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# INSULIN ORAL DELIVERY MAY BE POSSIBLE

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## Abstract:-

In the present review, the challenges for the oral delivery of insulin via different approaches is being studied. In the present study the oral absorption of insulin in body through oral route using different parameters of our body as well as of dosage forms which can increase the oral absorption of insulin is studied. From different insulin preparations which are prevalent it is concluded that insulin can be given in the form of a microsphere with the help of a insoluble resin, and embedded in the enteric coated tablets so that it can show absorption in the small intestine to achieve its absorption.

**Keywords:** Insulin, oral route, insoluble resin, enteric coated tablets.

## Introduction

Insulin, a major protein hormone consisting of 51 amino acids, is secreted by the β-cells of the pancreas and plays a crucial role in controlling diabetes.[1] The incidence of diabetes is growing rapidly both in the united states and worldwide. For example it is estimated that more than 180 million people worldwide are afflicted with diabetes, and the prevalence is expected to be more than double by the year 2030. In the UNITED STATES, approximately 21 million people are estimated to suffer from diabetes, and it is a major cause of morbidity and mortality.[2]

Diabetes is a chronic condition caused by a relative or an absolute lack of insulin. It's hallmark clinical characteristic are symptomatic glucose intolerance resulting in hyperglycemia and alteration in lipid and protein metabolism. Over the long term these metabolic abnormality contributes to the development of complication such as retinopathy, nephropathy and neuropathy.[3]

Genetically, etiologically and clinically diabetes is a heterogeneous group of disorders. Nevertheless most cases of diabetes mellitus can be assigned to type-1 & type-2 diabetes. The term gestational diabetes mellitus is used to describe glucose intolerance that cannot be ascribed specific genetic defect in β-cell function or insulin action; disease of exocrine pancreas; endocrinopathies; drug or chemical-induced; infections; and other genetic syndromes.

Approximately 5-10% of the diagnosed diabetic population has type-1 diabetes, which usually results from autoimmune destruction of the pancreatic β-cells. At clinical presentation these patients have little or no pancreatic reserve, have a tendency to develop ketoacidosis, and require exogenous in saline to sustain life. The incidence of sauto immune-mediated type-1 diabetes peaks during childhood and adolescence, but can occur at any age.[4]Administration of therapeutic peptide drugs such as insulin via the oral route, especially the gastrointestinal tract, represents one of the greatest challenges in modern pharmaceutical technology .[5] Successful delivery is difficult to achieve because these substances are too large and hydrophilic to readily across the intestinal mucosa. In addition, extensive enzymatic degradation by proteases is unavoidable before they reach their site of absorption .[6] One study estimated that the prevalence of diabetes in person over 65 years of age increased 62% from 2003 to 2004.[7]

Type-3 designation refers to multiple other specific cause of an elevated blood glucose; pancreatectomy, pancreatitis, non-pancreatic disease, drug therapy etc.

Type-4 is gestational diabetes (GDM) defined as any abnormality in glucose level noted for the first time during pregnancy. Gestational diabetes is diagnosed in approximately 4% of all pregnancies in the united states. During pregnancy the placenta and placental hormones creates an insulin resist0ance that is most pronounced in last trimester.[8]

Hyperglycemia (fasting plasma glucose >7.0 mmol/l, or plasma glucose >11.1 mmol/l 2 hours after meal ) occur because of uncontrolled hepatic glucose output and reduced uptake of glucose by skeletal muscles with reduced glycogen synthesis. When the renal threshold for glucose reabsorption is exceeded, glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which, in turn, results in dehydration, thirst and increased drinking (polydipsia ).[9]

### Insulin

Insulin was 1st discovered by Banting and Best in the year 1921 [10]. Insulin is a polypeptide hormone that travel around the blood stream; insulin has molecular weight of (pork): 5777.66 [11], (beef): 5733.61 [11], Human (semi synthetic, biosynthetic): 5807.69 [11], consisting of two

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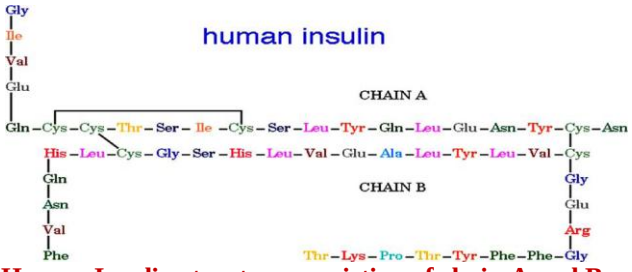
amino acid chains A & B . The A chain has 21 amino acids and B chain has 30 amino acids. The two chains are connected by two disulphide bridges, bonds formed between the sulphur atoms in the amino acid cystine. The A chain also has a third internal disulphide bridge. The disulphide bridges hold the molecule together. Although the amino acid sequence of insulin varies among species certain segments of the molecule are highly conserved, including the positions of the three disulphide bonds, both ends of the A chain and the C-terminal residues of the B chain .[12] The disulfide bridges and amino acid sequences are essential for insulin's biological activity . The helical structure of A12-18 is essential for biological activities of insulin. A8-10 is not much concerned with biological activities, but is much more important antigenically in binding to its antibodies.




**Human Insulin structure consisting of chain A and B**

**Mechanism Of Action/Effect**

Insulin acts on specific receptors located on the cell membrane of every cell, but their density depends on the cell type: liver and fat cells are very rich. The insulin receptor is a heterotetrameric glycoprotein consisting of 2 extracellular α and 2 transmembrane β subunits linked together by disulphide bonds. Binding of insulin to α subunits induce aggregation and internalization of the receptor along with the bound insulin molecules. This activates tyrosine kinase activity of the β sub units. In turn a cascade of phosphorylation and dephosphorylation reaction is set into motion resulting in stimulation or inhibition of enzymes involved in the rapid metabolic actions of insulin. Certain second messengers like phosphatidyl inositol triphosphate($PIP_3$ ) which are generated through activation of a specific $PI_3$-kinase also mediate the action of insulin on metabolic enzymes.[13]

Insulin is a polypeptide hormone that controls the storage and metabolism of carbohydrates, proteins, and fats. This activity occurs primarily in the liver, in muscle, and in adipose tissues after binding of the insulin molecules to receptor sites on cellular plasma membranes. Although the mechanisms of insulin's molecular actions in the cellular area are still being explored, it is known that cell membrane transport characteristics, cellular growth, enzyme activation and inhibition, and alterations in protein and fat metabolism are all influenced by insulin [14]. More specifically, insulin promotes uptake of carbohydrates, proteins, and fats in most tissues. Also, insulin influences carbohydrate, protein, and fat metabolism by stimulating protein and free fatty acid synthesis, and by inhibiting release of free fatty acid from adipose cells [14,15]. Insulin increases active glucose transport through muscle and adipose cellular membranes, and promotes conversion of intracellular glucose and free fatty acid to the appropriate storage forms (glycogen and triglyceride, respectively). Although the liver does not require active glucose transport, insulin increases hepatic glucose conversion to glycogen and suppresses hepatic glucose output.

Even though the actions of exogenous insulin are identical to those of endogenous insulin, the ability to negatively affect hepatic glucose output differs because a smaller quantity of exogenous insulin reaches the portal vein.[16]

**Challenges to Oral Insulin Delivery:**

Oral delivery of Insulin as a non-invasive therapy for Diabetes Mellitus is still a challenge to the drug delivery technology, since it is degraded due to the presence of enzymes in the acidic environment of stomach and also its absorption through the gastrointestinal mucosa is questionable. [17] Generally, peptides and proteins such as insulin cannot be administered via the oral route due to rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen, and poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity. [18,19] The oral bioavailability of most peptides and proteins therefore is less than 1%. The challenge here is to improve the bioavailability to anywhere between 30 – 50%. [20]

**Enzymatic Barrier:**

The harsh environment of the gastrointestinal tract (GIT) causes insulin to undergo degradation. This is because digestive processes are designed to breakdown proteins and peptides without any discrimination. Insulin therefore undergoes enzymatic degradation by pepsin and pancreaticproteolytic enzymes such as trypsin and α-chymotrypsin. Insulin can be presented for absorption only if the enzyme attack is either reduced or defeated.[21]

**Intestinal Transport of Insulin:**

Insulin has low permeability through the intestinal mucosa. There is no evidence of active transport for insulin. It has been found however that insulin delivery to the mid-jejunum protects insulin from gastric and pancreatic enzymes and release from the dosage form is enhanced by intestinal microflora. [22,23] Various strategies have been tried out to enhance the absorption of insulin in the intestinal mucosa and in some cases; they have proven successful in overcoming this barrier.

**Dosage form stability:**

The activity of proteins depends on the three-dimensional molecular structure. During dosage form development, proteins might be subject to physical and chemical degradation. Physical degradation involves modification of the native structure to a higher order structure while chemical degradation involving bond cleavage results in the formation of a new product. [22] If a protein needs to survive transit through the stomach and intestine, knowledge and assessment of stability parameters during formulation processing is of utmost importance.

**Approaches towards Oral Insulin Delivery Systems:**

Most peptides are not bioavailable from the GIT after oral administration. Therefore, successful oral insulin delivery involves overcoming the enzymatic and physical barriers

[23] and taking steps to conserve bioactivity during formulation processing. [24] In developing oral protein delivery systems with high bioavailability, various practical approaches might be most helpful.

1. Protecting insulin from enzymatic degradation by using antiproteolytic agents.
2. Promoting the gastrointestinal absorption of insulin through simultaneous use of a multitude of different penetration enhancers.
3. Chemical modification of insulin to improve its stability.
4. Bioadhesive delivery systems for enhancement of contact of the drug with the mucous membrane lining the gastrointestinal tract.

5. Carrier systems such as microspheres and nanoparticles which can improve the bioavailability of insulin.

**Enzyme Inhibitors:**

Insulin is degraded in the GIT by pepsin and other proteolytic enzymes. Enzyme inhibitors slow the rate of degradation of insulin which increases the amount of insulin available for absorption[19]. Administration of insulin via microspheres, together with the protease inhibitors like aprotinin, trypsin inhibitors, chymotrypsin inhibitors, Bowman –brik inhibitors could be found to be the most efficacious combination. The simultaneous release of these inhibitors and insulin in the intestine will prevent the proteolytic degradation and increases the bioavailability of insulin, In one such study gelatin microspheres containing trypsin inhibitors caused greater hypoglycaemic effect than microspheres without the same [25, 26]

**Limitations:** Formulations of insulin with protease inhibitors such as aprotinin have typically shown inconsistent effects; with in vitro and in vivo effects often being different. The use of enzyme inhibitors in long-term therapy however remains questionable because of possible absorption of unwanted proteins, disturbance of digestion of nutritive proteins and stimulation of protease secretion.[27]

**Penetration Enhancers:**

Penetration enhancers can increase the absorption of peptides and proteins in the gastrointestinal tract by their action on transcellular and paracellular pathways of absorption. Even if the intact molecule of insulin reaches the intestine, due to the large molecular size and relatively impermeability of the mucosal membrane it might not absorbed in sufficient concentration to
produce the required biological effect. One possible approach to overcome this drawback is to use penetration enhancers. **[**28] A number of absorption enhancers are available that causes these
tight junctions to open transiently allowing water-soluble proteins to pass. Absorption may be enhanced when the product is formulated with acceptable safe excipients. [29] These substances include bile salts, surfactants, trisodium citrates, chelating agents like EDTA [30] labrasol [31] Insulin transport across Caco-2 cells was shown to be dramatically increased by conjugation of insulin with TAT, a cell penetrating peptide (CPP). [31] Surfactants and fatty acids affect the transcellular pathway by altering membrane lipid organization and thus increase the absorption of drugs consumed orally. Bile salt micelles, EDTA and trisodium citrate have been reported to increase the absorption of insulin. Cyclodextrins have also been used to enhance the absorption of insulin from lower jejunal and upper ileal segments of rat small intestine.

**Limitations:** The drawbacks with penetration enhancers include lack of specificity, i.e., they allow all content of the intestinal tracts including toxins and pathogens the same access to the
systemic bloodstream [32] and risk to mucous membranes by surfactants and damage of cell membrane by chelators **[**33] Surfactants can cause lysis of mucous membrane and may thus
damage the lining of the gastrointestinal tract. Similarly, chelators such as EDTA cause depletion of $Ca^{2+}$ ions, which may in turn cause disruption of actin filaments and thus damage the cell membrane.

**Carrier Systems:**

The oral bioavailability of insulin can be enhanced by the use of novel carrier systems which deliver insulin to the target site of absorption. Liposomes, microspheres and nanoparticles have been developed for use as carrier systems for insulin.

**Liposomes:**

These are tiny spheres formed when phospholipids are combined with water. Encapsulating insulin in liposomes results in enhanced oral absorption of insulin. [34]
**Limitations:** The high doses of liposome-entrapped insulin required coupled with variability in glycemic response limits its use. [34] Other drawbacks include instability, leakage of entrapped drug, and low drug carrying capacity**.**

**Microspheres:**

Insulin can be encapsulated in a microcapsule or dispersed in a polymer matrix. Microspheres are prepared by emulsification using natural (gelatin or albumin) or synthetic polymers (polylactic or polyglycolic acid). [34,35] Insulin-loaded alginate microspheres complexed with cyclodextrins have an absorption enhancing effect leading to increase in bioavailability. [36,37]
In a recent study, Eudragit S100 microspheres on oral administration protected insulin from proteolytic degradation in the GIT and produced hypoglycemic effect. [38] Microspheres encapsulated with chitosan phthalate polymer protect the insulin from enzymatic degradation with an insulin-loading capacity of 62% and may be a potential carrier for oral insulin delivery. [39]

**Limitations:**The microspheres formed only show absorption in the small intestine for that we have to retain the microspheres in small intestine for maximum time which is not possible.

**Nanoparticles :**

Nanoparticles have been extensively studied as carriers for oral insulin delivery. [40] The nanoparticles protect insulin against in vitro enzymatic degradation. [41] Synthetic polymers used for nanoparticle formulation include polyalklylcyanoacrylate [42] polymethacrylic acid [43] polylactic-co-glycolic acids (PLGA) **[**44] Natural polymers used include chitosan, alginate, gelatin, albumin [45] and

lectin. [46] Chitosan has been proven to have good permeation enhancing abilities via the paracellular pathway. [47]

**Limitations**: The nanocapsules of insulin prepared using polyisobutyl cyanoacrylate as polymeric carrier showed initial low plasma concentration followed by higher plasma concentration after sometime, with no significant net enhancement of absorption. Hence, from carrier systems, insulin gets released slowly into intestinal lumen, with small amounts being absorbed.

**Chemical Modification:**
Modifying the chemical structure and thus increasing its stability is another approach to enhance bioavailability of insulin. An example of chemical modification is that of hexyl-insulin monoconjugate 2 (HIM-2) wherein a short chain polyethylene glycol (PEG) linked to an alkyl group is in turn linked to LYS-29 of the beta chain of insulin. Alteration of the physicochemical characteristics leads to enhanced stability and resistance to intestinal degradation of oral insulin [48]**,** Xia CQ et al recently demonstrated improved efficacy of orally administered insulin by conjugating insulin with transferrin through disulfide linkages. [49]

**Limitations:** Chemical modification does not always lead to improved oral absorption. For example, diacyl derivatives of insulin exhibited a higher proteolysis than native insulin in the
small intestine of rat under in vitro conditions.

**Bioadhesive Systems:**
Bioadhesive drug delivery systems anchor the drug to the gastrointestinal tract, and have been widely investigated to prepare sustained release preparations for oral consumption of drugs. The anchoring of the drug to the wall of the gastrointestinal tract increases the overall time available for drug absorption because the delivery system is not dependent on the gastrointestinal transit time for removal. Moreover, a drug administered through this method does not need to diffuse through the luminal contents or the mucus layer in order to reach mucosal epithelium lining the gastrointestinal tract. Because of intimate contact with the mucosa, a high drug concentration is
presented for absorption, and there is also the possibility of site-specific delivery if bioadhesion can be targeted to occur at a particular site in the gastrointestinal tract. Numerous mucoadhesive delivery systems like chitosan [50] sodium salicylate, and polyoxyethylene-9-lauryl ether [51] have been reported to improve the oral absorption of insulin. Carrier systems such as nanoparticles, microspheres and liposomes can also be used to improve the oral absorption of peptides and proteins.

**Limitations:** The bioadhesive systems may be affected by the mucous turnover of the gastrointestinal tract, which varies based on site of absorption. Moreover, directing a delivery
system to a particular site of adhesion in the gastrointestinal tract is yet to be achieved.

**Emulsions:**
Cho and Flynn [60] developed water-in-oil microemulsions in which the aqueous phase is insulin and oil phase is lecithin, non-esterified fatty acids and cholesterol in critical proportions. Invivo studies showed substantial reduction in blood glucose. These responded to changes in external environment suggesting potential application for oral insulin delivery. [52]

**Limitations:**The preparation of insulin emulsions is difficult and it may show first pass effect which is harmful for insulin absorption.

**Hydrogels:**
These are cross-linked networks of hydrophilic polymers, which are able to absorb large amounts of water and swell, while maintaining their three-dimensional structure .Complexation hydrogels are suitable candidates for oral delivery of proteins and peptides due to their abilities to respond to changes in pH in the GI tract and provide protection to the drugs from the harsh environment of the GI tract. [53]

**Limitations:** It get rapidly proteolysed, digested when it get swallowed and the major limitation is it is inactivated by liver and kidney.

**Previous Studies:-**

**1)Nanoparticles:-**
Biodegradable nanoparticles loaded with insulin–phospholipid complex were prepared by a novel reverse micelle–solvent evaporation method, in which soybean phosphatidylcholine (SPC) was employed to improve the liposolubility of insulin, and biodegradable polymers as carrier materials to control drug release. Intragastric administration of the 20 IU/kg nanoparticles reduced fasting plasma glucose levels to 57.4% within the first 8 h of administration and this continued for 12 h. PK/PD analysis indicated that 7.7% of oral bioavailability relative to subcutaneous injection was obtained.[54]

**2)Microspheres**:-
Micro spheres as oral delivery system for insulin: The feasibility of delivering insulin systemically by oral route using microspheres prepared with Eudragit L-100, sodium glycocholate and aprotinin was studied. Eudragit L-100 was used as carrier for micro spheres to give a site-specific release of insulin in the upper intestine; sodium glycocholate was used as penetration enhancer and aprotinin as a protease inhibitor.The in-vivo hypoglycemic effect study carried out using alloxan induced diabetic rats showed that the microspheres prepared with Eudragit L-100, 1% aprotinin and1% sodium glycocholate showed prolonged hypoglycemic effect for 4hours which was not even observed with subcutaneous bovine insulin injection .[54]

**3)Capsules:-**
Chitosan capsules for insulin delivery: Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides such as insulin.

Tozaki *et al*. developed colon-specific insulin delivery with chitosan capsules. *In vitro* drug release experiments from chitosan capsules containing 5(6)-carboxyfluorescein(CF) were carried out by rotating basket method with slight modifications. The intestinal absorption of insulin was evaluated by measuring the plasma insulin levels and its hypoglycemic effects after oral administration of the chitosan capsules containing insulin and additives. Little release of CF (5(6)-carboxyfluorescein) from the capsules was observed in an artificial gastric juice (pH= 1), or in an artificial intestinal juice (pH=7). However, the release of CF was markedly increased in the presence of rat caecal contents [55].

**4)Emulsion:-**

Water-in-oil type of emulsion: Human insulin was incorporated into a w/o emulsion by high-pressure homogenization.A fine stable dispersion of the aqueous phase was achieved and the emulsion was able to protect insulin against gastric degradation *in vitro* without further encapsulation.

5**)liposome:-**

Biocarrier insulin: In this system insulin was first entrapped in Liposome's. The preparation was developed using ghost erythrocytes as bio carriers for intraduodenal administration of insulin because proteolytic enzymes in duodenal region break erythrocytes. From such a system insulin was absorbed and showed its glucose lowering effects[56,57]

**6) Nanocapsule:-**

Nanocapsules for insulin delivery: Damge *et al.* [58] developed nanocapsules using biodegradable Polymer Poly (isobutyl / Cyanoacrylate) [mean size 220nm]. When administered orally by force-feeding to diabetic rats, insulin nanocapsules (12.5, 25, and 50 U/kg) decreased fasted glycemia 50-60% by day 2. This effect was maintained for 6 or 20 days with 12.5 or 50 U/kg, respectively. Only the dose of 100 U/kg decreased fed glycemia by 25% in diabetic rats. In normal rats, hyperglycemia induced by an oral glucose load was reduced by 50% with the same dose of oral insulin nanocapsules [58]. Sharma *et al.* [59] have loaded insulin in ceramic nanoparticles prepared from hydroxyapatite and encapsulated these particles in sodium alginate. *In vitro* release profile of insulin was carried out in simulated gastric (pH 1.2) and intestinal fluids (pH7.4). 100 mg of insulin loaded nano particle was introduced into 10 ml of respective medium. 0.1 ml of sample was withdrawn at various time intervals and evaluated for insulin using Lowry's method for protein estimation. Present investigation show that insulin loaded HA (hydroxyapatite) nanoparticle encapsulated in sodium alginate can effectively release almost 100 % of the drug in SIF [Simulated Intestinal Fluid] during a period of 2 hours. However, during the same period only 24-28 % of insulin was released in SGF [Simulated Gastric Fluid].[60]

## Conclusion:-

However, various dosage forms have been tried for delivering insulin but till now oral administration of insulin is not possible. From this study we can concluded that microspheres of insulin can be prepared and embedded in polymer matrix. After that these microspheres should be formulated in the form of enteric coated compressed tablets so that tablet should not be degraded by the gastric fluids and insulin should be released in the intestine by the matrix tablet in the form of microsphere. By this assumption we can say that Dream may come true of oral absorption of insulin.[61]

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