Lipid-Based Nanocarriers for Oral Delivery of Proteins and Peptides: Opportunities, Challenges, and Future Prospects

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ABSTRACT: Oral administration is the most common drug delivery route with high levels of patient acceptance. However, oral delivery of therapeutic proteins/peptides is extremely difficult, and improving the pharmacological bioavailability still is a challenging goal because of the poor membrane permeability, high molecular weight, and enzymatic degradation of these drugs. Lipid-based nanocarriers represent a viable means for enhancing the oral bioavailability of protein or peptide drugs while minimizing toxicity. Nowadays, like other macromolecules protein or peptide drugs are the promising candidates to be delivered employing liposomes and lipid nanoparticles including solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). Generally, these drug delivery strategies can reduce protein or peptide drug degradation and improve membrane permeability thus enhance bioavailability. This review demonstrates various lipid-based formulation strategies used for successful oral delivery of peptides and proteins, assesses the hurdles and delivery efficiency and their clinical implications.

Key words: Gastrointestinal tract; lipid nanoparticles; nanocarriers; oral delivery; peptide.

INTRODUCTION

From the perspective of various focal points, the oral route of drug administration is the most favored over that of others because of patient compliance, considerable resiliency in the design of formulation, and cost-effectiveness.[1] Recently, the enormous development in protein drug delivery has been attained which can be attributed to three major improvements: i) improved analytical methods for the detection of hormones and peptides, ii) large scale manufacturing of polypeptides due to enormous advancement in genetic engineering, and iii) better insight into the role of proteins in the pathophysiology of human ailments.[2] Proteins have turned into the choice of treatment for various human diseases because of their selectivity and capacity to provide effective therapy at a low concentration.[3]

The recent advancements of recombinant DNA technology have facilitated the production of high purity, quality, variety, and human-contaminant-free proteins in an abundant quantity. Therefore, pharmaceutical companies around the globe have developed protein oral drug delivery techniques for delivering active pharmaceutical ingredients (API) commercially (Table 1). Formulating peptides is a great challenge to pharmaceutical researchers because of their diverse unfavorable properties including macromolecular size, proneness to be degraded enzymatically, lower ability to withstand the low pH of the stomach, imperviousness through the intestinal membrane, short biological half-life, immunogenic nature, and the tendency to undergo conformational change and denaturation.[4] As reduced bioavailabilty of the proteinaceous drugs is due to their susceptibility to the enzymes and lower permeability through the intestinal wall, the challenge is to improve the plasma concentration of these agents from less than 1% to at any rate between 30–50% following oral administration.[5] Hence, unlike

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non-biologicals developing oral dosage forms for proteinaceous drugs requires special strategies, such as chemical modification, recruiting formulation vehicles, protease inhibitors, absorption enhancers, and mucoadhesive polymers which are currently under investigation.[6] Among the various formulation strategies, the incorporation of nanoparticles as a transporter has been able to attract considerable interest in this field of protein drug delivery. Nanoparticles as a drug delivery system are used to control molecule size, surface properties, and release of drug to accomplish the site-specific activity at the therapeutically optimal rate and dose regimen.[7,8] They have certain advantages including higher shelf-life during storage, stability *in vivo* after administration, and simplicity to scaling up without an aseptic procedure for oral administration.[9] Particularly, nanoparticles attached with peptidic ligands collectively can make a significant synergistic effect and hold out a considerable promise for the future.[10] This review focuses on the barriers to absorption for oral protein delivery, current techniques in protein delivery, lipid-based nanocarriers, and their suitability over other systems, and recent advancements in oral lipid-based nanocarriers for the delivery of protein or peptide drugs.

Table 1. Protein oral delivery technologies under development by companies.[14]

Company name	Product name	Protein drugs delivery strategies	Characteristics of the strategies and their advantages	Currently available Such products
Emisphere	Eligen®	Carrier molecules	Absorption is enhanced without altering the chemical form or pharmacological properties and the drug molecules cross the cell membrane through transcellular transport.	Calcitonin, GPL-1, PYY, insulin, growth hormone, parathyroid hormone, heparin.
Altus	CLEC®	Protein crystallization	Catalysts containing the enzyme alcohol dehydrogenase (ADH). Protein stabilization against proteolysis and self-digestion.	Calcitonin, other polypeptides, lipases, esterases, and proteases.
Generex	Oral-LynTM	Spray device and aerosol particles	Transported through penetration the buccal epithelium. Treatment of type 1 and 2 diabetes.	Insulin, macrotonin.
NOBEX/ Biocon	HIM2	Amphiphilic oligomers	Enzyme digestion inhibited and increased membrane permeation.	Insulin, enkephalin, Calcitonin.

BARRIERS TO ORALLY DELIVERED PROTEIN ABSORPTION

GI tract (GIT) itself acts as a barrier to the oral absorption of various drugs utilizing its physicochemical nature and hence before designing an oral protein/peptide formulation it is imperative to understand the effects of these factors which include highly variable pH, proteolytic enzymes, mucosal barrier, size and charge constraints (Figure 1).[11,12]

Effect of GI pH and enzymes. The conformation, solubility, and stability of protein and peptides are largely affected by the pH of the medium due to the presence of ionic groups on the amino acids. Intra or intermolecular change in the ionic concentration and extent of the hydrogen bonding capacity of the protein molecules significantly alters the three-dimensional structure of the protein. This in turn transforms the active state of the protein molecules to an inactive state and opens them up for rapid hydrolytic and/or enzymatic degradation.[12] The stomach produces gastric juice consisting of hydrochloric acid (HCl), potassium chloride (KCl), and sodium chloride (NaCl) and exhibiting a mean pH of 1.7 to the fluid in a fasted state which can increase up to 6.7 in the presence of food contents.[13] This acidic pH unfolds the three-dimensional structure of the protein, denatures it, and favors the enzymatic attack by pepsin causing proteolysis of proteins and peptides by pepsin into its constituents; aminoacids, dipeptides, what's more, tripeptides for

absorption.[14] The acidic pH of the stomach changes to pH 6 in the duodenum, 7.4 to the terminal ileum, and then drops down to 5.7 in the caecum, and 6.7 in the rectum.[15–17] These abrupt changes in pH found throughout the GI tract (GIT) therefore impose a major obstacle for the oral delivery of the protein drugs (Figure 1).
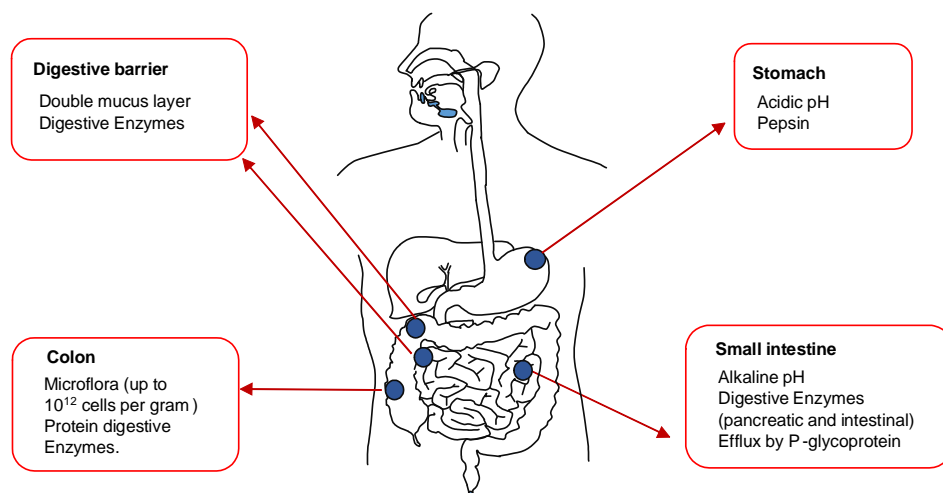

Digestive barrier

Double mucus layer
Digestive Enzymes

Stomach

Acidic pH
Pepsin

Colon

Microflora (up to 10^{12} cells per gram)
Protein digestive Enzymes.

Small intestine

Alkaline pH
Digestive Enzymes
(pancreatic and intestinal)
Efflux by P-glycoprotein

Figure 1. Major barriers to oral delivery of protein or peptide based drugs.

Table 2. A list of different proteases alongside their destinations of activity.[14,19]

Types	Enzymes	Major site of action
Gastric proteases	Pepsins (aspartic proteases)	Broad activity, hydrolyzes many peptide bond peptides
Brush border proteases	Aminopeptidase A Aminopeptidase N Aminooligopeptidase Dipeptidylaminopeptidase IV Carboxypeptidase	Aminopeptidases are N-terminopeptidases, degrading mostly 3–10 amino acid residue-dipeptides and amino acids
Cystosolic proteases	Di- and tripeptidase	2-3 aminopeptide amino acids
Intestinal pancreatic proteases	Trypsin (endopeptidase) α-chymotrypsin (endopeptidase) Elastase (endopeptidase) Carboxypeptidases (exopeptidase)	Peptide bonds of basic amino acids/ peptides Peptide bonds of hydrophobic amino acids/peptides Peptide bonds of smaller and nonaromatic amino acids/peptides A: C-terminal amino acid B: C-terminal basic amino acid
Brush border proteases	Aminopeptidase A Aminopeptidase N Aminooligopeptidase Dipeptidylaminopeptidase IV Carboxypeptidase	Aminopeptidases are N-terminopeptidases, degrading mostly 3–10 amino acid residue-dipeptides and amino acids

In addition to the unfavorable pH of the GIT, the presence of several proteolytic enzymes can also degrade orally delivered protein drugs. Endopeptidases such as trypsin, chymotrypsin, and elastase hydrolyze the bond interior to the terminal obligations of the peptide chain, while exopeptidases

including carboxypeptidase and aminopeptidase hydrolyze the bond connecting the NH_2-terminal or the COOH-terminal amino acid to the peptide chain (Figure 1).[18]   Table 2 shows different proteases alongside their destinations of activity.[14,19] Enzymatic degradation can happen at the lumen, brush outskirt, the cytosol of the enterocytes, and even in the lysosomes and other cell organelles.[20]

The partially digested protein then goes into the duodenum where it meets a higher pH. The abrupt change in the pH from around 2 in the stomach to 6 in the duodenum causes precipitation of peptides and proteins as this wide pH range covers the isoelectric purposes of numerous peptides and proteins.[21] These precipitated proteins don't quickly redissolve upon pH change.[22] On the other hand, the small intestine is the most significant spot for the absorption of food and drugs.   However, the enzymatic action of proteases in this region is higher than in some other sections of the GI tract.[23]   In the duodenum, the presence of pancreatic proteases including endopeptidases (trypsin, chymotrypsin, and elastase) and exopeptidases (aminopeptidases and carboxypeptidases) convert the ingested proteins and peptides into smaller peptides with 2–6 amino acid residues (Figure 1).[24] Aminopeptidases activity is found around 20–30% in some areas of the jejunum and ileum than in other neighboring zones. Because of this special property these areas, known as Peyer's patches, are potentially attracted sites for the delivery of proteins and peptides.[14,25,26] Besides the luminal portion of the small intestine, brush border, the microvilli-covered surface of cells in the small intestine, is another protein digestion site that contains enzymes such as alkaline phosphatase, sucrase, and as many as fifteen peptidases. Brush border enzyme action usually is more prominent in the duodenum and the jejunum than in the ileum while in the colon, the activity of membrane enzymes is significantly low.[12,18,27] This is primarily due to the difference in the available absorptive surface area as the microvilli become smaller and fewer while going down from the small intestine to the large intestine.[28] The process of protein and peptide degradation is not limited to the lumen and brush border of the small

intestine only but also continues in the cytosol of the enterocyte, in the lysosomes, and other cell organelles thus contributing to the poor bioavailability of the protein drugs (Figure 1).[29,30] The enzymatic activity of the peptidases differs between the brush border membrane and the cytosol of the enterocyte. Oligopeptides consisting of four or more amino acids are initially degraded by the brush-border membrane peptidases while those containing two/three amino acids are hydrolyzed by cytoplasmic peptidases.[26,31]   The presence of luminal pancreatic peptidases such as trypsin, chymotrypsin, and others residing in the brush border of the enterocytes may further digest the di/tripeptides which again are hydrolyzed inside the intestinal cells. Furthermore, in some cases, digestion of these biologicals also occurs inside the subcellular organelles such as endosomally internalized proteins undergo degradation within the lysosome.[12] It is estimated that lysosomes have an excess of 60 peptidases comprising of both endo and exopeptidases which collectively can act over the proteinaceous drugs to convert them into free amino acids.[29,32]

In 1981, Garrido *et al.* reported that, in the bypassed jejunum, the proteolytic activity of the peptidases was reduced leading to lower absorption of free amino acids and peptide solutions.[33] Interestingly, the absorption of large peptides and proteins was increased following administration in the ileum compared to oral ingestion suggesting the variations in the distribution of the proteolytic enzymes throughout the intestine.[29,34]   For this favorable property, the colon has been considered as a potential site for oral protein drug delivery. However, a substantial amount of microbial flora presents in this region producing a large number of peptidases capable of hydrolyzing proteins.[35]   In addition, the residual pancreatic enzymatic activities are still present in the colonic content.   Taken together, these factors impose challenges to the effective delivery and bioavailability of the proteins and peptide drugs.[29,36]

Mucosal barrier. Mucus plays an empirical role in the absorption and bioavailability of oral dosage

forms. Mucus is a physical barrier rather than chemical and composed of mainly secreted glycoprotein mucin. The thickness of the mucus layer varies with the thickest lining is found in the stomach and colon while in the small intestine the thickness depends on the extent of enzymatic activity.[37] The mucosal barrier comprises three protective layers providing the mucosal surface of the stomach with an additional defense mechanism. The first layer is a compact epithelial cell lining bound by tight junctions which protects the mucosal lining from vicious liquids. The second defensive layer is a special coating of mucus, secreted by surface epithelial cells and mucosal neck cells and completely wrapping the gastric mucosa with a protective gel-like layer. The third layer is made up of bicarbonate ions which are secreted by the surface epithelial cells.[38,39] One of the major components of the mucosal barrier is a hazy and fibrous layer of glycocalyx; chemically it is a weak acidic coat containing mucopolysaccharide and resides on top of the epithelial cells.[40] The top of the glycocalyx layer is lined by mucus secreted from the goblet cells. The mucus consists of mucin glycoproteins, enzymes, electrolytes, and water and exhibits cohesive and adhesive properties attributable to the presence of glycoproteins.[41–44]

The mucin and glycocalyx layers are the most important hindrances to peptides and proteins, which must be diffused first for arrival at the epithelial surface layer.[40] The viscosity and adhesive properties of these layers confer specific resistance to the diffusion of proteinaceous drugs[45]. Moreover, protein drugs transported to the mucosal surfaces are typically proficiently expelled by mucus clearance mechanisms [46] that persistently traps, expels pathogens and outside particles to shield the epithelial surface. This explains why poor tissue penetration is one of the greatest obstacles to orally administered drugs at present. Nanoparticles as drug transporters are a decent choice to penetrate the mucus layer and escape from the disposal activity of the mucus as well.[47]

Efflux pumps. Proteinaceous active transporters localized on the apical membrane of the mature epithelial cells responsible for the multidrug resistance in humans are called efflux pumps.[48] While some transporters facilitate absorption others such as P-glycoprotein (PGP), breast cancer protein, and multidrug resistance protein, etc. reduce the intestinal absorption of some drugs.[49] The mechanism by which most of the efflux pumps on the apical membrane reduces the absorption of different types of drugs includes the transportation of the drug molecules from the enterocytes back to the intestinal lumen.[50] Toxins, xenobiotics, lipids, peptides, and poorly water-soluble drugs, etc. are the substrates of such efflux pumps. So, these efflux pumps reduce the absorption of these types of drugs and thereby reduces their therapeutic activity also. For example, linear lipophilic and cyclic peptides (such as cyclosporine) are the substrates of PGP-I so, when these peptides are absorbed in GI the PGP-I pumps them back to the GI lumen.[51,52]

Particle size, surface charge, and solubility limitation. Physical properties of the proteins like size, charge, etc. limit the paracellular transport across the tight junctions and aqueous channels between the epithelial cells.[53] Paracellular protein transport is interdependent on its size and charge which means at constant charge, protein transport becomes size-dependent while the process is charge-dependent when size is constant.[54] Studies found that positively charged peptides are highly permeable and interact with the protein or lipid lining of the aqueous pores whereas, lipophilic peptides cross the lipid membrane through the transcellular pathway, for example, cyclosporine A.[54,55] Adjustment of the formulation and chemistry drastically interferes with the solubility of the drug and is preferred in pharmaceutical industries as size modulation is not a routine intervention. Salt formation of protein may enhance paracellular transport and thus increase the solubility while covalent attachment of protein with the hydrophilic or lipophilic polymer may increase the transcellular uptake of protein, for example, lipid derivative of insulin for sustained release.[56]

ROUTES OF PROTEIN AND PEPTIDE DRUG ADMINISTRATION

A major portion of the protein or peptide drugs are administered by the parenteral route and a large portion of them become ineffective as they are eliminated too quickly from the body. So, a site-specific delivery system that focuses on the delivery of low dose protein or peptide drugs to a particular body compartment without any undesirable side effects was initiated e.g. nasal and pulmonary delivery.[57] Table 3 illustrates some recent delivery systems of proteins and peptide drugs through various routes. Nevertheless, the oral route remains the most preferable route of drug delivery despite these recent advances to administer proteinaceous

compounds mainly due to the high patient compliance. The major drawback of oral delivery of protein or peptide drugs is poor absorption attributable to the factors discussed in the previous section. Absorption of these biologicals can be improved by by modifying the intestinal epithelial cells and targeting the M cells. Various strategies have been adopted in recent years to enhance the bioavailability of proteins such as chemical modification of the biological, absorption enhancers, mucous adhesion systems, and nanoparticle-based drug delivery.[58] However, this review focuses on the lipid-based nanoparticulate systems for delivering protein biologicals through the oral route.

Table 3. Different routes and approaches to deliver therapeutic protein.[57,132]

Routes of delivery	Formulation and device requirements
Direct injection: intravenous (i.v.), subcutaneous (s.c.), intramuscular (i.m.), intracerebral vein (i.c.v.)	Liquid or reconstituted solid (syringe), i.v. injected liposomes.
Depot system (s.c. or i.m.)	Biodegradable polymers, liposomes, permeable polymers (not degradable) microspheres, implants.
Pulmonary	Liquid or powder formulations, nebulizers, metered-dose inhalers, dry powder inhalers.
Oral	Solids, emulsions, microparticulates, nanoparticles, with or without absorption enhancers.
Nasal	Liquid, usually requires permeation enhancers, nanoparticles.
Transdermal	Iontophoresis, electroporation, chemical permeation enhancers, prodrugs, sonophoresis, transfersomes.
Buccal, rectal, vaginal,	Gels, suppositories, bioadhesives, microparticulates.

MECHANISM OF ABSORPTION OF PROTEIN OR PEPTIDE DRUG-LOADED NANO-PARTICLES THROUGH ORAL ROUTE

Protein and peptide drug-loaded nanoparticles can be absorbed through the gastrointestinal film through four modes: i) transcellular/transmembrane transport, ii) receptor-mediated transport, iii) carrier-mediated transport, and iv) paracellular transport (Figure 2).[6]

Transcellular/transmembrane transport. The transcellular/transmembrane pathway for absorption of nanoparticles involves transcytosis by which the molecules are diffused through the apical and basolateral membranes (Figure 2).[59] At first, the nanoparticles are endocytically taken up by the apical

membrane of the cells followed by transportation through the cells, and then discharged on the comparatively thinner basolateral membrane.[60] Finally, the transcellular transport ends up with the entrance of the absorbed particles into the blood.[6] This route is particularly favorable for lipophilic drugs due to the lipoid nature of the bilayered cell membrane (Figure 2). In addition, the size, charge, and hydrogen bonding potential may also affect the transcellular absorption of protein-based nanoparticles.[61] Intestinal epithelial cells and M cells are mainly responsible for the transmembrane uptake of the peptide nanocarriers. M cells are microfold cells and are mainly seen in the epithelium of the Peyer's patches representing only about 1% of the

total surface area of the intestine.[62] These cells are responsible for producing a mucosal immune response as they carry the antigenic particles from the lumen of the intestine to the lymphoid tissue. The M cells exhibit pronounced endocytic activity by a wide variety of adsorptive mechanisms such as clathrin-coated pits and vesicles, fluid-phase endocytosis, and phagocytosis which is conducive to the transport of peptide drugs, macromolecules, microorganisms as well as nanoparticles.[6] However, the intestinal enterocytes also have the ability to translocate particles although to a lesser extent attributable to their low endocytic activity.[63,64] The unique morphological features of the M cells including lack of a thick filamentous brush border glycocalyx, a thinner mucous gel layer, and presence of comparatively scanty, non-uniformly shaped microvilli of the apical surface further allow them to

easily fuse and contact the drug particles.[65] Hence, the mechanism of transmembrane transport of the protein-based nanoparticles through the M cells involves attachment of the molecules to the specific glycoproteins, endocytosed on the apical membrane, and being absorbed inside the M cells (Figure 2). Additionally, these cells have a hollow cavity on the basolateral side containing lymphocytes, dendritic cells, and phagocytes. The endocytosed nanoparticles are then released to this membrane and enter the systemic circulation. Due to the small size, massive surface area, and high attachment capacity, nanoparticles aggregate in the Peyer's patches from the lumen of the intestine suggesting the transcellular transport across the M cells is a potential mechanism of proteinaceous drug absorption through the oral route (Figure 2).[66]
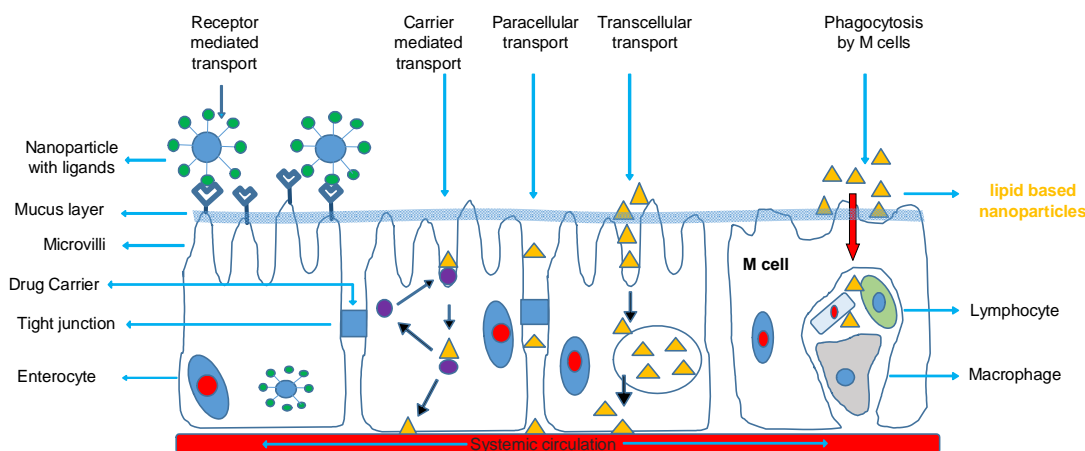

Figure 2. Routes of proteinaceous drug absorption.

Receptor-mediated transport. In the receptor-mediated pathway, the drug molecules bind to the membrane proteins or receptors as specific ligands or may themselves act as receptors which then bind to the surface attached ligands. This receptor-ligand complex is then incorporated inside the cell through the various processes of endocytosis including phagocytosis, pinocytosis, receptor-mediated endocytosis (clathrin-mediated), and potocytosis (nonclathrin-mediated) (Figure 2). The process of

drug transportation is specifically exploited for di- and tri-peptides and also for monosaccharides and amino acids. Receptor-mediated endocytosis isn't limited by the size of the drugs molecules rather dependent on the nature of the receptor and the ligands.[67] The endocytosed drugs are then taken up by the systemic circulation through two distinct absorption pathways: portal/hepatic circulation and intestinal lymphatic system. Portal circulation, due to its superior ability to transport both hydrophilic and

lipophilic drugs, represents the principal drug absorption pathway. Water-soluble molecules enter the liver through the hepatic portal vein and subsequently are transported to the systemic circulation passing through the hepatic artery and then to their sites of action. Conversely, lipophilic drugs (log *P*= 5) utilizing the intestinal lymphatic route have the advantage of avoiding the hepatic first-pass metabolism as they are directly emptied into the systemic blood flow through vena cava.

Carrier-mediated transport. Carrier-mediated transport is also known as facilitated diffusion or active absorption where particularly small hydrophilic molecules, di/tri-peptides (e.g., β-lactam antibiotics, angiotensin-converting enzyme (ACE) inhibitors, renin-inhibitors), monosaccharides, and amino acids are carried across the cells and then enter the systemic circulation from the basolateral membrane of the enterocytes with the help of membrane proteins or transporters.[26] This is an energy-dependent process which can occur even against a concentration gradient.

Paracellular transport. This is a passive diffusion process where molecules are transported through the aqueous pores/channels between adjacent epithelial cells. These pores occupy approximately 0.01-0.1% of the total surface area of the intestine corresponding to around 200 to 2000cm^2 area.[68] Small quantities of protein can be absorbed through this amount of space and provide their pharmacological activity.[53] Transport through this paracellular route is preferable for most low molecular weight water-loving molecules including small protein fragments and peptides.[59] The rate-limiting step in this process is transporting across the tight junctions between the epithelial cells. The epithelial tight junctions in the jejunum, ileum, and the colon of human intestine create aqueous pores with an average size of approximately 7–9 Å, 3–4 Å and 8–9 Å repectively.[69] This small size restrains the passage of solutes with a sub-atomic range surpassing 15 Å (around 3.5 kDa)[70] suggesting protein drug delivery through mucosal epithelia utilizing paracellular transport is critically controlled

(Figure 2). However, paracellular transport of proteins and peptides is dependent on the physicochemical properties, molecular size, shape, and overall charge of the molecules.[53]

NANOCARRIERS IN PROTEIN AND PEPTIDE DRUG DELIVERY SYSTEM

Different protein drug delivery systems attach the drug molecule to a suitable carrier system where *in vivo* fate of the drug molecules mainly depends on the properties of the carrier system. Microencapsulation, nanoencapsulation, and other techniques enveloping the bioactive therapeutic moiety enhance the therapeutic effect of the drug molecules by controlling the rate and site of drug release.[71] Some examples of the mostly employed particulate carrier systems for protein delivery include nanoparticles, polymeric hydrogels, microspheres, lipid-based drug delivery systems such as fat emulsions, liposomes, and solid lipid nanoparticles (SLN).[72]

The term "nano" refers to the size of the drug carriers that range from 1-100 nm.[73] Nanoparticles and microspheres are mainly studied for parenteral administration but in the case of mucosal administration, these are also suitable. Nanoparticle-based protein drug delivery covers different categories for example solid lipid nanoparticles, inorganic nanoparticles, polymeric nanoparticles, polymeric micelles, dendrimers, etc.[23] They prevent peptide degradation, prolong their action, and control their release properties from the formulation.[67] These properties depend on various factors like the size of the nanoparticle's surface nature namely, hydrophobicity/hydrophilicity, charge, polymer functional group, etc.[74] Parenterally administered protein drugs although have the advantage of improved bioavailability due to avoidance of the GIT-associated threats, rapid excretion hinders the sustained action of these drugs.[59] On the contrary, mucoadhesive oral nanoparticle-based proteins can adhere to the mucus layer of the intestine to enhance the retention of the drug at the absorptive site of GIT which, in turn, extends the action and achieves site-

specific delivery.[67] For example, chitosan, a biocompatible mucoadhesive polymer prolonged the retention of orally administered calcitonin in GIT.[75] Moreover, undesirable peak and trough concentrations of the drug can be avoided by proper excipient selection in nanoparticle-based oral protein delivery.[76]

Biodegradable polymers from natural or synthetic origin have extensively been studied to deliver proteinaceous drugs orally. Different delivery techniques and pharmaceutical applications are possible based on biodegradable materials used, degradation kinetics, and particle size distribution.[77] Biodegradable polyesters like poly (lactide) (PLA), poly (lactide-co-glycolide) (PLGA), poly-ε-caprolactone (PCL), and poly (orthoesters), chitosan, alginate, etc are most commonly used to formulate polymeric nanoparticles.[78] Among these biodegradable polymers poly (lactide-co-glycolide) (PLGA) is used to formulate protein or peptide nanomedicine successfully as it produces biodegradable monomers lactic acid and glycolic acid. Based on the formulation techniques polymeric nanoparticles are two types, nanospheres which are matrix type having an entire solid mass, and nanocapsules having a solid shell enveloping a liquid or semisolid core.[23] Specific physicochemical and biological properties need to be met by these polymers to deliver the drug. Structural modifications like grafting specific functional groups such as hydroxyl, carboxyl, amine, etc. on the surface of the polymer may be done to enhance the permeability of the drug molecule, target specific tissue, prolong duration action through mucoadhesion, etc.[79] Despite having tremendous benefits of polymeric NP's in peptide delivery; these carriers suffer from some drawbacks including toxic degradation, toxic monomer aggregation, residual material linked with them, and toxic degradation process [80]. There Another type of nanoparticle namely inorganic nanoparticle is now being investigated for oral nano-sized protein or peptide drug delivery.[81] However, in contrast to polymeric nanoparticles, inorganic nanoparticles are less employed for this purpose. Inorganic nanoparticles are manufactured from calcium, phosphate, ceramic, gold, etc. For example, calcium phosphate-PEG-insulin-casein (CAPIC) an oral insulin delivery system has been developed by BioSante Pharmaceuticals.[23] As inorganic nanoparticles are not metabolized rapidly or completely *in vivo*, some portion may pile up in the body, and toxicity may occur demanding extensive research on this type of nanoparticles.

LIPID-BASED NANOPARTICLES

An oral formulation of protein or peptide is limited by their poor stability and less permeable capacity in GIT (discussed earlier) which can be bypassed by formulating these agents into lipid-based nanoparticles as a delivery system.[82] Lipid-based nanoparticles are designed by incorporating drug molecules into the inert lipid carriers which are further stabilized by using surfactants.[83] This type of formulation is well tolerated due to the use of physiological lipids such as phospholipids, cholesterols, cholesterol esters, triglycerides, etc. Potential lipid-based carrier systems for controlled delivery of protein or peptide drugs include liposomes, solid lipid nanoparticles, oily suspensions, lipid implants, lipid microspheres, etc. Lipid-based carriers offer many advantages over other carrier systems including physiological stability and controlling the release of protein or peptide drugs because of their natural origin (mostly), manufacturing simplicity (compressing, moulding), being less prone to erosion like polymeric systems, slower water uptake which enhances protein stability, etc.[76] Protein drugs are highly fragile in GIT and hence physical encapsulation of these drugs and co-encapsulation of enzyme inhibitors reduce their degradation rate, enhance stability and retention time.[84] For example, solid lipid nanoparticles (SLN) can encapsulate protein drugs in their solid matrix thus reduces the degradation of the drugs in GIT.[77] Moreover, protein drugs are less bioavailable due to the poor permeability of the drugs. Recently various lipid compounds are used to encapsulate protein to reach the systemic circulation through the

transcellular pathway thus increase the bioavailability of the drugs.[85] For example, N-[8-(2-hydroxy benzoyl) amino] caprylate (SNAC) is used in Eligen® technology to improve the lipophilicity of

the protein and thus enhances their transcellular permeability.[86] Table 4 shows a summary of some lipid-based nanoparticle systems employed for the oral administration of proteins and peptides.

Table 4. A summary of some examples of lipid-based nanoparticles in oral delivery of protein/peptides use in clinical application.

Types of DDS	Compositions	Model Drug	References
Liposomes	Glycerylcaldityltetraether (GCTE) - liposomes	Vancomycin	133
	Lioposomes with 25% Tetra Ether Lipids (TELS)	Octreotide	134
	Biotinylated liposomes (BLPs)	Insulin	135
	Liposomes containing bioenhancer and tetraetherether lipids	Human Growth Hormone	136
	Glycerylcaldityltetraether (GCTE) - liposomes	Myrcludex B	137
	Chitosan-Thioglycolic acid – 6-mercaptonicotin amide – coated liposomes	Salmon Calcitonin	138
	Liposomes containg Sodium glycocholate (SGC), Sodium taurocholate (STC), etc.	Insulin	139,140
SEDDS/SNEDDS	Octreotide-Deoxycholate SEDDS	Octreotide	141
	Insulin-phospholipid complex loaded SNEDDS	Insulin	142
Proliposomes	Proliposomes encased in Eudragit S100	Insulin	143
SLN	Viscosity Enhancing Agent incorporated SLN nanoparticles	Insulin	144

FORMULATION OF NANOCARRIERS USING LIPIDS FOR THE ORAL DELIVERY OF PEPTIDES

Natural lipids are extensively used in pharmaceutical preparations due to their acceptable physicochemical and biopharmaceutical properties. Lipid excipients increase the absorption of different types of protein-drug through various mechanisms, reduces their enzymatic degradation, and control the release profile. In Table 5, some examples of commonly used lipid for oral delivery of proteins/peptides are shown.

Selection of lipids for lipid-based oral nanoparticles. A detailed knowledge on the chemistry and physicochemical properties of lipid is of imperative for effective protein or peptide drug formulation. Purity, chemical stability, solvent capacity, water miscibility, safety, the regulatory profile of lipids, digestibility, and the fate of digested products are considered during the selection of lipid for designing the formulations.[87] In order to develop emulsion type formulations, amphiphilic molecules exhibiting a hydrophobic region; one, two, or three hydrocarbon chain(s) of different lengths and a

differentiated polar head are commonly employed.[88] The solubilization of hydrophilic protein and peptide drugs in water can be improved by mixing them in a specific ratio with water and surfactant and then applying a phase diagram to formulate a water-in-oil-in-water emulsion.[89] In these emulsion-type systems, the oil phase usually comprises of triglycerides or mixed glycerides (a mixture of mono-, di- and triglycerides) possesing long-chain and/or medium-chain fatty acids. The protein entrapment efficiency of the triglycerides can be enhanced by by increasing the polarity of the oil phase through the incorporation of mono- and di-glycerides (polarity increases as triglycerides < diglycerides < monoglycerides).[90] By hydrolysing the triglycerides partially, a wide range of mixed glyceride excipients can be obtained that contain variable amounts of monoglycerides, diglycerides, and triglycerides. Free fatty acids, fatty alcohols, and phospholipids, etc. have also been used extensively in the design of lipid-based delivery carriers owing to their surface-active and penetration enhancing properties and their self-assembling capacity as well.[59]

THE FATE OF LIPID-BASED NANOCARRIERS INSIDE THE PHYSIOLOGICAL SYSTEM

The digestion of lipid formulation involves a physical breakdown and enzymatic hydrolysis of the triglyceride to diglyceride and fatty acid by gastric lipase from the stomach.[89] The secretion of bile salts and biliary lipids from the gallbladder stabilize the crudely emulsified lipid digestion products in the small intestine that are further digested by the pancreatic lipase/co-lipase digestive enzymes at the oil-water interface.[91] The product of lipid digestion is finally incorporated into bile salt micelles to form an intestinal mixed micellar phase with sufficient bile salt concentrations and the intestinal mixed micellar phase co-exists with several physical species in the small intestine, including multilamellar and unilamellar lipid vesicles, simple lipid solutions, and fatty acid soaps.[92] Shortly, during the digestive process, bilamellar vesicles usually transform into unilamellar vesicles and spontaneously dissolve into micellar and mixed micellar phases with an increase in the surfactant (bile salt)-to-lipid ratio.[93,94] A lipid-based formulation undergoes a similar mechanism of food-ingested lipids and can potentially generate immunogenic effects due to the absence of complement control proteins on the surface of the membrane.[95] Serum proteins (e.g. opsonin) prefer to

Table 5. Examples of commonly used lipids for oral peptide/protein delivery with their relevant properties.

Class	Example	Properties	Model drugs	References
Triglycerides	Long chain triglycerides (LCT) (Castor oil, soybean oil, tripalmitin, triolein)	GRAS, easily digested, and absorbed. Low self-dispersing capacity.	Salmon Calcitonin, Earthworm fibrinolytic enzyme, TAT (TAMRA labelled)	145–147
	Medium chain triglycerides (MCT) (Triglycerides of caprylic/capric acid)	GRAS status. Higher chemical stability than LCT. Good self-dispersing ability and better solvent capacity for hydrophilic molecules than LCT.		
Mono-, diglycerides	Glycerol monostearate, glycerol monooleate, glyceryl palmitostearate, mono/di-glycerides of caprylic acid	GRAS status. Better self-dispersing ability than LCT and MCT due to their amphiphilic nature	Lysozyme, Insulin, Erythropoietin (EPO)	106
Fatty acids	Stearic acid, oleic acid, linoleic acid, palmitic acid (subproducts of triglyceride degradation)	GRAS status. Surfactant properties and self-dispersing ability. Longer carbon chain hinders hydrophilic molecules encapsulation but enhance colloidal stability.	Leuprolide, Insulin, Thymopentin, Levothyroxine, Erythropoietin (EPO)	148,149
Fatty alcohols	Stearyl alcohol	Surfactant properties and self-dispersing ability. Used in mixtures with fatty acids to decrease lipids recrystallinisation.	Thymopentin, BSA, Erythropoietin (EPO)	100,146
Phospholipids	Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, dipalmitoyl-glycero-hosphocholine, dimyristoyl-phosphatidylcholine, distearoylphosphatidylethanolamine-PEG	Ionic amphiphiles, emulsifying and dispersion agents, self-assembling capacity into well-defined structures and bilayers	Insulin, Albumin, Epidermal Growth Factor, Leuprolide, Salmon Calcitonin	121,150–152

GRAS, generally regarded as safe

adsorb on hydrophobic rather than hydrophilic surfaces[96] and lead to their being taken up by the reticuloendothelial system (RES). Thus, polyethylene glycol (PEG) as a surfactant is added to the surface of the nanoparticles to give a hydrophilic property to prevent opsonin adsorption on the surface and

eventually the RES uptake of the nanoparticles.[97,98] Furthermore, PEGylation facilitates the paracellular transport of nanoparticles and decreases transendothelial electrical resistance (TEER) values of cells.[99] In the case of nano structured lipid carriers (NLC)s, the incorporation of endogenous lipids (both synthetic and natural lipids), prevents their uptake by macrophages.[100] For example, phagocytosis of NLCs is reduced due to the addition of high-density lipoprotein to NLCs that prevents apolipoprotein A-I (apoA-I) from binding to NLCs carrying tanshinone II-A) (a lipophilic cardiovascular drug).[101] The excipients of LBF (lipid, surfactants, and cosolvents) are degraded by lipases present in the GIT and lungs and considered harmless and usually recognized as a safe category (USFDA-approved).[102,103] Apart from this, fatty alcohols (e.g. stearyl alcohol) are degraded by a hepatic enzyme (e.g. fatty alcohol

dehydrogenase).[104] Such enzymatic decomposition breaks the structure of lipid-based nanoparticles and causes increased release of the drug.

RECENT ADVANCEMENTS IN ORAL DELIVERY OF PROTEIN OR PEPTIDES USING LIPID-BASED NANOCARRIERS

At present, lipid-based nanocarriers have drawn enormous attention in the oral delivery of proteins/peptides for their excellent capabilities in biocompatibility to cross the intestinal gastric/intestinal barrier without the degradation of the proteins/peptides. Figure 3 illustrates currently available approaches exploiting lipid-based nanocarriers for protein and peptide drug delivery via the oral route.
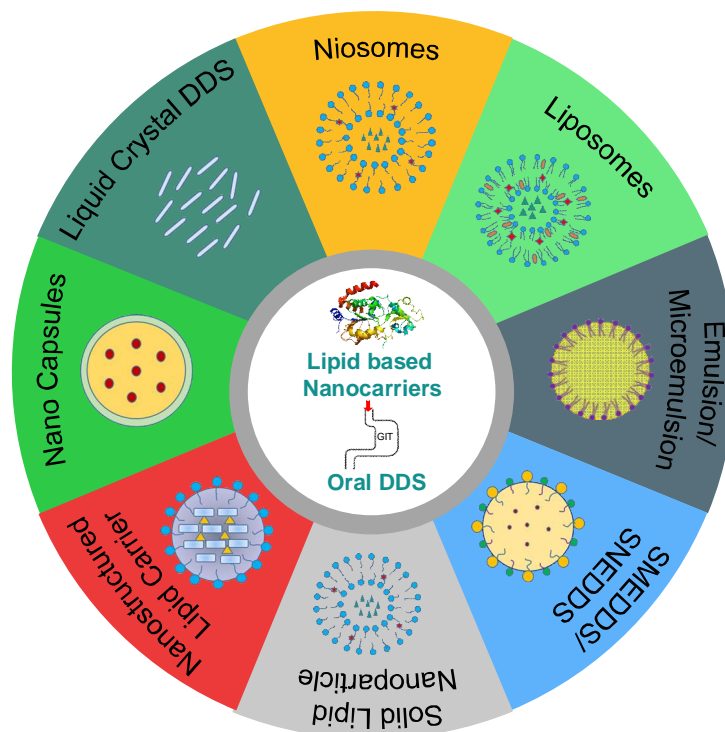

Figure 3. Currently available types of the lipid-based oral delivery of proteins/peptides.

Solid lipid nanoparticles (SLN). Natural, semi-synthetic, or synthetic lipids comprising triglycerides, partial glycerides, fatty acids, waxes, phospholipids,

and steroids, etc. that remain in the solid-state at ambient temperatures are dispersed in water with the help of an emulsifier which acts as solid lipid

nanoparticles (SLN).[105] The intrinsic lipid properties of SLNs can protect the protein or peptide drugs from enzymatic degradation and control the release from formulation.   The release properties of SLNs in highly composition-dependent and studies have shown that the degradation mechanism that controls the release of the peptide from triglyceride-based SLNs is lipase mediated.[106] Lipolysis and peptide diffusion mechanisms are the underlying mechanisms for peptide release from diglycerides-based SLNs while simple diffusion through the lipid channels is responsible for drug release from monoglyceride-

based SLNs.[89]   So, the peptide release rate follows the trend: monoglyceride > diglyceride > triglyceride, suggesting that a combination of different types of lipids can control the protein release from the formulations. The peptide molecules with the lipid components can control the release profile by ionic/hydrophobic interaction, for instance, the cationization of the peptide drug to enhance its entrapment owing to the ionic interaction with anionic lipids was found to impact the succeeding release.[106]   Different types of peptide release mechanisms are described in Figure 4.
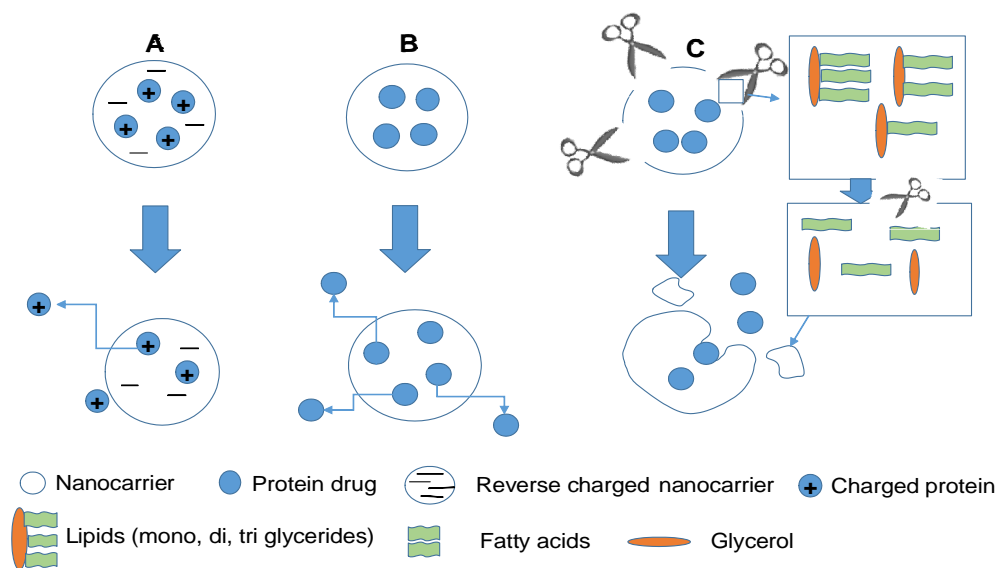

Figure 4. Peptide release mechanisms [153] from lipid based carriers based on (A) ionic disassociation followed by diffusion through lipid matrix channel, (B) simple diffusion through the lipid matrix channel, and (C) lipase-mediated degradation of the lipid matrix.

Therefore, the SLN formulation strategy can increase the hydrophobicity of peptides in the method of encapsulation, peptide-loaded reverse-micelles strategy as well as double emulsion technique to enhance the physical entrapment of the peptide molecules.[76] However, the release of the peptide can be controlled by using lipidic mixtures having distinct degradation profiles, lack of satisfactory evidence on contact with biological hurdles might justify the fact that they have not extended the clinical trials concerning utility for oral peptide and protein delivery.

Nanostructured lipid carriers (NLC). The colloidal carriers containing of a mixture of solid and

liquid lipids, and having an average particle size in the nanometer range are called NLC.[107] The ratio of solid lipids with liquid lipids ranges from 70:30 up to 99.9:0.1 and the melting point of the solid lipid decreases due to the presence of oil content.[108] NLC system can bypass the problems associated with the SLN system like elevated water content of SLN dispersions, drug expulsion during storage, and low payload for several drugs.[109]

Lipid drug conjugate (LDC) nanoparticles. Lower capacity to load the hydrophilic drugs is one of the major problems of SLN/NLC systems whereas highly potent low dose hydrophilic drugs can be incorporated into the solid matrix efficiently.[110]

Nowadays LDC nanoparticles have been developed with up to 33% drug loading capacity to overcome such problems and the preparation of LDC nanoparticles includes salt formation with a fatty acid followed by subsequent processing with aqueous surfactant using high-pressure homogenization.[110]

Liquid crystal drug delivery system. The liquid crystalline structures of some lipids due to their spontaneous self-assemble nature offer a prospective new class of sustained-release matrix named liquid crystal drug delivery system. The nanostructured liquid crystalline materials can act as a reservoir for gradual drug release in excess fluids such as the GIT or subcutaneous areas, thus they are highly stable to dilution. The rate of drug release is directly related to the nanostructure of the matrix. Nowadays, lyotropic liquid crystal systems have received considerable attention as drug delivery vehicles.[111] These drug delivery systems can be classified into lamellar (Lα), cubic, hexagonal mesophases, etc. Due to the ability to control the release of a wide range of bio-actives from low molecular weight drugs to proteins, peptides, and nucleic acids, reversed cubic (QII) and hexagonal mesophases (HII) have been extensively studied recently.[112]

Microemulsion and nanoemulsion. Two or more immiscible liquids are dispersed and thermodynamically stabilized by one or more suitable surfactants called microemulsions.[113] Both water-in-oil (W/O) and oil-in-water (O/W) microemulsions can be formed spontaneously as low/limited energy is required to produce a thermodynamically stable system. On the contrary, nanoemulsions (<200 nm droplets size) are thermodynamically unstable as high energy is required to produce a kinetically stable system.[73] Inadequate proportions of oily components, surfactant(s), and co-surfactant(s) are mixed to prepare microemulsion; whereas, high-energy processes (homogenizer, microfluidizers, or ultrasonicator), or lower-energy approaches including natural emulsification or phase inversion temperature methods, etc. are used to prepare nanoemulsion.[114] When hydrophilic peptides, MCL (most commonly),

and surfactants are mixed in a specific ratio to formulate W/O emulsion or multiple W/O/W emulsion, resulted in 80% entrapment efficiency for various peptidic drugs like insulin, sCT, and BSA.[83] Though the availability of studies on the mechanism of release of peptides from micro/nanoemulsions is limited, a study reported that the osmotically-driven swelling effect degrades emulsions and further causes drug release of emulsions containing MCT (medium-chain triglycerides/soybean oil), surfactants polysorbate 80, and cetyl PEG/PPG-10/1 dimethicone.[115]

Self-emulsifying/self-micro emulsifying/self-nano emulsifying drug delivery systems (SEDDS/SMEDDS/SNEDDS). Lipid-based nanosized droplets typically ranging from 0 to 250 nm are a promising carrier to decrease the degradation by GI fluid and enhance the oral absorption of protein or peptide drugs.[59] Self-emulsifying/self-micro emulsifying/self-nano emulsifying drug delivery systems (SEDDS/SMEDDS/SNEDDS) are thermodynamically and kinetically stable and can increase the oral bioavailability of drugs.[116] The bioavailability of saquinavir (HIV protease inhibitor) was 4% when first marketed as a hard gelatin capsule (Invirase®) but bioavailability became threefold higher than Invirase® when formulated as a self-nano emulsifying formulation (Fortovase®) containing medium-chain mono- and diglycerides, povidone, and α-tocopherol.[117]

Nanocapsules (NC). NC consist of a liquid core, normally an oil that acts as a drug reservoir, and one or more polymer coating layers encapsulating the core to control protein or peptide drug release as well as to permeate biological barriers.[7] Emulsification and polymer formation are the main two steps for NC formulation. Different strategies like the solvent displacement technique,[118] temperature cycling treatment,[119] and high-pressure or high-energy sources are used for emulsification. Whereas, polymer shell formation involves two main approaches (i) polymerization at the interface of the nanoemulsion[120] and (ii) polymer precipitation around oily nanodroplets. The versatile nature of

both the inner core and the polymer shell of NC technology and surfactant/s used to stabilize the NC may alter the encapsulation and release properties. For example, in the case of insulin-loaded PLA NC, it was reported that an increment in the amount of surfactant (sorbitan monostearate or sorbitan monooleate) was advantageous for the inclusion of insulin into polylactide NC[7]. Similarly, for both SLN and microemulsions, the employment of W/O/W emulsion core also successfully increased the peptide loading in this formulation. The release mechanism of peptides from NC involves the decrease of the NC size over time, which leads to the diffusion across the polymer shell and results in controlled release of the protein or peptide drugs whereas, the MW of the peptide was found to significantly affect the release rate, according to the following ranking: insulin (5.8 kDa) > OVA (45 kDa) > BSA (65 kDa) > urease (483 kDa)[121] Both encapsulation and release characteristics of protein or peptide drugs from the NC depend on the lipid composition of the oily core, lipid and polymer degradation, potential interaction with the peptide molecules, the affinity of the peptide for the polymer shell, diffusion across the oily medium and the polymer shell and disassociation of the peptide molecules from the counter-interacting parts.

Liposomes. Spherical-shaped concentric bilayered vesicles where an inner aqueous phase is covered by a lipid bilayer mainly hydrated phospholipids of natural or synthetic origin are called liposomes.[122] The average size of liposomes may range from tens of nanometers to several micrometers. The organic solvent is eliminated and lipids are rehydrated during the preparation of liposomes and involve diverse methods like the lipid film hydration method, the reverse-phase evaporation method, the solvent injection method as well as the detergent dialysis technique.[123] Liposomes possess the unique ability to encapsulate both hydrophilic and hydrophobic drugs due to their amphiphilic nature and encapsulate hydrophobic drugs more efficiently than other drug delivery systems. Peptides can be entrapped into the aqueous core of the liposomes and liposome-like nanostructures, such as niosomes and archeosomes, have also been proposed for the oral administration for example peptides and proteins such as insulin, sCT, albumin, adamantly tripeptides, globulin, leuprolide, and others have been entrapped into liposomes, with the final goal of enhancing oral bioavailability.[124]
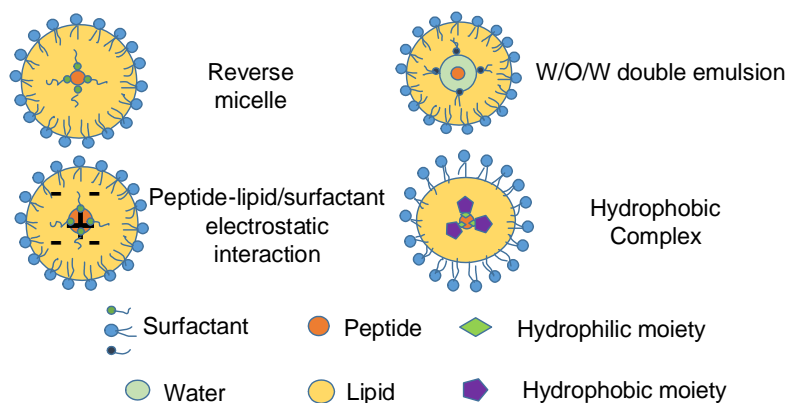

Figure 5. Demonstration of different strategies to improve the peptide loading capacity of lipid-based nanocarriers.[7]

Niosomes. Niosomes are structurally analogous to liposomes and composed mainly of non-ionic bilayer forming surfactants.[125] The synthetic surfactants used to prepare niosomes are less costly and have higher chemical stability than their naturally occurring phospholipid counterparts. Niosomes are produced by the hydration of synthetic non-ionic surfactants, with or without the incorporation of

cholesterol or other lipids.[125] They can be used for targeted drug delivery, increase the bioavailability of the drug and reduce the clearance like liposomes. As with liposomes, the composition of the bilayer and the method of production regulate the properties of the niosomes. Nowadays niosomes are used to deliver antigen and small molecules.[126]

In addition to the above mentioned lipid-based nanocarriers for the successful delivery of proteinaceous molecules, other strategies like the formation of W/O/W emulsions, hydrophobization of the peptides by reverse-micellization, complexation/ conjugation with lipophilic ingredients, etc. have been employed for increasing the peptide loading capacity of the lipid-based systems (Figure 5).

CHALLENGES AND OPPORTUNITIES IN ORAL DELIVERY OF PEPTIDES

Protein or peptide drugs are highly potent and target-specific but, *in vivo* drug delivery is a complex phenomenon because of their complex structure, poor stability, hydrophilicity, poor membrane permeability, separation, and storage problem.[127] Due to hydrophobic amino acids to the core and hydrophilic residues exposed on the surface most of the proteins show hydrophilic characteristics unless cyclization, amide formation, or ester formation block their amino and carboxyl termini.[59] For example, Cyclosporine is a cyclic peptide, which displays highly lipophilic characteristics.[128] Different levels of physiological barriers and gastric enzymes cause the inactivation of peptide drugs again, physical factors like pH, heat, moisture, etc. can limit the efficacy of these types of drugs.[19] Hence, oral administration of protein or peptide drugs is very challenging. Nowadays, different types of lipid-based drug delivery systems enhance solubility and membrane permeability, decrease enzymatic degradation, and ultimately increase the oral bioavailability of peptide drugs. For example, a lipid-based product of cyclosporine revolutionized the therapy of organ-transplant patients requiring chronic doses of this potent drug and it is now estimated that currently up to 4% of all drug products

marketed worldwide are formulated as lipid-based formulations.[128] As the physicochemical and biopharmaceutical properties of protein or peptide drugs create barriers to their oral absorption, alteration of such properties without adversely affecting their biological activity is a strategy in lipid-based peptide drug delivery technology. Different types of glycerides, surfactants, co-surfactants, and medium-chain diacylglycerols, etc. as excipients may improve membrane permeability and therefore enhance peptide drug bioavailability in different formulations. For example, permeation and stability are the major challenges for the oral absorption of peptides in GIT because of their hydrophilic nature, microemulsion (W/O) type formulation may protect them from degradation.[129] Though several drugs are successfully marketed as lipid-based preparations and the lipid-based drug delivery system (LBDDS) has a broad scope in terms of solubility and bioavailability enhancement, still this technology has some limitations such as the stability of lipid-based formulations, manufacturing methods, and the lack of a database considering the solubility of drugs in lipids.[130] To advance this technology proper regulatory guidelines for lipid-based formulations technology and further research are needed to be carried out in this field regarding the design of a proper *in vivo* model to correlate the data obtained *in vitro* studies to the actual *in vivo* experience.[131] As lipid-based drug delivery systems have been commercially employed on BCS class II compounds (poor solubility) with increased regulatory acceptance, the impact of lipid excipients on drug absorption has led to a improved interest in the potential application of LBDDS for BCS class III/IV compounds, such as hydrophilic peptides and proteins.[113]

CONCLUSION AND FUTURE PERSPECTIVES

The oral bioavailability of macromolecular drugs is limited due to several physiological barriers across the GIT including acid- and protease-mediated degradation, and poor intestinal permeability. Though lipid-based nanocarriers have emerged as a

potential oral DDS exhibiting promising *in vivo* results the translation of lipid-based oral formulations into clinical products is still rather challenging. A more integrated approach should be pursued to assess the weaknesses of the lipid-based oral DDS and should be critically correlated with their characteristics, the exact phenomena occurring immediately after the drug administration, and the mechanisms governing mucus permeation and intestinal drug absorption with the aid of predictive mathematical tools. New insights on the understanding of the interplay between nanocarriers, peptides, and physiological conditions in the intestine will be helpful in creating databases to summarize the existing data as well as to have a uniform model for both *in vitro* and *in vivo* tests.

Conflict of interest. The authors declare no conflict of interest.

ABBREVIATIONS

API, active pharmaceutical ingredient; BA, bioavailability; BSA, bovine serum albumin; CAPIC, calcium phosphate-PEG-insulin-casein; DDS, drug delivery system; GI, gastrointestinal; GIT, gastrointestinal tract; GRAS, generally regarded as safe; LBDDS, lipid-based drug delivery system; LCT, long-chain triglycerides; LDC, lipid drug conjugate; MCL, medium-chain lipid; MCT, medium-chain triglycerides; MW, molecular weight; NC, nanocapsules; NLC, nanostructured lipid carriers; PEG, polyethylene glycol; PEG, polyethylene glycol; PGP, P-glycoprotein; PK, pharmacokinetics; RES, reticuloendothelial system; SC, subcutaneous; SEDDS, self-emulsifying drug delivery systems; SLN, solid lipid nanoparticles; SMEDDS, self-micro emulsifying drug delivery systems; SNEDDS, self-nano emulsifying drug delivery system; TG, triglycerides.

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