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Nanoparticle Insulin Drug Delivery — Applications and New Aspects

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1. Introduction

Insulin is a hormone secreted from the β cells of the islets of Langerhans, specific groups of cells in the pancreas. Insulin is a protein consisting of two polypeptide chains, one of 21 amino acid residues and the other of 30, joined by two disulfide bridges. It was isolated in 1921 with its first clinical use in 1922 [1]. Insulin is prepared different techniques; One of these isolated from animals and the other is biotechnological preparation using with the recombinant DNA techniques [2, 3].

Insulin is a important player in the control of intermediary metabolism and profound effects on both carbohydrate and lipid metabolism. It has significant influence on protein and mineral metabolism [4, 5].

The traditional and most predictable method for the administration of insulin is by subcutaneous injections. This method is often painful and hence, deterrent to patient compliance especially for those requiring multiple dose injections of four times a day. Also, there have been reports of hypoglycemic episodes following multi dose injections of insulin [6, 7]. Several new approaches to the method have been adopted to decrease the suffering of the diabetic patients including the use of supersonic injector, infusion pump, sharp needles and pens. Some insulin delivery routs so problematic way for example oral administration; Oral delivery eliminates the pain caused by injection, psychological barriers associated with multiple daily injections. Oral delivery of insulin as a non-invasive therapy for Diabetes Mellitus is still a challenge to the drug delivery technology, because insulin is degraded by the enzymes in the acidic environment of stomach. Otherwise insulin delivery via transdermal delivery is so popular way of insulin administration but there are some disadvantages of this route, for example insulin molecular size and application problems etc. While some of them eased the pain encountered by the diabetic patients, they offer incomplete convenience. Even though

the ultimate goal would be to eliminate the need to deliver insulin exogenously and regaining the ability of patients to produce and use own insulin, new concepts are currently explored to deliver insulin using oral, pulmonary, nasal, ocular and rectal routes [8, 9].

The success of the route of administration is judged on the basis of its ability to elicit effective and predictable lowering of blood glucose level and therefore minimizing the risk of diabetic complications. It is clear that several difficulties have to overcome with the use of formulation and application devices technology [10, 11]. The various explored routes are reviewed in this chapter. On the other hand, the chapter is an attempt to illustrate the use of insulin drug delivery and their body route in diabetes management benefiting many diabetic patients with promising patient compliance.

Diabetes mellitus (DM) which is a metabolic disorder characterized by chronic hyperglycemia (increased blood and hepatic glucose levels) with disturbances in carbohydrate, fat and protein metabolism, resulted by diminished insulin secretion, impaired insulin action or both. It's expected to increase from 171 million in 2000 to 366 million by the year 2030 as predicted by the WHO so it continues to increase in prevalence and will become a serious threat of mankind health [12]. Insulin injections remain to be preferred approach for the treatment of insulin-dependent diabetes mellitus (T1DM) and for many patients non-insulin-dependent diabetes mellitus (T2DM) also. People with type 1 diabetes mellitus have an autoimmun mediated destruction of pancreatic islet beta-cells and insulin deficiency. T1DM usually occurs in children and young adults and require daily insulin administration by injection or an insulin pump for survival. On the other hand, insulin resistance (which is associated with excessive glucose production by the liver and impaired glucose utilization by peripheral tissue, especially muscle) is observed in T2DM. They have an impaired endogenous insulin secretion to deal with the increased blood glucose level and majority needs oral antidiabetic drugs. As the disease progresses, the pancreas looses its ability to produce insulin and necessity of insulin therapy increases [12, 13, 14].

Hyperglycemia, recurrence of ample fluctuation of blood glucose levels and insulin resistance can lead to long term complications such as micro and macrovascular. It is well known that improved metabolic control significantly reduces both microvascular (ie, retinopathy, nephropathy and neuropathy) or macrovascular [ie, cardiovascular disease (CVD), cerebrovascular accidents and peripheral vascular disease] complications in diabetes. The development of complications is a cause of considerable morbidity and increases disability and mortality for the individual with diabetes [15].

The conventional pharmacotherapies currently available for the treatment of type-2 diabetes include insulin sensitisers (metformin and thiazolidinediones), insulin secretagogues (sulphonylureas and glinides), alpha-glucosidase inhibitors, insulin and insulin analogues. Glucagon like peptide (GLP)-1 agonists and dipeptidyl peptidase (DPP)-4 inhibitors are the new therapies; that improve glycemic control have recently been developed [16, 17, 18, 19]. These therapies are proposed to treat the key metabolic abnormalities associated with T1DM and T2DM and minimize the side effects noted with conventional therapies. Also in development there are additional therapies that have effects on the kidney to promote glucose excretion [15]. SGLT-2 (proximal renal tubule) has high transport capacity for reabsorption of approximately
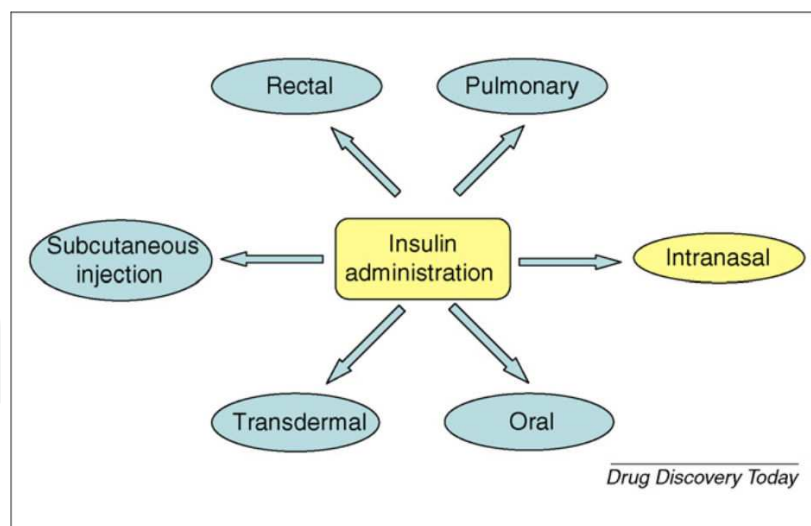
Figure 1. The main administration routes for insulin delivery (Reproduced with permission from Ref. [20], Copyright 2013 Elsevier)

90% of primarily filtered glucose. SGLT-2 inhibitors inhibit glucose reabsorption in proximal renal tubule. It results glycosuria leads to a decline in plasma glucose level. A wide variety of SGLT-2 inhibitors are currently under development with Dapagliflozin, Canagliflozin, Empagliflozin being the most advanced substances. Excretion of approximately 40% of primarily filtered glucose translates to a loss of 50–100 g glucose every day. The consequential decline in fasting and postprandial glucose leads to an HbA1c reduction of approximately 0.8%. The loss of energy substrate reduces body weight approximately 3 kg.

Current therapy for diabetes mellitus through oral anti-diabetic drugs and subcutaneous administration of insulin suffers from serious disadvantages, such as patient noncompliance and occasional hypoglycemia. Moreover, these approaches don't mimic the normal physiological fate of insulin release and doesn't provide better glucose homeostasis. In normal human physiology when the blood glucose level increases insulin releases from the pancreas, reaches to the hepatic portal vein and goes to liver which is its primary site of action. Subcutaneous administration of insulin moves firstly peripheral tissues and can produce peripheric hyperinsulinemia. In order to overcome the problems associated with parenteral administration of insulin, substantial progress has been made for insulin route such as ocular, vaginal, rectal, oral, pulmonary, transdermal, intranasal, and other routes (Figure 1) [20]. 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. Delivery of insulin via the ocular route was tested in animal models in combination with different absorption enhancers, with particular attention given to toxicity as polymers were added to overcome low absorption. Vaginal and rectal routes of insulin have also been evaluated but the absorption rate and bioavailability are poor due to the thick mucosal layers in these tissues. Lots of absorption enhancers (bile salts, chelating agents, surfactants, cyclodextrins, and dihydrofusidate) used but they couldn't prevent local reactions with severe complications.

Nasal delivery has also been evaluated because of the easy access, high vascularity and large absorption area associated with this route. Unfortunately, highly active mucociliary clearance in the nose hindered prolonged drug action resulting in poor bioavailability. Buccal and sublingual insulin administration provide better results due to the low levels of proteolytic enzyme activity, the high vascularization of the tissue, the large surface area for absorption and the ease of administration. Unlike other delivery routes, the gut is the natural route of nutrient absorption into the circulation. The fact that the gut presents the largest absorption surface of all routes provides better efficacy. However, the multiple layers of oral epithelial cells represent a significant GI barrier to drug penetration, which, coupled with the continuous flow of saliva, leads to poor efficacy.

Taking all of this account oral administration is considered to be the most safest and convenient which delivers the drug directly into the liver through portal circulation, where it inhibits hepatic glucose production. Hence by oral delivery to a greater extent the natural physiological route of insulin can be mimicked (Figure 2) [21]. The highly acidic environment in the stomach and the presence of proteolytic enzymes cause structural instability of the oral delivery of protein and peptide drugs including in the harsh environment of the gastrointestinal system [22, 23, 24]. These drugs should overcome some various GI barriers such as chemical, enzymatic and absorption barriers to obtain adequate bioavailability [25]. Different formulation of polymers for insulin delivery such as liposomes, microspheres, microemulsion and nanoparticles (NPs) have been investigated to circumvent these GI barriers [26, 27].
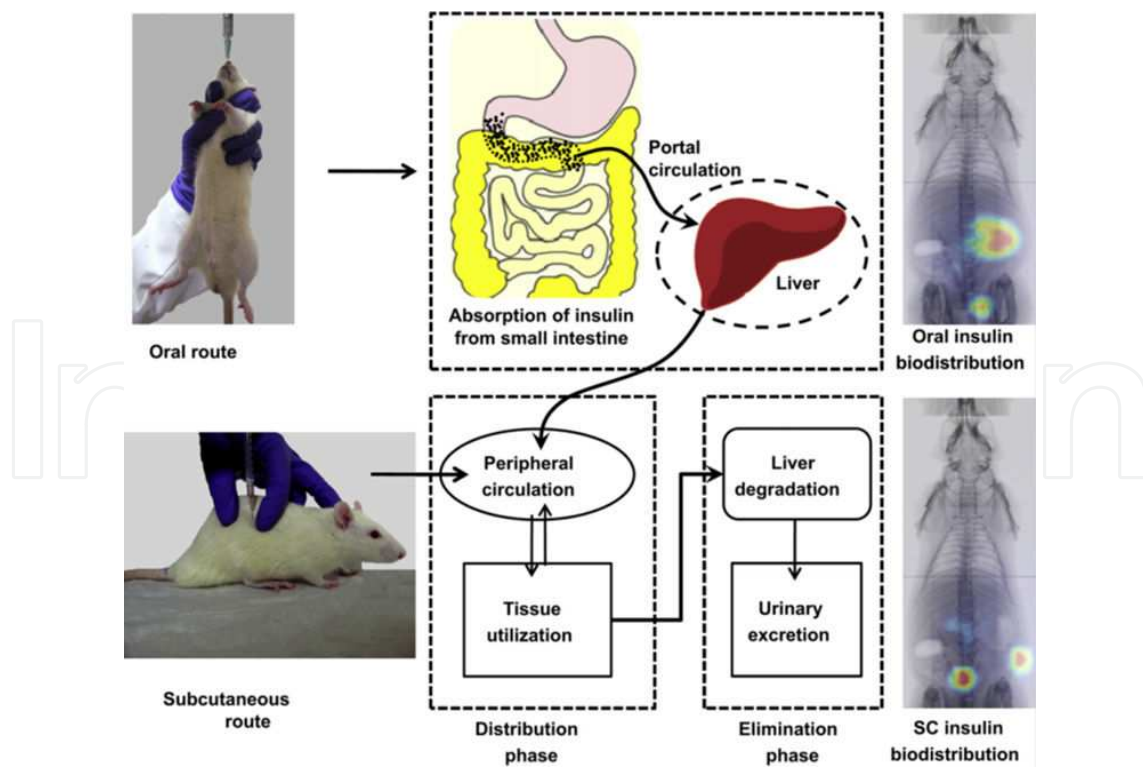

Figure 2. Schematic diagrams illustrating the absorption, distribution and elimination of aspart insulin following oral or subcutaneous (s.c.) administration to rats (Reproduced with permission from Ref. [21], Copyright 2013 Elsevier).

Among these approaches nanoparticular sysytems have attracted special interest because of providing the protection to the highly acidic medium in the stomach (preventing enzymatic degradation), prolonging intestinal residence time, increasing the permeability of drugs to systemic circulation (increasing absorption) and providing controlled-release properties for encapsulated drug [12, 28]. For the conventional medicine it is well understood the nanosize along with other characteristics does play an important role as evident from the improved bioavailability/pharmacological availability [29, 30]. Owing to the high surface area to volume ratio of NPs the window of absorption is also high in comparison with microparticles, this is an added advantage in improving the bioavailability of the administered drug [31, 32].

2. Delivery route of insulin

2.1. Oral delivery

Insulin therapy is effectively used in treatment of diabetes mellitus. Insulin is a key player in lowering blood glucose levels for type 1 diabetes and also required at later stages in type 2 diabetes patients. The widely accepted route for delivery of insulin is by parenteral adminis-tration but this delivery of insulin usually requires at least three or four daily insulin injections for good glycemic control. Consequently more acceptable different routes of insulin delivery have been searched to decrease suffering from discomfort, local pain, irritation, infection, immune reactions and lipoatrophy at the injection site of insulin. Oral delivery of insulin would deliver the drug directly into the liver through portal circulation and could mimic the phys-iological fate of endogenously secreted insulin [33, 34, 35]. However polypeptides, like insulin are degraded in the stomach pH and undergo proteolysis by enzymes in the gastrointestinal tract [22, 36]. Moreover the gastrointestinal mucosa has low permeability for large hydrophilic peptides.

In order to overcome the problems associated with parenteral administration of insulin several strategies that are based on nanotechnology has been developed to enhance the intestinal absorption of different protein and peptides. NPs consist of naturally occurring biodegradable polymers are widely investigated in this regard. They have emerged as potential carriers of several therapeutic agents for controlled drug delivery as well as the oral route of insulin. Various natural hydrophilic and hydrophobic polymers used as carrier of oral insulin such as chitosan, alginate, dextran sulphate, etc. are commonly used to prepare NPs.

2.1.1. Polymers used as matrices for oral insulin delivery

Over the past few decades, enhancing attention has been paid to the use of polymeric NPs either hydrophilic or hydrophobic as carriers for insulin delivery. Hydrophilic polymers are of particular interest due to their non-toxic, biocompatible, biodegradable and natural polymers. Among them, chitosan is widely used because of its ease of chemical modification and promising biological properties.

2.1.1.1. Hydrophilic polymers

Chitosan (CS): CS is well known naturally occuring copolymer of beta [1-4] linked and N-acetyl glucosamine and have been generally found in crustacean (crabs, shrimps and lobsters) shell and in some fungi or yeast. It is a biodegradable, biocompatible, non toxic, non-allergic easily absorbable natural hydrophilic polymer properties that have resulted in a wide array of applications in biomedical and drug delivery research [29, 30, 37]. Moreover it prolongs the intestinal residence time that shows its mucoadhesive property [38] (Figure 3). It has also been shown as a paracellular permeability enhancer by interacting with the TJ proteins occluding ZO-1 and opens the tight junctions between epithelial cells [34, 39, 40]. In addition to these properties it increases the stability of nanospheres and facilates effective encapsulation of proteins and drugs that make it as a suitable carrier material [38, 41, 42]. CS have been extensively used to develope new chitosan derivatized polymers. CS combined with poly(γ-glutamic acid) (γ-PGA) based insulin NPs are used as hydrophilic polymers for oral insulin delivery. *In vivo* preclinical studies of this formulations at a dose of 30 IU/kg in streptozotocin (STZ) induced diabetic rat models showed increased intestinal absorption of insulin from γ-PGA NPs. It has got long lasting hypoglycemic effect and 15% relative bioavailability compared to subcutaneous (sc) injection [43]. The same formulation filled in enteric coated capsules was even better at the same dose, showing 20% oral bioavailability. Also aspart insulin (=monomeric, 3 times faster than regular) is encapsulated in the same CS-γ-PGA; has got 15.7 % oral bioavailability [21, 44].

Moreover insulin loaded NPs with carboxylated chitosan and Poly-methyl methacrylate (PMMA) were developed to improve the insulin delivery via oral route. One of the most widely investigated polymer towards peptide delivery is acrylates which have high interest because of its pH sensitivity and carboxyl groups to enhance the bioadhesivity, alter the tight junction, chelate the Ca^{2+} there by inhibiting the proteolytic activity of proteases, etc. They evaluated their ability to reduce blood glucose levels in diabetic rats. *In vivo* experiments resulted in the reduction of blood glucose levels by 67% at a dose of 100 IU/kg and the pharmacological bioavailability of the 25 IU/kg at a dose of PMMA NPs was 9.7% [45, 46].

Chitosan with sodium alginate is being prepared another insulin loaded nanoparticle product which is used to improve the loading capacity and activity maintenance. It's observed that when insulin-loaded nanospheres (25, 50, 100 IU/kg) administered orally to diabetic rats they reduced glycaemia in a dose dependent manner. Their pharmacological availabilities are found 7.1, 6.8 and 3.4 %, respectively [33, 47, 48].

In addition hydroxypropyl methylcellulose phthalate (HPMCP) is a pH-sensitive polymer designed as an enteric coating material. It reduces the release of drug in acidic conditions and also to improve the colloidal stability of the particles. The release of insulin from CS/HPMCP NPs were significantly reduced at acidic pH and even after 6 h it was less about 25% only. Insulin was protected from enzymatic degradation in the case of CS/HPMCP in comparison with native chitosan particles. Insulin loaded chitosan and HPMCP NPs were orally administered to diabetic wistar rats. The pharmacological availability was 3.02% and 8.47%, respectively, for the chitosan and the modified NPs. In comparison to oral insulin solution the
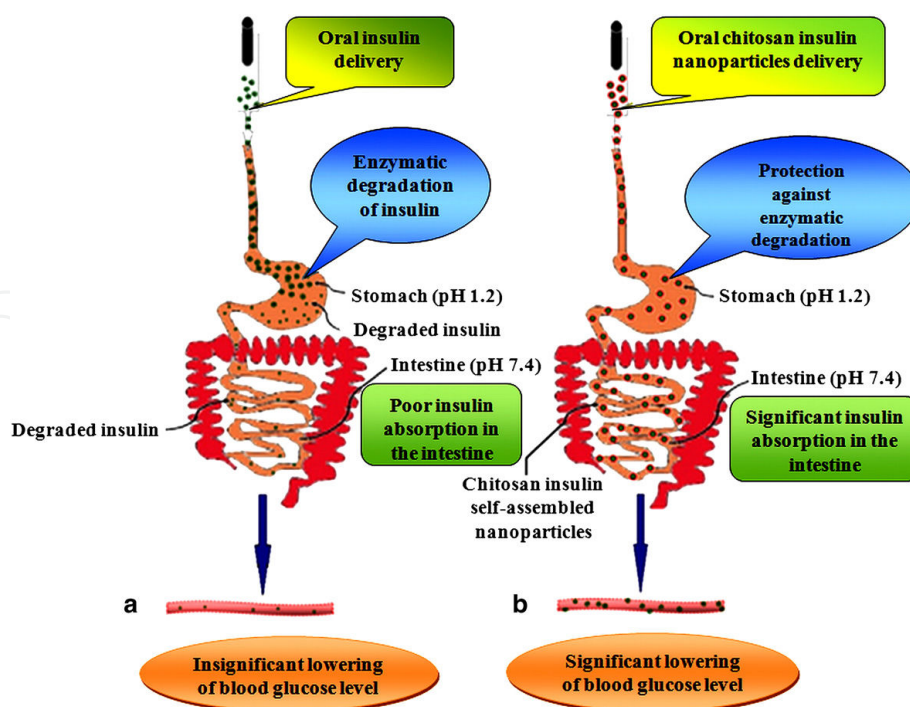
Figure 3. In-vivo efficiency of orally delivered insulin and chitosan/insulin self-assembled NPs (Reproduced with permission from Ref. [38], Copyright 2013 Elsevier).

hypoglycaemic effect was increased by 2.8 and 9.8-folds for the chitosan and the modified NPs, respectively [49, 50, 51].

Dextran sulphate-vitamin B12: Dextran sulphate is a non-toxic and highly water soluble different polymer used as matrices for oral delivery of insulin. Vitamin-B_{12} demonstrated as a ligand to enhance the uptake of the dextran NPs and their translocation across the gastrointestinal tract for high bioavailability. Insulin conjugated to dextran-vitamin B_{12} NPs to diabetic rats that had the least amount of cross linking were found to be most effective at lowering blood glucose levels (70-75%) in STZ induced diabetic rats. In addition the hypoglycemic effect lasted for 54 h. This modification showed the greatest hypoglycemic effect with a pharmacological availability of 29.4% (Table 1) [49, 52, 53].

2.1.1.2. Hydrophobic polymers

Poly (lactide-co-glycolide) (PLGA): Particles consisting of PLGA have been widely studied as therapeutic delivery vehicles owing to their biodegradabile and biocompatibile particles. The hydrophobic nature of PLGA matrices generally makes them incapable of entrapping water-soluble insulin. Intragastric administration of the insulin-loaded PLGA NPs (20 IU/kg) to diabetic rats reduced fasting plasma glucose levels to 57.4% within the first 8h of administration. The relative bioavailability of insulin following oral administration of NPs was 7.7% compared to subcutaneous injection of its solution. Star-branched PLGA (β-cyclodextrin-PLGA) NPs are highly promising for mitigating the burst effect and prolonging the release of insulin. Another study attempted to prevent the burst release of insulin in the stomach by

Particle	Size (nm)	Dose (IU/kg)	Pharmacological Activity (PA %)	Reference
CS-γPGA	218	30	15.1	
Entericcoated(EC)	233	30	20.1	21, 43, 44
Aspart Insulin	245	30	15.7	
CS-PMMA-PEG	1000	50	1.8	45
CS-PMMA	200-300	25	9.7	46
CS-NP	25-400	7, 14, 21	14, 15.6, 15.3	54
CS-dextran sulfate	500	50, 100	5.6, 3.4	55
CS/HPMCP	255	12.5	8.47	56
CS-Na alginate	748	25, 50, 100	7.1, 6.8, 3.4	57
B_{12}vitamin-dextran	192	20	29.4, 26.5	52, 53

Table 1. Hydrophilic Polymers.

using a cellulose derivative (hydroxypropyl methylcellulose phthalate, HPMCP) to prepare PLGA NPs. This modification reduced the initial release of PLGA NPs in simulated gastric fluid from 50% to 20%, and their relative bioavailability in diabetic rats was approximately 6.2% [49, 58, 59].

Poly lactide acide (PLA): PLA exhibit a strong affinity toward the small intestine due to their polyethylene oxide (PEO) blocks and a high permeation capability toward the cell membrane owing to their amphiphilic property. When orally treated with vesicular PLA NPs loaded with insulin to diabetic mice (50 IU/kg), the highest blood glucose reduction was achieved at 4.5 h. Although this effect lasted at least an additional 18.5 h, increasing the insulin concentration to 100 IU/kg did not enhance this hypoglycemic effect (hypoglycemic effect lasted for 23 hr) [60].

Poly-ε-caprolactone (PCL): NPs prepared with PCL and a monomeric form of insulin analog (aspart-insulin). Their results demonstrated that this formulation allows for preservation of biological activities of insulin, increase of serum insulin levels and improvement of the glycemic response. The maximum effect of reduction in hyperglycemia was found at 8 h after oral administration, which was more pronounced with aspart-insulin-loaded NPs (52%) at the dose of 50 IU/kg [61].

Lipidic polymers [*Solid lipid NPs (SLN)*]: Previous studies have demonstrated that nanoencapsulation of proteins in SLNs prolongs their blood residence time, modifies their biodistribution and improves their bioavailability [32]. Oral insulin delivery with SLNs administered to diabetic rats, their relative pharmacological bioavailability was 5.1% in comparison to SC injection of insulin; a considerable hypoglycemic effect was also observed during 24 h. To facilitate the transport of particles across the cellular barriers, in an another study the relative bioavailability increased to 7.1%. That study also suggested that increasing the drug entrap-

ment efficiency and utilizing protease inhibitors in SLNs may further enhance the bioavailability of insulin (Table 2).

Particle	Size (nm)	Dose (IU/kg)	BA (relative bioavailability)%	Reference
PLGA	150	20	40 7.7	46
PLGA-HPMCP	169	20	60 6.27	59
PLA		50	Hypoglycemic effect lasted for 23 h	60
PCL, Aspart insulin loaded	700	50	52/ 12-24 hr	61

Table 2. Hydrophobic Polymers.

2.2. Nasal delivery

Nasal administration has attracted a lot of interest as a highly efficient route for the systemic delivery of insulin. It has been well known that the pharmacokinetic profile of intranasal insulin resembles the pulsatile pattern of endogenous insulin secretion in healthy volunteers during meal times [62]. In addition it's considered as a promising route for the following reasons: the nose has relatively large surface area [150 cm^2] of absorption because of numerous microvilli, high vascularized subepithelial layer that passes directly into the systemic circulation, thereby avoiding the loss of drug by first pass metabolism in the liver, high permeability of the nasal epithelial membrane and lower enzymatic activity than the gastrointestinal tract. Although nasal administration of insulin has many advantages, there are also some barriers that limit the intranasal absorption of insulin. Macociliary clearance of formulations from the nasal cavity, low permeability of nasal mucosa to large molecules and the low bioavailability of insulin act as barriers to intranasal absorption. To overcome the various barriers by the nasal route, researchers have studied many extensive range of enhancers such as bile salts and derivatives, sodium lauryl sulfate, laureth-9, phospholipids, cyclodextrins, chitosan and enzyme inhibitors.

At first CS NPs is seemed to be the safest and most effective as a carrier for the nasal delivery of insulin. It protected insulin from degradation in the nasal cavity and increased intranasal absorption of insulin with its positive charge [20, 63]. Also PEG-grafted(g) have been used to enhance the solubility and improve the biocompatibility of CS. Insulin PEG-g with CS NPs administered intranasally to transport insulin across the nasal mucosa in rabbits [64]. However recent studies showed that insulin-CS solution formulation was more effective than the intranasal NP complex (Bioavailability 17 %, 3.6 % respectively) [65]. Because of NPs couldn't enhance the uptake of insulin; PEGylated trimethyl CS NP results was also not found significantly different from the insulin-CS solution formulation. Besides chitosan reduced gold NPs could enhance insulin transport into cells effectively. After insulin loaded gold Nps adminis-

tered to diabetic rats by intranasally blood glucose concentration was decreased by 20.27 % [66]. Moreover intranasal route of insulin loaded starch NPs containing sodium glycocholate which's used as mucoadhesive carrier, caused 70 % reduction of plasma glucose levels and significant hypoglycemia untill 6h in the STZ induced diabetic rats [67].

2.3. Pulmonary delivery

Pulmonary administration is one of the most promising alternative route of insulin delivery. The lungs offer a large and highly vascularised surface area for drug absorption approximately 80-140 m². Alveoles are covered by a very thin (0.1-0.2 mm) monolayer epithelium, that permits rapid drug absorption. The alveoli can be effectively targeted for drug absorption by drug delivery as an aerosol with a mass median aerodynamic diameter of less than 5µm. First pass metabolism in this administration avoids gastrointestinal system metabolism. Although metabolic enzymes are found in the lungs, their activities and pathways may be different from those found in the GIT and this makes the pulmonary route of many therapeutic proteins and peptides very promising [68, 69, 70].

There are variety of inhalation devices such as metered-dose inhalers or drug powder inhalers. Such as AERx®. Insulin Diabetes Management system developed by Novo Nordisk which delivers aerosol of human insulin; Exubera® developed by Nektar/Pfizer which uses a dry powder formulation. [36].

Dry powder inhalers are currently the most commonly used devices because of their stability and sterility to develope pulmonary insulin. The surfactants, bile salts and fatty acids have been evaluated as absorption enhancers which increase the permeability of drugs through the epithelial membranes. However polyoxyethylene (PE) oleyl ether showed good enhancement sorbitan trioleate exhibited moderate enhancing ability. The enhancing effects of glycerol trioleate, ethyl oleate, oleyl alcohol, palmitic acid and stearic acid were very low. In contrast liposomes are very effective pulmonary absorption enhancers for peptide and protein drugs. They've biogenic phospholipids and biocompatible, biodegradable and non immunogenic natural properties.

Experimental studies investigated that insulin could be efficiently encapsulated in liposomes which has approximately 1µm particle size. Liposome mediated pulmonary drug causes enhancement in drug retention time in the lungs and decreases side effects which results increased therapeutic effects. When aerolized insulin liposomes delivered by the inhalation route in mice caused significantly reduction in plasma glucose concentrations [71]. Insulin-calcium phosphate (CAP) and polyethylene glycol (PEG) particles were administered to the lungs by a route of administration of respiratory tract and these particles positively affected the disposition of the insulin in the lungs of rats [72]. Poly-lactide-co-glycolide (PLGA) particles have used to improve insulin loaded particles which has a mean diameter of 400 nm. After the pulmonary administration of insulin with PLGA nanospheres, blood glucose levels were significantly decreased and has got prolonged hypoglycemic response over 48 h in guinea pigs [73]. In another related study poly butyl cyanoacrylate NPs have been used given by pulmonary inhalation of insulin in the lungs resulted stable and prolonged pharmacological effect. A significant reduction in glucose levels were found and the relative pharmacological

bioavailability was 57.2 % [70]. Besides insulin/1, 2 dipalmitoyl phosphatidylcholine (DPPC) physical mixture used to enhance insulin absorption in pulmonary route of inhalation. This mixture caused higher blood glucose decrease because of their potentially effective, non-toxic and natural absorption enhancer property [74].

2.4. Buccal delivery

Insulin delivered by buccal route is through an aerosol spray into the oral cavity. It's absorbed through the inside of the cheeks and in the back of the mouth. The buccal mucosa is excellently accessible with surface area approximately 100-200 cm^2, lower risk to be traumatized and a relatively good permeability and perfusion [36, 75, 76, 77]. Several formulations and factors alone or in combination can influence release properties of buccal insulin delivery system. These formulations should contain absorption enhancers (such as surfactants, bile salts, chelators, sodium lauryl sulfate or fatty acids) to increase membrane permeability, enzyme inhibitors to protect the drug from degradation, protease inhibitors (aprotinin and sodium glycocholate) to function drug permeation across mucosa, lipophilicity modifications (conjugation with polymers) bioadhesive delivery systems (gels, films, patchs) and liposomal formulations [36, 76, 78]. Lysalbinic acid which is applied as an absorption enhancer was shown to enhance significantly buccal mucosa permeability for insulin. They investigated that it's a product of the alkaline hydrolysis of egg albumin and has no irritating or sensibilizing effect upon buccal use. Co-administration of lysalbinic acid and relatively small proteins such as insulin can increase insulin's permeability from the cheek mucosa of hamster [79].

In the last years a new innovative system has been developed by Generex Biotechnology Corporation (Toronto, Canada). It's based on a liquid formulation (Oral-Lyn®) of recombinant human insulin, absorption enhancers (which encapsulate and protect the Insulin molecules) and Rapid Mist® device (advanced buccal drug delivery technology). This device sends fastly small particles from an aqueous spray into the oral cavity. This allows rapid insulin absorption.

Oral-Lyn® has been evaluated in healthy persons and type I diabetes. It appears in the circulation within 10 min, the time to peak insulin concentration is is around 25 min. It has observed a more fast onset of action and less prolonged hypoglycemic action. Several studies in patients both type 1 and 2 diabetes demonstrated that this oral insulin can be efficient in controlling postprandial glucose levels. This new buccal insuin system needs further investigations in diabetic patients [35, 36, 76, 78].

2.5. Transdermal delivery

Transdermal insulin delivery is an appealing alternative to the invasive parenteral route of administration and other alternative routes of insulin such as pulmonary and nasal routes because the skin offers the advantages of an easy access and a very large surface area (1-2 m^2). It improves patient compliance and avoids both liver's first pass metabolism and degradation of drugs in gastrointestinal tract. The skin also represents an important painless interface for systemic drug administration. Despite these advantages the human skin limits permeation of foreign compounds especially large hydrophilic molecules like insulin. The

stratum corneum; which is the upper layer causes impermeability of the skin by its lipid-rich matrix. Several attemps have been made to overcome the skin barrier and to allow the transfer of large drugs such as insulin. They can be divided into chemical (liposome and chemical enhancers) and physical methods (mainly iontophoresis and sonophoresis).

2.5.1. Transdermal delivery methods

Chemical enhancers such as surfactants, fatty acids, fatty esters and azone-like compounds alter the lipid structure of the stratum corneum. They reduce its barrier properties and enhance its permeability for large molecule drugs that would not pass through the skin.

Iontophoresis is a non invasive technique used to increase transdermal insulin penetration through the skin by the application of a small electric current potential. Large drug molecules can be delivered in a shorter time with the help of this method and it increases drug's mobility.

Another non invasive technique *sonophoresis (ultrasound, phonophoresis)* which has been used to enhance (and or or delivery and activity of drugs) skin permeability to various low and high molecules weight drugs such as inulin. Low frequency ultrasound (20-160 kHz) decreases blood glucose levels both in animal and human studies [36, 72, 75].

Microneedles are minimally invasive painless and promising technology to deliver drugs into the skin without disruption of nerve endings. This technology create micronsized channels which interstitial fluid fills up the channels in the skin. It makes hydrophilic transport pathway, faciliates the stratum corneum barrier and increases skin permeability to large molecules [35, 36, 62, 75].

Also other methods have been investigated like microdermabrasion, pressure waves and electroporation but they're still in at a preliminary stage. Altogether chemical and physical methods they all need further investigations.

2.6. Ocular delivery

Ocular delivery is another the most promising and challenging delivery of ophthalmologically active peptides and proteins for the treatment of ocular diseases. The advantages of the ocular delivery are; less development of immunological reactions in eye tissues, less side effects, no tolerance and avoidance of hepatic first pass metabolism. While the enhancers such as saponin, dodecylmaltoside, tetradecylmaltoside, fusidic acid and glycocholate increases the systemic absorption of insulin in animals they may also increase the eye toxicity [36, 80, 81]. A series of alkylglycosides including tetradecyl-, tridecyl- and dodecylmaltoside and dodecylsucrose were potent stimulators of insulin absorption after topical ocular delivery in anesthetized rats when used at concentrations as low as 0.125 %. These are the most hydrophobic alkylglycoside reagents and were the most effective at enhancing systemic insulin absorption [82]. Moreover sucrose cocoate, a pharmacological excipient of cosmetic and dermatologic preparation was used to deter-

mine its possible absorption enhancer in ocular drug delivery. When insulin was delivered ocularly in the presence of 0.5 % sucrose cocoate, plasma insulin levels were significantly enhanced and blood glucose levels were reduced [83]. Because of this observation insulin-containing liposome was prepared to prolong the retention time of the formulation in the precorneal area [84]. This positively charged formulation decreased the blood glucose levels 65-70%.

More recently Gelfoam® an absorbable gelatin sponge ocular devices have been developed as insulin carriers for systemic administration of insulin. Although Gelfoam® containing 0.2 mg insulin has been showed prolonged systemic absorption of insulin within the desired therapeutic levels it may also cause long term toxicity such as slowing the tear production. Because of this toxicity sodium insulin and zinc insulin Gelfoam ocular devices have been developed and these devices were sufficient to control blood glucose levels (60 % of initial) for over 8 hours [62].

2.7. Vaginal delivery

In recent years numerous studies prove that vagina has got rich blood supply and large surface area that means good permeability and can be a potential route for systemic delivery to a wide range of compounds. The main advantages of vaginal drug route are avoidance of first pass metabolism, ease of administration and good permeability for low molecular weight drugs. For systemic delivery bile salts, dihydrofusidate, cyclodextrins, surfactants and chelating agents have been tested as enhancers to facilitate the rate of vaginal absorption but sometimes they induced several local reactions [36, 75].

2.8. Rectal delivery

Rectal route of delivery have been tested soon after the discovery of insulin but several investigators have met absorption problems through the mucosa. This administration's promising advantage is the possibility of avoiding, to some extent, the hepatic first-pass metabolism. Absorption promoters and surfactants were used to provide highest hypoglycemic effect in rectal insulin delivery. The most effective rectal absorption enhancer polyoxyethylene-9-lauryl ether (POELE) or sodium salicylate were used in insulin suppositories on diabetic dogs. It was investigated that hypoglycemic effect can be achieved about 50-55 % [85].

3. Conclusion

Over the last years numerous studies summerised polymeric NPs focused on different routes of insulin delivery. The association of insulin with NP formulations designed to protect insulin from degradation and enhance its uptake in the ileum. However, more research in this area is needed to achieve the goal that has plagued researchers for many

decades. At any rate, polymeric NPs for routes of insulin delivery seems to be the better alternative compared to others.

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