An overview of natural polymers for oral insulin delivery

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Current therapy for diabetes mellitus through oral anti-diabetic drugs and subcutaneous administration of insulin suffers from serious disadvantages, such as patient noncompliance and occasional hypoglycemia. Moreover, these approaches doesn't mimic the normal physiological pattern of insulin release. Oral route would be the most convenient and preferred route if it is available. Polymeric nano and/or microparticles, either natural or synthetic have been used as matrices for oral insulin delivery. Natural polymers are of particular interest due to their nontoxic, biocompatible, biodegradable and hydrophilic nature. Among the natural polymers used for oral insulin delivery, chitosan (CS) is widely explored owing to its ease of chemical modification and favorable biological properties. In addition, many advantages such as safety, biodegradability, widespread availability and low cost justify the continuing development of promising insulin delivery system based on CS.

Diabetes mellitus, a major endocrine disorder is characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism that results in defects in insulin secretion, insulin action or both [1]. Diabetes mellitus is the sixth most common cause of death in the world. Diabetes mellitus is classified into four categories: Type 1, 'insulin-dependent diabetes,' Type 2, 'noninsulin-dependent diabetes,' Type 3, 'other,' and Type 4, 'Gestational diabetes mellitus'. People with type 1 diabetes mellitus do not produce enough insulin to sustain life and become dependent on exogenous insulin for survival. By contrast, people with type 2 diabetes are not dependent on exogenous insulin for survival. Over time, many of these individuals will show decreased insulin production, thereby requiring exogenous insulin for adequate blood glucose control, especially during times of stress or illness. The World Health Organization (WHO) estimates the number of diabetics to be around 380 million [Diabetes: http://www.indiastat.com/health/16/diseases/77/diabetes/22070/stats.aspx] in 2030. India is host to the largest diabetic population in the world with an estimated 35 million people, amounting to 10% of the adult population followed by China and USA. Governments and various healthcare providers around the world are investing in health education, diagnosis and treatments for this chronic disorder. Consequently, it is one of the largest sectors in the global healthcare industry in terms of market value.

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Insulin

The discovery of insulin (Fig. 1), a pancreatic polypeptide hormone, by Banting and Best in 1921 revolutionized the treatment of diabetes. Potential routes for insulin administration are oral, pulmonary, buccal, rectal, transdermal, parenteral, nasal and vaginal [2]. Since the discovery of insulin, it is delivered to diabetic patients exclusively through the subcutaneous route. The usual duration of action is relatively short (i.e. four to eight hours) and therefore two to four daily injections are required for proper control of severe diabetic conditions. Although the parenteral route is satisfactory in terms of efficacy, the stress and discomfort of multiple daily injections provoked numerous attempts to develop a safe and an effective noninvasive route for insulin delivery [3]. Oral administration of insulin is a viable alternative to injections. In addition to patient compliance, on oral administration (Fig. 2), insulin is directly channeled from the intestine to the liver and a high level of insulin is reached in the portal blood, stimulating the physiological secretion pattern of the pancreas [4,5].

Before reaching the blood stream, insulin has to travel through three different organs through stomach, small intestine and the colon intact (i.e. they should not lose their conformation). After

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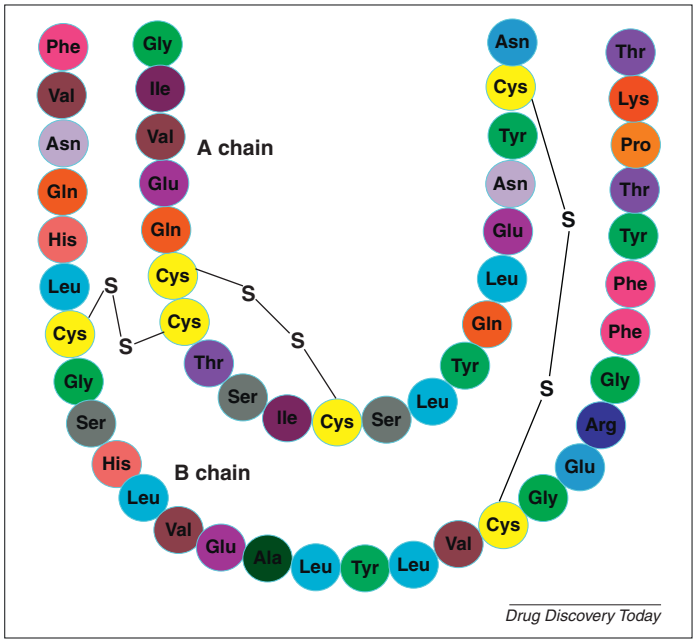

FIGURE 1

Structure of insulin.

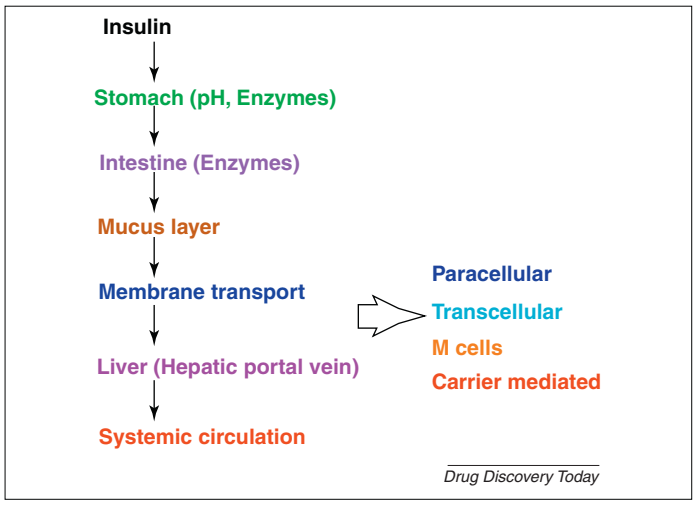

FIGURE 2

Route of oral insulin absorption into the bloodstream.

oral ingestion, insulin has to surpass gastric enzymes, such as pepsin, trypsin, chymotrypsin and carboxypeptidase in the duodenum. As the proteins reach the brush border they are acted upon by exopeptidases, in addition to insulin degrading enzymes present in the cytoplasm of absorptive cells. Three possible mechanisms can be suggested for intestinal uptake of insulin and/or insulin released from nano/microparticles: (i) uptake by paracellular pathway, (ii) transcytosis or receptor-mediated transcytosis, transport through the epithelial cells of the intestinal mucosa and (iii) lymphatic uptake through the M cells of the Peyer's patches mostly abundant in the ileum [6,7].

Attributes of ideal oral insulin carrier

A drug carrier for insulin should:

(i) Have increased resistance against gastrointestinal (GI) enzymes and pH gradients.

(ii) Provide a stable and biocompatible environment to ensure that the main fraction of insulin will be biologically active following encapsulation and stabilize and preserve physiological activity during both particle processing and insulin release.

(iii) Reduce or even better avoid enzymatic degradation and should increase insulin permeability within the intestinal membrane.

(iv) Once absorbed through the epithelial cell layer, released or particulate insulin can interact with cell-surface receptors or be captured by lymphatic cells, or pass through or be entrapped in the lymph nodes or transfer to the blood, provided that the particles remain intact and particle size are below a certain threshold limit [8,9].

(v) Prolong its intestinal residence time, thereby increasing the permeability of the mucosal epithelium to enhance the absorption of drug and providing the intact drug to the systemic circulation.

(vi) Deliver an accurate amount of insulin fast enough to control the glucose concentration in blood, and the function has to be reproducible each time insulin is delivered.

(vii) Be safe after oral administration.

Due to their large size, hydrophilicity, susceptibility to enzymatic degradation and poor absorption characteristics across the intestinal barrier, orally delivered insulin has low bioavailability [9]. The use of permeation enhancers, protease inhibitors, polymeric delivery systems, or chemical modification of proteins are the recent strategies being investigated to orally deliver proteins [10]. Most of these strategies have produced promising results, such as pH sensitivity, enzymatic inhibition among others, but the bioavailability is still low [11]. A combination of these strategies could result in the most successful method for oral protein delivery. Delivery systems containing mucoadhesive polymers promises several advantages that arise from localization at a given target site, prolonged residence time at the site of drug absorption and an intensified contact with mucosa, increasing the drug concentration gradient. Consequently, bioavailability of the drug is increased leading to improved patient compliance [12].

Polymers in oral insulin delivery

Over the past few decades, increasing attention has been paid to the use of polymeric nano/microparticles as carriers for oral insulin delivery [13]. A variety of both biodegradable and non-biodegradable polymers have been investigated, but non-biodegradable polymers pose problems of toxicity, difficulty in removal and also sustained release of insulin cannot be achieved using these polymers. Biodegradable polymers shield the encapsulated drug from the external harsh conditions and might also favor the uptake by the intestinal cells. Biodegradable polymeric particles will isolate the encapsulated drug from the external medium thereby protecting the peptide from the peptidases, enabling their uptake by enterocytes. Depending on the nature of the polymer, after absorption, polymeric particles will slowly degrade, providing a sustained and controlled release of the drug. Although in minute quantities, polymeric particles have been shown to cross the intestinal wall [14]. The size of the particles, surface charge and the nature of the

BOX 1

Natural polymers used for oral insulin delivery

1. Polysaccharides
- Chitosan
- Alginate
- Dextran
- Starch
- Pectin

2. Proteins
- Casein
- Gelatin

polymer are crucial parameters involved in particle uptake by the GI tract. Moreover, the physicochemical properties, drug release profile and biological behavior of polymeric particles can be easily modulated.

Natural polymers

Natural polymers are generally considered to be safe *in vivo*, and most of them are already in use as excipients in drug formulations. Nowadays, many natural polymers (polysaccharides and proteins) are being widely investigated as oral insulin delivery carriers (Box 1) by researchers worldwide (Fig. 3). Apart from being hydrophilic and biodegradable, polysaccharides exhibit enzymatic degradation behaviour and good biocompatibility. Polysaccharides can be easily modified chemically and biochemically, and are highly stable, safe, nontoxic, with gel forming properties, suggesting their suitability to be used for oral protein delivery. In addition to the possibility of administration through nonparenteral routes, polysaccharidic nanoparticles have the potential to retain protein stability and to increase the duration of the therapeutic effect of proteins [15]. Charged polysaccharides, such as chitosan (CS) and alginate can form polyelectrolyte complexes (PECs) by electrostatic interactions between oppositely charged groups resulting in ionpairing without altering the integrity of the polymer. Box 2 shows some of the properties of natural polymers used for oral insulin delivery.

Alginate

Alginates are widely used in biomedical applications owing to its mucoadhesive, biodegradable and biocompatible nature [16]. Low drug encapsulation efficiency of alginates can be improved by the interaction with polymers, such as CS, dextran sulfate, pectin and methylcellulose. Alginate–chitosan capsules have been studied for the development of oral insulin formulations [17]. However, the encapsulation efficiency of insulin in alginate–chitosan capsules is low. Ramdas *et al.* developed an oral formulation based on liposome encapsulated alginate CS gel capsules for insulin delivery [18], which helped to increase the encapsulation efficiency of insulin in alginate–chitosan capsules. The lipid exterior might help improve absorption across biological barriers, whereas the aqueous interior of the liposome will preserve the structure and conformation of insulin. Oral administration of lipoinsulin-loaded alginate–chitosan capsules was found to reduce blood glucose level in diabetic rats. Owing to the bioadhesive property of alginate, alginate coated lipoinsulin might anchor the lipoinsulin to the intestinal tract or increase the transit time of the formulation. Thus, the intimate contact with mucosa might help efficient absorption with increased bioavailability of insulin.

Starch

Starch, the second most abundant natural biopolymer is a versatile and inexpensive renewable agricultural material used for a variety of industrial and pharmaceutical applications [19]. Modified starch derivatives have been studied for use in oral insulin delivery systems. Mahkam *et al.* developed pH-responsive hydrogels containing pendant starch poly(CMS-co-MAA-co-MEG or PBD) by free-radical crosslinked copolymerization of methacrylic acid, poly(ethyleneglycol monomethyl ether methacrylate) (PEGMA), and carboxymethyl starch (CMS) for oral insulin delivery [20]. By increasing the methacrylic acid content in the copolymer, pH-sensitive hydrogels with improved optimal hydrolysis rates were obtained.

Dextran

Dextran is a nontoxic and highly water-soluble exocellular bacterial polysaccharide predominantly consisting of linear 1,6-linked glucopyranose units, with some degree of 1,3-branching. Dextrans conjugated with insulin have been studied for their pharmacokinetic and pharmacodynamic properties [21]. In an attempt to enhance nanoparticle absorption across GI tract Chalassani *et al.* optimized the effectiveness of VB_{12}–nanoparticle conjugates using different levels of cross-linking, linked with different VB_{12}-coating. VB_{12}–NPs conjugates (150–300 nm) showed profound (70–75% blood glucose reductions) and prolonged (54 hours) anti-diabetic effects with biphasic behavior in streptozotocin-induced diabetic rats. The biphasic behavior of the hypoglycemic effect might be presumed to the diurnal effects feed intake, glucose metabolism and insulin release from the crosslinked cores. The prolonged anti-diabetic activity for many hours might be presumed to the possibility that VB_{12}–dextran system might be trapped at glucose utilizing organs, where either intact conjugate is slowly internalized or releases free insulin. NPs with the low degree of cross-linking were found to be superior carriers, and were more effective with VB_{12} derivatives of carbamate linkage. The pharmacological availability of carbamate linked VB_{12} derivatives was found to be 29.4%, which was superior compared with NP conjugate of ester linked VB_{12} (1.5-fold) and relatively higher crosslinked particles (1.1-fold). These nanoparticle carriers demonstrated a similar oral insulin efficacy in congenital diabetic mice. Significant quantities of plasma insulin were found in both animal models [22,23].

Pectin

Pectin is an anionic, water soluble heterogeneous polysaccharide containing linear chains of α-(1 → 4)-D-galacturonic acid residues and 1,2 D-rhamnose with D-galactose and D-arabinose side chains [24]. Calcium salts of pectin have reduced solubility and matrix tablets prepared with calcium pectinate showed good potential to be used in colon-targeted drug delivery systems. The main drawback of calcium pectinate gel beads is their macroporous structure, which may cause low entrapment efficiency and fast release of incorporated drugs, especially for those of hydrophilic low molecular weight drugs. In a recent investigation, orally administered, insulin-loaded amidated pectin hydrogel beads produced sustained release of insulin, and also reduced plasma glucose concentration in streptozotocin-induced diabetic rats [25].

Gelatin

Gelatin, a natural polymer derived from collagen, is a nontoxic, noncarcinogenic and biodegradable biopolymer of immense biomedical and pharmaceutical utility. Goswami *et al.* developed

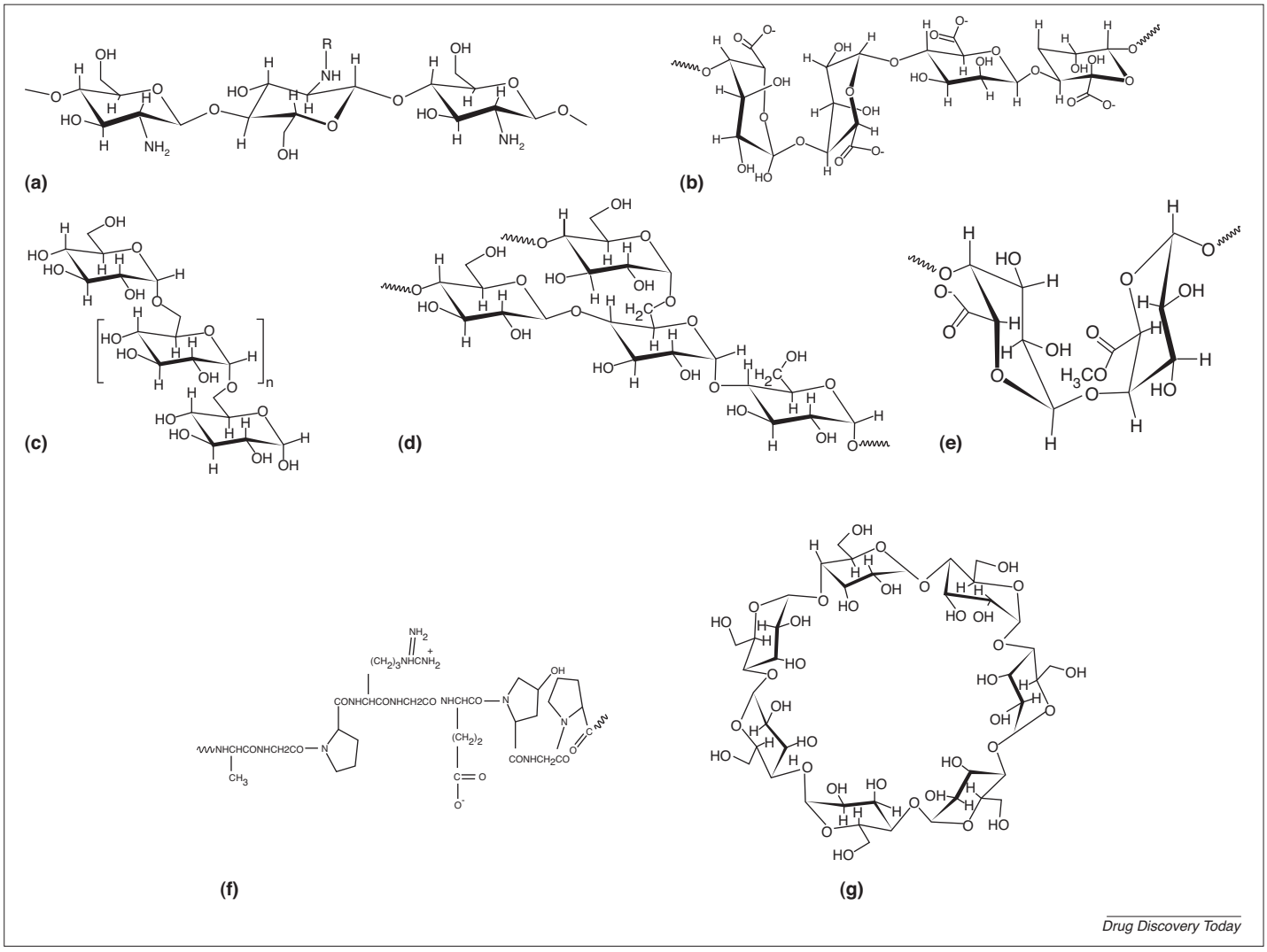

FIGURE 3

Chemical structure of natural polymers used for oral insulin delivery. **(a)**, **(b)**, **(c)**, **(d)**, **(e)**, **(f)** and **(g)** represents chitosan, alginic acid, dextran, starch, pectin, gelatin and cyclodextrin, respectively.

glutaraldehyde crosslinked gelatin nanoparticles, a swelling controlled release system for insulin that offered minimum release of insulin at gastric pH whereas optimum release was noticed at intestinal pH [26].

Casein

Casein is the predominant phosphoprotein, accounting for nearly 80% of proteins in milk. Morçol *et al.* developed calcium phosphate–poly(ethylene glycol)–insulin casein (CAPIC) particles for oral administration of insulin [27]. Owing to its non-degradability in acid and mucoadhesiveness, casein can protect insulin as it passes through the stomach and into the small intestine, and enables the drug to remain concentrated at the site of absorption. In a fasted diabetic mouse model, a single dose of CAPIC administered directly into the stomach could rapidly reduce the blood glucose levels by 80% within the first hour of treatment. In fed mice, CAPIC reduced 50% of blood glucose levels within three hours, and glucose returned to previous levels after five hours.

BOX 2

Characteristics of natural polymers used for oral insulin delivery

1. Cheap and often extracted from renewable resources
2. Biodegradable, nontoxic
3. Purity may vary
4. Induces a strong immunogenic response
5. Hydrophilic
6. Properties cannot be controlled
7. Only surface modification possible
8. Bioadhesive (e.g. chitosan and alginate)

Cyclodextrin

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six to eight glucose units linked by β-1,4-glucosidic bonds, resulting in the formation of toroidal molecules with internal hydrophobic cavities and external hydrophilic surface. The internal hydrophobic cavities in CDs can facilitate the inclusion of several guest molecules stabilized by noncovalent interactions [28]. CDs and their derivatives are extensively studied for oral administration of peptide and protein drugs. CD–insulin complex could stabilize insulin against aggregation, thermal denaturation and degradation. It could also enhance

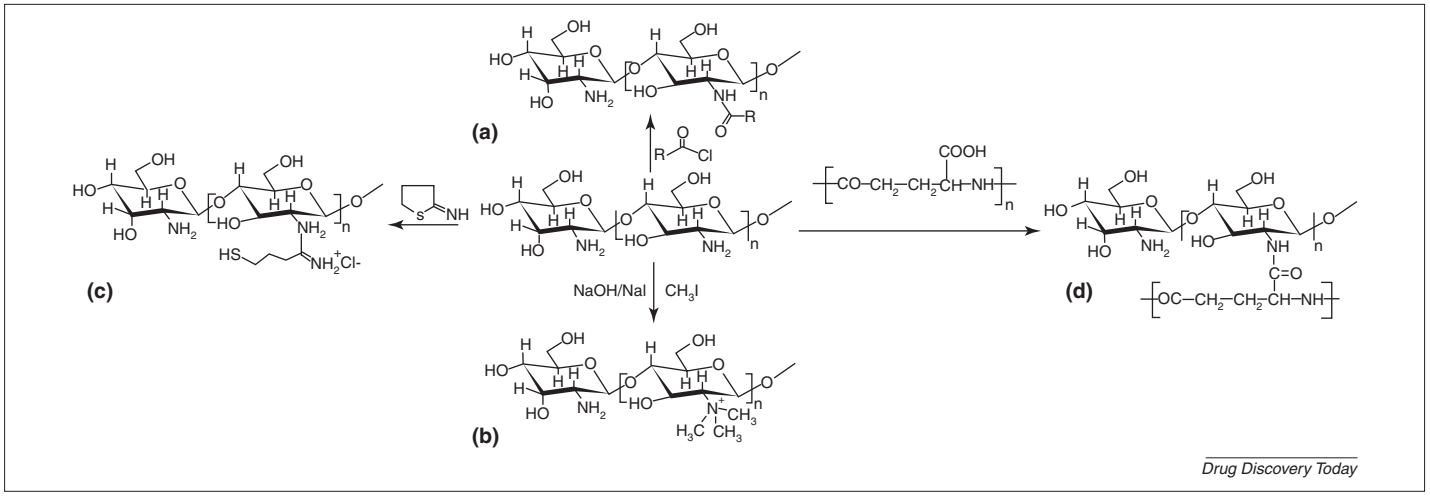

FIGURE 4

Structure of chitosan and its derivatives. **(a)**, **(b)**, **(c)** and **(d)** represents acylated chitosan, quaternized chitosan, thiolated chitosan and chitosan graft polyglutamic acid, respectively.

the absorption of insulin across the biological barriers by perturbing the membrane fluidity to lower the barrier function. However, unmodified CDs exhibits cytotoxicity and low water solubility, which limit their further pharmaceutical applications.

In an alginate and/or CS nanoparticle system, insulin was protected by forming complexes with cationic-β-cyclodextrin polymers (CPCDs). Owing to the electrostatic attraction between insulin and CPCDs, as well as the assistance of its polymeric chains, CPCDs could effectively protect insulin under simulated gastrointestinal (GI) conditions [29]. The cumulative insulin release in SIF was much higher (40%) than that without CPCDs (18%) because insulin was mainly retained in the core of the nanoparticles and well protected against degradation in simulated gastric fluid. The aggregation of the insulin molecules seems to be reduced by the complexation of the drug with βCD. CD complexes might help in enhancing drug stability and/or absorption, whereas the particulate delivery system might serve as a platform for the encapsulation of the complexed drugs [30].

Chitosan

CS, copolymer of β (1–4) linked glucosamine and N-acetyl glucosamine, have drawn considerable attention in pharmaceutical field owing to its excellent biocompatibility, biodegradability, and for ease of modification due to the presence of reactive surface functional groups [31]. Moreover, it has a unique feature of adhering to the mucosal surface and transiently opening the tight junctions between epithelial cells. When solubilized in dilute acid, CS becomes a cationic polymer, linear in structure, with a high positive charge density. CS is metabolized by lysozyme and breaks down slowly to harmless products (amino sugars) that are completely absorbed in the human body. CS solutions have been shown to increase trans and paracellular permeability in a reversible, dose-dependent manner [32]. The mechanism of action, which appears due to the positive charges on the CS, includes interactions with the tight junction proteins occludin and ZO-1, redistribution of F-actin, and slight destabilization of the plasma membrane [33]. Despite its favorable biological properties, CS is rarely used in oral administration of drugs due to its high solubility at low p H and limited capacity for controlling the release of drugs. To overcome this limitation, various chemical modifications of CS have been carried out through hydrophobic, hydrophilic and thiolation, among others. Due to the presence of highly reactive amino and hydroxyl functional groups, CS can be modified with different chemical entitities, thereby forming CS derivatives with desired properties, which may be suitable for oral drug delivery (Fig. 4). These modifications would not change the fundamental skeleton of CS but bring in new or improved properties for mucoadhesion and permeation enhancement, among others. CS is generally considered nontoxic and biodegradable, with an oral LD50 in mice of over 16 g/kg [34]. The nontoxic nature of CS, its capability to prolong the residence time in the GI tract through mucoadhesion, and its ability to enhance absorption by increasing cellular permeability have all been major factors contributing to its widespread evaluation as a component of oral dosage forms.

Chitosan–insulin conjugates

Chitosan–insulin nanoparticles administered orally at insulin doses of 50 U/kg and/or 100 U/kg were effective at lowering the serum glucose level of streptozotocin-induced diabetic rats [35]. Mitra *et al.* investigated the efficacy of nanoparticles formed by complex coacervation method using CS of various molecular weights and Eudragit L100-55 polymer [36]. As the molecular weight of CS increased, the amount of insulin released increased with respect to time. With an aim to improve bioavailability a new oral delivery system for insulin, based on a conjugate between insulin and low molecular weight chitosan (LMWC of 3,6, 9, and 13 k average MW) of narrow molecular weight distribution, was developed by Lee *et al.* Of those conjugates, LMWC (9k)-insulin exhibited the highest pharmacodynamic bioavailability of 3.7 [37].

Hydrophobic modification

Hydrophobic character of CS can be increased by covalent attachment of hydrophobic excipients. Hydrophobic interactions are believed to enhance the stability of substituted CS by reducing the hydration of the matrix thereby resisting the degradation by gastric enzymes [38]. Rekha *et al.* tried to establish the role of polymeric hydrophilic and/or hydrophobic balance on GI

absorption of insulin [39]. Laurylsuccinyl chitosan (LSC) particles were found to be highly mucoadhesive, which could be due to the hydrophobic interaction of lauryl groups to the hydrophobic domains of mucosa in addition to its negative ζ potential. The strong mucoadhesion of LSC particles might help in the direct delivery of insulin to the intestinal cell surface, thus reducing the susceptibility to the enzymatic degradation and thereby improving the bioavailability.

The bioadhesive property of CS can be enhanced by N-acylation with fatty acid chlorides. Fatty acids act primarily on the phospholipids component of the membrane thereby creating disorder and leading to increased permeability. Compared to the CS modified with short chain fatty acids, CS modified with higher fatty acids (eg. oleoyl chloride) showed better mucoadhesion property [40,41]. From these studies it seems that hydrophobically modified CS will be an interesting system for oral insulin delivery.

Thiolation

Nowadays, thiolated chitosans are gaining popularity because of their high mucoadhesiveness and extended drug release properties [42]. Thiolation can be achieved by the immobilization of thiol bearing moieties on the polymeric backbone of CS. Mucoadhesiveness of thiolated polymers is due to the formation of disulfide bonds with cysteine rich subdomains of mucus glycoproteins [43]. The permeation of paracellular markers through mucosa can be enhanced by utilizing thiolated instead of unmodified CS. In addition, thiolated chitosans display *in situ* gelling features. The permeation-enhancing effect seems to be based on the inhibition of protein tyrosine phosphatase, resulting in an opening of the tight junctions for hydrophilic macromolecules. This theory is supported by various *in vitro* and *in vivo* studies where significantly improved pharmacological efficacy and/or bioavailability of insulin was demonstrated [44]. Due to the inter- and intramolecular formation of disulfide bonds, a tight 3D network is formed which leads to high cohesiveness and allows a controlled drug release. In comparison to the unmodified polymers, thiomers exhibited a reversible opening of tight junctions, leading to a more pronounced permeation-enhancing effect.

Polyelectrolyte complex formation

PECs developed by mixing oppositely charged ions, provide an inexpensive, biocompatible, versatile alternative system to current polymeric delivery strategies that apply organic solvents as reaction environments [45]. The biodistribution study in a rat model showed that some of the orally administered CS polyglutamic acid NPs were retained in the stomach for a long duration, which might lead to the disintegration of NPs and degradation of insulin [46]. To overcome these problems, NPs were freeze dried and filled in an enteric-coated capsule. Upon oral administration, the enteric-coated capsule remained intact in the acidic environment of the stomach, but dissolved rapidly in the proximal segment of the small intestine. Consequently, all the NPs loaded in the capsule were brought into the small intestine, thus enhancing the intestinal absorption of insulin and providing a prolonged reduction in blood glucose levels. The relative bioavailability of insulin was found to be approximately 20%. In another study a self-assembled CS/γPGA (chitosan/poly-γ-glutamic acid) nanoparticles for oral administration of insulin was prepared by mixing poly-γ-glutamic acid solution with CS solution in the presence of $MgSO_4$ and sodium tripolyphosphate [47]. Oral administration of insulin-loaded NPs demonstrated a significant hypoglycemic action for at least ten hours in diabetic rats and the corresponding relative bioavailability of insulin was found to be 15.1%. In contrast to CS/γ-PGA NPs, trimethyl chitosan (TMC)/γ-PGA NPs may be a suitable carrier for transmucosal delivery of insulin within the entire intestinal tract where the pH values are close to the pK_a of CS. TMC/γ-PGA NPs had superior stability in a broader pH range compared to CS/γ-PGA NPs; the *in vitro* release profiles of insulin from both test NPs were significantly affected by their stability at distinct pH environments. Confocal Laser Scanning Microscopy confirmed that TMC/γ-PGA NPs opened the tight junctions of cell monolayers to allow the transport of insulin along the paracellular pathway at all test intestinal pH environments [48].

Quaternization

CS is not a suitable carrier for targeting protein drugs to specific sites of the intestine owing to the poor solubility at physiological pH values. TMC are drastically more soluble in neutral and alkaline environments of the intestine and hence are more efficient than CS for drug delivery and absorption across the intestinal epithelium. These derivatives are being extensively studied for oral insulin delivery [49]. The permeation-enhancing properties of these CS derivatives have been attributed to the ionic interactions with the tight junctions and cellular membrane components to increase the paracellular permeation of hydrophilic compounds. It was reported that N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride, a quaternized derivative of CS could improve the mucoadhesivity owing to the presence of positive charge and hydroxyl functional group on the side chain [50].

Both TMC and CS failed penetration enhancement toward ileum probably owing to a thicker mucus layer with barrier properties and a rich enzyme pool. TMC nanosystems with 35% quaternization degree exhibited good penetration-enhancement and mucoadhesive properties. Mucoadhesive properties increased the residence time of nanoparticles with intestinal epithelium, thereby offering more possibilities of nanoparticle internalization [51]. The mechanism of penetration enhancement involved paracellular pathway with enlargement of tight junctions for polymer solutions and CS NP, whereas endocytosis and/or internalization into duodenum and jejunum epithelial cells was confirmed only for nanoparticulate form [52].Oral and ileal administration of thiolated trimethylchitosan of molecular weight 200 kDa and 30% degree of quaternization [TMC-Cys (200,30)] NP led to notable hypoglycemic effects as compared to insulin solution, which lasted until eight hours and seven hours post-administration, with the maximum blood glucose reduction of 35% and 70%, respectively. Biocompatibility assessment revealed lack of toxicity of TMC-Cys NP [53].

Qian and co-workers reported that CS graft copolymer nanoparticles based on monomer methyl methacrylate (CM), *N*-dimethylaminoethyl methacrylate hydrochloride (CDM), and *N*-trimethylaminoethyl methacrylate chloride (CTM) enhanced the absorption and improved the bioavailability of insulin through the GI tract of normal male Sprague-Dawley (SD) strain rats to a greater extent than that of the phosphate buffer solution (PBS) of insulin [54]. Up to 100% insulin loading could be achieved, and

TABLE 1

Summary of *in vivo* efficacy of insulin-loaded natural polymer based particles on oral administration

Polymer	Size (nm)	Zeta (mV)	Animal model	Observations	Dose (IU/kg)	Bioavailability (BA)	Refs
Alginate crosslinked dextran sulfate poloxamer coated albumin	396	−36.6 to −44.5	Diabetic male Wistar rats	Sustained hypoglycemia for about 24 hours.	50	13	[58]
Alginate–dextran sulfate core, chitosan–polyethylene glycol-albumin coated nanospheres	≤1842	−7 ± 4	Diabetic male Wistar rats	Glycemic response was dose-dependent and lasted for at least 24 hours, with a maximal effect of 14 hours post-administration.	25 100	42 10	[59]
CAPIC	600		Diabetic mice	Sustained hypoglycemia for about 12 hours in fasted mice and about five hours in fed mice.	100	–	[27]
Alginate–dextran–chitosan	750		Diabetic male Wistar rats	Sustained hypoglycemia for about 18 hours.	50 100	6.8 3.4	[17]
Chitosan–TBA			Diabetic male Wistar rats	Blood glucose level decreased significantly for 24 hours.	11	1.69 ± 0.42%	[60]
Thiolated trimethyl chitosan	100–200	+12 to +18	Non-diabetic Sprague-Dawley rats	Notable hypoglycemic effect as compared with insulin solution, which lasted until eight hours and seven hours post-administration, respectively, with the maximum blood glucose reduction of 35% and 70%.	50	–	[52]
Chitosan–polyglutamic acid	218		Diabetic male Wistar rats	Hypoglycemic effect depends on copolymer composition two to ten hours post-administration.	30	15.1	[48]
Chitosan–polymethylmethacrylate	150–280		Non-diabetic Sprague-Dawley rats		100		[54]
Laurylsuccinylchitosan	315–1090		Diabetic male Wistar rats	Sustained reduction in blood glucose level for six hours from the initial value.	60	–	[39]
Dextran sulfate–chitosan	500	−20.6	Diabetic female Wistar rats	Hypoglycemic effect was observed for more than 24 hours.	50 100	5.6 3.4	[61]
Vitamin B12–dextran sulfate–chitosan	192			First phase-reduced glycemia for about 70% within five hours and reached basal levels within eight to ten hours. Second phase-sustained hypoglycemia for about 54 hours	20	26.5	[22]
Chitosan nanoparticle	25–400		Diabetic male Wistar rats	Prolonged hypoglycemic effect for 15 hours.	21	14.9	[62]
Aminoalkyl Vitamin B12 dextran sulfate chitosan	150–200		Diabetic female Wistar rats	70–75% blood glucose reduction	20	29.4	[23]

the NPs showed improved solubility in wide range of pH. A high encapsulation efficiency of insulin has been obtained, and *in vitro* release profiles of insulin from CDM and CTM nanoparticles showed an initial burst release followed by a slow and sustained release phase. Nearly all the loaded insulin was completely released from CM nanoparticles in six hours. Moreover, these nanoparticles provided a continuous release of the loaded insulin for up to four days. In this study the authors did not use any organic solvents or high-energy sources for insulin loading rather, free-radical polymerization was applied for the NP preparation.

As innovative attempt was made by Jin *et al.* by modifying TMC nanoparticles with cell penetrating peptide (CSK) to evaluate the efficacy of goblet cell targeting of nanoparticles for oral absorption of insulin. Insulin-loaded TMC–CSK nanoparticles not only promoted the uptake of nanoparticles in villi but also enhanced the permeation of the drug across the intestinal epithelium by clathrin or caveolae mediated endocytosis. Moreover, these nanoparticles could induce a hypoglycemic effect of 28% compared to unmodified TMC-insulin nanoparticles [55].

Chitosan-inhibitor conjugates

CS, due to its chelating ability with divalent metal ions is expected to inhibit protease degradation. Chelation of calcium further activates protein tyrosine kinases, which ultimately leads to the opening of tight junctions across the intestinal epithelium. Enzyme inhibitors conjugated directly to CS might improve drug bioavailability by localizing the inhibitory effect to the site of drug uptake, as well as by reducing toxicity [56]. The use of such chitosan-inhibitor conjugates may represent a valuable approach to improve protection from drug degradation and achieve more effective oral drug delivery. Recently, Su *et al.* reported that the immobilization of complexing agents, such as diethylenetriamine pentaacetic acid (DTPA) on CS/γPGA nanoparticles could significantly enhance the absorption of insulin throughout the entire small intestine, subsequently producing a significant and prolonged hypoglycemic effect [57].

Concluding remarks

For the past 88 years, since the introduction of insulin therapy, the management of blood glucose levels in diabetics has improved dramatically. However, replicating normal physiological patterns of insulin secretion through oral insulin administration is still at its infancy. Mucoadhesive polymeric nanoparticles, are nowadays an attractive option for increasing the bioavailability of insulin. Although various natural polymeric carriers have been developed for insulin release (Table 1), such systems have not shown sufficient bioavailability when administered orally. One of the main reasons for low bioavailability is due to their poor reproducibility, purity and degradability, which might lead to varied drug loading capacity and release kinetics. As the natural polymers can be extracted from different sources, purity of the polymers might vary, which might lead to altered physicochemical properties. The difference in experimental design and the chemicals used in induction of diabetes in different animal species could also account for the controversial observations between results. Furthermore no reports are available on the dose-responsive, toxicological and hematological effect of these particles on continuous administration. Besides increasing the bioavailability our aim should be to produce dose-dependent and reproducible formulations for successful oral insulin delivery. Therefore systematic evaluations of the matrix have to be carried out to make oral insulin delivery a reality.

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