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Review

Muco-Adhesive and Muco-Penetrative Formulations for the Oral Delivery of Insulin

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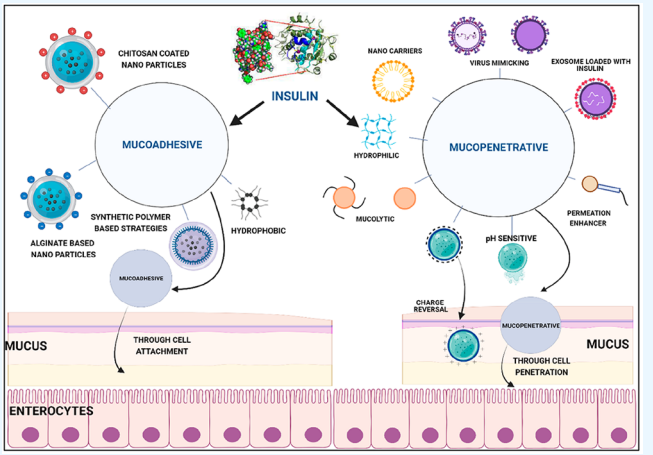
ABSTRACT: Insulin, a pivotal anabolic hormone, regulates glucose homeostasis by facilitating the conversion of blood glucose to energy or storage. Dysfunction in insulin activity, often associated with pancreatic β cells impairment, leads to hyperglycemia, a hallmark of diabetes. Type 1 diabetes (T1D) results from autoimmune destruction of β cells, while type 2 diabetes (T2D) stems from genetic, environmental, and lifestyle factors causing β cell dysfunction and insulin resistance. Currently, insulin therapy is used for most of the cases of T1D, while it is used only in a few persistent cases of T2D, often supplemented with dietary and lifestyle changes. The key challenge in oral insulin delivery lies in overcoming gastrointestinal (GI) barriers, including enzymatic degradation, low permeability, food interactions, low bioavailability, and long-term safety concerns. The muco-adhesive (MA) and muco-penetrative (MP) formulations aim to enhance oral insulin delivery by addressing these challenges. The mucus layer, a hydrogel matrix covering epithelial cells in the GI tract, poses significant barriers to oral insulin absorption. Its structure, composition, and turnover rate influence interactions with insulin and other drug carriers. Some of the few factors that influence mucoadhesion and mucopenetration are particle size, surface charge distribution, and surface modifications. This review discusses the challenges associated with oral insulin delivery, explores the properties of mucus, and evaluates the strategies for achieving excellent MA and MP formulations, focusing on nanotechnology-based approaches. The development of effective oral insulin formulations holds the potential to revolutionize diabetes management, providing patients with a more convenient and patient-friendly alternative to traditional insulin administration methods.



1. INTRODUCTION

Diabetes is one of the most common metabolic diseases affecting several million people across the world. As per a report from the International Diabetes Federation, there were 537 million adults living with diabetes in 2021 and this number is predicted to reach 783 million (1 in 8 adults) by 2045.[1] Diabetes is primarily classified as type 1 (T1D) and type 2 (T2D) with the latter contributing to almost 85−95% of all diabetes cases. However, the prevalence of T1D has increased and is expected to increase in the coming years.[2] T1D, a chronic autoimmune disorder mainly affecting the youth, is caused by the destruction of pancreatic β cells (β cells) producing insulin. Insulin therapy is one of the major treatment strategies for T1D, although other strategies such as insulin analogues, glucagon therapy, and metformin, among others, also exist.[3]

On the other hand, T2D occurs due to genetic, environmental, and lifestyle factors leading to β cell dysfunction and insulin resistance. In the case of T2D, despite enough insulin levels in the body, the target cells become unresponsive, thereby leading to hyperglycemia, which further aggravates β cell dysfunction. In persistent cases of T2D, insulin therapy is widely used. These huge demands for insulin are reflected in the high annual insulin sales. As per a report from Fortune business insights, the global human insulin market was evaluated to be USD 18.73 billion in 2022 and was projected to reach USD 21.04 billion by 2030.[4]

Insulin is an anabolic hormone that is responsible for the maintenance of glucose homeostasis. Insulin is a 51-amino acids-containing protein molecule that was obtained by cleavage of the C peptide chain from its precursor: the proinsulin molecule, which has three chains: chain A, chain B,

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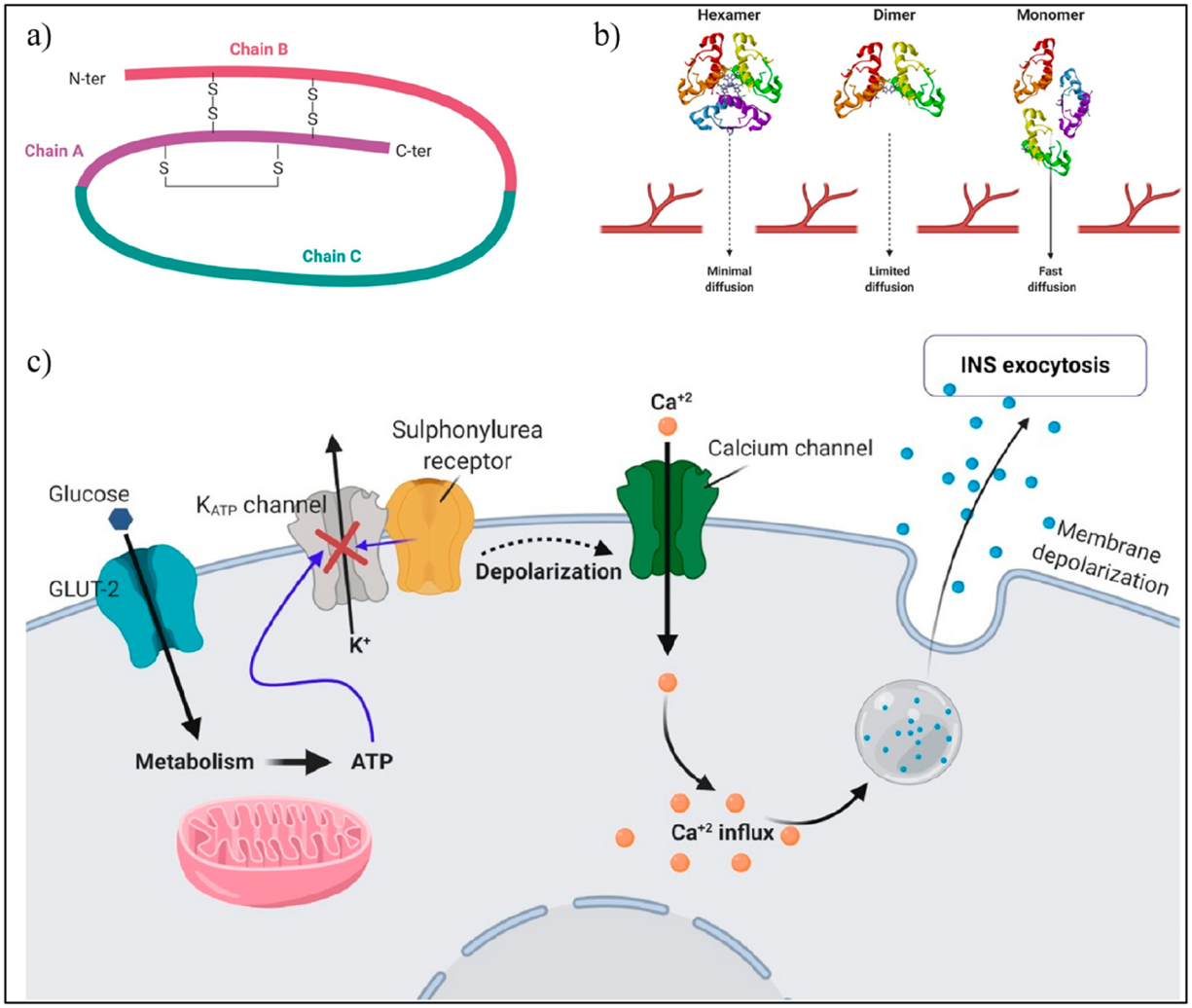

Figure 1. a) Structure of proinsulin; b) Three different forms of insulin and their rates of diffusion; c) Fate of physiological insulin: *Increase in blood sugar levels prompts pancreatic beta cells to release insulin via glucose metabolism, membrane depolarization, and calcium influx, resulting in lowering of blood sugar.* (Reprinted with permission from "The role of polysaccharides from natural resources to design oral insulin micro- and nanoparticles intended for the treatment of Diabetes mellitus: A review" by A. B. Meneguin, 2021, Carbohydrate Polymers, 256, 117504. Copyright 2021 Elsevier).

and C peptide. Figure 1a depicts the structure of proinsulin with all three chains. Also, insulin exists in three different states based on their concentration and external environment (buffer) in which they are dissolved. Insulin exists as a monomer (when its concentration is very low (around 10^{-6} M). However, at higher concentrations, they form dimers at neutral pH and form hexamers in the presence of zinc ions. Figure 1b shows the three forms of insulin and their effect on diffusion into circulation.

Insulin is produced by the β cells in the islets of Langerhans of the pancreas.[5] It functions by stimulating the conversion of glucose from the blood into energy or storage as glucagon. Any dysfunction in the β cells that impairs the activity of insulin can result in increased blood sugar levels, a condition known as hyperglycemia. This is a common characteristic of diabetic patients. It has been observed that insulin treatment for T2D patients is required in later stages of treatment. However, for T2D treatment, insulin therapy should be supplemented with certain dietary and lifestyle changes.

Insulin is typically delivered via one of the following delivery systems: syringes, jet injectors, insulin infusion pumps, and pens. Moreover, there are several formulations of insulin available in the market with varying duration of action: ultrarapid acting, rapid-acting, long-acting, short-acting, and premixed insulin, as reported in the review,[6] which determines the insulin's action onset time, peak time and duration of action. Also, it was reported that patients in need of insulin may have to be administered more than 60,000 insulin injections throughout their lives.[7] This leads to trypanophobia-related issues, especially poor patient compliance and hypoglycemia due to unrestricted injection. Thus, there is a need for more patient-friendly insulin delivery systems. The oral insulin formulations are key players when patient compliance is considered. Moreover, the physiological insulin which is made in the islets of Langerhans in the pancreas is secreted directly into blood vessels which flow into the hepatic portal circulation.[8] The advantage of oral administration of insulin over the other routes is that it can mimic the physiological fate of insulin, in addition to providing better glucose homeostasis, as shown in Figure 1c. However, there are several challenges associated with the oral delivery of insulin as explained in the section below.

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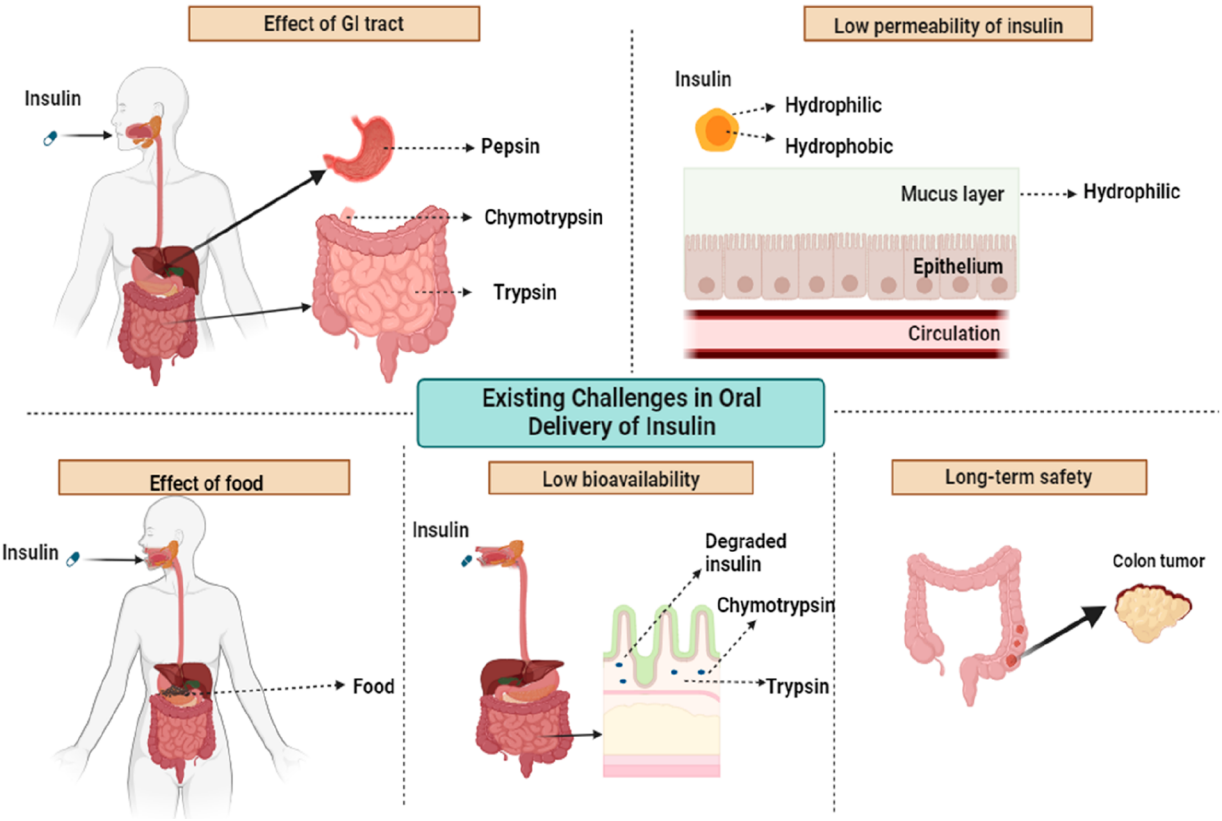

Figure 2. Existing challenges in the oral delivery of insulin (Created using BioRender.com).

2. EXISTING CHALLENGES IN THE ORAL DELIVERY OF INSULIN

Effect of the GI tract: Insulin gets degraded in the gastrointestinal tract (GI) due to its harsh environment. The proteins and peptides are broken down by digestive enzymes such as pepsin and pancreatic proteolytic enzymes such as trypsin and α-chymotrypsin produced in the GI tract without any discrimination. Most of the digestive enzymes are concentrated in the stomach and small intestine. The rest of the GI tract comprising the mouth, esophagus, colon, and rectum is relatively free of digestive activity. However, since the insulin gets degraded in the small intestine, it cannot enter the hepatic portal circulation.[8]

Low permeability of insulin: Insulin is a hydrophilic macromolecule, with some hydrophobicity in its interior regions.[9] Therefore, it is not possible for insulin to diffuse across epithelial cells through lipid-bilayer cell membranes and reach the bloodstream. This explains the low permeability of insulin through the intestinal mucosa.

Effect of food: Food affects the rate and extent of absorption and can increase or decrease the absorption of insulin delivered insulin. The various drug delivery strategies used for insulin delivery follow different rates and extent of absorption.[10] Hence, the optimal timing for oral insulin ingestion must be determined correctly. GI functions like bile acid secretion, gastric emptying, intestinal transit time, low pH of the stomach, and liver blood flow are also affected by the consumption of food.[11]

Low bioavailability: Orally administered insulin has a low bioavailability since most of the insulin ingested is not absorbed through the intestinal mucosa and remains in the gastrointestinal tract, where it is degraded by digestive enzymes[12] into its constituent amino acids. As a result, the physiological function of the insulin is lost, resulting in decreased bioavailability.

Long-term safety: The long-term safety of orally administered insulin must be considered when it is designed. Insulin is a mitogen and it has a high risk of causing several cancers including colon cancer.[13] This must be considered when designing oral insulin. Moreover, while insulin is not toxic on its own, the chemical compounds used in the various delivery systems such as excipients or absorption promoters must be thoroughly evaluated for its safety.[13] Figure 2 summarizes the above-mentioned challenges associated with the oral delivery of insulin.

To overcome the challenges mentioned above, several novel formulations are explored for insulin, which can either adhere to the mucous layer (muco-adhesive) or can penetrate the mucous layer and reach the circulation via the epithelial cell layer (muco-penetrative) or, in some cases, can have both the above-said properties (Table 1).

3. MUCUS LAYER IN THE GI TRACT

The mucus layer is a viscous gel-like structure coating the entire gastrointestinal tract, protecting the same from pathogens and other xenobiotics. However, this coating of mucus is not uniform throughout the GI tract. The mucus lining of the stomach and colon has two layers: an outer loosely attached layer and an inner firmly adhered layer; while that of the intestine has only one loosely attached layer. Figure 3a depicts the distribution of the mucus layer throughout the entire GI tract.

3.1. Components of Mucus. The gut mucosa particularly comprises mainly water and a multitude of other molecules

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Table 1. Summary of All Insulin-Based MA and MP Formulations, the Polymers Used, the Sizes of the Particles, and the Status of the Work[a]

Polymer/material used	Particle size	Surface charge	Insulin encapsulation efficiency	Status	Significance of study	ref
Chitosan and dextran sulfate	432.2 nm	−54.7 ± 0.458 mV	16.67%	In vitro	Sustained release for the first 10 h Low cytotoxicity toward MRC-5 lung fibroblast cells	91
Deacetylated chitosan and snail mucin	479.6−504.1 nm	22.1 to 31.2 mV	88.6−92.5%	In vivo	Nanoparticles observed to enter through the paracellular pathway Self-sustained release for 8 h Significant hypoglycemic effects on diabetic rats	20
Poly(methacrylic acid-g-ethyl-ene glycol) (P(MAA-g-EG))	NA	NA	96.1 ± 1.4%	In vivo	Immediate release within 30 min of ∼80% of insulin CPP-mediated paracellular entry	40
PEGylated liposome with B12 linkage	235.1 ± 2.2 nm	−13.4 ± 4.5	59%	In vivo	B-12 receptor-mediated transcytosis PK profile comparable to SC delivery	88
Alginate-coated Solid Lipid NP	323.8 nm	7.19 mV	17.19 ± 1.36%	In vivo	Sustained release for 6 h Hypoglycemic effect lasted for 10 h	35
Chitosan-coated Solid Lipid NP	395.3 nm	9.37 mV	20.53 ± 1.25%	In vivo	Sustained release for 8 h Hypoglycemic effects lasted for 8 h	92
Gum with dextran sulfate, chitosan, and albumin	622 nm	(−32.9)−(−20.6) mV	70%	Ex vivo	92.46 mN MA strength Sustained release for 6 h in simulated intestinal conditions	45
Eudragit, pectin, and sodium carboxymethylcellulose (SCMC)	Small patches of 5 × 3 mm (length × breadth)	NA	NA	In vitro + In vivo	Insulin-loaded MA patches 63% drop in blood glucose levels in 3 h	49
Zein and PEG	270 nm	(−38.9) ± (2.3) mV	81.1 ± 6 (μg/mg)	In vitro	Oral bioavailability 10%, 2.5-fold higher than for bare nanoparticles compared to other nanocarriers	84
Chitosan and snail mucin	37.5 ± 0.01 to 45.0 ± 0.12 μm	21.8 ± 0.1	>80%	In vivo	Higher encapsulation is achieved when the polymer and the drug have opposing charge significant reduction (>50%) in blood glucose levels and ensured prolonged effects of over 10 h	21
Chitosan and Liposomes	439.0 ± 12.3 nm	+60.5 ± 1.9 mV	73.33% ± 0.68%	In vivo	Insulin is shielded from the gastric environment by CS-coated liposomes, and their MA properties prolong their residence time in the mucosa.	22
Sucrose acetate isobutyrate (SAIB)	NA	NA		In vitro, In vivo	• Systemic effect of protein-based nanoparticles for GI absorption	46
Trimethyl chitosan	256.7 ± 4.9 nm	26.5 ± 1.1 mV	NA	In vitro	• Insulin rate is controlled at varying pH, increases permeability, enhances paracellular activity	26
Chitosan	560 ± 9 nm	+31 ± 3 mV	60%	In vitro, In vivo, ex vivo	• Reducing the glycemia in diabetic rats by up to 50% • Exerts mucoadhesiveness,	18
Baicalin	200−600 nm	−47.93 ± 4.71 mV	83.7%	In vitro, In vivo	• Nanoparticle is pH sensitive so it can control insulin release at varying pH • Enhances bioavailability	90
Cholic acid + hydroxypropyl methylcellulose (HPMCP)	239 nm	NA	90.9%	In vitro, In vivo	• Using enterohepatic circulation • maintained hypo-glycemic effect for more than 24 h	29
Gellan gum	1033−1197 nm	NA	55−91%	In vivo	• Sustained release up to 8 h under simulation • Mucus permeation to 73−86% ex vivo • Hypoglycemic effects up to 51% in 7 h	48
PLGA, chitosan, alginate	250 nm	−18.03 ± 2.78 mV	35%	In vivo	• Continuous release of insulin for 6 h • Hypoglycemic effect observed within 1 h in vivo	67
Chitosan, alginate	3 mm	NA	NA	In vitro	• Continuous increase of insulin release up to 35%	47
Chitosan and oligonucleotide	534 ± 24 nm	14.57 ± 1.1 mV	33% (prescaled up), 88% (scaled up)	In vitro	• Sustained release up to 88% for 10 h	86

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Table 1. continued

Polymer/material used	Particle size	Surface charge	Insulin encapsulation efficiency	Status	Significance of study	ref
					• All nanoparticles showed permeation through the transwell within 24 h	
Chitosan-mPEG	189.9 ± 5.71 nm	18.6 ± 0.70 mV	74.5 ± 0.95%	In vivo	• mPEG graft ratio of 10% showed the best results • mPEG enhanced mucus permeation	87
Baicalin-$AlCl_3$	100 nm	− 47.93 ± 4.71 mV	NA	In vivo	• Self-assembled NPs formed through coordination and hydrogen bonds • Downregulation of tight junctions achieved paracellular mode of entry	90
PLGA	200 nm	−20 mV	55%	In vitro	•45% insulin released at pH 6.8 •10% more mucus permeability of PLGA NP than unmodified NP	15
PBCA coated with FA-CS or HA	197.13 to 275.95 nm	−10.25 to −11.70 mV	99.87 to 99.91%	In vitro, in vivo	• Permeation percentage was more than 60% for all the graft ratios	53
PBCA	120−140 nm	−18 to (−10) mV	∼100%	In vivo	• Mass ratio-dependent properties •>60% mucus permeation in 10 min •>70% NPs retained for 12 h	85
Chitosan and alginate	100 to 300 nm	−25 mV to −16 mV	NA	In vitro, ex vivo	• Mucus penetration increased 1.6−2.5-fold when positive chitosan was coated with negative alginate	67
ENCP	236 ± 27 nm	+2 ± 2 mV		In vitro, ex vivo, in vivo	• Only ENCP permeated jejunal mucosa intact • ENCP showed 28 ± 9% uptake by intestinal epithelium	93
Milk-derived exosome	72.9 nm (dynamic light scattering) and 83.2 nm (nanoparticle-tracking analysis)	−9.8 mV	15.9%	In vitro, in vivo	• More sustained hypoglycemic effect compared to subcutaneous injection	58
PEC & SDS spray dried by CS/PVA	3.72 ± 0.03 to 4.63 ± 0.28 μm	−10.50 ± 1.03 to 7.59 ± 0.31 mV	NA	In vitro, in vivo	• PEC embedded in CS/PVA showed long-lasting hypoglycemic effect • The CS/PVA 1:1 group exhibited the highest relative pharmacological availability of 6.80%	65
PLGA and PEG linked by hydrazone bond	139 ± 1.10 nm	−32.8 ± 0.72 mV	48.03 ± 3.30%	In vitro, in vivo	• Hydrophilicity - hydrophobicity transition in response to acidic microenvironment at epithelial surface	62
Chitosan, biotin, PEC, HA	277.0 ± 12.7 nm	− 27.90 ± 0.23 mV	71.72 ± 0.48%	In vivo	• CS-Biotin 21.8%/Ins formulation showed the best hypoglycemic effect • In vivo hypoglycemic effect was dependent on the molecular weight of HA and biotin modification	52
Mesoporous silica	263.5 ± 3.021 nm	−0.49 mV	>80%	In vivo	• CPP5 mediated caveolae pathway of entry and enhanced trans-epithelial transport in vitro • Blood glucose reduced by 50%	54
DC modified MSN coated with SB12	2029 ± 267 nm	5.9 ± 0.39 mV	NA	In vitro, ex vivo, in vivo	• DC modified carrier avoided entry into lysosomes • Slow-release rate was achieved by the modification	70
DCDA-modified chitosan, octaarginine, and HA	187.17 ± 2.31 nm	−15.33 ± 0.45 mV	59.91 ± 1.53%	In vitro, in vivo	• HA coating increased the permeation percentage to 24.6% • Reversibly opened tight junctions • Sustained insulin release was observed in vitro -10% in SGF for 2 h and 58−64% in SIF for 6 h • Blood glucose level reduced to 38% in vivo	60
MSN modified with PLA−PEG and CPP	333.9 ± 134.1 nm	0.8 ± 4.0 mV	81.0 ± 1.9%	In vitro, in vivo	• Modification delayed the premature insulin release and reduced mucus trapping by 36% • PA% was 14.2 folds higher than oral insulin solution	69
DSPE-PCB micelles	25 ± 4 nm	−41 ± 4.6 mV	>98%	In vitro, in vivo, ex vivo	• Oral bioavailability was 42.6% • Penetrated across the epithelial cell layer proton-assisted amino acid transporter 1 (PAT1) mediated pathway	57
PC6 coated chitosan	233 ± 5 nm	12 ± 4 mV	80.5 ± 2.5%	In vitro, in vivo	• Mucus barrier and epithelial barrier were overcome at once due to the tight junction opening by PC6/CS NPs	66

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Table 1. continued

Polymer/material used	Particle size	Surface charge	Insulin encapsulation efficiency	Status	Significance of study	ref
					• Maintains certain blood concentration for a long time and has an oral bioavailability of 16.22%	
Cationic liposomes covered with BSA	194.9 nm	−10.93 mV	28.7% ± 5.1%	In vitro, ex vivo, in vivo	• Hypoglycemic effect persisted up to 12 h • Oral bioavailability was up to 11.9%	72
Alginate and chitosan	513 ± 19.00 to 522.50 ± 66.47 nm	−45.17 ± 2.38 to 3.77 ± 0.13 mV	44.38 ± 1.40%, 76.69 ± 10.92%, 44.87 ± 1.55%	In vitro, in vivo	• CCAB-embedded AC18N showed reduced drug release • Raised insulin level in the blood	64
HTCC coated with thiolated HA	102 ± 3 nm	−26.2 ± 1.0 mV	91.1 ± 0.4%	In vitro, ex vivo, in vivo	• Oral administration yielded a relative e bioavailability of 11.3%	68
PGA−PEG	202 ± 28 to 236 ± 27 nm	−44 ± 1 to +2 ± 3 mV	81 ± 4 to 99 ± 0%	In vitro, in vivo	• Cellular uptake of 47.59 ± 5.79%, highest ever to be reported in vitro • Did not translate to in vivo, moderate insulin response	73
PLGA with octa-arginine and phosphoserine	81.81 nm	−2.39 mV	≈ 40%	In vitro, in vivo	• Oral bioavailability of 5.96%, ∼1.9 fold higher than that of P-R8-INS NPs • Charge reversal-induced CPP-mediated cellular uptake and trans-epithelial transport	59
L-penetratin with HA coating	103.7 ± 3.5 nm	−19.7 ± 0.6 mV	96.6 ± 1.7%	In vitro, in vivo	• Sustained insulin release within 12 h • Relative bioavailability 11%, in comparison to s.c injection of free-form insulin	63
Alginate, lipid	124 ± 13 to 147 ± 27 nm	−14 ± 2 to −12 ± 1 mV	NA	In vivo	• Accumulation between 45 and 60 cm in the 100 cm length of small intestine	77
Polyurethane, chitosan, alginate	114 nm	32.5 mV	98.5%	In vivo	• Sustained release both in vitro and in vivo • Maximum insulin bioavailability of 11.6%	44
PAA	71.1 ± 9 nm	28.7 ± 3 mV	92 ± 12%	In vitro	•0.4−1:1 polymer: insulin mass ratio showed the best stability • Protection against trypsin and α-chymotrypsin	42
N, N-Dimethyl-N-Octyl Chitosan	150 ± 18.5 nm to 1846 ± 53.7 nm	+10.6 ± 1.27 mV to +25.3 ± 2.18 mV	17.0 ± 2.83% to 97.1 ± 1.94%	In vitro	• Significant release profile in the simulated intestine • No cytotoxicity with 5 h incubation	27
Chitosan, L-Phe, PEC, SDS	131.9 ± 1.1 nm	30.71 ± 4.31 mV	93.4 ± 2.6%	In vivo	• L-Phe graft ratio affected mucodiffusion • SDS provided stability and enhanced permeation through mucus • Hypoglycemic effects lasted up to 9 h	55

[a]This review covers various muco-adhesive (MA), muco-penetrative (MP), and hybrid type (MA and MP) formulations for the oral delivery of insulin. Before going into the formulation aspects, basics of structure, composition and functions of mucus layers in GI tract is discussed in detail.

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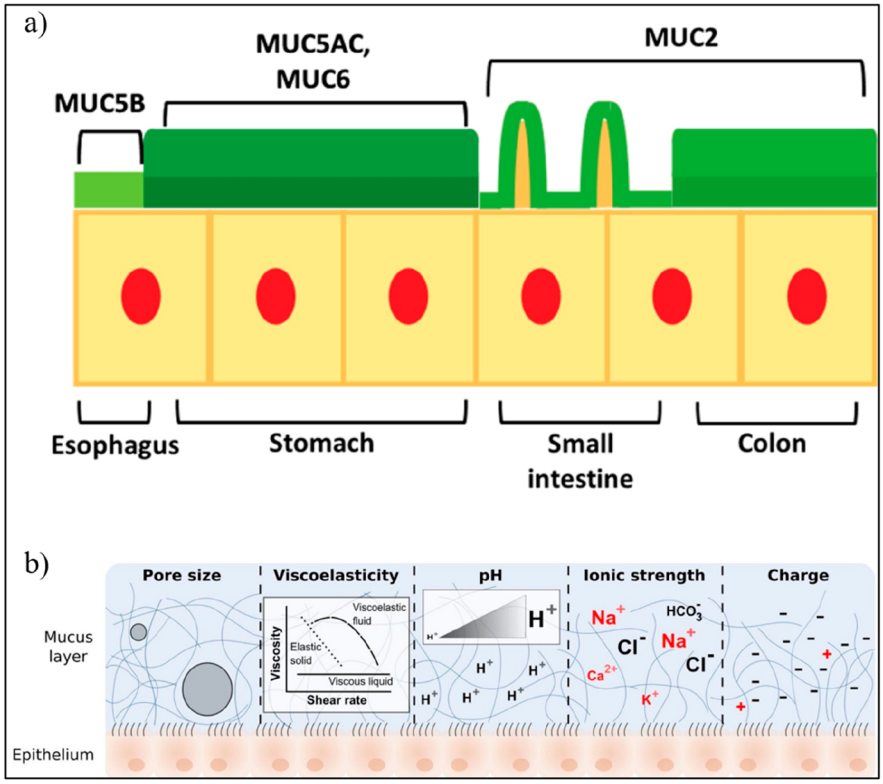

Figure 3. a) Schematic representation of the mucus layers across the GI tract, showing the two layers in the stomach and colon and a single mucus layer in esophagus and small intestine. Also, expression of different mucin proteins across the gastrointestinal tract are also elucidated; b) Important properties of mucus that drive their interactions with MA and MP formulations. (Reprinted in part from "Physicochemical properties of mucus and their impact on transmucosal drug delivery" by J. Leal, 2017, International Journal of Pharmaceutics, 532(1), 555−572; "Mucus interaction to improve gastrointestinal retention and pharmacokinetics of orally administered nano-drug delivery systems" by D. A. Subramanian, 2022, Journal of Nanobiotechnology, 20(1), 362. Copyright 2022 Elsevier)

Table 2. Properties of Mucus That Facilitate Mucoadhesive and Mucopenetrative Interactions

Properties of Mucus	For mucoadhesive formulations	For mucopenetrative formulations
Pore size	Smaller pore size provides a larger contact area for better adhesion	Larger pore size reduces steric hindrance for better penetration
Viscoelasticity	Higher viscoelasticity enhances mucus adherence	Lower viscoelasticity permeates penetration through mucus
pH	Lower pH increases viscoelasticity which enhances mucoadhesion	Neutral pH allows better diffusivity through reduced mucin interactions
Ionic strength	Lower ionic strength also increases viscoelasticity and causes mucus dehydration which enhances adherence to mucus	Higher ionic strength or high salt concentration increases the mobility of charged polymers through the charge-shielding effect
Charge	Negative charge	Neutral charge

such as mucin glycoproteins, globular proteins like immunoglobulins, nucleic acids, lipids, enzymes, cells, cell debris, and electrolytes.[14,15] The mucus and associated layer comprise three major types of cells−the intestinal epithelial cells forming the epithelial barrier, the goblet cells secreting mucus, and the M lymphatic cells[15] through which substances such as antigens penetrate.

The concentration of mucin protein (2−5%) in the mucus dictates its cross-linking and consequent viscoelastic properties. Mucins are glycoproteins with a protein backbone consisting of proline, serine, and threonine (PST) residue repeats interspersed with hydrophobic, cysteine-rich domains, facilitating polymerization and providing swelling and adhesive properties.[14] These proteins are heavily glycosylated with N-acetylgalactosamine (GalNac), N-acetylglucosamine (GlcNac), fucose, galactose (Gal), and sialic acid,[14] to prevent degradation in their digestive enzyme-prone environment. The mucus, once secreted, undergoes swelling to a 500-fold volume expansion. The presences of acidic glycosyl chains affect its conformation and gelling properties although water, lipids, and ions in the composition also significantly contribute toward its elasticity.[16] Moreover, it was also found that the mucin proteins secreted in the different regions of the GI tracts are different. MUC5B, a gel-forming mucin is found in the salivary gland and esophagus mucus; the mucus in the stomach consists of MUC5AC and MUC6; and finally, the mucus in the small intestine and colon has MUC2.[17]

The properties of the mucus that facilitate interactions with nanoparticles that enter the mucus layer are pore size, mucus turnover, viscoelasticity, pH, ionic strength, and charge, which are elucidated in Figure 3b[14] and Table 2. The mucus forms an essential layer in protecting the epithelial cells from damage due to the acidic pH of the stomach (pH 1−2), as well as preventing xenobiotics from entering the epithelial (pH 7) tract. In the context of drug delivery, it is important to know that the mucus has a high turnover rate to remove these "unwanted" particles including nanocarriers of oral drugs such as insulin. These particles interact primarily with the loosely

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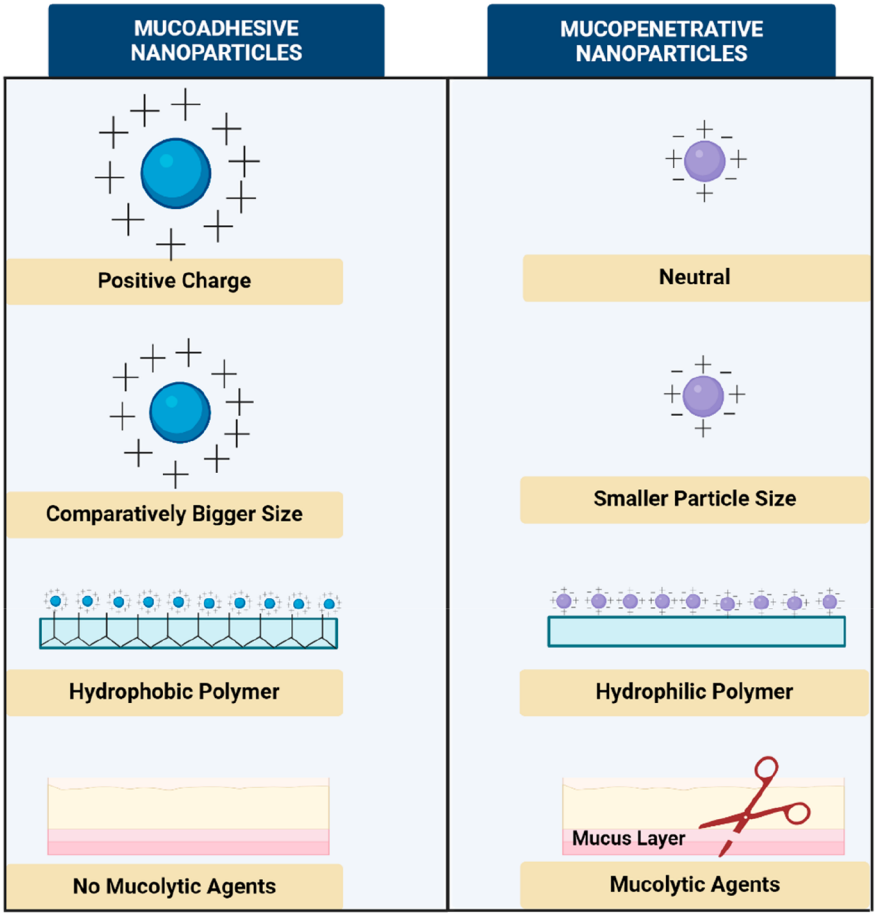

Figure 4. Comparison of Properties of MA and MP nanoformulation (Created using BioRender.com).

adherent mucus layer, which brushes them down the tract, preventing their interaction with the inner much firmly adherent layer.

Glycosidic branches interact using hydrogen bonding and electrostatic interactions while the lipid components interact using hydrophobic interactions with mucus entering particles. The inherent sialic acid and sulfate composition of mucus glycoprotein gives a net negative charge to the mucus, which facilitates better interaction with positively charged nanoparticles.[18] Particles that are smaller than the cross-linked mesh of the mucus can diffuse through the mesh, although other factors affect their diffusivity, as well. Nanoformulations, targeting improved oral insulin bioavailability, aim either to extend residence time within the mucus (muco-adhesive formulations) or to penetrate the mucus barrier and access circulation (muco-penetrative formulations). There are several reports on the utilization of MA and MP formulations for the enhanced drug uptake/release, which are mainly due to various intermolecular forces (including van der Waals, hydrogen bonding, and electrostatic interactions)[19] established between the formulations and mucin proteoglycans. Figure 4 highlights some key differences in the characteristics between these two types of formulations. The sections below give a detailed overview of the various muco-adhesive and muco-penetrative formulations for the oral delivery of insulin.

4. MUCOADHESIVE (MA) FORMULATIONS FOR INSULIN

4.1. Mucoadhesion Theory. According to the mucoadhesive theory, there are two steps to how particles adhere to the mucus layer.[17] The first step is the contact phase, wherein the movement of the gastrointestinal fluid contacts the particle with the mucus to prevent forces that repel adhesion. The second step is consolidation, which changes the mucus properties to strengthen the bond between the particles and the mucus to prolong the adhesion even in the strongly adherent layer. Consolidation can occur due to either the dehydration theory or the interpenetration theory. Dehydration theory follows the movement of water through the mucus layer, such that its lubrication is reduced to enhance adhesion. Interpenetration theory, on the other hand, involves strengthening the mechanical forces at the mucus-particle interface.

4.2. Strategies for Developing Mucoadhesive (MA) Formulations. MA formulations have a distinct positive charge that electrostatically interacts with the negative charge of mucin glycoproteins. Additionally, due to the nonspecific interactions of van der Waals forces and hydrogen bonding, there is a risk of rapid clearance of these particles during the frequent turnover of the loosely adherent mucus layer, thus not reaching the firmly adherent layer at the bottom. While the lipid-based formulations add to the mucoadhesion by increasing the retention times in the mucus, they are not as stable as the polymer-based formulations synthesized using

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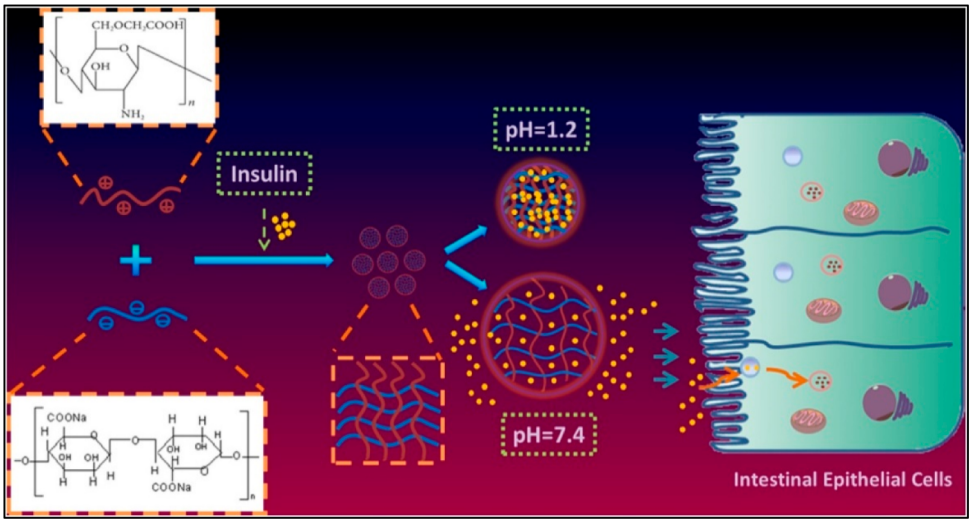

Figure 5. Demonstration of pH-modified nano hydrogel (O-carboxymethyl chitosan/sodium alginate) for augmented oral insulin delivery. (Reprinted from "pH-sensitive O-carboxymethyl chitosan/sodium alginate nano hydrogel for enhanced oral delivery of insulin" by H. Zhang, 2022, International Journal of Biological Macromolecules, 223, 433−445. Copyright 2022 Elsevier).

combinations of poly(lactic acid) (PLA), poly(sebacic acid) (PSA), poly(lactic-*co*-glycolic acid) (PLGA) and poly(acrylic acid) (PAA),[16] that facilitate polymer chain interpenetration. The following sections give a detailed overview of various strategies used for the construction of MA formulations.

4.2.1. Chitosan and Its Derivatives-Based Insulin Formulations. Chitosan, an exopolysaccharide from the shells of crustaceans is a commonly used polymer for mucoadhesion due to its positive charge, enhanced uptake by Peyer's patch due to its property to temporarily open the tight junctions in the mucosal cell membrane, biocompatibility, and its capacity to shield insulin from degradation.[18] MA nanoparticles based on mucin-chitosan complexes have shown ionic interaction between the positively charged chitosan and the negatively charged mucin, which forms a multidimensional entanglement network around the insulin. This network allows for gradual drug diffusion out of the release medium, resulting in sustained release and improved bioavailability.[20]

An insulin-encapsulated polymer matrix composed of positively charged chitosan was combined with negatively charged snail mucin proteins to enhance mucoadhesion.[20,21] While chitosan prolongs the bioavailability of encapsulated insulin, snail mucin acts as a medium onto which chitosan attaches to increase the residence time of the insulin along the walls of the gastrointestinal tract. Using techniques such as self-gelation and the double emulsion method, with higher chitosan concentrations, enhances the encapsulation efficiency of the system. The resulting irregularly shaped microparticles with a high positive surface charge indicated the presence of chitosan. The encapsulation and loading efficiency of insulin within the microparticles were found to be about 75% and 28%, respectively. The *in vitro* release studies demonstrated sustained release of over 80% of insulin over 12 h. In a diabetic animal model, the insulin-loaded microparticles significantly reduced blood glucose levels by over 50% compared to that of the control. The effect of the microparticles lasted for over 8 h, indicating their potential for sustained delivery of insulin.

Cationic liposomal nanoformulations with a chitosan coating provided an efficient vehicle for carrying insulin. Liposomes, due to their cationic lipids, interact with negatively charged insulin to encapsulate it and increase its retention time. Low molecular weight chitosan coating was used to improve the MA property. The short chains of chitosan were found to have increased interpenetration with mucin proteins. The diabetic blood glucose level significantly decreased after an hour when experimented in diabetic mice.[22]

In addition, the use of pH-sensitive polymers, such as poly(acrylic acid) (PAA), also facilitated electrostatic interactions by changing their charge in response to changes in pH, such as those that occur along the gastrointestinal tract.[23] Using trimethyl chitosan and fucoidan for enhancing the transepithelial permeation of insulin through the intestinal epithelial cell barrier has shown the potential for insulin delivery.[24]

Insulin-loaded nanoparticles cannot pass through tight junctions via the paracellular pathway. Upon entry into the GI tract, these particles adhere to and infiltrate mucus, leading to pH instability, which mediates their release into the bloodstream. Chitosan/poly(g-glutamic acid) as well as poly(isobutyl cyanoacrylate) nanoparticles aid GI absorption of insulin in the intestine in the presence of diethylene triamine penta-acetic acid. The interaction between the positively charged chitosan coating layer of the insulin-loaded poly(n-butyl cyanoacrylate) nanoparticles and the negatively charged mucus layer in the gastrointestinal tract was reported. The positively charged amino groups of chitosan can interact with the negatively charged sialic acid and sulfate groups in the mucus layer, leading to the formation of a stable MA bond. This electrostatic interaction between the chitosan coating layer and the mucus layer can prolong the residence time of the nanoparticles in the absorption site and enhance their absorption efficiency.[25]

Chemical modifications to chitosan such as methylation can enhance the mucoadhesion and absorption of insulin nanoparticles by increasing the solubility across both the acidic stomach and alkaline intestinal pH. When trimethylated chitosan in combination with hypoglycemia enabling fucoidan was used to encapsulate insulin, superior transepithelial absorption was observed, with modulated release at differing pH along the gastrointestinal tract.[26] Long-chain methylation (N, N-Dimethyl-N-Octyl) with lower molecular weight of chitosan had a better hydrophobicity as compared to trimethyl

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chitosan, which further enhances its epithelial absorption.[27] Acetylation also increases the interaction of the nanoparticles with the mucus layer. Using the polyelectrolyte complexation method, acetylated cashew gum and chitosan were combined to encapsulate insulin to obtain reduced blood glucose levels *in vivo* for up to 12 h postadministration.[28] Chitosan derivative made with cholic acid, quaternary ammonium, and hydroxypropyl methylcellulose phthalate (HPMCP) protected the loaded insulin from further degradation in the GI tract, with HPMCP increasing the mucoadhesion of the nanoparticle in the ileum. Moreover, the colic acid groups significantly improved the absorption of the nanoparticles in the ileum and liver. The hypoglycemic effect was sustained for over 24 h.[29]

4.2.2. Cellulose-Based Insulin Formulations. Cellulose-based formulations are a promising approach for oral insulin delivery. Cellulose is a natural polymer that is abundant, renewable, and biodegradable. They are also biocompatible and nontoxic, making them an ideal material for their application in drug delivery. Encapsulation of insulin with cellulose protects it from degradation in the gastrointestinal tract. It was also found that cellulose could control the release of insulin into the bloodstream, which can improve the efficacy of insulin therapy.

Gong et al.[30] designed pH-responsive carboxylated cellulose microspheres (CCMs) to enhance the oral bioavailability of insulin. The CCMs were prepared using the citric/hydrochloric acid hydrolysis method through the ionization of the carboxyl group, showing a controlled release of insulin in the bloodstream. Similarly, Li et al.[31,32] combined sodium carboxymethyl cellulose and poly(methacrylic acid) to create a hydrogel loaded with insulin which works according to the change in pH in the gastrointestinal tract as demonstrated with two different polymers: O-carboxymethyl chitosan and sodium alginate in Figure 5. Moreover, modification of chitosan with hydroxypropyl methylcellulose phthalate showed a 10-fold increase in hypoglycemic effect.[33]

4.2.3. Alginate-Based Insulin Formulations. Alginate is a natural polysaccharide that is biocompatible, biodegradable, and nontoxic making it suitable for oral delivery of insulin especially protecting the drug from degradation in the harsh gastrointestinal environment.[34] In the GI tract, alginate coating is effective over chitosan coating as the chitosan tends to get precipitated at pH 6−6.5 leading to its precipitation and further poor MA properties in the distal region of the GI tract. However, alginate interacts through the formation of hydrogen bonds with mucin glycoproteins while simultaneously protecting the encapsulated insulin.

To enhance the encapsulation efficiency of the carrier, solid lipid nanoparticles (SLN) were used. SLN particles with alginate coating showed sustained release, insulin permeation through the transcellular pathway, and significant hypoglycemic effect in diabetic rat models.[35]

In another study, self-nanoemulsifying drug delivery systems (SNEDDS) were developed by using a combination of surfactants. The MA properties were imparted through the coating of alginate and guar gum. Different nanoparticles with differing ratios of surfactants and mucoadhesives were tested in gastric and intestinal simulated environments. The formulation with soybean phosphatidylcholine and 0.05% sodium alginate showed the highest insulin bioavailability of 46.3%.[36]

4.2.4. Synthetic Polymer-Based Insulin Formulations. Synthetic polymer complexes demonstrated mucoadhesion through their properties of protease inhibition of the gastrointestinal enzymes and a mesh network that facilitates the slow and sustained release of insulin. Synthetic polymers such as poly(methacrylic acid-*co*-methyl methacrylate) [PDM], poly(acrylic acid-grafted-ethylene glycol [P(AA-*g*-EG)] and others have protease inhibitory properties, thereby protecting insulin from proteases as well as enhancing the mucoadhesion to offer a glue-like property to the mucus. Further modification of these particles with cell permeating molecules and size reduction enhanced their retention within the mucus layer and enabled cellular absorption, respectively.

PDM were reported to have been used as an MA carrier for insulin delivery. The PDM is modified with carboxylic acid groups to achieve mucoadhesion, which can interact with the mucus layer through hydrogen bonding and electrostatic interactions. Additionally, PDM is combined with another polymer, hyaluronic acid (HA), to improve the stability and versatility of the delivery system. The HA can also enhance the MA properties of the nanoparticles and improve their biocompatibility.[37]

P(AA-*g*-EG) hydrogels act as promising MA drug carriers with pH-responsive properties. The hydrogen bond between the PEG chains and the PAA backbone breaks when the pH becomes more basic due to ionization of the carboxylic groups. This resulted in swelling of the polymeric network and finally decomplexation of the polymeric network. These features allow the hydrogel network to transport medications or proteins to specific bodily regions depending on the pH.[38] Poly(methacrylic acid) grafted with poly(ethylene glycol) (P(MAA-*g*-EG)) hydrogel microparticles contain properties of sustained release and mucoadhesion in a pH-dependent manner and therefore are good carrier particles. The mucoadhesive properties come from the protective attributes of the small mesh size of the polymer network as well as their ability to inhibit calcium-dependent proteases that degrade insulin, by sequestration of calcium ions[39,40]

Thiolation and quaternization of polyelectrolyte complexes (PECs) such as poly(allylamine) enhance mucoadhesion through the formation of disulfide bonds between thiol groups of the PECs with the cysteine residues of the mucin proteins. Lower degrees of quaternization or introduction of a quaternary ammonium group to the polymer backbone[41] can reduce the interpenetration with the mucin proteins and limit steric hindrance, thereby enhancing mucoadhesion.[42]

Zhang et al.[43] designed an MA nanoparticle using alternate layering techniques of oppositely charged polymers like polygalacturonic acid (PGLA), chitosan, and alginate. The opposing charges of the layered structure gave pH sensitivity, since the electrostatic forces are greatly affected by changes in pH. The polysaccharide coating provided increased interaction with mucin, resulting in increased mucoadhesion. Moreover, the inherent hypoglycemic properties of PGLA further contributed to the reduction of blood glucose to 50%.

Core−shell nanoparticles were synthesized with polyurethane-chitosan (PU-CS) as the core and polyurethane-alginate (PU-ALG) blend forming the shell.[44] The alginate coat on the shell promoted mucus adherence, while chitosan promoted mucosal surface attachment through electrostatic interactions. Polyurethane contributed toward the sustainable swelling and release of insulin particles; however, it reduced the mucoadhesive property.

4.2.5. Miscellaneous Formulations. The polyelectrolyte complexation was performed with natural polymers as well

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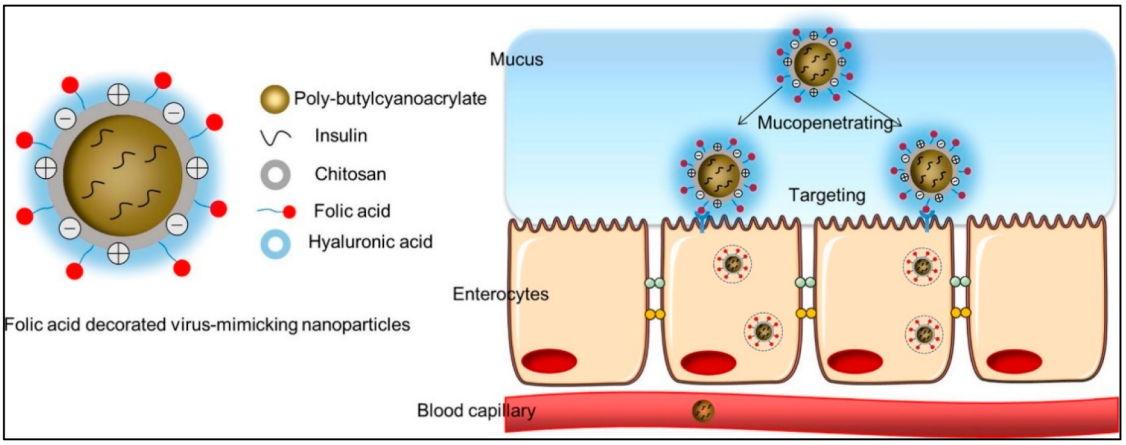

Figure 6. Demonstration of virus-mimicking nanoparticles as delivery vehicles for oral insulin (Reprinted from "Design of folic acid decorated virus-mimicking nanoparticles for enhanced oral insulin delivery" by Cheng, 2021, International Journal of Pharmaceutics, 596, 120297. Copyright 2021 Elsevier)

using ionotropic pregelation of *Sterculia striata* (SS) gum followed by electrolytic complexation of oppositely charged biopolymers such as dextran sulfate, chitosan, and albumin, to create a multilayer complex. Due to the presence of carboxyl and hydroxyl groups on the SS gum, its MA strength was observed to be high in *ex vivo* studies on pig intestinal mucosa studies. Insulin release kinetics in simulated intestinal conditions was more sustained showing an influence of pH on the association between insulin and the polymer matrix.[45]

A unique viscous biomaterial, sucrose acetate isobutyrate (SAIB), was used as an MA material to increase the retention time of insulin in the gastrointestinal tract. SAIB demonstrated temperature sensitivity wherein its elastic modulus drops with the temperature increase, thereby acting as a good carrier material to spread over the surface of the gastrointestinal tract and adhere to the mucus membranes. Its changing viscosity promoted a larger area of contact between the biomaterial and the mucus barrier.[46]

Protease inhibitors in combination with MA polymers are promising avenues in the development of oral insulin drug delivery systems. Protease inhibitors play a crucial role in preventing the degradation of insulin by digestive enzymes. Various protease inhibitors such as Aprotinin, Bacitracin, Bestatin, Bowman-Birk inhibitor, Chymostatin, and Leupeptin are being explored for this approach. MA polymers help insulin adhere to the lining of the gastrointestinal tract, which extends its residence time and enhances absorption. Some MA polymers that have been used for this purpose include Carbopol, chitosan, hyaluronic acid, poly(acrylic acid), poly-(methacrylic acid), and sodium alginate.[47] The properties of protection from degrading enzymes such as trypsin and chymotrypsin were also observed in natural polymers made of gellan gum, formed by retrograde mixing of high amylose starch with pectin and then encapsulating insulin using an ionotropic gelation technique. Gellan gum microparticles significantly reduced the transepithelial electrical resistance (TEER), indicating a 73−86% increase in permeation of entry through the paracellular route.[48]

A unique approach to using iontophoresis for the oral delivery of insulin demonstrates the potential of swallowable MA patches. This novel technology utilizes the electric gradient for the transport of insulin. The MA patches are released out of their enteric coating and swell at a concentrated location in the intestine to produce a gradient that stimulates insulin release. In vivo studies showed an impressive 63% drop in blood glucose levels with no associated tissue damage. This model has tremendous potential for translation into clinical devices.[49]

5. MUCOPENETRATIVE (MP) FORMULATIONS FOR INSULIN

For mucus penetration to occur, particles must be small in size to avoid steric hindrance and have a neutral charge with the absence of hydrophobicity to minimize interactions with the mucin and lipid components of the mucus and, thus, enable penetration. Greater surface density of targeting ligands such as lectins, invasins, and vitamin B12, along with permeation enhancers such as mucolytic drugs, promote diffusivity through the mucus layer, enabling access to the cells in the Peyer's patch. The attachment of any ligand allows specific binding with M cells or mucosal epithelial cells.[19] Often, the particles work through a viscous fingering phenomenon wherein lower viscosity fluid entering a higher viscosity fluid creates pressure-driven channels of low viscosity fluid that drive osmotically driven absorption and consequent absorption-driven endocytic entry into the enterocytes.[16]

5.1. Strategies for Developing Mucopenetrative Formulations. MP formulations primarily make use of their small size and neutral charge to avoid interactions with mucin proteins. Strategies highlighting these properties make use of encapsulations, altering overall hydrophilicity, reversing the charge, and using core-in-shell approaches. Some others utilize biomimetic and virus-mimicking strategies for effective penetration inside mucus. There are also permeation enhancers that aid in the process of penetration through mucus, as discussed below.

5.1.1. Permeation Enhancers. Permeation enhancers are substances that improve the absorption of insulin through the intestinal membrane, enhancing its bioavailability. They work by temporarily altering the properties of the membrane allowing better penetration of insulin molecules thus overcoming the gastrointestinal barriers.[50] Sodium caprate (C10) and sodium caprylate (C8) are some of the most commonly employed permeation enhancers that increase intestinal permeability. Polysorbate 80 (Tween 80) is a surfactant that is reported to increase the solubility and permeability of

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insulin.[51] Bile salts such as sodium taurocholate and sodium glycocholate, have also been used as permeation enhancers.[24]

5.1.2. Virus Mimicking Strategy. Hyaluronic acid (HA) coated virus-mimicking polyelectrolyte complex (PEC) was designed by loading insulin in biotin-decorated chitosan. Chitosan (CS) was a carrier for insulin, and it also protected it from enzymatic degradation. Also, biotin decoration on the surface of the particles helped to increase the uptake of these particles by enterocytes due to enriched biotin receptors on these cells. To facilitate mucus penetration, positively charged PEC was coated with negatively charged HA. Different biotin degrees of substitution and molecular weight of HA were considered, and the *in vivo* study indicated that the hypoglycemic effect was affected by both parameters. It was concluded from the report[52] that the CS-biotin21.8%/HA (200) PEC was the best formulation which gave the desired hypoglycemic effect.

Insulin-loaded poly(*n*-butyl cyanoacrylate) (PBCA) nanoparticles (NPs) coated with folic acid grafted chitosan (FA-CS) and HA mimicked the structure of the virus.[53] Butyl cyanoacrylate protected insulin and showed pH-responsive release characteristics in the gastrointestinal fluids, i.e., it prevented insulin release in the acidic environment, which is schematically represented in Figure 6. Folic acid was used as a targeting ligand to promote endocytosis of nanoparticles inside enterocytes. Mucus penetration study on porcine mucus revealed that the permeation percentage was more than 60%.

Mesoporous silica nanoparticles (MSN) with modification groups, negatively charged carboxyl groups, and positively charged cell-penetrating peptides (MSN-NH2@COOH/CPP5) were fabricated to achieve hydrophilic and electrically neutral surface properties of viruses.[54] CPP5 also promoted cellular uptake by the caveolae-mediated pathway and effectively enhanced the transepithelial transport. MSNs exhibited good physiochemical stability and biocompatibility. They protected proteins from degradation and had a large surface area, which resulted in high drug loading. In vitro investigation of mucin interaction with the NPs revealed that the aggregation rate of MSN-NH2@COOH/CPP5 was 20.63% and such a low aggregation rate showed that the interaction of the nanoparticles with mucin was less; therefore, they can penetrate the intestinal mucus layer efficiently. Transepithelial transport studies of MSNs using the Caco-2 cells monolayer model revealed that the transport of MSN-NH2@COOH/CPP5 was 2.0-fold higher than that of MSN-NH2@COOH and 2.4-fold higher than that of MSN@NH_2. The blood glucose level of diabetic rats was reduced by nearly 50% with the administration of insulin-loaded MSN-NH2@COOH/CPP5.

Liu et al.[55] utilized the characteristic negative−positive envelope on viruses that facilitate them to enter the intestinal epithelium through the mucus barrier to design a chitosan-*g*-N-Phe copolymer-based polyelectrolyte complex (PEC) with sodium dodecyl sulfate (SDS) coating as a nanoparticle carrier for oral insulin delivery. The addition of SDS coating proved to enhance the permeation of the nanoparticle beyond the mucus layer and act as a trypsin inhibitor to prevent the degradation of insulin within the intestinal epithelium. SDS facilitated the paracellular and transcellular routes of entry through epithelial cells and promoted the sustained release of insulin. The hypoglycemic effect lasted up to 9 h in rats and the pharmacological bioavailability was 5.8 ± 0.02%.

Virus capsid mimicking polymeric shell and biologic shell-based nanoparticles were formulated with insulin-loaded CS-Biotin as core coated by HA or alginate (Alg) (polymeric) or Streptavidin (SA) (biologic).[56] Polyglutamic acid (PGA) was added to the biologic-shell to mimic the virus structure since a positive zeta potential was observed in the case of only SA coating. Biologic shell complexes showed higher mucus penetration (>80%, 10 min) followed by Alg and HA-coated polymeric shell complexes. In vivo hypoglycemic study hinted that the biologic shell complex performed better in regulating the blood glucose level by decreasing it to almost 67% and a relative pharmacological availability of 5.1% was observed.

Capsid viruses have an external surface with equal densities of positive and negative charges, which result in unhindered diffusion through mucus. Virus-mimicking zwitterionic micelle DSPE-PCB (zwitterionic betaine polymer (polycarboxybetaine, PCB) conjugated to 1,2- distearoyl-*sn*-glycero-3 -phosphoethanolamine (DSPE) lipid) was found to be transported transcellularly rather than through tight junctions. The epithelial cell layer was penetrated by a pathway mediated by proton-assisted amino acid transporter 1 (PAT1) which is known to facilitate the penetration of betaine and its derivatives. DSPE-PCB/Insulin was freeze-dried and encapsulated with Eudragit L100−55 enteric-coating. The bioavailability was 42.6% on administration to diabetic rats. It was concluded that zwitterionic PCB particle diffuse faster than polyethylene glycol (PEG) through mucus since the ensemble-averaged geometric mean square displacement (MSD) of the zwitterionic particle was about 6.7 times the PEG.[57]

5.1.3. Exosome-Based Strategy. Exosomes showed high biocompatibility and long-circulating time and possess an intrinsic ability to encapsulate biological macromolecules. Taking advantage of this, Wu et al. fabricated insulin-loaded bovine milk-derived exosomes (EXO@INS) by a sonication method. Negatively charged phospholipids and hydrophilic proteins are the main components of the exosomal membrane, and they facilitate mucus penetration. EXOs are capable of deformation, which results in increased mucus penetration. EXO@INS (50 IU/kg) exhibited a stronger reduction in blood glucose level compared to subcutaneously injected insulin indicating the superior oral bioavailability of exosomes.[58]

5.1.4. Charge Reversal and Biomimetic Strategy. MP nanoparticles must possess hydrophilic and neutral charged surface to traverse across the mucus barrier, while hydrophobic and cationic surface for the epithelial barrier transition. Viruses can cross both these barriers due to the presence of densely coated anionic and cationic groups, which render a neutral charge, and evolved specialized proteins that can invade the host cell. Biomimetic NPs (P-R8-Pho NPs) with charge reversal ability were developed by coating Poly(lactic-*co*-glycolic acid) (PLGA) with cationic octa-arginine (R8) peptide and specific anionic phosphoserine (Pho).[59] R8, a cell penetrating peptide, is responsible for cellular uptake and trans-epithelial transport. Intestinal alkaline phosphatase (IAP), an enzyme expressed in the intestinal epithelium, functions as a catalyst for the hydrolysis of monophosphate esters which results in the dissociation of the anionic phosphoric acid. When the NP undergoes mucus penetration, enzyme-responsive hydrolysis of Pho, which is a substrate of IAP leads to the exposure of R8 thereby reversing the charge. The P-R8-Pho NPs achieved rapid mucus penetration and the permeability coefficient (Papp) was 12.57 × 10^{-6} cm/s which was 2.53-fold higher than P-R8 NPs. In vivo oral

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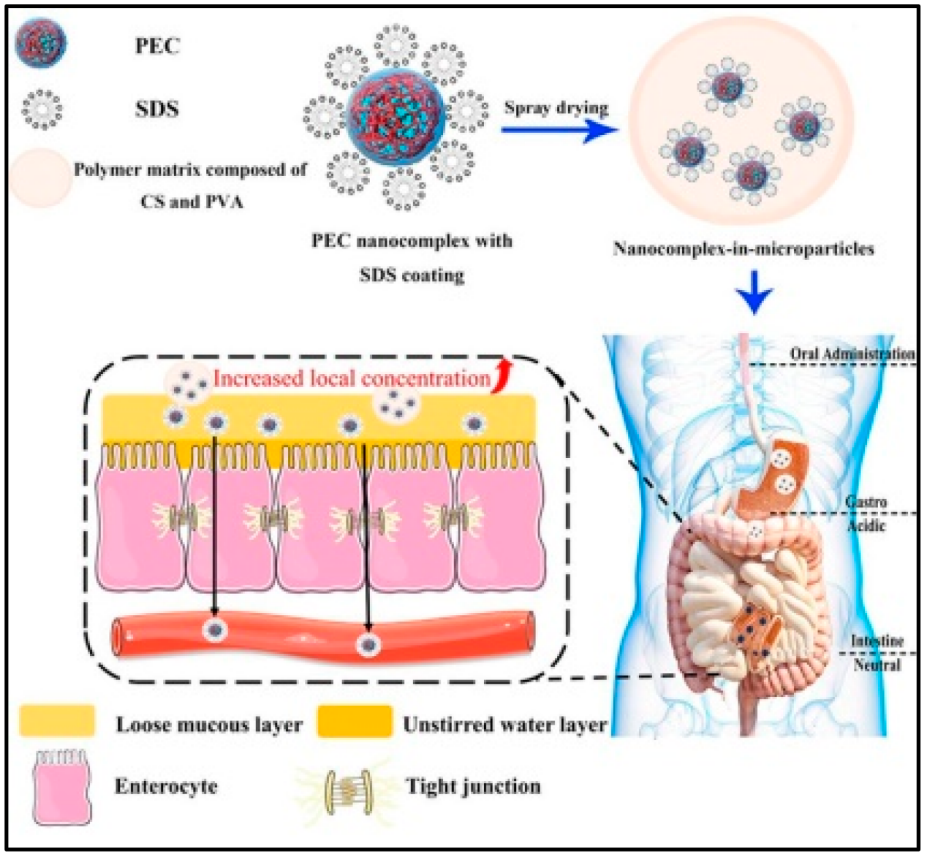

Figure 7. Demonstration of nanoin-microparticles as delivery vehicles for oral insulin (Reprinted from "Exploring the potential of redispersible nanocomplex-in-microparticles for enhanced oral insulin delivery" by Z. Ciu, 2022, International Journal of Pharmaceutics, 612, 121357. Copyright 2022 Elsevier).

administration resulted in a maximal blood glucose reduction of 32% and showed the highest relative oral bioavailability of 5.96%, which was ∼1.9 fold higher than that of P-R8-INS NPs.

Another approach was to modify the insulin nanocarriers by targeting peptides to enhance their mucus penetration and transepithelial absorption. Nanocarriers such as R8-modified PGA−PEG have shown promising results in oral insulin delivery. These nanocarriers are composed of poly(glycolic acid)-poly(ethylene glycol) (PGA−PEG) and are modified with octaarginine (R8), a cell-penetrating peptide (CPP).[60] The R8 modification enhances the uptake efficiency of the nanocarriers, allowing for improved mucus permeation and transepithelial absorption of insulin. The nanocarriers were designed to protect insulin from degradation in the acidic stomach environment and to release insulin in a controlled manner in the intestine. The R8-modified PGA−PEG nanocarriers have shown promising results in preclinical studies, demonstrating enhanced oral bioavailability and hypoglycemic effects in diabetic rats.[61] Similarly, HA-DCDA-CS-r8-INS NPs composed of insulin, Dicyandiamide Dicyandiamide-modified chitosan (DCDA-CS) to open the tight junctions reversibly thereby transporting NPs into the bloodstream via the paracellular pathway, cell-penetrating octaarginine (r8), and hydrophilic hyaluronic acid (HA) was developed. The addition of HA coating can significantly increase penetration percentage to 24.6% in mucus due to the shielding from external positive charges. In vivo hypoglycemic studies showed that HA-DCDA-CS-r8-INS NPs gradually reduced the blood glucose level to 38% within 6 h.

5.1.5. Nanoin-Microencapsulation Strategies. Li et al.[62] fabricated NPs made of Poly(lactic-*co*-glycolic acid) (PLGA) and polyethylene glycol (PEG) linked by hydrazone bond (PLGA-Hyd-PEG) and it was loaded with insulin and encapsulated into Eudragit L100-coated capsules which release the NPs at pH > 6 thereby protecting it from gastric activity. PEG serves as a hydrophilic shield for the hydrophobic PLGA core, thereby facilitating the rapid penetration of NPs into jejunal mucus. pH-mediated rapid cleavage of PEG and hydrolyzation of the hydrazone bond at pH 5.5 converted the NP surface from hydrophilic to hydrophobic, which resulted in the cell uptake on the jejunal epithelial surface. In vivo oral administration of the NPs resulted in a lowering of the blood glucose level to about 65% for up to 10 h.

Sequential flash nanocomplexation (FNC) technique was used to form nanocomplex with insulin and an L-Penetratin core coated with hydrophilic hyaluronic acid.[63] pH-sensitive hydroxypropyl methylcellulose phthalate (HPMCP) was used to encapsulate the NC to protect it in the gastric environment and deliver the NC to the small intestine. L-Penetratin enhances the trans-epithelial transport since it is a cell-penetrating peptide and HA helps in mucus penetration by electrostatic repulsion. NPs were released from the micro-capsule at pH 6.8, and the optimized microcapsule showed a relative bioavailability of 11% compared to subcutaneously injected free insulin.

Insulin-loaded alginate-C18 conjugate (AC18N) NP embedded in tripolyphosphate -cross-linked chitosan-oleic acid conjugate-coated calcium alginate beads (CCAB) was

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shown to enhance the blood glucose lowering extent of insulin synergistically. The bead prevented the premature release of insulin into the gastric medium. The negative charge and small size of the AC18N promoted mucus penetration.[64]

Redispersible nanocomplex-in-microparticles were prepared by loading insulin in Polyelectrolyte nanocomplex (PEC) with sodium dodecyl sulfate (SDS) coating and spray drying it with chitosan (CS)/ poly(vinyl alcohol) (PVA),[65] as shown in Figure 7. PVA exhibits excellent release regulation and film-forming ability. It forms a composite film with CS and helps in mucopenetration due to its hydrophilic and noncharged nature. CS of different molecular weights (50, 100, 150 kDa) and different CS/PVA ratios (1:4, 1:1, 4:1) were used and CS/PVA 1:4 and 1:1 groups showed enhanced mucus penetration of the in situ redispersed PEC and insulin permeation in intestine was in the order of CS/PVA 1:1 > 1:4 > 4:1. The highest relative pharmacological availability (6.80%) was exhibited by the CS/PVA 1:1 group which also demonstrated good enzymatic stability, enhanced mucus penetration and moderate insulin release rate.

5.1.6. Strategies That Alter Mucus−Nanoparticles Interaction. Zhou et al.[66] reported a thiolated-polymer based nano drug delivery system for oral insulin delivery. Insulin was loaded into chitosan nanoparticles which are coated with poly(acrylic acid)-cysteine-6-mercaptonicotinic acid-represented as PC6-CS NPs. The addition of PC6 attenuated the charge interaction between NPs and mucin, minimizing the interaction between mucin and nanoparticles. The presence of PC6 also facilitated the opening of tight junctions, as shown in Figure 8, thereby aiding insulin transport via the paracellular

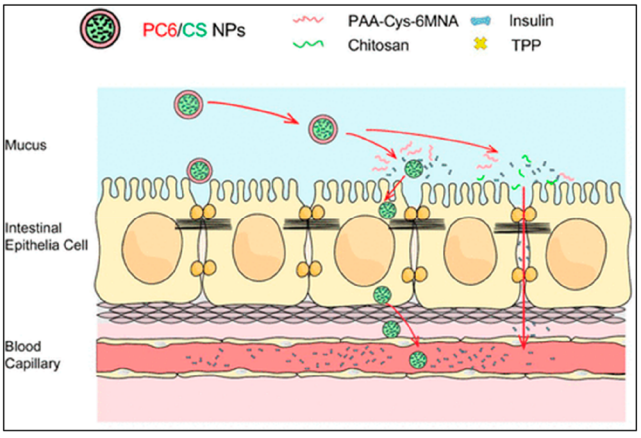

Figure 8. Schematic representation of the steps involved in the penetration of poly(acrylic acid)-cysteine-6-mercaptonicotinic acid-chitosan nanoparticles (PC6-CS NPs) across the mucus layer and enter the circulation through the paracellular pathway. (Reprinted (adapted) from "Thiolated Nanoparticles Overcome the Mucus Barrier and Epithelial Barrier for Oral Delivery of Insulin" by S. Zhou, 2020, Molecular Pharmaceutics, 17(1), 239−250. Copyright 2020 American Chemical Society)

pathway across epithelial cells. Importantly, upon traversing mucus, uncoating of the negatively charged PC6 from PC6-CS NPs due to its interaction with disulfide-containing proteins resulted in exposing the positively charged CS nanoparticles. This resulted in increased adherence and uptake of insulin-loaded CS nanoparticles by epithelial cells. The NPs showed long-term effects to reduce the blood glucose within 10 h and an oral bioavailability of 16.22% was observed.

Insulin-loaded poly(lactic-*co*-glycolic acid) (PLGA) NPs were prepared by the microfluidic technique and appended with heparin sulfate. PLGA can enhance the absorption of insulin by promoting the transcellular pathway of insulin. Heparin sulfate was added to facilitate the mucus penetration. It has mucolytic properties, which induce a shear thinning effect on the mucus by altering its structure via a charge−charge repulsion mechanism or by disruption of the intermolecular hydrogen bonds of the mucin. A moderate initial burst release was observed in the first stage (pH 2.2), following which a second burst release was detected after the pH was raised in the second stage (pH 6.8). Only a small percentage of insulin (∼15%) was released in the first stage, and it increased in the second stage reaching around 45% after 12 h. The initial burst release is due to the desorption of insulin molecules associated with PLGA NPs surface, whereas the latter burst is due to the ionization of PLGA at pH 6.8 which was higher than the pK_a of PLGA (pK_a = 3.8). This indicated that the PLGA protected insulin in the acidic environment.[15]

5.1.7. Core and Shell-Based Strategies. The core−shell nanocomplex (NC) with a positive core composed of insulin, chitosan, and sodium tripolyphosphate (TPP) and a negative shell made of alginate was reported.[67] Alginate was bound to the core by the electrostatic interaction between alginate's carboxyl group and the amino group of chitosan. The negatively charged alginate reduced the interaction between mucus and nanocomplex, thereby decreasing the mucus entrapment. On comparing the mucus penetration capacity *ex vivo*, it was found that the alginate-coated nanocomplex showed 1.6−2.5 times higher mucus penetration than the chitosan-insulin nanocomplex and increased amount of nanocomplex uptake by the intestinal villi.

A positively charged core made of insulin and N-(2-hydroxypropyl)-3-trimethylammonium chloride-modified chitosan (HTCC) was coated with thiolated hyaluronic acid (HA-SH) to give it a negative charge.[68] The mucus penetration was achieved by charge repulsion since the mucus is also negatively charged. The thiolated surface was shown to form disulfide bonds with mucin, thereby prolonging the intestinal retention time. Following the mucus penetration, the HA-SH coating gets dissociated, and the NC core gets deposited in the apical surface of the epithelial cells proceeding to transepithelial transportation. It showed a relative bioavailability of 11.3% compared to HA-coated NC and NC without coating.

5.1.8. Hydrophilicity-Based Strategies. INS@MSN@PLA−PEG-CPP, insulin-loaded mesoporous silica nanoparticles (MSNs) modified with poly(lactic acid)-methoxy poly(ethylene glycol) (PLA−mPEG) and cell-penetrating peptides (CPPs) were prepared to achieve hydrophilic and electroneutral properties.[69] PEG increased the hydrophilicity and decreased hydrophobic reactions with mucin, thereby decreasing mucus trapping by 36%. Low molecular weight protamine (LMWP) was chosen as the CPP to deliver insulin across intestinal barriers. According to the *in vivo* pharmacological studies, INS@MSN@PLA−PEG-CPP exhibited the fastest and most effective hypoglycemic effect as it reduced the blood glucose level to 48.78 ± 14.70% and its pharmacological availability was 14.2 folds higher than oral insulin solution.

Gao et al.[70] developed zwitterion-functionalized mesoporous silica nanoparticles (MSNs) modified with either deoxycholic acid (DC) or stearic acid (S). MSN-DC was coated with sulfobetaine 12 (SB12) and MSN-S was coated with either

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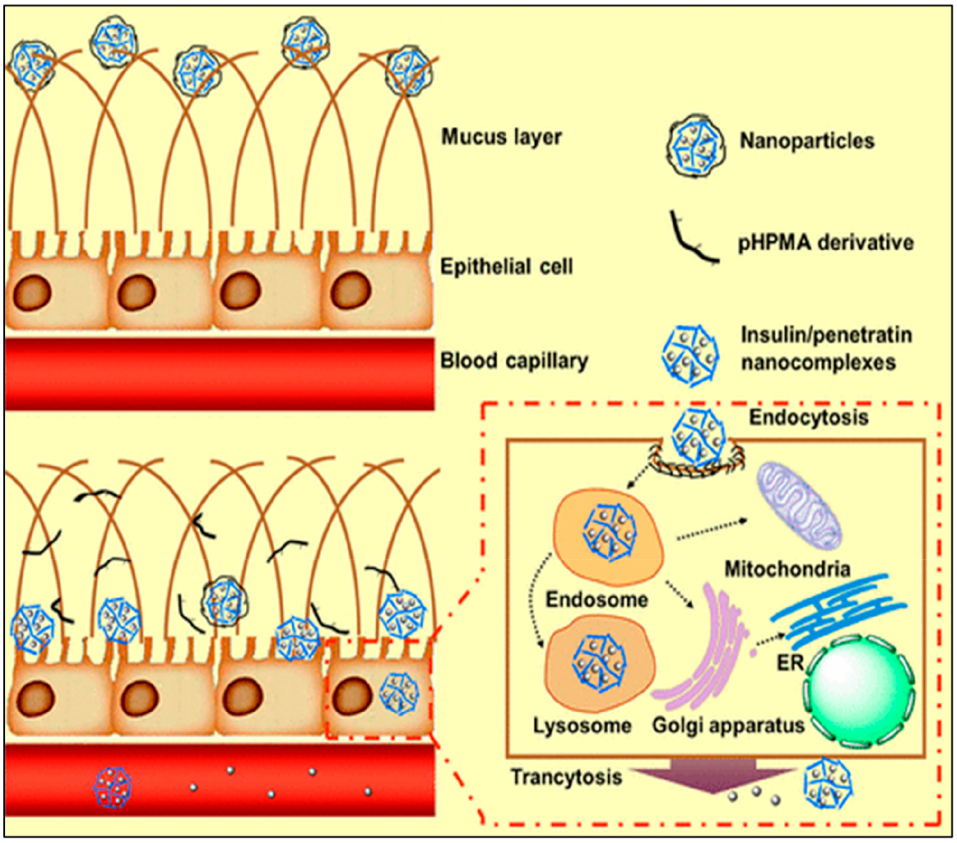

Figure 9. Schematic representation of the sequential steps that are involved when particles with both muco-penetrative and transepithelial cell entry properties are used in oral delivery. The top figure depicts the presence of a mucus-inert hydrophilic pHPMA layer over the nanoparticles which gives it MP properties, while in the bottom figure, the outer polymer layer is removed after penetration, showing the penetrating layer beneath, resulting in increased cellular uptake through endocytosis. (Reprinted (adapted) from "Overcoming the Diffusion Barrier of Mucus and Absorption Barrier of Epithelium by Self-Assembled Nanoparticles for Oral Delivery of Insulin" by W. Shan, 2015, ACS Nano, 9(3), 2345−2356. Copyright 2015 American Chemical Society.).

SB12, dilauroylphosphatidylcholine (DLPC), or Pluronic P123. Here SB12, DLPC, and P123 are hydrophilic and give an overall neutral charge. The DC coating had a hydrophobic surface, which facilitated epithelial absorption. Ex vivo permeation study revealed that the absorption of functionalized MSNs increased in all the intestine segments compared to unmodified MSN In a similar study, polyphosphoester and zwitterionic dodecyl sulfobetaine coated porous silica nanoparticles showed twice the mucin permeability coefficient and insulin oral bioavailability as compared to their nonzwitterionic counterparts.[71]

Diametrically opposite surface properties are required to achieve mucus penetration and increased epithelial uptake. Protein corona liposomes (PcCLs) were developed keeping this in mind. Bovine serum albumin (BSA) was adsorbed to cationic liposomes (CLs) to form PcCLs which were then electroporated with insulin.[72] The neutral charge and hydrophilic nature of the PcCLs facilitated mucus penetration. The hydrophobic nature of CLs and the anionic characteristics of cell membranes increased cellular uptake within intestinal cells. The behavioral study of PcCLs implied that BSA is hydrolyzed by enzymes as it crosses the mucus layer and CLs interact with the underlying epithelium, which resulted in improved transepithelial transport. The *in vivo* intrajejunal administration of PcCLs produced exceptional hypoglycemic effects that persisted for 12 h and the oral bioavailability of PcCLs was up to 11.9%.

5.1.9. pH-Responsive Insulin Release Strategy. Enveloped nano complexes (ENCPs) were made of insulin and octaarginine (r8), a permeation enhancer that was chemically conjugated with either lauric acid (C12) - (C12-r8) or cholesterol (Chol)-(Chol-r8).[73] PEGylated polymers enhance mucus penetration and prevent NP aggregation, and the carboxyl moieties of the PGA chain interact with the cationic insulin-r8 complex, thereby protecting the nanocomplex and projecting the PEG molecule toward the external phase. For this reason, the NPs were enveloped by negatively charged diblock (m[PEG]455-*b*-[PGA]10, methoxy-poly(ethylene glycol)-*block*-poly(L-glutamic acid sodium salt)) or branch type ([PGA]100-m[PEG]6 Poly(L-glutamic acid gamma- (omega-methoxyhepta (ethylene glycol)) sodium salt) PGA−PEGs). Since ENCPs enter enterocytes by endocytosis and the pH becomes more acidic along the endosomal pathway, the pH-based release of insulin was implemented here. At pH 4.0, both insulin and C12-r8 become positively charged, which leads to electrostatic repulsion and insulin release. The cellular uptake of PGA−PEG enveloped nanocomplexes (ENCPs) was 47.59 ± 5.79% which was the highest uptake ever to be reported *in vitro*. However, this result did not translate into enhanced insulin transport since only 2% of insulin was transported across the Caco-2 monolayer, and the *in vivo* oral administration also showed moderate response of insulin.

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6. HYBRID MECHANISMS OF ORAL INSULIN FORMULATIONS

6.1. Mechanism of Hybrid Formulations. The interactions between mucin and particle surface are highly influenced by the acidic pH of the gastrointestinal tract with enhanced cross-linkages[74] Amine and carboxyl groups promote mucoadhesion whereas PEGylation produces a brush-like conformation that prevents mucin-particle interaction thereby promoting mucopenetration. Other modifications such as acetylation of natural polysaccharides have also been shown to improve interactions of the particles with mucin.[28] Under acidic conditions, protonation of the mucin carboxyls masks electrostatic interactions but exposes hydrophobic domains. PEGylation and amine groups provide the penetration property.[75] A combination of alternate hydrophobic−hydrophilic layers, as well as positive−negative charges, and particle size are major contributing parameters to designing hybrid MA-MP carriers.[76] The hydrophobic outer layer adheres to the mucus and after gradual disintegration due to changes in pH or mucus turnover, the hydrophilic-coated MP particle gets released for mucus permeation and entry into cells, as demonstrated in Figure 9.[76]

6.2. Strategies Involving Hybrid Mechanism of Delivery. *6.2.1. Polymer-Coated Carrier-Based Strategies.* A noteworthy design of hybrid MA and MP nanovesicles involved a polymer−lipid double-encapsulated system wherein insulin-loaded lipid nanovesicles are encapsulated in a MA alginate carrier. The MA property of the alginate carrier comes from its ability to form carboxyl-hydroxyl hydrogen bonds with the mucin glycoproteins, which consequently increases the retention time of the vesicles in the mucus layer. However, once the alginate bead gets washed away due to mucus clearance, the nanovesicles are released, and owing to the mucopenetration provided by the hydrophilic (PEG) and hydrophobic poly(cholesteryl methacrylate) (PCMA) coblock polymer extension. These nanovesicles showed about 45−60% accumulation in the small intestine when administered orally in rats.[77]

Another study by Wu et al.[78] consisted of an alginate-based hydrogel as a carrier for insulin-encapsulated liposomes. In addition to the weaker noncovalent bonds between alginate and mucin, the cysteine-modification enhanced hydrogel-mucin interaction via disulfide linkages to prolong the retention time of the particles. The arginine-modified liposomes enabled energy-independent transport of the particles through direct hydrogen bonding with the proteoglycans on the enterocyte surface.

6.2.2. Ionic Liquid-Based Strategies. Ionic liquids (ILs) are green and tunable organic solvents, whose properties can be modulated through various combinations of cations and anions. They have good dissolution capacity and through their electrostatic interactions with the innate bile salt micelles along the gastrointestinal tract, ILs offer protection of carrier drug from the proteolytic enzymes.[79]

The Mitragotri group[80] designed an ionic liquid-based oral insulin formulation that consisted of chitosan and geranate (CAGE). Ionic liquid mixtures of eutectic solvents with organic cationic chitosan and anionic geranate provided both adhesive and penetrative properties, respectively. In vitro studies showed a concentration-dependent enhancement of paracellular transport across the Caco-2 cells. Such a medium also protects against insulin degradation by proteolytic enzymes in the intestine. In vivo studies showed a 65% reduction in blood glucose concentration.

Further work on this system[81] involved encapsulating CAGE into a poly(vinyl alcohol) (PVA) gel to create a patch that would promote sustained release of insulin at the surface of the enterocytes. Since the paracellular route-mediated transport of the cells is dependent on the CAGE concentration, repeated freeze−thaw cycles to synthesize FT-CAGE particles were attempted, showing enhanced mucus adhesion.

Another nano delivery system developed using ionic liquids was poly(lactide-*co*-glycolide) (PLGA) mixed with ILs nanocore embedded with insulin, with vitamin B12-conjugated chitosan coated using electrostatic interactions. These particles showed excellent bioavailability, entering the intestinal cells through intrinsic factor vitamin B12 receptors, clathrin-mediated endocytosis, or paracellular transport. The addition of vitamin B12 and the arginine present in the ILs both contribute to the cellular uptake of these particles, while chitosan promotes their movement through the mucus layer.[82]

6.2.3. Strategies Altering Polymer to Insulin Ratio. The high molecular weight of the insulin peptide (5.7 kDa) restricts its mobility through the intercellular gaps using the paracellular mode of transport.[83,84] Most of the insulin-loaded nanoparticles take the transcellular, absorption-mediated endocytosis, receptor-mediated endocytosis, or macropinocytosis pathways.[85]

However, this can be modulated by adjusting the polymer-to-insulin ratio. This parameter affects: (i) the extent of mucoadhesion and mucopenetration, (ii) drug loading capacity, and (iii) drug release rate. Polymers such as chitosan can not only adhere to the mucin due to its positive charge but can also enable the tight junctions to transiently open through interaction with the cytoskeletal proteins.[86] Wong et al.,[86] reduced the size of the insulin-loaded chitosan-PEGylated nanoparticles by using low molecular weight chitosan, to facilitate paracellular transport. The MP capacity was added through the attachment of the hydrophilic Dz13Scr oligonucleotide. Permeation assays showed almost 100% presence of nanoparticles in the receiving chamber of the permeation transwell system through primarily the absorption-mediated endocytic pathway.

Liu et al.[87] designed chitosan grafted polyethylene glycol monomethyl ether (mPEG) copolymers with different graft ratios of mPEG ranging from 5 to 18%. The formulation with 10% was observed to show the best mucus permeation *in vitro* and absorption *in vivo*. The hydrophilicity and steric hindrance provided by mPEG weakened the interaction between positively charged chitosan and negatively charged insulin, resulting in reduced drug loading. Increasing the graft ratio from 5% to 10%, decreased mucoadhesion as well as interaction with the mucus, thus enhancing mucus permeation, but increasing it further to 18% increased the mucus interaction.

A similar study with varying mass ratios of insulin and poly(*n*-butyl cyanoacrylate) (PBCA) was performed to tune the drug release rate of the self-polymerized nanoparticles. This system demonstrated controlled release at pH 6.8 and 7.4, with increased release when the amount of BCA was decreased. The highest concentration of plasma insulin was observed in diabetic rats with Ins/BCA = 2/10 NPs exhibiting a fast hypoglycemic effect, while Ins/BCA = 2/15 NPs showed a slow hypoglycemic effect.[85] Formulations with differing ratios of liposome, PEG, and B12, were synthesized where high

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molecular weight PEG increased the stability and retention time of the liposomes and, added with B12 ligand targeting, enhanced the overall mucus permeation and bioavailability of insulin. Lip−PEG-PB 0.125% was found to have the best bioavailability.[88]

6.2.4. Other Strategies. Cristian Reboredo et al.[83] transformed zein, which is a corn protein, into a nanoparticle coated with poly(ethylene glycol) and loaded with insulin (I-NP-PEG). The zein component because of its low solubility in water and lipophilicity added to the mucoadhesion while the PEG coating allowed the penetration through the mucus due to its very hydrophilic characteristic. Both PEG uncoated and coated zein nanoparticles were tested both *in vitro* and *in vivo* in Caco-2 and HT29-MTX cell lines and streptozotocin-induced diabetes rats, respectively. Since the bare nanoparticles without the PEG coating had only MA properties, they showed a 2.5-fold lower bioavailability than the coated nanoparticles. This can be explained by the contribution of the PEG coating toward providing an additional MP property through reduced fat accumulation facilitating the diffusivity through the mucus layer.

Chemical modifications, such as acetylation and thiolation of naturally occurring polysaccharides, can enhance the interactions of particles with the mucus. S-protection of thiolated cyclodextrins using polyethylene glycol (PEG) enhanced the retention time of particles in the mucus 11.2 folds. S-protection of free thiol groups is necessary to prevent undesired oxidation and reduce the reactivity of these particles such that they minimally react with mucins and diffuse through better. Instead of PEG, the use of 2-mercaptonicotinic acid enhances the mucoadhesion through electrostatic repulsive forces, hydrogen bonds, and hydrophobic interactions.[89]

An innovative multifunctional self-assembled nanoparticle synthesized using baicalin bonded with $AlCl_3$, showed pH-dependent insulin release, good MA properties as well as paracellular mode of transepithelial entry by modulation of the tight junctions.[90] These particles interact through hydrogen bonding and metal−ligand coordination bonds. Baicalin also had an additional property to regulate the expression of tight junctions, therefore, facilitating paracellular transport into enterocytes.

7. FUTURE PERSPECTIVES

Oral insulin delivery has been a long-standing goal of diabetes research. Over time, many promising oral insulin formulations have undergone clinical trials and shown positive results. However, despite these advancements, substantial challenges still exist that need to be overcome before oral insulin becomes a viable treatment option for patients with diabetes.[94] One of the primary challenges is that insulin can be broken easily by digestive enzymes. To overcome this challenge, researchers are developing new technologies such as polymeric nanoparticles, liposomes, and hydrogels to protect insulin from degradation and improve its absorption into the bloodstream.[95] Designing oral insulin formulations requires greater precision to release insulin at the right time and amount to achieve optimal glycemic control. Researchers are also exploring glucose-responsive insulin formulations that would release more insulin when blood sugar levels are high.[96,97]

Innovative formulations such as permeation enhancers, mucoadhesive nanoparticles,[59] and pH-sensitive polymers have helped overcome gastrointestinal barriers. Researchers have explored mucoadhesive drug delivery systems for effective

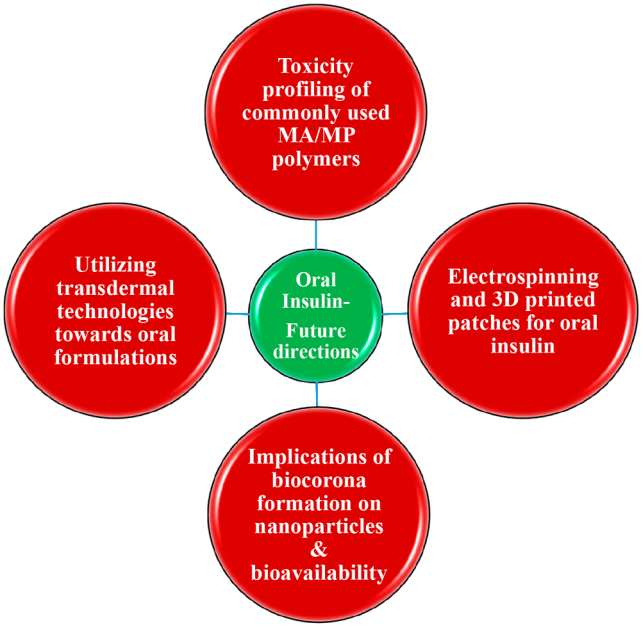

Figure 10. A schematic of plausible future research directions for oral insulin delivery systems

adherence to mucin-epithelial surfaces. Thiol group incorporation, lectin conjugation, and the use of biomaterials like sucrose acetate isobutyrate (SAIB) for enhanced mucoadhesion led to efficient drug absorption and improved glycemic control. Nanoparticles combined with hydrogels enhance insulin permeation.[59,98] However, the wide use of some MP polymers such as PEG becomes questionable since it has been observed to produce anti-PEG antibodies through complement activation.[99]

Further, transdermal delivery technologies such as iontophoresis and ionic liquids are explored in oral formulations to avoid gastrointestinal barriers and increase retention time.[79] Other emerging technologies, such as electrospinning and 3D printing, propel oral insulin delivery toward reality. Electrospinning offers control over nanofiber morphology and drug loading, enabling the design of mucoadhesive patches for sustained release.[100] Apart from electrospinning, 3D printing technology is also being utilized for the rapid synthesis of oral mucoadhesive patches. By customizing the physicochemical characteristics of the 3D printed biomaterial inks, it is possible to produce stretchable, mesh-like configurations[101] of polymer that can serve the purpose of mucoadhesion as well as accommodate sustained drug release.

3D printing allows for tailored biomaterial inks, creating stretchable, mesh-like configurations for mucoadhesion and sustained release.[101] These advancements showcase exciting possibilities, though challenges such as scalability, cost-effectiveness, and controlled nanofiber attributes persist. Despite the challenges, the future of oral insulin drug development is promising. Several oral insulin formulations are currently in clinical trials, and some have shown promising results. Other alternatives, such as liposome-encapsulated formulations like glycyrrhizin, exhibit promising hyperglycemia improvements, while small-molecule mimics like L-783,281 advance through Phase 2 trials.[102] However, not all efforts are successful, as evidenced by the Phase 3 failure of ORMD-0801, a hydrogel-based formulation.[103]

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The future of oral insulin drug development is very promising, as summarized in Figure 10. With continued research and development, oral insulin will likely become a viable treatment option for diabetes patients in the coming years. Oral insulin would be a much more convenient and less invasive way to deliver insulin than injectable insulin, and it could significantly improve the quality of life for millions of people with diabetes.

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Notes

The authors declare no competing financial interest.

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