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Review Article

NOVEL FORMULATION STRATEGIES FOR INSULIN DELIVERY

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ABSTRACT

Insulin is a hormone produced by pancreas that allows your body to utilize sugar (glucose) from carbohydrates in the food or to store glucose for future use. Successful Oral insulin delivery involves overcoming the enzymatic and physical barriers. Several companies across the globe are developing oral insulin based on different technology platforms. Insulin has been encapsulated in nanoparticles by muco-adhesive polymers such as chitosan, poly (lactic-co-glycolic acid) (PLGA) and alginate which prevent enzymatic degradation, and allow absorption across the epithelial layer in Peyer's patches.

Keywords: Novel Formulation Strategies, Insulin, hyperglycemia, Nanosphere-based oral insulin delivery

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Insulin[1]

Insulin is a peptide hormone that allows your body to consume glucose. Insulin helps keep blood sugar level from getting too high (hyperglycemia) or too low (hypoglycemia). The cells in your body need sugar for energy. However, sugar cannot go into most of your cells directly. After you eat food and your blood sugar level rises, cells in your pancreas (known as beta cells) are signaled to release insulin into your bloodstream. Insulin then attaches to and signals cells to absorb sugar from the bloodstream. Insulin is often described as a "key," which unlocks the cell to allow sugar to enter the cell and be used for energy.

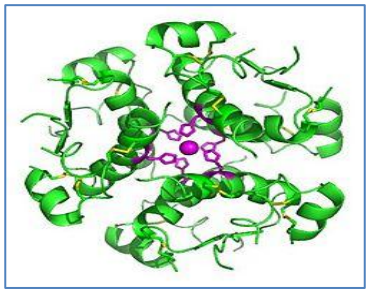
If you have more sugar in your body than it needs, insulin helps store the sugar in your liver and releases it when your blood sugar level is low or if you need more sugar, such as in between meals or during physical activity. Therefore, insulin helps balance out blood sugar levels and keeps them in a normal range. As blood sugar levels rise, the pancreas secretes more insulin.

If your body does not produce enough insulin or your cells are resistant to the effects of insulin, you may develop hyperglycemia (high blood sugar), which can cause long-term complications if the blood sugar levels stay elevated for long periods of time.

Structure of Insulin

High-resolution model of six insulin molecules assembled in a hexamer, highlighting the threefold symmetry, the zinc ion holding it together (pink sphere),

and the histidine residues (pink sticks) involved in zinc binding. Inactive insulin is stored in the body as a hexamer, while the active form is the monomer



Insulin Treatment for Diabetes[2]

People with type 1 diabetes cannot make insulin because the beta cells in their pancreas are damaged or destroyed. Therefore, these people will need insulin injections to allow their body to process glucose and avoid complications from hyperglycemia.

People with type 2 diabetes do not respond well or are resistant to insulin. They may need insulin shots to help them better process sugar and to prevent long-term complications from this disease. Persons with type 2 diabetes may first be treated with oral medications, along with diet and exercise. Since type 2 diabetes is a progressive condition, the longer someone has it, the more likely they will require insulin to maintain blood sugar levels.

Various types of insulin are used to treat diabetes and include[3, 4]

- **Rapid-acting insulin**: It starts working approximately 15 minutes after injection and peaks at approximately 1 hour but continues to work for two to four hours. This is usually taken before a meal and in addition to a long-acting insulin.
- **Short-acting insulin:** It starts working approximately 30 minutes after injection and peaks at approximately 2 to 3 hours but will continue to work for three to six hours. It is usually given before a meal and in addition to long-acting insulin.
- **Intermediate-acting insulin:** It starts working approximately 2 to 4 hours after injection and peaks approximately 4 to 12 hours later and continues to work for 12-18 hours. It is usually taken twice a day and in addition to rapid- or short-acting insulin.
- **Long-acting insulin:** It starts working after several hours after injection and works for approximately 24 hours. If necessary, it is often used in combination with rapid- or short-acting insulin.

Insulin can be given by a syringe, injection pen, or an insulin pump that delivers a continuous flow of insulin.

Recent advances in insulin

Researchers develop insulin substitute for treating diabetes orally.[5]

Researchers from Australia's Curtin University have found an insulin substitute to treat diabetes orally. A 3D molecular map of insulin was used to identify the key features that are needed for insulin's activity. A lead drug molecule that fitted the map and mimicked insulin in specific biological assays and animal models was found. Unlike insulin, the small drug molecule isn't broken down in the stomach so can be taken orally as a tablet. The insulin substitute could potentially replace the need for injections for sufferers of both type 1 and type 2 diabetes, because type 1 diabetics depend on insulin for their survival, the researchers plan to initially target type 2 diabetics prior to them developing full insulin dependency.

Advantages[6]

- Drug Delivery: it is a small, non-protein drug molecule for oral administration
- Convenience: it is taken in tablet form
- Safety: it circumvents the need for sterile needles
- Cost: it is cheaper to synthesize
- Ease of storage: it has a longer shelf life and does not require stringent, sterile conditions.

Oral Insulin: The Rationale for This Approach and Current Developments. [7]

This review addresses the physiological advantages that may be derived from oral insulin administration and examines the various technologies at the forefront of oral insulin delivery.

Current developments:

- Several companies across the globe are developing oral insulin based on different technology platforms. Generic to all these efforts is a system that provides protection of the insulin while in transit in the gastrointestinal tract and which can take the form of physical encapsulation or the creation of a modified insulin resistant to degradation.
- **Tablet designed to release in duodenum: -** In addition, some companies use an added component designed to enhance the transepithelial transport of the drug. Merrion Pharmaceuticals, a company based in Ireland, uses its GIPET® platform to deliver macromolecules and polypeptides, including insulin. The GIPET system is based on promoting drug absorption through the use of matrices consisting of medium-chain fatty acids and formulated as solid dosage forms. The matrices enjoy food additive status (Generally Recognized as Safe, GRAS) and are normal dietary components with long records of safe use. Insulin and the other ingredients are prepared as a physical mix and are formulated into a tablet designed to be released in the duodenum.
- **Recombinant human insulin to withstand enzymatic degradation in the stomach and facilitate its absorption: -** Biocon Limited, a biotechnology company located in India, is continuing the work of Nobex Corporation, developing oral insulin based on a modified form of insulin that possesses specific physicochemical characteristics that allow it to withstand enzymatic degradation in the stomach and facilitate its absorption. The conjugated insulin product (IN-105)

is recombinant human insulin conjugated covalently with a monodisperse, short-chain methoxy polyethylene glycol derivative that is crystallized and lyophilized into the dry active pharmaceutical ingredient after purification. An ascending dose study in type 2 diabetes has been presented.

- Diasome, a U.S.-based company, is employing a hepatic-directed vesicle (HDV) for insulin delivery. A HDV consists of liposomes (≤150 nm diameter) encapsulating the insulin, which also contain a hepatocyte-targeting molecule in their lipid bilayer. The targeting molecule directs the delivery of the encapsulated insulin to the liver cells and therefore relatively minute amounts of insulin are required for effect.
- Diabetology Limited, a U.K.-based company, is using its Axcess™ delivery technology system, which is based on a capsule containing a simple mixture of the drug, an absorption enhancer, and a solubilizer that allows absorption of the drug in the small intestine. The excipients used in the formulation are inert (GRAS) and are normal dietary components with long records of safe use. The company recently presented the results of a phase II, 10-day repeat-dose study of oral insulin in 16 patients with type 2 diabetes.
- Emisphere Technologies, a U.S.-based company, uses a system of carriers—designed low molecular weight chemical entities—that interact weakly and non-covalently with a protein drug. By altering the protein conformation and increasing its lipophilicity the carriers are able to enhance the transport of the protein across the gastrointestinal epithelium and into the bloodstream.
- Oramed platform technology is based on components aimed at providing protection of the protein during passage through the gastrointestinal tract in combination with an absorption enhancer. Oramed's protectants and absorption enhancers consist of known pharmacopeia adjuvant with a long safety track record. Oramed has completed phase I trials in healthy volunteers; results have shown that oral insulin delivered with their system is safe, well tolerated, and consistently leads to a desired reduction in glucose and C-peptide.[8]
- Of late, two other routes of insulin delivery systems—buccal and inhaled—are being advanced. Generex Biotechnology developed an oral–buccal insulin formulation whereby insulin is delivered directly into the mouth via a metered dose spray (RapidMist device, approved for use in Ecuador[9]). The insulin is not absorbed through the portal system but rather is systemic insulin. Generex's insulin has been approved and is available for clinical use in a few countries.
  MannKind cooperation has recently assumed the worldwide development and commercialization rights of Afrezzza (Human) inhaltion powder from Sanofi.
- Coromed is a privately funded biotechnology company. Their flagship product is called Alveair, and offers a 'needleless' alternative for insulin users. The device delivers a regulated blast of insulin, and Coromed[10] champion the fact that it has lower side-effect levels than injectable insulin.
- Nektar formed from Inhale Therapeutic Systems Inc, is another company that is promising oral insulin. It has collaboration with pharmaceutical corporation Pfizer and Sanofi-Aventis. The Nektar Pulmonary delivery system is one facet they have developed. Exubera, fast-acting dry powder insulin designed for inhalation has been developed and is being reviewed.[11]

Recent advances in oral insulin.[12]

- Protecting insulin from enzymatic degradation by using antiproteolytic agents.
- Promoting the gastrointestinal absorption of insulin through simultaneous use of a multitude of different penetration enhancers.
- Chemical modification of insulin to improve its stability.
- Bioadhesive delivery systems for enhancement of contact of the drug with the mucous membrane lining the gastrointestinal tract.
- Carrier systems such micro spheres and nanoparticles which can improve the bioavailability-of insulin.
- **Peptidase Inhibitors: -** Peptidase or protease inhibitors promote oral absorption of therapeutic peptides and proteins by reducing their proteolytic breakdown by enzymes in the gastrointestinal tract. Administration of insulin via microspheres together with the protease inhibitor aprotinin has been found to be the most efficacious combination involving protease inhibitors.
- **Chemical Modification:-** Modifying the chemical structure of a peptide or protein is another approach to enhance its bioavailability by increasing its stability in the face of possible enzymatic degradation and/or its membrane permeation. A diacyl derivative of insulin has been seen to maintain its biological activity and also have increased absorption from the intestine.

Oral insulin spray formulation and delivery system: [13]

- The system allows a liquid oral spray insulin formulation to be delivered into the mouth (administered to the buccal mucosa using a proprietary delivery system) via an aerosolized spray.
- The active pharmaceutical ingredient is recombinant human insulin; however, the formulation behaves in a fashion more similar to the synthetic fast-acting insulin analogues. One spray delivers approximately 10 U of insulin to achieve absorption of approximately 1 U of insulin systemically.
- The technology utilizes the formation of microfine micelles made from a combination of absorption enhancers that encapsulate and protect the insulin molecules for safe and effective delivery. The delivery device introduces a fine-particle aerosol at high velocity (100 mph; 160 km/h) into the

oropharyngeal cavity for local transmucosal absorption.

- The manually actuated mechanism incorporated into the device introduces the dose and standardized volume of the insulin formulation in relation to the patient's needs. Absorption is limited to the mouth with no entry of the product into the lungs.
- Although a thin membrane in the buccal mucosa composed of many superficial blood vessels guards the large surface area in direct contact with the circulation, the impelled micelles transverse the buccal mucosa superficial layers, and with the aid of the absorption enhancers, insulin molecules get rapidly absorbed into the bloodstream and appear in the circulation within 5–10 min after administration.

Cell penetrating peptide and silica-based nanoporous composites:[14]

- ❖ Silica-based, mucoadhesive oral insulin formulation with encapsulated-insulin/cell penetrating peptide (CPP) to overcome both enzyme and mucosal barriers.
- ❖ The CPPinsulin conjugates could facilitate cellular uptake of insulin while keeping insulin's biologic functions intact. The low molecular weight protamine (LMWP) behaves like a CPP peptide.
- ❖ The mucoadhesive properties of the produced silica-chitosan composites can be controlled by varying both the pH and composition; the composite consisting of chitosan (25 wt-%) and silica (75 wt-%) exhibited the greatest mucoadhesion at gastric pH.
- ❖ Furthermore, drug release from the composite network could also be regulated by altering the chitosan content. Overall, the universal applicability of these technologies can lead to development of a generic platform for oral delivery of many other bioactive compounds, especially for peptide or protein drugs which inevitably encounter the poor bioavailability issues.

Various approaches used in the development of oral insulin, and some of the recent data related to novel oral insulin preparation.[15]

- Insulin has been encapsulated in nanoparticles[16] by muco-adhesive polymers such as chitosan, poly (lactic-co-glycolic acid) (PLGA),[17] and alginate[18,19,20] which prevent enzymatic degradation, and allow absorption across the epithelial layer in Peyer's patches.[21,22]
- Insulin has also been co-administered with protease inhibitors such as bacitracin, sodium glycocholate and camostat mesilate which improve the absorption rate.[23]
- Insulin can also be modified by PEGylation, or by adding polyethylene glycol, which changes the pharmacokinetics, prevents enzymatic degradation, and increases absorption.[24]
- An insulin analogue, **IN-105**, delivered orally in form of tablets has been modified by linking a single shortchain amphiphilic oligomer, through a covalent non-hydrolysable amide bond, to the free amino acid group on the Lys-b29 residue of recombinant human insulin. This improves its solubility, stability and systemic absorption. Solubility is increased by PEGylation, while stability may be due to stearic hindrance. IN-105 is rapid acting oral insulin which can potentially find place in control of post prandial hyperglycemia. It is presently being studied in a phase 3 trial in type 2 diabetes, as well as a phase 1 trial in type 1 diabetes.[25]
- Another novel delivery mechanism is hepatic directed vesicles (HDV-I) loaded with insulin, which are <150 nm diameter liposomes that contain insulin attached a specific proprietary hepatocyte-targeting molecule (HTM). HDV-1 is stable at low pH, in blood, is able to avoid enzymatic degradation, and has high biopotency, exhibited by its low dose of 5 U.[26]
- Oral insulin in development for treatment of type 1 and type 2 diabetes is ORMD-0801. Capsules of ORMD-0801 have 8 mg insulin each.[27]

Nanospheres based oral insulin delivery: (Zinc insulin)[28]

- Zinc insulin is successfully encapsulated in various polyester and polyanhydride nanosphere formulations using Phase Inversion Nanoencapsulation (PIN). The encapsulated insulin maintains its biological activity and is released from the nanospheres over a span of approximately 6 h. A specific formulation, 1.6% zinc insulin in poly (lactide-co-glycolide) (PLGA) with fumaric anhydride oligimer and iron oxide additives has been shown to be active orally.
- This formulation is shown to have 11.4% of the efficacy of intraperitoneally delivered zinc insulin and is able to control plasma glucose levels when faced with a simultaneously administered glucose challenge. A number of properties of this formulation, including size, release kinetics, bioadhesiveness and ability to traverse the gastrointestinal epithelium, are likely to contribute to its oral efficacy.

Polylactide used to microcapsulate the insulin to avoid degradation by the enzymes in the GI.[29]

- Polylactide was prepared in order to microcapsulate the insulin to avoid the enzymes in the GI. Polylactide microcapsulated insulin (PLA-MCI) when spherical in shape (diameter 1.5-2.0 micron) the entrapment efficiency of insulin was 90% and the loading rate was 10% (W/W).
- PLA microcapsules are capable of protecting insulin from degradation by the proteolytic enzymes in the GI and of alleviating hyperglycemia for a prolonged period of time in diabetic rats. It may therefore be considered as a new carrier for oral insulin.

Oil-based formulations for oral delivery of Insulin:

- Insulin was solubilized in the form of anhydrous reverse micelles. Process involved micellar dissolution of insulin followed by freeze drying, then reconstitution of lyophilized product with an oil phase. These formulations were stable at room temperature for up to 12 months. No significant changes in the appearance were observed and no degradation products of insulin were detected during the course of the stability study.
- It was found that the efficacy of insulin oil solution (in diabetic/non diabetic rats) was dose dependent and insulin oil solution had the same efficacy as insulin emulsion with the same formulation composition. If ethylene-diaminetetraacetic acid (EDTA) was pre-delivered 40 min before the delivery of insulin oil solution, the hypoglycaemic effect of insulin oil solution was greatly enhanced, with an AUC (% glucose reduced) value increase from 28.5 ± 14.7 to 167.1 ± 72.3.
- The improvement of oral absorption induced by pre-delivery of EDTA might be attributed to enzyme inhibition, reduced gut mobility and the opening of paracellular routes.[30]

Zonula Occludens Toxin (Zot)[31]

- Aim of the study was to establish whether the permeabilizing effect of the toxin leads to increased intestinal absorption of macromolecules normally not absorbed when administered orally (insulin).
- To evaluate the transepithelial transport of insulin 10^{-11}M alone or in the presence of 1.1×10^{-10}M Zot, *in vitro* and *in vivo* experiments were performed on rabbit intestine. To evaluate the bioactivity of insulin after enteral co-administration with Zot, acute type 1 diabetic male BB/Wor rats were orally treated with insulin, with or without Zot, and the blood glucose levels of the rats were serially measured. Zot reversibly increases rabbit intestinal permeability to insulin by 72% (p=0.034) and immunoglobulins by 52% (p=0.04), *in vitro*. When tested *in vivo*, Zot induced a 10-fold increase of insulin absorption in both the rabbit jejunum and ileum, whereas no substantial changes were detected in the colon.
- In diabetic rats, bioavailability of oral insulin co-administered with Zot was sufficient to lower serum glucose concentrations to levels comparable to those obtained after parenteral injection of the hormone. The survival time of diabetic animals chronically treated with oral insulin+Zot was comparable to that observed in parenterally treated rats. This study offers a useful strategy for the oral delivery of drugs and proteins normally not absorbed through the gut.

1. Depomed has no patents granted in India; only 1 application is waiting for Examination (Acetaminophen). They have 2 products for Type 2 diabetes (Metformin HCl & Sitagliptin Metformin HCl).
2. EDTA chealates Mg, Ca invariably it acts as an inhibitor to the protein digesting enzymes, thus facilitating improvement of oral absorption [EDTA might be attributed to enzyme inhibition, reduced gut mobility and the opening of paracellular routes].
3. Solubilisation into the oil phase can avoid degradation of protein and the noncovalent coating of insulin molecules with lipophilic surfactant makes it possible to enhance permeation through the intestinal mucosa without introducing a new chemical entity.
4. Trypsin is produced in the pancreas, serving a vital role in digestion by breaking down proteins into smaller molecules the body can use. It is transported from the pancreas to the small intestine where its function is optimized by the organ's slightly alkaline environment.

   Trypsin has an optimal operating pH of about **7-8** (and reversibly inactivated at pH 4) and optimal operating temperature of about **37°C**.[32] The activity of trypsins is not affected by the inhibitor tosyl phenylalanyl chloromethyl ketone, TPCK, which deactivates chymotrypsin.

   Inhibitiors to prevent active trypsin are BPTI (bovine pancreatic trypsin inhibitor, or BPTI), SPINK1 (Pancreatic secretory trypsin inhibitor (PSTI) also known as serine protease inhibitor Kazal-type 1 (SPINK1) or tumor-associated trypsin inhibitor (TATI)) and α1-antitrypsin (Alpha-1 Antitrypsin (A1AT)). Use of such inhibitors can be highly damaging.
5. Trypsin is built with an extra piece of protein chain, this longer form of trypsin, called trypsinogen, is inactive and cannot cut protein chains. Then, when it enters the intestine, the enzyme **enteropeptidase** makes one cut in the trypsin chain, clipping off the little tail. This allows the new end of the chain to tuck into the folded protein and stabilize the active form of the enzyme. As extra insurance, the pancreas also makes a small protein, trypsin inhibitor that binds to any traces of active trypsin that might be present before it is secreted into the intestine. It binds to the active site of trypsin, blocking its action but not itself being cut into tiny pieces.
6. Inhibitors bind strongly to trypsin, blocking its active site and instantly forming an irreversible compound and halting digestion of certain proteins. However, if trypsin inhibitors (specifically KTI) are present, the majority of trypsin in the cycle of digestion is inactivated and ingested proteins remain whole. Effects of this occurrence include gastric distress and pancreatic hyperplasia (proliferation of cells) or hypertrophy (enlargement of cells).
7. In another separate experiment Influence of soybean-supplemented diet (naturally occurring purified trypsin inhibitors from soybean) on rat Pancreas and liver was conducted. This study demonstrated inhibition of growth in animals fed on trypsin inhibitor. The results of these experiments

confirm that the oral ingestion of raw soybean or purified trypsin inhibitors from varied sources is associated with enlargement of the pancreas in the rat (20% to 40% larger than normal) with an increased capacity to secrete pancreatic digestive enzymes. The liver was uninfluenced by the raw soybean diet.[33]

Metal binding in Insulin [34]

- Zinc plays an important role in insulin hexamerisation, which is closely related to some of the processes in insulin biosynthesis and storage.
- Zinc binds to insulin so that insulin is adequately stored in the pancreas and released when glucose enters the blood stream.[35]
- Zinc plays a clear role in the synthesis, storage and secretion of insulin as well as conformational integrity of insulin in the hexameric form.[36]

Zinc Insulin formulations.

Human insulins currently on the market include rapid-acting (*Regular*), intermediate acting (*NPH* and *Lente*) and long-acting (*Ultralente*) formulations. The former is a clear, colourless, aqueous solution buffered at neutral pH (7-7.8). Meta-cresol is added as preservative, glycerol as tonic stabiliser, as well as zinc chloride. Hexamers, made stable by zinc ions.

Absorption of *NPH* is delayed by protamine, a protein extracted from the nucleus of fish sperm, where its role is to stabilise DNA. The commercial form is *Isophane-NPH* insulin, a white suspension of orthorhombic crystals containing 0.9 molecules of protamine and two atoms of zinc per hexamer. In the crystal, protamine regulates interactions between dimers and hexamers. The vehicle is water buffered at pH 6.9-7.5. Phenol or metacresol are added as preservatives. Insulin crystals are insoluble in water and tend to precipitate to the bottom of the vial, which has to be tipped various times to resuspend them before use. *NPH* is longer-acting: in fact its blood absorption begins 1.5 h after subcutaneous ***injection***; it has a peak plasma concentration at 4 to 12 h and disappears within 24 h.

Effects if Proteolysis is hampered.[37]

- Acidity is created through the digestion of protein. Therefore a protease deficiency results in an alkaline excess in the blood. This alkaline environment can cause anxiety and insomnia.
- In addition, since protein is required to carry protein-bound calcium in the blood, a protease deficiency lays the foundation for arthritis, osteoporosis and other calcium-deficient diseases.
- Because protein is converted to glucose upon demand, inadequate protein digestion leads to hypoglycemia, resulting in moodiness, mood swings and irritability.
- Protease also has an ability to digest unwanted debris in the blood including certain bacteria and viruses. Therefore, protease deficient people are immune compromised, making them susceptible to bacterial, viral and yeast infections and a general decrease in immunity.

Oral Liposomes

Issues Related to Oral Liposomes

The problems associated with administering liposomes orally are as follows:

1. Stability and Consistency:

Liposomal formulations are vesicular systems which are suspended in physiological media. However, liposomal suspensions are highly unstable over longer duration of time (<3 weeks).

Physical stability always poses a big issue in developing liposomal formulations for oral delivery.

Remedy:

a. Lyophilization of Liposomal suspension overcomes the physical instability of the system. The lyophilized liposomes are needed to be reconstituted with sterile water for oral delivery at the patient's end.

b. PROLIPOSOMES (Discussed in detail in ANNEXURE-I)

2. GIT Physiological Barriers:

a. Gastric pH:

Liposomal formulations are susceptible to hydrolysis due to acidic pH of stomach. Lipids are more prone to acidic hydrolysis and hence the morphology of the liposomes gets distorted which then causes pre burst release of entrapped molecules. The un-buffered pH of the stomach ranges from 1.5 to 2.5 thus causing chemical instability in the liposome membrane surface.

Remedy:

a) Developing liposomes with acid stable lipids.[38]

b) Microencapsulation of the prepared liposomes with polymers which are acid stable[39] (Eg. Polyacrylates, polycarbophils, carbopol 934 etc.)

b. Gastric and Intestinal Enzymes:

Most of the lipids used in the preparation of Liposomes are esters of higher fatty acids including phospholipids viz. Phosphatidylcholines and phosphatidylethanolamines (soyalecithins-synthetic and non-synthetic sources). Gastric pepsins and intestinal trypsins and chymotrypsins are hydrolases which break lipid esters and dysfunctionalize liposomes into distorted structures.

Lipases are prime hydrolases of lipids which are being secreted into small intestine. Lipases also cause hydrolytic degradation of liposomes.

c. Bile acids :

Bile acids are biosurfactants secreted from Gall bladder which are responsible for emulsifying lipophilic moieties reaching small intestine. Uncoated liposomes which are lipophilic in their surface characteristics are easily interacted by bile acids thereby causing

destabilization of vesicular structure of liposomes and premature expulsion of entrapped molecules. [40,41] Hence, these released molecules are no longer protected and are thus exposed to luminal intestinal enzymes (hydrolases) causing their further degradation.

Remedy:

1. Incorporation of exogenous Bile salts in the developed liposomes (eg. Sod taurocholate, Sod glycocholate and Sod cholate)

Bile salts used in the liposomes cater many significant advantages which are mentioned as under and then detailed in the **ANNEXURE (ANX-I)**

a. Bile salts in the liposomes inhibit bile acid related destabilization of uncoated liposomes.

b. Bile salts inhibits intestinal enzymes and thus enhances both liposomal and entrapped molecules' stability,

c. Bile salts acts as permeation enhancer for the liposomes and thus enhances transfection efficiency of the entrapped molecules throughout GIT into the blood stream.

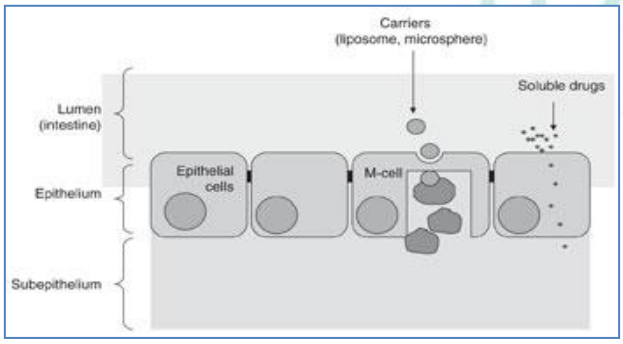
2. **Coating of liposomes with hydrophilic polymers:**

Hydrophilic polymeric coating of liposomes decreases its proneness for interaction with bile acids in small intestine.

A smart choice of polymers such as polyacrylates which are hydrophilic enteric polymers prevents both bile acid induced destabilization by providing hydrophilicity to the liposomal surface and further gives gastric pH protection to the surface of liposomes (being enteric releasing polymer).

ANNEXURE-I

1. **Intracellular transfection of liposomes (permeation) through enterocytes.**



Pathways of drug absorption across the intestinal epithelium[42]

2. **Use of Liposomes To Aid Intestinal Absorption of Entrapped Insulin In Normal And Diabetic Dogs:[43]**

The effectiveness of liposomes in aiding intestinal absorption of entrapped insulin was studied in normal and diabetic dogs. lntraduodenal administration of free insulin (490 and 1630 U) or free insulin (88 U) plus empty liposomes to normal conscious dogs produced no change in plasma immunoreactive insulin or glucose. Administration of 40-80 U insulin entrapped in liposomes composed of either phosphatidylcholine, distearoylphosphatidylcholine, or dipalmitoyl-phosphatidylcholine with cholesterol and dicetyl-phosphate (in the ratio 10:2:1 by weight) to normal dogs produced substantial rises in peripheral plasma immunoreactive insulin after 45-60 min. However, the magnitude of these rises was neither reproducible nor dose-dependent. No fall in plasma glucose was observed, lntraduodenal administration of 50-100 U insulin entrapped in liposomes to diabetic dogs also produced rises in plasma immunoreactive insulin levels after 45-60 min but again these rises were not dose-related. However, unlike the results in normal dogs, a small fall in plasma glucose followed the plasma immunoreactive insulin rise in diabetic dogs. This glucose fall was not dose-dependent nor was it related to the magnitude of the rise in plasma immunoreactive insulin.

3. **Oral delivery of insulin by using surface coating liposomes: Improvement in stability of insulin in GI tract.**

The potency of surface coated liposomes with some materials was investigated for oral delivery of peptide drugs. *In vitro* release of insulin, a model peptide, from liposomes in the bile salts solution was markedly reduced by coating the surface with the sugar chain portion of mucin (Mucin-Lip) or polyethylene glycol (PEG-Lip). Encapsulation of insulin into Mucin-Lip and PEG-Lip completely suppressed the degradation of insulin in the intestinal fluid, whereas uncoated liposomes suppressed it only partially. These results demonstrated that surface coating liposomes with PEG or mucin gained resistance against digestion by bile salts and increased the stability in the GI tract. Administration of insulin encapsulated in positively charged liposome caused the rapid decrease in the plasma glucose level which recovered to the control level within 3 h. In contrast, PEG-Lip and Mucin-Lip caused a gradual decrease in the glucose level after administration. The hypoglycemic effect by PEG-Lip lasted for much longer duration than that of uncoated liposomes. The slow release of insulin from the surface coating liposomes achieved the longer duration of oral hypoglycemic activity.[44]

4. **Enhanced oral absorption of insulin-loaded liposomes containing bile salts: A mechanistic study:**

A bile salt-reinforced liposome (BS-liposomes) system composed of soybean phosphatidylcholine and three types of bile salts (sodium glycocholate, sodium taurocholate and sodium deoxycholate) was developed for the oral delivery of insulin. The prepared BS-liposomes showed good protection of insulin against enzymatic degradation by pepsin, trypsin and chymotrypsin in vitro and a sustained hypoglycemic effect for 24 h with oral bioavailability of 10.2% in diabetic rats. Some studies in the field of oral vaccination[45] revealed that incorporation of bile salt in liposomal formulation could stabilize the membrane against the detrimental effects of bile acids in the

gastrointestinal tract (GIT)[46]. Besides, the presence of bile salts possibly enhances the internalization of BS-liposomes due to their absorption-enhancing effect.

5. Hypoglycemic activity and oral bioavailability of insulin-loaded liposomes containing bile salts in rats: The effect of cholate type, particle size and administered dose:[47]

The addition of bile salts to the lipid bilayers of the liposomes was believed to make the vehicles resistant to the detrimental effects of physiological bile salts in the GI tract and thus protect entrapped molecules from enzymatic degradation. Furthermore, bile salts embedded in the lipid bilayers of liposomes may perform membrane-destabilizing effect upon contact with the intestinal epithelia and facilitate internalization of the particles. A pro-liposome formulation of bilosomes (bile salt containing liposomes) has shown preliminary enhancing effect on oral bioavailability of a model peptide drug salmon calcitonin.[48,49] Oral administration of these liposomes elicited mild and prolonged hypoglycemic effect in parallel with increase in blood insulin levels for a duration of over 20 h, peaking at about 8–12 h. Mechanistic study supports absorption of intact liposomes that encapsulate (Recombinant) rhINS rather than free rhINS. The hypoglycemic effect and oral bioavailability of the liposomes containing either bile salts or cholesterol were in the order of SGC > STC > CH > SDC, which is attributable to the better protective effect on encapsulated rhINS against enzymatic degradation. The highest oral bioavailability of 8.5% and 11.0% can be achieved by SGC-liposomes in non-diabetic and diabetic rats, respectively.

6. Liposomes containing Sodium glycocholate (SGC) as potential oral insulin delivery system: preparation, in vitro characterization, and improved protection against enzymatic degradation.

The optimal formulation showed an insulin entrapment efficiency of 30% ± 2% and a particle size of 154 ± 18 nm. Transmission electron micrographs revealed a nearly spherical and deformed structure with discernable lamella or sodium glycocholate liposomes. Sodium glycocholate liposomes showed better protection of insulin against enzymatic degradation by pepsin, trypsin and α-chymotrypsin than liposomes containing the bile salt counterparts of sodium taurocholate and sodium deoxycholate.

Proliposomes[50]

For liposomes to enter the market, they must be stable during the storage period, and remain intact before reaching their targeted tissues to produce action. Various approaches have been used to overcome these problems, some of which include control of particle size and lamellarity, altering the lipid composition, electrostearic stabilization and lyophilization etc. **Lyophilization** is the only technique which produces liposomes with long term stability profile.

One of approaches which helped in overcoming the stability issue associated with liposome and led to the development of a new drug delivery system is the Proliposome (PL**).**

Proliposomes (PLs) are dry, free-flowing granular products composed of drug(s) and phospholipid(s) which upon addition of water, disperse to form a multi-lamellar liposomal suspension. It is one of the most cost-effective and widely used methods for producing commercial liposome products. It is based upon the intrinsic property of hydrated membrane lipids to form vesicles on contact with water. Being available in dry powder form, they are easy to distribute, transfer, measure and store making it a versatile system.

Liposomes can either be formed in vivo under the influence of physiological fluids or can be formed in vitro prior to administration using a suitable hydrating fluid. The liposomes formed on reconstitution are similar to conventional liposomes and more uniform in size. [51, 52]

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