Carrier-Based Systems as Strategies for Oral Delivery of Therapeutic Peptides and Proteins: a mini-review

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Abstract

Peptides and proteins play an essential role in biological systems and the human body. Deficiency or dysfunction of peptides and proteins such as insulin can lead to various illnesses. The therapeutic use of peptides and proteins in some illnesses such as diabetes, cardiovascular disease, autoimmunity, and cancer, among others, is highly considered. Peptides and proteins have large molecular structures and are generally hydrophilic, and maintaining their spatial composition or tertiary configurations are necessary for their pharmacological activities. Maintaining the stability of the protein structure and composition is therefore very important and necessary to maintain functionality. There are different routes for peptides and proteins administration such as injection (subcutaneous, intravenous, and intramuscular), oral, nasal, pulmonary, rectal, and ocular routes. The oral administration route is better accepted by the patient due to safety and ease of use. However, oral administration often is unsuitable because of the physicochemical properties and macromolecular structure of proteins and the presence of gastrointestinal (GI) enzymes under an acidic environment within the stomach. Proteins and peptides undergo enzymatic degradation, tertiary structural alteration, and low absorption. This necessitates the need to use alternative oral drug delivery systems for peptides and proteins that maintain their overall structure and enhances their absorption and bioavailability. In this article, we review carrier-based oral drug delivery systems that were suitable for peptides and proteins.

Keywords: Peptides and proteins; Oral delivery; Carrier-based; Bioavailability

1. Introduction

Peptides and proteins have many functions, including acting as receptors, functioning as transporters, controlling gene expression, and facilitating intracellular and extracellular reactions, all of which play broad and varied roles in living organisms (Doostmohammadi et al. 2019; Leader et al. 2008). They are generally proposed as significant contributors to the production of new therapeutic agents for several human diseases such as diabetes, cardiovascular disorders, autoimmunity diseases, cancers, among many others (Liu et al. 2018; Tan et al. 2010). Recombinant peptides and proteins, therapeutic synthetic peptides, and over 1000 proteins and peptides have been frequently used therapeutically and in clinical trials (Leader et al. 2008). Some biopharmaceuticals, including recombinant therapeutic proteins, monoclonal and polyclonal antibodies, enzymes, and peptides, were reported among the top 10 pharmaceutical products market (Sengupta and Kulkarni 2013). At present, the peptides and proteins-based drug market are estimated to be worth >$40 billion annually, representing about ten percent of the ethical pharmaceutical market. This sales volume has been steadily increasing faster than other pharmaceutical products, and success rates are now twice that of small molecule drugs available for the marketing of biologics (Sachdeva 2017). There are many administration routes for peptides and proteins based drugs, and due to higher safety and better compliance, significant attention has been devoted to developing the oral administration route (Liu et al. 2018). However, peptides and proteins face mounting challenges during oral delivery as they are hydrophilic substances that cannot transfer through the lipophilic cell membrane. They also are macromolecules that typically have a molecular size > 500 Da, minimizing their paracellular transmission across tight junctions. Besides, their absorption rate and half-life in the body is severely reduced in the GI tract due to enzymatic degradation and denaturation in the highly acidic environment. This makes it challenging to deliver them through oral

administration and creates the need to use some novel delivery system (Liu et al. 2018; Mahajan et al. 2014).

A successful peptides and protein delivery system should be able to preserve the protein activity at the highest level and have characteristics such as high entrapment and drug loading capacity, good stability, and constitutes a simple and repeatable preparation process (Zhu et al. 2016). Some carrier-based delivery systems such as Nanoparticle, Nanoemulsion, and Lipid bilayer vesicles have been developed recently. In this review, we focus on several proteins and peptides suitable oral delivery systems and strategies.

2. Therapeutic peptides and proteins

Peptides and proteins affect many biological processes, functioning at specific sites as endocrine or paracrine signals and others as neurotransmitters or growth factors. They are very specific in action compared to smaller molecules when used as a potential drug (Uhlig et al. 2014). Some peptides and proteins have a pharmacological effect and are used as a drug in the treatment of some diseases such as cancer, cardiovascular disease, diabetes, multiple sclerosis, and rheumatoid arthritis (Burmester and Pope 2017; Ciplea et al. 2020; Kurrikoff et al. 2019; Recio et al. 2017; Sanchez et al. 2016; Shen et al. 2019). They also have antioxidant, antimicrobial, and anti-inflammatory effects (Cicero et al. 2017). The approval rate of protein and peptide related drugs has increased substantially since the approval of recombinant human insulin (Humulin®) in 1982 (Walsh 2000). In 2014, the total number of approved biopharmaceutical products in the United States and the European Union grew by 246, and global sales of biopharmaceutical medicines reached $445 billion in 2019 (Sengupta and Kulkarni 2013). However, some challenges still exist in peptides and proteins administration, including lower stability of protein-based drug product, degradation and aggregation of proteins, low absorption and bioavailability, degradation by enzyme and acid in GI track, and short half-life, which makes it necessary to repeat administrations to achieve the desired effects

(Ibraheem et al. 2014; Wang and Ohtake 2019). Some methods and delivery systems have been developed to optimize the physicochemical properties of proteins, protect them and deliver them to their targets within the biological systems (Ibraheem et al. 2014).

3. Common peptides and proteins delivery routes

Therapeutic peptides and proteins were typically delivered by different administration routes that showed in figure 1, such as subcutaneous, intravenous, and intramuscular injections, oral, nasal, pulmonary, rectal, and ocular routes (Gedawy et al. 2018; Ibraheem et al. 2014). Administration of peptides and protein by injection routes can often cause some problems like pain. Also, therapeutic peptides and proteins are quickly eliminated from the blood flow, which makes it necessary to repeated doses of drugs, thus poorly tolerated by some patients (Allahyari and Javadzadeh 2019; Cacciatore et al. 2016; Ibraheem et al. 2014). For patients, injection routes are painful, and they are often costly and can cause harmful and unwanted adverse effects. As a result, studies have concentrated their attention on developing more effective, safer, and simpler alternative routes for their delivery, leading to the development of non-invasive protein drug delivery systems (Allahyari and Javadzadeh 2019; Ibraheem et al. 2014; Zaric et al. 2019). For both local and systemic effects, nasal peptides and protein delivery can be used. However, it is usually limited because of the physical barrier of the nasal epithelium, rapid mucociliary clearance, and a limited number of drugs administered via this route (Chen et al. 2018; Wei et al. 2020). The rectal route can also be used for local and systemic therapy but is still limited because of some disadvantages such as low peptides and protein absorption through the rectal epithelium and overall low bioavailability (Ibraheem et al. 2014). There are many benefits to the ocular route of protein delivery as it is simpler and quicker than conventional injection routes. The ocular administration of protein drugs protects them by avoiding the first-pass metabolism. First-pass metabolism is one cause of low bioavailability

of peptides and proteins when administered by the oral route (Allahyari and Javadzadeh 2019; Cao et al. 2019; Gedawy et al. 2018; Ibraheem et al. 2014; Mandal et al. 2018). However, some reasons limit ocular peptides and protein administration, including poor bioavailability due to low permeability of peptides and protein through eye membrane and enzymes in the ocular tissue. The ocular route, therefore, is not the optimal route for systemic peptides and protein delivery (Allahyari and Javadzadeh 2019; Mandal et al. 2018). Although oral bioavailability of protein drugs is limited, the oral administration of protein remains an interesting and attractive alternative to conventional parenteral protein delivery, as it is a painless and much easier administration route (Allahyari and Javadzadeh 2019; Cao et al. 2019; Gedawy et al. 2018). The design of oral delivery system, which can protect the peptides and proteins, improving their absorption and bioavailability without interfering or interacting with its biological activity, has become attractive and impotent in protein-based delivery systems strategy.

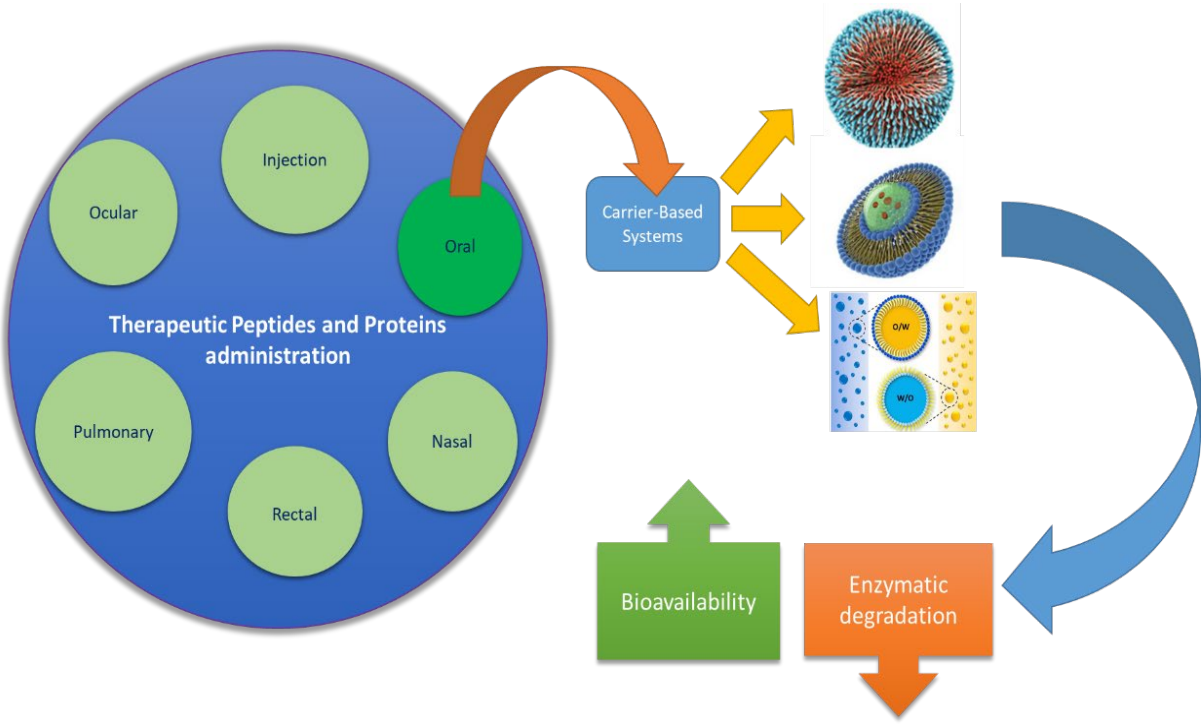

Figure 1. Therapeutic peptides and proteins administration routes and schematically carrier-based system that used in oral delivery.

4. Oral protein delivery system

The oral route has attracted the most attention among the different administration routes due to its specific benefits, including controllable delivery, ease of use, feasibility for solid product, and better patient compliance (Cao et al. 2019; Liu et al. 2018). Protein stability is also one of the significant challenges facing a product's successful entry into the oral delivery systems market. The drug delivery system that protects peptides and protein against intestinal degradation and can increase intestinal permeability is valuable to enhance their oral bioavailability and overall activity (Rao et al. 2008). To enhance the stability and bioavailability of therapeutic peptides and proteins, many studies have focused on the use of carrier-based delivery systems such as Nanoparticles, Nanoemulsion and Lipid bilayer vesicles that schematically showed in figure 1. (Al-Remawi et al. 2017; Cao et al. 2019; Gedawy et al. 2018; Lee et al. 2019; Liu et al. 2018; Shalaby and El-Refaie 2018).

5. Nanoparticle carrier-based delivery system

The main objectives in such a system often are to monitor particle size and surface properties in the design of nanoparticles as a delivery system (Jahanshahi et al. 2005). To achieve the site-specific action of the drug at the therapeutically optimal rate, the release kinetics of the drug, and dose regimens are critical (Soppimath et al. 2001).

Some advantages of using nanoparticles as a method for drug delivery are:

- ✓ Surface morphology, size and properties of nanoparticles can be easily manipulated to obtain both active and passive compounds targeting the administration routes.
- ✓ Nanoparticles can control drug's release during transport and at the target of the drug, altering the delivery of the drug by the organ and subsequent clearance of the drug to improve its therapeutic effectiveness and minimize any adverse effects.

- ✓ The drug delivery system may be used for several administration routes, including oral, parenteral, nasal, and intraocular. (Mohanraj and Chen 2006).

Drug delivery efficiency is directly related to particle size because particle size can increase bioavailability and allow more effective direct intracellular delivery (Galindo-Rodriguez et al. 2005). Some studies have found that the amount of nanoparticles crossing the intestinal epithelium is greater than the number of microspheres and that the transport includes not only epithelial M cells but also normal enterocytes (Jung et al. 2000). Nanoparticles allow peptides and protein drugs to cross through the cell membrane, bind, and encapsulate within a matrix that protects them against enzymatic and hydrolytic destruction (Mohanraj and Chen 2006). Tian et al. (2018), developed a core-shell nanoparticle vehicle as oral insulin delivery that combines the ability to penetrate mucus, enhanced gastrointestinal retention and transepithelial transport properties to improve bioavailability. Chitosan modified with N-(2-hydroxy)-propyl-3-trimethylammonium chloride was used to prepare nanoparticles due to its positive charge structure of the quaternary amine groups, independent of environmental conditions. The surface of nanoparticles was coated with polyanionic thiolated hyaluronic acid. The average size was 75 to 200 nm and the polydispersity index (PDI) varied from 0.23 to 0.40. Cumulative drug release from modified nanoparticles at pH 7.4 by 24 h was 74.2%. This strategy showed improved intestinal retention, increased ability to mucus penetration, and transepithelial transportation (Tian et al. 2018). Xu et al. (2018) used solid lipid nanoparticles for the oral delivery of insulin. Nanoparticles consisted of a solid-lipid shell and an aqueous core loaded with hemagglutinin-2 peptide and insulin, respectively. Size, PDI, and encapsulation efficacy of insulin nanoparticles were 171.2 nm, 0.23 and 97.93%, respectively. Approximately 40% of encapsulated insulin was released from the nanoparticles within 2 h. To increase serum insulin concentration and produce an excellent hypoglycemic response, the nanoparticle formulation

was quite effective (Xu et al. 2018b). Some recent studies about fabrication the oral delivery system for peptides and proteins are reported in Table.1

6. Nanoemulsion carrier-based delivery system

Nanoemulsions are drug delivery systems in which immiscible liquids are mixed to form a single-phase by adding suitable surfactants or mixing to produce a droplet, otherwise known as a micelle with a diameter range of approximately 0.1-100 nm (Sun et al. 2012). Due to unique structure and properties, their small droplet size carrier packages, the possibility of manufacturing with biocompatible materials, enhanced dissolution rate, solubility, diffusion through an unstirred aqueous layer and improved mucosal permeability, attention to such systems of nanoemulsions has increased recently (Gao et al. 2011; Gurpreet and Singh 2018; Rai et al. 2018; Singh et al. 2017). Nanoemulsions have a protective effect on the GI environment, improving the membrane fluidity, and transient opening of tight junctions caused by lipid constituents or surfactants components of these nanoemulsion formulations. It has also been commonly used to enhance the stability of drugs such as peptides and proteins (Jirwankar et al. 2020; Khaleel Basha et al. 2020; Li et al. 2013). Sun et al. (2012) designed a nanoemulsion delivery system for oral delivery of bovine serum albumin as a model protein drug. This formulation has demonstrated more than 90% encapsulation efficiency and substantially increased protein stability while preserving high levels of bioactivity of the drug (Sun et al. 2012). Li et al. (2013) developed modified nanoemulsions with alginate/chitosan for oral delivery of insulin. The in vitro analysis showed well-preserved nanoemulsion integrity in simulated gastric fluids and noted responses in both control and diabetic rats groups (Li et al. 2013).

7. Lipid bilayer vesicles carrier-based delivery system

Liposomes and niosomes are one or more lipid bilayer vesicles. The lipid bilayers in liposome and noisome are formed by the self-assembly of phospholipids and non-ionic surfactants, respectively. Both hydrophilic and hydrophobic molecules are encapsulated in liposome and niosomes. Studies show that liposomes and niosomes can be prescribed by different routes of administration, such as the oral route (Ge et al. 2019; Nguyen et al. 2016; Niu et al. 2014; Shalaby and El-Refaie 2018; Tan et al. 2010). Entrapment of peptides and protein in lipid bilayer vesicles can protect them from enzymatic and acid degradation, increase absorption and bioavailability in the oral prescription that schematically shows in Figure 2. (Liu et al. 2020; Nguyen et al. 2016; Pardakhty et al. 2007; Tan et al. 2010). Varshosaz et al. (2003) used Sorbitan Monoester as a non-ionic surfactant to preparative niosomes for oral delivery, the result showed niosomal formulations of insulin are stable and protects insulin from trypsin and α-chymotrypsin (Varshosaz et al. 2003). Al-Remawi et al. (2017) prepared Chitosan/lecithin liposomes as oral delivery systems for insulin. The result showed oral liposomal formulations were more effective than the oral formulations of free insulin in rats (Al-Remawi et al. 2017). In liposome or niosome type, some modifications such as chitosan coating, PEGylated, Folate targeted, etc., increases their potential for oral delivery (Al-Remawi et al. 2017; Moghassemi et al. 2015; Shalaby and El-Refaie 2018; Yazdi et al. 2020).

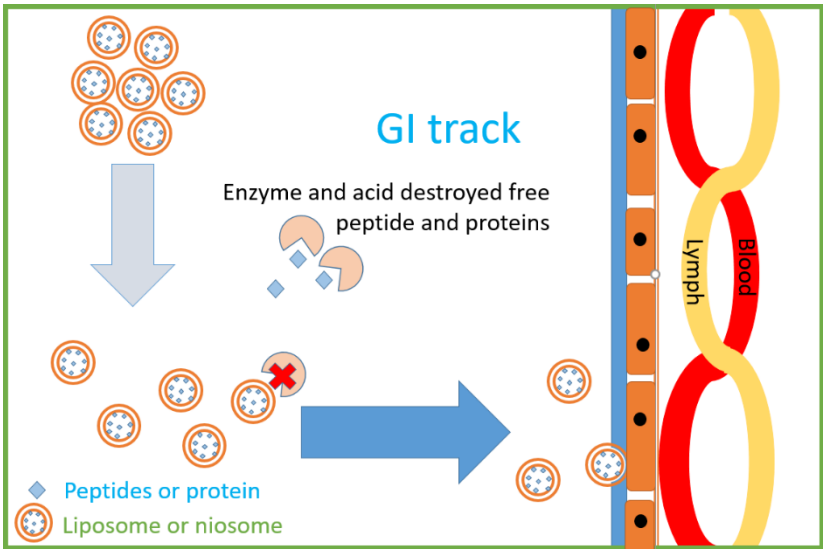

Figure 2. Schematically shows lipid bilayer vesicles can protect from GI enzyme, acid environment and increase absorption of peptides and proteins

Table 1. Some recent studies in preparation or use of the oral delivery systems for peptides and proteins

Peptides and proteins	**Carrier type**	**Main Material**	**Main observation**	**Reference**
Bovine serum albumin (model protein vaccine)	Modified Nanoparticles	Chitosan and Eudragit® L100	Significantly protect the protein against the enzymes and gastric environment	(Xu et al. 2018a)
Insulin	Nanoparticles	Chitosan	The blood glucose level through oral administration effectively controlled in diabetic rats	(He et al. 2017)
Glucagon like peptide-1 and Dipeptidyl	Nanoparticles	Poly(lactic-co-glycolic acid) and Polyvinyl alcohol	Blood glucose levels have reduced following oral administration of the nanoparticles	(Araújo et al. 2016)

peptidase-4 inhibitor				
Insulin	Modified Nanoparticles	Poly(lactic-co-glycolic acid), Cationic octa-arginine peptide and Specific anionic phosphoserine	Administration of insulin-loaded NPs have higher oral bioavailability in diabetic rats	(Wu et al. 2018)
Interferon alpha	Nanoparticles	Chitosan	Nanoparticles increase the oral bioavailability of interferon alpha that can be found in plasma	(Cánepa et al. 2017)
Cp1-11 peptide/insulin	Nanoparticles	Chitosan and Alginate	Nanoparticles can protect insulin against proteases in the gastrointestinal tract that led to degradation	(Chen et al. 2019)
Insulin	Nanoparticles	Silica	The permeation of nanoparticles enhanced both in vivo and in vitro study without toxic effect	(Lamson et al. 2020)

Ovalbumin	Nanoparticles	Cyclodextrin and Chitosan	Nanoparticles could enhance intestinal absorption	(He et al. 2019)
Insulin	Modified Nanoparticles	Deoxycholic acid and Chitosan	Degradation of insulin in the epithelium is significantly prevented	(Fan et al. 2018)
Recombinant Human Insulin	Modified Liposome	Egg phosphatidylcholine and Cholesterol	In diabetic mice reduction of blood sugar in 1 h and maintained up to 8 h	(Shalaby and El-Refaie 2018)
Insulin	Noisome	Olyoxyethylene alkyl ether surfactants or Sorbitan monostearate and Cholesterol	Decreased levels of blood glucose and elevated serum insulin in animal models	(Pardakhty et al. 2011)
Long tumor peptide and the	Liposome	1,2-distearoyl-sn-glycero-3-phosphocholin and Cholesterol	Positive therapeutic results were demonstrated in mouse model	(Naciute et al. 2020)

Toll-like receptor ligand Pam2Cys				
Cyclosporine A	Noisome	Tween 60, Span 60 and Cholesterol	The bioavailability of niosomal formulation of Cyclosporine A is increased with sustained release profile in albino rabbits	(Rehman et al. 2021)
Insulin	Noisome	Span 60 and Cholesterol	Niosomal formulation was able to facilitate insulin transport across intestinal membrane models	(Moghassemi et al. 2015)
Insulin	Modified Liposome	Hydrogenated soya phosphatidylcholine, Methoxy polyethylene glycol	Liposomes significantly increased insulin absorption through intestinal epithelia	(Yazdi et al. 2020)

		distearylphosphatidylethanolamine and Cholesterol		
Insulin	Liposome	Egg yolk lecithin, Cholesterol and 1,2-dioleoyl-3-trimethylammonium-propane	Remarkable ability to penetrate mucus and intestinal epithelial absorption.	(Wang et al. 2019)
Salmon calcitonin	Nanoemulsion	Sodium oleate andSodium deoxycholate	Formulations significantly protected peptides from degradation by enzymes	(Jirwankar et al. 2020)
Insulin	Nanoemulsion	Alginate/Aloe vera	Insulin translocation in the cell monolayer is very promising.	(Khaleel Basha et al. 2020)
Teriparatide	Nanoemulsion	Oleic Acid, Labrasol and Plurol oleique	A sustained release profile was achieved following the oral administration and	(Altaani et al. 2020)

			improvement effects in osteoporosis animal model	
Insulin	Modified nanoemulsion	Phospholipid and Ethyl oleate	Higher oral bioavailability than that of oral insulin solution in diabetic rats observed	(Hu et al. 2019)
Coenzyme Q10	Nanoemulsion	Soybean oil, Lecithin and Octenyl succinic anhydride modified starch	Bioavailability increased 1.8-fold, compared with coenzyme Q10 dissolved in oil	(Niu et al. 2020)
MAGE1-HSP70/SEA (protein vaccine)	Modified Nanoemulsion	Soybean oil, Pluronic 188, Span 20, Tomato lectin	The levels of antibody induced by antigen in Nanoemulsion via the oral route was higher than other group	(Long et al. 2019)

Conclusion

Oral administration is one of the most widely used routes of prescribing drugs due to its greater compliance in most patients and fewer side effects. Peptides and proteins, due to their hydrophilic structure and large molecules, and their sensitivity to degradation by enzymes and acids, cannot be adequately absorbed and have high bioavailability when used without any carrier or delivery systems by the oral route. Injection routes are more commonly used for proteins administration. The use of new carrier-based drug delivery systems, however, can be a suitable alternative for oral peptides and protein delivery with increased stability, enhance uptake, and bioavailability. Studies on carrier-based drug delivery systems such as nanoparticles, nanoemulsions, and bilayer vesicle systems demonstrate the ability of these systems to improve the oral delivery of peptides and proteins such as insulin, teriparatide, interferon alpha, and coenzyme Q10. The use of these systems results in increased absorption, protected, and reduces the effect of degrading enzymes and acid on peptides and protein finally increases their bioavailability. Therefore, with the existing studies in this field, it seems that in the future, carrier-based drug delivery systems can be used for oral administration of peptides and proteins.

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Compliance with Ethical Standards

Conflict of interest

The authors declare that there are no conflict of interest.

Reference:

Al-Remawi M, Elsayed A, Maghrabi I, Hamaidi M, Jaber N (2017) Chitosan/lecithin liposomal nanovesicles as an oral insulin delivery system Pharm Dev Technol 22:390-398 doi:10.1080/10837450.2016.1213745

Allahyari S, Javadzadeh Y (2019) Non-Invasive and Less Degradative Ways of Insulin Administration Pharm Sci 25:85-92 doi:10.15171/ps.2019.14

Altaani BM, Almaaytah AM, Dadou S, Alkhamis K, Daradka MH, Hananeh W (2020) Oral Delivery of Teriparatide Using a Nanoemulsion System: Design, in Vitro and in Vivo Evaluation Pharm Res 37:80 doi:10.1007/s11095-020-02793-0

Araújo F et al. (2016) In vivo dual-delivery of glucagon like peptide-1 (GLP-1) and dipeptidyl peptidase-4 (DPP4) inhibitor through composites prepared by microfluidics for diabetes therapy Nanoscale 8:10706-10713 doi:10.1039/c6nr00294c

Burmester GR, Pope JE (2017) Novel treatment strategies in rheumatoid arthritis The Lancet 389:2338-2348 doi:https://doi.org/10.1016/S0140-6736(17)31491-5

Cacciatore I, Ciulla M, Fornasari E, Marinelli L, Di Stefano A (2016) Solid lipid nanoparticles as a drug delivery system for the treatment of neurodegenerative diseases Expert Opin Drug Deliv 13:1121-1131 doi:10.1080/17425247.2016.1178237

Cánepa C, Imperiale JC, Berini CA, Lewicki M, Sosnik A, Biglione MM (2017) Development of a Drug Delivery System Based on Chitosan Nanoparticles for Oral Administration of Interferon-α Biomacromolecules 18:3302-3309 doi:10.1021/acs.biomac.7b00959

Cao S-j, Xu S, Wang H-m, Ling Y, Dong J, Xia R-d, Sun X-h (2019) Nanoparticles: Oral Delivery for Protein and Peptide Drugs AAPS PharmSciTech 20:190 doi:10.1208/s12249-019-1325-z

Chen J, Hu L, Yang G, Hu Q (2018) Current Therapeutic Strategy in the Nasal Delivery of Insulin: Recent Advances and Future Directions Curr Pharm Biotechnol 19:400-415 doi:10.2174/1389201019666180619145429

Chen X, Ren Y, Feng Y, Xu X, Tan H, Li J (2019) Cp1-11 peptide/insulin complex loaded pH-responsive nanoparticles with enhanced oral bioactivity Int J Pharm 562:23-30 doi:https://doi.org/10.1016/j.ijpharm.2019.03.020

Cicero AFG, Fogacci F, Colletti A (2017) Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review Br J Pharmacol 174:1378-1394 doi:https://doi.org/10.1111/bph.13608

Ciplea AI et al. (2020) Monoclonal antibody treatment during pregnancy and/or lactation in women with MS or neuromyelitis optica spectrum disorder Neurol Neuroimmunol Neuroinflamm 7:e723 doi:10.1212/NXI.0000000000000723

Doostmohammadi M, Ameri A, Mohammadinejad R, Dehghannoudeh N, Banat IM, Ohadi M, Dehghannoudeh G (2019) Hydrogels For Peptide Hormones Delivery: Therapeutic And Tissue Engineering Applications Drug Des Devel Ther 13:3405-3418 doi:10.2147/DDDT.S217211

Fan W et al. (2018) Functional nanoparticles exploit the bile acid pathway to overcome multiple barriers of the intestinal epithelium for oral insulin delivery Biomaterials 151:13-23 doi:https://doi.org/10.1016/j.biomaterials.2017.10.022

Galindo-Rodriguez SA, Allemann E, Fessi H, Doelker E (2005) Polymeric nanoparticles for oral delivery of drugs and vaccines: a critical evaluation of in vivo studies Critical reviews™ in therapeutic drug carrier systems 22

Gao F et al. (2011) Nanoemulsion improves the oral absorption of candesartan cilexetil in rats: Performance and mechanism J Control Release 149:168-174 doi:10.1016/j.jconrel.2010.10.013

Ge X, Wei M, He S, Yuan WE (2019) Advances of Non-Ionic Surfactant Vesicles (Niosomes) and Their Application in Drug Delivery Pharmaceutics 11 doi:10.3390/pharmaceutics11020055

Gedawy A, Martinez J, Al-Salami H, Dass CR (2018) Oral insulin delivery: existing barriers and current counter-strategies J Pharm Pharmacol 70:197-213 doi:https://doi.org/10.1111/jphp.12852

Gurpreet K, Singh S (2018) Review of nanoemulsion formulation and characterization techniques Indian J Pharm Sci 80:781-789

He M, Zhong C, Hu H, Jin Y, Chen Y, Lou K, Gao F (2019) Cyclodextrin/chitosan nanoparticles for oral ovalbumin delivery: Preparation, characterization and intestinal mucosal immunity in mice Asian J Pharm Sci 14:193-203 doi:https://doi.org/10.1016/j.ajps.2018.04.001

He Z et al. (2017) Scalable fabrication of size-controlled chitosan nanoparticles for oral delivery of insulin Biomaterials 130:28-41 doi:https://doi.org/10.1016/j.biomaterials.2017.03.028

Hu X-B, Tang T-T, Li Y-J, Wu J-Y, Wang J-M, Liu X-Y, Xiang D-X (2019) Phospholipid complex based nanoemulsion system for oral insulin delivery: preparation, in vitro, and in vivo evaluations Int J Nanomedicine 14:3055

Ibraheem D, Elaissari A, Fessi H (2014) Administration strategies for proteins and peptides Int J Pharm 477:578-589 doi:https://doi.org/10.1016/j.ijpharm.2014.10.059

Jahanshahi M, Zhang Z, Lyddiatt A Subtractive chromatography for purification and recovery of nano-bioproducts. In: IEE Proceedings-Nanobiotechnology, 2005. vol 3. IET, pp 121-126

Jirwankar P, Shah D, Shao J (2020) Protection of Protein Drugs by Self-Emulsified Nanoemulsion Against Proteolysis J Pharm Sci 109:2615-2621 doi:10.1016/j.xphs.2020.05.005

Jung T, Kamm W, Breitenbach A, Kaiserling E, Xiao J, Kissel T (2000) Biodegradable nanoparticles for oral delivery of peptides: is there a role for polymers to affect mucosal uptake? European Journal of Pharmaceutics and Biopharmaceutics 50:147-160

Khaleel Basha S, Syed Muzammil M, Dhandayuthabani R, Sugantha Kumari V (2020) Development of nanoemulsion of Alginate/Aloe vera for oral delivery of insulin Materials Today: Proceedings doi:https://doi.org/10.1016/j.matpr.2020.04.138

Kurrikoff K, Aphkhazava D, Langel Ü (2019) The future of peptides in cancer treatment Curr Opin Pharmacol 47:27-32 doi:https://doi.org/10.1016/j.coph.2019.01.008

Lamson NG, Berger A, Fein KC, Whitehead KA (2020) Anionic nanoparticles enable the oral delivery of proteins by enhancing intestinal permeability Nat Biomed Eng 4:84-96 doi:10.1038/s41551-019-0465-5

Leader B, Baca QJ, Golan DE (2008) Protein therapeutics: a summary and pharmacological classification Nat Rev Drug Discov 7:21-39 doi:10.1038/nrd2399

Lee SH, Song JG, Han H-K (2019) Development of pH-responsive organic-inorganic hybrid nanocomposites as an effective oral delivery system of protein drugs Journal of Controlled Release 311-312:74-84 doi:https://doi.org/10.1016/j.jconrel.2019.08.036

Li X et al. (2013) Nanoemulsions coated with alginate/chitosan as oral insulin delivery systems: preparation, characterization, and hypoglycemic effect in rats Int J Nanomedicine 8:23-32 doi:10.2147/IJN.S38507

Liu C, Kou Y, Zhang X, Cheng H, Chen X, Mao S (2018) Strategies and industrial perspectives to improve oral absorption of biological macromolecules Expert Opin Drug Deliv 15:223-233 doi:10.1080/17425247.2017.1395853

Liu Y et al. (2020) Insulin-lipid complex, preparation method therefor, and preparation thereof. Google Patents,

Long P et al. (2019) Tomato lectin-modified nanoemulsion-encapsulated MAGE1-HSP70/SEA complex protein vaccine: Targeting intestinal M cells following peroral administration Biomed Pharmacother 115:108886 doi:https://doi.org/10.1016/j.biopha.2019.108886

Mahajan A, Rawat AS, Bhatt N, Chauhan MK (2014) Structural modification of proteins and peptides Indian J Pharm Educ Res 48:34-47

Mandal A, Pal D, Agrahari V, Trinh HM, Joseph M, Mitra AK (2018) Ocular delivery of proteins and peptides: Challenges and novel formulation approaches Adv Drug Del Rev 126:67-95 doi:https://doi.org/10.1016/j.addr.2018.01.008

Moghassemi S et al. (2015) Uptake and transport of insulin across intestinal membrane model using trimethyl chitosan coated insulin niosomes Mater Sci Eng C 46:333-340 doi:https://doi.org/10.1016/j.msec.2014.10.070

Mohanraj V, Chen Y (2006) Nanoparticles-a review Tropical journal of pharmaceutical research 5:561-573

Naciute M, Niemi V, Kemp RA, Hook S (2020) Lipid-encapsulated oral therapeutic peptide vaccines reduce tumour growth in an orthotopic mouse model of colorectal cancer Eur J Pharm Biopharm 152:183-192 doi:https://doi.org/10.1016/j.ejpb.2020.04.020

Nguyen TX, Huang L, Gauthier M, Yang G, Wang Q (2016) Recent advances in liposome surface modification for oral drug delivery Nanomedicine 11:1169-1185 doi:10.2217/nnm.16.9

Niu M et al. (2014) Enhanced oral absorption of insulin-loaded liposomes containing bile salts: A mechanistic study Int J Pharm 460:119-130 doi:https://doi.org/10.1016/j.ijpharm.2013.11.028

Niu Z, Acevedo-Fani A, McDowell A, Barnett A, Loveday SM, Singh H (2020) Nanoemulsion structure and food matrix determine the gastrointestinal fate and in vivo bioavailability of coenzyme Q10 J Control Release 327:444-455 doi:https://doi.org/10.1016/j.jconrel.2020.08.025

Pardakhty A, Moazeni E, Varshosaz J, Hajhashemi V, Rouholamini Najafabadi A (2011) Pharmacokinetic study of niosome-loaded insulin in diabetic rats Daru : journal of Faculty of Pharmacy, Tehran University of Medical Sciences 19:404-411

Pardakhty A, Varshosaz J, Rouholamini A (2007) In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin Int J Pharm 328:130-141 doi:https://doi.org/10.1016/j.ijpharm.2006.08.002

Rai VK, Mishra N, Yadav KS, Yadav NP (2018) Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications J Control Release 270:203-225 doi:https://doi.org/10.1016/j.jconrel.2017.11.049

Rao SVR, Yajurvedi K, Shao J (2008) Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of protein drugs: III. In vivo oral absorption study International Journal of Pharmaceutics 362:16-19 doi:https://doi.org/10.1016/j.ijpharm.2008.05.015

Recio C, Maione F, Iqbal AJ, Mascolo N, De Feo V (2017) The Potential Therapeutic Application of Peptides and Peptidomimetics in Cardiovascular Disease Front Pharmacol 7 doi:10.3389/fphar.2016.00526

Rehman MU et al. (2021) Oral bioavailability studies of niosomal formulations of Cyclosporine A in albino rabbits Pak J Pharm Sci 34:313-319

Sachdeva S (2017) Peptides as 'Drugs': The Journey so Far Int J Pept Res Ther 23:49-60 doi:10.1007/s10989-016-9534-8

Sanchez L, Wang Y, Siegel DS, Wang ML (2016) Daratumumab: a first-in-class CD38 monoclonal antibody for the treatment of multiple myeloma J Hematol Oncol 9:51 doi:10.1186/s13045-016-0283-0

Sengupta S, Kulkarni A (2013) Design Principles for Clinical Efficacy of Cancer Nanomedicine: A Look into the Basics ACS Nano 7:2878-2882 doi:10.1021/nn4015399

Shalaby TI, El-Refaie WM (2018) Bioadhesive Chitosan-Coated Cationic Nanoliposomes With Improved Insulin Encapsulation and Prolonged Oral Hypoglycemic Effect in Diabetic Mice J Pharm Sci 107:2136-2143 doi:https://doi.org/10.1016/j.xphs.2018.04.011

Shen L et al. (2019) Insulin treatment and clinical outcomes in patients with diabetes and heart failure with preserved ejection fraction Eur J Heart Fail 21:974-984 doi:https://doi.org/10.1002/ejhf.1535

Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, Chourasia MK (2017) Nanoemulsion: Concepts, development and applications in drug delivery J Control Release 252:28-49 doi:https://doi.org/10.1016/j.jconrel.2017.03.008

Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE (2001) Biodegradable polymeric nanoparticles as drug delivery devices Journal of controlled release 70:1-20

Sun H et al. (2012) Development and characterization of a novel nanoemulsion drug-delivery system for potential application in oral delivery of protein drugs Int J Nanomedicine 7:5529-5543 doi:10.2147/IJN.S36071

Tan ML, Choong PFM, Dass CR (2010) Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery Peptides 31:184-193 doi:https://doi.org/10.1016/j.peptides.2009.10.002

Tian H et al. (2018) Uniform Core–Shell Nanoparticles with Thiolated Hyaluronic Acid Coating to Enhance Oral Delivery of Insulin Adv Healthc Mater 7:1800285 doi:https://doi.org/10.1002/adhm.201800285

Uhlig T et al. (2014) The emergence of peptides in the pharmaceutical business: From exploration to exploitation EuPA Open Proteomics 4:58-69 doi:https://doi.org/10.1016/j.euprot.2014.05.003

Varshosaz J, Pardakhty A, Hajhashemi V-i, Najafabadi AR (2003) Development and Physical Characterization of Sorbitan Monoester Niosomes for Insulin Oral Delivery Drug Deliv 10:251-262 doi:10.1080/drd_10_4_251

Walsh G (2000) Biopharmaceutical benchmarks Nat Biotechnol 18:831-833 doi:10.1038/78720

Wang A et al. (2019) Protein Corona Liposomes Achieve Efficient Oral Insulin Delivery by Overcoming Mucus and Epithelial Barriers Adv Healthc Mater 8:1801123 doi:https://doi.org/10.1002/adhm.201801123

Wang W, Ohtake S (2019) Science and art of protein formulation development Int J Pharm 568:118505 doi:https://doi.org/10.1016/j.ijpharm.2019.118505

Wei X, Duan X, Zhang Y, Ma Z, Li C, Zhang X (2020) Internalization Mechanism of Phenylboronic-Acid-Decorated Nanoplatform for Enhanced Nasal Insulin Delivery ACS Appl Bio Mater 3:2132-2139 doi:10.1021/acsabm.0c00002

Wu J, Zheng Y, Liu M, Shan W, Zhang Z, Huang Y (2018) Biomimetic Viruslike and Charge Reversible Nanoparticles to Sequentially Overcome Mucus and Epithelial Barriers for Oral Insulin Delivery ACS Appl Mater Interfaces 10:9916-9928 doi:10.1021/acsami.7b16524

Xu B, Zhang W, Chen Y, Xu Y, Wang B, Zong L (2018a) Eudragit® L100-coated mannosylated chitosan nanoparticles for oral protein vaccine delivery Int J Biol Macromol 113:534-542 doi:https://doi.org/10.1016/j.ijbiomac.2018.02.016

Xu Y, Zheng Y, Wu L, Zhu X, Zhang Z, Huang Y (2018b) Novel Solid Lipid Nanoparticle with Endosomal Escape Function for Oral Delivery of Insulin ACS Appl Mater Interfaces 10:9315-9324 doi:10.1021/acsami.8b00507

Yazdi JR et al. (2020) Folate targeted PEGylated liposomes for the oral delivery of insulin: In vitro and in vivo studies Colloids Surf B Biointerfaces 194:111203 doi:https://doi.org/10.1016/j.colsurfb.2020.111203

Zaric BL, Obradovic M, Sudar-Milovanovic E, Nedeljkovic J, Lazic V, Isenovic ER (2019) Drug Delivery Systems for Diabetes Treatment Curr Pharm Des 25:166-173 doi:10.2174/1381612825666190306153838

Zhu X et al. (2016) Polymeric nanoparticles amenable to simultaneous installation of exterior targeting and interior therapeutic proteins Angew Chem 128:3370-3373