International Journal of Modern Biology and Medicine, 2014, 5(1): 5-16

International Journal of Modern Biology and Medicine **ISSN: 2165-0136**

Journal homepage: www.ModernScientificPress.com/Journals/IJBioMed.aspx *Florida, USA*

Review

Oral Protein-Drug Delivery Systems Suitable for Systemic Circulation

Ibrahim Sani *, Abubakar Abdulhamid, Fatima Bello

Department of Biochemistry, Kebbi State University of Science and Technology, Aliero, P.M.B. 1144, Birnin Kebbi, Kebbi State, Nigeria

* Author to whom correspondence should be addressed; E-Mail: isani76@gmail.com; Tel.: +2348037731851.

Article history: Received 18 January 2014, Received in revised form 20 February 2014, Accepted 22 February 2014, Published 28 February 2014.

Abstract: Till recently, injection remains the most common means of administering therapeutic proteins and peptides because of their poor oral bioavailability. Currently, there is a high level of interest in the use of the oral route as a portal for protein drug entry to the systemic circulation. As a site for drug delivery, the oral route offers advantages over the conventional parenteral and other alternative routes of drug administration. It provides high patient compliance, ease of administration, less expensive and ability to terminate delivery when required. The oral route appears to be a potential site for drug delivery to the systemic circulation. However, this site is associated with limitations that restrict its use as a route for the systemic delivery of drugs. The low permeability of the membranes that line the oral route results in a low flux of the drugs. Designing and formulating a polypeptide drug delivery through the gastrointestinal tract has been a persistent challenge because of their unfavourable physicochemical properties, which includes enzymatic breakdown, poor membrane permeability and large molecular size. Various strategies are currently under investigation which enhances drug penetration to improve bioavailability from less than 1% to at least 30-50%. These strategies involve chemical modification of the protein drugs, protease inhibitors, absorption enhancers, formulation vehicles and mucoadhesive polymeric systems. These pharmaceutical approaches which overcome various physiological barriers, help to improve oral bioavailability and ultimately achieve

formulation goals for oral protein drug delivery.

Keywords: protein drugs; drug delivery systems; bioavailability; oral route; parenteral route; thiolated-chitosans.

1. Introduction

Macromolecules are high molecular weight compounds composed of same or different subunits known as monomers. They can either be synthetic or biological in origin. The biological macromolecules (known as biomolecules) are synthesized in the biological systems (e.g. human body and other microorganisms). Proteins, peptides, nucleic acids, lipids and carbohydrates are examples of biological macromolecules (Muller, 2010).

Proteins that are engineered in the laboratory for pharmaceutical use are known as therapeutic proteins. The majority of biopharmaceuticals marketed to date are recombinant therapeutic protein drugs (Chaudhury and Das, 2010).

Due to the advances in the field of biotechnology, production of these proteins and peptides in economic large-scale mainly from microorganisms make them readily available for various therapeutic applications in the field of medicine and clinical studies. These proteins and peptides are currently considered of therapeutic value that can be used as drugs of choice for the treatment of various diseases because of their exquisite specificity and bioactivity (Park *et al*., 2011).

Today the therapeutic proteins that are used to relieve patients suffering from many conditions include among others (Lee, 2002): (1) various cancers (monoclonal antibodies, interferons), (2) heart attacks, strokes, cystic fibrosis, Gaucher's disease (enzymes, blood factors), (3) diabetes (insulin), (4) anaemia (erythropoietin), and (5) haemophilia (blood clotting factors).

Until recently, the parenteral administration (subcutaneous, intramuscular or intravenous route) of these therapeutic proteins into the body remains the most widely used due to their efficiency in achieving high bioavailability and rapid onset of action after the drug administration, but patient compliance with these parenteral routes is considerably very poor and generally affects the therapeutic value of the drugs especially for long term management of certain diseases (Shaji and Patole, 2008).

Compared with the parenteral route of drug administration, oral route remains simpler and more convenient for drug delivery (Park *et al*., 2011). This is attributed to its easy for paediatric use and other numerous advantages, such as high patient compliance, easy drug administration, highly economical methods of production, ease of approvals from regulatory bodies, safer for risk of infections via inappropriate use or reuse of needles and presence of gastrointestinal tract (GIT) with

high surface area for the drug absorption (Chaudhury and Das, 2010).

The main obstacle for oral therapeutic proteins delivery is poor bioavailability due to several unfavourable physicochemical properties of the proteins drugs, such as incomplete or low absorption through the GIT, susceptibility to enzymatic degradation or inactivation, denaturation of the drug or drug-carriers due to varying pH of the stomach, lack of lipophilicity, short plasma half-life, immunogenicity, large molecular size, and the tendency to undergo aggregation and adsorption (Shaji and Patole, 2008; Chaudhury and Das, 2010).

Multitude of strategies is highly needed for efficient design and formulation of oral proteins and peptide drug delivery system which is capable of stabilizing the drug for easy oral administration, protect the drug from extreme acidity, gastric and intestinal proteases, and also facilitate aqueous solubility of the drug at near-neutral pH, enhance its penetration through the lipid layer so as to enable the protein drug to cross the intestinal and basal membranes into the blood circulation.

2. Overview of the Oral Drugs Delivery

Drugs are transported from the mouth as the site of administration to their target sites within the body through intestinal and cellular barriers. The inner lining of the intestine is covered by a single layer of epithelial cells referred to as enterocytes, which are interconnected by protein complex known as tight junctions. Drugs taken orally can be transported from the intestinal lumen into the blood circulation by either paracellular or trans-cellular movement. The trans-cellular movement involves the passage of the drugs through cells, which is the most common pathway of drug transport, while the paracellular involves passage of drugs (that are too polar to pass across the lipoidal cell membrane) through the tight junctions between the cells. Proteins and other macromolecules cannot easily pass through either of the ways to the blood circulation unless efforts in overcoming the intestinal and cellular barriers have been undertaken (Muller, 2010).

3. Pharmaceutical Approaches for Effective Oral Protein Delivery

Various strategic procedures were carried out for many years aiming at improving the therapeutic proteins bioavailability. The approaches normally used for the oral protein delivery systems formulation consist of using formulations that protect the protein drugs from acid and proteases in the GIT and enhance their permeation, and also using excipients like mucoadhesive polymers, absorption or permeation enhancers, and enzyme inhibitors (Park *et al*., 2011). Another approach is by chemical modification of the proteins and their hydrophobisation or lipidisation to improve their enzymatic stability and membrane penetration (Shaji and Patole, 2008).

3.1. Absorption Enhancers

Absorption enhancers such as bile salts, Mg^{2+} or Ca^{2+}- chelating agents, lipids and surfactants, have been employed (as part of the formulation) in improving the protein drugs permeation obstacles through the intestinal wall (Park *et al*., 2011).

Surfactants such as saponin, laureth-9 and sodium lauryl-sulphate facilitate the trans-cellular movement of drugs by temporary disruption of the intestinal barrier, making the cellular lipid membrane more permeable; but this as a limitation, can affect the integrity of the membrane (Shaji and Patole, 2008).

Chelating agents like EDTA exert their action by complex formation with magnesium or calcium ions and disrupt the tight junctions for the paracellular movement enhancement of the hydrophilic protein drugs (Shaji and Patole, 2008).

Lipids that composed of long alkyl chains, such as fatty acids and phospholipids, like sodium caprate, acyl carnitine and sodium laurate enhance protein drugs absorption through transient opening of tight junctions without obvious harmful effects to the intestinal mucosa (Shaji and Patole, 2008; Park *et al*., 2011).

Another permeation enhancer is *Zonula occludens* toxin which is known to be safest and most effective, by exerting its effect via transient alteration of the intestinal epithelia tight junctions for transport of the drug proteins through the mucosal barriers (Shaji and Patole, 2008; Muller, 2010).

Protein drugs co-administration with carrier molecules is another recently developed option for the use of absorption enhancers, in which partially unfolded protein conformations are temporarily stabilized by the transport carriers causing exposure of their hydrophobic side chains which aids their lipid solubility and facilitate their absorption through the lipid bi-layer (Schatz and Dobberstein, 2006).

Emisphere Technologies Inc. has developed an oral insulin drug delivery system (Eligen^{TM}) by creating a weak non-covalent association between the insulin drug and an absorption enhancer, sodium N-[8-(2-hydroxybenzoyl)aminocaprylate] (SNAC). The insulin-SNAC association is reversed upon crossing the intestinal barrier into the blood stream, and has been proven capable of increasing the oral bioavailability of insulin, and this formulation can also be used for protein drugs with molecular weight ranging from 0.5 kDa to 150 kDa (Park *et al*., 2011).

The drawback in the use of these absorption enhancers is the co-transportation of undesirable molecules present in the GIT into the blood circulation (Muller, 2010).

3.2. Chemical Modification

Small amphiphilic molecules can be used to modify protein drugs through conjugation to enable the absorption of the hydrophilic proteins and peptides (Shen, 2003). Protein-cobalamin

conjugates have been proposed so as to aid and increase the oral protein bioavailability by facilitating absorption through the vitamin B_{12} absorption pathway, which enables the absorption of the conjugate together with the protein drug. But a major limitation that affects this particular method is less availability or low number of intrinsic factor receptors in the GIT, because the conjugate has to interact with an intrinsic protein factor to aid its absorption, and this can really affect the efficiency of this approach (Shen, 2003).

Recently, NOBEX and Biocon have covalently modified insulin drug for oral delivery by introducing hydrophobicity to the protein (insulin) through conjugation with lipophilic moiety thereby enhancing its stability and increase its cellular absorption. Their formulation, hexyl-insulin mono-conjugate 2 (HIM2) has single amphiphilic oligomer chemically attached to the primary amine group of the Lys-29 residue in the β-chain of the human insulin, and it has shown of been capable of resisting enzymatic attack, and its absorption has been facilitated (Park *et al*., 2011).

3.3. Formulation Vehicles/Carrier Delivery Systems

Several formulation vehicles have been utilized for adequate protection of protein drugs from been affected by proteolysis and acidic nature of the GIT. These vehicles such as liposomes, nanoparticles, microspheres and emulsions can target intestinal specific site drug delivery with adequate control in the release rate and also enhance its permeation across the intestinal mucosa (Shaji and Patole, 2008; Park *et al*., 2011).

3.3.1. Microspheres

These are produced from synthetic or natural polymers. An example is P(MAA-g-EG) microsphere which is also a bio(muco)adhesive polymeric system. The pH variation effect from stomach to the intestine on the oral bioavailability of protein and peptide drugs together with their proteolytic degradation can be prevented by using pH-sensitive microspheres as oral delivery vehicles. Lowman *et al*. (2009) investigated the use of microspheres as oral insulin delivery vehicles, where they encapsulated insulin into P(MAA-g-EG) microsphere and observed an increased in bioavailability in diabetic and healthy rats. The advantage of these microspheres is that they restrict the release of protein drugs to the favourable and conducive area of the GIT via shrink-and-swell mechanism.

3.3.2. Emulsions

These also provide protection to protein drugs from proteolysis in the GIT. The permeation enhancement of the drug depends on the nature of the emulsifying agent, size of the particles in the disperse phase, type of lipid phase, pH and drug stability.

Toorisaka *et al.* (2003) have formulated oral insulin drug delivery system using solid-in-oil-in-water (S/O/W) emulsion, where a surfactant-insulin complex was dispersed into the oil phase and this prevented proteolysis and increase the absorption through the intestinal mucosa. They investigated the hypoglycaemic effect of this formulation over several hours after the oral administration to diabetic rats.

The limitations associated with their formulation are instability when kept for long period and the need for it to be stored at low temperature (Eiichi *et al.*, 2005). But these limitations could be tackled through development of dry emulsion oral drug delivery systems (Eiichi *et al.*, 2005). To improve its efficiency, the dry emulsion formulation is further enteric coated with a pH-responsive polymer which shows sensitivity against change in external pH and presence of lipase under the simulated gastro-intestinal conditions (Park *et al*., 2011).

3.3.3. Nanoparticles

These are polymeric solid particles with range of size between 10 to 100 nm that allow protein drugs encapsulation within a matrix and provide them with protection against enzymatic and hydrolytic degradation (Lee, 2002). Several authors have shown the ability of these nanoparticles to be absorbed intact by the epithelial lining of the intestine mainly via the Peyer's patches with subsequent systemic distribution (Sakuma *et al* 2001; Pinto Reis *et al*, 2006; Lee and Yuk, 2007). It was noticed that peptides and proteins encapsulated within these particles show less sensitivity to enzymatic proteolysis with increased permeation through the GIT via their interaction with polymers when compared to their native counterpart. Uptake of nanoparticles such as polystyrene/chitosan/PLA-PEG is shown to be affected by certain factors, such as the nanoparticles size, their surface charge, the nature of their dynamic interaction in the GIT and their ligands (Park *et al*., 2011). Major limitations associated with the use of nanoparticles are possibility of aggregation, absence of precise drug release control, low incorporation efficiency of hydrophilic drugs and tendency of non-degradable particles accumulation in tissues (Morishita, 2006).

3.3.4. Liposomes

These are also used in the formulation of oral drug delivery systems especially for the protein drugs in order to enhance their permeation from GIT into the blood stream for systemic circulation. Liposomal drug delivery systems containing the protein drug and permeation enhancer show remarkable increase in the oral bioavailability of the protein drugs (Degim *et al.*, 2004). It was noticed that when these formulation vehicles are administered orally, they are subjected to degradation by pancreatic lipase, bile salts and gastric acidity. But there are several attempts to improve the stability of these liposomes by polymer incorporation at their surface or employing gastro-intestinal-resistant

lipids (Rick, 2005).

Shaji and Patole (2008) mentioned that encapsulation of insulin in PEG or mucin coated liposomes acquired resistance against digestion by bile salts and increased its stability in the GIT, which was identified by noticing gradual decrease in the plasma glucose level in diabetic rats after oral administration.

An example of oral-protein polymer-coated liposomes drug delivery system is Orasome™ which is a proprietary stable liposome technology developed by Endorex Corporation in promoting oral bioavailability of many therapeutic hormonal proteins and peptides, like insulin and growth hormone (Park *et al*., 2011).

3.4. Mucoadhesive Polymeric Systems

These in fact are the most promising approach compared to several approaches where site specific drug delivery can be achieved. These polymeric systems (e.g. carboxymethyl-cellulose and poly-[methacrylic acid-g-ethylene glycol], P(MAA-g-EG)) are mostly synthetic derivatives of cellulose or polyacrylic acid which are capable of adhering to the mucin layer of the mucosa at the site of the drug uptake and protect the protein from metabolism before absorption in the GIT, thereby increasing the drug residence time resulting in the increase of the oral protein drugs bioavailability (Lee and Yuk, 2007). These polymers exhibit behaviour of swelling in respond to changes in many factors they come across, such as electric field, ionic strength, pH, enzymes, temperature or light (Park *et al*., 2011). Designing mucadhesive controlled release systems can be achieved for co-release of drug and inhibitor simultaneously with efficient protein protection. P(MAA-g-EG) is a pH sensitive mucoadhesive polymeric drug delivery system utilized for good protection of the protein drug from proteolysis in the stomach and upper portion of the intestine (Lee and Yuk, 2007).

Mucoadhesive polymers when used alone in the formulation of oral protein drug delivery cannot efficiently provide total protection to the drug against enzymatic proteolysis, instead, they have to be conjugated with protease inhibitor or chemically modifying them for the perfect protection to be achieved (Lee and Yuk, 2007; Park *et al*., 2011). Hence, mucoadhesive polymeric drug delivery systems provide high drug concentration gradient as driving force for absorption, and their interaction with absorption membrane, enhances permeation (Aidoo, 2009).

The limitation of this strategy is associated to the expensive costs of certain enzyme inhibitors.

3.5. Enzyme Inhibitors

Enzymatic proteolysis is one of the major obstacles that face oral protein delivery. In overcoming this barrier of protein degradation, protease inhibitors are needed in order to obtain an

effective therapeutic bioavailability of the oral protein drugs (Park *et al*., 2011).

The selection of such inhibitors depends on the structure of the proteins and the information regarding the specificity of the proteases for the assurance of the stability of the protein drugs in GIT. The amount of inhibitors that could be co-administered is essentially important for the intestinal permeability of the protein or peptide drug (Bernkop, 2008).

When considering insulin as a protein drug, it usually undergoes degradation by serine proteases, trypsin, α-chymotrypsin and thiol metalloproteinase (Shaji and Patole, 2008). Presence of excipients that inhibit these enzymes has shown a remarkable stability of the insulin, examples of these enzyme inhibitors include pancreatic inhibitor, FK-448, soybean trypsin inhibitor, P-chloromeribenzoate, aprotinin, camostat mesylate, bacitracin, and another new class of inhibitors, such as chicken and duck ovomucoids were also discovered (Agarwal *et al.*, 2001).

Inactivation of the local proteolytic enzymes by pH manipulation is another alternative approach, in which sufficient amount of buffer that lowers the intestinal pH blow 4.5, is needed to deactivate trypsin, chymotrypsin and elastase (Shaji and Patole, 2008).

Because of the nature of enzyme distribution and quantities, the use of enzyme inhibitors alone for the oral protein delivery cannot be highly effective; in order to increase the efficiency, a formulation of polymer and enzyme inhibitor conjugate system has shown to promote the oral bioavailability of the protein drugs (Lee, 2002; Park *et al*., 2011).

Major limitations concerning the use of enzyme inhibitors are induction of severe side effects especially in long term therapy and excessive high cost (Park *et al*., 2011).

4. Multifunctional Oral Protein Drug Delivery System

After critical analysis of each individual strategy briefly discussed above in trying to overcome the challenges facing the oral macromolecular drug delivery, and their individual limitations ranging from high cost of production to severe side effects especially on long term therapy; that can affect the patient's conveniency, damage to intestinal mucosa and less efficiency of some approaches due to limited number of properties or features that help in enhancing the oral bioavailability of the protein drugs, a question has now been raised, that, which strategy or approach is suitable to be designed that can overcome all the above mentioned limitations of the other approaches so as to achieve the target goal of enhancing an effective increase in the bioavailability of the oral macromolecular drug delivery systems?

The answer to the above question is employing the use of a polymeric drug delivery system that exhibits multifunctional properties such as mucoadhesion, enzyme inhibition, permeation enhancement, high buffering properties and can additionally provide controlled and/or targeted drug

release.

The polymeric system considered possessing the above mentioned multifunctional properties and suitable for this issue is chitosan polymeric drug delivery system due to its abundance in nature; as structural component of exo-skeleton of crustaceans and also found in molluscs, insects and fungi (George and Abraham, 2006). The next reason for considering chitosan is its other suitable properties, such as biocompatibility and biodegradability, pH-sensitiveness, mild gelation conditions and ease of chemical modifications when improving its properties is desired (George and Abraham, 2006; Vigl, 2009). Another reason is that chitosan is a cationic polymer which consists of β-1-4 D-glucosamine units (Vigl, 2009), which can interact ionically with the anionic sub-structures of sialic acid residues on the intestinal mucus layer (Aidoo, 2009).

In order to improve the chemical and mechanical properties of chitosan for the purpose of enhancing the bioavailability of the oral protein drug delivery, it can be chemically modified by thiolation through immobilisation of thiol bearing moieties on the chitosan backbone to yield thiolated-chitosan, such as chitosan-thioethylamidine (TEA) (Kafedjiiski *et al*., 2005).

As mentioned earlier, chitosan exhibits mucoadhesive properties, and this can be improved by the thiolation of the chitosan, hence, formulation design involving the thiolated-cationic-chitosan has the capability to be covalently anchored in the intestinal mucus layer through disulphide bonds between the cysteine-rich domains of the mucus and the thiol groups attached to the chitosan (George and Abraham, 2006). In comparison to the corresponding non-modified chitosans, the mucoadhesion of the thiolated-chitosans is stronger because the disulphide linkages of the sub-structures of mucins are susceptible to reductive disruption by the thiols (Vigl, 2009).

The mucoadhesive thiolated-chitosans allow a close and prolonged contact of the delivery system with the mucosa which can lead to high local drug concentration that can eventually facilitate its permeation through the mucus and the absorption membrane due to generation of high drug concentration gradient on the mucosa (Vigl, 2009). Apart from increasing the active drug concentration at the site of absorption, other benefits considered to be gained through close contact of the thiolated-chitosans with the mucosa are protection of the incorporated protein drug from luminal degrading enzymes and enhancement of the drug uptake due to the permeation enhancing properties of the chitosan polymeric system.

High buffer capacity is another feature of multifunctional cationic chitosan polymeric systems (Vigl, 2009). Therefore, these thiolated-chitosans act as ion-exchange resins capable of maintaining a stable pH level inside the polymeric network over a certain period, and this contributes to the stability of the incorporated macromolecular drug against pH-dependent denaturation and enzymatic degradation.

Chitosan exhibits a pH-sensitive behaviour, which dissolves easily at low pH while insoluble at

higher pH ranges (George and Abraham, 2006). But thiolation changes this behaviour of the chitosan so as to be stable at low pH and dissolves at higher pH ranges (Vigl, 2009), this means that thiol modification of chitosan improves its stability in the stomach and subsequent controlled delivery of the incorporated macromolecular drugs in the intestine.

The mechanism of pH-sensitive response of the thiolated-chitosan drug delivery system is shrinking in the acidic environment of the stomach due to the formation of intermolecular polymer complexes, thereby protecting the integrated drug from acidic and enzymatic degradation. While in the neutral and basic pH of small intestine, dissociation of the complexes causes swelling of the thiolated-chitosans, which eventually results in the release of the drug (Vigl, 2009; Muller, 2010).

Permeation studies across Caco-2 cells, which serve as a model of intestinal epithelium, demonstrate a strong permeation-enhancing effect of chitosan accompanied by a decrease in the trans-epithelial resistance, hence, indicating loosening of the tight junction (Smith *et al*., 2004). The positive charges of the chitosan interact with the negatively charged residues on the cell surface, which results in conformational changes in the structural organization of tight junction-associated proteins (Vigl, 2009). Hence, the thiolation of the mucoadhesive cationic-chitosan improves its permeation-enhancing properties (via inhibitory effect of thiol groups on tyrosine phosphatase enzyme, which mediates the closing of tight junction) and facilitates the paracellular route of absorption of the hydrophilic therapeutic proteins thereby increasing their bioavailability.

4. Conclusions

Multifunctional thiolated-chitosan drug delivery system is a promising system toward increasing the bioavailability of the therapeutic proteins after an oral administration because of the following reasons: (1) protection of the incorporated drug against pH- and enzymatic degradation along its way down the GIT, (2) provision of sustainable drug release at target site due to its cohesive properties, (3) increase in the residence time of the drug delivery system, (4) provision of high drug concentration gradient at the target site which will serve as a driving force for absorption, and (5) tight contact with the absorption barrier due to mucoadhesion that can facilitate permeation.

References

Agarwal, V., Nazzal, S., Reddy, I. K., and Khan, M. A. (2001). Current status of the oral delivery of insulin. *Pharm. Technol.*, **10**: 76-90.

Aidoo, S. (2009). Bioadhesive polymers. [Online] [Accessed 3rd March, 2011], at: www.userweb.port.ac.uk/~raldom/mucoadhesives/bioadhesives.ppt

Bernkop, S. A. (2008). The use of inhibitory agents to overcome the enzymatic barrier to perorally

administered therapeutic peptides and proteins. *J. Control Release,* **52**: 1-16.

Chaudhury, A., and Das, S. (2010). Recent advancement of chitosan-based nanoparticles for oral controlled delivery of insulin and other therapeutic agents. *AAPS Pharm. Sci. Tech.*, **12**(1): 10-20.

Degi, Z., Unal, N., Essiz, D., and Abbasoglu, U. (2004). The effect of various liposome formulations on insulin penetration across Caco-2 cell monolayer. *Life Sci.,* **75**: 2819-2827.

Eiichi, T., Masakazu, H., Noriho, K., Hiroshige, O., Yuko, K., and Masahiro, G. (2005). An enteric-coated dry emulsion formulation for oral insulin delivery. *J. Control Release,* **107**: 91-96.

George, M., and Abraham, T. E. (2006). Polyionic hydrocolloids for the intestinal delivery of protein drugs: Alginate and chitosan – a review. *J. Control Release,* **114**: 1-14.

Kafedjiiski, K., Krauland, A. H., Hoffer, M. H., and Schnurch, A. B. (2005). Synthesis and in vitro evaluation of a novel thiolated chitosan. *Biomaterials,* **26**: 819-826.

Lee, H. J. (2002). Protein drug oral delivery: The recent progress. *Arch. Pharm. Res.*, **25**: 572-584.

Lee, K. Y., and Yuk, S. H. (2007). Polymeric protein delivery systems. *Prog. Polym. Sci.*, **32**: 669-697.

Lowman, A. M., Morishita, M., Kajita, M., Nagai, T., and Peppas, N. A. (2009). Oral delivery of insulin using pH-responsive complexation gels. *J. Pharm. Sci.,* **88**: 933-937.

Morishita, M., and Peppas, N. A. (2006). Is the oral route possible for peptide and protein drug delivery? *Dr. Disc. Tod.,* **11**: 905-910.

Muller, G. (2010). Oral delivery of protein drugs: Driver for personalised medicine? *Curr. Iss. Mol. Biol.,* **13**: 13-24.

Park, K., Kwon, I. C., and Park, K. (2011). Oral protein delivery: Current status and future prospect. *React. Funct. Polym.,* **71**: 280-287.

Pinto Reis, C. M. S., Neufeld, R. J., Ribeiro, A. J., and Veiga, F. (2006). Nanoencapsulation II: Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. *Nanomed.: Nanotech., Biol., Med.,* **2**: 53-65.

Rick, S. (2005). Oral protein and peptide drug delivery. In: *Drug Delivery: Principles and Applications,* Binghe, W., Teruna, S., and Richard, S. (eds.), Wiley Interscience, New Jersey, p. 189

Sakuma, S., Hayashi, M., and Akashi, M. (2001). Design of nanoparticles composed of graft copolymers for oral peptide delivery. *Adv. Drug Deliv. Rev.,* **47**: 21-37.

Schatz, G., and Dobberstein, B. (2006). Common principles of protein translocation across membranes. *Science,* **271**: 1519-1526.

Shaji, J., and Patole, V. (2008). Protein and Peptide Drug Delivery: Oral approaches. *Ind. J. Pharm. Sci.,* **70**: 269-277.

Shen, W. (2003). Oral peptide and protein delivery: Unfulfilled promises? *Dr. Disc. Tod.,* **8**: 607-608.

Smith, J., Wood, E., and Dornish, M. (2004). Effect of chitosan on epithelial cell tight junctions. *Pharm. Res.,* **21**: 43-49.

Toorisaka, E., Ono, H., Arimori, K., Kamiya, N., and Goto, M. (2003). Hypoglycaemic effect of surfactant-coated insulin solubilised in a novel solid-in-oil-in-water (S/O/W) emulsion. *Int. J. Pharm.*, **252**: 271-274.

Vigl, C. (2009). Multifunctional Polymeric Excipients in Oral Macromolecular Drug Delivery. In: *Oral Delivery of Macromolecular Drugs,* Springer U.S., New York, pp 137-152.