by phospholipid micelles. Furthermore, conjugation of polyethylene glycol (PEG) to phospholipid micelles can provide steric stabilization and develop self-assembling Sterically Stabilized Micelles (SSM) [279, 280]. By incubation of peptides in such PEG-ylated micelles, self-association of PEG corona and peptides can induce peptide transition from unstable unordered conformation to stable alpha helical conformation which is a desirable conformation for many peptides such as secretin/ glucagon peptide family [281]. The most important phospholipid used in the SSM is the sodium salt of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG2000) which is a biocompatible pharmaceutical ingredient [282]. In addition to phospholipid micelles, polymeric micelles with a size ranging from 100-200 nm have been widely used as drug delivery system. To date, numerous studies have been undertaken to enhance the stability and bioactivity of peptides and proteins by micelles. Zhen et al., for instance, developed an oral delivery system for insulin by utilizing N-octyl-N-arginine chitosan micelles, which exhibited increased uptake rate from Caco-2 monolayer and promoted oral bioavailability [191]. In another study Bahman et al. prepared micelles by using polystyrene Co-maleic acid (SMA) and encapsulated insulin into SMA micelles. Insulin loaded micelles efficiently stimulated glucose uptake in HepG-2 hepatic cells and were transported across the Caco-2 epithelial cells by 46% and across intestinal

epithelium by 22% [188]. Furthermore, Wang et al. developed an anhydrous reverse micelle system (ARM) for oral delivery of insulin, achieving a pronounced reduction in plasma glucose level of fasted diabetic rats. As the administered formulation was an oily solution containing reverse micelles, however, the system does not fall under the definition of lipid-based nanocarriers [104]. In Table 12 an overview about peptide and protein loaded micelles for oral administration is provided. Similar to liposomes, micelles do not provide a sufficient protective effect for incorporated peptide and protein drugs towards proteases. Only when appropriate additional auxiliary agents are incorporated in these nanocarriers a protective effect can be provided [283].

Tab. 12: List of peptide and protein loaded micelles for oral administration.

Peptide/protein	Outcome	Animal model	Hydrophobic counter ion	Mean size (nm)	Ref
Cyclosporine A	In-vitro permeation of Cyclosporine A loaded polymeric micelles through Caco-2 cells was 1.8 and 2.3-fold improved in absence and presence of intrinsic factor, respectively.	-	-	33 ± 3	[195]
Exendin-4	In-vitro permeation of Exendin-4 (Ex-4) loaded micelles through Caco-2 cells was 2.8-fold improved. Oral administration of Ex-4 loaded micelles exhibited a hypoglycemic effect with a bioavailability of 12.7%.	Mice	-	250	[185]
Insulin	In-vitro permeation of Insulin loaded polymeric micelles through Caco-2 cells was 2.3-fold improved. Oral administration of insulin loaded polymeric micelles in rats provided a hypoglycemic effect for up to 3 h.	Rat	-	179.7	[188]
Insulin	In-vitro permeation of Insulin loaded arginated chitosan micelles through Caco-2 cells was 22.3-fold improved. Significant hypoglycemic response was observed by oral administration of Insulin loaded arginated chitosan micelles.	Rat	-	327	[191]
Insulin	Oral administration of insulin loaded micelles showed prolonged hypoglycemic response with relative bioavailability of 7.05%.	Rat	-	106.8-184	[284]
Insulin	Oral administration of freeze-dried powder of insulin loaded zwitterionic micelles showed an oral bioavailability >40%.	Mice	-	15-30	[285]

6. Comparison with other technologies

In comparison to other technologies for oral peptide and protein delivery lipid-based nanocarriers offer several advantages as highlighted in Tab. 12. From the industrial perspective in particular the

high flexibility in excipients that can be chosen from the inactive ingredients in approved drug products list provided by the FDA [7] and the high freedom to operate as the entire technology cannot be protected by patents due to massive prior art, are advantageous. The likely greatest disadvantage of lipid-based nanocarriers is that the technology cannot be applied to all kind of peptides. If a peptide drug exhibits simply no or a too low number of charged groups the formation of suitable HIP is not feasible and consequently the lipophilic character of the peptide cannot be raised sufficiently. Just anionic substructures on the therapeutic peptide are not advantageous either, as in this case cationic hydrophobic counter ions are required. Although certain cationic surfactants such as benzalkonium chloride or cetyl pyridinium chloride are registered as preservatives and antiseptics, their use in HIP might cause nonetheless at least in case of chronic treatments safety concerns. Newer biodegradable cationic surfactants are so far not commercially available [61, 62, 286]. Depending on the progress made on an independent pathway for approval or assessment of novel auxiliary agents, that is currently elaborated by the FDA [287] however, they might get registered in the near future. Hydrophobic H-bond pairing technologies are still in their infancy in order to compensate these shortcomings of HIP. In contrast, most other oral delivery technologies such as permeation enhancers or multifunctional polymers are much more independent from the chemical structure of therapeutic peptides. Strengths and weaknesses of lipid-based nanocarriers in comparison to other technologies are summarized in Tab. 13

Tab. 13. Strengths and weaknesses of lipid-based nanocarriers in comparison to other technologies

	Technology overview	Strengths in comparison to lipid–based nanocarriers	Weaknesses in comparison to lipid–based nanocarriers	References
Hydrophilic nanocarriers	• Peptide and protein drugs are incorporated in hydrophilic nanocarriers	• Most hydrophilic nanocarriers are stable towards lipases • A controlled drug release can be achieved in particular by making use of ionic interactions	• Minor protective effect towards proteases • A fusion process with cellular membranes is unlikely • Long-term exposure of these nanoparticles in GI tract is still a concern	[288, 289]
Enzyme inhibitors	• GI peptidases and proteases are inhibited by the co-administration of enzyme inhibitors	• Provide a protective effect towards enzymatic degradation in the entire intestinal environment and not just within the delivery system	• Enzyme inhibitors cause various local and systemic side effects • Even when they do not exhibit any side effects, they were shown to cause in case of	[290-292]

			repeated dosing hyperplasia and hypertrophy of the pancreas due to feedback regulations; as a consequence of pancreatic carcinoma cannot be excluded • They address just one barrier but not all of them	
Permeation enhancers	• The absorption of peptide drugs is enhanced by the co-administration of permeation enhancers	• Can be applied for all kind of peptide drugs • By synchronizing their delivery with the peptide drug their potential can be further improved	• Permeation enhancers are extensively diluted all over the GI-tract • Most of them are rapidly absorbed leaving the peptide drug alone behind in the GI-tract • They address just one barrier but not all of them	[26, 293]
Multifunctional polymers	• Provide an intimate contact with the intestinal mucosa because of mucoadhesive properties (I), exhibit enzyme inhibitory (II) and permeation enhancing properties (III)	• In case of non-biodegradable polymers systemic toxic side effects can be excluded as these excipients are too big to be absorbed	• Can only to a minor extent reach the absorption membrane as they cannot permeate mucus gel layer sufficiently • Improvement in oral bioavailability is comparatively low	[239, 294, 295]
Ionic liquids	• Ion pairs that are liquid at room temperature	• Provide paracellular absorption enhancement	• A liquid state instead of a solid state at room temperature does not provide any benefit from the drug delivery point of view • Counter ions such as choline are from the safety point of view problematic	[296]
Microneedle systems	• Dissolvable microneedles inject the peptide drug across the GI-mucosa into the systemic circulation	• The enzymatic, mucus and epithelial barrier can be overcome in a much more efficient manner • There is no limitation in peptide size; even proteins and antibodies can be delivered	• 'Robotic pills' are comparatively complex delivery systems • The amount of peptide drug that can be administrated is limited to 5 mg • The repeated damage of the mucosa might cause safety concerns	[297-299]
Chemical modification	• Amino acid modification and backbone modification	• Less prone to degradation by proteolytic enzymes	• Alternation of peptide bioactivity • Difficult to scale up	[300]

7. Future perspectives

The potential of lipid-based nanocarriers for oral peptide and protein drug delivery can be significantly increased by optimizing these delivery systems based on the knowledge already available. As many properties such as a sufficiently high lipophilic character of HIP (log D >3), stability

towards lipases, high mucus permeating properties and absorption enhancing properties have to be brought together in one formulation, however, this is not an easy task. It will take time to identify the most suitable approaches and combinations for this optimization process. In our opinion further progress will mainly be made by more efficient combinations of already well-established excipients and auxiliary agents rather than by the design of new ones. By the introduction of additional functionalities the efficacy of lipid-based nanocarriers for oral peptide delivery will be further improved. In particular for an enhanced drug absorption additional functionalities are helpful as the epithelial barrier is the most challenging barrier of all. It is known from numerous studies that the type and composition of excipients has a great impact on the performance of lipid-based nanocarriers on the absorption membrane. So far, however, we are aware of only a very few details how certain excipients are involved in interactions with the cellular membrane. The available data show that effects of excipients such as those of cell penetrating peptides, of cholic acid for cholic acid receptor-mediated uptake and of paracellular permeation enhancers can be transferred to lipid-based nanocarrier systems [199, 200, 301, 302]. Making use of such additional beneficial functionalities will further improve the overall potential of lipid-based nanocarriers for oral peptide delivery.

More recently lipid-based nanocarriers converting their surface charge from negative to positive were introduced for oral drug delivery. Because of a negative surface charge these nanocarriers can efficiently permeate the anionic mucus gel layer. Having reached the underlying epithelium they are able to convert their charge from negative to positive providing intensified interactions with the negatively charged cell membrane and in the following improved drug uptake. The concept is mainly based on phosphate ester surfactants that assemble on the surface of lipid-based nanocarriers with their polar head group being orientated towards the aqueous phase. These phosphate substructures provide a negative surface charge. On the absorption membrane they are cleaved off by the membrane bound enzyme alkaline phosphatase and released from the surface of these nanocarriers. The surface charge converts consequently from negative to positive. Wolf et al., for instance,

designed SEDDS containing a phosphorylated PEG-surfactant showing a change in zeta potential from -15.1 mV to +6.5 mV in the presence of alkaline phosphatase. These SEDDS showed a 3-fold higher mucus permeability than SEDDS having been preincubated with phosphatase [303]. A similar concept was followed by Le et al.. Cell penetrating peptide (CPP) decorated SEDDS were coated with a polyphosphate in order to mask the cationic charges of this auxiliary agent. On the epithelium the polyphosphate was cleaved to monophosphates by intestinal alkaline phosphatase resulting in a conversion of zeta potential from -14.1 mV to +4.2 mV. As these monophosphates were released from the CPP it regained its cationic character and provided a 4-fold improved cellular uptake on Caco-2 cells [304]. Following the same concept, Wu et al. designed charge converting nanocarriers for insulin delivery exhibiting a 1.9-fold higher oral bioavailability with this system in comparison to similar nanocarriers that did not exhibit charge converting properties [182].

The larger a peptide is the more challenging it is to mask all hydrophilic substructures on its surface via hydrophobic ion pairing. Nevertheless, lipid-based nanocarriers might also work for large protein drugs. At least their local delivery in the GI-tract seems feasible. Being incorporated in the oily phase the protein is protected towards an enzymatic degradation. As in the mucus gel layer the enzymatic activity is comparatively much lower, the therapeutic protein might remain active there. Large protein drugs such as anti-TNF-alpha antibodies for treatment of colitis or Crohn's disease, beta-lactamase for treatment of lactose intolerance or food allergens for de-sensibilization might be interesting candidates for such developments. Even the systemic delivery of large proteins via the oral route seems feasible. Horseradish peroxidase with a molecular mass of approximately 40 kDa was ion paired with docusate and incorporated in SEDDS. Studies on Caco-2 cell monolayer and on freshly excised rat intestine showed a 4 times and 2.5 times higher membrane permeation of the protein with this formulation compared to the free protein [94]. Although this study has to be regarded as an initial step towards oral delivery of large proteins, it is nevertheless encouraging.

8. Conclusion

Within the last decade the number of lipid-based nanocarriers for oral delivery of peptide and protein drugs increased tremendously and some of these delivery systems have already proven their efficacy in clinical trials. In contrast to most other technologies all four main barriers including the enzymatic -, sulfhydryl barrier -, mucus – and epithelial barrier can be properly addressed with lipid-based nanocarriers. Moreover, based on our knowledge about these barriers and on shortcomings of lipid-based nanocarriers having been used so far, the potential of these delivery systems can be substantially further improved. In particular their protective effect towards proteases can be optimized by a higher control of drug release and the right combination of excipients hindering these enzymes to interact with them and their payload. Although mucus permeating properties of lipid-based nanocarriers are already high, they can still be further improved. Muco- and bioinert surfaces are of hindrance once these carriers have reached the absorption membrane, as they limit interactions with cellular membranes. In particular surface decorations providing both high mucus permeating properties and intensive interactions with the cellular membrane are therefore likely key to success. Furthermore, a deepened knowledge about the fate of lipid-based nanocarriers on the absorption membrane will contribute to the design of more efficient delivery systems. Being on the one hand aware of the recent progress made in this field and keeping on the other hand these future opportunities for improvements in mind, lipid-based nanocarriers will further shape the landscape of oral peptide and protein drug delivery.

References

[1] I. Vecchio, C. Tornali, N.L. Bragazzi, M. Martini, The discovery of insulin: an important milestone in the history of medicine, Frontiers in endocrinology, 9 (2018) 613.
[2] U.S.F.a.D. Administration, Human insulin receives FDA approval, FDA Drug Bull, 12 (1982) 18-19.
[3] D.J. Craik, D.P. Fairlie, S. Liras, D. Price, The future of peptide-based drugs, Chemical biology & drug design, 81 (2013) 136-147.
[4] A. Mahmood, A. Bernkop-Schnürch, SEDDS: A game changing approach for the oral administration of hydrophilic macromolecular drugs, Advanced Drug Delivery Reviews, 142 (2019) 91-101.
[5] E. Moroz, Oral delivery of macromolecular drugs: Where we are after almost100 years of attempts, Advanced Drug Delivery Reviews, 101 (2016) 108-121.
[6] P. Fonte, F. Araújo, S. Reis, B. Sarmento, Oral insulin delivery: how far are we?, Journal of diabetes science and technology, 7 (2013) 520-531.
[7] FDA, Inactive Ingredient Search for Approved Drug Products, FDA, https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.
[8] J. Griesser, G. Hetényi, M. Moser, F. Demarne, V. Jannin, A. Bernkop-Schnürch, Hydrophobic ion pairing: key to highly payloaded self-emulsifying peptide drug delivery systems, International journal of pharmaceutics, 520 (2017) 267-274.
[9] E. Muntoni, E. Marini, N. Ahmadi, P. Milla, C. Ghè, A. Bargoni, M.T. Capucchio, E. Biasibetti, L. Battaglia, Lipid nanoparticles as vehicles for oral delivery of insulin and insulin analogs: preliminary ex vivo and in vivo studies, Acta diabetologica, 56 (2019) 1283-1292.
[10] C.M. Meaney, C.M. O'Driscoll, A comparison of the permeation enhancement potential of simple bile salt and mixed bile salt: fatty acid micellar systems using the CaCo-2 cell culture model, International journal of pharmaceutics, 207 (2000) 21-30.
[11] M.E. Lane, C.M. O'Driscoll, O.I. Corrigan, Quantitative estimation of the effects of bile salt surfactant systems on insulin stability and permeability in the rat intestine using a mass balance model, Journal of pharmacy and pharmacology, 57 (2005) 169-175.
[12] S. Maher, T.W. Leonard, J. Jacobsen, D.J. Brayden, Safety and efficacy of sodium caprate in promoting oral drug absorption: from in vitro to the clinic, Advanced drug delivery reviews, 61 (2009) 1427-1449.
[13] S.D. Luzio, G. Dunseath, A. Lockett, T.P. Broke-Smith, R.R. New, D.R. Owens, Comparison of an Oral Insulin (Capsulin) and Aetrapid During an Isoglycaemic Clamp Study in Persons with Type 2 Diabetes, Diabetes, 56 (2007).
[14] N. Easa, R.G. Alany, M. Carew, A. Vangala, A review of non-invasive insulin delivery systems for diabetes therapy in clinical trials over the past decade, Drug Discovery Today, 24 (2019) 440-451.
[15] M. Kidron, I.M. Raz, M. Wolfensberger, H. Schwob, C. Schruefer, Pharmacokinetics (PK) and pharmacodynamics (PD) of oral insulin in healthy subjects, American Diabetes Association 68th Annual MeetingSan Francisco, CA.
[16] E. Arbit, M. Kidron, Oral insulin delivery in a physiologic context, Journal of diabetes science and technology, 11 (2017) 825-832.
[17] M. Durán-Lobato, A.M. López-Estévez, A.S. Cordeiro, T.G. Dacoba, J. Crecente-Campo, D. Torres, M.J. Alonso, Nanotechnologies for the delivery of biologicals: historical perspective and current landscape, Advanced Drug Delivery Reviews, (2021) 113899.
[18] S. Faulkner, EnterisBioPharma touts more Phase II data for oral endometriosis therapy, Drug Delivery Business, 201/8.
[19] A.T. Zizzari, D. Pliatsika, F.M. Gall, T. Fischer, R. Riedl, New perspectives in oral peptide delivery, Drug discovery today, 26 (2021) 1097-1105.
[20] S.P. Hämmerle, L. Mindeholm, A. Launonen, B. Kiese, R. Loeffler, E. Harfst, M. Azria, M. Arnold, M.R. John, The single dose pharmacokinetic profile of a novel oral human parathyroid hormone formulation in healthy postmenopausal women, Bone, 50 (2012) 965-973.

[21] L.B. Tankó, Y.Z. Bagger, P. Alexandersen, J.P. Devogelaer, J.Y. Reginster, R. Chick, M. Olson, H. Benmammar, L. Mindeholm, M. Azria, Safety and efficacy of a novel salmon calcitonin (sCT) technology-based oral formulation in healthy postmenopausal women: acute and 3-month effects on biomarkers of bone turnover, Journal of bone and mineral research, 19 (2004) 1531-1538.
[22] M.A. Karsdal, K. Henriksen, A.C. Bay-Jensen, B. Molloy, M. Arnold, M.R. John, I. Byrjalsen, M. Azria, B.J. Riis, P. Qvist, Lessons learned from the development of oral calcitonin: the first tablet formulation of a protein in phase III clinical trials, The Journal of Clinical Pharmacology, 51 (2011) 460-471.
[23] J.P. Gleeson, K.C. Fein, K.A. Whitehead, Oral delivery of peptide therapeutics in infants: Challenges and opportunities, Advanced Drug Delivery Reviews, 173 (2021) 112-124.
[24] T.D. Brown, K.A. Whitehead, S. Mitragotri, Materials for oral delivery of proteins and peptides, Nature Reviews Materials, 5 (2020) 127-148.
[25] A.C. Anselmo, Y. Gokarn, S. Mitragotri, Non-invasive delivery strategies for biologics, Nature Reviews Drug Discovery, 18 (2019) 19-40.
[26] S. Maher, R.J. Mrsny, D.J. Brayden, Intestinal permeation enhancers for oral peptide delivery, Advanced drug delivery reviews, 106 (2016) 277-319.
[27] A.L. Lewis, N. McEntee, J. Holland, A. Patel, Development and approval of rybelsus (oral semaglutide): ushering in a new era in peptide delivery, Drug delivery and translational research, (2021) 1-6.
[28] J.D. Bucheit, L.G. Pamulapati, N. Carter, K. Malloy, D.L. Dixon, E.M. Sisson, Oral semaglutide: a review of the first oral glucagon-like peptide 1 receptor agonist, Diabetes technology & therapeutics, 22 (2020) 10-18.
[29] P.R. Beauchesne, N.S.C. Chung, K.M. Wasan, Cyclosporine A: a review of current oral and intravenous delivery systems, Drug development and industrial pharmacy, 33 (2007) 211-220.
[30] S. Noble, A. Markham, Cyclosporin, Drugs, 50 (1995) 924-941.
[31] S. Maher, D.J. Brayden, Overcoming poor permeability: translating permeation enhancers for oral peptide delivery, Drug Discovery Today: Technologies, 9 (2012) e113-e119.
[32] L. Heinemann, Y. Jacques, Oral insulin and buccal insulin: a critical reappraisal, Journal of diabetes science and technology, 3 (2009) 568-584.
[33] J.O. Morales, D.J. Brayden, Buccal delivery of small molecules and biologics: of mucoadhesive polymers, films, and nanoparticles, Current opinion in pharmacology, 36 (2017) 22-28.
[34] S.L. Samson, L.B. Nachtigall, M. Fleseriu, M.E. Molitch, A. Giustina, W.H. Ludlam, G. Patou, A. Haviv, N. Biermasz, L. Kennedy, One-Year Outcomes of the Open-Label Extension of CHIASMA OPTIMAL, a Phase 3 Study of Oral Octreotide Capsules in Acromegaly, Journal of the Endocrine Society, 5 (2021) A515.
[35] D.J. Brayden, S. Maher, Transient Permeation Enhancer®(TPE®) technology for oral delivery of octreotide: a technological evaluation, Expert Opinion on Drug Delivery, 5 (2021) 1501-1511.
[36] M. Durán-Lobato, Z. Niu, M.J. Alonso, Oral delivery of biologics for precision medicine, Advanced Materials, 32 (2020) 1901935.
[37] K. Shailubhai, V. Palejwala, K.P. Arjunan, S. Saykhedkar, B. Nefsky, J.A. Foss, S. Comiskey, G.S. Jacob, S.E. Plevy, Plecanatide and dolcanatide, novel guanylate cyclase-C agonists, ameliorate gastrointestinal inflammation in experimental models of murine colitis, World journal of gastrointestinal pharmacology and therapeutics, 6 (2015) 213.
[38] L. Dossche, R. Michelet, P. De Bruyne, C. Van Herzeele, E. Gasthuys, S. Rittig, A. Vermeulen, J.V. Walle, Desmopressin oral lyophilisate in young children: new insights in pharmacokinetics and pharmacodynamics, Archives of Disease in Childhood, 106 (2021) 597-602.
[39] H. Iyer, A. Khedkar, M. Verma, Oral insulin–a review of current status, Diabetes, Obesity and Metabolism, 12 (2010) 179-185.
[40] J.R. Kintzing, J.R. Cochran, Engineered knottin peptides as diagnostics, therapeutics, and drug delivery vehicles, Current opinion in chemical biology, 34 (2016) 143-150.
[41] F. Bahman, K. Greish, S. Taurin, Nanotechnology in insulin delivery for management of diabetes, Pharmaceutical nanotechnology, 7 (2019) 113-128.

[42] P. Vass, B. Démuth, E. Hirsch, B. Nagy, S.K. Andersen, T. Vigh, G. Verreck, I. Csontos, Z.K. Nagy, G. Marosi, Drying technology strategies for colon-targeted oral delivery of biopharmaceuticals, Journal of Controlled Release, 296 (2019) 162-178.
[43] A. Bernkop-Schnürch, C.E. Kast, D. Guggi, Permeation enhancing polymers in oral delivery of hydrophilic macromolecules: thiomer/GSH systems, Journal of Controlled Release, 93 (2003) 95-103.
[44] S.T. Buckley, T.A. Bækdal, A. Vegge, S.J. Maarbjerg, C. Pyke, J. Ahnfelt-Rønne, K.G. Madsen, S.G. Schéele, T. Alanentalo, R.K. Kirk, Transcellular stomach absorption of a derivatized glucagon-like peptide-1 receptor agonist, Science translational medicine, 10 (2018).
[45] M.K. Marschütz, A. Bernkop-Schnürch, Oral peptide drug delivery: polymer–inhibitor conjugates protecting insulin from enzymatic degradation in vitro, Biomaterials, 21 (2000) 1499-1507.
[46] J.F. Woodley, Enzymatic barriers for GI peptide and protein delivery, Critical reviews in therapeutic drug carrier systems, 11 (1994) 61-95.
[47] A. Bernkop-Schnürch, The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and proteins, Journal of Controlled Release, 52 (1998) 1-16.
[48] S. Chakraborty, D. Shukla, B. Mishra, S. Singh, Lipid–an emerging platform for oral delivery of drugs with poor bioavailability, European Journal of Pharmaceutics and Biopharmaceutics, 73 (2009) 1-15.
[49] T. Schmitz, C.W. Huck, A. Bernkop-Schnürch, Characterisation of the thiol-disulphide chemistry of desmopressin by LC, mu-LC, LC-ESI-MS and Maldi-Tof, Amino Acids, 30 (2006) 35-42.
[50] M. Ijaz, S. Bonengel, O. Zupančič, M. Yaqoob, M. Hartl, S. Hussain, C.W. Huck, A. Bernkop-Schnürch, Development of oral self nano-emulsifying delivery system (s) of lanreotide with improved stability against presystemic thiol-disulfide exchange reactions, Expert opinion on drug delivery, 13 (2016) 923-929.
[51] A. Bernkop-Schnürch, R. Fragner, Investigations into the diffusion behaviour of polypeptides in native intestinal mucus with regard to their peroral administration, Pharmacy and Pharmacology Communications, 2 (1996) 361-363.
[52] M.D. Donovan, G.L. Flynn, G.L. Amidon, Absorption of polyethylene glycols 600 through 2000: the molecular weight dependence of gastrointestinal and nasal absorption, Pharmaceutical research, 7 (1990) 863-868.
[53] A.L. Smart, S. Gaisford, A.W. Basit, Oral peptide and protein delivery: intestinal obstacles and commercial prospects, Expert opinion on drug delivery, 11 (2014) 1323-1335.
[54] D.J. Brayden, T.A. Hill, D.P. Fairlie, S. Maher, R.J. Mrsny, Systemic delivery of peptides by the oral route: formulation and medicinal chemistry approaches, Advanced drug delivery reviews, 157 (2020) 2-36.
[55] O. Zupančič, A. Bernkop-Schnürch, Lipophilic peptide character – What oral barriers fear the most, Journal of Controlled Release, 255 (2017) 242-257.
[56] FDA, Applications Covered by Section 505(b)(2), https://www.fda.gov/regulatory-information/search-fda-guidance-documents/applications-covered-section-505b2, 1999.
[57] I. Freije, S. Lamouche, M. Tanguay, Review of drugs approved via the 505 (b)(2) pathway: Uncovering drug development trends and regulatory requirements, Therapeutic innovation & regulatory science, 54 (2020) 128-138.
[58] K.D. Ristroph, R.K. Prud'homme, Hydrophobic ion pairing: encapsulating small molecules, peptides, and proteins into nanocarriers, Nanoscale Advances, 1 (2019) 4207-4237.
[59] A. Adjei, S. Rao, J. Garren, G. Menon, M. Vadnere, Effect of ion-pairing on 1-octanol-water partitioning of peptide drugs. I: the nonapeptide leuprolide acetate, International journal of pharmaceutics, 90 (1993) 141-149.
[60] O. Zupančič, A. Partenhauser, H.T. Lam, J. Rohrer, A. Bernkop-Schnürch, Development and in vitro characterisation of an oral self-emulsifying delivery system for daptomycin, European Journal of Pharmaceutical Sciences, 81 (2016) 129-136.
[61] I. Shahzadi, M.H. Asim, A. Dizdarević, J.D. Wolf, M. Kurpiers, B. Matuszczak, A. Bernkop-Schnürch, Arginine-based cationic surfactants: Biodegradable auxiliary agents for the formation of

hydrophobic ion pairs with hydrophilic macromolecular drugs, Journal of colloid and interface science, 552 (2019) 287-294.
[62] M. Kurpiers, J.D. Wolf, H. Spleis, C. Steinbring, A.M. Jörgensen, B. Matuszczak, A. Bernkop-Schnürch, Lysine-Based Biodegradable Surfactants: Increasing the Lipophilicity of Insulin by Hydrophobic Ion Paring, Journal of Pharmaceutical Sciences, 110 (2021) 124-134.
[63] R. Bordes, K. Holmberg, Amino acid-based surfactants–do they deserve more attention?, Advances in Colloid and Interface Science, 222 (2015) 79-91.
[64] H. Liang, D. Harries, G.C.L. Wong, Polymorphism of DNA–anionic liposome complexes reveals hierarchy of ion-mediated interactions, Proceedings of the National Academy of Sciences, 102 (2005) 11173-11178.
[65] S.V.R. Rao, J. Shao, Self-nanoemulsifying drug delivery systems (SNEDDS) for oral delivery of protein drugs: I. Formulation development, International Journal of Pharmaceutics, 362 (2008) 2-9.
[66] R. Ismail, T.N.Q. Phan, F. Laffleur, I. Csóka, A. Bernkop-Schnürch, Hydrophobic ion pairing of a GLP-1 analogue for incorporating into lipid nanocarriers designed for oral delivery, European Journal of Pharmaceutics and Biopharmaceutics, 152 (2020) 10-17.
[67] J.W. Dobbins, H.J. Binder, Effect of bile salts and fatty acids on the colonic absorption of oxalate, Gastroenterology, 70 (1976) 1096-1100.
[68] N. Muranushi, E. Mack, S.W. Kim, The effects of fatty acids and their derivatives on the intestinal absorption of insulin in rat, Drug development and industrial pharmacy, 19 (1993) 929-941.
[69] F. Hintzen, G. Perera, S. Hauptstein, C. Müller, F. Laffleur, A. Bernkop-Schnürch, In vivo evaluation of an oral self-microemulsifying drug delivery system (SMEDDS) for leuprorelin, International Journal of Pharmaceutics, 472 (2014) 20-26.
[70] H. Sang Yoo, T. Gwan Park, Biodegradable nanoparticles containing protein-fatty acid complexes for oral delivery of salmon calcitonin, Journal of Pharmaceutical Sciences, 93 (2004) 488-495.
[71] H.D. Lu, P. Rummaneethorn, K.D. Ristroph, R.K. Prud'homme, Hydrophobic ion pairing of peptide antibiotics for processing into controlled release nanocarrier formulations, Molecular pharmaceutics, 15 (2018) 216-225.
[72] K. Ristroph, M. Salim, B.K. Wilson, A.J. Clulow, B.J. Boyd, R.K. Prud'homme, Internal liquid crystal structures in nanocarriers containing drug hydrophobic ion pairs dictate drug release, Journal of Colloid and Interface Science, 582 (2021) 815-824.
[73] C. Dumont, V. Jannin, A. Beloqui, V. Préat, H. Fessi, S. Bourgeois, In vitro evaluation of leuprolide-containing solid lipid-based nanosuspensions: ability to encapsulate, release, protect and permeate in the gastrointestinal tract, ratio, 1 2.
[74] S. Bonengel, M. Jelkmann, M. Abdulkarim, M. Gumbleton, V. Reinstadler, H. Oberacher, F. Prüfert, A. Bernkop-Schnürch, Impact of different hydrophobic ion pairs of octreotide on its oral bioavailability in pigs, Journal of controlled release, 273 (2018) 21-29.
[75] J. Griesser, G. Hetényi, H. Kadas, F. Demarne, V. Jannin, A. Bernkop-Schnürch, Self-emulsifying peptide drug delivery systems: How to make them highly mucus permeating, International Journal of Pharmaceutics, 538 (2018) 159-166.
[76] O. Zupančič, G. Leonaviciute, H.T. Lam, A. Partenhauser, S. Podričnik, A. Bernkop-Schnürch, Development and in vitro evaluation of an oral SEDDS for desmopressin, Drug Delivery, 23 (2016) 2074-2083.
[77] S. Bashyal, J.-E. Seo, T. Keum, G. Noh, S. Lamichhane, S. Lee, Development, Characterization, and Ex Vivo Assessment of Elastic Liposomes for Enhancing the Buccal Delivery of Insulin, Pharmaceutics, 13 (2021) 565.
[78] J. Liu, T. Gong, C. Wang, Z. Zhong, Z. Zhang, Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine-based mixed micelles: preparation and characterization, International journal of pharmaceutics, 340 (2007) 153-162.
[79] T. Keum, G. Noh, J.-E. Seo, S. Bashyal, S. Lee, In vitro and ex vivo evaluation of penetratin as a non-invasive permeation enhancer in the penetration of salmon calcitonin through TR146 buccal cells and porcine buccal tissues, Pharmaceuticals, 13 (2020) 408.

[80] D.J. Brayden, V. Stuettgen, Sodium glycodeoxycholate and sodium deoxycholate as epithelial permeation enhancers: in vitro and ex vivo intestinal and buccal bioassays, European Journal of Pharmaceutical Sciences, 159 (2021) 105737.
[81] T. Uchiyama, T. Sugiyama, Y.S. Quan, A. Kotani, N. Okada, T. Fujita, S. Muranishi, A. Yamamoto, Enhanced permeability of insulin across the rat intestinal membrane by various absorption enhancers: Their intestinal mucosal toxicity and absorption-enhancing mechanism of n-lauryl-β-d-maltopyranoside, Journal of pharmacy and pharmacology, 51 (1999) 1241-1250.
[82] S. Guarini, W. Ferrari, Structural restriction in bile acids and non-ionic detergents for promotion of heparin absorption from rat gastro-intestinal tract, Archives internationales de pharmacodynamie et de thérapie, 271 (1984) 4-10.
[83] S. Bashyal, J.-E. Seo, Y.W. Choi, S. Lee, Bile acid transporter-mediated oral absorption of insulin via hydrophobic ion-pairing approach, Journal of Controlled Release, 338 (2021) 644-661.
[84] D. Dahlgren, M. Sjöblom, M. Hedeland, H. Lennernäs, The in vivo effect of transcellular permeation enhancers on the intestinal permeability of two peptide drugs enalaprilat and hexarelin, Pharmaceutics, 12 (2020) 99.
[85] O. Zupančič, J. Rohrer, H. Thanh Lam, J.A. Grießinger, A. Bernkop-Schnürch, Development and in vitro characterization of self-emulsifying drug delivery system (SEDDS) for oral opioid peptide delivery, Drug development and industrial pharmacy, 43 (2017) 1694-1702.
[86] E. Shefter, M. Manning, Ion-pairing of drugs for improved efficacy and delivery, WO9408599, (1994).
[87] E. Muntoni, L. Anfossi, P. Milla, E. Marini, C. Ferraris, M.T. Capucchio, E. Colombino, L. Segale, M. Porta, L. Battaglia, Glargine insulin loaded lipid nanoparticles: Oral delivery of liquid and solid oral dosage forms, Nutrition, Metabolism and Cardiovascular Diseases, 31 (2021) 691-698.
[88] R.D. Vaishya, A. Mandal, M. Gokulgandhi, S. Patel, A.K. Mitra, Reversible hydrophobic ion-paring complex strategy to minimize acylation of octreotide during long-term delivery from PLGA microparticles, International journal of pharmaceutics, 489 (2015) 237-245.
[89] S. Bashyal, J.-E. Seo, T. Keum, G. Noh, Y.W. Choi, S. Lee, Facilitated permeation of insulin across TR146 cells by cholic acid derivatives-modified elastic bilosomes, International journal of nanomedicine, 13 (2018) 5173.
[90] Z. Degim, N. Ünal, D. Eşsiz, U. Abbasoglu, The effect of various liposome formulations on insulin penetration across Caco-2 cell monolayer, Life sciences, 75 (2004) 2819-2827.
[91] K.-H. Song, S.-J. Chung, C.-K. Shim, Enhanced intestinal absorption of salmon calcitonin (sCT) from proliposomes containing bile salts, Journal of Controlled Release, 106 (2005) 298-308.
[92] B.S. Ramakrishna, M. Mathan, V.I. Mathan, Alteration of colonic absorption by long-chain unsaturated fatty acids: influence of hydroxylation and degree of unsaturation, Scandinavian journal of gastroenterology, 29 (1994) 54-58.
[93] R.S. Kalhapure, C. Mocktar, D.R. Sikwal, S.J. Sonawane, M.K. Kathiravan, A. Skelton, T. Govender, Ion pairing with linoleic acid simultaneously enhances encapsulation efficiency and antibacterial activity of vancomycin in solid lipid nanoparticles, Colloids and Surfaces B: Biointerfaces, 117 (2014) 303-311.
[94] T.N.Q. Phan, B. Le-Vinh, N.A. Efiana, A. Bernkop-Schnürch, Oral self-emulsifying delivery systems for systemic administration of therapeutic proteins: science fiction?, Journal of drug targeting, 27 (2019) 1017-1024.
[95] I. Shahzadi, I. Nazir, T.N.Q. Phan, A. Bernkop-Schnürch, About the impact of superassociation of hydrophobic ion pairs on membrane permeability, European Journal of Pharmaceutics and Biopharmaceutics, 151 (2020) 1-8.
[96] S. Bashyal, J.-E. Seo, T. Keum, G. Noh, S. Lamichhane, J.H. Kim, C.H. Kim, Y.W. Choi, S. Lee, Facilitated Buccal Insulin Delivery via Hydrophobic Ion-Pairing Approach: In vitro and ex vivo Evaluation, International Journal of Nanomedicine, 16 (2021) 4677.
[97] R. Wibel, J.D. Friedl, S. Zaichik, A. Bernkop-Schnürch, Hydrophobic ion pairing (HIP) of (poly) peptide drugs: Benefits and drawbacks of different preparation methods, European Journal of Pharmaceutics and Biopharmaceutics, 151 (2020) 73-80.

[98] S. Sun, N.A. Liang, Y. Kawashima, D. Xia, F. Cui, Hydrophobic ion pairing of an insulin-sodium deoxycholate complex for oral delivery of insulin, International journal of nanomedicine, 6 (2011) 3049.
[99] R. Gaudana, V. Khurana, A. Parenky, A.K. Mitra, Encapsulation of protein-polysaccharide HIP complex in polymeric nanoparticles, Journal of drug delivery, 2011 (2011).
[100] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Canadian journal of biochemistry and physiology, 37 (1959) 911-917.
[101] H.D. Williams, L. Ford, S. Lim, S. Han, J. Baumann, H. Sullivan, D. Vodak, A. Igonin, H. Benameur, C.W. Pouton, Transformation of biopharmaceutical classification system class I and III drugs into ionic liquids and lipophilic salts for enhanced developability using lipid formulations, Journal of pharmaceutical sciences, 107 (2018) 203-216.
[102] P. Li, L. Ford, S. Haque, M.P. McInerney, H.D. Williams, P.J. Scammells, P.E. Thompson, V. Jannin, C.J.H. Porter, H. Benameur, Lipophilic Salts and Lipid-Based Formulations: Enhancing the Oral Delivery of Octreotide, Pharmaceutical Research, (2021) 1-13.
[103] Y. Xu, M. Van Hul, F. Suriano, V. Préat, P.D. Cani, A. Beloqui, Novel strategy for oral peptide delivery in incretin-based diabetes treatment, Gut, 69 (2020) 911-919.
[104] T. Wang, N. Wang, A. Hao, X. He, T. Li, Y. Deng, Lyophilization of water-in-oil emulsions to prepare phospholipid-based anhydrous reverse micelles for oral peptide delivery, European journal of pharmaceutical sciences, 39 (2010) 373-379.
[105] P. Khatri, J. Shao, Impact of digestion on the transport of dextran-loaded self-emulsified nanoemulsion through MDCK epithelial cell monolayer and rat intestines, International journal of pharmaceutics, 536 (2018) 353-359.
[106] I. Nazir, I. Shahzadi, A. Jalil, A. Bernkop-Schnürch, Hydrophobic H-bond pairing: a novel approach to improve membrane permeability, International journal of pharmaceutics, 573 (2020) 118863.
[107] Y. Wang, C. Shi, Q. Gan, Y. Dai, Separation of amino acids by polymeric reversed micelle extraction, Separation and purification technology, 35 (2004) 1-9.
[108] I. Nazir, M.H. Asim, A. Dizdarević, A. Bernkop-Schnürch, Self-emulsifying drug delivery systems: Impact of stability of hydrophobic ion pairs on drug release, International journal of pharmaceutics, 561 (2019) 197-205.
[109] N. Samiei, S.M. Foroutan, F. Razipour, A. Zarghi, A. Shafaati, An investigation into the ability of alendronate ion pairs to increase oral absorption, International journal of pharmaceutics, 527 (2017) 184-190.
[110] J.M. Miller, A. Dahan, D. Gupta, S. Varghese, G.L. Amidon, Quasi-equilibrium analysis of the ion-pair mediated membrane transport of low-permeability drugs, Journal of Controlled Release, 137 (2009) 31-37.
[111] S.A. Megwa, S.E. Cross, M.W. Whitehouse, H.A.E. Benson, M.S. Roberts, Effect of ion pairing with alkylamines on the in-vitro dermal penetration and local tissue disposition of salicylates, Journal of pharmacy and pharmacology, 52 (2000) 929-940.
[112] G. Hetényi, J. Griesser, M. Moser, F. Demarne, V. Jannin, A. Bernkop-Schnürch, Comparison of the protective effect of self-emulsifying peptide drug delivery systems towards intestinal proteases and glutathione, International Journal of Pharmaceutics, 523 (2017) 357-365.
[113] T. Karamanidou, K. Karidi, V. Bourganis, K. Kontonikola, O. Kammona, C. Kiparissides, Effective incorporation of insulin in mucus permeating self-nanoemulsifying drug delivery systems, European journal of pharmaceutics and biopharmaceutics, 97 (2015) 223-229.
[114] J. Liu, C. Hirschberg, M. Fanø, H. Mu, A. Müllertz, Evaluation of self-emulsifying drug delivery systems for oral insulin delivery using an in vitro model simulating the intestinal proteolysis, European Journal of Pharmaceutical Sciences, 147 (2020) 105272.
[115] M.J. Ansari, M.K. Anwer, S. Jamil, R. Al-Shdefat, B.E. Ali, M.M. Ahmad, M.N. Ansari, Enhanced oral bioavailability of insulin-loaded solid lipid nanoparticles: pharmacokinetic bioavailability of insulin-loaded solid lipid nanoparticles in diabetic rats, Drug delivery, 23 (2016) 1972-1979.

[116] M. Niu, Y. Lu, L. Hovgaard, W. Wu, Liposomes containing glycocholate as potential oral insulin delivery systems: preparation, in vitro characterization, and improved protection against enzymatic degradation, International journal of nanomedicine, 6 (2011) 1155.
[117] N. Lupo, C. Steinbring, J.D. Friedl, B. Le-Vinh, A. Bernkop-Schnürch, Impact of bile salts and a medium chain fatty acid on the physical properties of self-emulsifying drug delivery systems, Drug Development and Industrial Pharmacy, 47 (2021) 22-35.
[118] K. Senior, Bilosomes: the answer to oral vaccine delivery?, Drug Discovery Today, 6 (2001) 1031-1032.
[119] S. Xie, L. Zhu, Z. Dong, X. Wang, Y. Wang, X. Li, W. Zhou, Preparation, characterization and pharmacokinetics of enrofloxacin-loaded solid lipid nanoparticles: influences of fatty acids, Colloids and Surfaces B: Biointerfaces, 83 (2011) 382-387.
[120] H. Yuan, S.-P. Jiang, Y.-Z. Du, J. Miao, X.-G. Zhang, F.-Q. Hu, Strategic approaches for improving entrapment of hydrophilic peptide drugs by lipid nanoparticles, Colloids and surfaces B: Biointerfaces, 70 (2009) 248-253.
[121] M.H. Richards, C.R. Gardner, Effects of bile salts on the structural integrity of liposomes, Biochimica et Biophysica Acta (BBA)-General Subjects, 543 (1978) 508-522.
[122] J.D. Friedl, C. Steinbring, S. Zaichik, N.N. Le, A. Bernkop-Schnürch, Cellular uptake of self-emulsifying drug-delivery systems: polyethylene glycol versus polyglycerol surface, Nanomedicine (Lond), 15 (2020) 1829-1841.
[123] Z. Zhang, F. Gao, S. Jiang, L. Chen, Z. Liu, H. Yu, Y. Li, Bile salts enhance the intestinal absorption of lipophilic drug loaded lipid nanocarriers: mechanism and effect in rats, International journal of pharmaceutics, 452 (2013) 374-381.
[124] X. Hu, J. Zhang, Z. Yu, Y. Xie, H. He, J. Qi, X. Dong, Y. Lu, W. Zhao, W. Wu, Environment-responsive aza-BODIPY dyes quenching in water as potential probes to visualize the in vivo fate of lipid-based nanocarriers, Nanomedicine, 11 (2015) 1939-1948.
[125] R. Mahjub, F.A. Dorkoosh, M. Rafiee-Tehrani, A. Bernkop Schnürch, Oral self-nanoemulsifying peptide drug delivery systems: impact of lipase on drug release, Journal of microencapsulation, 32 (2015) 401-407.
[126] H.Y. Xue, H.L. Wong, Tailoring nanostructured solid-lipid carriers for time-controlled intracellular siRNA kinetics to sustain RNAi-mediated chemosensitization, Biomaterials, 32 (2011) 2662-2672.
[127] M.A. Maier, M. Jayaraman, S. Matsuda, J. Liu, S. Barros, W. Querbes, Y.K. Tam, S.M. Ansell, V. Kumar, J. Qin, Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics, Molecular Therapy, 21 (2013) 1570-1578.
[128] P.C. Christophersen, L. Zhang, A. Müllertz, H.M. Nielsen, M. Yang, H. Mu, Solid lipid particles for oral delivery of peptide and protein drugs II–the digestion of trilaurin protects desmopressin from proteolytic degradation, Pharmaceutical research, 31 (2014) 2420-2428.
[129] D.J. McClements, Edible lipid nanoparticles: digestion, absorption, and potential toxicity, Progress in lipid research, 52 (2013) 409-423.
[130] G. Leonaviciute, O. Zupančič, F. Prüfert, J. Rohrer, A. Bernkop-Schnürch, Impact of lipases on the protective effect of SEDDS for incorporated peptide drugs towards intestinal peptidases, Int J Pharm, 508 (2016) 102-108.
[131] Y.E. Arnold, G. Imanidis, M. Kuentz, In vitro digestion kinetics of excipients for lipid-based drug delivery and introduction of a relative lipolysis half life, Drug development and industrial pharmacy, 38 (2012) 1262-1269.
[132] N.J. Koehl, R. Holm, M. Kuentz, V. Jannin, B.T. Griffin, Exploring the impact of surfactant type and digestion: Highly digestible surfactants improve oral bioavailability of nilotinib, Molecular Pharmaceutics, 17 (2020) 3202-3213.
[133] X. Wang, S. Jiang, X. Wang, J. Liao, Z. Yin, Preparation and evaluation of nattokinase-loaded self-double-emulsifying drug delivery system, Asian Journal of Pharmaceutical Sciences, 10 (2015) 386-395.

[134] Y. Rosiaux, V. Jannin, S. Hughes, D. Marchaud, Solid lipid excipients as matrix agents for sustained drug delivery, Excipient Applications in Formulation Design and Drug Delivery, (2015) 237-271.
[135] D. Irby, C. Du, F. Li, Lipid–Drug Conjugate for Enhancing Drug Delivery, Molecular Pharmaceutics, 14 (2017) 1325-1338.
[136] T. Kauss, A. Gaubert, L. Tabaran, G. Tonelli, T. Phoeung, M.-H. Langlois, N. White, A. Cartwright, M. Gomes, K. Gaudin, Development of rectal self-emulsifying suspension of a moisture-labile water-soluble drug, International journal of pharmaceutics, 536 (2018) 283-291.
[137] J.-T. Lo, B.-H. Chen, T.-M. Lee, J. Han, J.-L. Li, Self-emulsifying O/W formulations of paclitaxel prepared from mixed nonionic surfactants, Journal of pharmaceutical sciences, 99 (2010) 2320-2332.
[138] S. Singh, M. Bajpai, P. Mishra, Self-Emulsifying Drug Delivery System (SEDDS): An Emerging Dosage Form to Improve the Bioavailability of Poorly Absorbed Drugs, Critical Reviews™ in Therapeutic Drug Carrier Systems, 37 (2020).
[139] G.F. Balata, E.A. Essa, H.A. Shamardl, S.H. Zaidan, M.A.S. Abourehab, Self-emulsifying drug delivery systems as a tool to improve solubility and bioavailability of resveratrol, Drug design, development and therapy, 10 (2016) 117.
[140] N. Akhtar, A. Ahad, R.K. Khar, M. Jaggi, M. Aqil, Z. Iqbal, F.J. Ahmad, S. Talegaonkar, The emerging role of P-glycoprotein inhibitors in drug delivery: a patent review, Expert opinion on therapeutic patents, 21 (2011) 561-576.
[141] A. Ethier, P. Bansal, J. Baxter, N. Langley, N. Richardson, A.M. Patel, The role of excipients in the microstructure of Topical semisolid drug products, The Role of Microstructure in Topical Drug Product Development, Springer2019, pp. 155-193.
[142] N.D. Pujara, Self emulsifying drug delivery system: A Novel approach, International journal of current pharmaceutical research, 4 (2012) 18-23.
[143] F. Prüfert, U. Hering, S. Zaichik, N.-M.N. Le, A. Bernkop-Schnürch, Synthesis and in vitro characterization of a preactivated thiolated acrylic acid/acrylamide-methylpropane sulfonic acid copolymer as a mucoadhesive sprayable polymer, International journal of pharmaceutics, 583 (2020) 119371.
[144] R. Chintalapudi, T. Murthy, K.R. Lakshmi, G.G. Manohar, Formulation, optimization, and evaluation of self-emulsifying drug delivery systems of nevirapine, International journal of pharmaceutical investigation, 5 (2015) 205.
[145] T. Huo, C. Tao, M. Zhang, Q. Liu, B. Lin, Z. Liu, J. Zhang, M. Zhang, H. Yang, J. Wu, Preparation and comparison of tacrolimus-loaded solid dispersion and self-microemulsifying drug delivery system by in vitro/in vivo evaluation, European Journal of Pharmaceutical Sciences, 114 (2018) 74-83.
[146] O.M. Feeney, H.D. Williams, C.W. Pouton, C.J.H. Porter, 'Stealth'lipid-based formulations: Poly (ethylene glycol)-mediated digestion inhibition improves oral bioavailability of a model poorly water soluble drug, Journal of Controlled Release, 192 (2014) 219-227.
[147] I. Shahzadi, A. Fürst, P. Knoll, A. Bernkop-Schnürch, Nanostructured Lipid Carriers (NLCs) for Oral Peptide Drug Delivery: About the Impact of Surface Decoration, Pharmaceutics, 13 (2021).
[148] A. Christiansen, T. Backensfeld, W. Weitschies, Effects of non-ionic surfactants on in vitro triglyceride digestion and their susceptibility to digestion by pancreatic enzymes, European Journal of Pharmaceutical Sciences, 41 (2010) 376-382.
[149] A. Speranza, M.G. Corradini, T.G. Hartman, D. Ribnicky, A. Oren, M.A. Rogers, Influence of emulsifier structure on lipid bioaccessibility in oil–water nanoemulsions, Journal of agricultural and food chemistry, 61 (2013) 6505-6515.
[150] M.H. Michaelsen, S.D.S. Jørgensen, I.M. Abdi, K.M. Wasan, T. Rades, A. Müllertz, Fenofibrate oral absorption from SNEDDS and super-SNEDDS is not significantly affected by lipase inhibition in rats, European Journal of Pharmaceutics and Biopharmaceutics, 142 (2019) 258-264.
[151] J. Chamieh, A.D. Tarrat, C. Doudou, V. Jannin, F. Demarne, H. Cottet, Peptide release from SEDDS containing hydrophobic ion pair therapeutic peptides measured by Taylor dispersion analysis, International journal of pharmaceutics, 559 (2019) 228-234.

[152] S. Pinsuwan, A. Li, S.H. Yalkowsky, Correlation of octanol/water solubility ratios and partition coefficients, Journal of Chemical and Engineering Data, 40 (1995) 623-626.
[153] A. Bernkop-Schnürch, A. Jalil, Do drug release studies from SEDDS make any sense?, Journal of Controlled Release, 271 (2018) 55-59.
[154] A.M. Jörgensen, J.D. Friedl, R. Wibel, J. Chamieh, H. Cottet, A. Bernkop-Schnürch, Cosolvents in Self-Emulsifying Drug Delivery Systems (SEDDS): Do They Really Solve Our Solubility Problems?, Molecular Pharmaceutics, 17 (2020) 3236-3245.
[155] W. Shan, X. Zhu, M. Liu, L. Li, J. Zhong, W. Sun, Z. Zhang, Y. Huang, Overcoming the diffusion barrier of mucus and absorption barrier of epithelium by self-assembled nanoparticles for oral delivery of insulin, ACS nano, 9 (2015) 2345-2356.
[156] L.M. Ensign, B.C. Tang, Y.-Y. Wang, A.T. Terence, T. Hoen, R. Cone, J. Hanes, Mucus-penetrating nanoparticles for vaginal drug delivery protect against herpes simplex virus, Science translational medicine, 4 (2012) 138ra179-138ra179.
[157] Q. Xu, L.M. Ensign, N.J. Boylan, A. Schön, X. Gong, J.C. Yang, N.W. Lamb, S. Cai, T. Yu, E. Freire, J. Hanes, Impact of Surface Polyethylene Glycol (PEG) Density on Biodegradable Nanoparticle Transport in Mucus ex Vivo and Distribution in Vivo, ACS Nano, 9 (2015) 9217-9227.
[158] S.S. Olmsted, J.L. Padgett, A.I. Yudin, K.J. Whaley, T.R. Moench, R.A. Cone, Diffusion of macromolecules and virus-like particles in human cervical mucus, Biophyisical journal, 81 (2001) 1930-1937.
[159] H.M. Yildiz, C.A. McKelvey, P.J. Marsac, R.L. Carrier, Size selectivity of intestinal mucus to diffusing particulates is dependent on surface chemistry and exposure to lipids, Journal of drug targeting, 23 (2015) 768-774.
[160] K. Maisel, L. Ensign, M. Reddy, R. Cone, J. Hanes, Effect of surface chemistry on nanoparticle interaction with gastrointestinal mucus and distribution in the gastrointestinal tract following oral and rectal administration in the mouse, Journal of Controlled Release, 197 (2015) 48-57.
[161] L. Wu, W. Shan, Z. Zhang, Y. Huang, Engineering nanomaterials to overcome the mucosal barrier by modulating surface properties, Advanced drug delivery reviews, 124 (2018) 150-163.
[162] K. Maisel, M. Reddy, Q. Xu, S. Chattopadhyay, R. Cone, L.M. Ensign, J. Hanes, Nanoparticles coated with high molecular weight PEG penetrate mucus and provide uniform vaginal and colorectal distribution in vivo, Nanomedicine, 11 (2016) 1337-1343.
[163] S. Dünnhaupt, O. Kammona, C. Waldner, C. Kiparissides, A. Bernkop-Schnürch, Nano-carrier systems: strategies to overcome the mucus gel barrier, European Journal of Pharmaceutics and Biopharmaceutics, 96 (2015) 447-453.
[164] H. Yuan, C.-Y. Chen, G.-h. Chai, Y.-Z. Du, F.-Q. Hu, Improved transport and absorption through gastrointestinal tract by PEGylated solid lipid nanoparticles, Molecular pharmaceutics, 10 (2013) 1865-1873.
[165] H. Friedl, S. Dünnhaupt, F. Hintzen, C. Waldner, S. Parikh, J.P. Pearson, M.D. Wilcox, A. Bernkop-Schnürch, Development and evaluation of a novel mucus diffusion test system approved by self-nanoemulsifying drug delivery systems, Journal of pharmaceutical sciences, 102 (2013) 4406-4413.
[166] J. Wu, W. Lin, Z. Wang, S. Chen, Y. Chang, Investigation of the Hydration of Nonfouling Material Poly(sulfobetaine methacrylate) by Low-Field Nuclear Magnetic Resonance, Langmuir, 28 (2012) 7436-7441.
[167] G. Gregoriadis, B.E. Ryman, Liposomes as carriers of enzymes or drugs: a new approach to the treatment of storage diseases, Biochemical Journal, 124 (1971) 58P-58P.
[168] O. Lieleg, R.M. Baumgärtel, A.R. Bausch, Selective filtering of particles by the extracellular matrix: an electrostatic bandpass, Biophys Journal, 97 (2009) 1569-1577.
[169] W. Shan, X. Zhu, W. Tao, Y. Cui, M. Liu, L. Wu, L. Li, Y. Zheng, Y. Huang, Enhanced Oral Delivery of Protein Drugs Using Zwitterion-Functionalized Nanoparticles to Overcome both the Diffusion and Absorption Barriers, ACS Applied Materials & Interfaces, 8 (2016) 25444-25453.
[170] Z. Yu, W. Fan, L. Wang, J. Qi, Y. Lu, W. Wu, Effect of surface charges on oral absorption of intact solid lipid nanoparticles, Molecular pharmaceutics, 16 (2019) 5013-5024.

[171] X. Hu, W. Fan, Z. Yu, Y. Lu, J. Qi, J. Zhang, X. Dong, W. Zhao, W. Wu, Evidence does not support absorption of intact solid lipid nanoparticles via oral delivery, Nanoscale, 8 (2016) 7024-7035.
[172] S. Alqahtani, A. Alayoubi, S. Nazzal, P.W. Sylvester, A. Kaddoumi, Nonlinear absorption kinetics of self-emulsifying drug delivery systems (SEDDS) containing tocotrienols as lipophilic molecules: in vivo and in vitro studies, The AAPS journal, 15 (2013) 684-695.
[173] S. Martins, S. Costa-Lima, T. Carneiro, A. Cordeiro-da-Silva, E.B. Souto, D.C. Ferreira, Solid lipid nanoparticles as intracellular drug transporters: an investigation of the uptake mechanism and pathway, International journal of pharmaceutics, 430 (2012) 216-227.
[174] Q. Li, D. Xia, J. Tao, A. Shen, Y. He, Y. Gan, C. Wang, Self-assembled core-shell-type lipid-polymer hybrid nanoparticles: intracellular trafficking and relevance for oral absorption, Journal of pharmaceutical sciences, 106 (2017) 3120-3130.
[175] T. Wang, Y. Luo, Biological fate of ingested lipid-based nanoparticles: current understanding and future directions, Nanoscale, 11 (2019) 11048-11063.
[176] A. Beloqui, M.Á. Solinís, A.R. Gascón, A. del Pozo-Rodríguez, A. des Rieux, V. Préat, Mechanism of transport of saquinavir-loaded nanostructured lipid carriers across the intestinal barrier, Journal of Controlled Release, 166 (2013) 115-123.
[177] K.S. Kim, K. Suzuki, H. Cho, Y.S. Youn, Y.H. Bae, Oral Nanoparticles Exhibit Specific High-Efficiency Intestinal Uptake and Lymphatic Transport, ACS Nano, 12 (2018) 8893-8900.
[178] C.M. O'Driscoll, Lipid-based formulations for intestinal lymphatic delivery, European Journal of Pharmaceutical Sciences, 15 (2002) 405-415.
[179] S. Chaudhary, T. Garg, R.S.R. Murthy, G. Rath, A.K. Goyal, Recent approaches of lipid-based delivery system for lymphatic targeting via oral route, Journal of drug targeting, 22 (2014) 871-882.
[180] S. Kalepu, M. Manthina, V. Padavala, Oral lipid-based drug delivery systems–an overview, Acta Pharmaceutica Sinica B, 3 (2013) 361-372.
[181] H.M. El-Laithy, E.B. Basalious, B.M. El-Hoseiny, M.M. Adel, Novel self-nanoemulsifying self-nanosuspension (SNESNS) for enhancing oral bioavailability of diacerein: simultaneous portal blood absorption and lymphatic delivery, International journal of pharmaceutics, 490 (2015) 146-154.
[182] J. Wu, Y. Zheng, M. Liu, W. Shan, Z. Zhang, Y. Huang, Biomimetic viruslike and charge reversible nanoparticles to sequentially overcome mucus and epithelial barriers for oral insulin delivery, ACS applied materials & interfaces, 10 (2018) 9916-9928.
[183] A. Zhou, T. Lu, L. Wang, C. Lu, L. Wang, M. Wan, H. Wu, Lymphatic transport of puerarin occurs after oral administration of different lipid-based formulations to unconscious lymph duct-cannulated rats, Pharmaceutical development and technology, 19 (2014) 743-747.
[184] M.C. Tekeli, Y. Aktas, N. Celebi, Oral self-nanoemulsifying formulation of GLP-1 agonist peptide exendin-4: development, characterization and permeability assesment on Caco-2 cell monolayer, Amino Acids, 53 (2021) 73-88.
[185] C. Chen, X. Zhu, Y. Dou, J. Xu, J. Zhang, T. Fan, J. Du, K. Liu, Y. Deng, L. Zhao, Exendin-4 loaded nanoparticles with a lipid shell and aqueous core containing micelles for enhanced intestinal absorption, Journal of biomedical nanotechnology, 11 (2015) 865-876.
[186] P. Li, A. Tan, C.A. Prestidge, H.M. Nielsen, A. Müllertz, Self-nanoemulsifying drug delivery systems for oral insulin delivery: In vitro and in vivo evaluations of enteric coating and drug loading, International Journal of Pharmaceutics, 477 (2014) 390-398.
[187] Y. Xu, Y. Zheng, L. Wu, X. Zhu, Z. Zhang, Y. Huang, Novel solid lipid nanoparticle with endosomal escape function for oral delivery of insulin, ACS applied materials & interfaces, 10 (2018) 9315-9324.
[188] F. Bahman, S. Taurin, D. Altayeb, S. Taha, M. Bakhiet, K. Greish, Oral Insulin Delivery Using Poly (Styrene Co-Maleic Acid) Micelles in a Diabetic Mouse Model, Pharmaceutics, 12 (2020) 1026.
[189] J. Hecq, K. Amighi, J. Goole, Development and evaluation of insulin-loaded cationic solid lipid nanoparticles for oral delivery, Journal of Drug Delivery Science and Technology, 36 (2016) 192-200.
[190] R.B. Anchan, M. Koland, Oral Insulin Delivery by Chitosan Coated Solid Lipid Nanoparticles: Ex vivo and in vivo Studies, Journal of Young Pharmacists, 13 (2021).

[191] Z.-H. Zhang, S. Abbad, R.-R. Pan, A.Y. Waddad, L.-L. Hou, H.-X. Lv, J.-P. Zhou, N-octyl-N-arginine chitosan micelles as an oral delivery system of insulin, Journal of biomedical nanotechnology, 9 (2013) 601-609.
[192] N. Li, X.-R. Li, Y.-X. Zhou, W.-J. Li, Y. Zhao, S.-J. Ma, J.-W. Li, Y.-J. Gao, Y. Liu, X.-L. Wang, The use of polyion complex micelles to enhance the oral delivery of salmon calcitonin and transport mechanism across the intestinal epithelial barrier, Biomaterials, 33 (2012) 8881-8892.
[193] S.V.R. Rao, K. Yajurvedi, J. Shao, Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of protein drugs: III. In vivo oral absorption study, International journal of pharmaceutics, 362 (2008) 16-19.
[194] N. Shrestha, O. Bouttefeux, K. Vanvarenberg, P. Lundquist, J. Cunarro, S. Tovar, G. Khodus, E. Andersson, Å.V. Keita, C.G. Dieguez, The stimulation of GLP-1 secretion and delivery of GLP-1 agonists via nanostructured lipid carriers, Nanoscale, 10 (2018) 603-613.
[195] M.F. Francis, M. Cristea, F.M. Winnik, Exploiting the vitamin B12 pathway to enhance oral drug delivery via polymeric micelles, Biomacromolecules, 6 (2005) 2462-2467.
[196] S.J. Lee, P.H. Schlesinger, S.A. Wickline, G.M. Lanza, N.A. Baker, Simulation of fusion-mediated nanoemulsion interactions with model lipid bilayers, Soft Matter, 8 (2012) 3024-3035.
[197] D. Hoekstra, O.C. Martin, Transbilayer redistribution of phosphatidylethanolamine during fusion of phospholipid vesicles. Dependence on fusion rate, lipid phase separation, and formation of nonbilayer structures, Biochemistry, 21 (1982) 6097-6103.
[198] J. Yang, A. Bahreman, G. Daudey, J. Bussmann, R.C.L. Olsthoorn, A. Kros, Drug delivery via cell membrane fusion using lipopeptide modified liposomes, ACS central science, 2 (2016) 621-630.
[199] K. Bunchongprasert, J. Shao, Cytotoxicity and permeability enhancement of Capmul® MCM in nanoemulsion formulation, International journal of pharmaceutics, 561 (2019) 289-295.
[200] B. Wahlang, D. Kabra, Y.B. Pawar, K. Tikoo, A.K. Bansal, Contribution of formulation and excipients towards enhanced permeation of curcumin, Arzneimittelforschung, 62 (2012) 88-93.
[201] A.A. Khan, J. Mudassir, N. Mohtar, Y. Darwis, Advanced drug delivery to the lymphatic system: lipid-based nanoformulations, International journal of nanomedicine, 8 (2013) 2733.
[202] N.A. Mabbott, D.S. Donaldson, H. Ohno, I.R. Williams, A. Mahajan, Microfold (M) cells: important immunosurveillance posts in the intestinal epithelium, Mucosal immunology, 6 (2013) 666-677.
[203] Y. Aramaki, H. Tomizawa, T. Hara, K. Yachi, H. Kikuchi, S. Tsuchiya, Stability of liposomes in vitro and their uptake by rat Peyer's patches following oral administration, Pharm Res, 10 (1993) 1228-1231.
[204] G.A. Ramaldes, M.A. Pereira, P.T.d. Castro, L.M. Vieira, V.N. Cardoso, Uptake of liposomes by Peyer´ s patches following per os administration in mice, Revista Brasileira de Ciências Farmacêuticas, 38 (2002) 173-182.
[205] A. Bargoni, R. Cavalli, O. Caputo, A. Fundarò, M.R. Gasco, G.P. Zara, Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats, Pharmaceutical research, 15 (1998) 745-750.
[206] H. Tomizawa, Y. Aramaki, Y. Fujii, T. Kara, N. Suzuki, K. Yachi, H. Kikuchi, S. Tsuchiya, Uptake of phosphatidylserine liposomes by rat Peyer's patches following intraluminal administration, Pharmaceutical research, 10 (1993) 549-552.
[207] H. Harde, M. Das, S. Jain, Solid lipid nanoparticles: an oral bioavailability enhancer vehicle, Expert Opinion on Drug Delivery, 8 (2011) 1407-1424.
[208] Y. Aramaki, H. Tomizawa, T. Hara, K. Yachi, H. Kikuchi, S. Tsuchiya, Stability of liposomes in vitro and their uptake by rat Peyer's patches following oral administration, Pharmaceutical research, 10 (1993) 1228-1231.
[209] J.B. Prajapati, S.D. Verma, A.A. Patel, Oral bioavailability enhancement of agomelatine by loading into nanostructured lipid carriers: Peyer's patch targeting approach, International journal of nanomedicine, 13 (2018) 35-38.
[210] M. Ramırez, L. Amate, A. Gil, Absorption and distribution of dietary fatty acids from different sources, Early human development, 65 (2001) S95-S101.

[211] P. Ganesan, D. Narayanasamy, Lipid nanoparticles: Different preparation techniques, characterization, hurdles, and strategies for the production of solid lipid nanoparticles and nanostructured lipid carriers for oral drug delivery, Sustainable Chemistry and Pharmacy, 6 (2017) 37-56.
[212] D.J. McClements, J. Rao, Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity, Critical reviews in food science and nutrition, 51 (2011) 285-330.
[213] V. Ghosh, S. Saranya, A. Mukherjee, N. Chandrasekaran, Antibacterial microemulsion prevents sepsis and triggers healing of wound in wistar rats, Colloids and Surfaces B: Biointerfaces, 105 (2013) 152-157.
[214] M.J. Lawrence, G.D. Rees, Microemulsion-based media as novel drug delivery systems, Advanced drug delivery reviews, 45 (2000) 89-121.
[215] G. Amoabediny, F. Haghiralsadat, S. Naderinezhad, M.N. Helder, E. Akhoundi Kharanaghi, J. Mohammadnejad Arough, B. Zandieh-Doulabi, Overview of preparation methods of polymeric and lipid-based (niosome, solid lipid, liposome) nanoparticles: A comprehensive review, International Journal of Polymeric Materials and Polymeric Biomaterials, 67 (2018) 383-400.
[216] D. Shukla, S. Chakraborty, S. Singh, B. Mishra, Lipid-based oral multiparticulate formulations–advantages, technological advances and industrial applications, Expert opinion on drug delivery, 8 (2011) 207-224.
[217] E. Pupo, A. Padrón, E. Santana, J. Sotolongo, D. Quintana, S. Dueñas, C. Duarte, C. Maria, E. Hardy, Preparation of plasmid DNA-containing liposomes using a high-pressure homogenization–extrusion technique, Journal of controlled release, 104 (2005) 379-396.
[218] M.A. Iqbal, S. Md, J.K. Sahni, S. Baboota, S. Dang, J. Ali, Nanostructured lipid carriers system: recent advances in drug delivery, Journal of drug targeting, 20 (2012) 813-830.
[219] S. Tokunaga, H. Tashiro, K. Ono, T. Sharmin, T. Kato, K. Irie, K. Mishima, T. Satho, T.M. Aida, K. Mishima, Rapid production of liposomes using high pressure carbon dioxide and direct ultrasonication, The Journal of Supercritical Fluids, 160 (2020) 104782.
[220] L. Pavoni, D.R. Perinelli, G. Bonacucina, M. Cespi, G.F. Palmieri, An overview of micro-and nanoemulsions as vehicles for essential oils: Formulation, preparation and stability, Nanomaterials, 10 (2020) 135.
[221] J. Flanagan, H. Singh, Microemulsions: a potential delivery system for bioactives in food, Critical reviews in food science and nutrition, 46 (2006) 221-237.
[222] Y. Singh, J.G. Meher, K. Raval, F.A. Khan, M. Chaurasia, N.K. Jain, M.K. Chourasia, Nanoemulsion: Concepts, development and applications in drug delivery, J Control Release, 252 (2017) 28-49.
[223] L. Battaglia, M. Gallarate, R. Cavalli, M. Trotta, Solid lipid nanoparticles produced through a coacervation method, Journal of microencapsulation, 27 (2010) 78-85.
[224] M. Gallarate, L. Battaglia, E. Peira, M. Trotta, Peptide-Loaded Solid Lipid Nanoparticles Prepared through Coacervation Technique, International Journal of Chemical Engineering, 2011 (2011) 132435.
[225] M.A. Bianco, M. Gallarate, M. Trotta, L. Battaglia, Amphotericin B loaded SLN prepared with the coacervation technique JOURNAL OF DRUG DELIVERY SCIENCE AND TECHNOLOGY (2010) 20, Journal of Drug Delivery Science and Technology, 20 (2010) 187-191.
[226] P. Chattopadhyay, R. Huff, B.Y. Shekunov, Drug encapsulation using supercritical fluid extraction of emulsions, Journal of Pharmaceutical Sciences, 95 (2006) 667-679.
[227] S.R. Mudshinge, A.B. Deore, S. Patil, C.M. Bhalgat, Nanoparticles: emerging carriers for drug delivery, Saudi pharmaceutical journal, 19 (2011) 129-141.
[228] A. Laouini, C. Jaafar-Maalej, I. Limayem-Blouza, S. Sfar, C. Charcosset, H. Fessi, Preparation, characterization and applications of liposomes: state of the art, Journal of colloid Science and Biotechnology, 1 (2012) 147-168.

[229] M. Cagel, F.C. Tesan, E. Bernabeu, M.J. Salgueiro, M.B. Zubillaga, M.A. Moretton, D.A. Chiappetta, Polymeric mixed micelles as nanomedicines: Achievements and perspectives, European Journal of Pharmaceutics and Biopharmaceutics, 113 (2017) 211-228.
[230] H. Zhang, L. Zhao, L. Chu, X. Han, G. Zhai, Preparation, optimization, characterization and cytotoxicity in vitro of Baicalin-loaded mixed micelles, Journal of colloid and interface science, 434 (2014) 40-47.
[231] D.W. Deamer, Preparation and properties of ether-injection liposomes, Annals of the New York Academy of Sciences, 308 (1978) 250-258.
[232] P. Stano, S. Bufali, C. Pisano, F. Bucci, M. Barbarino, M. Santaniello, P. Carminati, P.L. Luisi, Novel camptothecin analogue (gimatecan)-containing liposomes prepared by the ethanol injection method, Journal of liposome research, 14 (2004) 87-109.
[233] I. Maji, S. Mahajan, A. Sriram, P. Medtiya, R. Vasave, D.K. Khatri, R. Kumar, S.B. Singh, J. Madan, P.K. Singh, Solid self emulsifying drug delivery system: Superior mode for oral delivery of hydrophobic cargos, Journal of Controlled Release, (2021).
[234] D.E. Dobry, D.M. Settell, J.M. Baumann, R.J. Ray, L.J. Graham, R.A. Beyerinck, A model-based methodology for spray-drying process development, Journal of pharmaceutical innovation, 4 (2009) 133-142.
[235] D.K. Tan, M. Maniruzzaman, A. Nokhodchi, Advanced pharmaceutical applications of hot-melt extrusion coupled with fused deposition modelling (FDM) 3D printing for personalised drug delivery, Pharmaceutics, 10 (2018) 203.
[236] K.A. Gaidhani, M. Harwalkar, D. Bhambere, P.S. Nirgude, Lyophilization/freeze drying–a review, World journal of pharmaceutical research, 4 (2015) 516-543.
[237] G. Leonaviciute, A. Bernkop-Schnürch, Self-emulsifying drug delivery systems in oral (poly) peptide drug delivery, Expert opinion on drug delivery, 12 (2015) 1703-1716.
[238] J. Kubackova, O. Holas, J. Zbytovska, B. Vranikova, G. Zeng, P. Pavek, A. Mullertz, Oligonucleotide Delivery across the Caco-2 Monolayer: The Design and Evaluation of Self-Emulsifying Drug Delivery Systems (SEDDS), Pharmaceutics, 13 (2021) 459.
[239] D. Sakloetsakun, S. Dünnhaupt, J. Barthelmes, G. Perera, A. Bernkop-Schnürch, Combining two technologies: Multifunctional polymers and self-nanoemulsifying drug delivery system (SNEDDS) for oral insulin administration, International journal of biological macromolecules, 61 (2013) 363-372.
[240] L. Anil, K. Kannan, Microemulsion as drug delivery system for Peptides and Proteins, Journal of Pharmaceutical Sciences and Research, 10 (2018) 16-25.
[241] P. Joyce, T.J. Dening, T.R. Meola, H.B. Schultz, R. Holm, N. Thomas, C.A. Prestidge, Solidification to improve the biopharmaceutical performance of SEDDS: Opportunities and challenges, Advanced drug delivery reviews, 142 (2019) 102-117.
[242] H. Etezadi, A. Maleki, J.D. Friedl, A. Bernkop-Schnürch, Storage stability of proteins in a liquid-based formulation: Liquid vs. solid self-emulsifying drug delivery, International Journal of Pharmaceutics, 590 (2020) 119918.
[243] J.D. Friedl, A.M. Jörgensen, B. Le-Vinh, D.E. Braun, M. Tribus, A. Bernkop-Schnürch, Solidification of self-emulsifying drug delivery systems (SEDDS): Impact on storage stability of a therapeutic protein, Journal of Colloid and Interface Science, 584 (2021) 684-697.
[244] B. Singh, S. Bandopadhyay, R. Kapil, R. Singh, O.P. Katare, Self-emulsifying drug delivery systems (SEDDS): formulation development, characterization, and applications, Critical Reviews™ in Therapeutic Drug Carrier Systems, 26 (2009).
[245] K. AboulFotouh, A.A. Allam, M. El-Badry, A.M. El-Sayed, Role of self-emulsifying drug delivery systems in optimizing the oral delivery of hydrophilic macromolecules and reducing interindividual variability, Colloids and Surfaces B: Biointerfaces, 167 (2018) 82-92.
[246] C. Menzel, T. Holzeisen, F. Laffleur, S. Zaichik, M. Abdulkarim, M. Gumbleton, A. Bernkop-Schnürch, In vivo evaluation of an oral self-emulsifying drug delivery system (SEDDS) for exenatide, Journal of Controlled Release, 277 (2018) 165-172.

[247] T.N.Q. Phan, R. Ismail, B. Le-Vinh, S. Zaichik, F. Laffleur, A. Bernkop-Schnürch, The Effect of Counterions in Hydrophobic Ion Pairs on Oral Bioavailability of Exenatide, ACS Biomaterials Science & Engineering, 6 (2020) 5032-5039.
[248] E.-l. Ma, H. Ma, Z. Liu, C.-x. Zheng, M.-x. Duan, In vitro and in vivo evaluation of a novel oral insulin formulation, Acta pharmacologica sinica, 27 (2006) 1382-1388.
[249] J. Liu, U. Werner, M. Funke, M. Besenius, L. Saaby, M. Fanø, H. Mu, A. Müllertz, SEDDS for intestinal absorption of insulin: Application of Caco-2 and Caco-2/HT29 co-culture monolayers and intra-jejunal instillation in rats, International journal of pharmaceutics, 560 (2019) 377-384.
[250] A.J. Almeida, E. Souto, Solid lipid nanoparticles as a drug delivery system for peptides and proteins, Advanced drug delivery reviews, 59 (2007) 478-490.
[251] A.J. Almeida, S. Runge, R.H. Müller, Peptide-loaded solid lipid nanoparticles (SLN): influence of production parameters, International Journal of Pharmaceutics, 149 (1997) 255-265.
[252] C. Dumont, V. Jannin, C. Miolane, Q. Lelong, J.-P. Valour, S. Urbaniak, H. Fessi, S. Bourgeois, A proof-of-concept for developing oral lipidized peptide Nanostructured Lipid Carrier formulations, Journal of Drug Delivery Science and Technology, 54 (2019) 101394.
[253] F.Q. Hu, Y. Hong, H. Yuan, Preparation and characterization of solid lipid nanoparticles containing peptide, International journal of pharmaceutics, 273 (2004) 29-35.
[254] M. Üner, Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): their benefits as colloidal drug carrier systems, Die pharmazie-an international journal of pharmaceutical sciences, 61 (2006) 375-386.
[255] R.H. Müller, S.A. Runge, V. Ravelli, A.F. Thünemann, W. Mehnert, E.B. Souto, Cyclosporine-loaded solid lipid nanoparticles (SLN®): Drug–lipid physicochemical interactions and characterization of drug incorporation, European journal of pharmaceutics and biopharmaceutics, 68 (2008) 535-544.
[256] R.H. Müller, S. Runge, V. Ravelli, W. Mehnert, A.F. Thünemann, E.B. Souto, Oral bioavailability of cyclosporine: solid lipid nanoparticles (SLN®) versus drug nanocrystals, International journal of pharmaceutics, 317 (2006) 82-89.
[257] N. Zhang, Q. Ping, G. Huang, W. Xu, Y. Cheng, X. Han, Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin, International journal of pharmaceutics, 327 (2006) 153-159.
[258] M. Gallarate, L. Battaglia, E. Peira, M. Trotta, Peptide-loaded solid lipid nanoparticles prepared through coacervation technique, International Journal of Chemical Engineering, 2011 (2011).
[259] L. Wang, C.-Y. Wang, Y. Zhang, H.-J. Fu, Y. Gao, K.-R. Zhang, Preparation and characterization of solid lipid nanoparticles loaded with salmon calcitonin phospholipid complex, Journal of Drug Delivery Science and Technology, 52 (2019) 838-845.
[260] C. Chen, T. Fan, Y. Jin, Z. Zhou, Y. Yang, X. Zhu, Z.-r. Zhang, Q. Zhang, Y. Huang, Orally delivered salmon calcitonin-loaded solid lipid nanoparticles prepared by micelle–double emulsion method via the combined use of different solid lipids, Nanomedicine, 8 (2013) 1085-1100.
[261] S. Morel, E. Ugazio, R. Cavalli, M.R. Gasco, Thymopentin in solid lipid nanoparticles, International journal of pharmaceutics, 132 (1996) 259-261.
[262] J. Parmentier, B. Thewes, F. Gropp, G. Fricker, Oral peptide delivery by tetraether lipid liposomes, International journal of pharmaceutics, 415 (2011) 150-157.
[263] M. Werle, H. Takeuchi, Chitosan–aprotinin coated liposomes for oral peptide delivery: development, characterisation and in vivo evaluation, International journal of pharmaceutics, 370 (2009) 26-32.
[264] E. Yamazoe, J.-Y. Fang, K. Tahara, Oral mucus-penetrating PEGylated liposomes to improve drug absorption: Differences in the interaction mechanisms of a mucoadhesive liposome, International Journal of Pharmaceutics, 593 (2021) 120148.
[265] M. Niu, Y.n. Tan, P. Guan, L. Hovgaard, Y. Lu, J. Qi, R. Lian, X. Li, W. Wu, Enhanced oral absorption of insulin-loaded liposomes containing bile salts: a mechanistic study, International journal of pharmaceutics, 460 (2014) 119-130.
[266] C. Hui, H. Huang, A study on chitosan-coated liposomes as a carrier of bovine serum albumin as oral protein drug, Journal of Dispersion Science and Technology, (2021) 1-10.

[267] K. Gradauer, J. Barthelmes, C. Vonach, G. Almer, H. Mangge, B. Teubl, E. Roblegg, S. Dünnhaupt, E. Fröhlich, A. Bernkop-Schnürch, R. Prassl, Liposomes coated with thiolated chitosan enhance oral peptide delivery to rats, Journal of Controlled Release, 172 (2013) 872-878.
[268] K. Suzuki, K.S. Kim, Y.H. Bae, Long-term oral administration of Exendin-4 to control type 2 diabetes in a rat model, Journal of Controlled Release, 294 (2019) 259-267.
[269] J.C. Byeon, S.-E. Lee, T.-H. Kim, J.B. Ahn, D.-H. Kim, J.-S. Choi, J.-S. Park, Design of novel proliposome formulation for antioxidant peptide, glutathione with enhanced oral bioavailability and stability, Drug delivery, 26 (2019) 216-225.
[270] J. Parmentier, G. Hofhaus, S. Thomas, L.C. Cuesta, F. Gropp, R. Schröder, K. Hartmann, G. Fricker, Improved oral bioavailability of human growth hormone by a combination of liposomes containing bio-enhancers and tetraether lipids and omeprazole, Journal of pharmaceutical sciences, 103 (2014) 3985-3993.
[271] M. Niu, Y. Lu, L. Hovgaard, P. Guan, Y. Tan, R. Lian, J. Qi, W. Wu, Hypoglycemic activity and oral bioavailability of insulin-loaded liposomes containing bile salts in rats: the effect of cholate type, particle size and administered dose, European journal of pharmaceutics and biopharmaceutics, 81 (2012) 265-272.
[272] X. Zhang, J. Qi, Y. Lu, W. He, X. Li, W. Wu, Biotinylated liposomes as potential carriers for the oral delivery of insulin, Nanomedicine, 10 (2014) 167-176.
[273] H. He, Y. Lu, J. Qi, W. Zhao, X. Dong, W. Wu, Biomimetic thiamine-and niacin-decorated liposomes for enhanced oral delivery of insulin, Acta Pharmaceutica Sinica B, 8 (2018) 97-105.
[274] T.I. Shalaby, W.M. El-Refaie, Bioadhesive chitosan-coated cationic nanoliposomes with improved insulin encapsulation and prolonged oral hypoglycemic effect in diabetic mice, Journal of pharmaceutical sciences, 107 (2018) 2136-2143.
[275] E. Campione, C. Lanna, T. Cosio, L. Rosa, M.P. Conte, F. Iacovelli, A. Romeo, M. Falconi, C. Del Vecchio, E. Franchin, Pleiotropic effect of Lactoferrin in the prevention and treatment of COVID-19 infection: randomized clinical trial, in vitro and in silico preliminary evidences, BioRxiv, (2020).
[276] G. Serrano, I. Kochergina, A. Albors, E. Diaz, M. Oroval, G. Hueso, J.M. Serrano, Liposomal lactoferrin as potential preventative and cure for COVID-19, International journal of research in health science 8(2020) 8-15.
[277] K. Cholkar, A. Patel, A. Dutt Vadlapudi, A. K Mitra, Novel nanomicellar formulation approaches for anterior and posterior segment ocular drug delivery, Recent patents on nanomedicine, 2 (2012) 82-95.
[278] A. Patel, K. Cholkar, A.K. Mitra, Recent developments in protein and peptide parenteral delivery approaches, Therapeutic delivery, 5 (2014) 337-365.
[279] B. Ashok, L. Arleth, R.P. Hjelm, I. Rubinstein, H. Önyüksel, In vitro characterization of PEGylated phospholipid micelles for improved drug solubilization: effects of PEG chain length and PC incorporation, Journal of pharmaceutical sciences, 93 (2004) 2476-2487.
[280] L. Vukovic, F.A. Khatib, S.P. Drake, A. Madriaga, K.S. Brandenburg, P. Král, H. Onyuksel, Structure and dynamics of highly PEG-ylated sterically stabilized micelles in aqueous media, Journal of the American Chemical Society, 133 (2011) 13481-13488.
[281] A. Krishnadas, H. Onyuksel, I. Rubinstein, Interactions of VIP, secretin and PACAP1-38 with phospholipids: a biological paradox revisited, Current pharmaceutical design, 9 (2003) 1005-1012.
[282] K. Esparza, D. Jayawardena, H. Onyuksel, Phospholipid micelles for peptide drug delivery, Pharmaceutical Nanotechnology, Springer2019, pp. 43-57.
[283] C. Katsuda, K. Niiyama, E. Obana, T. Yamamoto, Y. Katou, M. Kataoka, K. Ohkura, Y. Shinohara, Specific formation of trypsin-resistant micelles on a hydrophobic peptide observed with Triton X-100 but not with octylglucoside, Biochimica et Biophysica Acta (BBA)-Biomembranes, 1798 (2010) 2090-2093.
[284] P. Zhang, Y. Xu, X. Zhu, Y. Huang, Goblet cell targeting nanoparticle containing drug-loaded micelle cores for oral delivery of insulin, International journal of pharmaceutics, 496 (2015) 993-1005.

[285] X. Han, Y. Lu, J. Xie, E. Zhang, H. Zhu, H. Du, K. Wang, B. Song, C. Yang, Y. Shi, Z. Cao, Zwitterionic micelles efficiently deliver oral insulin without opening tight junctions, Nature Nanotechnology, 15 (2020) 605-614.
[286] I. Shahzadi, A. Jalil, M.H. Asim, A. Hupfauf, R. Gust, P.A. Nelles, L. Knabl, A. Bernkop-Schnürch, Lipophilic arginine esters: The gateway to preservatives without side effects, Molecular pharmaceutics, 17 (2020) 3129-3139.
[287] FDA, Novel Excipient Review Pilot Program, FDA, https://www.fda.gov/drugs/development-approval-process-drugs/novel-excipient-review-pilot-program.
[288] A. Bernkop-Schnürch, Reprint of: Nanocarrier systems for oral drug delivery: Do we really need them?, European Journal of Pharmaceutical Sciences, 50 (2013) 2-7.
[289] Q. Zhu, Z. Chen, P.K. Paul, Y. Lu, W. Wu, J. Qi, Oral delivery of proteins and peptides: Challenges, status quo and future perspectives, Acta Pharmaceutica Sinica B, (2021).
[290] D. Kumar Malik, S. Baboota, A. Ahuja, S. Hasan, J. Ali, Recent advances in protein and peptide drug delivery systems, Current drug delivery, 4 (2007) 141-151.
[291] A. Yadav, S. Mohite, Recent advances in protein and peptide drug delivery, Research journal of pharmaceutical dosage forms and technology 2020; 12 (3): 205, 212 (2020).
[292] D.J. Drucker, Advances in oral peptide therapeutics, Nature reviews Drug discovery, 19 (2020) 277-289.
[293] J.R. Jørgensen, M.L. Jepsen, L.H. Nielsen, M. Dufva, H.M. Nielsen, T. Rades, A. Boisen, A. Müllertz, Microcontainers for oral insulin delivery–in vitro studies of permeation enhancement, European Journal of Pharmaceutics and Biopharmaceutics, 143 (2019) 98-105.
[294] S. Volpi, U. Cancelli, M. Neri, R. Corradini, Multifunctional Delivery Systems for Peptide Nucleic Acids, Pharmaceuticals, 14 (2021) 14.
[295] H.F. Darge, E.Y. Hanurry, Y.S. Birhan, T.W. Mekonnen, A.T. Andrgie, H.-Y. Chou, J.-Y. Lai, H.-C. Tsai, Multifunctional drug-loaded micelles encapsulated in thermo-sensitive hydrogel for in vivo local cancer treatment: Synergistic effects of anti-vascular and immuno-chemotherapy, Chemical Engineering Journal, 406 (2021) 126879.
[296] A. Banerjee, K. Ibsen, T. Brown, R. Chen, C. Agatemor, S. Mitragotri, Ionic liquids for oral insulin delivery, Proceedings of the National Academy of Sciences, 115 (2018) 7296-7301.
[297] T. Liu, M. Chen, J. Fu, Y. Sun, C. Lu, G. Quan, X. Pan, C. Wu, Recent advances in microneedles-mediated transdermal delivery of protein and peptide drugs, Acta Pharmaceutica Sinica B, (2021) 2326-2343.
[298] M.H. Asfour, Advanced trends in protein and peptide drug delivery: a special emphasis on aquasomes and microneedles techniques, Drug delivery and translational research, (2020) 1-23.
[299] E. Caffarel-Salvador, S. Kim, V. Soares, R.Y. Tian, S.R. Stern, D. Minahan, R. Yona, X. Lu, F.R. Zakaria, J. Collins, A microneedle platform for buccal macromolecule delivery, Science Advances, 7 (2021) eabe2620.
[300] S.T. Buckley, F. Hubálek, U.L. Rahbek, Chemically modified peptides and proteins-critical considerations for oral delivery, Tissue barriers, 4 (2016) e1156805.
[301] Y. Li, C. Zhu, Mechanism of hepatic targeting via oral administration of DSPE–PEG–cholic acid-modified nanoliposomes, International journal of nanomedicine, 12 (2017) 1673.
[302] A. Mahmood, F. Prüfert, N.A. Efiana, M.I. Ashraf, M. Hermann, S. Hussain, A. Bernkop-Schnürch, Cell-penetrating self-nanoemulsifying drug delivery systems (SNEDDS) for oral gene delivery, Expert opinion on drug delivery, 13 (2016) 1503-1512.
[303] J.D. Wolf, M. Kurpiers, R.X. Götz, S. Zaichik, A. Hupfauf, D. Baecker, R. Gust, A. Bernkop-Schnürch, Phosphorylated PEG-emulsifier: Powerful tool for development of zeta potential changing self-emulsifying drug delivery systems (SEDDS), European Journal of Pharmaceutics and Biopharmaceutics, 150 (2020) 77-86.
[304] N.-M.N. Le, C. Steinbring, B. Le-Vinh, A. Jalil, B. Matuszczak, A. Bernkop-Schnürch, Polyphosphate coatings: A promising strategy to overcome the polycation dilemma, Journal of Colloid and Interface Science, 587 (2021) 279-289.