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Review

Optimising oral systems for the delivery of therapeutic proteins and peptides

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Therapeutic proteins/peptides are mostly administered as parenteral (injectable) preparations as a result of their poor oral bioavailability which is due to degradation by proteolytic enzymes, poor membrane permeability and large molecular size. However, the oral route would be preferred to the parenteral administration because it is more convenient for self-administration, non-invasive and more patient friendly. Consequently, efforts have intensified over the past two decades to maximize the extent of absorption of protein and peptide drugs in order to achieve optimum bioavailability via the oral route. A suitable oral delivery system should retain the drug and maintain its integrity until it gets to the region of maximum absorption where the protein/peptide is released. It would be advantageous for such a delivery system to be capable of attaching itself to the absorptive cells in that region during the course of drug release by means of specific interactions with the tissue components. Furthermore, movement of drug should be independent of prevailing factors in the gut during passage. This review examines the various efforts and strategies that have been used to pursue the goals of effective oral peptide delivery, progress made so far, as well as current trends and future prospects. Relevant issues and phenomena such as membrane permeability control, intestinal absorption, paracellular pathway and targeting have also been discussed.

Key words: Protein and peptide delivery, delivery systems, oral administration, targeting, intestinal absorption.

INTRODUCTION

Over the last two decades, the field of biomedical research has witnessed dramatic advances in the understanding, diagnosis, and treatment of human diseases. These developments have been fueled by an increased awareness of the essential roles played by endogenous proteins and peptides in the regulation and integration of life processes (Samonen, 1985). For example, in the form of skin, hair, cartilage and muscle, proteins hold together, protect, and provide structure to the body of a multicellular organism. In the form of enzymes, hormones, antibodies and globulins, they catalyze, regulate and protect the body chemistry while in the form of haemoglobin, myoglobin and various lipoproteins, they effect the transport of oxygen and other chemical substances within the body (Schwendeman et al., 1996; Adessi and Sotto., 2002). The increasing importance of proteins and peptides can be attributed to three main developments. First, improved analytical methods have fostered the discovery of numerous hormones and peptides that have found applications as biopharmaceuticals. Second, molecular biology and genetic engineering have enabled the large-scale production of polypeptides previously available only in small quantities. Third, there is now a better understanding of the role of regulatory proteins/peptides in the pathophysiology of human diseases. Consequently, pharmaceutical scientists are now routinely using specific peptide sequences as lead structures for drug development. (Adessi and Sotto, 2002, 2004).

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Despite the high level of activity in peptide-based drug research, several obstacles hinder the development of peptides into useful therapeutically deliverable agents, the most important of which is imparting good bioavailability while maintaining pharmacological efficacy (Humphrey and Ringrose, 1986). This problem stems from the unique structural features of peptides which are directly linked to their high instability in biological milieu, rapid elimination from plasma, poor transportability across membranes and ease of metabolism either within the gastrointestinal tract or within the cells lining the tract (Davis et al., 1986; Schwendeman et al, 1996).

ORAL DELIVERY OF PROTEINS AND PEPTIDES

A primary objective of oral delivery systems is to protect protein and peptide drugs from acid and luminal proteases in the GIT. More recent efforts seem to focus on site-specific delivery systems. The site could be an organ, a cell subset or even an intracellular region with the objective of restricting the distribution of the peptide to the specific target site. This should allow for an increase in efficacy with an attendant decrease in toxicity (Arhewoh and Okhamafe, 2004).

Various systems for achieving site-specific delivery of orally administered protein/peptide drugs have been addressed in recent years including coating systems based on pH changes and enzymatic activity of intestinal microflora (Macloid et al., 1999; Stubbe et al., 2001), nanoparticles, (Shinji et al., 1997), liposomes (Kimura and Saishin, 1985), matrix devices (Krishnaiah et al., 2001) and conjugate (degradable prodrug) formation (Yano et al., 2002; Arhewoh and Okhamafe, 2004) . All of these have produced variable release profiles, often because the transit time through the colon can vary substantially from as low as 6 to as high as 30 h. Several of these approaches are somewhat complex and if they were to be translated into actual manufacture of oral delivery systems, the products would be expensive and, therefore, unaffordable in most developing countries. In our laboratories, preliminary studies on microcapsules of chitosan-alginate modified with selected excepients such as HPMCAS, talc, microcrystalline cellulose, polymeta-crylates and pectins were carried out. Protein release was determined at different pH media spanning the pH range of the gut (Figure 1). It was observed that microcapsules modified with talc and microcrystalline cellulose had higher protein retention in the core in all the pH tested (Okhamafe et al., 1996; Ahonkhai et al., 2005; Arhewoh et al., 2005). This shows that modification of the core of chitosan-alginate microcapsules could facilitate drug targeting to the colon.

ORAL GENE DELIVERY

In vivo production of proteins through oral gene therapy has also been investigated (Rothman et al., 2005).

Rather than administering the protein itself, the DNA plasmid that codes for it is swallowed. This then attaches to the cells in the small intestine where it manufactures the required protein drug using available materials in the cell. The protein is thereafter absorbed into the blood stream. The gene has a short half-life and hence must be administered regularly to be effective. The advantage is that it provides for safe, easily managed treatment unlike most gene-based therapies currently available. It is also hoped that this system will be relatively free from ectopic expressions usually experienced with gene therapies since the bulk of the administered genes remain within the intestine and is passed out with feaces along with sloughed cells of the intestinal epithelium.

MEMBRANE PERMEABILITY CONTROL

It is important that a successful oral delivery system incorporating a membrane would exhibit a permeability, which can be modulated controlled by factors favourable to the targeting of the drug to the specific site of absorption. To achieve this, suitable polymer additives may be used. The benefits of this approach have been highlighted by Okhamafe and Goosen (1993). Membrane permeability control is also a critical factor in living cell encapsulation technology and in the development of bioartificial organs. Furthermore, membrane modulation is often employed to improve the strength and stability of the microcapsule itself (Okhamafe and Goosen, 1999). Ahonkhai et al. (2005) recently found that by incorporating polymers or solid fillers in the core of chitosan-alginate microcapsule, the release of a model protein, bovine serum albumin (BSA) can be modulated such that 70% of the protein is delivered to the desired absorption site in the gut 9 h later (see Figure 1). Related applications include the pre-purification and concentration of products of cell encapsulation, release into the circulatory system of hormonal and enzymatic products generated by bioartificial organs which are protected from components of the immune system, principally immunoglobulins. Some relevant parameters to be considered in membrane permeability control are highlighted in Table 1.

PROTEIN/PEPTIDE ABSORPTION

Intestinal absorption

The primary function of the intestine is the digestion and absorption of food substances including proteins. Within the intestinal lumen, pancreatic endopeptidases chiefly trypsin, elastase and chymotrypsin together with the exopeptidases, carboxyl peptidase A and B, produce amino acids and peptides typically 2 to 6 residues in length. The efficient absorption of an intact peptide, therefore, is the exception rather than the rule. Examples

Table 1. Critical factors affecting microcapsule membrane permeability.

Capsule wall	Solute	Capsule	Process factor
Polymer molecular weight	Size	Swelling	Polymer concentration
Chemical structure	Shape	Size	Reaction time
Multiple membrane	Electric charge	Shape	Additives
Physical integrity			Purity
Thickness			PH

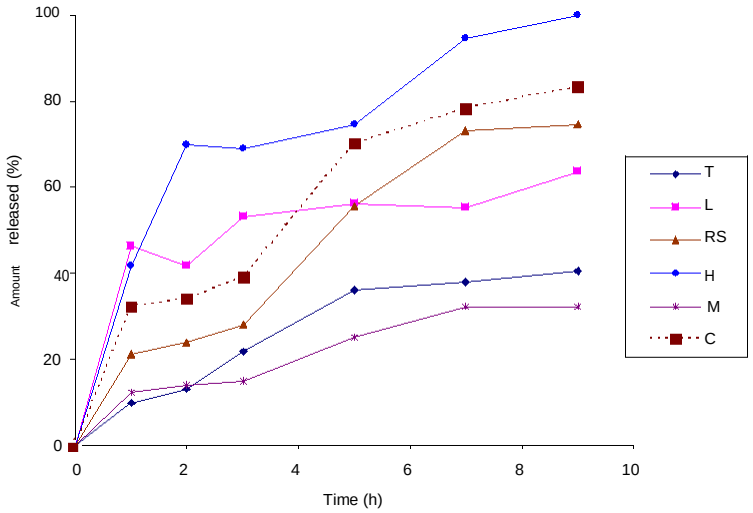

Figure 1. Protein (BSA) release at pH 1.2 from microcapsules modified with different additives – Talc (T), Eudragit L100 (L), Eudragit RSPM (RS), HPMCAS (H), microcrystalline cellulose (M) and Control (i.e., unmodified) (C)

orally absorbed, biologically active peptides are rare. However, both thyrotrophin-releasing hormone (TRH) (Yokohama et al., 1984) and the luteinizing hormone-releasing hormone (LHRH) analogue, leuprolide (Gonzalez-Barcena et al., 1975) were reported to exert biological activity when given orally in rat and man. The activity of these two proteins is a reflection of their extreme potency as only a small portion of TRH was actually absorbed in rat and man. It was further observed that in the rat, absorption is limited to the upper region of the intestine and is mediated by a Na^+ dependent, peptide transport system (Yokohama et al., 1984). Remarkably, absorption of DN- 1417, a close structural analog of TRH, is not active, but occurs by passive diffusion in all parts of the intestine. Furthermore, only 1% is absorbed in rat and 10% in dog. This example illustrates the sensitivity of transport proteins to structural modifications in their substrates (Kimura 1984).

Colonic absorption

Most of the water and electrolytes in chyme, which pass through the ileocecal valve into the large intestine, are absorbed in the colon, usually leaving less than 100 ml of fluid to be excreted in the feces. Most of the absorption in the large intestine occurs in the proximal one half of the colon, giving this portion the name, absorbing colon. The mucosa of the large intestine, like that of the small intestine, has a high capability for active absorption of sodium (Guyton and Hall, 1996). Numerous bacteria, especially colon bacilli, are present normally in the absorbing colon (Wilson and Basit, 2005). They are capable of digesting small amounts of cellulose, in this way providing a few calories of nutrition to the body each day. Substances formed as a result of bacterial activity are vitamin K, vitamin B_{12}, thiamin, riboflavin and various gases that contribute to flatus in the colon, especially carbon dioxide, hydrogen and methane. It is based on this bacterial activity that the colon can be used as a site of drug delivery whereby polymer- coated drugs are targeted to the colon. The bacteria in the colon degrade the polymer thus releasing the drug where it can either exert local action or be absorbed into systemic circulation (Stubbe at al., 2001; Wilson and Basit, 2005).

Colonic transit is an important factor in the absorption of drugs that either act locally in the colon or are

absorbed into systemic circulation from the colon. The time at which a delivery system arrives at the colon depends mainly on the gastric emptying rate (Davies et al., 1991). The arrival time for a tablet or capsule in the colon can range from about 5 h in the fasted condition to 13 h or longer in the fed condition. In healthy volunteers, the colonic transit of tablet or capsules releasing drug over a prolonged period probably delivers most of its active ingredients to the colon rather than to the upper GI tract (Hardy et al., 1989).

Lymphatic absorption

Drugs administered through the gastro-intestinal tract are normally transported into systemic circulation via the portal vein. As a consequence, compounds can sometimes undergo extensive metabolism during the first pass through the liver. Some well-known drugs, such as lignocaine, are almost totally metabolized by this process with a consequence that little or none is available to the general circulation. Other routes, such as rectal, buccal, nasal and transdermal, avoid the first pass effect but do not provide the convenience of oral delivery. If a drug is absorbed through the lymphatic system rather than by the portal circulation, it will find its way into the blood via the thoracic duct and will, therefore, avoid the first pass effect. A drug absorbed lymphatically is incorporated into chylomicrons (and other lipoproteins) produced by the fat digestion process. Various surfactant solubilised systems and other enhancing agents would appear to direct drugs to the lymphatic route and there are even claims that particulates can be taken up this way (Leferve and Joel., 1977; Khoo et al., 2001).

The conjugation of large molecular weight drugs with polymers, such as dextran, appears to be an interesting strategy for enhancing the lymphatic uptake of anticancer agents (Muranishi et al., 1987). In this case the lymphatics could well be the target site (e.g., for treatment of metastatic spread) rather than the systemic circulation and beyond.

PATHWAY FOR PROTEIN/PEPTIDE ABSORPTION

Identifying a region in the intestine that favours protein/peptide absorption is a crucial step in the design of oral delivery systems for protein drugs. The intestinal barrier is of major importance. Furthermore, regional variations in the penetration barriers to peptides may result in regional differences in their absorption (Kompella and Lee, 2001) . There are certain factors that affect the permeability of molecules through the intestinal barrier and these include:

Physicochemical properties of the drug molecules
Characteristics of the intestinal barrier
Transport mechanism
Intestinal fluid composition
Disease state

Targeting the drug to specific cells along the lining of the intestine may be useful in facilitating protein/peptide absorption, for instance, M-cells located on the dome epithelium of gut-associated lymphoid tissues are known to be capable of sampling macromolecular antigen from the lumen through an endocytic pathway (Keljo and Hamilton, 1983; Kompella and Lee, 2001) . The M-cells are located on Peyer's patches and the possibility of administering microparticles orally to target this site has been suggested (Eldridge et al., 1990). The researchers administered 20 mg of microspheres containing the fluorescent dye, coumarin-6 to non- anesthesized mice. At 48 h, the mice were killed and three representative Peyer's patches, together with the first mesenteric lymph node proximal to the appendix and spleen, were excised for microscopic observation. The number of absorbed microspheres was counted in frozen sections using a florescence microscope. The percentage of ingested dose was not determined; however, of the microspheres investigated, only those composed of polystyrene, poly (methylmethacrylate), poly (hydroxylbutyrate), poly (D, L-lactide), poly (L-lactide) and poly (D, L-lactide-coglycolides) were absorbed into the Peyer's patches of the small intestine, while those composed of ethyl cellulose, cellulose acetate hydrogen phthalate and cellulose triacetate were not. Microsphere uptake occurred only in Peyer's patches and was restricted to those microspheres up to 10 μm in diameter. Absorptive cells located on the ideal epithelium are known to be capable of sampling luminal peptide growth factors (Gonnela et al., 1987) bile acids (Ho, 1987) and cyanocobalamine (Doscherholmen et al., 1971)

From recent advances in protein absorption studies have emerged a new system termed 'Active Peptide Transport' (APT) (Stevenson and Keon, 1998). APT describes a new formulation that is designed to "actively" open alternative absorptive pathways in the body. These alternative pathways make transporting proteins to body cells and the muscles faster and more efficient. APT is important because it is designed to allow the body to absorb peptides faster and more efficiently. The body has three different protein transport systems that it uses for muscle growth. The first system uses free-form amino acids. This is a very inefficient system because the free-form amino acids must compete with one another for absorption. The second system involves small, short peptide chains of 2 to 3 amino acids linked together. These are called di- and tri-peptides. Di- and tri-peptides are absorbed into the blood stream through the transcellular pathways. The third system involves large molecular weight peptides and is unique to whey protein. This is not used when casein, egg white or soy proteins are digested. It should be noted that not all proteins stimulate all three protein transport systems. In fact, only specially made designer proteins stimulate the pathways. APT with full spectrum whey peptides is designed to

facilitate the stimulation of all three transport systems and can only be found in designer protein. These APT full spectrum whey peptides are a complete spectrum of very low, low, medium and high molecular weight whey peptides.

Paracellular pathway

The paracellular pathway is found along the intestinal wall and is used by the second and third transport systems as an alternative pathway for peptide absorption. This pathway is normally closed off to entry of peptides and nutrients by a special cell called “tight junction” (Stevenson and Koan 1998). In a current model of a tight junction, two major integral membrane proteins are found - occludin and claudin - each with four membrane spanning alpha-helices. The junction depends upon extra cellular calcium to maintain integrity. The permeability properties of tight junctions vary considerably in different epithelia and epithelial cells can transiently alter their tight junctions in order to allow increased flow of solutes and water through breaches in the junction barriers (doscherholmen et al., 1971; Fanning et al., 1998). The tight junctions usually prevent the transport of protein through the paracellular pathway. However, research has shown that specific amounts of key ingredients, the same ingredients found in designer protein’s APT, help the tight junctions to open (Fanning et al., 1998). Naturally, with both the transcellular and paracellular pathways opened, protein is potentially absorbed faster and in greater amounts. Furthermore, unique protein with molecular weight larger than the di- or tri-peptides absorbed by the second transport system may be able to pass through intact (Stevenson and Koan, 1998; Muranishi and Yamamoto, 1994)

TARGETING

Existing methods for selective targeting are based on chemical conjugation of therapeutic and diagnostic agents or their carriers to cell specific targeting molecules (Gaidamakova et al., 2001; Pastorino et al., 2001). These methods are limited by potential damage to targeting molecules that can be inflicted by the conjugation procedure. In addition, conjugation procedures have to be developed on case - by - case basis (Amidon, 1995). In order to avoid this problem, a new approach has been developed to constructing molecular vehicles for target-mediated delivery of therapeutics and diagnostic agents. In this approach, the targeting molecule is expressed as a fusion protein containing a recognition tag. The recognition tag is defined as a protein or peptide that can bind non-covalently with another peptide or protein (adapter). In turn, the adapter is chemically conjugated to a carrier of therapeutics or diagnostics. The assembled molecular delivery vehicle contains a carrier - adapter conjugate, which bound non-covalently to a recognition tag fused to the targeting protein (Gaidamakova et al., 2001).

Another approach towards delivering drugs into cells is the concept of loligomers. Loligomers are synthetic peptides composed of a branched polylysine case harboring identical arms, each carrying peptide signals guiding their import and localization into cells. The most important advantages of loligomers include:

The multivalent presentation of targeting signals resulting from a tentacular arrangement. Multivalency increases the efficiency of import and intracellular routing signals as compared to similar linear peptides.

Another advantage is that it reduces and delays the impact of peptide degradation in terms of cellular processing and compartmentalization (Borkx et al., 2002).

The vectorial delivery of nucleus – directed loligomers into cells has recently been confirmed by microscopy and flow cytometry studies (Borkx et al., 2002). Practical uses of loligomers include photosenstizers for use in photodynamic therapy and the incorporation of cytotoxin T-lymphocyte epitopes with a view to creating synthetic vaccines. Branched peptide such as loligomers represents simple and versatile molecular vehicles with potential applications in a wide variety of drug design approaches.

Delivery to cancer cells

Cancer treatment has always posed a problem. The effectiveness of conventional solid tumor treatment is limited by the systemic toxicity and lack of specificity of chemotherapeutic agents. Barriers are also frequently hampering targeting of drugs and toxins to solid tumors and their microenvironment. Present treatment modalities are frequently insufficient to eliminate viable cancer cells without exceeding the limits of toxicity to normal tissue. The coming generation of cancer therapeutics depends on the precise targeting and sustained release of anti-tumor agents to overcome their limitations.

Phage-derived peptides for targeting of doxorubicin conjugates to solid tumors have been designed (Schatzlein et al., 2001). Nano-conjugates are low molecular weight conjugates of a small drug or toxin and a targeting ligand coupled through a cleavable linker group. They offer potential advantages for tumor specific delivery in diffusion – limited situations. As a model, a doxorubicin conjugate targeted to the transferring receptor (TFR) was chosen. A library of phage expressing a cyclic nanopeptide was panned against TFR. The apparent affinity of phages determined by surface plasmon resonance (SPR) increased with each cycle of the panning procedure. After five rounds, approximately 80% of phages expressed the same

peptide, which mediated a 30 to 50-fold increased receptor specific cellular uptake of the phages. The corresponding peptide was synthesized using solid phage peptide chemistry on a sulphonamide based safety catch resin. Crude mixtures of the peptide, as well as transferrin itself, were able to inhibit the phage uptake significantly.

Chitosan nanoparticles encapsulating dextran – doxorubicin conjugate has been used as carrier for targeting tumors (Mitra, 2001). Doxorubicin (DXR) commonly used in cancer therapy is known to produce undesirable side effects such as cardiotoxicity. To minimize these, attempts were made to couple the drug with dextran (DEX) and then to encapsulate this drug conjugate in hydrogel nanoparticles. By encapsulation of the drug conjugate in biodegradable, biocompatible long circulating hydrogel nanoparticles, it further improved the therapeutic efficacy of the conjugate. The size of these nanopaticles, as determined by quasi-elastic light scattering, was found to be 100 $\pm$ 10 nm in diameter, which favours the enhanced permeability and retention (EPR) effect as observed in most solid tumors. The anti tumor effect of these DEX – DXR nanoparticles, was evaluated in macrophage tumor cells implanted in Balb/c mice. The *in vivo* efficacy of these nanoparticles as antitumor drug carriers was determined by tumor regression and increased survival time as compared to drug conjugate and free drug. These results suggest that encapsulation of the conjugate in nanoparticles not only reduces side effects, but also improves its therapeutic efficacy in the treatment of solid tumor. Preliminary studies to evaluate the potentials of chitosan particles as carriers for doxorubicin was also carried out and it was concluded that it was feasible for the chitosan nanoparticles to entrap the basic drug, doxorubicin, and to deliver it into the cell in its active form (Janes et al., 2001).

Water -soluble polymers have been used to target tumors. The rationale for the use of water soluble polymers for anticancer drug delivery includes the potential to overcome some forms of anti-drug resistance and preferential accumulation in tumors due to enhanced permeability and retention (EPR) effect, biorecognizability and targetability (Kopeck et al., 2001).

FINAL REMARKS

Several sites in the GIT have been investigated by researchers for oral delivery of therapeutic proteins and peptide drugs but no major breakthrough with broad applicability to diverse proteins and peptides has been achieved. Nonetheless, the oral route has distinct advantages over the parenteral route which is invasive and inconvenient for repeated use. Delivery systems for oral administration of therapeutic substances have been developed for site-specific delivery in GIT region. Of these regions, the colon is often preferred for the delivery of peptide drugs because of slow transit, low volume and a lack of vigorous stirring, leading to an ability to create local conditions favourable to stabilization and absorption enhancement. In addition, the colonic region has a high presence of microbial anaerobic organisms providing reducing conditions and sufficient area to partially compensate for low peptide mucosal permeability and a lack of digestive enzymes (proteases).

It is expected that in the foreseeable future more focus will be on the colon as a region for site-specific delivery of therapeutic substances (proteins, peptides, genes, etc) that are unstable in the other regions of the GIT in order to optimize convenience, therapeutic benefits and safety.

REFERENCES

Adessi C,Sotto C (2002). Converting a peptide into a drug: strategies to improve stability and bioavailability. Curr. Med. Chem 9 (9) 963-978

Adessi C , Sotto C. (2004). Strategies to improve stability and bioavailability of peptide drugs. Frontiers in Med. Chem. 1 (1) 513-527.

Ahonkhai EI, Arhewoh IM,Okhamafe AO (2005). Studies on protein release from chitosan-alginate microcapsules modified for oral vaccine delivery with selected additives. (Unpublished)

Amidon G (1995). Drug Delivery Design. Pharm.l News, 2 (6). 32 – 33.

Arhewoh IM and Okhamafe AO (2004). An overview of site-specific delivery of orally administered proteins/peptides and modelling considerations. JMBR, 3(1) 7-20

Arhewoh IM, Ahonkhai EI,Okhamafe AO. (2005). Studies on protein release from chitosan-alginate microcapsules modified with pectin. (Unpublished)

Borkx RD, Bisland SK,Gariepy J (2001) Designing Peptide- based scaffolds as drug delivery vehicles, J. Controlled Release. 78 (1-3) 115 – 123.

kompella UB, Lee VHL. Adv Drug Del Rev 46 (2001) 211 – 245.

Davis SS, Hardy JG, Fara JW (1986). Transit of pharmaceutical dosage form through the small intestine. Gut, 27. 886-892.

Davis SS, Illum L, Tomlinson E (1986). Delivery systems for peptide drugs. NATO ASI Series A. Life Sciences. Plenum Press, New York. 125-368.

Doscherholmen A, Macmalion J,Ripley D (1971). Impaired absorption of egg and vitamin B_{12} in postgastrectomy and achlorhydric patients, J. Lab clin. Med. 78, 839 – 840.

Eldridge JH, Hammond CJ, Meulbroek A, et al. (1990) Controlled vaccine release in the gut associated lymphoid tissues. In. Orally administered biodegradable microspheres target the Peyer's patches. J. Controlled release 11 (1990) 205-214.

Fanning AS, Jameson BJ, Jesaitis LA (1998). The tight junction protein zo-1 establishes a link between the transmembrane protein occludin and the actin cytoskeleton.J Biol Chem. 273 (45) 29745-29753.

Gaidamakova EK, Backer MV, Backer JM (2001). Molecular vehicle for target–mediated delivery of therapeutics and diagnostics, J Controlled Release. 74 (1-3) 314 – 347.

Gonnela PA, Siminoski K, Ri A (1987). Transepithelial transport of epidermal growth factor by absorptive cells of suckling rat ileum. J. Clin Invest. 50, 22-32.

Gonzalez Barcena D, Kastin AJ, Miller MC (1975). Stimulation of leutinizing hormone release after oral administration of an analogue of LH releasing hormone. Lancet, 2; 1126.

Guyton AC, Hall JE. (1996) Textbook of med Physiol. 7th Edn, 709-880.

Hardy JG, Davis SS, Wilson CG (1989) Drug delivery to the gastrointestinal tract. Gut 83-95, 229-235.

Humphrey MJ, Ringrose PS (1986). Peptides and related drugs: a review of their absorption, metabolism and excretion. Drug Met Rev. 17, 283.

Janes KA, Fresneau MP, Marazuela Al (2001) Chitosan Nanoparticles as delivery systems for doxorcubicin, J. Controlled Release. 73 (2-3)

255 – 267.
Keljo DJ,Hamilton JR. (1983) Quantitative determination of macromolecular transport rate across intestinal Peyer's patches Am J. Physiol. 244,G637 – G644.
Khoo SM,Edwards GA, Porter CJH, Charman WN (2001). A conscious dog model for assessing the absorption, enterocyte-based metabolism, and intestinal lymphatic transport of halofantrine. J. Pharm Sci 90: 1599-1607,
Kimura T (1984). Transmucosal absorption of small peptide drugs. Pharm Int, 5, 75.
Kimura T,Saishin I. (1985) 1818 – 1824. In: Encyclopedia of controlled drug delivery, Edith Marthiowitz. (Edn) John Wiley and Sons Inc. New York. 2 (1999), 739 – 740.
Kompella UB,Lee VHL (2001). Delivery systems for penetration enhancement of peptide and protein drugs: design considerations. Adv Drug Del Rev 46; 211-245.
Kopeck J, Kopekova P, Minko T, (2001) Water-soluble polymers in tumor targeted delivery, J Controlled Release. 74 (1-3) 147 – 158.129.
Krishnaiah YSR, Veer RV, Dinesh KB, (2001) Development of Colon Targeted Drug Delivery Systems for Mebendazole. J Controlled Release 77(1-2): 87-95.
Leferve ME, Joel DD (1977). Intestinal absorption of particulate matter, life sci 21, 1403 – 1403. In S. Sakuma et al, advanced drug delivery reviews 47 (2001) 21- 37.
Mitra S, Gaur U, Ghosh PC, Maitra An (2001). Tumor targeted delivery of encapsulated dextrian doxorubicin conjugate using chitosan nanopoarticles as carriers, J. Controlled Release. 74 (1-3). 317 – 323.
Muranishi S, Takada K, Hoshikawa H, Murakami M (1987) Enhanced absorption and lymphatic transport of macromolecules via the rectal route. In Delivery system for Peptide drugs. Davis SS et al (Eds). Plenum Press. New York 177-189.
Muranishi S, Yamamoto A (1994). Mechnism of absorption enhancement through the gastrointestinal epithelium. In: Drug absorption enhancement. Taylor and Francis (Eds) 67-100.
Okhamafe AO, Goosen MFA (1993). Control of membrane permeability in Microcapsules. In; Fundamentals of animal cell encapsulation and immobilization. Goosen Mattheus (Edn) CRC Press, Inc. USA.
Okhamafe AO,Goosen MFA (1999). Modulation of membrane permeability. In; Cell encapsulation technology and therapeutics. Goosen Mattheus (Edn) Birkhäuser Boston, USA. 53-62
Okhamafe AO, Amsden B, Chu W, Goosen MFA (1996) Modulation Of Protein release from chitosan-alginate microcapsules modified with the pH sensitive polymer-hydroxylpropyl methylcellulose acetate succinate (HPMCAS). J. Microencapsulation. 13, 497-508.
Pastorino F, Stuart D, Ponzoni M, Allen TM (2001). Targeted delivery of antisense oligonucleotides in cancer. J. Controlled Release. 14 (1-3) 69 – 75.
Rothman S, Tseng H. Goldfine I (2005). Oral gene therapy: a novel method for the manufacture and delivery of protein drugs. 7; (3) 549-557.
Schatzlein AG, Rutherford C, Corrihons F,Moore BD (2001) Phage derived peptides for targeting of doxorubicin Conjugates to solid tumours. J Controlled Release. 74 (1-3) 357 – 362.
Schwendeman SP, Cardamone M, Bradon MR, (1996). Stability of proteins and their delivery from biodegradable polymer microspheres. In: Microparticulate systems for the delivery of protein nd vaccines. Mracel Dekker.
Shinji S, Suzuki N, Kikuchi H, Hiwatori K, (1997). Oral peptide delivery using nanopaticles composed of novel graft copolymers having hydrophobic backbone and hydrophilic branches. Int J. Pharm . 149, 93-106.
Stevenson BR, Keon BH (1998). The tight junction: morphology to molecules. Annu Rev Cell Dev Biol 14, 89-109.
Stubbe B, Martis B, Moonter GV (2001) *In vitro* evaluation of azo containing polyscaccharide gels for colon delivery. J. Controlled Release. 75 (1-2): 103-114.
Wilson PJ, Basit AW(2005). Exploiting gastrointestinal bacteria to target drugs to the colon: An in vitro study using amylose coated tablets. Int J. Pharmaceutics 300(1-2) 89-94.
Yano H, Hirayama F, Kamada M (2002). Colon specific delivery of prednisolone-appended-cyclodestrin conjugate: Alleviation of systemic side effect after oral administration. J Controlled Release. 79(1-3): 103-112.
Yokohama S, Yamashita K, Toguchi H, Takeuchi J, Kitamon N. (1984). Absorption of thyrotropin – releasing hormone after oral administration of TRH tartrate monohydrate in rat, dog and Human. J. Pharm Dyn. 7; 101.