

REVIEW

Developments in encapsulation of insulin: Is oral delivery now possible?

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Abstract: This review presents the possibilities of oral delivery of insulin. Insulin, being readily destroyed/transformed by the proteolytic enzymes and first-pass effects in the digestive system, has mainly been administered through injection, such as intravenous injection and transdermal injection. With developments in the material sciences, appropriate encapsulation methodologies have been developed that could be employed to protect insulin from the digestive effects of the human GI system, and thereby have opened a gateway of research exploring the oral route of insulin delivery. One approach is to incorporate insulin into an emulsion with an appropriate oil-phase, which protects the insulin from degradation. Coating with natural or synthetic polymeric materials, or with lipids, followed by size-reduction to 100-1000 nm is applied as another common approaches of insulin encapsulation. Other approaches like liposomes, nanogels, *etc.* are also being explored. This review gives a summary of methods of preparation as well as *in vitro* and *in vivo* bioavailability of insulin through these methods. It is observed that the oral bioavailability of insulin intake has increased from about 0.1% to about 20% for encapsulated insulin.

Keywords: insulin, oral delivery, encapsulation, materials

1 Introduction

Diabetes is a worldwide disease that causes millions of deaths annually^[1]. The inherent cause of diabetes is the inability of the body to produce or utilize insulin, a polypeptide hormone produced naturally by the pancreatic beta cells of a healthy individual on rise in blood glucose level (after digestion of food)^[2]. This compound controls blood glucose levels by protecting the islet beta cells, promoting the uptake and utilization of glucose in tissues and cells and accelerating glycogen, fatty acid and protein synthesis^[3]. Non-availability of enough insulin during diabetes causes hyperglycemia which leads to various derived disorders, such as diabetic ketoacidosis, hyperosmolar hyperglycemic state, cardiovascular disease, stroke, chronic kidney disease, foot ulcers, *etc.*^[4]. In contrast, if blood glucose level is low, the amount of insulin in the blood will decrease and then glycogen will breakdown to glucose^[2]. Normally, diabetes can be classified into three types: type 1 diabetes

means patients produce no or very little insulin in their bodies^[5]; type 2 diabetes means patients cannot use insulin efficiently though insulin is produced at normal levels^[6]; and a third type of diabetes, also known as gestational diabetes mellitus, which often occurs during pregnancy and disappear after childbirth^[3].

Data published by WHO showed that almost 3 million people over the world die from diabetes each year, with 95% of the cases attributed to type 2 diabetes^[7]. What's more alarming is the fact that if diabetes is not controlled appropriately, the number of death would be 380 million in the year of 2025^[8]. To prevent that from happening, people should pay more attention to the diagnosis and prevention of diabetes.

Insulin (INS) was first isolated and identified from the pancreas in 1922 by Banting and Best, which significantly improves the living standard of patients with diabetes, especially those with type 1. With a molecular weight of about 6000, it is considered a high-molecular-weight protein that can easily be decomposed by the proteolytic enzyme and eliminated by the first-pass effect of the liver. Additionally, due to its high molecular weight, it can easily be aggregated, which makes it hard to be absorbed. In order to overcome such negative effect, people are administered with insulin through injection especially for the intravenous route^[9,10]. Patients with type I diabetes need to be injected with insulin throughout their whole life period once they are diagnosed. This has brought great pain and inconvenience

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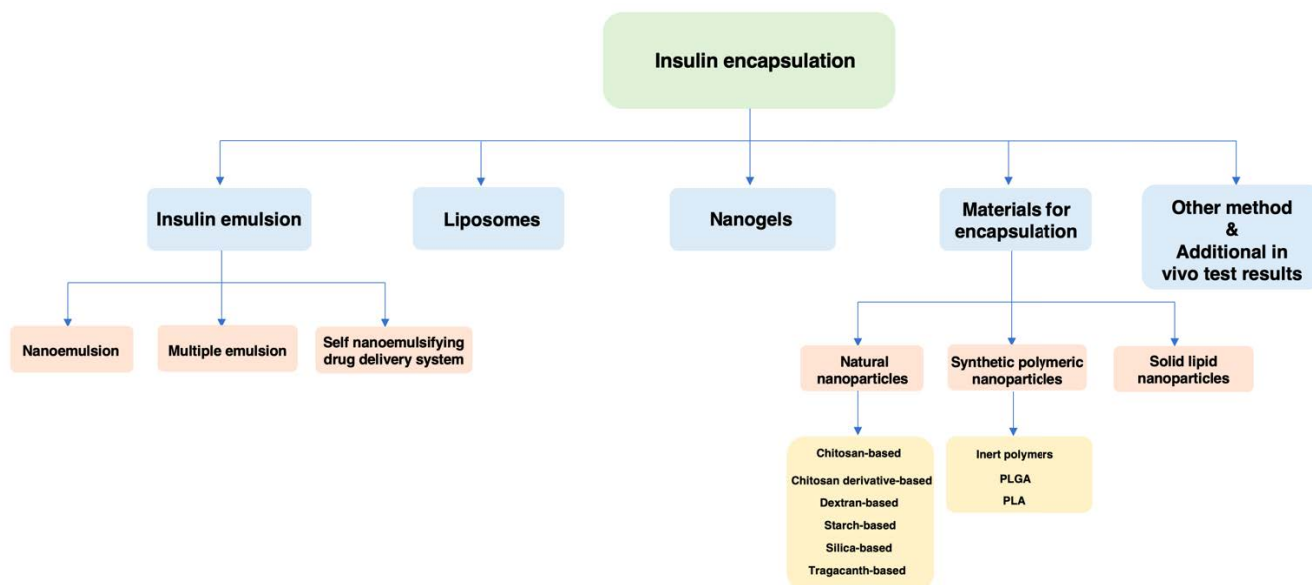


Figure 1. Scope of types and fabrication methods of insulin encapsulation covered in this review

nience to patients, accompanied by various adverse reactions. To reduce the negative effect and enhance the living standard of the patients, new methods of administration are being explored. Despite the appearance of the insulin pump, also known as continuous subcutaneous insulin infusion (CSII), it still brings various health risks to patients due to pump failure, insulin infusion set (IIS) blockage, in-fusion site problems, insulin stability issues, user error, *etc.*^[11] Therefore, researchers have started to pay more attention to oral intake of insulin. However, the bioavailability of taking insulin orally is less than 0.1%^[12], which increases only slightly with use of enzyme inhibitors like those identified from DNA-templated macro-cycle libraries^[13], some absorption enhancers like Gelucire 44/14 *etc.*^[14] A more promising way to enhance the bioavailability of insulin is to encapsulate it with appropriate materials.

Nanoemulsions (NEs), liposomes (LIs) and nanogels are three efficient encapsulation techniques. They can encapsulate and protect the insulin from degradation in the stomach and release them in specific parts of the digestive tract. In this review, developments and approaches of encapsulation of insulin is discussed and emphasized. This review mainly focuses on recent studies on methods and materials used for encapsulating insulin and some in-vivo results of current studies (shown in Figure 1).

2 Insulin emulsion

An emulsion is a fine dispersion of minute droplets of two insoluble and immiscible liquids. Insulin emul-

sion involves dissolving insulin with other liquids and homogenizing the mixture to increase phase dispersion, followed by solvent evaporation the method is often referred to as 'solvent evaporation method'. As defined by International Union of Pure and Applied Chemistry (IUPAC), emulsions are 'clear, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant, often in combination with a cosurfactant'. The aqueous phase contains salt or other ingredients, and the 'oil' could be a complex mixture of different hydrocarbons and olefins. In the case of nano-emulsion, an aqueous solution of insulin is dissolved in an organic solution of polymers. In the case of multiple emulsion, insulin is first dissolved in the aqueous phase, then mixed into oil phase to form water-in-oil primary emulsion, which is then re-emulsified into an oil phase for the formation of the double emulsion. Multiple emulsions provide better encapsulation efficiency with prolonged drug release due to better protection of dissolved drug by 2 layers. In addition, self nano-emulsifying drug delivery systems is another potential method to encapsulate insulin. Depending on the mode of delivery, these emulsions could be lyophilized or spray-dried into nanoparticles, or given directly, or converted into gels. Thus, often a combination of emulsion method with other methods is also demonstrated. The three most common emulsion method of preparation are introduced in this section.

2.1 Nano-emulsions

In contrast to ordinary emulsions, nano-emulsion is a colloidal dispersion system of 10-100 nm emulsion

droplets dispersed in another liquid. They form upon simple mixing of the components and do not require the high shear conditions generally used for the formation of ordinary emulsions. As an oral insulin carrier, aside from good permeability because of their small size, nano-emulsion can also invert phase upon changing the amount of disperse phase and ambient temperature (*e.g.* body temperature), thereby releasing drugs to the bloodstream and inhibit diabetes. The key to forming nano-emulsion is to select an appropriate aqueous phase, oil phase, emulsifier, and co-emulsifier so that the activity of drugs can be maintained in the process of preparation.

Different materials are used to prepare nano-emulsions, such as lecithin by Cilek *et al.* and Eudragit S100 by Jain *et al.*^[15,16] Cilek *et al.* found that the size of nano-emulsion largely dependent on the purity of lecithin and the mixing ratio between surfactant (lecithin) and co-surfactant (alcohol)^[15]. Besides, the nature of nano-emulsion does not change with the storage temperature^[15,16]. Jain *et al.*^[16] prepared nano-emulsion using Eudragit S100 (an acrylic resin pH regulating and releasing agent), and added Polysorbate-20 and Polyvinyl alcohol (PVA) to internal and external water phases as dispersants and stabilizers respectively. After it was given to rabbits orally, their blood glucose dropped to $(76 \pm 3) \%$ within 2 hours and the effect lasted 6 hours^[16]. Thus, this kind of nano-emulsion could be an oral carrier for insulin and could be a potential mean to encapsulate the same. However, duration of activity of nano-emulsions is shorter compared with nanoparticles. Consequently, other effective emulsion techniques are being explored.

Barbari *et al.*^[17] developed a novel nano-emulsion based method to produce ultra-small, water-dispersed nanoparticles from chitosan. The nano-sized (50 nm) water pools of the water-in-oil nano-emulsion served as 'nano-containers and nano-reactors', which could enhance the efficiency of the chitosan nanoparticles (discussed in Section 3). Under this situation, the chitosan nanoparticles could tolerate an extended pH range and showed more ability to penetrate through the Caco-2 cell (15%-19% bioavailability). However, they did not show the *in-vivo* results, which is important to establish this method as the standalone method of insulin administration.

2.2 Multiple emulsion

In order to enhance the efficiency of the emulsion, a bilayer or multilayer emulsion offers the potential to control the diffusion rate of drugs more effectively, sustainably and with better control. On the other hand, multiple emulsion can keep protein and polypeptide drugs in the

internal water phase well and avoid the damage of digestive enzymes in the gastrointestinal tract. The release mechanism of W/O/W insulin from the emulsion is that emulsion particles are diluted, followed by expanding and bursting in a hypotonic environment, during which insulin can be released^[18].

Mutaliyeva *et al.*^[19] developed an approach to encapsulate insulin by a modified double emulsion method (preparative procedure shown in Figure 2). The results showed that this method could optimize the physicochemical properties of insulin-loaded Ch-Xan Gum nanoparticles with a process yield of 65.677.5%, and association efficiency of 85.3%. Additionally, it could control the release of insulin in 3 hours. Cournarie *et al.*^[20] compared the properties of emulsion systems prepared with medium-chain triglyceride and long-chain triglyceride respectively. Long-chain triglyceride had higher viscosity and surface tension. After being taken by mice orally, the insulin emulsion showed a good curative effect. Insulin multiple-emulsions prepared with medium-chain triglyceride had a smaller particle size and was more stable compared to long-chain triglyceride preparations. In order to prepare multiple emulsion with a better effect, these two substances could be combined. Toorisaka *et al.*^[21] reported that W/O/W insulin multiple emulsion shows long-term hypoglycemic activity. They also found that adding vegetable oil to multiple emulsion as a surfactant and using pH-sensitive polymer as an enteric coating material produces a better insulin release behavior. Thus, multiple emulsion methods is now regarded as an established method in literature, and above recent researches demonstrate an emerging potential of this technique being used for oral insulin delivery.

2.3 Self-nanoemulsifying drug delivery system (SNEDDS)

Self nano-emulsifying drug delivery systems (SNEDDS) are emulsion systems that are designed to be spontaneously favorable towards emulsion formation. This happens because of extremely low interfacial tension between the oil and aqueous phases, with the presence of surfactants and co-surfactants. The application of SNEDDS is generating increasing attention because of its excellent bioavailability. A wide range of aqueous phase, oil phase, surfactants and co-surfactants are used by different groups of researchers^[22], and the ratio of oil to Smix (surfactant, solvent and co-solvent) is optimized. Miglyol 840, Captex 355 and Capmul MCM, Lauroglycol FCC, Labrafil M1944CS, Oleic acid, Peceol and Caproyl-90 can potentially be used as oil phases, Tween 80, cremophor EL and DSAB, Transcutol HP can function as surfactants and PEG-400

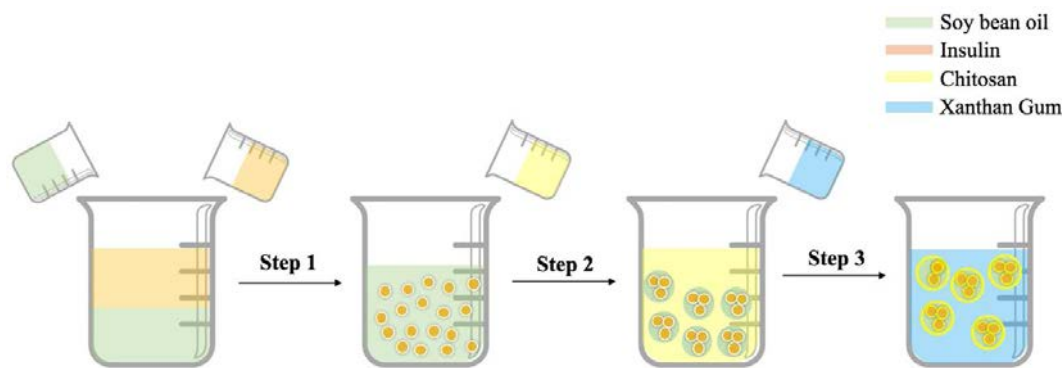


Figure 2. The preparative procedure of insulin encapsulation: (step 1) Emulsification; (step 2) Re-emulsification producing a double emulsion; (step 3) Addition of Xanthan Gum to stabilize the droplets of double emulsion with biopolymers shells^[19]

and Propylene glycol function as co-surfactants. The small droplet-size of less than 200 nm allows for easier cellular uptake. In these research papers, optimized formulations are explored by comparing the emulsification properties as emulsification time and size of droplets, by means of experimental design^[22].

Sakloetsakun *et al.* combined the use of SNEDDS with encapsulated chitosan nanoparticles, taking advantage of the mucoadhesive properties exhibited by chitosan^[22]. SNEDDS has proved to be associated with a small particles size of around 100 nm and high entrapment efficiency of higher than 70%, even up to 90% or higher in some cases. Karamanidou *et al.* also used SNEDDS method to form of an INS/ dimyristoyl phosphatidyl-glycerol (DMPG) ionic complex with 70.89% encapsulation efficiencies. *In vitro* test showed that the developed SNEDDS formulation for oral insulin delivery protected the therapeutic protein from enzymatic degradation by intestinal enzymes (*i.e.*, trypsin, a-chymotrypsin)^[23]. These findings showed that the developed SNEDDS formulation could be considered as a novel delivery system for the oral administration of insulin.

3 Liposomes

The negatively charged liposome is one of the components of most biofilms. It consists of phospholipid bilayers and stays in the form of colloidal dispersion in aqueous buffers^[24]. Liposome mainly consists two-part, one being the inner part, with polar headgroups of phospholipids and the other one being the hydrophobic carbohydrate tails^[25]. After some specific compounds, both hydrophobic and hydrophilic, are encapsulated by the liposomes, it can help these compounds deal with some enzymatic degradation effects. If liposome is combined with biological cells and wraps compounds synchronously, the entrapment efficiency of will be im-

proved and the stability will be optimized^[26,27]. Additionally, it can also help these compounds be released to some target organs. Liposome performs as a carrier and releases insulin through fusion and surface adsorption of intestinal cytomembrane^[28]. The size of the liposome varies a lot by different purposes, ranging from 40-50 nm to 1-2 μm ^[29]. One apparent goal of encapsulating some compounds like insulin into the liposome is that it can enhance the therapeutic of these compounds in many ways. The high affinity of biological lipid membrane can help big molecules permeate somatic cells. Liposome combined with insulin is known as HDVI (hepatocyte directed vesicle with insulin) and can be hepatocyte-targeted. It was of great significance to recover liver function and circulatory function of patients with diabetes as soon as possible^[26]. What's more, because of its biodegradable effect and high bioaffinity, it can be safely utilized as a material of food application *in vivo*^[30].

In order to make it easily used in food administration, it should be designed properly. The key strategy of designing the liposome is controlling the releasing time and location. Lipid composition, surface charge, and vesicular size can all affect this goal tremendously. Moreover, as the fluidity and the rigidity of the bilayer can also affect the release and the control of liposomal compounds like insulin, many studies have been conducted on the effects of phospholipids at different phase transition temperatures on fluidity, and the effects of lipid composition on the rigidity of liposomes^[31].

To make the liposome safer for oral delivery of insulin, Cui *et al* raised a special modified liposome to load insulin. The protective ability of this kind is generated by ergosterol (Er-Lip), which is a type of botanical steroidal, instead of cholesterol. The protective mechanism was similar to that of liposomes containing sodium glycocholate (Sgc-Lip) and superior to that of liposomes containing cholesterol (Ch-Lip)^[32]. In addition, the blood glucose level can decrease to about 50% of the

initial level after this kind of liposomes was taken orally, which was significantly superior to the *in vivo* effect of the Ch-Lip and similar to the Sgc-Lip^[32]. The results of their study showed that liposomes being used to protect insulin for food administration could be both effective and safe.

Zhang *et al.* studied active ingredients in the liposome. Liposome containing DPPC (dipalmitoyl phosphatidylcholine)-cholesterol is used and added with MC (methylcellulose) as a mucosal adhesive. After the liposome being orally administered to rats, it is observed that the blood glucose is dropped. The role of phospholipid component (a composite of DPPC, cholesterol and MC) was very crucial. Besides, pH value, active sites of pro-tamine sulfate and liposome also mattered a lot^[33].

Interestingly, one recent study produced a unique bile acid conjugated partially uncapped liposome to deliver insulin. They first made the chondroitin sulfate-gaurocholic acid coated, insulin-loaded partially uncapped liposome (IPUL-CST). The insulin-loaded partially uncapped liposome (IPUL) was first made by dimethyl dioctadecyl ammonium bromide (DDAB), deoxycholic acid (DOCA), and 10 nm size of superparamagnetic iron oxide nanoparticles dissolved in chloroform. After insulin was loaded into this liposome, the insulin-loaded liposome was then coated with a chondroitin sulfate-TCA conjugate (CST). This unprecedented uncapped liposome can increase the oral bioavailability of insulin to 34% lasting 16 hours^[34].

As a carrier, the problems of not being stable enough, easy to aggregate and coalesce, making liposome hard to absorb wrapped drugs. People tried different methods to overcome these defects, such as adding a second surfactant to the bimolecular film of the liposome, modification with charged chemicals or chemical groups, linking lipid molecules that constitute membrane structure by covalent bonds and production of the polymeric liposome, *etc.* To improve the stability of drug-loaded liposome, scientists linked the outer end of each lipid molecule that formed liposome with covalent bonds, forming polymeric liposome on the surface of the membrane. Recently, Degim *et al.* pointed out that liposome insulin modified by wheat germ agglutinin (WGA) had good absorptivity. The ileum is the best part to absorb liposome insulin. However, the protein degradation and epithelial permeation were main factors affecting the mucosal absorption of insulin^[35]. Wang *et al.* studied the oral absorption mechanism of insulin liposome at the cellular level and found that insulin wrapped by liposome can be absorbed both intracellularly and extracellularly. By comparing intracellular absorption of insulin and insulin liposome using ELISA, it turned out that the permeability

of the latter was significantly higher than that of the former one, suggesting that liposome entrapment can promote the intracellular absorption of insulin^[36].

4 Nanogel

Gel particle is a kind of three-dimensional reticular spatial structure obtained by physical crosslinking. Due to its high hydrophilia, good biocompatibility, it is becoming one of the most promising oral delivery biomaterials. It has recently widely been used in drug delivery and tissue engineering^[37]. Gel particle is crosslinked by weak non-covalent. Therefore, its physical crosslinking can be destroyed under shear effect. Additionally, the hydrogel viscosity can be recovered after it is kept static for a while. Accordingly, it has a reversible transition behavior. This thixotropic property allows the supramolecular gel particles to gradually dissolve and release the drug through oral administration, which can achieve the goal of sustained release drugs in a period of time. These gel particles can also stabilize water-soluble compounds, especially for reducing the enzymatic hydrolysis of peptide compounds like the insulin, and are often used as the study of controlled release systems for water-soluble compounds^[38].

Sibiya *et al.* wrapped human insulin using intestinal biodegradable galacturonic acid gel particles (pectin-insulin, PI particles) and drenched diabetic mice. The results showed that PI particles not only released insulin persistently but also reduced the concentration of blood glucose with concentration-dependency. Moreover, this method also can protect the kidney during the oral application. Thus, it can be seen that this kind of hydrogel had a great prospect and it should encourage further evaluations and development in this method to make it into food administration^[35].

Xu *et al.* found that the hypoglycemic effect of P (MAA-g-EG) copolymer hydrogel containing insulin was significantly correlated to its dose. This pH-sensitive hydrogel was expected to be used as a drug carrier^[40]. Yamagata *et al.* studied the protection mechanism of this hydrogel. P(MAA-g-EG) hydrogels protected internal insulin by forming a copolymerization complex in gastric juice. In intestinal juice, the hydrogel can significantly reduce the degradation rate and the level of the calcium ion in insulin. The protection efficiency of insulin depended on the carboxyl fragment in polymers.

On the other hand, the degradation efficiency to inhibit insulin in intestinal juice was significantly correlated to calcium ion in this kind of hydrogel, suggesting that the loss of calcium ion played an important role in inhibiting the activity of intestinal enzyme. The results showed that

P(MAA-g-EG) hydrogel can be a very good protective carrier in gastrointestinal absorption of insulin^[41].

Currently, hydroxyethyl methacrylate (HEMA), one low fouling material, was employed to produce one novel nanogel through microemulsion polymerization method. Compared to oral insulin administration, this special nanogel had both higher value of cumulative hypoglycemic effect and lower minimum blood glucose (nearly 50% of basal level), which can last for 12 hours^[45]. Thus, nanogel was proved to be an alternative way to delivery insulin via oral administration.

5 Other method

In recent years, EligenTM technology has brought up more and more attention. A compound with low molecular weight is used as carrier. The non-covalent interaction between this carrier and insulin is very weak. It can improve the lipophilicity of insulin and its ability to permeate gastrointestinal epithelium. EligenTM technology is a kind of macromolecule-delivering platform technology developed by Emisphere Technologies, Inc. Emisphere delivery agents, which are based on sodium N-[8-(2-hydroxy-benzoyl)aminocaprylate] (SNAC), interact with the drug molecules to create a weak, non-covalent association. In this situation, the drug remaining chemically unmodified^[46]. After loading of specific compounds in this way, they can be transported across the epithelial membrane^[47]. The mechanism of this method is that due to the weak association between carriers and drugs, the interaction is reversible. In addition, drugs released are supposed to be in the therapeutically active state. Unlike the other traditional penetration enhancers, which may cause some damage to the intestinal epithelium once they penetrate through it, Emisphere delivery agents were purport to keep the intestinal epithelium intact when it goes through the intestinal epithelium and this delivery system are suitable to various groups of compounds whose molecular weight range from 500 to more than 150,000 Daltons. Consequently, this technique could be a potential method to deliver insulin and make it into food.

Starokadomskyy *et al* used N-[8-(2-hydroxybenzoyl) amino] caprylate (SNAC) as a carrier to study how insulin was absorbed by crossing caco-2 monolayer cells. The results showed that SNAC could increase the permeability of insulin by about 10 times, without affecting the permeability of mannitol or damaging cytomembrane. It could also protect insulin from being degraded by protease^[48]. The feasibility of EligenTM technology for delivering insulin orally have been proved both in humans and animals. However, it does not mean that this tech-

nology is perfect, since it may cause some consumer feel nausea. Thus, if researchers want to use this technology as food application, some specific corrigent is necessary^[49-55].

Interestingly, one new ingestible self-orienting system for oral delivery of insulin was developed. It was the first time that the injection method was involved in the oral delivery. Alex *et al.* developed a special pill called ingestible self-orienting millimeter-scale applicator (SOMA) that autonomously positioned itself to engage with GI tissue inspired by the leopard tortoise's ability to passively reorient. It then deployed milliposts fabricated from active pharmaceutical ingredients (insulin) directly through the gastric mucosa while avoiding perforation (Figure 3)^[56]. Inside the pill was a 7 mm long conical cylinder with a conical front end pressed by insulin. The conical front end was aligned with the pill in the direction of gastric mucosa. The cylindrical part under the cone was made of cellulose, and the bottom of the pill was supported by a compressed spring. The cylindrical body was filled with rapidly soluble sugars between the cylindrical body and the small hole. After the pill was standing in the gastric mucosa, the sugar dissolved as the pill got in contact with the gastric mucosa, which will cause a reduction of pressure at the conical front. As a result, the conical front containing insulin would be ejected by the spring that was relaxed due to the pressure difference. The conical front would then dissolve as well, followed by the releasing of insulin (Figure 3). By optimizing the shape and mass center of the pill, the gravity center of the pill was always outside the fulcrum as long as the bottom plane did not contact the surfaces of the gut, and finally stood on the surface of the gastric mucosa. Although the area of the gastric mucosa can be horizontal or moderately inclined, the pill can still stand on it by itself (Figure 3). As a result, when the blood concentration of insulin was more than 20 times lower than that of conventional injection, it was still enough to exert 40% hypoglycemic effect^[56]. However, some weaknesses of this research should be mentioned. First, the blood sugar with the 40% reduction, in the research, referred to fasting blood sugar, which is often several times lower than postprandial blood sugar. That means that the absolute amount of insulin absorbed was not enough to control postprandial blood sugar, while the control of postprandial blood sugar, not basic blood sugar control, is more needed for non-injection insulin administration. Moreover, slow dissolution of the conical end would cause springs to be released slowly, which might possibly lead to the elevation of the pills, especially in the case of inclining.

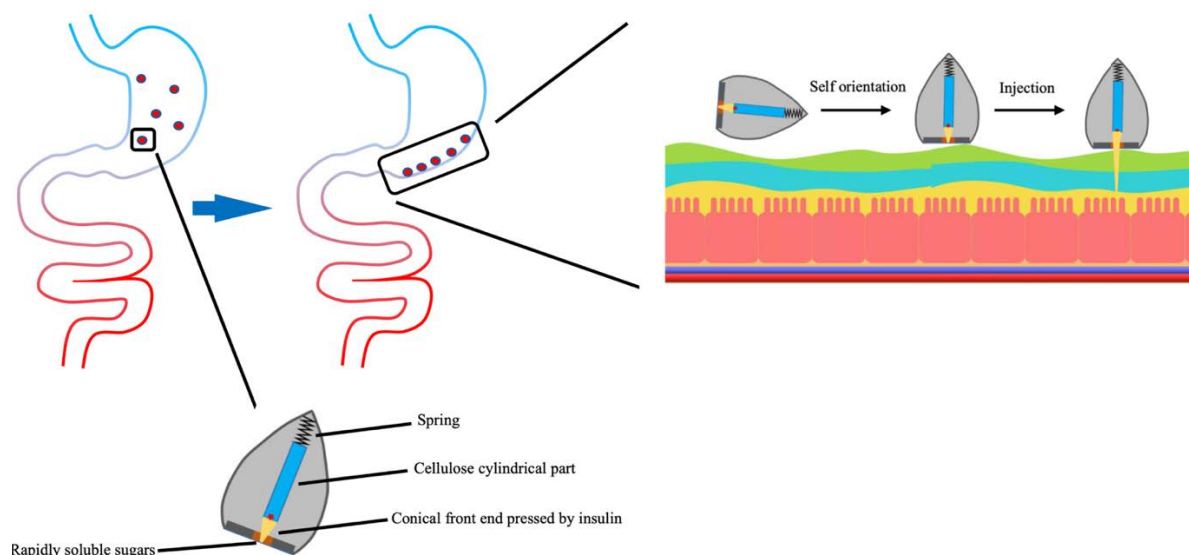


Figure 3. The mechanisms of ingestible self-orienting millimeter-scale applicator

6 Materials used for encapsulation

6.1 Natural Polymeric materials

Natural Polymeric nanoparticles are the nanoparticles made by natural materials. This kind of nanoparticles often has low toxicity. They are therefore used to encapsulate insulin recently (some of them are shown in Table 1). These materials are comprehensively introduced in this section.

6.1.1 Chitosan-based materials

Chitosan (CS) is a kind of polysaccharide consisting of partially deacetylated chitins. It is a polymer extracted from the shells of crustaceans and insects (shown in Figure 3)^[57]. The physical and chemical properties of CS are affected by relative molecular weight, deacetylation degree and the pH. Since CS is positively charged and the mucous layer is negatively charged, CS is easily adsorbed mucosally and can prolong the retention time of drugs in small intestine^[58]. Besides, CS can reversibly open tight junctions between Caco-2 monolayer cells (shown in Figure 4), reduce their transmembrane resistance and increase paracellular permeability^[59].

PA-CS prepared by Mukhopadhyay *et al.* was pH-sensitive and can make drugs invariant in the gastrointestinal tract. Following oral administration to diabetic mice at 50 IU/kg, the relative bioavailability was 11.78%. There was no severe systemic toxicity^[60]. A type of pH-sensitive nanoparticle, PMAA-CS-PEG, was used for oral delivery of insulin, as well. The entrapment efficiency of this nanoparticle was up to 99.9%. The average particle size was 172 nm. The release of insulin in the intestine was twice as much as that in the stom-

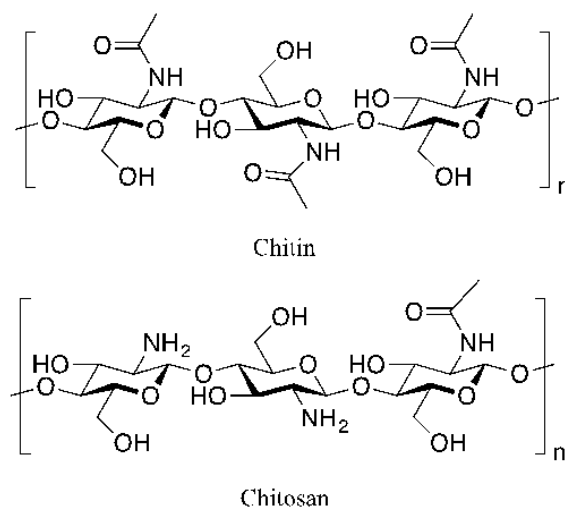


Figure 4. Chemical structures of chitin and chitosan

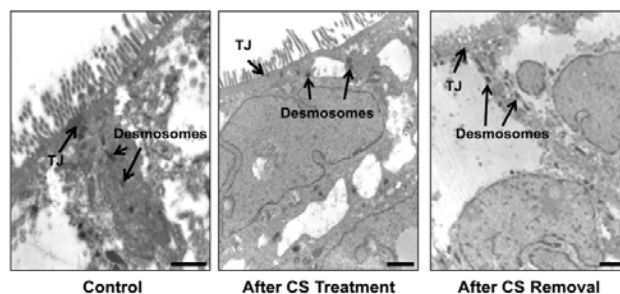


Figure 5. TEM micrographs of the polarized Caco-2 cells before CS treatment (control), after CS treatment and following CS removal (scale bars: 0.5μm)^[59]

Table 1. Natural polymeric nanoparticles prep method, physical properties and results

Preparation method	Particle size	Efficiency / in vitro observation / in vivo observation	Reference
Chitosan modified with valine by conjugating the terminal carboxyl groups of carboxymethyl chitosan (CMCS) to the amino groups of 3-APBA and L-Valine to synthesize CMCS-PBA-LV	190.1 nm	EE: 67% LC: 9.8% Only a release of 16.6% in simulated gastric fluid	[49]
Insulin loaded into the nanocarrier			
Polyelectrolyte complexation is formed between insulin and chitosan/pectin, without the usage of chemical crosslinkers.	240–1900 nm	Production yield maximum: 34.0% EE highest: 62.0% Stable in simulated gastric fluid (pH=1.2) Controlled release over 2h in simulated intestinal fluid (pH=6.8) EE: 50-64% LC: 13-19% In glucose-free medium, after 2h, release is 16% for PMAPBA1/chitosan-SH NPs, 51% for PMAPBA1/chitosan NPs.	[50]
Poly(3-methacrylamido phenylboronic acid) (PMAPBA) complexed with thiolated chitosan (chitosan-SH) forming cross-linked shell.	240-300 nm	At pH=7.4, Initial burst release observed in medium with different concentrations of glucose. Release after 9 h in 0, 1.0, and 3.0 mg/mL glucose concentration were 20, 28, and 97%	[51]
Polyurethane (PU) is incorporated into shell of NPs with alginate (ALG) and chitosan (CS)	90–110 nm	EE: more than 90% insulin At pH=1.2, slight insulin release (PU-ALG/CS 13.7 %, ALG/CS 15.77% and PU-ALG 14.17%) up to one hour	[52]
Insulin cross-linked with chitosan shell		Improved insulin bioavailability (10.36%) Moderate release at pH=6.8 for PU-ALG/CS NPs up to 50% at 10h, sustained release at pH=7.4	
Calcium/alginate gel was formed from water/oil (W/O) nanoemulsion with decrease in pH.	313.2 ± 2.8 nm (ADS-NPs), 338.8 ± 5.8 nm (CS-NPs), 300.8 ± 3.8 nm (ALB-NPs)	EE: 72.4 ± 3.3% (ADS-NPs), 21.9 ± 2.8% (CS-NPs), 30.7 ± 3.4% (ALB-NPs) DL: 10.1 ± 2.8% (ADS-NPs), 4.8 ± 0.4% (CS-NPs), 6.2 ± 1.4% (ALB-NPs) Decrease in efficiency due to extra coating step	[53]
Insulin was entrapment in the NPs matrix with the presence of dextran sulfate.		In SGF (pH=1.2), up to 35% release for 2h, in comparison to 80% for free insulin	
The dual coating was applied by polyelectrolyte complexation.		Release only increased when raising pH from 5.5 to 7.4 Release was more sustained at pH 7.4 than 5.5 for both CS-NPs and ALB-NPs	
Insulin-SeNP is fabricated by ionic cross-linking/ <i>in situ</i> reduction technique.	Approximately 120 to 160 nm depending on different chitosan/insulin ratio, pH and Se concentration	EE: around 80% for most types of particles Insulin-SeNPs showed release of 30% within 12 h in both pH 1.2 HCl solution and pH 6.8 PBS. However, Insulin-SeNPs release was 55% in aerobic condition	[54]
Prepared by emulsification-internal gelation technology.	293±4 nm (5 min ultrasonication+co surfactant)	EE: 99.3%±0.5% In SGF (pH=1.2), bare release during the first 2 hours, followed by a burst release of insulin was noticed under basic conditions (pH 6.8) simulating the intestinal environment	[55]
Insulin complexed with nanoparticle matrix polymers, alginate–dextran sulfate and the complex was pH-stabilized due to electrostatic effect.	248±2 nm (10 min+cosurfactant)	Release almost finished in the following 3 hours	
Insulin is first conjugated with LMWP, which then made into NPs through W/O/W double emulsion	253.8 ± 6.4 nm	EE: 49.3 ± 2.1% DL: 8.2 ± 0.4% Burst release within 1h followed by slow release until 6h. P _{app} value of insulin loaded in NPs treated with LMWP is 1.5 times higher than that of NPs without LMWP treatment.	[56]

ach^[61]. Su *et al.* prepared DTPA- PGA-CS nanoparticles. When pH was greater than seven, the nanoparticles gradually expanded and degraded. By opening the tight junction between intestinal epithelial cells, the absorption of insulin in small intestine can be promoted with prolong hypoglycemic duration^[62].

6.1.2 Chitosan derivative based materials

The PKa value of CS is about 6.5, and the deprotonated form of CS is insoluble in neutral or alkaline environment. At a pH of 7.4, CS will lose mucus adsorption and the ability to open tight junctions^[63]. The physical and chemical properties of CS, such as water solubility, mucus adsorption and intestinal permeability can be enhanced to some extent by chemical modifications. Several CS derivatives have been synthesized and prepared, including trimethyl CS (TMC), sulfhydryl CS and β -cyclodextrin-CS.

Methyl was introduced to CS to make TMC, to improve the solubility of CS in neutral or alkaline environments. By modifying TMC using a targeting polypeptide-CSK polypeptide, we can get CSK-TMC-insulin nanoparticles. Compared with unmodified TMC-insulin nanoparticles, they had lower cell toxicity. Since CSK and intestinal goblet cells have a very good affinity, namely, CSK-TMC nanoparticles are targeted at goblet cells, they can improve the permeability of nanoparticles in the small intestine. In a hypoglycemic experiment, following oral administration of CSK-TMC nanoparticles to diabetes rats (50 IU/kg), the blood glucose level fell by 28% within 3h, 1.4 times as fast as TMC nanoparticles, and the relative bioavailability was 1.5 times as high as TMC nanoparticles^[64]. Liu *et al.* prepared trimethyl CS nanoparticles (T-NPs) and made trimethyl CS nanoparticles (P-T-NPs) using water-soluble poly (N-2 hydroxypropyl methacrylate) as the coating material. Compared with the former, the latter had good mucosa permeability and can better open tight junctions between cells and promote the transcellular transport of nanoparticles. 4 hours after oral administration of P-T-NPs to diabetic rats (50 IU/kg), the blood glucose concentration fell by 36%. The hypoglycemic effect was significantly higher than the same dose of T-NPs. There was no hypoglycemia. The bioavailability was 2.8 times that of T-NPs^[65].

Mercaptide polymer possesses a mercapto structure and is absorbed via mucous. It can form sulfhydryl disulfide with mucin, closely attached to the mucous layer of the small intestine and prolong the effect of insulin. Besides, mercaptide can enhance permeability by inhibiting protein tyrosine phosphatase^[66]. Yin *et al.* prepared N-trimethyl chitosan-cysteine nanoparticles (TMC-CysNPs) (the synthetic route of self-assembled

TMC-CysNP are shown in Figure 5), forming disulfide bonds with the mucous layer. Therefore, mucous adsorption of TMC-CysNP was 2.1 ~ 4.7 times as high as that of TMCNPs. During the *in vivo* studies, TMC-CysNPs can better promote the permeability of insulin in the small intestine of rats and prolong hypoglycemic duration^[67].

Cyclodextrin-insulin compounds can protect insulin from aggregating, degrading and denaturing and make it exist stably. By lowering biological barrier function, researchers can promote the absorption of insulin^[68]. However, cyclodextrin matrix has renal toxicity and poor water solubility, which restricts its role as a pharmaceutical preparation. Researchers can overcome the above problems by preparing cyclodextrin derivatives, such as sulfobutyl- β -cyclodextrin and hydroxypropyl- β -cyclodextrin. Zhang *et al.* prepared positively charged β -cyclodextrin-modified CS nanoparticles. The entrapment efficiency was 87%. Since insulin mainly existed in the chitosan-alginate nucleus and had electrostatic interactions with -cyclodextrin, it wouldn't degrade in intestinal juice^[69].

6.1.3 Dextran-based materials

Dextran is a good carrier for entrapping protein and can provide a stable environment with biocompatibility. The bioactivity of insulin can be maintained during the preparation of nanoparticles and the release of insulin. Besides, the bioactive components in dextran directly combine with targeting ligands, therefore, can solve the poor permeability of polypeptide and protein. In order to further improve the absorption of nanoparticles in the gastrointestinal tract, Chalasani *et al* made dextran and epichlorohydrin into nanoparticles by means of emulsification and cross-linking. The surface of nanoparticles was modified with succinum glycoside and combined with VB12. The nanoparticles can protect 65% 83% of insulin from being destroyed by intestinal protease^[70]. Other Reis *et al.* found that entrapping insulin with alginate-dextran can inhibit the release of insulin in acidic conditions and increase release in neutral conditions and demonstrated that nanoparticle-entrapped insulin had bioactivity using both *in vivo* and *in vitro* bioassays^[71]. Zhenqing *et al.* prepared insulin into Insulin-loaded Sanguis Draxonis nanocapsules (ISDN) using deposition technology. The stability test showed that the activity of most of the insulin in ISDN not only sustained in a solution containing proteolytic enzyme at 37°C for 30 minutes but also sustained at 25°C for 6 months. The ISDN was administered to diabetic mice by 25, 50 and 75ug/kg. Eight hours later, the level of blood glucose fell to (60.5 \pm 2.7) %, (52.6 \pm 2.3) % and (47.3 \pm 3.1) % that of the level before the administration. Addition-

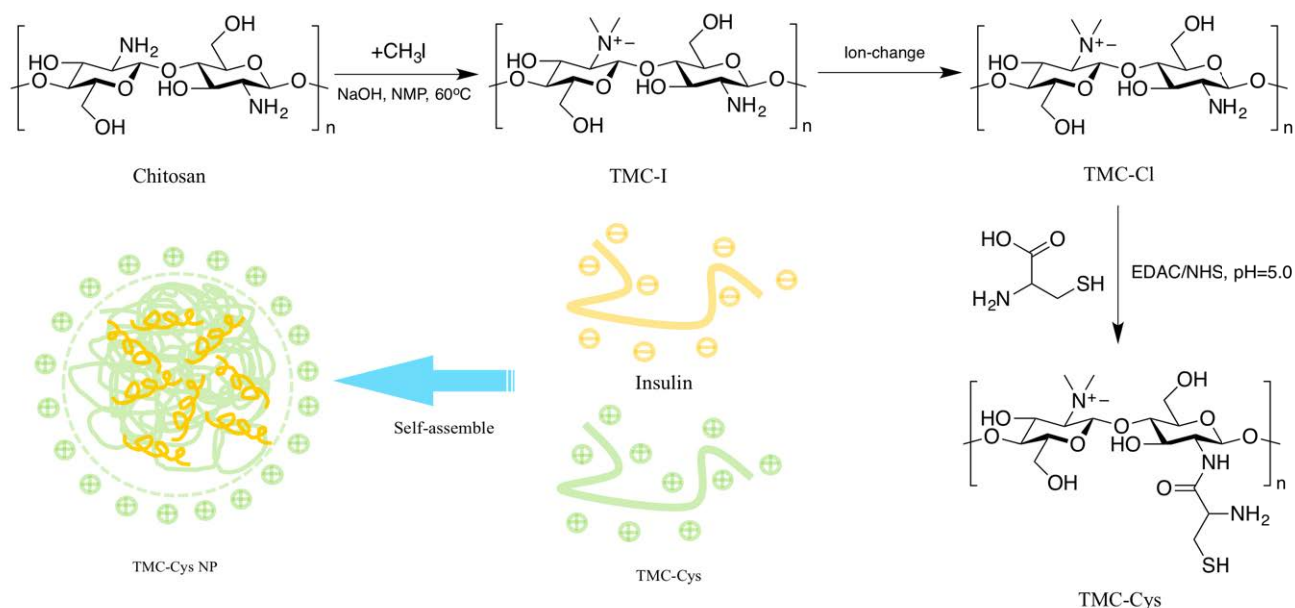


Figure 6. Synthetic route of TMC-Cys and representation of the formation of self-assembled TMC-Cys NP

ally, the reducing effect of blood glucose could last 2 to 4 days, suggesting that ISDN was a stable and effective delivery system of insulin^[72].

Recently, fatty acids and cholic acid were also used to produce chitosan derivative based nanoparticles. Cholic acid modified N-(2-hydroxy)-propyl-3-trimethylammonium chitosan chloride (HTCC) was reacted with lauric acid (LA) and oleic acid (OA) through the amidation reaction to produce HTCC-LA and HTCC-OA. The nanoparticles were produced by the electrostatic and hydrophobic interactions between insulin and the HTCC-LA and HTCC-OA. The particle size was about 280nm with 98% entrapment efficiency. After they were administrated by diabetic mice, the bioavailability of insulin increased 233% and 311% compared with free insulin group. The results showed that fatty acid-modified chitosan could also be an effective delivery system of insulin^[73]. The other novel chitosan derivative based nanoparticles was produced by enterohepatic circulation of bile acids and quaternary ammonium modified chitosan derivative and hydroxypropyl methylcellulose phthalate (HPMCP). The particle size of this kind of nanoparticles was 239 nm with loading efficiency of 90.9%. The *in-vivo* test showed that the hypoglycemic effect can last for more than 24 hours and their bioavailability was 30% even compared with injection^[74]. These results showed that more novel compounds can be selected to modify chitosan to achieve effective delivery systems of insulin.

6.1.4 Starch-based materials

As a type of naturally biodegradable renewable resource, starch is non-toxic, widely-sourced, non-immunogenic, stable in air and compatible with most drugs. It is widely applied in the pharmaceutical field. But some of its inherent characteristics greatly limit its application, such as insolubility in cold water, the instability of starch paste and the tendency of aging and aging under heat, acid and shear action, poor film-forming property and transparency and poor freeze-thaw stability, *etc.* Therefore, it is necessary to modify the original starch to improve its structure and property, promote its function and expand the application scope^[75]. Using starch nanoparticles as the backbone and polyglutamic acid as the branch chain, Zhang *et al.* made a new oral delivery system of protein drugs (SNP-g-PGA). SNP-g-PGA is pH-sensitive and has good stability in acid conditions^[76].

6.1.5 Silica-based materials

Silicon is used to entrap insulin because of its porosity, strong absorbability, superior permeability and slow release, *etc.* Mesoporous silica nanoparticles (MSNs) have good biocompatibility and cellular internalization. An *in vivo* experiment showed that these nanoparticles can improve the permeability of insulin in ileal villus, thereby lowering the blood glucose level^[77]. Zhao *et al.* prepared silica nanoparticles coated by hydroxypropyl methylcellulose phthalate (Ins-SiO₂-HP55). Following oral administration to diabetic rats (30 IU/kg), the blood glucose level remained at levels of 4.85 ~ 2.67 mmol/L,

significantly lower than the normal level^[78]. Considering the function of pure silica is single, Lee *et al* may combine it with the functional polymer or small organic molecules using physical or chemical methods in order to promote the functionality and specificity of functional and intelligent nanoparticles^[79].

6.1.6 Tragacanth-based materials

Biopolymers such as tragacanth, an anionic polysaccharide gum, can be a kind of alternative polymeric carrier for physiologically important peptides and proteins. It can form a polyelectrolyte complex with the insulin-like the chitosan-based nanoparticles. However, current research found the tragacanth-based nanoparticles showed better mucoadhesion than chitosan-based nanoparticles. In addition, Its stability also cannot be affected by the temperature and shearing^[80]. Therefore, tragacanth-based nanoparticles can be a potential method to encapsulate the insulin.

6.2 Synthetic polymeric materials

Fabrication methods associated with nanoparticles made with synthetic polymers are usually double emulsion-solvent evaporation method brought up above. The recent trend of development is to add modifications of nanoparticles, in order to improve encapsulation efficiency, release profile or cell permeability, *etc.* Lal *et al.* applied montmorillonite (Mt) as organic layer of emulsion, which is not only non-toxic and bio-inert itself, but also show excellent adsorption properties of toxins sourced from diet, bacteria, *etc.*^[81] Optimal formulation processes high encapsulation efficiency and decreased burst release in more sustainable ways. Cell-penetrating peptides (R8, Tat and penetratin) and secretion peptide were studied by Zhu *et al.*, and the resulting nanoparticles are proved to have greater permeability with the additional use of secretion peptide compared to penetratin alone^[82]. Liu *et al.* modified nanoparticles with targeting ligand FQSIYPpIK (FQS), by coating FQS modified trimethyl chitosan chloride to cores of insulin-loaded micelles^[83]. In the experiment performed by Sun *et al.*, the nanoparticles initially prepared are of no difference from emulsion-solvent evaporation method, achieving PLGA coated nanoparticles, which then were further encapsulated into pH-sensitive hydroxypropyl methylcellulose phthalate (HP55) by spray drying method^[84].

6.2.1 Inert polymers

Due to the destruction and degradation of insulin in the digestive tract, one of the major challenges of encapsulation it is to overcome the gastrointestinal barriers. The best way to do this is via encapsulating insulin with enteric capsules (using spray-drying for example) or turning them into pellets for oral use. Inert polymer

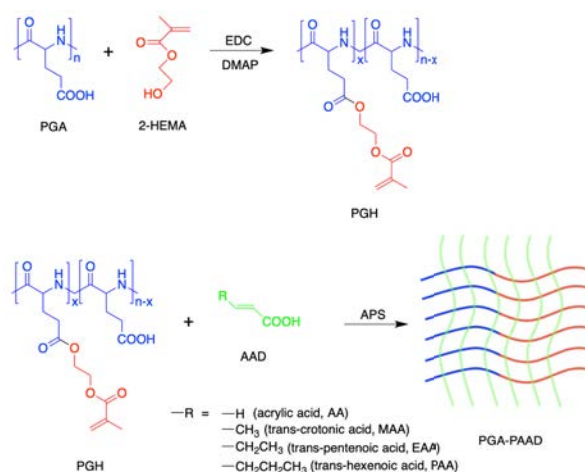


Figure 7. The structure of PGA-PAAD

materials with polyacrylic acid and its ester derivatives (PAAD) have evolved as good candidates for the coating material. The carboxylic groups in PAAD could be a potential inhibitor of some proteolytic enzymes responsible for insulin degradation in GI tract by binding to available calcium ions (Ca^{2+}) critical to the degrading action of these enzymes^[85]. Moreover, this group renders enteric properties to such coatings (*i.e.* protection of insulin in stomach's acidic environment, releasing them under low acid conditions in the small intestine). PAAD is stable in acid environment such as the stomach, followed by releasing insulin into small intestine, which makes itself an appealing candidate for encapsulation^[86]. The amount of coating materials used and their effect on the rate of drug release are also inconsistent among different studies. Besides, permeation performance varies among different inert polymer materials. Thus, it is necessary to design a specific PAAD that can deliver insulin properly.

PAAD has been used for encapsulation of insulin by many groups^[86-88]. Gao *et al* have developed a specific hydrogel made by 4 types of PAAD together with a kind of poly-glutamic acid (PGA) (shown in Figure 6) to control the release of insulin to targets. *In vitro* test indicated that the release rate of insulin was quite lower in AGF (pH = 1.2) in the presence of pepsin. By contrast, insulin can be steadily released in AIF (pH=6.8) in the presence of pancreatin. *In vivo* test showed that after diabetic rats were given insulin-loaded by this material, the hypoglycemic effect was ameliorated and could last for 7 hours^[87].

In addition, Zhang *et al.* raised another special PAAD called CMC-g-PAA (Carboxymethyl chitosan-g-polyacrylic acid). In *in vitro* test, the result also showed it was very stable in acid condition and could release insulin in neutral environment (shown in Figure 7). The *in*

in vivo test showed that after the diabetes mice were administered with 100.0 IU/kg of insulin-loaded-CMC-g-PAA hydrogel, their blood glucose decreased by $34.1 \pm 4.7\%$ and the effect could last for about 9 hours, which was longer than the previous one^[88]. Thus, this material can be a potential encapsulating material for insulin and can potentially play a leading role to make insulin into some food products.

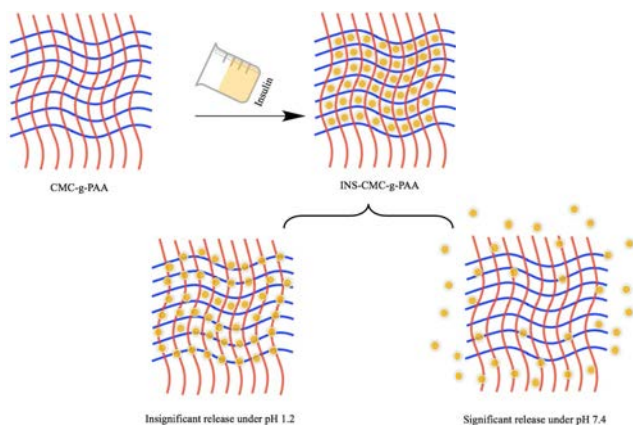


Figure 8. The result of the *in vitro* test of the CMC-g-PAA

6.2.2 PLGA

Poly (lactic-co-glycolic acid) (PLGA) is polymerized by lactide (LA) and glycolide (GA) and widely studied in terms of oral insulin preparations. Modification of PLGA nanoparticles with cell penetrating peptides and secrete peptides can help insulin permeate intestinal epithelial cells, thus improves its bioavailability. Research on PLGA nanoparticles suggested that PLGA nanoparticles had uniform sizes and high entrapment efficiency. Burst release could be reduced as well. After Diabetic rats were given PLGA nanoparticles at 10 IU/kg, their blood glucose fell more significantly within 14 ~ 16 hours than those taking uncoated nanoparticles^[82]. Intestinal epithelial cells contain lots of folate (FA) receptors. The particle size of specific PLGA nanoparticles (FA-PEG-PLGA nanoparticles) prepared by Jain et al using multiple emulsion solvent evaporation was 260 nm. The entrapment efficiency was $(87.0 \pm 1.92)\%$. Compared with the subcutaneous injection of insulin solution, oral administration (50 IU/kg) would not cause as much hypoglycemic shock to diabetic rats. The hypoglycemic duration was about 3 times that of subcutaneous injection. Compared with PEG-PLGA nanoparticles, it had better hypoglycemic effect^[89]. Additionally, Malathi *et al.* prepared natural water-soluble VE and PLGA nanoparticles coated with emulsified polyethylene glycol (TPPLG nanoparticles). The maximum entrapment efficiency was $(78.6 \pm 1.2)\%$. After taking PLGA (70/30) nanoparticles, blood glucose level fell at

a rate of 3 times as in the case of oral insulin solution. As reported by histopathological studies, TPPLG nanoparticles could recover damages to liver, kidney and pancreas caused by streptozotocin, suggesting that nanoparticles had both biocompatibility and repair effect^[90].

However, depending on the different synthetic process, the relative molecular weight of PLGA and molar ratios of LA and GA differ from each other as well. By changing the ratios of LA and GA, degradation of polymer and subsequent release rate of drug *in vivo* can be controlled. The metabolites of PLGA *in vivo* are CO_2 and water, which are non-toxic and non-irritant and have good biodegradability^[91].

6.2.3 PolyLactic Acid (PLA)

PLA Vesicles are a special kind of nanoparticle with a bilayer structure. It can entrap both hydrophobic and hydrophilic drugs and control drug release effectively, so as to achieve the purpose of sustained release. Xiong et al synthesized two kinds of bilayer vesicles, which were hydrophilic inside and hydrophobic outside. The first kind of the bilayer vesicle was taking PLA-F127-PLA (the formula and the synthetic route of PLA-F127-PLA is shown in Figure 8) as a carrier of insulin. The *in vivo* experiment found that within 4.5 h, the blood glucose concentration fell from 18.5 mmol/L to 5.3 mmol/L. Five hours later, the blood glucose concentration was at the minimum level of 4.5 mmol/L and remained for at least 18 hours^[92]. The other kind, PLA-P85-PLA, was synthesized (the formula and the synthetic route of PLA-P85-PLA is shown in Figure 8) as a carrier of insulin. An *in vitro* release experiment showed that after 7.5 hours of administration, insulin was completely released. Following oral administration to diabetic mice (200 IU/kg), the blood glucose concentration reached the minimum level, 15% of the original blood glucose concentration in 2.5 hours. 10.5 hours later, it rose to 31% of the original and remained at this level for about 14 hours^[93]. Chen *et al* prepared insulin polylactic acid nanoparticles (INS-PLA-NPs) by 'solvent-nonsolvent' method. The average particle size was (84.34 ± 1.476) nm. The average coating rate was $(65.93 \pm 1.345)\%$. The average drug loading rate was (0.62 ± 10.03) U/mg. After INS-PLA-NPs were administered to diabetic mice at 50, 60 and 80 IU/kg respectively, their blood glucose all fell significantly^[94]. Therefore, with respect to blood glucose level control, different PLA vesicles have an impact and can be used as a potential encapsulation method for oral intake. Similar to PLGA, the *in vivo* mesostate of PLA is lactic acid and the metabolites are CO_2 and water. It shows good biodegradability. However, the high number of ester bonds in PLA worsen its hydrophilicity and lowers biocompatibility. Immune rejection is another con-

cern, resulting in inflammation and infection, followed by thrombosis and even lesions^[95].

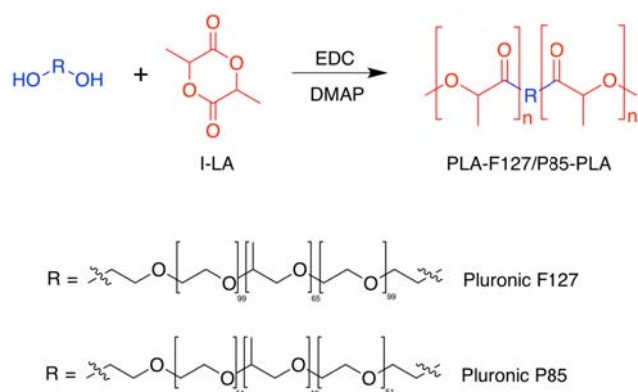


Figure 9. Molecular Formulas and synthetic routes of PLA-F127-PLA and PLA-P85-PLA

6.3 Solid lipid materials

Using solid phase lipid as the oil phase, the entrapment efficiency of solid lipid nanoparticles was proved to be lower compared to polymeric nanoparticles, as shown in the Table 2 below. This is due to the hydrophobic nature of the lipid^[96]. Double emulsion-solvent evaporation is the technique mostly used in solid lipid nanoparticles.

7 Absorption mechanisms of different encapsulation methods

The goal of all the methods (nano-emulsions, multiple emulsions, liposomes and nanogels) is to safely transport insulin through the stomach, help it to intactly penetrate through the intestinal mucosa and epithelial cells and release it into the blood (Figure 10). Thus, they are more efficacious ways to deliver insulin compared with other oral consumption. Compared with other methods, multiple emulsions have a two-step release mechanism. They can first release the inner small particles after they go through the stomach and then the insulin can be carried by the small particle to penetrate the intestinal layers and released into the blood (Figure 10)^[83]. For liposomes, an additional releasing behavior should also be mentioned. As the lipid bilayer of liposomes can fuse with other bilayers such as cell membrane, it can fuse to the cell membrane and the insulin can be endocytosed by the cell directly (Figure 10).

8 Additional in-vivo test results of most recent research with highlights

Various efforts have been made in order to enhance the hypoglycemic effect. This is often achieved by slight

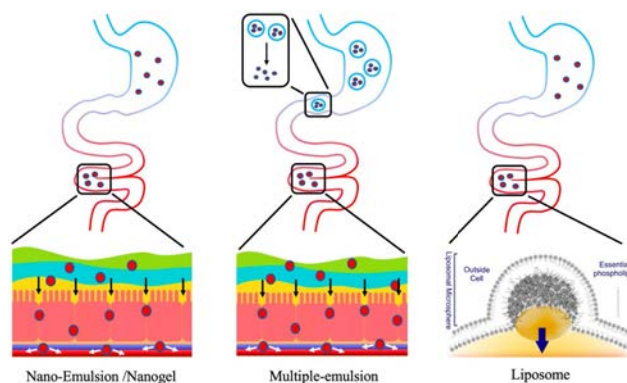


Figure 10. Absorption mechanisms of different encapsulation techniques

modification of the structure of natural polymers used in polymeric nanoparticles or the core of solid lipid nanoparticles. Hypoglycemic effect is most often illustrated by bioavailability or pharmacological availability (PA). Being supplementary to the *in vivo* test results incorporated above, in this section, a brief overview is provided in light of structural characteristics, permeability tests in monolayer caco-2 cells where applicable, as well as test results *in vivo*.

L-Valine was incorporated into chitosan-based nanoparticles in order to facilitate the absorption at the site of small intestine and the relative pharmacological availability was found to be much higher with a value of $7.55 \pm 1.32\%$ compared to that of free insulin (less than 0.8%)^[82]. The combination of N-trimethylated chitosan (TMC) and N-(2-hydroxypropyl) methacrylamide (HPMA) polymer (pHPMA) derivative could promote the mucosal adhesiveness and permeability at the same time, giving final bioavailability as high as 8.56% ^[65]. Chitosan nanoparticles with trans-activating transcriptional peptide (Tat), a cell-penetrating peptide being incorporated, had hypoglycemic effect of 6.89 and 1.79 times higher than that of PVA-NPs and CS-NPs respectively. Moreover, it was worth noticing that this type of nanoparticles can reach the colon with an ideal physiological environment for absorption^[82]. Another study relevant to Tat showed 3, 4, 2.16 and 1.73 folds for cellular transport, cellular uptake, pharmacological availability and bioavailability was achieved respectively for Eudragit S100 (ES)-Tat-chitosan nanoparticles, compared to nanoparticles with ES coating only. It was also worth noticing from the *in vivo* test that maximum hypoglycemic effect was achieved at 4 hours at level of 66.06% , which lasted for 12 hours^[101]. N-(2-hydroxy)-propyl-3-trimethylammonium chloride modified chitosan (HTCC-CA) nanoparticles, targeting liver, could achieve a time as long as 2-11 hours is

Table 2. Solid lipid nanoparticles prep method, physical properties and results *in vitro*

Preparation method	Particle size	Efficiency / <i>in vitro</i> observation / <i>in vivo</i> observation	Reference
Solid lipid nanoparticles achieved by w/o/w double emulsion-solvent evaporation, with the incorporation of three types of hydrophilic viscosity-enhancing agent (VA) to SLN cores	insulin-VEN: 205. ± 23.4 (before incubation) 210.3 ± 9.5 (after incubation at pH=2.5) 208.1 ± 24.2 (after incubation at pH=6.8)	EE: 54.5% (70% w/w PG) No peaks generated by degradation products were shown in the chromatograph Relative bioavailability of 5.1%	[97]
Forming cationic solid lipid nanoparticles (cSLN) using positively-charged DOTAP as organic phase and double emulsion-solvent evaporation.	217-289 nm	LD: 30% to 42% Burst release (35 ± 4% for F1 and 46 ± 5% for F3) in the first 30 min, followed by more sustained release until 300 min Transport monolayer cell rises from 1.16 ± 0.27% for free insulin to 2.89 ± 0.39% for cSLN EE: 23-56%	[98]
SLNs were made by double emulsion solvent evaporation (w/o/w) method, using glyceryltrimyristate (Dynasan 114) as lipid phase and soy lecithin and polyvinyl alcohol as primary and secondary emulsifier	91 ± 5.12 to 250 ± 6.12 nm	Apparent permeability of 16, compared twice as much as pure drug approximately five times of pure insulin solution when loaded in SLN (8.26% versus 1.7% only <i>In vitro</i> : Between 2 and 3 h, burst release of 22% and 50% in pH 1 and 7.4 respectively pH 7.4, 67% release in 12 h; pH 1, 40% release in 12 h.	[99]
Materials: VB12-stearate + sol-gel + phospholipids Method: double emulsion and solvent-evaporation and hydrogel technique	367.8 ± 12.6 nm	EE: 55.9% <i>In vitro</i> : lower burst release of 10% in the first 2 h <i>In vivo</i> : PA = 9.31% Effect up to 12 h	[100]

obtained for maintaining blood glucose level of lower than 70%^[102]. Folic acid was also used for enhancing cell permeability by promoting endocytosis. Blood glucose level of 50% of the initial is maintained for 12 hours with the bioavailability of 7.77% and pharmacological availability of 7.22% being obtained^[103]. The use of hydroxypropyl- β -cyclodextrin on chitosan nanoparticles could achieve bioavailability of 20.32%, due to the protective effect of hydroxyl and carboxyl group. However, the lowering of blood glucose level was not at high extent, though it could be maintained for 6 hours^[104]. Mediated by SAR6EW, a novel cell-penetrating peptide, the cellular uptake was greatly improved, the blood glucose level dropped to 49.3% in 3 hours and maintained at level of 81.6% for more than 8 hours^[105]. With deoxycholic acid modification of the chitosan nanoparticles, the relative bioavailability rose from 7.2% to 15.9% by exploiting bile acid pathway. The hypoglycemic effect lasted for up to 12 hours with a constant decrease in blood glucose level to about 50% of the original^[106]. The incorporation of cysteine or cell penetrating peptide exhibited 17 and 12 permeability

across intestinal cells, with 1.86 to 2.03 relative bioavailability^[53]. Insulin loaded alginate nanoparticles could increase the blood flow at site of action, with the bioavailability of 20.2%^[107]. The application of dextran-b-poly(lactide-co-glycolide) polymersome also enhanced adhesiveness to GI tract, with permeability proved to be 16.89% and 9.34% for two types of DEX nanoparticles. Hypoglycemic effect also lasted for more than 12 hours, with blood glucose level decreased to a quarter of the original level^[108].

Similar modifications have also been applied to solid lipid nanoparticles. Viscosity-enhancing agents have been applied to solid lipid nanoparticle core. This was mainly to enhance encapsulation efficiency by preventing drugs from going to the aqueous phase. Concerning bioavailability, there was not much effect^[97]. The development of cationic solid lipid nanoparticle using cationic lipid for better bioadhesion properties^[98]. Another study employing glyceryltrimyristate (Dynasan 114) as lipid phase and soy lecithin and polyvinyl alcohol as primary and secondary emulsifier could produce nanoparticles with 5 times relative bioavailability (8.26%) of that for

free insulin (1.7%)^[109]. Vitamin B12 could be used to promote transport efficiency across the membrane, and therefore Vitamin B12 coated solid lipid nanoparticles could achieve pharmaceutical availability of 9.31%^[110].

9 Conclusions and Future Trends

In recent years, research on insulin encapsulation is becoming more and more mature for oral consumption. As the encapsulation methods can protect the insulin from the enzymes and acid condition, the bioavailability of the insulin can be maintained through the gastrointestinal route. With the improvement of the carrier materials, the sorbafacient and the preparative methods, the bioavailability of insulin's oral preparations is increasing so that there is a higher likelihood of oral delivery. However, related issues still exist, which hinders it from being ideal for consumption: (1) in the preparation and storage of the oral insulin, how to maintain the physiological activity of it, especially for the storage process? (2) how to further reduce the inactivating effects of the digestive enzyme and the first-pass effect? (3) how to stabilize the configuration of insulin, which can reduce the multimerization of insulin molecular to promote the absorption of insulin in the intestine? Currently, insulin encapsulation is focused on inert polymer materials, some different kinds of nanoparticles, various emulsions, gel particle, liposomes and some other technologies. Taking this into consideration, some specific nanoparticles, the pH-sensitive hydrosol and the Eligen™ technology seems to be the more promising methods to encapsulate insulin effectively. If appropriate absorbefacients, crosslinking agents and enzyme inhibitors are selected in the combination of control of the accurate release time, targeting location and the duration time of insulin, bioavailability will certainly be improved, which is a key factor for optimal encapsulation of insulin for oral delivery. Besides, encapsulated insulin can also be used for buccal mucosal administration. The particle size of the them are small, and the contact area with the mucosa is large, which is favorable for rapid release and absorption^[111]. What's more, the self-orienting millimeter-scale applicator (SOMA) proved the feasibility of the combination of the oral delivery and the injection method. This is perhaps the future of insulin oral delivery.

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