Oral delivery of peptides and proteins using lipid-based drug delivery systems

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Introduction: In order to successfully develop lipid-based drug delivery systems (DDS) for oral administration of peptides and proteins, it is important to gain an understanding of the colloid structures formed by these DDS, the mode of peptide and protein incorporation as well as the mechanism by which intestinal absorption of peptides and proteins is promoted.

Areas covered: The present paper reviews the literature on lipid-based DDS, employed for oral delivery of peptides and proteins and highlights the mechanisms by which the different lipid-based carriers are expected to overcome the two most important barriers (extensive enzymatic degradation and poor transmucosal permeability). This paper also gives a clear-cut idea about advantages and drawbacks of using different lipidic colloidal carriers ((micro)emulsions, solid lipid core particles and liposomes) for oral delivery of peptides and proteins.

Expert opinion: Lipid-based DDS are safe and suitable for oral delivery of peptides and proteins. Significant progress has been made in this area with several technologies on clinical trials. However, a better understanding of the mechanism of action *in vivo* is needed in order to improve the design and development of lipid-based DDS with the desired bioavailability and therapeutic profile.

Keywords: drug delivery systems, (micro)emulsions, liposomes, mucoadhesion, particles, permeability, SLN, stability

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1. Introduction

1.1 Oral delivery of peptides and proteins

Over the years, more than 100 biopharmaceutical products have been approved and are generating a revenue of more than US$56 billion [1]. The rapid advances within the biotechnology sector have led to an increase in the number of peptide and protein drug candidates in research and development against severe diseases such as cancer, diabetes, osteoporosis, growth hormone deficiency and infectious diseases [2]. Today, peptide and protein drugs are mainly administered by injection. However, oral administration is generally preferred both by the patient and the pharmaceutical industry, due to more convenient dosing resulting in better compliance, and also reduced cost of production. Approaches to develop oral delivery systems for peptides and proteins are currently receiving a lot of interest both in the industry, and in the academic world. Though oral delivery of peptides and proteins is challenging and often results in very low bioavailability due to poor hydrolytic and enzymatic stability as well as poor permeability across the gastrointestinal (GI) mucosa. Various approaches have been used to overcome these obstacles, mainly including physiologically functional excipients such as chemical absorption enhancers, enzyme inhibitors and mucoadhesive polymers, and

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Article highlights.

- Lipid-based drug delivery systems (DDS) have been shown to improve the oral absorption of peptide and protein drugs by increasing the stability, the permeability and/or the retention time in the gastrointestinal (GI) tract.
- Different types of lipid-based DDS have been used for oral peptides and proteins delivery, including solid lipid core particles, liposomes and (micro)emulsions.
- Solid lipid core particles have potential as oral DDS for peptides and proteins due to increased drug stability by physical protection inside the solid lipid core, ease of industrial scale-up and feasible incorporation of peptides and proteins by the double emulsion technique (w/o/w).
- Liposomes can encapsulate hydrophilic peptide and proteins in the aqueous core, where they are protected from degradation by the phospholipid bilayer. But liposomes have shown poor colloidal stability in the GI tract, due to enzymatic degradation and interactions with bile salts and other lipids that can protrude the vesicle structures.
- The potential of (micro)emulsions (w/o, w/o/w) for oral delivery of peptides and proteins is based on incorporation into the aqueous core of the (micro) emulsions and can thus achieve high drug loading of the DDS.
- Self-micro-emulsifying DDS (SMEDDS)/self-nano-emulsifying DDS (SNEDDS) has the advantage over solid lipid core particles DDS of being dosed as a capsule containing a preconcentrate, and lack of heating during the production process. However, the hydrophilic peptide or protein needs to be complexed with lipids prior to be incorporated into the SMEDDS/SNEDDS preconcentrate.

This box summarizes key points contained in the article.

different drug delivery systems (DDS) ((micro)emulsions, liposomes and solid lipid nanoparticles (SLN)) [3-5]. Through the years, many reviews have focused on the effect of applying specific excipients used to improve the oral absorption of peptides and proteins by increasing the membrane permeability, decreasing the rate of degradation and/or increasing the residence time at the absorption site [6-12]. There are quite a few review papers comprising various vehicles that have been designed to improve the oral bioavailability of peptides and proteins, such as, mucoadhesive polymeric systems [13-16], particulate carrier delivery systems [17-22] and targeted delivery systems [23,24]. However, as per the authors' knowledge, there are no reviews on the variety of lipid-based DDS employed for oral delivery of peptides and proteins, even though this is an area that received increased interest over the past years.

Therefore, the objective of this article is to review the literature on lipid-based DDS that have been used to overcome various physiological challenges encountered by orally administered peptide and protein drugs, and special focus is on the mechanism of absorption enhancement by different lipid-based systems.

1.2 Absorption of peptides and proteins from the GI tract

By nature, peptides and proteins are nutrients and are designed to be degraded by peptidases and proteases in the GI tract prior to absorption into the systemic blood circulation. The extensive enzymatic degradation in both GI lumen and at the mucosal membrane complicates the oral delivery of the intact peptide or protein. If the enzymatic barrier is overcome, the poor membrane permeability of peptides and proteins will often constitute the second major problem; on one hand, their high aqueous solubility/hydrophilicity makes them non-ideal candidates for transcellular diffusion across the lipophilic plasma membranes, and on the other hand, peptides and proteins usually have large molecular size, which limit their paracellular permeation (**Figure 1**). The paracellular pathway, which comprises aqueous channels for molecular diffusion between enterocytes, may be a dominant pathway for hydrophilic peptides and proteins [25], yet the aqueous channels have been estimated to have a pore radius of around 0.52 nm in humans [26], which limits diffusion of large molecules. For instance, the human insulin monomer (Mw 5808 Da) has a radius of about 1 nm [27], and the radius of bovine serum albumin (BSA) (Mw 66.5 kDa) is 4 nm [28].

Moreover, it has been reported that there is a strong correlation between the transcellular flux and the total number of hydrogen bonds, which the drug could potentially make with water [29]. Water molecules form a close, hydrogen-bonded layer with the peptide backbone and the hydrophilic side chain amino acid groups of the soluble proteins. These hydrogen bonds must be removed before peptide/protein can permeate by transcellular diffusion [29]. Cellular uptake into the intestinal epithelium by other mechanisms than diffusion, that is, endocytosis, may also happen to a limited extend if the protein is incorporated in a particle of the right size (e.g., 100 nm vesicles is suitable for clathrin-mediated endocytosis) or in the right molecular structure [30] and may potentially lead to transcellular transport (**Figure 1**). In this case, protein drugs will be exposed to the harsh intracellular environment with increased degradation, which may result in that no drug is absorbed into the systemic circulation.

The absorption of peptide and protein drugs from the GI tract is not only determined by the proteolytic degradation of the drug and the drug's physical membrane permeability mentioned above, but also related to the formulated lipid-based DDS (e.g., the content of lipids in the formulation and associated digestion hereof).

1.3 Lipid digestion in the GI tract and its influence on peptides and proteins absorption

Lipid digestion is initiated in the stomach by gastric lipase catalyzing the removal of one fatty acid from triglycerides, forming free fatty acids and diacylglycerides. However, the main part of lipid digestion takes place in the small intestine facilitated by pancreatic lipase and co-lipase, producing

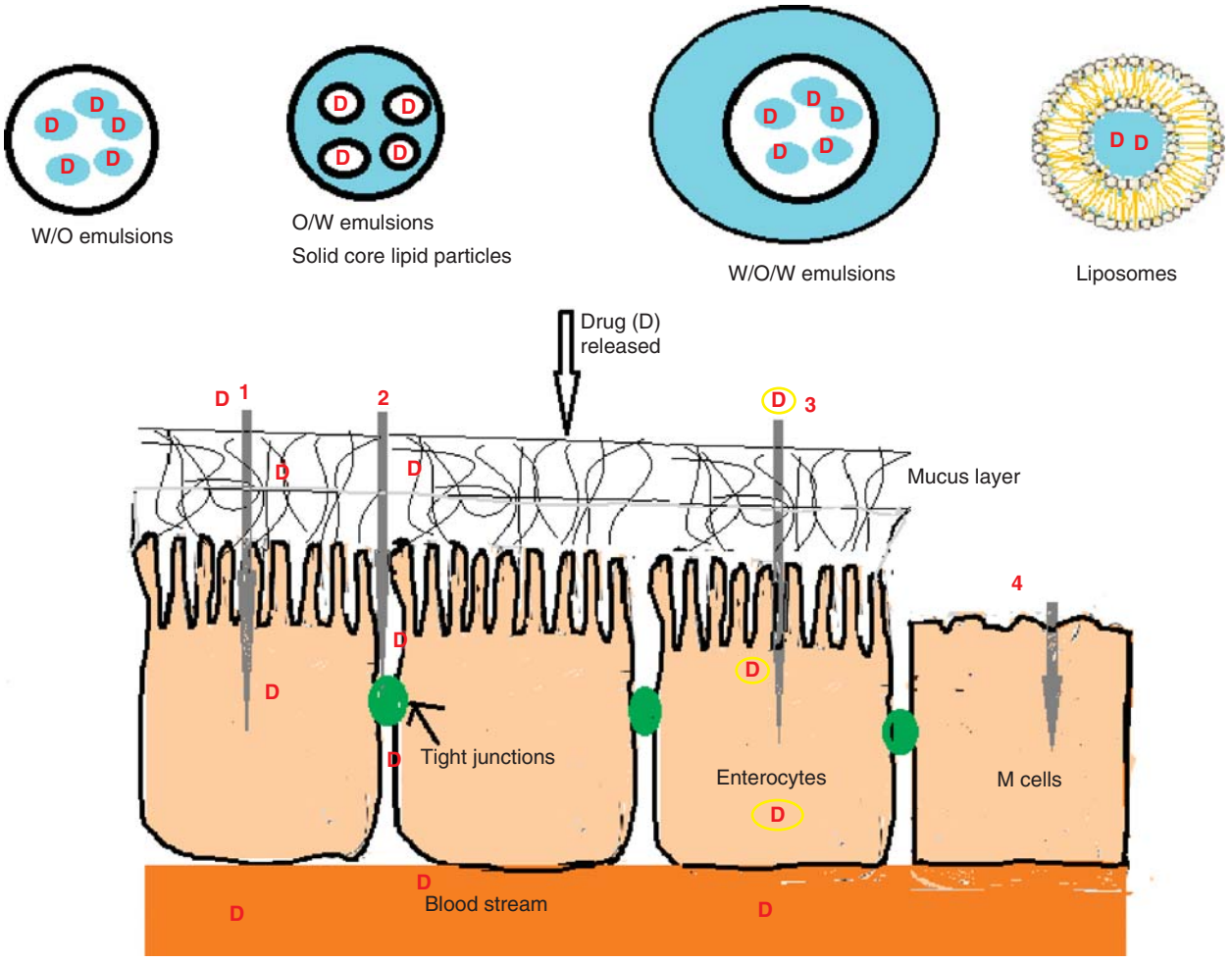

Figure 1. The absorption enhancement mechanisms of lipid-based DDS. 1) Transcellular transport; 2) paracellular transport; 3) transcytosis; 4) uptake by M cell.

more free fatty acid and monoacylglyceride (digestion products). In addition, lipid-based DDS intake can at certain levels of intake induce gall bladder contraction in humans and thus increase the level of bile salt/phospholipid (BS/PL) micelles in the small intestine [31], which together with the formed digestion products will form uni- and/or multilamellar vesicles [32]. Further, it has recently been reported that structures with hexagonal phases will be formed during *in vitro* lipolysis of self-micro-emulsifying DDS (SMEDDS) [33]. Under these circumstances, on one hand, the hydrophilic peptides and proteins hypothetically might be encapsulated in the hydrophilic core of the vesicles or hexagonal phases, thus avoiding contact with the digestion fluid, and reducing further drug degradation during absorption. On the other hand, the formation of vesicles together with the BS/PL may facilitate the transcellular diffusion by increasing the lipid membrane disorder or transcytosis of the drug compound, which potentially resulting in improved epithelial permeability, although the exact mechanism involved in these absorption processes is not very well understood [34]. Furthermore, the digestion products and BS/PL are by themselves good absorption enhancers, which will affect paracellular transport of the drug compound by transiently opening tight junctions. The exact mechanisms of absorption enhancers will be discussed in Section 3.2.

2. Lipid-based DDS

Lipids encompass a very heterogenous group of chemical compounds, ranging from triglycerides to hydrophilic surfactants [31]. According to the lipid classification system developed by Small lipids can be categorized based on their physical interaction with water, into polar and non-polar lipids [35]. Lipid-based DDS are usually composed of polar lipids such as phospholipids, triglycerides and fatty acids (**Table 1,2,3,4**), which are biocompatible and biodegradable, making them ideal drug delivery vehicles. Lipid-based DDS are increasingly employed for the oral administration of peptide and protein drugs even though literature data on their fate in the GI tract are lacking. Generally, peptides and

Table 1. Solid lipid core particles as DDS for peptide and protein drugs.

Peptide/Protein	Solid lipid core particles	Excipients used	Characterization	Production procedure
Thymopentin [38]	SLN	Lecithin, stearic acid, TDC, $NaC_{16}P$	Size (nm) 100, 200 E.E. (%) 5.2, 1.7	Warm microemulsion (o/w with ionic pair or w/o/w)
Lysozyme [94]	SLN	Witepsol® E85 (hard fats comprises glycerides of plant origin), Softisan® 142 (hydrogenate coco-glycerides), Monosteol™ (propylene glycol palmitostearate), Superpolystate™ (propyleneglycol 300 mono-, di-stearate), poloxamers 182 and 188, Tween and SC	Size (nm) 549, 644 Zeta potential (mV) -11, -9.8 E.E. (%) 43.2, 59.2	High-pressure homogenization cold dispersion
Albumin [95]	SLN	DPPE/PEG, stearate/PEG	N.A.	Adsorption onto SLN
Insulin [96]	Polymer-coated SLN	Tripalmitin, lecithin, PEG-2000 and -4500 stearate, poloxamer 188	Size (nm) 223 – 395 Zeta potential (mV) -17.2 to -38.9	Double emulsion technique
Gonadorelin [97]	SLN	Monostearin, polyvinyl alcohol	Size (nm) 421.7 Zeta potential (mV) -21.1 E.E. (%) 50.4 – 69.4	Solvent displacement
Salmon calcitonin [40]	Polymer-coated SLN	Tripalmitin, lecithin, chitosan, PEG-2000 and -4500 stearate, poloxamer 188	Size (nm) 226 – 537 Zeta potential (mV) -50.3 to 29.2 E.E. (%) > 90, 30.7	Solvent emulsification-evaporation
Insulin [67]	SLN	Soya lecithin, stearic acid, PE, poloxamer 188	Size (nm) 50.2 – 68.0 Zeta potential (mV) -13.0 to -46.3 E.E. (%) 17.9 – 37.8	Warm microemulsion (w/o/w)
Human insulin [22]	SLN	CP, poloxamers 407	Size (nm) 361 Zeta potential (mV) -3.4 E.E. (%) 43	Solvent emulsification-evaporation
Porcine insulin [39]	SLN	SPC, poloxamer 188, stearic and palmitic acid, SC	Size (nm) 114.7 Zeta potential (mV) -51.4 E.E. (%) 97.8	Reverse micelle-double emulsion technique
Bovine insulin and hGH [66]	SLN	Tristearin, Tween 80 PC, PEG-5000	Size (nm) 197 E.E. (%) 57, 48	Supercritical CO_2 (PGSS)
LHRH [98]	Liposheres	Stearic acid, lecithin, butyric acid	Size (nm) 300 E.E. (%) 90	Warm microemulsion (w/o/w)
[Bovine insulin [99]	LM	CP, GMS, TDC, lecithin	Size (nm) 1335 – 1526 Zeta potential (mV) -10 to -12 E.E. (%) 78 – 84	Solvent emulsification-diffusion
[BSA [100]	Lipid microparticles	Trimyristin Gelucire® 50/02 (mixture of glycerides and fatty acid esters)	Size (μm) < 50 E.E. (%) 13 – 62	Supercritical CO_2

BSA: Bovine serum albumin; CP: Cetylpalmitate; DDS: Drug delivery systems; DPPE: Dipalmitoylphosphatidylethanolamine; E.E.: Encapsulation efficiency; GMS: Glyceryl monostearate; hGH: Human growth hormone; LM: Lipid microparticles; LRHR: Luteinizing hormone-releasing hormone; $NaC_{16}P$: Sodium hexadecyl phosphate; PC: Phosphocholine; PE: Phosphatidyl-ethanolamine; PGSS: Particle from gas saturated solution; SC: Sodium cholate; SLN: Solid lipid nanoparticles; SPC: Soybean phosphocholine; TDC: Taurodeoxycholate sodium salts.

Table 2. Liposome DDS for peptide and protein drugs.

Peptide/Protein	Liposomes	Excipients used	Characterization	Production procedure
Bovine insulin [101]	Surface-coated liposomes	DPPC, CHOL, DSPE-PEG 2000	Size (nm) 348, 479, 453 E.E. (%) 31.4, 35.3, 37.7	Film hydration method
Insulin [72]	Double liposomes	HSPC, stearic acid, PS	Size (μm) 2.1 – 9.3 E.E. (%) 5.7 – 27.3	Glass filter method
Adamantyltripeptides [102]	Liposomes	EPC, CHOL, stearic acid, DCPh	N.A.	Reverse-phase evaporation
Insulin [44]	Lectin-modified liposomes	PE	Size (nm) 166.2 – 194.1 Zeta potential (mV) -2.1, 8.7 E.E. (%) 30.3 – 82.5	Reverse-phase evaporation
Salmon calcitonin [103]	Chitosan-coated liposomes	DSPC, CHOL, DCPh, chitosan	Size (nm) 207.4 – 769.9 Zeta potential (mV) 34.8, 30.3	Film hydration method
Epidermal growth factor [73]	Multivesicular liposomes	DMPG, DOPC, triolein, CHOL	Size (μm) 1.4 E.E. (%) 28.7 – 60.0	Two-step (w/o/w) double emulsification process
Leuprolide [45]	*O*-PSCG-coated liposome	PC, CHOL	Size (nm) 112.2 – 168.9 Zeta potential (mV) -8.8 to -20.1 E.E. (%) 29.4 – 37.1	Film hydration method
Salmon calcitonin [104]	Chitosan-coated liposomes	DSPC, DCPh, CHOL, chitosan	Size (nm) 305.2 Zeta potential (mV) 50 to -35 E.E. (%) 87	Film hydration method
Human insulin [74]	Niosomes	Brij series (PEG ester with different fatty acids), CHOL	Size (μm) 4.2 – 41.8 E.E. (%) 17.4 – 40.2	Film hydration method
Salmon calcitonin [90]	Pectin–liposome nanocomplexes	DSPC, SA, CHOL	Size (nm) 844 – 1298 Zeta potential (mV) +20 to -60) E.E. (%) 49.7	Film hydration method
Insulin [75]	Archaeosomes	PLFE extracted from sulfolobus acidocaldarius	Size (nm) 269.0 Zeta potential (mV) -36.2 E.E. (%) 18.6	Reverse-phase evaporation

CHOL: Cholesterol; DCPh: Dicetyl phosphate; DDS: Drug delivery systems; DMPG: Dimyristoylphophatidylglycerol; DOPC: Dioleoylphosphatidylcholine; DSPC: Distearoylphosphatidylcholine; DSPE-PEG: Distearoylphosphatidylethanolamine-poly-(ethylene glycol); E.E.: Encapsulation efficiency; EPC: egg Phosphatidylcholine; HSPC: Hydrogenated soybean phosphatidylcholine; PLFE: Polar lipid fraction E; PS: Phosphatidylserine; PSCG: Palmitoylscleroglucan; SA: stearylamine.

proteins can be encapsulated into either the lipid core of solid lipid core particles or the aqueous core of liposomes. In addition, they can also be encapsulated into the aqueous phase of (micro)emulsions or being lipidified and loaded into the lipid phase of the DDS. In addition, only non-convalent modification of peptides and proteins by the use of lipids will be discussed in the present review.

In the following section, examples on: i) solid lipid core particles, including mainly SLN, but also lipid microparticles (LM), ii) liposomes, iii) (micro)emulsions and iv) lipid-based DDS loaded with lipidified peptides and proteins are discussed separately. Besides the above-mentioned lipid-based DDS, self-assembled liquid crystalline systems (hexagonal and cubic phase, etc.) also have shown potential as lipid-based DDS for peptides and proteins for sustained drug release [21]. However, in most cases they have been employed for parental or topical delivery. There are several reviews on liquid crystals for peptides and proteins delivery [20,21], in which general knowledge about the systems and possible application are well described. Due to the very limited number of studies available for liquid crystalline DDS for oral delivery of peptides and proteins, they will not be further described in the present reviews, but some of them will be mentioned in Section 3 [36,37].

2.1 Solid lipid core particles

Solid lipid core particles here include SLN, LM and Liposphere™, and each of them is described below.

SLN are spherical particles made from lipids or lipid blends (solid at body temperature) and with a mean diameter

Table 3. (Micro)emulsion DDS containing peptide and protein drugs in the aqueous phase.

Peptide/Protein	(Micro)emulsions	Excipients used	Characterization	Production procedure
Human insulin [48]	Emulsion (w/o)	SPC, olive oil, Cremophor® (ethoxylated castor oil), Migyol® 812 (C8 – C12 medium chain triglycerides)	Size (nm) 34.3	High-pressure homogenization
Salmon calcitonin [84]	Emulsion (o/w)	Migyol® 812, (C8 – C12 medium chain triglycerides) EPC, Tween 80, tocopherol acid succinate	Size (nm) 110	High-pressure homogenization
Salmon calcitonin [52]	Emulsion (w/o/w)	Paraffin, CHOL, sorbitan oleate, polyglyceryl-3 polyricinoleate	Size (μm) 14 E.E. (%) 92.1	Two-step emulsifcation method
[Human insulin 68]	Nanocapsules in microemulsion	C8, C10 mono-, di- and triglycerides, sorbitan mono-oleate, Tween 80	Size (nm) 200, 210 E.E. (%) 84.3, 94.7, 97.2	Interfacial polymerization
[Human insulin 56]	Emulsion (o/w)	Lauric, palmitic, stearic, palmitoleic, linoleic acid, SGC	N.A.	Grinded and dried
EFE-d [51]	Microemulsion (w/o)	C8, C10 triglyceride, PEG-6 dioleate, PEG-8 glyceryl caprylate/caprate	Size (nm) 6.9	N.A.
Insulin [53]	Self-emulsifying DDS (o/w)	PEG-8 glyceryl caprylate/caprate, PEG-3-oleate	Size (nm) 264.7 E.E. (%) 99.2	w/o
Bovine insulin [50]	Microemulsion (w/o)	Triacetin, PG, didoceyldimethyl ammonium bromide	Size (nm) 161.7 E.E. (%) 85	Low shear reverse micelle

CHOL: Cholesterol; DDS: Drug delivery systems; EFE-d: Earthworm fibrinolytic enzyme; E.E.: Encapsulation efficiency; EPC: Egg phosphatidylcholine; PG: Propylene glycol; SGC: Sodium glycocholic acid.

between approximately 50 and 1000 nm. The production procedure of SLN containing peptides and proteins is often based on the double emulsion technique (w/o/w) (**Table 1**) employing heating and/or high pressure. A typical w/o/w procedure for preparing SLN includes two steps: i) primary emulsification (w/o): an aqueous solution of the protein drug is emulsified into an oil phase; ii) re-emulsification: the primary emulsion (w/o) is further emulsified into a second aqueous phase containing surfactant to form a w/o/w double emulsion. There are several studies employing SLN as a DDS for oral delivery of peptide and protein drugs (**Table 1**). The lipids used in the SLN are often long chain oils like stearate, palmitate and their derivatives, such as, tripalmitin and polyethylene glycol (PEG) stearate. Usually, peptide and protein drugs can be dissolved in the water phase and then encapsulated by the oil phase, or solubilized in the melted lipids by the formation of hydrophobic complexes with lipophilic excipients. However, the encapsulation efficiency (E.E.) values may be very low, for example, thymopentin-loaded SLN had an E.E. of less than 10%, both when prepared by the o/w or the w/o/w procedure [38], in the first case the thymopentin was dispersed in oil and then emulsified in the water phase to form an o/w emulsion, in the second case the w/o/w was prepared as described above. The E.E. value depends not only on the therapeutic protein and choice of excipients, but can also be affected by the processes employed, as demonstrated by Liu *et al.* These authors were able to increase E.E. up to 97.8% by applying the reverse micelle technique [39]; sodium cholate (SC) and soybean phosphatidylcholine (SPC) were employed to improve the solubility of insulin in oil prior to further loading into a mixture of stearic acid and palmitic acid. In addition, effective encapsulation can be achieved by increasing the interaction between the peptide drugs and the lipids. In Garcia-Fuentes's group, high E.E. (> 90%) of calcitonin in SLN was achieved by incorporating tripalmitin and lecithin, negative charged lipids expected to be interacting with the cationic residues of calcitonin [40].

LM is similar to SLN, but larger sizes may be obtained, and the types of lipids used are often solid lipids including trimyristin, glyceryl monostearate (GMS), etc. The production procedures also differ a lot among the different LM DDS.

Examples of solid lipid core particles also include the Liposphere technology described by Domb as a particulate dispersion of solid spherical particles with a particle size between 0.2 and 100 μm in diameter consisting of a solid hydrophobic lipid core (e.g., triglycerides or fatty acid derivatives stabilized by a monolayer of phospholipids) [41].

Generally, solid lipid core particles are considered good oral DDS for peptides and proteins due to increased drug stability by physical protection inside the solid lipid core, a ease of industrial scale-up and feasible incorporation of peptides and proteins by the double emulsion technique (w/o/w).

Table 4. Lipid-based DDS containing peptide and protein drugs in the oil phase.

Peptide/Protein	DDS	Excipients used	Characterization	Production procedure
Surfactant-coated insulin [55]	s/o/w	Soybean oil, sucrose erucic/lauric ester	Size (μm) 1.0 E.E. (%) 97.5	High-pressure homogenization
Surfactant-coated insulin [71]	s/o/w dry emulsion	Soybean oil, sucrose erucic/lauric ester, Cremophor® EL (ethoxylated castor oil), glycerin, HPMCP	Size (μm) 50	High-pressure homogenization
β-Lactamase [62]	SNEDDS	Different oils, PG, PEG-400, SPC, Cremophor EL (ethoxylated castor oil)	Size (nm) 22 – 50	ARMs technique
Porcine insulin [59]	Oil solution	SPC, MCT	Size (nm) 23	ARMs technique
Insulin [58]	Oil solution	EPC, PC blends with Lyso-PC, ursodeoxycholate, C8, C10 partial glycerides	Size < 10 μm	ARMs technique

ARMs: Anhydrous reverse micelles; BSA: Bovine serum albumin, DDS: Drug delivery systems; E.E.: Encapsulation efficiency; EPC: Egg phosphatidylcholine; HPMCP: Hydroxypropyl methylcellulose phthalate; MCT: Medium chain triglyceride; PC: Phosphatidylcholine; SNEDDS: Self-nano-emulsifying DDS; s/o/w: Solid-in-oil-in-water emulsion; SPC: Soybean phosphatidylcholine.

2.2 Liposomes

Liposomes are concentric bilayered vesicles in which an aqueous volume, containing the peptide/protein drug, is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. There are different preparation methods for liposomes, such as the film hydration method, reverse-phase evaporation and the glass filter method. Among these methods, there is a need to introduce organic solvents to dissolve the phospholipids during the liposome production, which may have deleterious effect for peptide and protein drugs [42]. As a result of the preparation procedure and the type of phospholipid used, liposomes may have different physical properties. MLV (multilamellar vesicles), SUV (small unilamellar vesicles) and LUV (large unilamellar vesicles) are reported in literature [43]. As shown in **Table 2**, the lipids used for liposomes are often biocompatible, such as phosphatidylcholine (PC), phophatidylglycerol (PG) and phosphatidylethanolamine (PE). The particle size, charge and E.E. of hydrophilic drugs in liposomes vary quite a lot, due to the use of different excipients and/or the variation in preparation methods. For example, Zhang *et al.* modified liposomes with different lectins, resulting in insulin-loaded liposomes with size range from 166.2 to 194.1 nm, a zeta potential from -2.1 to +8.7 mV and an E. E. from 30.3 to 82.5 (%) [44]. Other groups prepared negatively charged *o*-palmitoylscleroglucan (*O*-PSCG)-coated liposomes with different excipients resulting in liposomes with different sizes (112.2 – 168.9 nm), zeta potential values from -8.8 to -20.1 mV and varying degree of leuprolide encapsulation (%) (29.4 – 37.1) [45]. Chitosan-coated liposomes also have been developed and showed an E.E. of 87% for calcitonin, sizes between 200 and 300 nm and zeta potential values from -35 to +50 mV (**Table 2**) [46,47].

The advantages of liposomes as peptide and protein DDS are as follows: they can enclose the hydrophilic macromolecule in their internal aqueous compartment surrounded by the bilayer of phospholipids, which lead to a high drug E.E. and decrease the initial drug release [44]. And their size, charge and E.E. of hydrophilic drugs can be optimized by addition of other excipients to the lipid mixture prior to liposome preparation and/or by using different preparation methods (**Table 2**).

2.3 (Micro)emulsions

Microemulsions are clear, stable, isotropic liquid mixtures of oil, water and surfactant, frequently in combination with a co-surfactant. The aqueous phase may contain salt(s) and/or other ingredients, and the 'oil' may actually be a complex mixture of different hydrocarbons. Microemulsions form on simple mixing of the components and can be either direct (oil dispersed in water, o/w) and reversed (water dispersed in oil, w/o). Since the existence of a microemulsion is dependent on a specific ratio between the ingredients, including water, dilution of the microemulsion can lead to disintegration.

In contrast to microemulsions, emulsions have larger droplet sizes, and are often prepared by high-pressure homogenization and other emulsification methods which need energy input (**Table 3**) and are also characterized by being thermodynamically unstable. Lipids used in emulsion systems employed for peptides and proteins delivery are often medium chain fatty acids and derivatives, such as medium chain triglycerides; they are less lipophilic than long chain lipids and are therefore easier to emulsify. Further, medium chain fatty acids are also considered to be good permeability enhancers (**Table 3**). Peptides and proteins are often loaded into the aqueous phase of the emulsion-based systems (**Table 3**). One special case is the nanocapsules in microemulsion (emulsion–polymer complex DDS), which the w/o microemulsion (drug in the water phase) is polymerized with butyl cyanoacrylate monomer, using the w/o microemulsion as scaffold and producing the nanocapsules in oily microemulsion with high E.E. (> 80%). Peptide and protein drugs can also be loaded into the water phase of the w/o and w/o/w (micro)emulsion systems and achieve high

drug loading of the DDS [48-51], for instance, the salmon calcitonin can be loaded into the water phase of the w/o/w emulsion with a E.E. of 95% [52]. In another example by Ma *et al.*, a SMEDDS with a final high E.E. of 99.2% was prepared by dispersing concentrated insulin solution (60 mg/ml) into the oil phase of a SMEDDS preconcentrate [53]. A broad size range from few nm to μm is observed from **Table 3**, depending on the emulsion type and preparation procedure. Usually, the double emulsion (w/o/w) has a larger size, for example, 14 μm for salmon calcitonin-loaded emulsion, compared with emulsions (**Table 3**).

In general, the potential of emulsion-based systems (w/o, w/o/w) for oral delivery of peptides and proteins is usually based on the aqueous solubility of the peptide and protein drug and thus achieve high drug loading of the DDS and the (micro)emulsion's good membrane interaction property [54]. However, the shearing force (and resulting heat) required to form crude emulsions may destabilize and denature the protein during the preparation of emulsion. Use of high amounts of surfactants and co-surfactants to form the microemulsion will reduce the need for high shearing force, which may avoid denaturing the protein by the shearing force.

2.4 Lipid-based DDS loaded with lipidified peptides and proteins

Examples of lipid-based DDS loaded with lipidified peptides and proteins (**Table 4**) include emulsions, oil solution, self-nano-emulsifying DDS (SNEDDS), etc. Generally, peptide and protein drugs can be complexed with lipids and thus becoming more lipophilic prior to be incorporated into the lipid phase with a high E.E. For example, Toorisaka *et al.* coated insulin with a surfactant, sucrose erucic ester and subsequently dispersed the coated insulin in soybean oil to form a solid-oil-water (s/o/w) emulsion [55]. Another example is a system composed of different fatty acids and sodium glycocholate (SGC), in which insulin is associated with the fatty acid and SGC mixture and then dried [56]. The peptide and protein drug can be surrounded or complexed by an amphiphilic molecule, for example, a surfactant like SPC and subsequently dissolved into the oil-based vehicle, which is a typical anhydrous reverse micelles (ARMs) technique. The ARMs are stable systems containing non-aqueous phase, in which the hydrophilic drugs (peptides/proteins) are surrounded by hydrophobic surfactants [57]. By using ARMs technique, the peptide/protein can form the complex with the hydrophobic surfactant and can be dissolved into lipid-based DDS. As mentioned above, the oils used in the lipid-based DDS are often medium chain oils due to their potential permeability-enhancing effect and easier emulsification compared with long chain oils [58,59].

Contrary to the emulsions, the SNEDDS delivery systems are isotropic mixtures of oil, surfactant, co-surfactant, co-solvents and the drug and spontaneously form a nanoemulsion in the GI tract on oral administration [60]. Further, compared with emulsions, SNEDDS preconcentrate do not contain a water phase, which may enhance the stability of peptide and protein drugs during storage. A recently published patent describes how SNEDDS can be employed for oral delivery of insulin and insulin derivatives [61]. Moreover, fluorescent-labeled β-lactamase was loaded into SNEDDS via the ARMs technique described above. In that study, the protein was dispersed into the phospholipids suspension, which was lyophilized prior to dissolving the lyophilized powder in the SNEDDS preconcentrate [62].

3. Mechanisms of oral bioavailability enhancement for peptides and proteins

As mentioned previously, various strategies have been employed to overcome enzymatic and permeability barriers in the GI tract, such as the use of enzyme inhibitors, and co-administration absorption enhancers with peptides and proteins as well as chemical modification of the drugs. Moreover, as described above, many different types of lipid-based DDS have been studied for oral peptides and proteins delivery. In those studies, several DDS approaches have been identified as important for the absorption enhancement of lipid-based DDS, indicated in **Table 5** as 1, 2 and 3 (approach 1, increased stability of peptide and protein drugs; approach 2, membrane permeability enhancement; approach 3, increased retention time at absorption sites). In the following, the three approaches will be discussed.

3.1 Increased stability of peptide and protein drugs

Most peptide and protein drugs are fragile and easily degraded in the GI tract. Proteolysis generally occurs in the stomach fluid by simple acid hydrolysis or catalyzed by pepsin, as well as in the small intestine by various proteases located in the lumen, mucosa, membrane and cytosol of the enterocytes [6,11].

At present, multiple strategies have been employed to overcome the instability of peptide and protein drugs in the GI tract, such as co-administration of enzyme inhibitors [63], encapsulation in carriers to avoid the exposure to the enzymes [9,36,37] and structure modification of the peptide and protein drug [64]. In general, the lipid-based DDS can increase intact peptides and proteins' residence time in the GI tract by physical encapsulation of the drug and co-encapsulation of enzyme inhibitors in DDS [65]. For instance, experiments showed that the cubic phase can protect peptides against enzymatic degradation in simulated intestinal fluid by incorporating them in the hydrophilic region of the cubic phase [36]. In addition, another group also demonstrated that the cubic phase offered the protection of a metaloenzyme in gastric environment [37]. For example, SLN can encapsulate the peptide and protein drug in the solid matrix and thereby decrease the drug degradation in the GI tract (**Table 5**) [22,66,67]. Moreover, it has been addressed that the emulsions (w/o, w/o/w, s/o/w, etc.) can be used to protect the drug against degradation by either physical encapsulation and/or loading together with enzyme inhibitors [48,50,55,68]. In **Table 5**, surfactant-coated fluorescein isothiocyanate

Table 5. The mechanisms of lipid-based DDS to improve oral absorption for peptide and protein drugs.

Lipid-based DDS	Model drug	Approaches	Mechanisms
Niosomes [74]	Human insulin	1	Entrapment of insulin in bilayer structure of nisomes protected it against proteolytic degradation
Double liposomes [72]	Insulin	1	Insulin encapsulated in liposomes was protected from enzymatic proteolysis and aprotinin was employed as an enzyme inhibitor
Surface-coated liposomes [101]	Bovine insulin	3	The surface-coated liposomes can interact with the mucus layer and slow the transit rate in the GI tract
Archaeosomes [75]	Insulin	1	Unique lipid structures conferred stability on archaeosomes and low leakage of drug. Further, the negatively charged surface of archaeosomes was also responsible for keeping membrane integrity
Self-emulsifying DDS [53]	Insulin	1 and 2	The DDS was stable and protected from degradation. Increased paracellular permeation by opening of tight junctions
Microemulsion (w/o) [50]	Bovine insulin	1, 2 and 3	Lipid coating protected the drug in aqueous core of microemulsion. Permeability was altered by DMAB. Reduced interfacial tension between the DDS and mucosal layers
s/o/w emulsion [55]	Surfactant-coated insulin	1 and 2	A sharp particle size distribution result in high stability of the emulsion for a long time. The degraded fatty acids form soybean oils form micelles good for absorption of the surfactant-coated insulin
Emulsion (w/o) [48]	Human insulin	1	The emulsion was able to protect insulin against gastric degradation *in vitro* without further encapsulation. Simultaneous incorporation of aprotinin as a protease inhibitor increases the stability of the peptide
[Polymer-coated SLN [96]	Insulin	1 and 2	Lipids have the stabilizing effect on proteins and the absorption promoting effect *in vivo*. Coating nanoparticle with polymers and decreased enzymatic degradation
WGA-modified SLN [67]	Insulin	1 and 3	The WGA-modified SLN was able to protect insulin against pepsin degradation *in vitro*, due to WGA's good resistance to acidic pH and enzymatic degradation. Increase the adhesive interaction of carriers with cell membranes
SLN [22]	Human insulin	1 and 2	The SLN lipid matrix was able to protect insulin from degradation in GI tract. Hydrophobic SLN facilitate the uptake by intestinal cells
SLN [66]	Bovine insulin and hGH	1	The SLN is claimed to be stable in GI tract and protect the protein from degradation in the stomach
LM [99]	Bovine insulin	1	The LM protected insulin against pepsin degradation *in vitro*
Pectin–liposome nanocomplexes [90]	Salmon calcitonin	3	The pectin can adhere to the mucus layer and prolong retention in the intestinal mucosa
Chitosan-coated liposomes [104]	Salmon calcitonin	1 and 3	Good mucoadhesive of polymer. Encapsulation of drug within the liposomal particles, avoiding drug degradation
Emulsion (o/w) [87]	Salmon calcitonin	1, 2 and 3	Adherence of the emulsion droplets to the epithelium mucosa. The protease inhibition and absorption enhancement properties of polyacrylic acid
Emulsion (w/o/w) [52]	Salmon calcitonin	1	Incorporating drug in the inner aqueous phase of a w/o/w emulsion to protect the peptide from enzymatic degradation. Drug was further protected by incorporating the protease inhibitor, aprotinin, in the outer aqueous phase
Emulsion (w/o) [51]	EFE-d	1 and 2	The surfactant in emulsion-induced membrane structure and fluidity changes, resulting in enhanced permeability
O-PSCG-coated liposome [45]	Leuprolide	1	The stability of liposome in GI tract was increased by PSCG coating and decreased the degradation in GI fluids
Multivesicular liposomes [73]	rhEGF	1	Multivesicular liposomes were able to suppress the enzymatic degradation and release the protein in a sustained manner

DDS: Drug delivery system; DMAB: Didoceyldimethylammonium bromide; EFE: Earthworm fibrinolytic enzyme; GI: Gastrointestinal; hGH: LM: Lipid microparticles; LMWH: Low molecular-weight heparin; PSCG: Palmitoylscleroglucan; rhEGF: Recombinant human epidermal growth factor; SLN: Solid lipid nanoparticles; s/o/w: Solid-in-oil-in-water emulsion; TMC: *N*-Trimethyl chitosan chloride; WGA: Wheat germ agglutinin.

(FITC)-labeled insulin was found to disperse well in the oil phase with a narrow droplet size distribution and no leak from the emulsion in 10 h [55]. Sharma *et al.* developed a microemulsion (w/o) loaded with bovine insulin and enhanced the relative bioavailability 10 times, compared with insulin solution [50]. According to these authors, one possible absorption enhancement mechanism could be the protection by the microemulsion and the enhanced stability of insulin. Dogru *et al.* incorporated the peptide calcitonin in the inner aqueous phase of a w/o/w emulsion and the protease inhibitor, aprotinin, in the outer aqueous phase; and this improved the oral absorption as verified by an increased hypocalcemic effect using a rat model [49]. In addition, a simple emulsion (w/o) containing both insulin and aprotinin have been developed and showed good resistance of the peptide toward degradation in gastric media [48]. However, the drawback of this liquid emulsion was the physical–chemical instability during long-term storage and the general requirement for storage at low temperature, which argued for the preparation of a dry emulsion. Dry emulsion DDS are typically prepared of o/w emulsions containing a soluble or an insoluble solid carrier in the aqueous phase by spray drying [69] or lyophilization [70]. The dry emulsions further have the advantage of physical stability and ease of administration in capsules and tablets. In a study done by Toorisaka *et al.*, a pH-responsive polymer (hydroxypropyl methylcellulose phthalate (HPMCP)) was employed to make an enteric-coating emulsion. Enteric-coating techniques have been used to avoid proteolytic degradation of insulin in the stomach [71].

Liposomes can also encapsulate peptide and protein drugs and thereby increase their stability *in vivo* [72,73]. However, the problem with the liposome is its colloidal instability during transport through the environment of the GI tract. Therefore, many kinds of modified liposomes, with increased stability, have been prepared and have been shown to protect macromolecular drugs from degradation by enhancing both delivery system and drug stability in the GI tract. Examples are polymer-coated liposomes and pectin–liposome nanocomplexes (**Table 5**). It was thus reported that the colloidal stability of liposomes in the GI tract was increased by *O*-PSCG coating resulting in a decreased degradation of leuprolide in GI fluids as compared with non-coated liposomes [45]. Another example is the low molecular weight chitosan (LCS)-coated liposomes, which resulted in a 14.2% absolute bioavailability after intragastric administration of calcitonin (500 IU/kg) to rats, compared with 0.1% for calcitonin solution [47]. In that study, it was shown that the oral bioavailability could be enhanced due to the LCS's enzyme inhibition effect, irrespective of whether the peptide was encapsulated or not [47]. In addition to modified liposomes, other kinds of vesicle-based systems show higher colloidal stability. For example, preparation of niosomes with a mixture of cholesterol and non-ionic surfactants protected the drug against proteolytic degradation *in vitro* and only 26.3% of the drug was released during 24 h in simulated intestinal fluid [74]. Another group designed insulin-containing archaeosomes using polar lipid fraction E from sulfolobus acidocaldarius and found that the archaeosomes had better stability in simulated GI fluids without enzymes, compared with a conventional liposome prepared by egg phosphatidylcholine (EPC) and cholesterol. Further, a diabetic mouse study showed lower levels of blood glucose for the mice dosed archaeosomes, as compared with the conventional liposome [75].

3.2 Membrane permeability enhancement

Peptide and protein drugs are believed to primarily permeate through the epithelial membrane via the paracellular route, in order to reach the blood circulation [11] as many peptide and protein drugs are hydrophilic, which complicate their transcellular passage through the lipophilic membrane. However, paracellular transport through tight junctions is often limited for peptide and protein drugs, due to both the small area and the small channel of tight junctions [76]. Therefore, much attention has been directed toward increasing both paracellular and transcellular permeability of peptide and protein drugs, mainly including non-covalent modification of the peptide and protein with lipids and employing permeability enhancers.

Currently, various types of lipophilic compounds have been used to lipidize peptides and proteins and thus enhance the transcellular passage of the drug. As previously mentioned, a well-known case is the use of *N*-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC), a caprylic acid modified compound, which is applied in the Eligen® Technology. SNAC has been shown to form a complex with peptide drugs and increase the overall drug lipophilicity, thus finally improve the transcellular permeability [77].

The application of permeability enhancers, including surfactants, polymers and fatty acids, is one of the most popular strategies to increase oral bioavailability [10,24]. Among permeation enhancers, lipid excipients, including medium chain fatty acid, fatty acid modified compounds and phospholipids are one of the most used categories. Lipid excipients have been described to improve both the paracellular and transcellular transport by different mechanisms of action, including changes in the enterocyte membrane fluidity, decrease the viscosity of the mucus lining the epithelial membrane and opening of tight junctions [81]. For example, the hypoglycemic and hypocalcemic effects of oil-based DDS loaded with insulin and calcitonin, respectively, have been ascribed to the permeability-enhancing effect of lysophospholipids and medium chain fatty acids [20,58]. Co-administrated polyunsaturated long chain fatty acids can also act as good absorption enhancers. For example, omega-3 and omega-6 fatty acids used in the Pheroid™ vesicles (a DDS, which comprises essential and plant fatty acids, being emulsified in water saturated with nitrous oxide) was shown to increase the permeability of salmon calcitonin across the intestinal membrane [79].

With regards to the permeation enhancement, special focus has been on the use of medium chain fatty acids, which have

been shown to mediate destabilization and solubilization of membranes and open the tight junctions, both mechanisms leading to increased transport of drugs. The mechanism of tight junction opening of caprate acid (C10) has been shown to be mediated by changing the perijunctional actin and also changing the localization of zonula occludens-1 (ZO-1), occludin and claudin-1. Furthermore, C10 can also mobilize intracellular Ca^{2+} from stores in Caco-2 cells, or causing a reduction in intracellular adenosine triphosphate (ATP), which can also lead to enhanced paracellular permeability [80]. A study on sodium caprate (C10) suggested that this enhancer may reversibly open the tight junctions, thereby increasing the permeation of macromolecules by paracellular transport [81]. Another example is a microemulsion (w/o) with C8, C10 triglyceride polyglyceryl-6 dioleate and PEG-8 glyceryl caprylate/caprate developed by Cheng *et al.* They showed that the w/o microemulsion induced a higher intestinal membrane permeability of the earthworm fibrinolytic enzyme (EFE-d) by using a Franz diffusion cells with isolated rat intestinal tissue and also promoted a 208-fold higher intraduodenal bioavailability when compared with a drug solution [51]. The GI Permeation Enhancement Technology (GIPET®) I, II, III, which is a solid-dose (enteric-coated tablets)/microemulsion-based technology mainly based on medium chain fatty acids or their derivatives' permeability-enhancing effect [82]. Either the enteric-coated tablets or the microemulsions in an enteric-coated soft gel capsule can achieve the oral bioavailability of 5 – 13% for a range of peptide and protein drugs, compared with the normal low bioavailability < 1%. Moreover, addition of polyethylene glycosylated C8 and C10 fatty acids to SMEDDS were shown to open the tight junctions between Caco-2 cells. This increased the permeability of the paracellular marker ranitidine 20-fold compared with a control microemulsion without the C8 and C10 derivatives, while no changes occurred in the permeability of the transcellular marker propranolol, and this finally resulted in a 15.2% bioavailability of this SMEDDS in comparison with subcutaneous injection in diabetic beagle dogs [53].

3.3 Increased retention time at absorption sites

Another frequently used strategy for enhancing oral absorption of peptide and protein drugs is to increase the retention time at the absorptive site in the GI tract. This can be done by including mucoadhesive polymers in the DDS, in such a way that the DDS adheres to the mucus layer lining to the intestine. By increasing the residence time, the absorption may be improved under the condition that the peptide and protein drugs are not degraded. Chitosan is known as a good biocompatible and mucoadhesive polymer, and is included in one of the examples of a mucoadhesive DDS for oral peptide and protein delivery [83]. In that study, it was found that chitosan-coated liposomes prolonged retention of polymers and calcitonin in the GI tract, owing to the mucoadhesive and mucus penetration properties of the polymer [83]. In addition, Thongborisute *et al.* reported that only when the peptide drug was encapsulated by chitosan-coated liposomes, the oral absorption of calcitonin could be improved to an oral bioavailability of 8.3%, compared with a bioavailability of 0.1% if the drug was absorbed to the surface of chitosan-coated liposomes [47]. Furthermore, submicron emulsion droplets with polyacrylic acid have been shown to adhere to the epithelial mucosa, resulting in a 14.7% relative bioavailability on intracolonic administration (10 IU/kg) to Sabra rats. The bioavailability enhancement was attributed to both adherence of the polymer-modified emulsion droplets to the epithelial mucosa, as well as the protease inhibition and absorption enhancement properties of the polyacrylic acid applied polymer [87]. Similarly, another study also showed that the polyacrylic acid adhered strongly to mucosa obtained from rat stomach and small intestine and *in vivo* study showed that the GI transit time of polyacrylic acid-coated microspheres was prolonged, compared with that of the polyglycerol ester of fatty acid-based microspheres [85]. Other studies on mucoadhesive particulate systems, developed to increase the residence time of both drugs and enhancers, have been reviewed [13,89]. For example, Thirawong *et al.* designed the pectin–liposome nanocomplexes, which can increase the retention time in mucus layers. The performed *ex vivo* mucoadhesive test by using confocal laser scanning microscopy suggested that pectin could prolong retention of FITC-dextran 4000 (FD4) or FD4-loaded liposomes and allow them to penetrate into GI epithelial cells, especially at duodenum and jejunum parts of the small intestine (**Table 5**) [87].

In addition, surface modification of the lipid-based DDS by polymers can influence the fate of the lipid-based DDS in the GI tract and the extent of absorption by the above-mentioned three approaches. For instance, the polymer-coated liposomes can improve the stability of both the liposome and the drug in GI tract [45,47]; the chitosan and polyacrylic-coated liposomes can be mucoadhesive and increase the retention time [47,87]. Moreover, the chitosan-coated SLN can avoid phagocytosis after intestinal uptake, compared with the non-coated SLN [91].

4. Conclusion

Over the past decades, various safe and highly potent peptide and protein drugs have been developed. However, effective delivery of these drugs into the human body by the oral route remains a major challenge, even though the employment of lipid-based DDS shows promising potential. The present paper reviews the types of lipid-based DDS for oral delivery of peptides and proteins, mainly including solid lipid core particles, liposomes, (micro)emulsions and self-emulsifying systems. Some of the benefits of using lipid-based carriers are their biocompatibility, potential permeability-enhancing effect and the ease of modifying the composition of the DDS. In conclusion, numerous lipid-based DDS have been designed to improve the oral absorption of peptide and protein drugs by increasing the stability, the permeability and/or the retention time in the GI tract.

5. Expert opinion

Quite a number of lipid-based DDS have been developed and investigated for oral peptides and proteins delivery. At present, very few lipid-based DDS with peptides and proteins are on the market for oral delivery, with the exceptions of Sandimmune® and Neoral® containing the cyclic peptide cyclosporine, even though many technologies are recently patented and some lipid-based DDS are in clinical trials. For example, the Emisphere Co. employed SNAC in the formulation for non-covalent interaction with peptide, and a Phase I clinical trial for potential glucagon-like peptide-1 (GLP-1) drug is presently ongoing. However, a similar project with calcitonin for osteoporosis treatment was recently abandoned, due to the failure of reaching the clinical end points in a Phase III study. Another example is the sodium caprylate suspension of octreotide in hydrophobic oil developed by Chiasma Co., which is now in Phase III clinical trial.

In the SLN DDS, basically, the liquid lipid of an emulsion is replaced by a solid lipid. Incorporation of the peptide and protein drugs into the solid lipid core may improve their stability and thus increase the level of permeated amount after oral administration. This is mainly due to a fixation of the drug in the solid lipid core, which will avoid an exposure to the GI fluid to some extent, and finally decrease the drug degradation. However, for preparation of SLN via the high-pressure homogenization method, the lipid needs to be melted at least 5 – 10°C above their melting point during preparation, which can lead to peptide and protein degradation and loss of drug into the aqueous phase [92]. By nature, lipids that are solid at room temperature often contain long chain saturated fatty acids, which render the core of solid lipids very hydrophobic, and complicate the incorporation of hydrophilic peptides and proteins, often resulting in very low E.E. [38]. In order to enhance the E.E. of peptides and proteins into the melted solid lipid mixture, a protein–lipid complex can be pre-formed, for example, by reverse micelle-double emulsion technique (**Table 1**).

Another frequently used approach for oral delivery of peptides and proteins is liposomes and they can encapsulate the hydrophilic drugs in the aqueous core. Liposomes have been reported to protect the peptide and protein from degradation, due to the protection by the phospholipids bilayer [90]. However, liposomes often show colloidal instability in the GI tract. This is due to interactions with bile salts and other lipids that can protrude the vesicle structures, thereby disrupting the phospholipids bilayer. Further, phospholipase A2 is secreted from the pancreas and will hydrolyze phospholipids in the liposomes, which also will destroy the vesicular structure. Thus, there is a need to stabilize the liposome in order to protect them from fast degradation in the GI tract. This can be done by selection of phospholipids or other possible liposomal components that are not easily hydrolyzed, or by modifying the liposomal surface by polymer coating or ligand modification (e.g., lectin) [44,94], the modification process being rather complicated and may induce further safety issue to consider.

The applicability of (micro)emulsions as oral DDS is motivated by the following reasons: first, there are different kinds of emulsions (w/o, o/w, w/o/w), which can be applied for both hydrophilic and hydrophobic peptides and proteins [92]; second, they can easily be prepared either by homogenization or spontaneous emulsification, taking the physical instability of the peptide and protein drugs into consideration. Moreover, the (micro)emulsions may improve the oral drug absorption, due to the small particle size, permeability- and solubility-enhancing effect and the protection of drug against enzymatic degradation by the oil droplet, if the drug is encapsulated into either the oil phase (o/w) or the inner water phase (w/o and w/o/w). However, the (micro)emulsions are usually digested very fast *in vivo* and lost the ability to protect peptide and protein drugs. In this case, the enzyme inhibitor can also be loaded into the (micro)emulsions together with the drug, in order to increase the protection of drug. Recently, SMEDDS/SNEDDS for poorly soluble drugs have attracted attention as DDS for oral delivery of peptide and protein drugs. They are attractive for peptide and protein drug delivery for a number of reasons. For example, i) the mild condition of preparation, minimizing the drug loss during the preparation, ii) the barrier of the oil that offer protection against GI tract degradation of peptides and proteins, iii) the permeability-enhancing properties, due to the component used and iv) substantially higher stability of the drug itself due to the anhydrous nature [62,93]. To load the hydrophilic peptides and proteins into the lipophilic oil mixture, the ARMs technique (**Table 4**) can be employed or by complexing the peptide and protein drug with lipophilic surfactants (**Table 3**). The essential part is how lipophilic the peptide and protein drug can become by these approaches in order to get enough drug loading for oral dosing. The SMEDDS/SNEDDS can also be digested rather fast in GI tract, with the risk of peptide and protein degradation prior to absorption. Enteric coating of capsules loaded with SMEDDS/SNEDDS can be used to avoid gastric degradation of peptides and proteins.

Overall, solid lipid core particles DDS and SMEDDS/SNEDDS seem to be advantageous with regard to the following aspects: a relatively ease of industrial scale-up and the avoidance of use of organic solvents as compared with liposome DDS. However, solid lipid core particles DDS consist of solid lipid particles dispersed in an aqueous phase, which complicates dosing. The aqueous phase can be removed by lyophilization or spray drying, but quality control of this process is usually difficult and expensive. SMEDDS/SNEDDS has the advantage over solid lipid core particles DDS of being dosed as a capsule containing a preconcentrate, which is a simple lipid solution. The lack of heating in the production process also provides a better stability of the peptide or protein drug.

In conclusion, oral delivery of peptides and proteins has shown to be feasible by application of lipid-based DDS. Significant progress has been made in this area with several technologies on clinical trials. However, a better understanding of the mechanism of action *in vivo* is needed in order to

improve the design and development of lipid-based DDS with the desired bioavailability and therapeutic profile.

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