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An extensive analysis of oral insulin: Application for oral insulin delivery

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Abstract: The development of dependable and effective oral insulin delivery systems has long been a challenge for pharmaceutical researchers because of several obstacles, such as mucus barriers, enzyme breakdown, and stomach acidity. However, several strategies were developed to enhance membrane permeability and biological activity while preventing insulin breakdown in the gastrointestinal tract (GIT). The potential of chitosan polymer-based carriers to preserve insulin in the gastrointestinal tract and efficiently transport it across the intestinal mucosa, thereby increasing its bioavailability, has led to extensive research on these strategies.

Chemical and physical changes have been made to chitosan to improve its capabilities, and recently, chitosan beads, microparticles, and nanoparticles have shown promise as oral insulin delivery (OID) systems. An overview of the many forms of diabetes mellitus, insulin production, and gastrointestinal obstacles to oral insulin is made easier by this article. Also covered are the drawbacks of subcutaneous insulin delivery as well as alternative administration methods. The difficulties of safely delivering insulin through the GIT and methods to increase its bioavailability are emphasized, as the GIT is the best and most practical oral administration route. This analysis also emphasizes the latest developments in chitosan-based OID carriers and their possible uses in the future.

Keywords- Oral Insulin, Diabetes mellitus, Chitosan, GIT, Microparticle

Introduction: Diabetes mellitus has become one of the most devastating diseases of modern history. According to the International Diabetes Federation's estimates, there were 366 million people with diabetes in 2011, and this number is expected to reach 532 million by 2030. and the fact that the economic burden of diabetes represents approximately 6% of the total health budget of developed countries [2]. Surprisingly, insulin, the most effective diabetes treatment, has not gained widespread use. While insulin can be used alone in the therapy of diabetes without any oral antidiabetic drug, the reverse is not true [3]. Insulin therapy is commonly delayed despite the dire consequences, partly due to the inconvenience and complications associated with insulin administration by injection.

Thus, over the last decade, the focus has shifted from developing insulin alternatives to developing alternative delivery methods.

Invasive parenteral (injected) insulin suffers from poor patient compliance due to needle phobia, pain, skin bulges, allergic reactions, common infections, and stress generated from the difficult long-term regimen of insulin therapy [4, 5]. Moreover, many patients still experience hypoglycemic episodes despite easier glucose monitoring options. Parenteral insulin is also associated with nonphysiological delivery to the wrong target

tissues, poor pharmacodynamics, non-ideal initiation, and weight gain [3, 6]. Developments in biotechnology have led to the development of alternatives to parenteral delivery, although these developments have not been rapid enough to meet the pressing demand.

The goal of an alternative delivery route is to reach the bloodstream by noninvasive means, which is inaccessible for a protein drug due to the multiple physicochemical barriers. Oral insulin delivery is still the greatest challenge in the biomedical field [24]. Primarily, the gastrointestinal tract (GI) serves as a significant barrier to insulin reaching the site of absorption due to the stomach's harsh acidic pH and the proteolytic enzymes present in the gastrointestinal tract [25, 26]. The successful oral administration of insulin encounters various challenges, including a harsh acidic stomach, extensive enzymatic degradation by proteolytic enzymes in the GI tract, the mucosal surface of the intestine, and the tight junctions between epithelial cells [4]. Therefore, to achieve successful oral administration, nanoparticles of insulin and biodegradable polymer are investigated. The first report suggested that insulin-entrapped poly(isobutyl cyanoacrylate) (PIBCA) nanocapsules showed up to 20 days of hypoglycemic effects in a diabetic animal model after oral administration [27]. Insulin-loaded poly(lactide-co-glycolide) nano formulations [28, 29] and poly(fumaric-co-sebacic) anhydride [30] nano formulations also managed to control the plasma glucose level in an animal model after peroral administration. Pan et al. also reported a significant lowering of serum glucose levels in diabetic rats by chitosan/insulin nanoparticles synthesized by the ionotropic gelation method [31].

In the present study, we propose the use of chitosan nanoparticles for oral insulin delivery. Therefore, the present work aims to encapsulate insulin into chitosan by self-assembled method and to study the morphological characteristics of the synthesized nanoparticles. In addition, we also intend to investigate the in vitro release of insulin and to determine its efficiency in lowering blood glucose in an alloxan-induced diabetic animal model.

The goal of an alternative delivery route is to reach the bloodstream by noninvasive means, which is inaccessible for a protein drug due to the multiple physicochemical barriers. Including those arising in the innate immune system. Scientists are trying to evade these barriers efficiently through ocular, vaginal, rectal, oral (buccal, gastrointestinal (GI), and sublingual), nasal, and other routes [7, 8]. The barriers to reaching the bloodstream are either physical, such as poor absorption at barrier surfaces, or chemical, such as pH inactivation and enzymatic degradation. Fig. 1 presents possible hurdles for oral insulin delivery.

How Oral Insulin Works: Oral Insulin works by two Different methods

Oral insulin delivered by nanoparticles

Scientists at the University of Sydney have developed a method of taking insulin in a capsule or even inside a piece of chocolate. The insulin is enclosed in tiny nanocarriers, which are 1/10,000th the width of a human hair. The nano-carriers are so small that they can’t be seen under a regular microscope.

These nano-carriers help deliver insulin directly to the liver, where it is needed most, and release it only when blood sugar levels are high. This targeted delivery reduces the risk of side effects, such as low blood sugar (hypoglycemia), which can happen with traditional insulin injections.

The new method mimics how the body naturally uses insulin, leading to better blood sugar control and fewer complications. Insulin doesn’t need refrigeration, and taking it is much easier and more discreet than injections.

“To make the oral insulin palatable, we incorporated it into sugar-free chocolate,” said Nicholas J. Hunt at the University of Sydney, who leads the project with Victoria Cogger. “This approach was well received.”

The method has been successfully tested on mice, rats, and baboons and has shown promising results. Mice and rats did not have low blood sugar (hypoglycemia), which is a potentially dangerous side effect that can happen with traditional insulin injections. Human trials are set to begin in 2025, with hopes that this new form of insulin will be available to everyone within 2-3 years.

Sublingual insulin drops with a cell-penetrating peptide

Researchers at the University of British Columbia have developed insulin drops that are placed under the tongue. This delivery method makes it easier and more convenient for diabetes patients to manage their blood sugar levels.

The drops work thanks to a special cell-penetrating peptide developed by Professor Shyh-Dar Li and his team. The peptide, sourced from fish byproducts, opens a pathway for insulin to pass through the lining of the mouth and into the bloodstream. Normally, insulin is destroyed in the stomach or struggles to enter the bloodstream because it's a large molecule. But with this peptide, insulin can quickly and effectively reach the blood.

Cell-penetrating peptides are becoming a powerful and flexible tool to help proteins and other molecules move through different protective barriers in the body. These special peptides work by creating pores on the surfaces of cells, allowing proteins to enter more easily.

In preclinical tests in mice, drops developed by Dr. Li's lab have proved to be just as effective as traditional insulin injections, without the pain or complications of needles and evidence of toxicity. This breakthrough could make managing diabetes simpler and more comfortable for millions of patients.

Diabetes Mellitus

Type 1 Diabetes Mellitus: Type 1 diabetes is a disease that occurs when your body makes little or no insulin. Insulin is a hormone that helps blood glucose, or blood sugar, get into the body's cells for energy. When your body doesn't have enough insulin, glucose in your blood can't get into the cells. As a result, your cells lack energy, your blood glucose level goes up, and you develop diabetes.

A problem with the body's immune system causes type 1 diabetes. Your immune system normally fights infections. Type 1 diabetes develops when the body's immune system destroys the cells in the pancreas that make insulin. When the immune system attacks healthy cells in your body, it's called autoimmunity. Type 1 diabetes is an autoimmune disease.

Most people with type 1 diabetes need to take insulin every day to manage their blood glucose levels and stay alive. You may be able to prevent or delay health problems that can result from type 1 diabetes by learning to manage your blood glucose level, consuming healthy foods and drinks, and getting regular physical activity.

Type 2 Diabetes Mellitus

Type 2 diabetes, the most common type of diabetes, is a disease that occurs when your blood glucose, also called blood sugar, is too high. Its occurrence is commonly caused by two main factors:

(1) Failure to respond to insulin by insulin-sensitive tissues

(2) Impairment of insulin secretion and action by pancreatic β-cells (Figure 1) [15, 16]. Impaired resistance to insulin action occurs when target cells cannot respond to the circulating insulin [17]. T2DM patients also have a significant risk of infections brought on by an abnormal immune system, impaired glucose control, and diabetic neuropathy. These are either common mild infections, such as external otitis, cystitis, pneumonia, enteric infections, appendicitis, and peritonitis, or rare severe infections like emphysematous pyelonephritis [18].

Blood glucose is your main energy source and comes from your food. Insulin, a hormone made by the pancreas, helps glucose get into your cells to be used for energy. In type 2 diabetes, your body doesn't make enough insulin or does not use insulin well. Too much glucose then stays in your blood, and not enough reaches your cells.

Developments in insulin delivery

Receiving several injections daily in the early days of insulin therapy was inconvenient for many people. As a result, scientists started trying to figure out how to make insulin last longer. Researchers at Nordisk Insulin Laboratories, Drs. Hans Christian Hagedorn and B. Norman Jensen, in 1936, suggested that adding protamine derived from the sperm of a type of American trout might extend the effects of injectable insulin.

The University of Toronto's Scott and Fisher found that adding zinc to insulin guaranteed its stability and extended its effects. 3 Later on, ZPI insulin was created, which is a combination of porcine insulin, zinc, and protamine. In order to create stable combinations with fast-acting insulin, Nordisk developed neutral protamine hagedorn (NPH), the first crystalline (protamine-isophane) insulin, in 1946. The Lente insulin formulations were first introduced in the middle of the 1950s and included varying proportions of crystalline and amorphous zinc insulin. Patients were then presented with a large selection of insulin formulations that were both fast-acting and long-acting.

The creation of purified nanocomponent (MC) insulin and later human insulin was a major advancement, though, as the majority of patients developed antibodies to the foreign bovine or porcine insulins. Proinsulin and other immunogenic peptides were removed from the preparation as much as feasible when purified MC insulin from animal pancreas was released in 1973. The production of recombinant human insulin has been made possible by the development of genetic engineering. In 1978, Genentech used recombinant DNA technology to create synthetic "human" insulin in Escherichia coli bacteria 7. In addition to ensuring the future supply of insulin, the development of human insulin genetic engineering allowed for the restructuring of the insulin molecule to overcome some of its therapeutic drawbacks.

Oral insulin delivery approaches

By mimicking the physiological destiny of insulin in the body, oral insulin preserves a wide surface area available for absorption in the gut and improves glucose homeostasis. Tightly packed epithelial cells that make up the gastrointestinal tract (GIT) can restrict the oral absorption of proteins like insulin. Despite numerous attempts to administer insulin orally, it has proven challenging to influence its stability and pharmacological effect. Numerous attempts have been made to produce oral insulin using hydrogels, liposomes, microspheres, and nanoparticles (NPs). Micro- and nanospheres of chitosan (CS) have shown promise as formulations for insulin mucosal transport. Figure 1 highlights some innovative oral insulin administration strategies. {Draw later}

Types of insulin products

The primary way that insulin products are classified is based on their action profiles: • Insulin lispro, insulin aspart, and insulin glulisine are examples of rapid-acting insulin analogues that start working 15–30 minutes after injection and peak between 30 and 90 minutes later. When administered subcutaneously, their effective duration of action lasts 4 to 5 hours.

The duration of action of short-acting analogues, such as normal (soluble) insulin, is 6 to 8 hours. They start to work 30 to 60 minutes after subcutaneous injection and peak 2 to 3 hours later.

• Because protamine is added to conventional insulin during manufacturing, intermediate-acting analogues like NPH (isophane) insulin have delayed absorption. About two to four hours after the subcutaneous injection, NPH insulin starts to work. It has an effective duration of action of 10 to 16 hours, with its greatest effect occurring approximately 6 to 10 hours after injection.

• Ultraleft insulin and other long-acting insulin analogues start to function 4–6 hours and remain active for 24–28 hours.

Different types of stimulus-responsive insulin delivery systems

The use of stimuli-responsive polymeric carriers (Fig. 2) that can release encapsulated insulin in response to changes in environmental stimuli or external activation could create a less invasive or non-invasive system

for the smart delivery of insulin from the body's reservoir. Encapsulation of insulin in these stimuli-responsive vehicles may eliminate the need for improvements in patient safety, frequent subcutaneous injections, and compliance.

pH-responsive insulin release

From extremely acidic (pH 1.0–3.0) in the stomach to 6.0–6.5 in the duodenum and to neutral or slightly alkaline (pH 7.0–7.5) in the jejunum and ileum, the gastrointestinal tract's pH value quickly increases [19]. Because they undergo structural changes in response to environmental pH fluctuations, which change the polymer's solubility and cause the hydrogel to swell, pH-sensitive polymers seem to be good choices for this application. When it enters the small intestine, the pH-responsive carrier offers regulated release of the cargo at neutral pH and is stable, protecting the encapsulated therapeutic protein from the acidic conditions of the stomach environment [20].

This mechanism regulates the release of insulin based on blood glucose levels. Insulin delivery techniques have utilized pH-responsive carriers, such as alginate/κ-carrageenan composite hydrogel beads [21].

Enzyme-responsive insulin release

The selective catalysis of enzymes changes the design of materials with macroscopic features. Due to the enzyme's high selectivity, mild in vivo operating conditions, and crucial role in numerous biological pathways, this kind of sensitivity is distinct. An enzyme-sensitive substrate and an additional component that regulates or guides interactions that result in macroscopic transitions are typically seen in enzyme-responsive compounds [22]. Functional groups must be incorporated into a substance under enzymatic conditions to impart enzyme sensitivity. In a biological setting, glucose oxidase, the most widely employed glucose-sensitive moiety, can enzymatically convert glucose to gluconic acid. When glucose oxidase oxidizes glucose, it often consumes local oxygen and produces a lot of H_2O_2, which renders the enzyme inactive [19].

Ultrasound-responsive insulin release

Genes and medications can be delivered to target tissues by ultrasound-responsive compounds, and sonication releases them at certain locations. Chitosan microgels were used to incorporate insulin-loaded nanocapsules [23]. Nano capsule-encapsulated insulin can passively diffuse from the nanoparticles while being held in place by the microgel. Following sonication, the microgel's insulin can be quickly released to regulate blood sugar levels. Polymer-coated bubbles/emulsions (nanodroplets, microbubbles, nanoemulsions, and nanobubbles), polymer vesicles/micelles, and polymer hydrogels are examples of frequently used polymer-based materials that respond to ultrasound. [2-(diethylamino) ethyl methacrylate-stat-2-tetrahydrofuranyloxy) ethyl methacrylate] Poly(ethylene oxide)-block-poly Insulin delivery methods have made use of ultrasound-responsive polymers, such as [PEO-b-P(DEA-stat-TMA)]. [24].

Glucose-responsive insulin release

More and more attention is being paid to closed-loop-based smart insulin administration, which can produce insulin by imitating the pancreatic β-cells in response to hyperglycemia [25]. These closed-loop delivery systems usually include two modules: one for glucose monitoring and the other for glucose-induced insulin release. An electromechanical insulin pump that combines an external insulin infusion pump with a continuous glucose monitor is one prominent example. With these wireless, portable, and wearable devices, insulin infusion rates can be changed in response to blood glucose level readings from glucose sensors. On the other hand, a lot of research has been done on synthetic smart insulin delivery devices that use formulation and material design to provide closed-loop delivery [26].

In order to regulate blood glucose, these chemically closed-loop systems, which are often based on glucose-responsive molecules, may recognize rises in blood glucose levels and react by releasing specific amounts of insulin [19]. 3. One glucose-responsive polymer for insulin administration is acrylamidophenylboronic acid [26].

Temperature-responsive insulin release

Temperature is the most widely used stimulus in environmentally responsive polymer systems. The temperature changes are relatively easy to control as well as easily adaptable in vivo and in vitro [27]. One of the unique characteristics of temperature-responsive polymers is the presence of a critical solution temperature. The critical solution temperature is the temperature at which the polymer and the solution (or other polymer) intermittently change with the composition of the phase [28]. The triblock copolymer of poly(ethylene glycol)-poly(ε-caprolactone)-poly(ethylene glycol) (PEG-PCL-PEG) is an example of a temperature-responsive polymer used in insulin delivery systems [29].

Near-infrared (NIR)-responsive insulin delivery -

An interesting strategy for controlled subcutaneous insulin delivery is based on an NIR-activated device consisting of a drug reservoir covered with an impermeable ethyl cellulose membrane containing gold nanoparticles [18]. Under the influence of NIR radiation, the gold nanoparticles are heated, resulting in the reversible collapse of the interconnected polymer nanoparticle network. By modulating the irradiation, a reproducible, repeatable, and customized method of insulin dose was created according to the needs of diabetic rats after subcutaneous injection [30]. Reduced graphene oxide (rGO) was introduced as an NIR-responsive material. Poly(ethylene glycol) dimethacrylate-based hydrogels containing rGO are an example of an NIR-responsive polymer used in insulin delivery systems [31].

Electrical-responsive insulin delivery systems

Electrical potential has also been used as a trigger to activate insulin release [32]. When the potential pH of the hydrogel medium is positive or negative, the chitosan/layered double hydroxide shifts, resulting in faster insulin release. By tuning the electrical signal, different release rates can be achieved under physiological conditions. The rate of insulin release is regulated by the addition of various anions due to their interaction with ionic sites. The ability of insulin to bind to the surface is strongly influenced by external potential stimuli and pH [32]. Electro-responsive PAA and polymethacrylic acid hydrogels are examples of electro-responsive polymers used in insulin delivery systems [34].

Magnet-responsive insulin delivery

Magnetic fields can be used to target and increase the residence time of magnetically responsive particles to improve delivery efficiency upon oral administration. An external magnetic field can localize magnetite-containing insulin carriers to the intestinal region, in which state the amount of insulin can be significantly increased, resulting in improved hypoglycemic effects [18]. Poly(ethyleneimine)/Fe3O4 is an example of a magnetic nanoparticle used in insulin delivery systems [34].

Release and uptake of insulin from novel formulations

Numerous factors, such as the kind of composition, the ratio of composition, the physical or chemical interaction between components, and manufacturing techniques, influence the release of drugs from polymeric nanocarriers. Drug release from vehicles can be categorized into four groups according to their mechanism: accelerated release, solvent chemical interaction, and diffusion. [71] In capsule-like devices, where the medication is dissolved or distributed in a core, diffusion-controlled drug release takes place. It was released by diffusion through a polymeric matrix (monolithic systems) or inert water-insoluble polymeric membranes (reservoir systems). [72] Drug release behavior from the delivery carriers may be impacted when a solvent is transported into the drug delivery systems. Osmotic and swelling-controlled releases are examples of solvent-controlled releases. Water moves from the carrier with a low drug concentration to the center of the carrier with a high drug concentration in an osmotic-controlled release system, which is a semi-permeable polymer membrane-packed carrier. The essential component of a swelling-controlled system is a polymer material with a three-dimensional cross-linked network structure, such as a hydrogel, where the mesh size regulates the behavior of drug release. [73] When certain physiological cues are triggered, smart polymeric drug delivery devices can release encapsulated pharmaceuticals at the right time and site of action. A tiny input can cause

these polymers to react, changing their structure and characteristics on a macroscopic level. [74] [75] Carriers composed of labile bonds can also be used to take advantage of certain stimuli (such as pH or enzymes), which

Endocytosis was responsible for the time-dependent absorption of insulin. The NPs were exocytosed basolateral as a result of the intracellular flow. Insulin-loaded NPs were adsorbed on the surface of Caco-2 cells, and most of them were internalized, according to confocal microscopy. The literature has documented NPs' intracellular or even nuclear localizations in Caco-2 cells. [76] Insulin-loaded NPs were internalized by cells using a variety of transcellular mechanisms, including receptors, caveolae, micropinocytosis, and microtubule-mediated endocytosis. [77]

Oral Insulin

The formidable task of administering insulin orally has been pursued relentlessly over the last several decades to alleviate the pain and stress caused by insulin injections experienced by millions of diabetic patients worldwide. A number of different methods have been explored to improve the oral bioavailability of proteins. These concepts have focused on overcoming the barriers to intestinal absorption, or the digestive enzymes. [35]

Many attempts have been made to use absorption promoters or permeation enhancers, like detergents, fatty acids, or bile salts, which can permeabilize the mucous and epithelial layers and open the intercellular tight junctions. An obvious disadvantage to this type of system is its potential for toxicity and local damage. Encapsulation of the insulin within enteric capsules or microspheres has also been carried out [36]. Protease inhibitors, such as sodium glycocholate, aprotinin, bacitracin, soybean trypsin inhibitor, camostat mesilate, and chromistan, have been administered concurrently with the oral insulin formulations to stop the degradation by digestive enzymes, and these can be combined with any of the other methods of insulin delivery. [37]

Non-invasive insulin delivery by oral route has been recognized as the most preferred by patients for many years [38]. This route consists of the lips to the oropharynx (100 cm^2) [39]. The oral mucosa is made of epithelium, connective tissue, lamina propria, and adipose tissue. Saliva dilutes, and the mucin in saliva creates a barrier for insulin permeation [40]. The absorption of insulin occurs by permeability across the mucosa (Fig. 3). Insulin cannot reach the epithelial cells due to the mucin blanket, which takes the insulin and further reduces the absorption of the drug significantly [39]. In oral delivery of insulin, the thickness of the mucosa plays an important role. With the increase in thickness of the mucosa, the permeability of insulin lowers drastically [41]. However, a low total surface area in the range of 100–200 cm^2 is the major limitation of this method for oral drug delivery. On the other hand, insulin is susceptible to hepatic first-pass metabolism and will be metabolized in the gastrointestinal (GI) tract mucosa [39]. There are different types of particles that are applied for oral insulin delivery.

The most common carriers are listed below:

PEG nanoparticles are synthetic polymers currently prominent for medication delivery applications in cancer therapy [42]. PEG is a protein-resistant, extremely water-soluble, nontoxic, nonimmunogenic, and hydrophilic polymer. It is the ideal polymer for biological inclusion because of these characteristics. To administer therapeutic drugs under a variety of physiological settings, PEG offers a protective covering of nanocarriers [43]. Conjugating PEG with a bioactive material permits more control over the therapeutic agent's optimal size, physicochemical characteristics, and in vivo retention duration. Proteins and antibodies separate different nanoparticles and biomolecules, which the mononuclear phagocytosis system can remove more quickly [43]. By forming a strong barrier surrounding the nanocarriers, the PEG layer on the nanoparticles stops the photochemical reactions of the particles and increases the amount of time they remain in the body. PEG nanoparticles have better serum stability, good biocompatibility, and suitable physicochemical characteristics [44].

The effectiveness of PEG-capped poly(lactic-co-glycolic acid) (PLGA) nanoparticles emulsified with d-tocopherol poly(ethylene glycol) 1000 succinate (TPGS) as possible drug carriers for oral insulin delivery was investigated [45]. When compared to diabetic rats given free insulin, these nanoparticles dramatically decreased serum glucose, and when insulin-loaded PLGA nanoparticles were used, the decrease was three times greater. The findings show that TPGS-emulsified PEG-capped PLGA nanoparticles administered orally can effectively reduce serum glucose levels in less than a day. According to histopathological research, these nanoparticles have regenerative and biocompatible properties and can restore damage caused by streptozotocin to the liver, kidney, and pancreas. It was determined that PEG might serve as a possible medication carrier for insulin administered orally.

Liposomes: The most widely used nanocarriers for targeted drug delivery are liposomes, which have been thoroughly studied [47]. By stabilizing therapeutic chemicals, removing obstacles to cellular and tissue absorption, and enhancing compound distribution to target areas in vivo, they enhanced medicines for a variety of biomedical applications. Phospholipid vesicles with distinct water gaps composed of one or more concentric lipid bilayers are known as liposomes [48]. A large range of medications can be encapsulated by these vesicles due to the liposomal system's special capacity to eliminate hydrophilic and lipophilic substances. It is possible to confine hydrophilic molecules in the aqueous center while introducing hydrophobic molecules into the bilayer membrane. Liposomes have many benefits as a drug delivery method, such as self-assembly, biocompatibility, the capacity to carry vast amounts of medication, and a variety of physicochemical and biophysical characteristics that can be altered to regulate their biological characteristics [48]. Compounds encapsulated in liposomes are shielded from circulation-borne dilution, degradation, and inactivation. Because liposomes typically include naturally occurring phospholipids, they are widely regarded as pharmacologically inactive and have low toxicity [50].

In one investigation, phosphatidyl ethanol produced by phospholipase D, which is catalyzed by the trans phosphate dilation of trans phosphatidylcholine, was used to construct a liposomal carrier for the oral administration of insulin [50]. Rats with a blood glucose level of 270 mg/100 ml were given the produced liposome orally at a dosage of 12 IU/kg body weight. All forms of liposomes administered orally caused hyperinsulinemia. Blood glucose levels dropped along with hyperinsulinemia brought on by liposomes containing dipalmitoyl phosphatidyl ethanol.

Nanoparticle

Insulin can be protected from enzymatic and chemical degradation by being encapsulated in NPs, and polymeric NPs can significantly increase absorption through the paracellular pathway and improve uptake by the small intestinal epithelial cells, according to the development of NP-based insulin delivery systems. [51] Most NPs utilized as drug delivery systems are between 70 and 300 nm in size, with the largest being around 1 μm. [52] The NPs injected with 10 IU/mL insulin had an average particle size of 551.67 nm. Using glucose-responsive polymeric nanoparticles (NPs) to create an oral insulin platform has the following benefits:

They prevent hypoglycemia and hyperglycemia by avoiding intestinal epithelial barriers, protecting the gastrointestinal tract (GIT) from the integrity and bioavailability of loaded insulin, and only releasing insulin when blood glucose levels are reached.

Stimulus-sensitive nanoparticles (NPs) are one of the smart/intelligent systems in site-specific drug delivery because they can respond to environmental stimuli and deliver bioactive molecules to a particular location in the human body when necessary. [53]. NPs have been employed as medication carriers due to their biocompatibility, biodegradability, and ability to stop insulin from breaking down. [54] The NPs derived from chitosan and its modifications, including aminated chitosan, arginine chitosan, carboxymethyl chitosan, glycolic chitosan, mannosylated chitosan, succinyl chitosan, and thiolate chitosan, are promising in addition to other polymeric vehicles that allow the encapsulation of insulin for oral administration. [55] Enhanced drug delivery, high stability, high carrier capacity, and the ability to combine hydrophilic and hydrophobic molecules are some of the primary technological advantages of using NPs as drug carriers. [56] However, there are disadvantages as well, like toxicity, cost, and regulatory challenges.

Solid lipid nanoparticles (SLNs) are a significant class of NPs that are frequently employed in oral protein administration among the many carriers. They were made by a w/o/w double emulsion method. SLNs aggregated when their colloidal stability in simulated gastric juice (SGF, pH 2) was assessed, but they did not change in neutralized simulated gastric fluid (SGF). With or without trypsin, the various SLNs did not significantly aggregate or disintegrate in the simulated intestinal fluid (SIF, pH 6.8). About 40% of the insulin was released from INS HA2-O-SLNs (an aqueous core loaded with insulin) in two hours, according to the in vitro insulin release profile. The insulin release profile from INS HA2-W-SLNs was longer-lasting than that of INS HA2-O-SLNs and INS SLNs (SLNs without HA2 peptide). After 12 hours of incubation, only around 55% and 35% of the encapsulated insulin were released from the INS HA2-W-SLNs in pH 6.8 and pH 5.5 buffers, respectively, compared to 70% and 50% of the INS SLNs and INS HA2-O-SLNs. [57]

Co-modified colon-specific cell-penetrating peptide nanoparticles (CS-CPP-NPs) were created by employing CPPs and amphipathic CS derivatives (ACS) as delivery vehicles. The in vitro release of ACS-modified NPs (N-trimethyl-N-octyl CS (TOCS-NPs), N-trimethyl-N-dodecyl CS (TDCS-NPs), CPPs-modified NPs, Tat-commodified polylactic-co-glycolic acid (PLGA) (TDCS-Tat-NPs), R8-co-modified PLGA-poly (lactic-co-glycolic acid) (TDCSR8-NPs), or penetrating-co-modified PLGA (TDCS-Pen-NPs)) did not significantly alter the drug-release behavior of the NPs in SGF. Furthermore, it was discovered that polyvinyl alcohol (PVA)-NPs had a significant burst release, reaching 40% after 0.5 hours. With no appreciable change, ACS-modified NPs showed a cumulative drug release of roughly 20% in SIF. PVA-NPs, on the other hand, showed a noticeable burst release, reaching 50% after an hour and over 80% after 48 hours. The CPP-modified NPs showed no appreciable burst release and a 20% cumulative release after 48 hours. [58]

Enough insulin was encapsulated in acid-resistant metal-organic framework nanoparticles (UiO-68-NH2), and transferrin was utilized to adorn the exterior with targeting proteins. With a high oral bioavailability of 29.6%, the transferrin-coated nanoparticles successfully penetrated the intestinal epithelium and controlled insulin release in physiological conditions via a receptor-mediated transcellular route. [59] Insulin was injected between the layers of stacked nanosheets to create gastro-resistant imine-linked-covalent organic framework (n-COF) nanoparticles. Insulin protection and glucose-responsive release in digestive juices were both demonstrated in vitro using insulin-loaded nCOF [60]. The two-fold modified functional NP (PG-FAPEP) was developed to overcome significant absorption obstacles. The in vivo investigations further demonstrated that PG-FAPEP exhibited a high oral insulin bioavailability of 14.3% and was able to reach the intestinal epithelium through lysosomal escape, folate receptor-mediated endocytosis, and proton-coupled oligopeptide transporter-mediated exocytosis. [61] To address major absorption barriers, a twofold modified functional NP (PG-FAPEP) was created. The in vivo studies further demonstrated that PG-FAPEP could enter the intestinal epithelium through proton-coupled oligopeptide transporter-mediated exocytosis, lysosomal escape, and folate receptor-mediated endocytosis, with a high oral insulin bioavailability of 14.3%. [61]

Hydrogel-κ-carrageenan and alginate were combined to create a pH-responsive composite hydrogel bead, which was then tested as an oral insulin delivery vehicle [62]. Because of the electrostatic interaction between the positively charged insulin and the negatively charged sulfate group of the κ-carrageenan, the hydrogel beads formed at pH 1.2 were able to successfully retain insulin. Insulin was released slowly at pH 7.4, and the release profile got closer to zero-order kinetics as the amount of κ-carrageenan employed to produce the hydrogel beads increased. Alginate/κ-carrageenan composite hydrogel beads are a potential oral insulin delivery technology, according to the data. Chitosan-coated calcium alginate beads were used to encapsulate insulin [63].

In simulated stomach (pH 7.5) and intestinal (pH 1.2) fluids, its release from alginate-chitosan-glutaraldehyde and alginate-chitosan beads was investigated. The intestinal fluid was shown to be superior by comparing the release quantities and the ionic interaction between the alginate chitosan matrix and the medium pH. Additionally, the breakdown of the released insulin was examined. After six hours of incubation, the granules stayed stable, and the amount of undigested insulin was adequate for physiological circumstances. Thus, this technique can be suggested for oral insulin delivery.

Salecan is a non-ionic carbohydrate that is derived from polysaccharides. The physicochemical features of this polysaccharide are significant. Properties [64]. Salecan, for instance, possesses excellent rheological

qualities, high viscosity yield values, high pseudoplasticity, and the capacity to create high viscosity solutions at low shear pressures and concentrations. Additionally, salecan has appealing biological properties such as biodegradability and biocompatibility [65]. Because of these qualities, salecan is the preferred material for a variety of applications, such as water purification, medication delivery, tissue engineering, and biosensors [65]. Additionally, the backbone of salecan can be readily adjusted chemically and biochemically since it contains several derivable groups (such as hydroxyl groups), which increases the flexibility and convenience of creating specific materials with a desired function.

Salecan polysaccharides are highly appealing for the creation of biomaterials because of all these benefits. The primary drawback of salecan-based hydrogels as a delivery system is the challenge of managing their deterioration. Insulin was administered orally using a hydrogel based on salecan. According to the in vitro insulin release profile, entrapped insulin release was considerably enhanced at neutral pH but inhibited in acidic environments. When diabetic rats were given insulin-loaded salecan hydrogel orally, their fasting blood glucose levels steadily dropped over six hours. The outcomes mimic future advancements in salecan-based hydrogels as vehicles for regulated insulin delivery following oral administration [66].

Thiol-functionalized hydrogel microparticles based on polymethacrylic acid/PEG/chitosan were used to create an oral insulin delivery system [67]. By grafting cysteine onto the hydrogel's active surface carboxyl groups, thiol modification was accomplished. This work shows that hydrogel thiolation aided by microparticles is a viable oral delivery method for peptides and proteins.

Microsphere

Drugs are uniformly distributed throughout the polymer matrix of microspheres. Before being formed into microspheres, insulin is typically dissolved in the polymer solution 3. The resulting insulin-loaded microspheres had a particle size of 5.25 µm. [68]. Insulin and polyvinyl sulfate potassium film layers were alternately deposited on the surface of polylactic acid microspheres to successfully create the insulin microspheres. The insulin-loaded microspheres remained comparatively stable for 12 hours, and the amount of insulin released from them was negligible in the SGF solution. But after 6 hours, the accumulated release rate of the insulin in the insulin-loaded microspheres reached about 90%, indicating that they are promising for oral insulin delivery. The insulin was released gradually into the PBS. [68]

Glycidyl methacrylate-dextran/concanavalin (Dex-GMA/Con A)-based glucose-responsive microspheres based on concanavalin (Con)-sugar affinity were utilized to encapsulate insulin and offer a chemically regulated insulin delivery function. A high-speed shear emulsion-based cross-linking technique was used to create insulin-loaded glucose-responsive microspheres. A scaffold-based synthetic artificial pancreas was created by first creating glucose-responsive, insulin-loaded microspheres using a high-speed shear emulsion-based cross-linking technique and then integrating them into CS hydrogels. In vitro insulin release from integrated scaffolds and free microspheres responded to variations in glucose concentration by exhibiting a proportional bolus and basal release rate and amount.

Thus, scaffold-based synthetic artificial pancreases have potential for use in insulin delivery applications. [69] Microspheres containing various mixtures of eudragit RL100 and RS100-loaded insulin have been created utilizing the solvent evaporation procedure. This stable formulation has strong bioadhesion and good encapsulation efficiency. The highest in vitro release, 9% and 87%, respectively, was observed at pH 1.2 and 7.2. [70]

To improve the oral bioavailability of insulin, a more environmentally friendly method was used to create pH-responsive carboxylate cellulose microspheres (CCMs) by hydrolyzing with citric and hydrochloric acid. The oral bioavailability of insulin is ensured by the pH sensitivity of CCMs. Insulin was released at 48.87% and 85.12% in artificial intestinal fluid (AIF) and artificial gastric fluid (AGF), respectively, based on in vitro release assays.

Potential Problems with Oral Insulin

The optimal timing for oral insulin ingestion depends at least in part on the technology used for drug delivery and will need to be determined for each oral insulin in development. Furthermore, the food effect is likely to determine how oral insulin will be used and for what indications. For example, the rate and extent of absorption of an oral drug are often affected by food and may differ if the drug is administered shortly before or after a meal (fed conditions) as opposed to administration under fasting conditions.

Another problem is that the bioavailability of every polypeptide and protein delivery system created to date is comparatively low. Significant intra- and inter-subject variability is predicted by low bioavailability. Increasing the amount of insulin in the dose form is one method to decrease unpredictability. Commercial factors made such a proposal unfeasible for insulin until recently. However, at the moment, the price and availability of insulin can justify such a tactic. Additionally, low bioavailability suggests that the majority of the insulin consumed is not absorbed and stays in the digestive system. Proteases and peptidases are likely to break down insulin that is stored in the gastrointestinal system.

However, whether insulin, a recognized mitogen linked to an elevated risk of several malignancies, including colon cancer, may raise the incidence of cancer when taken orally is a question that will need to be addressed in long-term safety studies.

Lastly, even though insulin may not be hazardous in and of itself, the chemical substances used as excipients or absorption promoters in the different delivery methods must be found to be safe and effective in long-term clinical and toxicological investigations.

Barriers for oral absorption of insulin (oral insulin 3 paper)

Insulin has several oral absorption challenges in the GIT, mostly enzymatic, physical, and chemical. Oral drugs go via the gastrointestinal tract, adhere to the mucous layer, cross the intestinal epithelium, and enter the bloodstream. However, insulin has a very poor oral bioavailability (less than 1%) due to its instability in the GIT and limited epithelial penetration. The remarkable change in pH from 1.0 to 2.5 in the stomach to 7.5 in the terminal ileum creates a chemical barrier to the insulin taken orally.

The main enzymes involved in the breakdown of proteins in the GIT are pepsin in the stomach, pancreatin in the small intestine (which includes trypsin, chymotrypsin, and elastase), aminopeptidases in the brush border membrane, and specific enzymes in the cytosol. Furthermore, the liver's enzymes will probably break down any insulin that survives the aforementioned proteases. Moreover, insulin molecules' negative charge in the small intestine hinders their ability to pass through the mucus layer, which is known to be negatively charged. [78] [79] Table 1 provides a summary of the physiological obstacles to oral insulin administration and the strategies to get beyond them.

Physiological Barrier	Constitution	Mechanism to overcome	Reference
Digestive enzyme degradation	Trypsin, pepsin, elastase, carboxypeptidase, and chymotrypsin	employing a gastro-resistant architecture, having a hydrophobic effect, and a shielding effect	[80][81]
Degradation caused by stomach acid	pH 1-2 gastric acid	pH responsiveness and an acid-resistant polymer coating	[82][83][84]
Retention by the barriers of the mucus layer	Electrolytes, Glycoproteins, Lipids, and Water	The charge-reversing and mucus-inert electroneutral surface	[85][86]
Intestinal epithelial cell layer retardation	Tight junction, lysosome breakdown, basolateral circulation, and apical endocytosis	Permeation is improved, and active transportation is increased.	[87][88]

Future perspectives

Insulin is primarily used to treat diabetes mellitus (DM), especially type 1, which is one of the most prevalent and deadly chronic illnesses. Insulin therapy compliance and adherence are now low because of local

hypertrophy and fat deposits caused by numerous daily injections. Noninvasive alternative techniques have been created and studied in an effort to counteract the drawbacks of invasive delivery and enhance patient compliance. However, there are currently no oral insulin drugs available on the market, and no formulation has been able to effectively negotiate all therapeutic obstacles.

Future research on toxicology, blood insulin levels, and dose-dependent therapeutic efficacy should be carried out to bolster the oral insulin delivery system's feasibility. If insulin delivery nanosystems could overcome the aforementioned challenges, diabetes mellitus patients could avoid the inconvenience of giving insulin injections.

Conclusions

In this work, we suggest delivering insulin orally using chitosan nanoparticles. Thus, the current study intends to investigate the morphological properties of the produced nanoparticles and encapsulate insulin into chitosan using the self-assembled approach. Furthermore, in an animal model of diabetes produced by alloxan, we plan to examine the in vitro release of insulin and assess its effectiveness in reducing blood glucose. Finding the best insulin delivery system is challenging due to the complicated requirements of diabetics, high expense, low permeability and bioavailability, instability, and adverse effects.

Refference

1)Rathmann W, Giani G. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:2568 9.

2)Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047 53.

3)Sabetsky V, Ekblom J. Insulin: a new era for an old hormone. Pharmacological Research 2010;61:1 4.

4)Khafagy el-S, Morishita M, Onuki Y, Takayama K. Current challenges in non-invasive insulin delivery systems: a comparative review. Advanced Drug Delivery Reviews 2007;59:1521 46.

5)Kennedy FP. Recent developments in insulin delivery techniques. Current status and future potential. Drugs 1991;42:213–27.

6)Al-Tabakha MM, Arida AI. Recent challenges in insulin delivery systems: a review. Indian Journal of Pharmaceutical Sciences 2008;70:278 86.

7)F. Cui, K. Shi, L. Zhang, A. Tao, Y. Kawashima, J. Control. Release 114 (2006) 242–250.

8)K. Sonaje, K.J. Lin, S.P. Wey, C.K. Lin, T.H. Yeh, H.N. Nguyen, C.W. Hsu, T.C. Yen, J.H. Juang, H.W. Sung, Biomaterials 31 (2010) 6849–6858

9)El-S. Khafagy, M. Morishita, Y. Onuki, K. Takayama, Adv. Drug Deliv. Rev. 59 (2007) 1521–1546.

10)C. Damgé, C. Michel, M. Aprahamian, P. Couvreur, Diabetes 37 (1988) 246–251.

11)J.M. Barichello, M. Morishita, K. Takayama, T. Nagai, Drug Dev. Ind. Pharm. 25 (1995) 471–476.

12)G.P. Carino, J.S. Jacob, E. Mathiowitz, J. Control. Release 65 (2000) 261–269.

13)E. Mathiowitz, J.S. Jacob, Y.S. Jong, G.P. Carino, D.E. Chickering, P. Chaturvedi, C.A. Santos, K. Vijayaraghavan, S. Montgomery, M. Bassett, C. Morrell, Nature 386 (1997) 410–414.

14)Y. Pan, Y.J. Li, H.Y. Zhao, J.M. Zheng, H. Xu, G. Wei, J.S. Hao, F.D. Cui, Int. J. Pharm. 249 (2002) 139–147.

15)Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of type 2 diabetes mellitus. Int J Mol Sci. 2020;21(17):6275.

16)Song M, Wang H, Chen K, Zhang S, Yu L, Elshazly EH, et al. Oral insulin delivery by carboxymethyl-β-cyclodextrin-grafted chitosan nanoparticles for improving diabetic treatment. Artif Cell Nanomed Biotechnol. 2018 Nov 12;46(sup 3):S774–82 [cited 2023 Apr 29] https://pubmed.ncbi.nlm.nih.gov/30280608/.

17)Goyal R, Jialal I. Type 2 diabetes. Vol. 3; 2023. p. 8–17. StatPearls. http://www.ncbi.nlm.nih.gov/pubmed/29219149.

18)Pivari F, Mingione A, Brasacchio C, Soldati L. Curcumin and type 2 diabetes mellitus: Prevention and treatment. Nutrients. 2019;11:1837.

19)Y.J. Lin, F.L. Mi, P.Y. Lin, Y.B. Miao, T. Huang, K.H. Chen, C.T. Chen, Y. Chang, H. W. Sung, Strategies for improving diabetic therapy via alternative administration routes that involve stimuli-responsive insulin-delivering systems, Adv. Drug Deliv. Rev. 139 (2019) 71–82,

20)J. Xie, A. Li, J. Li, Advances in pH-sensitive polymers for smart insulin delivery, Macromol. Rapid Commun. 38 (2017) 1700413,

21)H.-P. Lim, C.-W. Ooi, B.-T. Tey, E.-S. Chan, Controlled delivery of oral insulin as part using pH-responsive alginate/κ-carrageenan composite hydrogel beads React. Funct. Polym. 120 (2017) 20–29,

22)D. Roy, J.N. Cambre, B.S. Sumerlin, Future perspectives and recent advances in stimuli-responsive materials, Prog. Polym. Sci. 35 (2010) 278–301

23)J. Di, J. Yu, Q. Wang, S. Yao, D. Suo, Y. Ye, M. Pless, Y. Zhu, Y. Jing, Z. Gu, Ultrasound-triggered noninvasive regulation of blood glucose levels using microgels integrated with insulin nanocapsules, Nano Res. 10 (2017) 1393–1402

24)P. Wei, E.J. Cornel, J. Du, Ultrasound-responsive polymer-based drug delivery systems, Drug Deliv. Transl. Res. 11 (2021) 1323–1339,

25)Y. Zhang, J. Yu, A.R. Kahkoska, J. Wang, J.B. Buse, Z. Gu, Advances in transdermal insulin delivery, Adv. Drug Deliv. Rev. 139 (2019) 51–70

26)J. Wu, D.H. Bremner, H. Li, X. Sun, L. Zhu, Synthesis and evaluation of temperature- and glucose-sensitive nanoparticles based on phenylboronic acid and N-vinylcaprolactam for insulin delivery, Mat. Sci. Eng. C 69 (2016) 1026–1035

27)D.P. Huynh, G.J. Im, S.Y. Chae, K.C. Lee, D.S. Lee, Controlled release of insulin from pH/temperature-sensitive injectable pentablock copolymer hydrogel, J. Control. Release 137 (2009) 20–24,

28)D.T. Nguyen, V.H.G. Phan, D.S. Lee, T. Thambi, D.P. Huynh, Bioresorbable pH and temperature-responsive injectable hydrogels-incorporating electro-sprayed particles for the sustained release of insulin, Polymer. Degraded. Stab. 162 (2019) 36–46,

29)S. Payyappilly, S. Dhara, S. Chattopadhyay, Thermo-responsive biodegradable PEG-PCL-PEG based injectable hydrogel for pulsatile insulin delivery, J. Biomed. Mater. Res. Part A 102 (2014) 1500–1509,

30)E.S. Gil, S.M. Hudson, Stimuli-responsive polymers and their bioconjugates, Prog. Polym. Sci. 29 (2004) 1173–1222,

31)F. Teodorescu, Y. Oz, G. Qu´eniat, A. Abderrahmani, C. Foulon, M. Lecoeur, R. Sanyal, A. Sanyal, R. Boukherroub, S. Szunerits, Photothermally triggered on-demand insulin release from reduced graphene oxide modified hydrogels, J. Control. Release 246 (2017) 164–173,

32) N. Hosseini-Nassab, D. Samanta, Y. Abdolazimi, J.P. Annes, R.N. Zare, Electrically controlled release of insulin using polypyrrole nanoparticles, Nanoscale 9 (2017) 143–149,

33)S. Mallawarachchi, A. Mahadevan, V. Gejji, S. Fernando, Mechanics of controlled release of insulin entrapped in polyacrylic acid gels via variable electrical stimuli, Drug Delivery Translation. Res. 9 (2019) 783–794

34)A. Espona-Noguera, J. Etxebarria-Elezgarai, L. Saenz del Burgo, A. Ca˜nibano- Hern´andez, H. Gurruchaga, F.J. Blanco, G. Orive, R.M. Hern´andez, F. Benito- Lopez, J. Ciriza, L. Basabe-Desmonts, J.L. Pedraz, Type 1 diabetes mellitus reversal via implantation of magnetically purified microencapsulated pseudoislets, Int. J. Pharm. 560 (2019) 65–77,

35)Carino GP, Mathiowitz E. Oral insulin delivery. Adv Drug Deliv Rev. 1999,35(2-3) 249-257

36) Touitou E, Rubinstein A. Targeted enteral delivery of insulin to rats. Int J Pharm 1986; 30:95-99.

37)Morishita M, Morishita I, Takayama K, et al. Site-dependent effect of aprotinin, sodium caprate, sodium EDTA, and sodium glycocholate on intestinal absorption of insulin. Biol Pharm Bull. 1993;16: 68–72.

38)A. Chaudhury, S. Das, Recent advancement of chitosan-based nanoparticles for oral controlled delivery of insulin and other therapeutic agents, AAPS Pharm SciTech 12 (2011) 10–20,

39)M. Boushra, S. Tous, G. Fetih, K. Korzekwa, D.B. Lebo, H. Yi, H. Lun, Development and evaluation of viscosity-enhanced nanocarrier (VEN) for oral insulin delivery, Int. J. Pharm. 511 (2016) 462–472,

40)A. Vaidya, S. Mitragotri, Ionic liquid-mediated delivery of insulin to buccal mucosa, J. Control. Release 327 (2020) 26–34,

41)A.H. Shojaei, R.K. Chang, X. Guo, B.A. Burnside, R.A., Couch, Systemic drug delivery via the buccal mucosal route, Pharm. Technol. 25 (2001) 70–81.

42)N.A. Singh, A.K.A. Mandal, Z.A. Khan, Fabrication of PLA-PEG nanoparticles as delivery systems for improved stability and controlled release of catechin, J. Nanomater. 2017 (2017) 690-714,9,

43)B. Pelaz, P. Del Pino, P. Maffre, R. Hartmann, M. Gallego, S. Rivera-Fern´andez, J. M. De La Fuente, G.U. Nienhaus, W.J. Parak, Surface functionalization of nanoparticles with polyethylene glycol: effects on protein adsorption and cellular uptake, ACS Nano 9 (2015) 6996–7008,

44)M. Anbarasu, M. Anandan, E. Chinnasamy, V. Gopinath, K. Balamurugan, Synthesis and characterization of polyethylene glycol (PEG) coated Fe3O4 nanoparticles by chemical co-precipitation method for biomedical applications, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 135 (2015) 536–539,

45)S. Malathi, P. Nandhakumar, V. Pandiyan, T.J. Webster, S. Balasubramanian, Novel PLGA-based nanoparticles for the oral delivery of insulin, Int. J. Nanomedicine 10 (2015) 2207–2218,

46)L. Sercombe, T. Veerati, F. Moheimani, S.Y. Wu, A.K. Sood, S. Hua, Advances and challenges of liposome-assisted drug delivery, Front. Pharmacololy. 6 (2015) 286

47)C. Bombelli, A. Stringaro, S. Borocci, G. Bozzuto, M. Colone, L. Giansanti, R. Sgambato, L. Toccaceli, G. Mancini, A. Molinari, Efficiency of liposomes in the delivery of a photosensitizer controlled by the stereochemistry of a gemini surfactant component, Mol. Pharm. 7 (2010) 130–137,

48)T.T. Pham, C. Jaafar-Maalej, C. Charcosset, H. Fessi, Liposome and niosome preparation using a membrane contactor for scale-up, Colloids Surf. B Biointerfaces 94 (2012) 15–21

49)L. Sercombe, T. Veerati, F. Moheimani, S.Y. Wu, A.K. Sood, S. Hua, Advances and challenges of liposome-assisted drug delivery, Front. Pharmacol. 6 (2015) 286,

50)M.A. Kisel, L.N. Kulik, I.S. Tsybovsky, A.P. Vlasov, M.S. Vorob'yov, E.A. Kholodova, Z.V. Zabarovskaya, Liposomes with phosphatidylcholine as a carrier for oral delivery of insulin: studies in the rat, Int. J. Pharm. 216 (2001) 105–114,

51)Zhang T, Tang JZ, Fei X, et al. Can nanoparticles and nano protein interactions bring a bright future for insulin delivery? Acta Pharm Sin B 2021; 11(3): 651–67.

52)Hecq J, Amighi K, and Goole J. Development and evaluation of insulin-loaded cationic solid lipid nanoparticles for oral delivery. J Drug Deliv Sci Technol 2016; 36: 192–200.

53) Jamwal S, Ram B, Ranote S, et al. New glucose oxidase immobilized stimuli-responsive dextran nanoparticles for insulin delivery. Int J Biol Macromol 2019; 123: 968–978.

54)Chen X, Ren Y, Feng Y, et al. Cp1-11 peptide/insulin complex loaded pH-responsive nanoparticles with enhanced oral bioactivity. Int J Pharm 2019; 562: 23–30.

55)Tsai LC, Chen CH, Lin CW, et al. Development of multifunctional nanoparticles self-assembled from trimethyl chitosan and fucoidan for enhanced oral delivery of insulin. Int J Biol Macromol 2019; 126: 141–150.

56)Gelperina S, Kisich K, Iseman MD, et al. The potential advantages of nanoparticle drug delivery systems in the chemotherapy of tuberculosis. Am J Respir Crit 2005; 172(12): 1487–1490.

57)Xu Y, Zheng Y, Wu L, et al. Novel solid lipid nanoparticle with endosomal escape function for oral delivery of insulin. ACS Appl Mater Interfaces 2018; 10(11): 9315–9324.

58)Guo F, Ouyang T, Peng T, et al. Enhanced oral absorption of insulin using colon-specific nanoparticles co-modified with amphiphilic chitosan derivatives and cell-penetrating peptides. Biomater Sci 2019; 7(4): 1493–1506.

59)Zou JJ, Wei G, Xiong C, et al. Efficient oral insulin delivery enabled by transferrin-coated acid-resistant metal-organic framework nanoparticles. Sci Adv 2022; 8(8): eabm4677.

60)Benyettou F, Kaddour N, Prakasam T, et al. In vivo oral insulin delivery via covalent organic frameworks. Chem Sci 2021; 12(17): 6037–6047.

61)Xi Z, Ahmad E, Zhang W, et al. Dual-modified nanoparticles overcome sequential absorption barriers for oral insulin delivery. J Control Release 2022; 342: 1–13.

62)H.-P. Lim, C.-W. Ooi, B.-T. Tey, E.-S. Chan, Controlled delivery of oral insulin as part using pH-responsive alginate/κ-carrageenan composite hydrogel beads, React. Function. Polymer. 120 (2017) 20–29,

63)S. ¨Onal, F. Zihnioğlu, Encapsulation of insulin in chitosan-coated alginate beads: oral therapeutic peptide delivery, Artificial. Cells. Blood Substitute. Immobility. Biotechnology. 30 (2002) 229–237,

64)X. Qi, W. Wei, J. Li, G. Zuo, X. Pan, T. Su, J. Zhang, W. Dong, Salecan-based pH sensitive hydrogels for insulin delivery, Mol. Pharm. 14 (2017) 431–440,

65)X. Qi, T. Su, X. Tong, W. Xiong, Q. Zeng, Y. Qian, Facile formation of salecan/ agarose hydrogels with tunable structural properties for cell culture, Carbohydr. Polym. 224 (2019), 115208,

66)X. Qi, Y. Yuan, J. Zhang, J.W.M. Bulte, W. Dong, Oral administration of salecan-based hydrogels for controlled insulin delivery, J. Agric. Food Chem. 66 (2018) 10479–10489,

67)S. Sajeesh, C. Vauthier, C. Gueutin, G. Ponchel, C.P. Sharma, Thiol functionalized polymethacrylic acid-based hydrogel microparticles for oral insulin delivery, Acta Biomater. 6 (2010) 3072–3080,

68)Zhang H, Wang W, Li H, et al. Microspheres for the oral delivery of insulin: preparation, evaluation and hypoglycaemic effect in streptozotocin-induced diabetic rats. Drug Dev Ind Pharm 2018; 44(1): 109–115.

69)Xi Z, Ahmad E, Zhang W, et al. Dual-modified nanoparticles overcome sequential absorption barriers for oral insulin delivery. J Control Release 2022; 342: 1–13.

70)Momoh MA, Jackson TC, Musiliu AO, et al. Microspheres of insulin-Eudragit complex: formulation, characterization, and in vivo studies. Afr J Pharm 2017; 11(29): 327–341.

71)Lee JH and Yeo Y. Controlled drug release from pharmaceutical nanocarriers. Chem Eng Sci 2015; 125: 75–84.

72)Adepu S and Ramakrishna S. Controlled drug delivery systems: current status and future directions. Molecules 2021; 26(19): 5905.

73)Son GH, Lee BJ, and Cho CW. Mechanisms of drug release from advanced drug formulations such as polymeric-based drug-delivery systems and lipid nanoparticles. J Pharm Investigation 2017; 47: 287–296.

74) James HP, John R, Alex A, et al. Smart polymers for the controlled delivery of drugs–a concise overview. Acta Pharm Sin B 2014; 4(2): 120–127.

75) Kalaydina RV, Bajwa K, Qorri B, et al. Recent advances in "smart" delivery systems for extended drug release in cancer therapy. Int J Nano med 2018; 13: 4727–4745.

76)Reix N, Parat A, Seyfritz E, et al. In vitro uptake evaluation in Caco-2 cells and in vivo results in diabetic rats of insulin-loaded PLGA nanoparticles. Int J Pharm 2012; 437(1–2): 213–220.

77)El Leithy ES, Abdel-Bar HM, and Ali RA. Folatechitosan nanoparticles triggered insulin cellular uptake and improved in vivo hypoglycemic activity. Int J Pharm 2019; 571: 118708.

78) Zhang T, Tang JZ, Fei X, et al. Can nanoparticles and nano protein interactions bring a bright future for insulin delivery? Acta Pharm Sin B 2021; 11(3): 651–67.

79)Seyam S, Nordin NA, and Alfatama M. Recent progress of chitosan and chitosan derivatives-based nanoparticles: pharmaceutical perspectives of oral insulin delivery. Pharmaceuticals 2020; 13(10): 307.

80)Choudhury RR and Mukherjee R. Polymeric nanoparticle-based oral insulin delivery system. Biomed Res 2022; 33(2): 30–36.

81)Benyettou F, Kaddour N, Prakasam T, et al. In vivo oral insulin delivery via covalent organic frameworks. Chem Sci 2021; 12(17): 6037–6047.

82)Zhang T, Tang JZ, Fei X, et al. Can nanoparticles and nano protein interactions bring a bright future for insulin delivery? Acta Pharm Sin B 2021; 11(3): 651–67.

83)Gong Y, Mohd S, Wu S, et al. pH-responsive cellulose-based microspheres designed as an effective oral delivery system for insulin. ACS Omega 2021; 6(4): 2734–2741.

84)Mohanty AR, Ravikumar A, and Peppas NA. Recent advances in glucose-responsive insulin delivery systems: novel hydrogels and future applications. Regen Biomater 2022; 9: rbac056.

85)Seyam S, Nordin NA, and Alfatama M. Recent progress of chitosan and chitosan derivatives-based nanoparticles: pharmaceutical perspectives of oral insulin delivery. Pharmaceuticals 2020; 13(10): 307.

86)Pratap-Singh A, Guo Y, Baldelli A, et al. Concept for a unidirectional release mucoadhesive buccal tablet for oral delivery of antidiabetic peptide drugs such as insulin, glucagon-like peptide 1 (GLP-1), and their analogs. Pharmaceutics 2023; 15(9): 2265

87)Olorunsola EO, Davies KG, Ibiang KP, et al. Prosochit®-based Nanoparticulate system of insulin for oral delivery: design, formulation, and characterization. J Appl Pharm Sci 2023; 13(3): 044–052.

88)Wang T, Shen L, Zhang Y, et al. "Oil-soluble" reversed lipid nanoparticles for oral insulin delivery. J Nanobiotechnology 2020; 18: 98.

89)Giovannucci E. Insulin and colon cancer. Cancer Causes Control. 1995;6(2):164-79.

90)Argiles JM, Lopez-Soriano FJ. Insulin and cancer (Review). Int J Oncol. 2001;18(4):683-7.

91)Ish-Shalom D, Christoffersen CT, Vorwerk P, Sacerdoti-Sierra N, Shymko RM, Naor D, De Meyts P. Mitogenic properties of insulin and insulin analogues mediated by the insulin receptor. Diabetology. 1997;40 Supply 2: S25-31.