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Various insulin delivery systems for management of diabetes mellitus

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Abstract

Insulin, a crucial polypeptide drug in management of diabetes, is degraded by the gastrointestinal enzymes and in the acidic harsh conditions of the stomach, following oral administration. Subcutaneous injection, although being effective, has several challenges and drawbacks. In the current study, the manuscript started by introduction about diabetes mellitus, its types, the complications of it on different body systems. Also, the manuscript discussed the role of insulin to regulate blood glucose concentration. The types of therapeutic insulin were discussed. Nanoparticle delivery systems used for delivery of insulin were mentioned in details such as liposomes, polymeric micelles, solid lipid nanoparticles and Nano-gels. Finally, the different routes of insulin administration are addressed such as subcutaneous, oral, rectal, pulmonary, buccal and vaginal routes.

Keywords:
Insulin; Types of therapeutic insulin, Insulin complications, Nanoparticle delivery systems. Different routes of insulin administration

Introduction

Diabetes Mellitus

Diabetes is a chronic condition in which the body cannot use properly the energy from food. The name ‘diabetes mellitus’ and ‘diabetes insipidus’ are unrelated, although both conditions cause frequent urination and constant thirst. Diabetes mellitus results from the disability of the body to use blood glucose for energy and as a result the urine in this type is sweet because of the glucose content [1, 2]. Diabetes is an increase in blood glucose levels above normal values. Diabetes occurs as a result of deficiency of insulin production from the pancreas, the cells cannot use insulin (insulin resistance), or combination of these. Since cells cannot take in the glucose, it builds up in blood resulting in hyperglycemia.

Types of Diabetes Mellitus

There are two main types of diabetes: Type 1 diabetes and type 2 diabetes [3].

Type 1 diabetes

Type 1 occurs due to severe insulin deficiency leading to insulin-dependent diabetes. In this type the body produces antibodies which attack the insulin producing cells of the pancreas. It is more common during childhood. This type is an idiopathic diabetes. There are no predisposing factors, but both genetic and environmental factors can be involved. Those patients should take insulin injections to control blood glucose levels.

Type 2 diabetes

Type 2 diabetes occurs because the pancreas does not produce enough insulin and the cells do not respond to the produced insulin (insulin resistance). Type 2 diabetes is attributed to obesity and is more common in adults. Type 2 diabetes can be controlled with diet and exercise, and occasionally medication. This type is more prevalent than type 1 diabetes [1, 4].

Complications of diabetes

If diabetes is not controlled, it can cause many complications damaging the blood vessels, nerves and organs [5-9].

Microvascular complications

Microvascular diseases include retinopathy, nephropathy and neuropathy. Diabetic retinopathy is due to damage of the blood vessels of the retina which results in focal blurring, retinal detachment and partial or total vision loss. Diabetic nephropathy occurs because the blood vessels of the kidneys are damaged leading to chronic kidney disease and renal failure.

Macrovascular complications

Moreover, diabetes if left uncontrolled will cause macrovascular diseases such as; angina pectoris and myocardial infarction, transient ischemic attacks and strokes and peripheral arterial disease.

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Infections

Diabetic patients are susceptible to immune dysfunction because of the reduced efficiency of white blood cells in fighting bacterial and fungal infections.

Acute metabolic complications

There are also acute complications from diabetes mellitus such as hypoglycemia and ketoacidosis. Hypoglycemia is caused by insufficient food intake, excessive exercise, or due to intake of exogenous insulin, insulin secretagogues or any other oral hypoglycemic agents. Another acute complication is ketoacidosis. Ketoacidosis is more common with type 1 diabetes. It occurs due to insulin deficiency and the production of excessive amounts of the counter regulatory hormones. The blood becomes acidic due to the large production of ketones [1, 10, 11].

Role of glucose

In healthy people, blood glucose levels increase after meals, but it is restored after about 2-4 hours. Glucose is an essential source of energy in many tissues. Red blood cells and brain cells use it almost exclusively to produce energy. Maintained blood glucose concentrations are controlled by the action of two hormones; insulin and glucagon which have antagonistic action to one another. Insulin is produced by the β cells of the pancreatic islets, otherwise glucagon is produced by the α cells. The pancreas produces more insulin and less glucagon when the blood glucose concentrations are high and the excess glucose will be converted into glycogen and stored in the liver. On the other hand, low blood glucose levels lead to the production of more glucagon and less insulin and thus stimulating the breakdown of glycogen to glucose which re-enters the blood stream. This process of maintaining blood glucose levels within a narrow range is called "glucose homeostasis. Glucose homeostasis is performed mostly by the liver which stores glucose (as glycogen) in the post absorptive state and it is released into the circulation between meals (**Figure 1**). Homeostasis is maintained when the rate of glucose utilization by peripheral tissues is balanced with the rate of glucose production. The balancing of glucose production and utilization depends imperatively upon endocrine regulation by insulin and its counter-regulatory hormones.

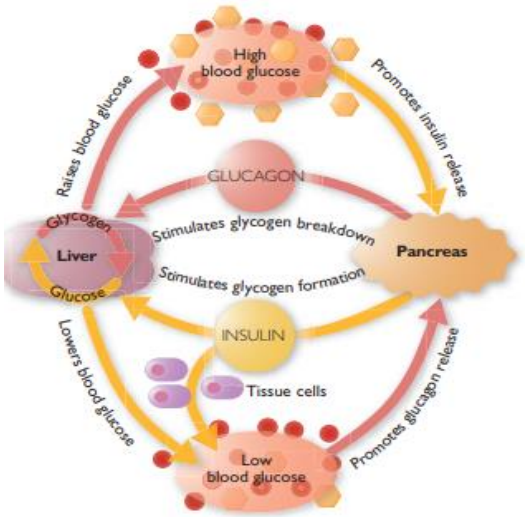

Figure 1. Glucose homeostasis is controlled by the antagonistic actions of two hormones: insulin and glucagon [11].

Role of insulin

Insulin is the principal hormone that regulates blood glucose concentration. It is responsible for both storage and the controlled release of energy. Any kind of insulin deficiency or loss of response to insulin will result in diabetes. Insulin is secreted by the β cells of the islets of Langerhans in the pancreas and it enters the portal circulation which takes it to the liver, the main target organ of insulin action. As insulin is secreted endogenously, the liver is exposed to three times higher insulin levels than other tissues because when insulin is secreted into the portal venous system, it traverses the liver prior to reaching the systemic circulation. So about 50% of insulin is degraded in the process of 'first pass'. In healthy subjects, insulin levels are low during fasting, but after eating insulin secretion rapidly rises[1].So, insulin is the maestro that regulates blood glucose concentration.

The structure of insulin

Insulin is a peptide hormone and is composed of 51 amino acids arranged in two chains that are connected together by two disulfide bonds. The A chain is composed of 21 amino acids, while the B chain is 30 amino acids. Initially, insulin is synthesized as a precursor of 110 amino acids and form preproinsulin. Then, it undergoes post-translational modifications and 24 amino acids are cleaved to form proinsulin. Thereafter, it is stored within the β cell inside secretory granules. Finally, The C peptide is cleaved by proteolysis and this yields the mature insulin and the C peptide in equimolar amounts. The C peptide has unknown physiological function and it is slightly extracted by the liver, therefore this peptide is measured and used as an index of endogenous insulin secretion [1, 12, 13]. Diabetic patients are treated with insulin that mimics the action of the endogenously secreted insulin. Many insulin analogues are synthesized by modifying slightly the insulin molecule structure to alter the pharmacokinetic properties of insulin.

Types of therapeutic insulin

Therapeutic insulin analogues include [1, 12, 13]:

1. Short acting regular insulin

This type has a delayed onset of action and reaches the peak 2-3 hours after injection and may cause hypoglycemia due to its continual action several hours later. Hexamers are formed after injection then dissociate into dimers and monomers. So, this type should be taken 30 minutes before the meal [14-16].

2. Rapid-acting insulin analogs

In this type, the structure of insulin is modified to alter its pharmacokinetics by decreasing the formation of hexameric structures after injection so achieving more rapid dissolution and absorption into the bloodstream. Thus, upon switching from regular to rapid-acting insulin analog the dose of insulin should be reduced as it causes higher peak plasma concentrations. Rapid-acting insulin analogs include insulin lispro (Humalog), insulin aspart (Novorapid or Novolog) and insulin glulisine (Apidra).

a. Insulin Lispro (Humalog)

This is the first generation of the rapid-acting insulin analogue [17]. It is formed upon reversing the B28 (proline) and B29 (lysine) amino acid sequence of insulin. It is absorbed faster than regular insulin and has higher peak plasma levels and shorter duration of action due to reduction in self-association. Thus, it can be injected before and immediately after a meal. It acts within 15 min, reaches maximum peak value in approximately 1 hour and disappears within 2-4 hours after subcutaneous injection. Insulin lispro achieved better postprandial glucose levels and less hypoglycemic events compared with regular insulin.

b. Insulin Aspart (Novolog)

In this type the amino acid B28 (proline) is substituted with aspartic acid thus, reducing self-association of the molecule and avoid forming any hexamers [18, 19]. When given intravenously, it had a safety profile very close to that of human insulin. Its absorption is twice as fast as human insulin and reaches maximum plasma levels twice as high whereas it has shorter duration of action.

c. Insulin Glulisine (Apidra)

Here the amino acid asparagine at B3 position is substituted with lysine and the lysine at B29 position is replaced with glutamic acid [19]. It has rapid onset and shorter duration of action than human insulin. The structure of this insulin causes it to self-associate into dimers in the absence of ligands and the isoelectric point is shifted to lower values (pH 5.1; human insulin, pH 5.5) and thus increasing the solubility at the physiological pH.

3. Intermediate-acting insulin

This type dissolves slowly after subcutaneous injection as it is a suspension formed from the addition of protamine or zinc to human insulin resulting in crystal formation. NPH (neutral protamine Hagedorn) insulin is formed by mixing soluble insulin and protamine protein and form stable mixtures [20, 21]. The onset of its action is about 2 hours, gives a peak plasma concentration in 6-14 hours and has duration of action approximately 10-16 hours. A dose given at bedtime can be used as basal insulin, or a dose taken in the morning can be used as basal and prandial insulin. Regular insulin or rapid- acting insulins can be combined with NPH insulin. Zinc insulins are insulin crystals formed from excess zinc added to insulin. It has a delayed absorption and prolonged duration of action depending on the size of the crystal. This form cannot be used with soluble insulin due to the presence of excess zinc in the vial.

4. Long-acting insulin

a. Insulin Glargine (Lantus)

Two structural modifications are formed to bring about a stable molecule. The B chain at the C-terminus is elongated by the addition of two arginines resulting in shifting the isoelectric point of the insulin from a pH of 5.4 to 6.7 [22]. Due to this change the insulin becomes less soluble at physiological pH and more soluble at acidic pH and is formulated at a pH of 4. The second structural modification is substituting asparagine at position 21 of the A chain with glycine. This substitution results in stabilization of the hexamer structure and prevents dimerization. When insulin glargine is injected into subcutaneous tissue the acidic solution is neutralized and precipitated at physiological pH and absorbed slowly from the site of injection. Precipitates of insulin glargine are released and continue for about 24 hours in a manner similar to that achieved by a continuous subcutaneous insulin infusion. Insulin glargine should not be mixed with other insulin because its pharmacokinetics and pharmacodynamics will be altered and this will affect its absorption profile. Its onset of action is approximately 2 hours and its duration of action is 20-24 hours. Insulin glargine provides basal insulin supply and consistent rate of absorption without peak actions resulting in reduced nocturnal hypoglycemia when given at bed time compared with NPH insulin.

a.

b. Insulin Detemir (Levemir)

In this type the B30 amino acid is deleted and a C14 fatty acid side chain is attached to the B29 lysine amino acid [23, 24]. It associates with albumin in the subcutaneous tissues after injection so, it is slowly absorbed. After reaching the bloodstream it rebounds with albumin retarding its distribution to peripheral tissue. Its onset of action is about 2 hours and its duration remains 16-24 hours. It can be taken once or twice daily.

c. Insulin Degludec (Tresiba)

It is an ultra-long-acting insulin formed from omitting the B30 amino acid and linking a glutamic acid spacer which links a 16-carbon fatty di-acid chain to the B29 amino acid [18, 25]. After subcutaneous injection it forms soluble multi-hexamers releasing insulin monomers slowly into the bloodstream, thus providing a prolonged duration of action and can be injected only three times a week. Its half-life is 25 hours and its action is maintained more than 42 hours. Detemir is not associated with weight gain. In comparison to basal insulin, it was observed that insulin glargine and insulin detemir result in less nocturnal hypoglycemia and decreased the rate of hypoglycemic events compared to NPH. Also, in a study of patients with type 2 diabetes to differentiate between the effect of using U-100 insulin glargine and U-300 insulin glargine it was shown that U-300 insulin glargine causes less nocturnal hypoglycemia.

Nanoparticle delivery systems used for delivery of insulin

Focusing on noninvasive insulin administration routes has become a crucial requirement for many medical and pharmaceutical researchers to improve patient compliance and reduce pain and other side effects caused by injection. Different nanoparticle materials have been successfully applied for loading of antidiabetic drugs (curcumin and berberine) with remarkable results *in vivo* in controlling high blood glucose levels [26-33]. Nanoparticles are particles in the size range between 1 and 100 nanometers and are comprised of carbon, metal, metal oxides or organic materials. The nanoparticles have a unique physical, chemical and biological properties at nanoscale in comparable to their respective particles at larger scales. This phenomena is mainly attributed to the relatively higher surface area to the volume, enhanced reactivity or stability in chemical processes, and increased mechanical strength, *etc*. [34]. There are different types of nano-drug delivery systems which are constructed from different materials (**Figure 2**) [35].

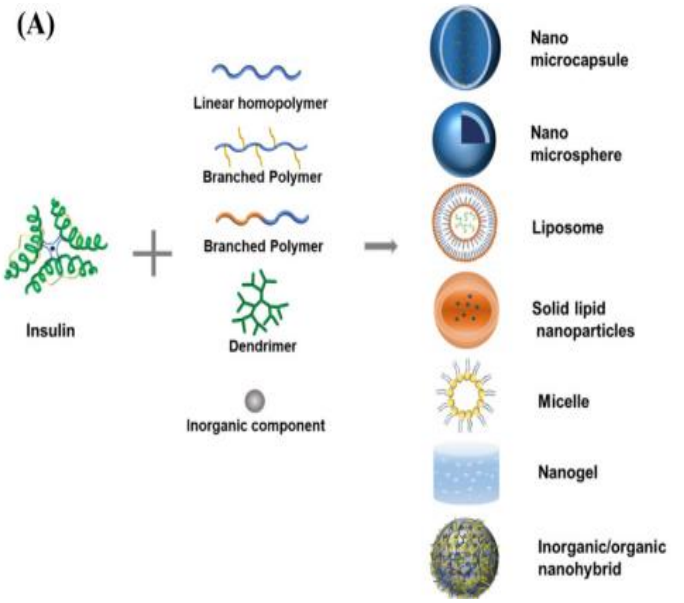

Figure 2. Nanomaterials of various compositions that have been utilized for various biomedical applications [35]

In the following sections, the different insulin delivery nanosystems are discussed.

1. Liposomes

Liposomes are small spherical vesicles of size ranging from 25 nm to 2.5 µm. They are constituted from one or more phospholipids bilayers enclosing a water core. The polar groups of the phospholipid bilayers are positioned to the inner and the outer phase is aqueous. Liposomes have several advantages such as low toxicity, high biocompatibility, biodegradability, reproducibility and non-immunogenicity [35, 36]. Wang et al. prepared protein corona liposomes for efficient delivery of oral insulin by overcoming mucus and epithelial barriers. Cationic liposomes (CLs) are prepared by thin film hydration method. The carrier materials used to prepare CLs are egg yolk lecithin (EPC), cholesterol, and the cationic lipid, DOTAP. Bovine serum albumin (BSA) is adsorbed on the surface of CLs to form protein corona liposomes (PcCLs) (**Figure 3**). The PcCLs could enhance the oral bioavailability of insulin [37]. *In vitro* and *in vivo* experiments revealed that the uptake amounts and transepithelial permeability of PcCLs were 3.24 and 7.91 times higher than that of free insulin, respectively. Intrajejunal administration of PcCLs produced high hypoglycemic effects in Type 1 diabetic rats and increased the oral bioavailability up to 11.9% [35, 37]. Kim et al. used a conventional thin film rehydration method to prepare an uncapped positive charged liposomal nanoparticle (IPUL-CST) and the particle size was approximately 200 nm [38]. The materials used were dimethyloctadecylammonium bromide (DDAB), deoxycholic acid (DOCA), and supramagnetic iron oxide nanoparticles (SPION) with the diameter of 10 nm. The uncapped structure was developed by dispersing superparamagnetic iron oxide naoparticles in lipossomes, permitting magnetic shear stress to squeeze the liposomal surface and tear it forming open led bilayer holes. Thus, insulin was encapsulated not only in the outside of the liposomes but also in the internal space of the liposomes. Insulin was loaded by diffusion and electrostatic interactions. The cationic insulin loaded liposomes were then encapsulated by complexation with the anionic chondroitin sulfate-taurocholic acid coupling (CST). This increased the active transport of IPUL-CST using the apical sodium-dependent bile acid transporter mediated intestinal uptake and lymphatic transport pathways. It was demon 3strated that the IPUL-CST was delivered to the circulation by absorption with the distal ileum via the lymphatic pathway in vivo absorption pathway. The apparent bioavailability after oral administration of this insulin-loaded liposome was approximately 34%, and the blood glucose level was decreased at least 16 h after oral administration [35, 38].

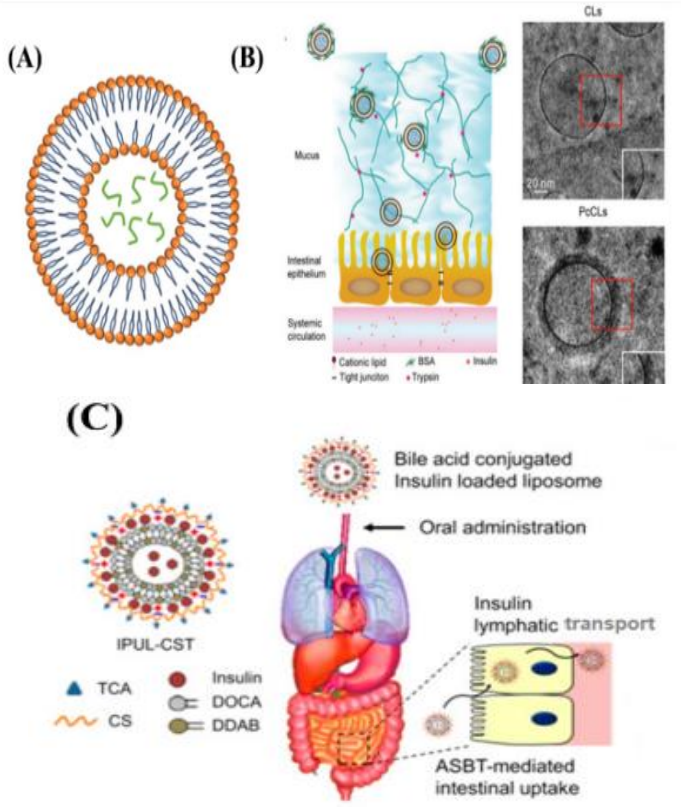

Figure 3. (A) The structure of liposomes. (B)TEM images of CLS and PcCLs, and schematic diagram for the process of the transport of the PcCLs through the mucus layers and epithelial barrier. (C) Schematic diagram of IPUL-CST and its intestinal uptake and lymphatic transport [35].

Several nano-liposomal drugs for oral insulin delivery have been approved for marketing due to their biocompatibility, such as paclitaxel liposomes. Liposomes have a high safety profile so they are broadly used. Still, liposomes have low encapsulation efficiency attributed to its lipophilicity. Because of poor thermodynamic stability and short half-lives of liposomes, nano-liposomes are more appropriate to be used for preparing quick acting insulin preparations [35].

2. Polymer Micelles

Micelles are synthetic amphiphilic colloids [35]. The size range of that colloidal dispersion ranges from 5 to 100 nm. Amphiphilic polymers can be self-assembled in aqueous environments forming a core-shell structure (**Figure 4**). This core can be either fluid or solid core. If the formed core is solid, then it is nanospheres. If the formed core is liquid, then it is called polymer micelles. The core of the micelle is made up of a dense region forming the hydrophobic part. Poorly water-soluble drugs are encapsulated in the core due to hydrophobic interactions. Whereas, the outer surface of PM is hydrophilic [39]. The

hydrophilic blocks that are used for preparing polymeric micelles are polyoxyethylene, polyethylene glycol, polyvinylpyrrolidone. While, the hydrophobic materials are polylactic acid, methyl methacrylate, polystyrene, polypropylene, *etc.* [35]. Polymeric micelles are capable of protecting cargoes from sudden release and enzymatic degradation in gastric juice, and then release insulin in the environment of the intestine, thereby, increasing intestinal permeability and improving the efficiency of insulin delivery. The pH-sensitive polymer micelles are of great significance for diabetes mellitus therapy as they can minimize the burst release under acidic conditions in the stomach, promoting their adhesion and increasing their residence time in the intestine [35, 40]. Hu et al. developed smart pH-sensitive cationic polymer micelles having a core/shell structure which can be self-assembled in the aqueous environment [40]. The micelles were made up of methyl methacrylate (MMA, hydrophobic unit) and methacrylic acid (MAA, pH-sensitive and hydrophobic unit) forming the core. The surface was wrapped with hydrophilic and pH-sensitive poly(2-aminoethylmethacrylate) (PAEMA) [35, 40]. The PAEMA segments provided a protective barrier to enhance the stability of the micelles. When the amine residues of PAEMA were exposed to the acidic environment of the stomach they were drastically protonated acquiring a positive surface. Enhanced drug permeability and bioavailability were achieved by gaining the mucoadhesion function thus opening tight junctions in the intestinal wall [35, 40-43].

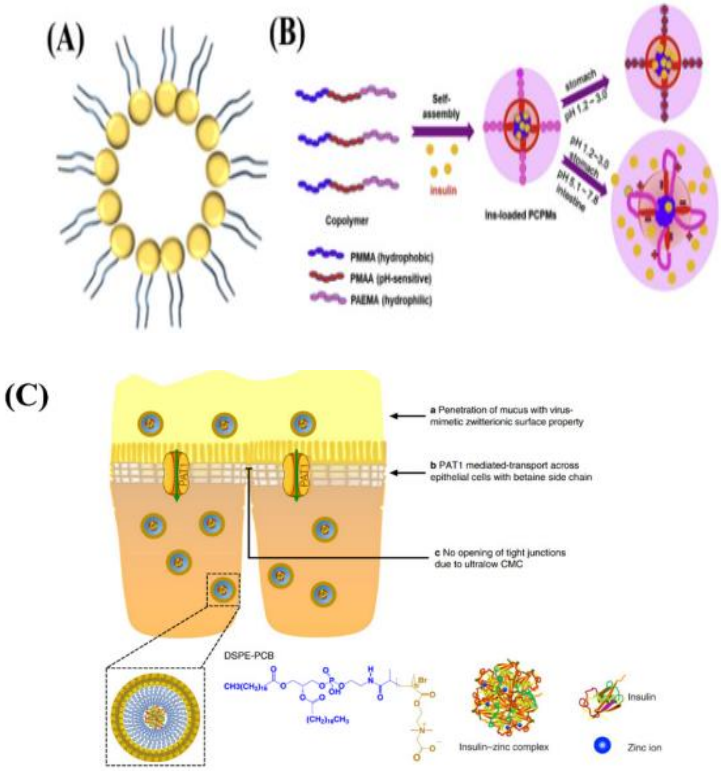

Figure 4. (**A**) Structure of micelles. (**B**) Schematic representation of Ins-loaded PCPMs and its pH-triggered release. (**C**) Schematic representation of DSPE-PCB micelles for oral delivery of insulin [35].

3. Solid Lipid Nanoparticles (SLNs)

They are colloidal particles in the size range between 10 and 1000 nm. SLNs are constituted from natural or synthetic lipids, such as lecithin, fatty acids, fatty alcohols, and other lipid-like materials, and the drug is embedded in the lipid core (**Figure 5**). There are several advantages of SLNs which include low toxicity, avoidance of organic solvent, excellent biocompatibility and biodegradability and high entrapment efficiency of hydrophobic pharmaceuticals. However, SLNs have several disadvantages, such as poor drug loading capacity, short *in vivo* life span, and low physical stability [35, 44]. The drug content in such formulations is low because the drugs have to be soluble in lipids and the preparation technology has many limitations [35]. There are several methods for SLN preparation, such as high shear homogenization, hot homogenization, cold homogenization, ultrasonication or high speed homogenization, solvent emulsification, microemulsion, spray drying method, and double emulsion method [35, 44]. Boushra et al. developed viscosity-enhanced nanocarrier for oral delivery of insulin [45]. This SLN system was prepared from soy-lecithin. The viscosity of this system was enhanced by adding a hydrophobic viscosity enhancer (VA) to the SLN core. Thus, the disadvantage of low encapsulation efficiency by SLN of hydrophilic pharmaceuticals was solved. The oral administration of insulin VEN had demonstrated good hypoglycemic effect in fasted rats with a relative bioavailability of 5.1% [35, 45]. Xu et al. prepared novel SLN with endosomal escape function to avoid the lyso-endosomal degradation in epithelial cells, which is one of the most significant barriers [46]. The shell of these solid nanoliposomes is constituted from soy lecithin and contains endosomal escape factor hemagglutinin-2 peptide (HA2). The core was aqueous and loaded with insulin. Those SLN were prepared by ultrasonication. During intracellular trafficking of SLNs, it was observed that the shell containing HA2 could successfully avoid lysosomal degradation of epithelial cells. Moreover, the amount of insulin that was accumulated in the basolateral side of epithelial cells was much more than that of free insulin. Those nanoliposomes had excellent hypoglycemic response after oral administration in diabetic rats [35, 46]. SLN is better than liposomes in terms of stability and preparation method is simpler. But, the encapsulation efficiency of hydrophilic drugs, such as insulin is low. So, further studies are still needed for the development of oral insulin preparations [35].

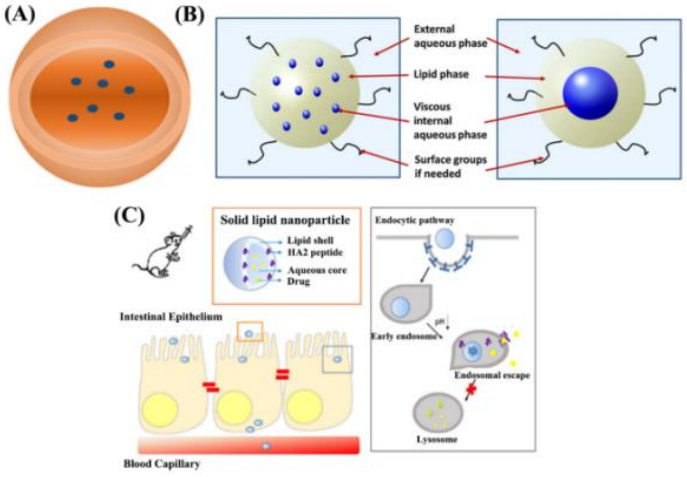

Figure 5. (**A**) Structure of SLNs. (**B**) Schematic representation of possible structures of VEN. (**C**) Schematic diagram of SLN and its behavior in intestinal epithelium [35].

4. Nanogels

Nanogels are nanosized hydrogels with a 3D network structure, formed from physically or chemically cross-linking of one or more hydrophilic polymer networks. When nanogels dispersed in aqueous media, their swollen networks become soft and can accommodate a required volume of water. However, they do not dissolve in water. Nanogels are efficient carriers to encapsulate hydrophilic insulin. Modifications of the monomer polymer make the nanomaterial sensitive to pH value, temperature, and glucose to be released [35, 47]. Li et al. prepared smart responsive nanogels of approximately 200 nm using microemulsion radical polymerization method [48]. The materials used are the pH-sensitive polymer, ethylene glycol dimethacrylate (EGDMA), and the glucose-sensitive monomer, 4-vinylbenzeneboronic acid (VPBA). Hydrophilic phenylboronic acid-glucose complexes were formed as the glucose concentration was increased, and the hydrogel size increased too. Upon exposure to the pH environment of the small intestine, electrostatic repulsion between the polymer chains occurred as the carboxyl group of acrylic acid lost its protons. This nanogel system was further modified with polyethylene glycol-folic acid to produce an encapsulated three-dimensional (3D) HA hydrogel system thus, overcoming multiple barriers and providing multi protection for insulin during the transport process. The polyethylene glycol-folic acid promoted the penetration of the nanogel through the folate receptor on epithelial cells by receptor mediated endocytosis. The HA hydrogel system produced obvious hypoglycemic effect upon oral administration to diabetic rats [35, 48]. There are several benefits of nanogel drug delivery systems, such as being biocompatible, highly hydrophilic, and reducing the toxicity of drugs. On the other hand, hydrogels have poor storage stability resulting in difficulty to maintain drug perseverance [35, 47].

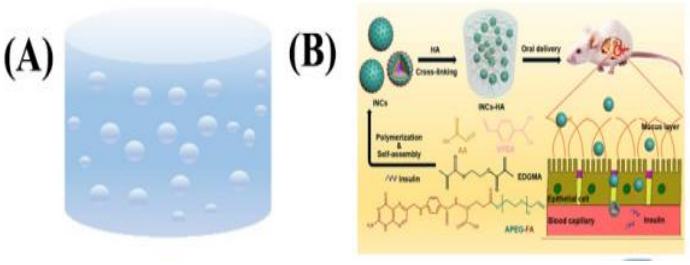

Figure 6. (**A**) Structure of nanogel. (**B**) Schematic representation of insulin-loaded glucose-responsive nanocarriers further encapsulated into hyaluronic acid (HA) hydrogel for oral delivery of insulin [35].

Routes of insulin administration

Since diabetes mellitus results from lack of insulin and defect in its function, the ideal treatment is to provide diabetics with exogenous insulin that mimics normal insulin function. There are several routes for delivering insulin. The following sections will discuss the different routes of insulin administration and the advantages and disadvantages of each route.

1. The subcutaneous route

The subcutaneous route is the most common administration route. However, this route has many disadvantages, such as patient's incompliance from daily injections, hypertrophy and fat deposition at the administration site, peripheral hyperinsulinemia, physiological stress, high cost and risk of infection. Moreover, unlike physiologically secreted insulin, subcutaneous administration bypasses the liver resulting in peripheral hyperinsulinemia. In normal individuals the levels of circulating insulin are decreased by 50% due to the hepatic first-pass metabolism. Other routes of insulin administration include oral, buccal, nasal, pulmonary, transdermal, ocular, rectal and vaginal administration.

2. The oral route

The oral administration of insulin can avoid many of the drawbacks of the subcutaneous insulin as it enters the portal circulation and reaches the liver before reaching the systemic circulation [28-33, 49-53]. As a result, this route does not cause peripheral hyperinsulinemia as it mimics the pathway of the physiologically secreted insulin. On the other hand, this route has low bioavailability as insulin is degraded in the acidic environment of the stomach and it is exposed to the proteolytic enzymes in the luminal cavity resulting in inactivation and digestion. Even so if the drug survives these conditions, it will have poor permeability across intestinal epithelium and cannot penetrate the mucus layer covering the absorptive surfaces of the gastrointestinal tract (GIT) and reach the epithelial cells to enter the bloodstream due to its high molecular weight and hydrophilic characteristics [54-56]. While the oral route of insulin administration is the most acceptable route and it would greatly improve patient compliance many efforts have been made to overcome the previous drawbacks. Manufacturing a novel oral insulin formulation is still the Holy Grail that many pharmaceutical companies are racing to achieve. This route would not just avoid the patient to be subjected to needles, but also it mimics the endogenous insulin pathway as it would enter the hepatic portal system before reaching systemic circulation. Nanotechnology enabled to design various insulin delivery systems to protect insulin from any damaging effects, modify the release profile of insulin from these nanocomposites and/or deliver them to target absorption sites, thus increasing the bioavailability of orally administered insulin. Several studies created nano-formulations based on chitosan (CS) and CS-derivatives, dextran and lipid-based formulations such as coating the drug with liposomes and polymer films. For example, in a study, insulin-chitosan complexes were developed using chitosan/heparin multilayer coatings which are deposited onto insulin-chitosan microparticulate cores in the presence of poly(ethylene)glycol (PEG) in the precipitating and coating solutions [57]. The addition of PEG improved the loading efficiency of insulin. The results showed that nanolayer encapsulation increased the loading capacity of insulin up to 90% compared with non-encapsulated insulin. Also, the blood glucose level was reduced up to 50% in a sustained and dose dependent manner in streptozotocin-induced diabetic mice [55, 57].

In another study trying to deliver insulin orally, a nanoparticle was formulated using poly(lactic acid)-*b*-poly(ethylene glycol) conjugated to the Fc fragment of IgG, which binds with high affinity to the neonatal Fc receptor (FcRn) [58]. The FcRn mediates the transport of immunoglobulin G antibodies across epithelial barriers. *In vitro* and *in vivo* models showed efficient transport of the nanoparticles across the intestinal epithelium and binding with high affinity to FcRn at acidic pH. This model nanoparticle insulin therapy was orally administered at a dose of 1.1 IU/kg to mice and induced a significant hypoglycemic response that was maintained for more than 10 hours [58, 59].

Despite many of those studies were very promising, however the blood glucose lowering effect by entrapped insulin was variable and unpredictable upon oral administration. As a result, variability in insulin absorption throughout the GIT would happen. Owing to these problems, oral insulin still does not serve as a suitable route of administration of injectable insulin.

3. The rectal route

Rectal insulin delivery is another alternative route of administration. It avoids the patient to be subjected to the harmful invasive techniques. Moreover, it avoids many of the problems associated with oral route such as difficulty in swallowing, protection from degradation by the proteolytic enzymes due to the absence of enzymes in the rectum and the harsh acidic conditions of the stomach and avoiding first-pass metabolism if the drug is administered at appropriate distance in the rectum. However, the bioavailability of rectal drug absorption is often lower than oral drug delivery due to the relatively small surface area available for drug uptake. Other disadvantages of the rectal route are inconvenience to some patients and erratic and unpredictable drug absorption as it may be expelled after insertion. Anatomically, the rectum comprises the distal part of the large intestine. The rectum is about 12-19 cm long and it deviates forming three lateral veins; the superior hemorrhoid vein which drains into the inferior mesenteric and portal system, the middle and inferior veins drain from the lower rectum directly into systemic venous circulation. This is why the rectal route somehow avoids first-pass metabolism and facilitate systemic absorption owing to this porto-systemic shunting. However, if the drug reaches the end of the colon, it may be lost in the portal circulation. The rectal epithelium is composed of a single layer of columnar cells along with goblet cells that are responsible for mucous secretion. The rectal surface area is about 200-400 cm^2. This organ does not contain villi or microvilli and its pH is around 7.2-7.4. The mucous layer is about 100 µm thick and it protects the surface epithelia and acts as a barrier for drug absorption. The absorption mechanisms from the rectum are transcellular or paracellular, but no carrier mediated transport has been reported [54-56, 60, 61]. Anyhow, rectal administration of insulin has lower bioavailability compared to subcutaneous administration. So, many absorption enhancers are incorporated in suppositories and designing mucoadhesive formulations to improve the delivery and sustain the release of the formulation. This is indicated in many studies. For example, in a study showing the hypoglycemic response in diabetic and non-diabetic rats upon administration of insulin, the surfactant Cetomacrogol 1000 was incorporated into the microenemata and the results showed that the hypoglycemic response was affected by both the concentration of the surfactant and the insulin dose [54, 62]. In another study showing the effects of glyceryl esters of acetoacetic acid on rectal absorption of insulin it was shown that the level of glucose in the blood was decreased upon administration of insulin suppository containing glyceryl-1,3-diacetoacetate or 1,2-isopropylideneglycerine-3-acetoacetate in rabbits. However, this effect was suppressed upon addition of calcium or magnesium in the suppository. This indicates that the adjuvant interacts with the calcium and magnesium ion located in the rectal membrane and enhances the absorption of insulin [54, 63].

4. The pulmonary route

Pulmonary (inhaled) insulin is another route of insulin administration which has several advantages such as being non-invasive technique. Also, the inhaled drugs are absorbed onto a large surface area as they are absorbed into the alveolar capillary network. Additionally, the alveoli have thin diffusion barrier and the drug is targeted there using an aerodynamic which has a small diameter less than 5 µm. Another main point is that pulmonary drug delivery bypasses first-pass metabolism and the proteolytic enzymes and the harsh conditions in the GIT associated with the oral route. Despite the presence of enzymes in the lungs, those enzymes are involved in other metabolic pathways different from those in the GIT. In a study of using insulin microcrystals with a mean diameter of 3 µm which is given to STZ-induced diabetic rats via the pulmonary route, it causes hypoglycemia lasted for more than 13 hours, as compared to the insulin solution. The hypoglycemic effect was attributed to the sustained release profile of insulin from the microcrystals which were deposited greatly throughout the whole lung [55, 64].

Hyaluronic acid (HA) is a polymer with very useful properties helping in pulmonary delivery of insulin. It has mucoadhesive properties that help in prolonging the contact between the drug with the absorption sites in the lung. In a study a dry powder formed from hyaluronic acid (HA) and insulin were co-sprayed to the lungs of male Beagle dogs. Then, excess zinc ions or hydroxypropyl cellulose (HPC) were added to the dry powder to modify its properties. The results showed that HA formulations comprising insulin extended the mean residence time and the terminal half- life when compared to spray dried pure insulin. Moreover, addition of zinc ions or hydroxypropyl cellulose increased the mean residence time about 9 fold higher and the half- life more than 2 folds as compared to spray dried pure insulin [55, 65].

Absorption enhancers were also involved in many experimental studies to improve insulin delivery by this route. Many studies suggested that phospholipids improved absorption of insulin *via* the pulmonary route. 1,2-dipalmitoyl phosphatidylcholine (DPPC) was incorporated with insulin and to blank liposomes. These mixtures were administered to anaesthetized rats. The results indicated that DPPC increased the hypoglycemic effect compared to insulin and liposome mixture`[55, 66].

5. Buccal route

In this route, insulin is delivered using an aerosol into the oral cavity which is then absorbed through the inner surfaces of the cheeks and the back of the mouth. This is distinguished from oral delivery as in oral delivery the drug is absorbed throughout the GIT. Also, it is distinguished from the pulmonary route where the drug is absorbed in the lungs. The buccal mucosa is highly vascularized, relatively immobile and recovers quickly after exposure to stress. Accordingly, this route is suitable for administration of retentive dosage forms. Drugs administered by thus route enter the systemic circulation through the internal jugular vein bypassing hepatic first-pass metabolism resulting in high bioavailability. Moreover, this route has low enzymatic activity, suitable for drug excipients that can irritate the mucosa, painless, permeation enhancer/enzyme inhibitor or pH modifier can be included in the formulation, and various designs of multidirectional or unidirectional release systems for local or systemic action could be accomplished [55, 67]. Buccal mucosa represents a very important topical route for the delivery of protein and peptide drugs. However, peptides are not often well absorbed through mucosa due to their molecular size, hydrophilicity and the low permeability of the membrane. The mechanism of transport of the peptides through the buccal

mucosa occurs by passive diffusion and is often accompanied by varying degrees of metabolism. To improve the buccal absorption of peptides penetration enhancers are used to increase membrane permeability and/or enzyme inhibitors are added to improve the stability [67, 68]. Other strategies include chemical modifications to improve the stability by minimizing enzymatic degradation. Also, small peptides can be derivatized to produce prodrugs which possess appropriate physicochemical properties comparable to the parent compound by providing a peptide which is more lipophilic therefore facilitating absorption. Derivatization may also protect small peptides against degradation by peptidases present at the mucosal membrane barrier. Few studies have been carried out to demonstrate the effect of the absorption enhancers on peptide transport across the buccal mucosa. There are several classes of enhancers which include surfactants (anionic and non-ionic), bile salts, chelators, fatty acids and alcohols. The penetration enhancer must be non-irritant, non-toxic and physiologically inactive. Penetration enhancers improve the absorption of peptides by changing the rheology of the mucous layer as it reduces the viscosity and/or elasticity of the mucous layer. In addition, they increase membrane fluidity thus facilitating transcellular transport through interaction with their lipidic or proteic membrane components. Moreover, it facilitates paracellular transport [68].

Surfactants are considered to be the most suitable agent for buccal delivery. Although the widespread of surfactant use, it can induce various side effects depending on the type of surfactant, the concentration and duration of exposure. These side effects include; protein denaturation or extraction, enzyme inactivation, swelling of tissue and extraction of lipid components. Sodium lauryl sulfate (SLS) is an ionic surfactant which affects both the protein and lipid structures of the membrane by expansion of intercellular spaces and insertion of SLS molecules into the lipid structure. SLS is proved to be efficient in the improvement of buccal absorption of human calcitonin and insulin [68]. In a study using laurenth 9 as a non- ionic surfactant it was shown that laurenth 9 was a very effective absorption promoter that increases the absorption of insulin through buccal mucosa when used at 5%. This effect wasn't due to its hydrophilic-lipophilic balance (HLB). Rather, this effect was a consequence of the ether link formed between the hydrophobic and hydrophilic portions [69].

Bile salts are another class of natural or semi-synthetic surfactants. Depending on their lipophilicity they enhance the absorption of insulin across the mucous membranes. The lipophilicity/hydrophilicity ratio directly affects the efficiency of insulin delivery. Bile salts solubilize the epithelial lipids, maybe by micellization, thus increasing membrane permeability. Membrane permeability effects have been reported to be reversible and dependent on their concentrations [68]. In a study of using sodium glycholateas absorption promoter it was shown that sodium glycholate effectively promoted the absorption of insulin from the oral mucosa by about 0.5% compared with the amount absorbed upon intramuscular injection of insulin [70].

Another method for improving the absorption of peptides through biological membranes is the chemical modifications of peptides to produce prodrugs and analogues. By this way the peptide is protected against enzyme degradation at the mucosal barrier and rendering the peptide more lipophilic, thus improving peptide transport [68].

6. The vaginal route

The human vagina is a potential non-invasive route for the delivery of topically or systemically acting drugs. This site is very promising for systemic drug delivery due to its large surface area and rich blood supply because of the presence of dense networks of blood vessels (**Figure 7**). The main advantages of vaginal drug delivery over conventional drug delivery systems are avoidance of hepatic first pass metabolism, high permeability for low molecular weight drugs including both hydrophilic and hydrophobic. Even in case of high molecular weight drugs such as peptides and proteins, it has very suitable permeability. Other advantages include reduction in GI side effects observed with orally administered medications. In contrast to parenteral route, it is pain-free and reduces the chance of developing infections. However, even with all the advantages of a vaginal application, there are several drawbacks that include; it is gender specific, personal hygiene and influence of sexual intercourse [71-74]. Moreover, there is a considerable variability in the rate and extent of absorption of the vaginally administered drugs due to the changes in the epithelium thickness of the vagina [74]. Changes in the vaginal membrane thickness are observed during the menstrual cycle and in postmenopausal women whose vaginal epithelium thickness is reduced [73].

The human vagina is an elasticated fibromuscular tubular organ that extends from the vulva to the cervix which is 6-10 cm long. It is located between the cervix, urinary bladder and the rectum. The vaginal wall is composed of three layers: epithelial layer, which is the inner mucosal layer and composed mainly of non-keratinized squamous cells, muscular coat built of smooth muscle cells and external membrane (tunica adventia) that is filled with collagen. The inner surface of the vagina has numerous folds, which are called rugae. The rugae plays a significant role in maintaining tension and stiffness of the whole organ and increases the surface area of the vaginal wall [27-30]. The presence of smooth elastic fibers in the muscular coat also confers excellent elasticity to the vagina. Moreover, the elasticity is further increased due to the presence of loose connective tissue of tunica adventia. There is copious network of blood vessels that supply blood to the vagina which include a plexus of arteries extending from the internal iliac artery, uterine, middle rectal and internal pudental arteries. There is a large venous plexus surrounding the vagina that eventually empties into the internal iliac veins. As a result, drugs absorbed from the vagina does not undergo first-pass metabolism as blood leaving the vagina enters the peripheral circulation via that rich venous plexus which empties into the internal iliac veins [74]. Despite the absence of any secretion glands or goblet cells, the vagina is continuously irrigated by a large amount of secretions that possess vital physiological role. The vaginal fluid is constituted from cervical and vestibular glands secretions, exfoliated epithelium cells, transudation from the blood vessels with desquamated vaginal cells, leucocytes and secretions from the endometrium and fallopian tubes [72, 74]. The vaginal fluid also contains organic compounds and inorganic ions such as proteins (*e.g.*, secretory immunoglobulin A, mucin, immune and exfoliated epithelial cells), carbohydrates, urea, glucose, lactic acid, acetic acid, potassium, sodium, calcium and chloride. In normal physiological conditions the vaginal fluid is thick, clear, or slightly opaque but in case of bacterial, fungal or other infection, the fluid is creamy, clumpy, green, or yellow with a specific odor [71]. The amount and composition of the vaginal fluid change throughout the menstrual cycle. The amount of fluid produced by postmenopausal women is 50% less compared to that produced by women of reproductive age. Sexual arousal also affects the volume and composition of vaginal fluid and so affects the drug release pattern from vaginal delivery systems [74]. Sexual arousal causes an increase in vasocongestion and blood pressure

in the blood vessels which in case permits greater fluid transudation onto the epithelial surface leading to lubrication [72]. The predominant probiotic microbe in the vagina is Lactobacillus. It produces lactic acid through the fermentation of glucose, thus maintaining an acidic vaginal pH in the range of 3.5-4.5. There are several other mechanisms that are involved in the protection of the vagina from exogenous pathogens such as producing antimicrobial bacteriocins, peroxidases, and other organic acids. The microbes are also systematically removed due to exfoliation of the vaginal epithelial cells wall continuously [71, 72]. Moreover, Lactobacilli has another protective mechanism by creating biofilms across the epithelium. Once the bacteria are matured, it produces extracellular polymeric substances (EPS) aiding with colonization of more lactobacilli. In adequate quantity, Lactobacilli are abundant and gain the advantage over other microbes for the resources present on the vaginal epithelium and consequently subside the presence of pathogenic anaerobes [72].

Despite the several challenges to deliver the drug across the vaginal tissues, vaginal drug delivery is a very promising route for drug administration. Like other mucosal surfaces the drug needs to be dissolved from the formulation and diffuses across the gel forming mucus network (GFM) and extracellular polymeric substances (EPS) of the biofilm to be absorbed. Vaginal absorption of drugs has several mechanisms depending on the physicochemical properties of the drug: (1) transcellular route and it is a concentration dependent diffusion mechanism and the drug crosses from the superficial glycogen filled cells till it reaches the lamina propria which is rich in blood vessels allowing for drug clearance, (2) the paracellular or intercellular route by diffusing between the tight junctions of the epithelial cells and (3) vesicular or receptor mediated transport [72].

Using mucoadhesive drug delivery systems has a significant effect on the effectiveness of the vaginal formulations. Adhesion to the mucous membranes permits the formulation to stays longer at the delivery site and allows for a more intimate contact with the site of action. Properly designed mucoadhesive drug delivery systems are of great importance in the case of systemic delivery. Mucoadhesive systems have the capability to minimize the distribution of active pharmaceutical ingredients (API) throughout the vaginal cavity and promote its penetration into the systemic circulation [71].

The vaginal mucous membrane is composed essentially of water, inorganic salts, carbohydrates, lipids, salts, DNA, enzymes, and mucins. So, its characteristics is very similar to the properties of hydrogels [71]. Mucin is a member of glycoproteins and is responsible for forming a hydrogel layer on the external mucosa. Mucoadhesion is the process of adhesion and binding of the polymers with the vaginal mucosa through physical and chemical interactions. There are three stages for the connection and formation of mucoadhesive interactions between the polymers and the mucosal layer. Firstly, wetting, hydration and swelling of the polymer will initiate the intimate contact with the tissues. Secondly, connection and interpenetration of the polymer chains with the chains of the mucin. Thirdly, the interpenetrated chains will form weak chemical bonds, originating from electrostatic attraction, hydrophobic interactions, van der Waals forces and hydrogen bonds [71]. Generally, the stronger the mucoadhesion capability of the polymer is, the residence time of the drug formulation at the application site will be longer. The physicochemical properties of the polymer greatly affect the mucoadhesion capability of the vaginal formulations. These physicochemical properties include its molecular weight, degree of ionization and the presence of functional groups [71]. An increase in the molecular mass of the polymer chain results in an increase in the mucoadhesiveness of a polymer. Moreover, the presence of hydroxyl, carboxyl and amino groups within the polymer structure results in formation of hydrogen bonds between the functional groups of the polymers and the mucosal layer. Generally, the stronger the hydrogen bonding, the stronger is the adhesion. Additionally, the presence of charged functional groups among the polymer chain greatly affects the strength of bioadhesion. Anionic polyelectrolytes create stronger bioadhesion than neutral polymers [75].

Mucoadhesive polymers are categorized into three different groups: 1. Polymers that exhibit adhesive properties after being in contact with water environment, 2. Polymers that interact with mucus membranes by non-specific binding such as non-covalent or electrostatic, 3. Polymers that are capable of binding to specific receptors on the surface of the mucosa [71].

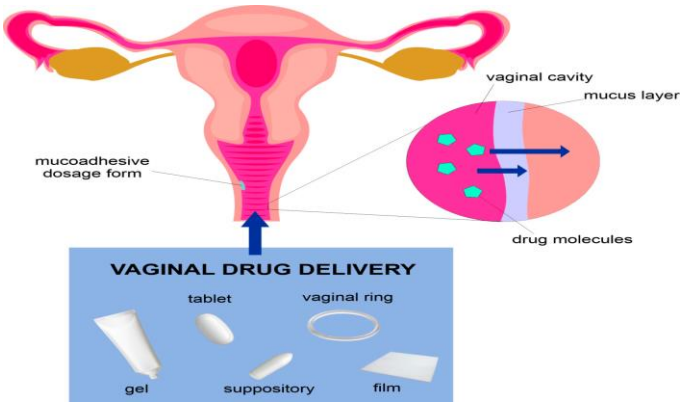

Figure 7. Vaginal drug delivery route of administration.

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