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REVIEW ARTICLE OPEN ACCESS

Breaking Biological Barriers: Organic Nanocarriers for Multiroute Transmucosal Insulin Delivery

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ABSTRACT

The clinical use of therapeutic peptides such as insulin remains constrained by inefficient delivery routes that fail to achieve sufficient drug levels at target tissues. Moreover, the lifelong reliance on injectable formulations imposes considerable physical discomfort and psychological burden on patients. In response, a wide range of innovative strategies have emerged to provide less invasive, more patient-friendly, and highly bioavailable alternatives. Among these, transmucosal insulin delivery has gained particular attention due to the rich vascularization of mucosal surfaces, which enables rapid systemic absorption while avoiding the limitations of parenteral administration. Recent advances in nanoscience and nanotechnology have accelerated the development of novel delivery systems capable of protecting insulin from hostile physiological environments and enhancing its transport across mucosal barriers. In this review, we summarize the progress of organic nanosystems, specifically lipid-based and polymer-based nanocarriers, as promising platforms for noninvasive insulin administration via nasal, ocular, buccal, sublingual, and inhalation routes.

1 | Introduction

Insulin is a polypeptide hormone produced by beta cells of the pancreatic islets, made of 51 amino acids [1, 2]. It comprises two polypeptide chains held together by sulfide bonds and has a cationic charge at lower pH [3]. Due to its amino acid composition,

insulin falls on the margin between a large polypeptide and a small protein. It is considered the most widely used peptide therapeutic for diabetes, playing a vital role in controlling and maintaining cell growth, proliferation, and carbohydrate, fat, and protein metabolism [4, 5]. Insulin promotes the uptake of glucose from the blood into fat, liver, and skeletal muscle cells [6].

Hatem Mohamed and Nadine Wafik Nabih contributed equally to this work.

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Diabetes mellitus is a pervasive, insidious, and lifelong disorder caused by insufficient insulin secretion from the pancreas (Type 1) or reduced insulin response in cells (Type 2) [7]. According to the World Health Organization, it is the most common metabolic disease, threatening the health of about 830 million people worldwide, with the majority living in low- and middle-income countries [8–10]. Insulin is considered the main therapeutic management for diabetes mellitus Type 1 and is frequently required in late stages of diabetes mellitus Type 2 [11]. To date, subcutaneous injection remains the conventional route for daily insulin delivery for Type 1 diabetes. The parenteral route provides optimal drug absorption; however, it is associated with pain at the injection site, local tissue infection, poor patient compliance, and high medication cost [10]. Hence, there is a surge in developing more patient-friendly insulin delivery systems.

Although insulin is mainly recognized as a therapeutic agent in diabetes management, it has also been investigated across a wide range of other biomedical research fields. During the last decade, there has been a significant increase in the topical application of insulin for the treatment of corneal ulcers [12], since insulin enhances corneal epithelial healing and nerve repair in diabetic mice and facilitates the healing of epithelial defects unresponsive to conventional treatments [13].

Moreover, insulin has shown positive effects in patients with Alzheimer's disease (AD). Clinical studies have documented significant impairments in brain glucose utilization and in sensitivity to insulin and insulin-like growth factor stimulation that coincide with the progression of AD (Figure 1). Administration of insulin can improve memory recall, ameliorate memory deficits, and reduce the progression of hypometabolism in the brain. However, a significant obstacle to drug transport into the brain is the blood–brain barrier (BBB), which makes insulin administration very challenging [14].

Over the past 30 years, the potential of transmucosal drug delivery has driven extensive research aimed at developing a new generation of therapeutics superior to conventional oral tablet formulations. The development of drug delivery systems has been motivated by the high vascularization and accessibility of

various mucosal surfaces [15]. The mechanism of transmucosal drug transport varies among different mucosal tissues and can be broadly categorized into passive and active transport pathways [16]. Passive transport is further divided into paracellular transport, involving the movement of drugs through tight junctions, and transcellular transport [17]. Transcellular transport involves movement across the epithelial cell membrane and requires a concentration gradient [18].

On the other hand, active transport requires carrier-mediated transport and endocytosis. Carrier-mediated transport employs specialized carrier proteins to facilitate the translocation of drugs across the epithelial membrane against a concentration gradient. In contrast, endocytosis is an energy-dependent intracellular process that involves the uptake of drugs into vesicles, their internalization, and subsequent release on the other side of the cell (Figure 2) [16].

Conversely, the benefits of transmucosal delivery are hindered by the mucus layer itself. Mucus is a complex, viscoelastic gel acting as a protective layer covering much of the soft tissue in our bodies. Consequently, numerous nanomedicine-based strategies have been developed to overcome the mucus barrier and enhance drug efficacy and bioavailability, making the design of delivery systems a central focus in mucosal nanomedicine research [19].

2 | Nanoplatforms

Nanotechnology has become an integral component of modern biomedicine, enabling the design of nanoscale materials and delivery systems that improve the diagnosis, prevention, and treatment of various diseases by boosting bioavailability, enabling targeted delivery, and controlling the release of therapeutic agents [20–24].

Recently, advances in nanotechnology have significantly facilitated the transmucosal delivery of peptide-based drugs. Generally, nanocarriers are categorized into three main classes: organic, inorganic, and hybrid. Inorganic nanosystems, such as metallic nanoparticles (NPs) [25], quantum dots, magnetic NPs, and silica [26], offer excellent structural stability and tunable optical and electronic properties. However, their limited biodegradability can raise concerns about potential toxicity in patients [27]. Organic NPs consist of self-assembled macromolecular structures that, despite their relatively limited physicochemical stability, possess remarkable biocompatibility and biodegradability. They offer versatile surface functionalization with targeting ligands and can encapsulate both hydrophilic and hydrophobic therapeutic agents, enabling a controlled and sustained drug-release profile. Organic NPs are further divided into lipid-based nanocarriers and polymer-based ones. Conversely, hybrid NPs were engineered to integrate the benefits of both organic and inorganic systems. They display improved biocompatibility, targetability, and stability despite their complex synthesis and high production costs [28, 29].

2.1 | Lipid-Based Nanocarriers

Lipid-based nanocarriers are promising delivery systems in the clinical setting. They offer several advantages, making them potential nanocarriers for peptide-based drugs, including adaptability, higher payload capacity, and biocompatibility [30]. The selection and engineering of lipid carriers demand careful

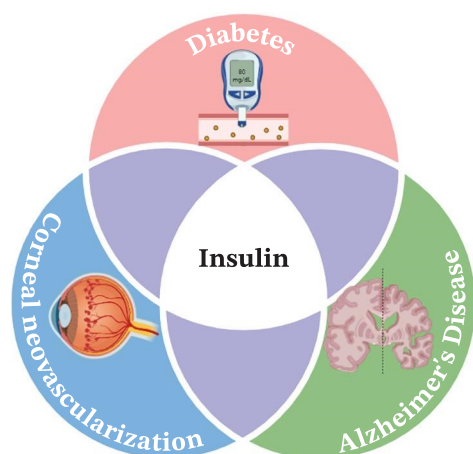


FIGURE 1 | Insulin serves as a therapeutic modality not only for the management of diabetes, but also in corneal neovascularization and AD. This illustration has been created by BioRender. <https://www.biorender.com/>.

Transmucosal Drug Transport

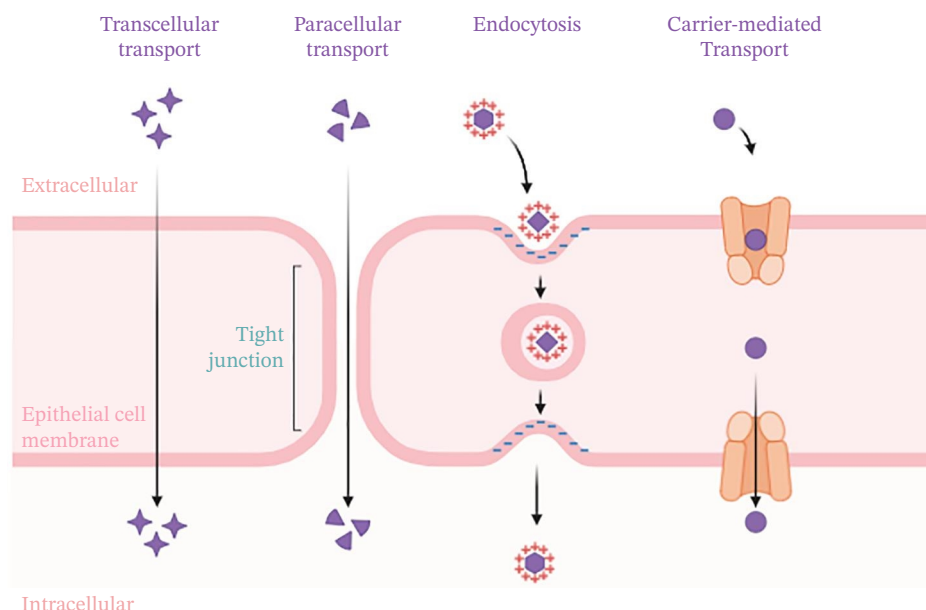


FIGURE 2 | Schematic presentation of the transmucosal transport main mechanisms: transcellular transport, paracellular transport, endocytosis, and carrier-mediated transport. This illustration has been created by BioRender. <https://www.biorender.com/>.

tuning, since they directly influence transmucosal transport kinetics. Lipid composition, release profiles, mucosal compatibility, and the inclusion of edge activators for deformability or PEGylation for stealth must all be finely tuned to achieve balanced encapsulation efficiency (EE%), mucosal adhesion, and release kinetics [28, 29]. Lipid-based nanocarriers include liposomes, solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), transferosomes, nanoemulsions (NEs), micelles, etc.

2.1.1 | Liposomes

Liposomes are small, rounded vesicles with a size range between 50 and 500 nm. They consist of a phospholipid bilayer enclosing an aqueous core, which partitions hydrophobic drugs into the bilayer, while hydrophilic agents localize in the core. Liposomes are readily prepared, biocompatible, and can be functionalized with ligands to enhance their transmucosal uptake. Unfortunately, they are susceptible to drug leakage and oxidative breakdown of unsaturated lipids during storage and require stabilizers or PEGylation to extend their shelf life [31–34].

2.1.2 | SLNs, NLCs, and NEs: Solid and Hybrid NPs

SLNs consist of a solid lipid core stabilized by one or more surfactants to enhance colloidal stability and control drug delivery. Yet, their use is mostly hindered by drug expulsion during storage due to lipid crystallization [35].

NLCs represent an advancement over SLNs. They incorporate both solid and liquid lipids, thereby diminishing crystallinity, increasing drug loading, and preventing expulsion, features particularly beneficial for chronic therapies that necessitate extended stability and storage [36].

NEs are oil-in-water dispersions having a droplet size typically < 200 nm [37]. They offer excellent mucosal permeability and scalable manufacturing, making them particularly suitable for rapid-onset drug delivery applications. Compared to SLNs/NLCs, NEs show limited sustained-release properties and require reformulation for chronic therapy (e.g., by adding gelling agents to extend retention) [38–40].

2.1.3 | Deformable Vehicles, Transferosomes

Transferosomes are ultradeformable liposomes that enclose at least one inner aqueous compartment, the activator of the edge [41].

The latter is typically a single-chain surfactant, such as sodium cholate (SC) or Span 80, that disrupts lipid bilayer packing and increases flexibility. This deformability facilitates tight junction penetration and enhances mucosal permeability, enabling efficient transport of drugs or other therapeutic agents across biological membranes. Nevertheless, transferosomes may cause irritation due to their high surfactant content and often show short-term stability, requiring careful optimization of lipid-to-surfactant ratios [42].

2.2 | Polymer-Based Nanocarriers

Polymeric NPs are nanocarriers ranging in size from 10 to 1000 nm and are widely used in drug delivery [43]. They include active substances either encapsulated into their matrix or surface-adsorbed onto the polymeric core. In addition, their surfaces could be decorated with various functional groups, thereby improving cargo stability and prolonging their half-lives [44].

2.2.1 | Poly(lactic-co-glycolic) Acid (PLGA) NPs

PLGA is frequently used to synthesize polymeric NPs owing to its biodegradability, biocompatibility, and favorable physicochemical properties [45]. The surface functionalization of PLGA NPs with polyethylene glycol (PEG) was reported to provide several benefits, as follows:

- 1. It improves the water solubility of NPs and the hydrophobic drugs they load.
- 2. It increases the surface charge and the steric hindrance on the PLGA NPs, thereby prolonging their stability and reducing their aggregation.
- 3. It confers a stealth effect, extending the systemic circulation time of their cargos by counteracting opsonization and subsequent clearance by the reticuloendothelial system [44].

2.2.2 | Chitosan NPs

Chitosan is a semisynthetic linear copolymer originating from *N*-deacetylated chitin, and comprising a certain number of *N*-acetyl-*D*-glucosamine and β -linked units [45]. It is a biodegradable, biocompatible polyaminosaccharide with remarkable physicochemical and mechanical characteristics [46]. The chain length and average molecular weight of chitosan can be controlled by enzyme-mediated decomposition or acid hydrolysis [47]. Modifying chitosan NPs with PEG results in a promising nanoplatform for effective chemotherapeutic delivery, capable of reducing their recognition by the reticuloendothelial system, thereby extending their circulation time [48, 49].

2.2.3 | Polymeric Micelles

Micelles are polymeric NPs formed by the self-assembly of amphiphilic block copolymers in aqueous media, thereby creating a hydrophobic core for drug encapsulation and a hydrophilic shell for stability and solubility [50]. Micelles have been shown to enhance the apparent water solubility of hydrophobic drugs, addressing major hurdles in effective drug delivery [51].

Nanotechnological strategies, such as the use of chemical enhancers, lipids, and polymer-based nanocarriers, are among the most frequently used approaches for peptide delivery, as their tunable size and surface functionalization enable enhanced pharmacokinetics, prolonged systemic half-life, and improved target specificity [52]. Table 1 compares the major organic nanoplatforms used in improving insulin delivery through the transmucosal membranes.

While comparisons between studies should be made carefully due to variability in formulation composition, experimental

TABLE 1 | Comparison of the major organic nanocarriers involved in transmucosal insulin delivery.

Nanocarrier platform	Major advantages	Major limitations	Most suitable transmucosal routes	Translational potential
Liposomes	Excellent biocompatibility, ability to encapsulate hydrophilic insulin, ease of surface modification	Drug leakage, limited storage stability	Buccal, nasal, ocular	High
Transfersomes	High deformability and enhanced mucosal penetration	Surfactant-associated instability and potential irritation	Buccal, nasal	High
SLNs	Good physical stability and controlled release	Drug expulsion during lipid crystallization	Oral, nasal	Moderate to high
NLCs	Higher drug-loading capacity and improved storage stability compared to SLNs	More complex formulation optimization	Oral, nasal	High
Nanoemulsions	Excellent permeability enhancement and scalable manufacturing	Limited sustained-release capability	Buccal, nasal, pulmonary	Moderate to high
Chitosan nanoparticles	Strong mucoadhesion, permeation enhancement, biocompatibility	Batch-to-batch variability and limited mechanical stability	Nasal, oral, buccal	High
PLGA nanoparticles	Controlled release, excellent biodegradability, regulatory acceptance	Relatively complex manufacturing processes	Oral, nasal	High
Polymeric micelles	Improved solubility and stability of encapsulated therapeutics	Potential dilution-induced instability	Oral, ocular	Moderate

models, and evaluation methods, several conclusions can be drawn. Transfersomes and elastic liposomes (ELs), as deformable lipid-based systems, have been shown to enhance transmucosal permeation by increasing membrane flexibility consistently. On the other hand, polymeric-based nanosystems, including chitosan and PLGA NPs, provided prolonged residence time, increased stability against degradation, and controlled-release capabilities. NLCs also improved loading capacity while increasing stability. Overall, the choice of nanocarrier appears to be route-dependent and should be optimized with consideration for permeability enhancement, insulin protection, formulation stability, and translational potential.

In the following sections, we will discuss organic nanocarriers investigated via various transmucosal routes that have recently been explored for noninvasive insulin delivery.

3 | Buccal/Sublingual Routes

Physiological and physicochemical barriers, including gastrointestinal tract (GIT) pH, degradative enzymes, and the low insulin membrane permeability, are the major obstacles to effective oral insulin delivery [53]. Consequently, the buccal and sublingual routes have recently attracted significant interest due to their rich vascularization, which drains directly into the systemic circulation; decreased enzymatic drug degradation; avoidance of the hepatic first-pass effect; and increased patient compliance [54]. In addition, they provide a fast onset of action, versatility in designing dosage forms, and are painless and simple to eliminate in cases of mucosal irritation [55]. In contrast, the buccal route has its own limitations. Low surface area for absorption, intercellular lipids, and continuous saliva production are all factors that may accelerate drug removal from the site of absorption. Recently, several studies have demonstrated the promise of nanomedicine as a basis for developing buccal delivery systems to overcome its bioavailability limitations.

For example, using the thin-film hydration method, SC-incorporated ELs (SC-ELs) and sodium-glycodeoxycholate-incorporated ELs (SGDC-EL) encapsulating insulin were developed, and their permeability across porcine buccal tissues was assessed (Figure 3). The SC-EL and SGDC-EL nanocarriers had

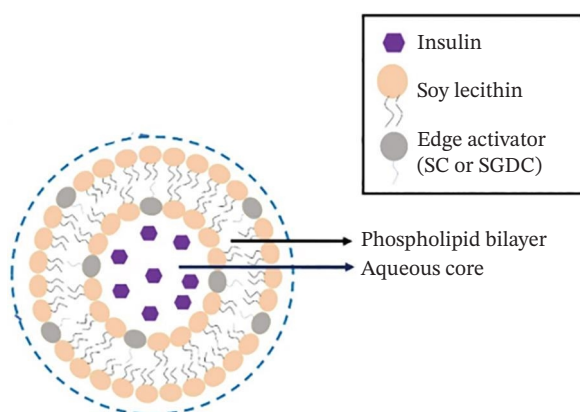


FIGURE 3 | Structure of the liposomes holding insulin (SC-EL or SGDC-EL), with the lipid bilayer made of soy lecithin and the edge activator, and the insulin in the core. This figure has been adapted from Ref. [56]. Permission from MDPI, copyright (2021).

a particle size (PS), a polydispersity index (PDI), a zeta potential (ZP), an EE%, and a drug-loading capacity (DLC%) of 144.40 ± 3.42 and 150.40 ± 3.50 nm, 0.187 ± 0.016 and 0.126 ± 0.015 , 67.37 ± 2.10 and 71.64 ± 1.25 mV, $76.45 \pm 8.84\%$ and $79.41 \pm 5.07\%$, and $0.137 \pm 0.016\%$ and $0.143 \pm 0.009\%$, respectively. Following their administration, SGDC-EL improved insulin permeation, exhibiting a 4.33-fold increase in the permeability coefficient relative to the insulin solution and a 3.70-fold increase relative to SC-EL. This underscores that the primary driver of improvement was the deformability of ELs, as the SGDC-EL's relative deformability index of 1.57 ± 0.05 was 1.41-fold greater than that of the SC-EL. Nevertheless, further investigations are required to clarify the underlying mechanism of insulin transport [56].

Another study also employed bile salts as permeation enhancers since they are regarded as biomimetic surfactants due to their high biodegradability and biocompatibility. Bashyal et al. [57] aimed to design hydrophobic ion-pairing (HIP) nanocomplexes (C1 and C2) using anionic bile salts and cationic peptides (Figure 4). The PSs were as follows: 364.37 ± 11.79 nm for C1 and 256.73 ± 17.74 nm for C2. In addition, their PDI values were 0.005 ± 0.000 and 0.189 ± 0.014 , respectively, indicating monodispersity of the C1-nanocomplex. The ZP of C1 was -0.26 ± 0.95 mV, while that of C2 was -0.13 ± 0.53 mV, and their complexation efficiency (CE%) was 92.71 ± 2.64 and 99.28 ± 0.13 , respectively. The C1-nanocomplex was superior, as evidenced by a significant increase in insulin permeability across TR146 human buccal cell layers and porcine buccal tissue, with permeability coefficients 3.00- and 51.76-fold higher, respectively, than those of insulin solution. Additionally, C1 exhibited huge uptake and installation of insulin in TR146 cells and porcine buccal tissue. It showed intense fluorescence in TR146 cells and a progressive inward shift in fluorescence intensity across buccal tissue over time, which was more pronounced than that observed with C2 or free insulin [57].

A year later, Guo et al. [58] introduced a temperature-sensitive gel loaded with an insulin-phospholipid complex encapsulated in deformable nanovesicles (IPC-DNV-TSG) and compared the delivery efficacy of both IPC-DNV and IPC-DNV-TSG using a TR146 cell model (Figure 5). DNV had a PS of 85.84 ± 2.38 nm, a ZP of -26.2 ± 0.5 mV, and an EE% of $77.47\% \pm 3.85\%$. The 2% w/v gelatin gel remained stable at 4°C for 3 months and maintained

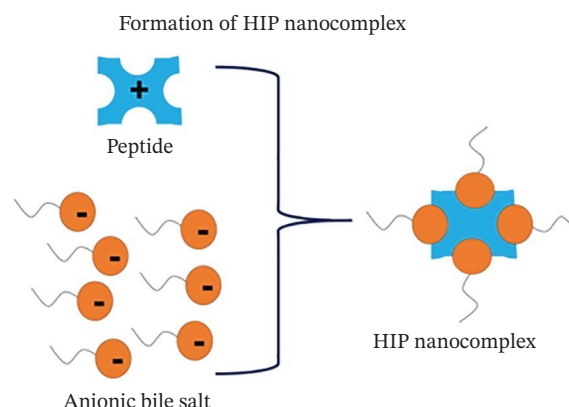


FIGURE 4 | Structure of HIP nanocomplexes (C1 and C2) made of anionic bile salts and cationic peptides. This figure has been adapted from Ref. [57]. Permission from Taylor and Francis, copyright (2021).

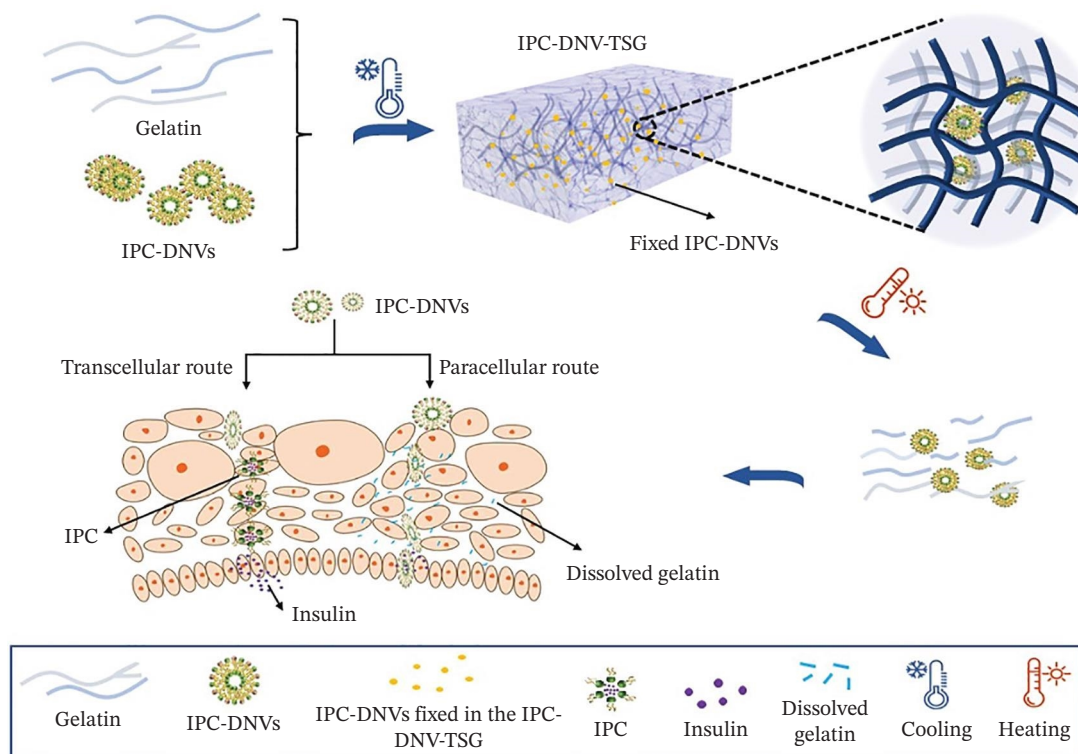


FIGURE 5 | Structure of the temperature-sensitive gel loaded with insulin–phospholipid complex encapsulated in deformable nanovesicles. Following administration, IPC-DNV-TSG crossed the mucosal barrier more efficiently, whereas IPC-DNV showed higher cellular uptake, resulting in comparable hypoglycemic activity for both. This figure has been adapted from Ref. [58]. Permission from MDPI, copyright (2022).

a remarkable hypoglycemic effect. Both formulations showed good cell viability. However, cellular uptake at 2 h was lower for IPC-DNV-TSG than for IPC-DNVs, as confirmed both qualitatively by confocal laser scanning microscopy (CLSM) and quantitatively by fluorescence-activated cell sorting (FACS). Surprisingly, cumulative transport at 4 h was not significantly different between the two groups. This could be attributed to the hydrophilicity, cell adhesion properties, biocompatibility of gelatin, and viscosity of IPC-DNV-TSG. Although IPC-DNV-TSG showed slightly reduced cellular uptake, it crossed the mucosal barrier more efficiently via the paracellular pathway, compensating for this deficiency. The *in vivo* hypoglycemic efficacy curves verified these results. At the early stage, the hypoglycemic effect of IPC-DNV-TSG was slightly weaker than that of IPC-DNVs; however, by 6 h, its bioavailability was not significantly different from that of IPC-DNVs [58].

Overall, nanocarriers enhance mucoadhesion, membrane permeability, and insulin residence time at the absorption site, thereby improving drug stability, absorption, and bioavailability.

All data regarding the type of nanocarrier, size, PDI, ZP, EE%, DLC%, cell lines, intended use, and advantages of the sublingual/buccal nanovehicles are summarized in Table 2.

4 | Nasal Route

Direct nose-to-brain transport relies on the anatomical connection between the nasal cavity and the brain, primarily via the olfactory and trigeminal pathways, as well as through systemic absorption via peripheral circulation. In this context, nasal insulin has attracted substantial attention compared to nonparenteral routes, as the nasal epithelium provides a large surface area for absorption,

the subepithelial layer is highly vascularized, and the endothelial membrane is porous, collectively contributing to bypassing pre-systemic metabolism and enhancing bioavailability [59].

However, there are several barriers to the absorption of drugs administered intranasally. The presence of an active mucociliary clearance mechanism, the reduced permeability of the nasal epithelium, which makes the absorption of high-molecular-weight drugs difficult, and the limited volume that can be administered are all contributing factors that limit absorption from the nasal cavity and decrease bioavailability [60]. Thus, to overcome the poor bioavailability, novel drug delivery systems, including NPs and nanoplatforms, have been suggested as follows.

To address the imperative need for a patient-convenient, non-invasive insulin therapy, Zuben et al. [39] developed insulin-loaded liposomes using a thin-film hydration technique and functionalized them with the cell-penetrating peptides (CPPs) TAT and penetratin (PNT). They compared the performance of the three formulations. The free, TAT-functionalized, and PNT-functionalized liposomes had a PS of 109.3 ± 1.04 , 110.4 ± 1.37 , and 101.5 ± 1.57 nm, respectively; a ZP of -49.6 ± 1.33 , -33.9 ± 1.10 , and -20.6 ± 1.30 mV, respectively; and a PDI of less than 0.4 for all formulations. Additionally, both CPP-functionalized formulations remained stable and free from phase separation over a 90-day stability period. However, the EE% (measured indirectly) was 72% for nonfunctionalized liposomes, but decreased to 60% and 71% when functionalized with PNT and TAT, respectively, along with lower insulin release values. Furthermore, after 24 h of permeation through porcine nasal mucosa, 83.96% of insulin from the solution, 63.32% from the TAT formulation, and 57.11% from the PNT formulation permeated. This

TABLE 2 | Overview of nanoformulations administered sublingually/buccally, detailing nanocarrier type, physicochemical characteristics (size, PDI, ZP, EE%, and DLC%), cell lines, intended use, and reported therapeutic advantages.

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
Liposomes	SC-EL and SGDC-EL: 144.40 ± 3.42 and 150.40 ± 3.50, respectively.	SC-EL and SGDC-EL: 0.187 ± 0.016 and 0.126 ± 0.015, respectively.	SC-EL and SGDC-EL: 67.37 ± 2.10 and 71.64 ± 1.25, respectively.	SC-EL and SGDC-EL: 76.45 ± 8.84 and 79.41 ± 5.07, respectively.	SC-EL and SGDC-EL: 0.137 ± 0.016 and 0.143 ± 0.009, respectively.	Porcine buccal tissues.	—	SGDC-EL improved insulin permeation, with a 4.33-fold increase in the permeability coefficient compared with the insulin solution and a 3.70-fold increase compared with the SC-EL	[56]
Nanocomplexes	C1 and C2: 364.37 ± 11.79 and 256.73 ± 17.74, respectively.	C1 and C2: 0.005 ± 0.000 and 0.189 ± 0.014, respectively.	C1 and C2: −0.26 ± 0.95 and −0.13 ± 0.53, respectively.	—	—	TR146 and porcine buccal tissue.	—	C1 exhibited a large increase in insulin uptake and installation.	[57]
DNVs	85.84 ± 2.38	—	−26.2 ± 0.5	77.47 ± 3.85	—	TR146 cells.	Diabetes	The gel formation was stable during refrigerated storage for 3 months.	[58]

indicates that electrostatic interactions and the subsequent complex formation between insulin and the CPPs reduced the drug's incorporation into the liposomes, thereby hindering the peptides' absorption-enhancing activity [61].

Since insulin's macro-polypeptide nature negatively influences its chances of reaching the brain, SLNs and PLGA NPs were promising carriers for insulin's intranasal delivery to the brain in AD. Hence, Akel et al. [62] formulated and characterized uncoated and chitosan-coated insulin-loaded SLNs and PLGA NPs with pleasant physicochemical properties, supporting intranasal applicability. The PS of PLGA NPs and SLNs was 135 ± 1.17 nm and 99.1 ± 5.3 nm, respectively. At the same time, the chitosan-coated SLNs and PLGA NPs showed a slight increase in PS (174.6 ± 10.7 nm and 145.2 ± 6.2 nm, respectively), facilitating both nose-to-brain transport via the olfactory nerve and receptor-mediated endocytosis. The uncoated SLNs and PLGA NPs showed negative ZP values of -28.2 ± 1.8 and -42.3 ± 1.5 , respectively, whereas chitosan coating shifted the ZP to $+58.4 \pm 0.7$ and $+61.3 \pm 0.5$ for PLGA NPs and SLNs, respectively. The EE% (assessed directly) of all prepared NPs was approximately 80%, while the DLC% averaged approximately 27%. All insulin-loaded nanocarriers demonstrated structural stability, improved mucoadhesion, nasal diffusion, and drug-release rate in vitro compared with native insulin, with chitosan-coated SLNs showing the greatest superiority. Studies on human RPMI 2650 nasal epithelial and human hCMEC/D3 brain endothelial cells confirmed the safety and intranasal applicability of NPs. Insulin-loaded NPs demonstrated enhanced permeability across the nasal mucosa, an effect further promoted by the chitosan coating. However, native insulin exhibited greater BBB penetration than the NP-based formulations. Thus, an ideal nose-to-brain formulation can be attained using a mixture of native insulin and insulin-chitosan SLNs to ensure both immediate efficacy and prolonged drug release [62].

Another attempt was made by Nojoki et al. [63], who encapsulated insulin in chitosan-coated transfersomes (Transfersulin). The nanovesicles, synthesized by the film hydration method, had a PS of 137.9 ± 28.2 nm, a PDI of 0.20, a ZP of $+23.4$ mV, an EE% of $65.1 \pm 0.9\%$ (assessed directly), and a DLC% of $9.1 \pm 0.4\%$, respectively. As confirmed by circular dichroism and fluorescence spectroscopy, insulin's structural integrity and stability were kept intact following the encapsulation. In vivo, insulin nasal uptake was confirmed by fluorescence imaging. The nanosystems revealed increased intracellular uptake, sustained drug release, and, consequently, more potent neuroprotective effects. Additionally, evaluation of the spatial and memory learning course using the Morris water maze showed a decrease in the escape latency time below 10 s. Meanwhile, histological examination of the hippocampus supported the mentioned behavioral findings [63].

Zuben et al. [64] reported loading insulin into liposomes functionalized with CPPs (TAT and PNT), which were added to a hydrogel prepared from an aqueous solution of hydroxyethylcellulose (HEC). Liposomes were prepared by hydrating a thin lipid film and exhibited a uniform size. Rheological analysis showed that the formulations behaved as non-Newtonian fluids, exhibiting pseudoplastic flow characteristics, which persisted even after liposome addition. Regarding insulin release, the liposomal gel promoted greater drug retention by providing an additional barrier to release through the

hydrogel. In contrast, TAT and PNT exhibited slower drug release due to complexation with insulin. The in vitro release kinetics of insulin from all formulations conformed to Weibull's mathematical model, achieving around 90.74% release from the HEC-based hydrogel formulation, 59.69% from liposomal insulin in HEC, 42.46% from TAT-functionalized liposomes in HEC, and 41.83% from PNT-functionalized liposomes in HEC, respectively. In diabetic rats with baseline glucose levels of about 460 mg/dL, serum insulin levels were reduced to approximately 241 mg/dL for HEC liposomal insulin after 360 min, 325 mg/dL for HEC TAT-functionalized liposomes after 180 min, and 304 mg/dL for HEC PNT-functionalized ones after 120 min [64].

To protect insulin from enzymatic degradation, a self-emulsified nanoemulsion (SEN) loaded with insulin into the lipid droplets (oil phase) was developed. SEM enabled insulin's absorption through the mucus gel barrier, facilitating both transcellular and paracellular permeation. The insulin-soy-L-phosphatidylcholine (SPC) complex was obtained by the solid dispersion method, with a PS of 32–42 nm and a PDI of 0.250 ± 0.07 . Following in vitro testing in human nasal epithelial cells, the insulin-loaded SEN significantly ($p < 0.01$) enhanced insulin permeation, resulting in a 3-fold increase compared to insulin solution alone. When evaluated intranasally in anesthetized rats, the C_{max} and bioavailability (relative to subcutaneous delivery) of the insulin-loaded SEN were 255.9 μ U/mL and 68%, respectively. In contrast, the insulin solution resulted in a C_{max} of only 5.8 μ U/mL and 5% relative bioavailability. Intranasal administration of 3.6 IU/kg insulin-loaded SEN resulted in a remarkable 70% drop in plasma glucose levels, with the hypoglycemic effect persisting throughout the 4-h experimental duration. Additionally, the nasal mucosa's histological examination showed no signs of toxicity [65].

In another noninvasive approach for insulin delivery to the CNS, Jamshidnejad-Tosaramandani et al. [66] designed insulin-loaded chitosan NPs. The latter were spherical with a PS of 165.3 nm, a PDI of 0.24, a ZP of $+21.6$ mV, an EE% of 69.05% by indirect assessment, and a DLC% of 27.97%. Mucotoxicity studies on the ovine nasal epithelium (OVINAE) confirmed that the NPs granted mucoadhesion while lacking any significant toxicity to the nasal tissue. Insulin release from the NPs exceeded 90%, showing a biphasic pattern characterized by an initial burst within the first 15 min, followed by sustained release over 4 h [66].

To restore brain-glucose balance as a therapeutic approach for AD, insulin-chitosan polyelectrolyte complexes were prepared and stabilized around NE droplets and administered nasally. NEs had a PS of 145 ± 2 nm, a PDI of 0.29, a ZP of $+12.7 \pm 0.5$ mV, a drug content of 1.25 ± 0.15 mg/mL, and an association efficiency of $69 \pm 8.6\%$. NEs retained their optimal nanotechnological performance after incubation under simulated nasal conditions (pH 5.5, 37°C) and after 30 days of storage at 8°C. They enabled accelerated insulin's mucosal permeation in comparison to the free peptide [11].

In 2023, Das et al. [67] designed an intranasally administered insulin-loaded arginine-coated self-emulsifying nanoglobule system (INS-LANano). The addition of a biodegradable cationic surfactant, lauroyl ethyl arginate (LAE), resulted in the creation of positively charged nanoglobules with L-arginine on their surface, which electrostatically attached to the negatively charged insulin helices (Figure 6). INS-LANano measured 23.38 nm in diameter with a PDI of 0.089 and a ZP of $+0.118$ mV.

In vitro, these nanoglobules enhanced insulin permeability by 15.2% compared to insulin solution across the human airway epithelial cell (Calu-3) monolayer, while in vivo administration in diabetic rats at a dose of 2 IU/kg insulin resulted in rapid insulin absorption, reaching a significantly higher $C_{\max}$ of 14.3 mU/L and a relative bioavailability of 23.3% [67].

A combinational mucoadhesive NPs based on trimethyl chitosan, encapsulating rivastigmine, an acetylcholinesterase inhibitor, along with insulin, was designed for the management of AD. Chitosan NPs were prepared by ionic gelation and had a PS of 187.00 nm, a PDI of 0.29, a ZP of 17.6 mV, and EEs% (via indirect assessment) of $58.00 \pm 0.5\%$ and $47.8 \pm 0.2\%$ for rivastigmine and insulin, respectively. Drug transport efficiency was significantly higher across sheep nasal mucosa with NPs than with drug solutions, achieving 73.3% for rivastigmine and 96.9% for insulin, thereby exceeding the efficiencies of the drug solutions. Moreover, histological studies demonstrated no significant toxicity in sheep nasal epithelium samples [68].

Another promising, patient-friendly platform for intranasal insulin delivery was developed using Zein-Eudragit NPs via the liquid-liquid dispersion method. Zein, a hydrophobic plant-derived protein, is well recognized for its capacity to form controlled-release NPs, whereas Eudragit imparts a strong positive surface charge, facilitating improved electrostatic interactions with the negatively charged mucin in the nasal cavity. NPs exhibited a mean PS, PDI, ZP, and EE% (assessed indirectly) of less than 200 nm, below 0.3, +30 mV, and 42%, respectively. NPs remained stable for up to 60 days when stored under refrigerated conditions. The in vitro release profile using the Franz diffusion cell system showed a biphasic release pattern. The latter consisted of an initial burst effect, followed by a gradual release, with almost 60% of the encapsulated insulin released over 24 h. The in vitro mucoadhesion study further confirmed the ability of these NPs to prolong their residence time within the nasal cavity [69].

A recent study examined the intranasal delivery of insulin-coated PLGA NPs and mucoadhesive chitosan-coated PLGA NPs for AD management. The prepared PLGA NPs had a PS of 268 ± 10 nm and a PDI of 0.06 ± 0.025 , both of which increased after chitosan coating to 332 ± 8 nm and 0.161 ± 0.007 , respectively, with a ZP of $+21.5 \pm 1$ mV. Additionally, the EE% (by quantifying insulin in the supernatant) and DLC% for the PLGA NPs were $72.3 \pm 3.3\%$ and $3.4 \pm 0.15\%$, respectively, whereas for the chitosan-coated NPs, they were $61.3 \pm 4.7\%$ and $2.09 \pm 0.15\%$, respectively. Both types of NPs showed a biphasic release pattern, with an initial burst release of 45.4% for PLGA NPs and 8% for chitosan-coated NPs. Cytotoxicity tests on the Neuro-2a cell line derived from mouse brain tissue showed that PLGA NPs had cell viability ranging from 85% to 100%, whereas chitosan-coated NPs resulted in lower viability. Ex vivo testing of the NPs' effect on insulin permeability through sheep nasal mucosa revealed a fourfold increase with PLGA NPs and a 16-fold increase with chitosan-PLGA NPs compared to insulin solutions. Additionally, the mean brain concentration-time profiles showed significantly higher AUC values for intranasal delivery compared to intravenous administration. Specifically, the AUC increased by 1.37, 1.46, and 1.88 times for insulin solution, PLGA NPs, and chitosan-coated PLGA NPs, respectively. The AUC values were 13,596.92 $\mu\text{IU/mL}\cdot\text{min}$ for chitosan-PLGA NPs, 10,534.15 $\mu\text{IU/mL}\cdot\text{min}$ for PLGA NPs, and 9903.78 $\mu\text{IU/mL}\cdot\text{min}$ for the insulin solution, confirming the improved brain delivery provided by the NP formulations [70].

In summary, novel nanoplatform strategies utilize nasal formulations to deliver drugs to the brain by increasing brain drug concentrations, bypassing the BBB, and minimizing disruption to systemic circulation.

All data regarding the type of nanocarrier, size, PDI, ZP, EE%, DLC%, cell lines, intended use, and advantages of the nasal nanovehicles are summarized in Table 3.

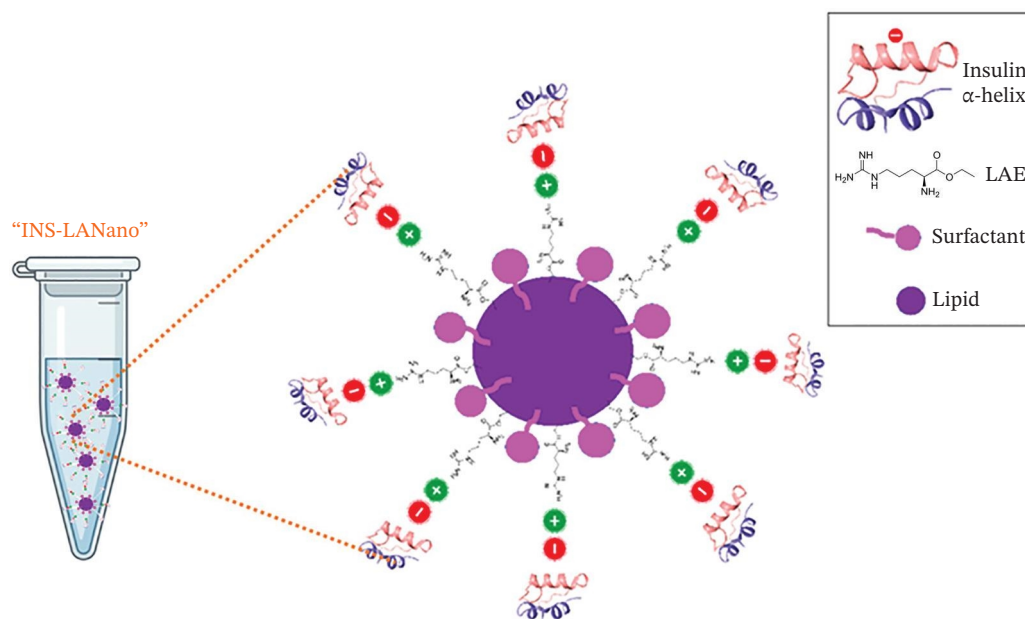


FIGURE 6 | Schematic illustration of the design of INS-LANano, having a lipid core attached to surfactant, LAE, and insulin. This figure has been adapted from Ref. [67]. Permission from MDPI, copyright (2023).

TABLE 3 | Overview of nanoformulations administered nasally, detailing nanocarrier type, physicochemical characteristics (size, PDI, ZP, EE%, and DLC%), cell lines, intended use, and reported therapeutic advantages.

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
Liposomes	Free, TAT, PNT: 109.3 ± 1.04, 110.4 ± 1.37, and 101.5 ± 1.57, respectively.	Less than 0.4.	Free, TAT, PNT: -49.6 ± 1.33, -33.9 ± 1.10, and -20.6 ± 1.30, respectively.	Free, TAT, PNT: 72, 71, 60, respectively.	—	Porcine nasal mucosa from domestic pigs.	—	Stability over 90-day storage.	[61]
SLNs and PLGA NPs	PLGA NPs and SLNs: 135 ± 1.17 and 99.1 ± 5.3, respectively. Chitosan-coated SLNs and PLGA NPs: 174.6 ± 10.7 and 145.2 ± 6.2, respectively.	—	SLNs and PLGA NPs: -28.2 ± 1.8 and -42.3 ± 1.5, respectively; chitosan-coated PLGA NPs and SLNs: +58.4 ± 0.7 and +61.3 ± 0.5, respectively.	Around 80.	Average 27.	RPMI 2650 and hCMEC/D3 cells.	AD	All insulin-loaded nanocarriers exhibited structural stability, enhanced mucoadhesion, nasal diffusion, and drug-release rate compared to native insulin, with chitosan-coated SLNs showing the greatest superiority.	[62]
Transferosomes	137.9 ± 28.2	0.20	+ 23.4	65.1 ± 0.9	9.1 ± 0.4	Tested in vivo on adult Wistar rats.	AD	The nanosystems revealed increased intracellular uptake, sustained drug release, and, consequently, more potent neuroprotective effects.	[63]
Liposomes	—	—	—	—	—	Tested in vivo on Wistar rats.	Diabetes	The release kinetics of insulin reached 59.69% from liposomal insulin in HEC, 42.46% and 41.83% from TAT- and PNT-functionalized liposomes in HEC, respectively.	[64]

(Continues)

TABLE 3 | (Continued)

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
SENs	32–42	0.250 ± 0.07	—	—	—	Human nasal epithelial cells.	Diabetes	The $C_{\max}$ and bio-availability of the insulin-loaded SEN were 255.9 $\mu\text{U/mL}$ and 68%, respectively, compared with subcutaneous delivery. Intranasal administration of 3.6 IU/kg insulin-loaded SEN resulted in a remarkable 70% drop in plasma glucose levels, with the hypoglycemic effect persisting throughout the 4-h experimental duration.	[65]
Chitosan NPs	165.3	0.24	+21.6	69.05	27.97	OVINAE.	—	Insulin release from the NPs exceeded 90%, with an initial burst within the first 15 min, followed by sustained release over 4 h.	[66]
Chitosan-coated NEs	145 ± 2	0.29	+12.7 ± 0.5	—	—	Porcine nasal mucosa.	AD	NEs exhibited great properties before and after incubation in a simulated nasal fluid (pH 5.5, 37°C), as well as after 30 days of storage at 8°C, and promoted faster mucosal permeation of insulin than the free peptide.	[11]

(Continues)

TABLE 3 | (Continued)

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
Self-emulsifying nanoglobules	23.38	0.089	+0.118	—	—	Calu-3 cell line.	Diabetes	In vitro, they increased insulin permeability by 15.2% compared to the insulin solution alone. In vivo administration of insulin to diabetic rats at a dose of 2 IU/kg resulted in rapid absorption, with a significantly higher $C_{\max}$ of 14.3 mU/L and a relative bioavailability of 23.3%.	[67]
Chitosan NPs	187.00	0.29	17.6	58.00 ± 0.5 and 47.8 ± 0.2 for rivastigmine and insulin, respectively.	—	Sheep nasal epithelium samples.	AD	NPs showed a transport efficiency of 73.3% for rivastigmine and 96.9% for insulin, thereby surpassing those of the drug solutions. Histological studies demonstrated no significant toxicity.	[68]

(Continues)

TABLE 3 | (Continued)

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
Zein-Eudragit NPs	Less than 200	under 0.3	+30	42	—	—	Diabetes	The in vitro release profile showed a biphasic pattern: An initial burst followed by slower release, with almost 60% of the encapsulated insulin released over 24 h. The in vitro mucoadhesion study further confirmed the ability of these NPs to prolong their residence time within the nasal cavity.	[69]
PLGA NPs and chitosan-PLGA NPs	PLGA NPs and chitosan-PLGA NPs: 268 ± 10 and 332 ± 8, respectively.	PLGA NPs and chitosan-PLGA NPs: 0.06 ± 0.025 and 0.161 ± 0.007, respectively.	+21.5 ± 1	PLGA NPs and chitosan-PLGA NPs: 72.3 ± 3.3 and 61.3 ± 4.7, respectively.	PLGA NPs and chitosan-PLGA NPs 3.4 ± 0.15 and 2.09 ± 0.15, respectively.	Neuro-2a cell line.	AD	The AUC values were 13,596.92 µIU/mL-min for chitosan-PLGA NPs, 10,534.15 µIU/mL-min for PLGA NPs, and 9903.78 µIU/mL-min for the insulin solution, confirming the improved brain delivery provided by the NP formulations.	[70]

5 | Ocular Route

Studies on the systemic delivery of insulin have shown that the conjunctival sac and nasal cavity cannot achieve sufficient plasma levels due to low bioavailability and short corneal residence time. NPs represent a comfortable drug delivery system with prolonged action in the ophthalmic field. This is due to the reduction of the PS in ocular formulations, which can lead to a threefold increment in the postinstillation concentration of the delivered drug [71].

For instance, the bead mill method was used to prepare an ophthalmic dispersion of protamine zinc insulin NPs with and without polyacrylic acid for the management of diabetes. NPs exhibited a PS of 80–550 nm and a ZP of +6 to +12 mV. Testing the NPs on the human corneal epithelial cell (HCEC) line HCE-T confirmed no significant corneal tissue toxicity. The plasma insulin concentrations in rabbits receiving the NP formulation were significantly higher than those in rabbits receiving only insulin suspension. In addition, insulin levels were further enhanced by increasing the amount of polyacrylic acid in the formulation. Moreover, a single instillation before the oral glucose tolerance test effectively mitigated the rapid increase in plasma glucose levels [72].

In 2023, a combination treatment was developed using trimethyl chitosan and liposomes for the codelivery of vascular endothelial growth factor small-interfering RNA (siVEGF) and insulin as a therapeutic intervention for alkali-burned corneas (Figure 7). Liposomes were prepared by a reversed-phase evaporation method. They had a diameter of 216.63 ± 4.51 nm, a ZP of $+12.40 \pm 0.40$ mV, and insulin EE% and DLC% of $64.75 \pm 6.22\%$

and $25.90 \pm 2.49\%$, respectively, after loading and chitosan coating. The liposomes showed ideal sustained-release behavior, with cumulative insulin release reaching $83.54 \pm 1.56\%$. In vitro, upon testing on HCECs, siVEGF–chitosan insulin liposome (siVEGF–TIL) achieved a 2.06-fold stronger VEGF down-regulation at 100 nM compared to 50 nM, with cell viability reaching $141.29 \pm 15.42\%$. Additionally, VEGF and CD31 levels were reduced to 6.70 ± 0.60 and 4.03 ± 0.55 pg/mg protein, respectively. In vivo, the corneal opacity score decreased to 0.5 ± 0.45 , and the corneal tissues were almost transparent following 14 days of treatment. Overall, siVEGF–TIL inhibited oxidative stress, inflammation, and corneal neovascularization, maintained corneal transparency, and accelerated epithelialization [73].

To promote corneal injury healing and reduce inflammation, insulin was encapsulated into PLGA NPs coated with chitosan via a double emulsion technique (Figure 8). Coated NPs had a size of 213.9 ± 7.5 nm, a PDI of less than 0.2, a ZP of $+11.80 \pm 2.08$ mV, an EE% of $50.28 \pm 2.49\%$, and a DLC% of $4.42 \pm 0.22\%$. Cytotoxicity tests on human umbilical vein endothelial cells (HUVECs) showed high cell viability. In vitro studies indicated that the NPs significantly decreased tumor necrosis factor alpha (TNF- α) and interleukin-6 (IL-6) expression. Additionally, they inhibited vascular endothelial cell migration, tubular structure formation, and increased cellular uptake and adhesion to the cornea, with 64.2% fluorescence visualized at 3 h. In vivo, chitosan–PLGA NPs markedly reduced neovascularization to $20.9 \pm 2.5\%$. Quantitative proteomic analysis showed that insulin treatment decreased FTO expression in the neovascularized corneal tissue [13].

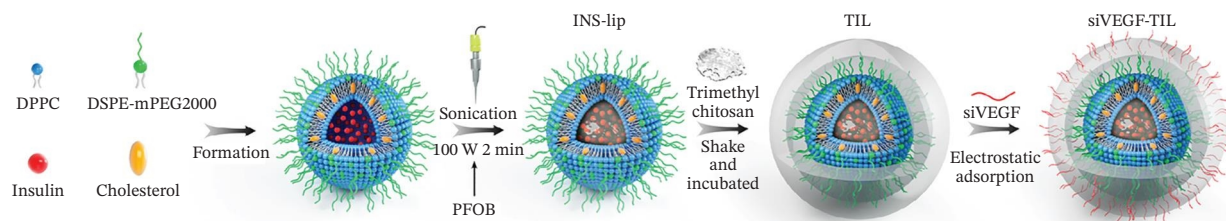


FIGURE 7 | Schematic illustration of the synthetic steps of siVEGF–TIL. DPPC, DSPE–mPEG2000, cholesterol, and insulin were combined to form liposomes. Then, the latter were coated with trimethyl chitosan and attached to siVEGF. This figure has been adapted from Ref. [73]. Permission from Wiley, copyright (2023).

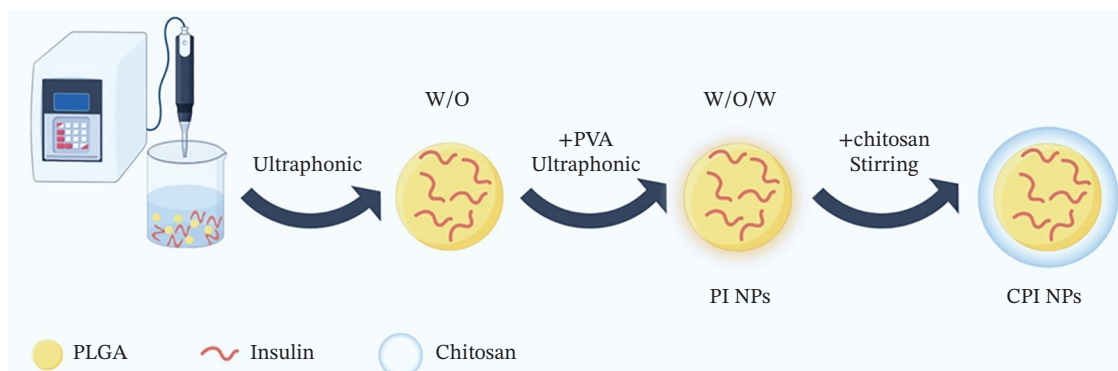


FIGURE 8 | Synthesis of chitosan-coated PLGA NPs. PLGA and insulin were mixed and sonicated to obtain a water-in-oil emulsion. The primary emulsion was further mixed with polyvinyl alcohol via ultrasonication to produce a water-in-oil-in-water emulsion. Finally, chitosan was added to coat the final NPs. This figure has been adapted from Ref. [13]. Permission from RSC, copyright (2025).

TABLE 4 | Overview of the ocular nanoformulations, detailing nanocarrier type, physicochemical characteristics (size, PDI, ZP, EE%, and DLC%), cell lines, intended use, and reported therapeutic advantages.

Type of nanocarriers	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
Protamine zinc insulin NPs	80–4550	—	+6 to +12	—	—	HCE-T cells.	Diabetes	The plasma insulin concentrations in rabbits receiving the NP formulation were significantly higher than those in rabbits receiving only insulin suspension, and the insulin levels were further enhanced with the amount of polyacrylic acid in the formulation. Also, a single instillation before the oral glucose tolerance test effectively mitigated the rapid rise in plasma glucose levels.	[72]
Trimethyl chitosan-coated liposomes	216.63 ± 4.51	—	+12.40 ± 0.40	64.75 ± 6.22	25.90 ± 2.49	HCECs.	Corneal neovascularization	VEGF and CD31 levels were reduced to 6.70 ± 0.60 and 4.03 ± 0.55 pg/mg protein, respectively. Corneal opacity score decreased to 0.5 ± 0.45, and the corneas were almost completely transparent after 14 days of treatment. Overall, siVEGF-TIL inhibited oxidative stress, inflammation, and corneal neovascularization, maintained corneal transparency, and accelerated epithelialization.	[73]
Chitosan-coated PLGA NPs	213.9 ± 7.5	Less than 0.2	+11.80 ± 2.08	50.28 ± 2.49	4.42 ± 0.22	HUVECs.	Corneal neovascularization	The NPs decreased TNF-α and IL-6. They inhibited vascular endothelial cell migration, tubular structure formation, and increased cellular uptake and adhesion to the cornea, with 64.2% fluorescence visualized at 3 h. In vivo, chitosan-PLGA NPs markedly reduced neovascularization to 20.9 ± 2.5%.	[13]

In conclusion, insulin-loaded nanocarriers for ophthalmic administration offer several benefits, including increased insulin delivery to ocular tissues, protection against enzymatic degradation, and drug stability in the ocular tissues. Nanocarriers also increase corneal permeability and the drug's residence time on the ocular surface, providing a noninvasive route for drug delivery to ocular tissues without injection.

All data regarding the type of nanocarrier, size, PDI, ZP, EE%, DLC%, cell lines, intended use, and advantages of the ocular nanovehicles are summarized in Table 4.

6 | Inhalation Route

The inhalation route has become a promising delivery method capable of achieving systemic therapeutic effects comparable to those of the intravenous route while bypassing the absorption barriers associated with less invasive administration methods. This may result from their ability to circumvent the typical oral delivery obstacles that hinder absorption, such as dietary factors, interpatient metabolic differences, harsh gastrointestinal conditions, intestinal mucus, and epithelial barriers. Additionally, the rapid blood circulation via the pulmonary route allows for

effective deposition and penetration of the administered drugs. However, challenges such as drug clearance by lung proteases and the mucociliary system, and poor absorption through the epithelial layer to reach the deep lung parenchyma, have made it imperative to encapsulate peptide-based drugs in nanocarriers to withstand the lung's clearance mechanisms [74].

For example, insulin-loaded SLNs were prepared using the double emulsion method with Witepsol (W) and Softisan (S) lipids for diabetes management. SLNs had a PS ranging from 227.03 ± 4.74 to 325 ± 3.74 nm, a ZP ranging from -23.36 ± 0.17 to -33.5 ± 0.75 mV, a PDI ranging from 0.144 to 0.202, and an EE % ranging between $70 \pm 1.09\%$ and $72 \pm 1.08\%$. The W and WS NPs exhibited an initial burst release of approximately $10 \pm 3.46\%$ and $20 \pm 1.60\%$ within 48 h, followed by a sustained and controlled release of $61.90 \pm 3.50\%$ and $82.79 \pm 10.08\%$, respectively, over 10 days. The aerodynamic features measured by the cascade impactor indicated that the particles are suitable for deep lung deposition and alveolar absorption of insulin for diabetes therapy. However, more investigations are needed to confirm the effectiveness of SLNs in in vivo animal models [75].

Another study conducted by Yu et al. [76] aimed to offer insights into the structure and formulation optimization of pulmonary

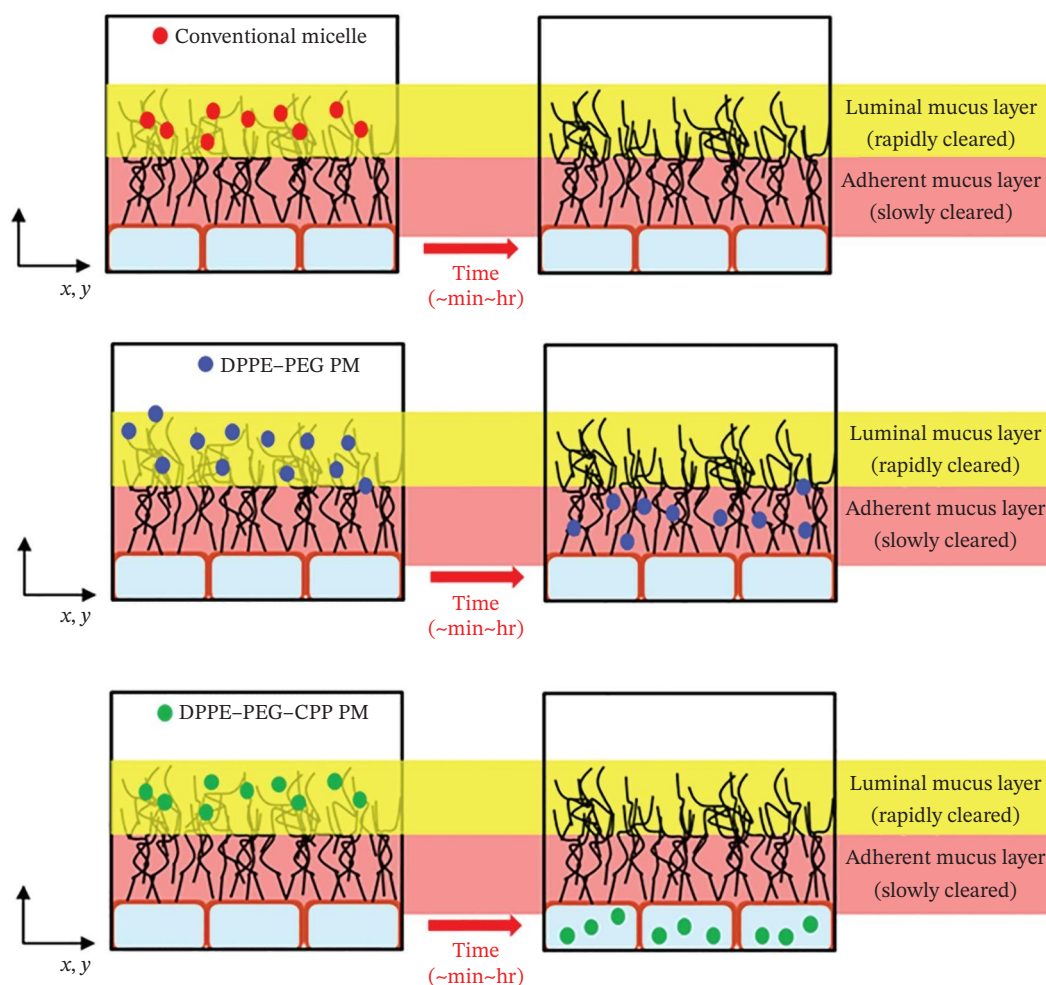


FIGURE 9 | Schematic design of the insulin-loaded polymeric micelles comparing conventional micelles and DPPE-PEG-modified micelles. In contrast to conventional micelles, the incorporation of PEG allows the micelles to diffuse more efficiently, and the CPP facilitates stronger interactions with cell membranes and enhances cellular uptake. This figure has been adapted from Ref. [77]. Permission from Taylor and Francis, copyright (2024).

TABLE 5 | Overview of the inhaled nanoformulations, detailing nanocarrier type, physicochemical characteristics (size, PDI, ZP, EE%, and DLC%), cell lines, intended use, and reported therapeutic advantages.

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC %	Cell line	Intended use	Advantages	Reference
SLNs	227.03 ± 4.74 to 325 ± 3.74	0.144 to 0.202	−23.36 ± 0.17 to −33.5 ± 0.75	70 ± 1.09 to 72 ± 1.08	—	—	Diabetes	The aerodynamic features measured by the cascade impactor indicated that the particles are suitable for deep lung deposition and alveolar absorption of insulin.	[75]
DNVs	80–490	—	Between −34.62 and −49.40	63.82–92.85	—	—	Diabetes	Within the drug load range of 3–5 mg/mL, formulations with lower drug loads demonstrated superior hypoglycemic efficacy, and the area above the blood glucose decline curve with time for nebulized DNVs was 2.32 times greater than that of the insulin solution.	[76]
Micelles	71.75 ± 1.08 nm (PEG2k) and 94.60 ± 1.14 (CPP5k).	—	—	From 61.93 ± 1.66 to 68.08 ± 5.45.	—	Calu-3 and A549 cell lines.	Diabetes	The incorporation of PEG allows the micelles to diffuse more efficiently by forming a hydrophilic outer shell that reduces interaction with mucus components. On the other hand, the CPP facilitates stronger interactions with cell membranes and enhances cellular uptake.	[77]

delivery carriers. Hence, a series of DNVs encapsulating insulin was designed with different key quality attributes to investigate their effect on aerosol generation, macrophage phagocytosis, and bloodstream penetration. The results indicated that within a size range of 80–490 nm, the DNVs with the smaller PS readily evaded macrophage clearance, resulting in a more potent *in vivo* hypoglycemic effect. DNVs' ZP ranged between −34.62 and −49.40 mV, while their EE% ranged between 63.82% and 92.85%. Moreover, the deformability itself improved nebulization implementation and reduced macrophage phagocytosis. Furthermore, within the drug load range of 3–5 mg/mL, formulations with lower drug loads demonstrated superior hypoglycemic efficacy, and the area above the blood glucose decline curve with time for nebulized DNVs was 2.32-fold higher than that of the insulin solution [76].

In another study, insulin-loaded polymeric micelles were synthesized using Soluplus combined with DPPE-PEG of two molecular weights (PEG2k and PEG5k) and DPPE-PEG conjugated with the CPP oligoarginine (R9), forming CPP2k and CPP5k micelles for effective pulmonary insulin delivery (Figure 9). The polymeric micelles exhibited PSs ranging from 71.75 ± 1.08 nm (PEG2k) to 94.60 ± 1.14 nm (CPP5k). Insulin EE% ranged between $61.93 \pm 1.66\%$ and $68.08 \pm 5.45\%$. *In vitro*, PEG2k and CPP2k exhibited sustained-release profiles, releasing about 40% of insulin cumulatively over the test period. At 1 s, PEG-coated micelles demonstrated roughly 100 times higher mean-squared displacement in mucus compared to noncoated micelles, indicating better mucus penetration. Pharmacokinetic evaluation revealed that PEG2k and CPP2k micelles reached C_{max} values of 220.6 ± 1.48 and 223.6 ± 28.48 mIU/L, respectively. In diabetic rats, these formulations led to significantly lower blood glucose levels of 162.3 ± 27.54 mg/dL (PEG2k) and 147.67 ± 33.56 mg/dL (CPP2k) 2 hours after administration. To conclude, testing in Calu-3 and A549 (human alveolar epithelial cells) demonstrated that incorporating PEG enables the micelles to diffuse more efficiently by forming a hydrophilic outer shell that reduces interactions with mucus components, while the CPP promotes stronger interactions with cell membranes and enhances cellular uptake [77].

To conclude, pulmonary nanocarrier-based insulin offers more than just a promising treatment for diabetes mellitus, owing to its noninvasive delivery and its potential to enhance metabolic regulation. It has been shown to improve patient compliance and drug absorption due to the rich blood supply and large lung surface area, making this route of administration as effective as the traditional subcutaneous insulin regimen.

All data regarding the type of nanocarrier, size, PDI, ZP, EE%, DLC%, cell lines, intended use, and benefits of the inhalation nanovehicles are summarized in Table 5.

7 | Oral Route

Orally administered insulin has received outstanding attention due to its admirable characteristics. It provides therapeutic benefits by mimicking the natural physiological mechanism of endogenous insulin. Unfortunately, the therapeutic accessibility of oral insulin is severely hampered by harsh biological barriers, such as gastrointestinal enzymes and a wide pH range, resulting in bioavailability of less than 2% [10]. Additionally, due to its high molecular weight, it faces multiple challenges, including poor absorption, poor adhesion, and a short duration of action [78]. People with diabetes have long eagerly anticipated the prospect of an orally bioavailable form of insulin, which could relieve them from the inconvenience of daily injections. Hence, numerous oral insulin nanocarrier-based delivery strategies have been explored [79].

Reboredo et al. [80] designed bare zein NPs loaded with insulin, some of which were coated with PEG, and evaluated the hypoglycemic effects of both. The PEG coating did not change their physicochemical properties; both had a PS, a PDI, a ZP, an EE%, and an insulin payload of approximately 270 nm, 0.16, −39.2 mV, 81%, and 80 g/mg. Cytotoxicity assays conducted on the human colon carcinoma HT29-MTX cells and Caco-2 cells showed no toxicity after 24 h of treatment. In *Caenorhabditis elegans* treated with Orlistat as a positive control, PEG-modified NPs caused a greater reduction in fat accumulation than uncoated zein NPs. Additionally, PEG coating significantly increased oral insulin bioavailability and enhanced hypoglycemic effects, likely because PEG can penetrate the mucus layer and reach enterocytes, while mucoadhesive zein NPs tend to stay trapped in the mucus. This was supported by their notable hypoglycemic activity, with PEG NPs showing an oral bioavailability of 10.2% and coated NPs at 4.2% in diabetic rat models [80].

Recently, Li et al. [81] provided a novel perspective into the development of nanoplateforms that respond to the mildly acidic microenvironment of the jejunal epithelial surface. The fabrication of a pH-sensitive core-shell architecture for an oral insulin system, loaded as a freeze-dried powder into enteric-coated capsules, was enabled by incorporating a biodegradable copolymer composed of PEG and PLGA building blocks linked by a hydrazone bond (PLGA-Hyd-PEG). This novel nanoplateform underwent a transition from hydrophilic to hydrophobic at the epithelial surface. The latter exhibited a PS of PLGA-Hyd-PEG

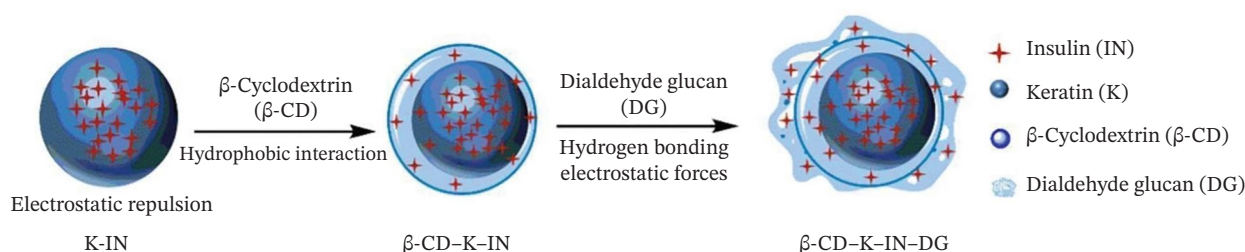


FIGURE 10 | Design of dual β-cyclodextrin/dialdehyde glucan-coated keratin NPs (β-CD-K-IN-DG) for successful oral insulin administration in diabetic patients. This figure has been adapted from Ref. [78]. Permission from Elsevier, copyright (2024).

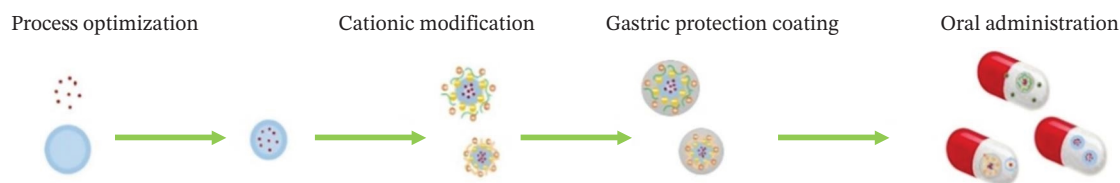


FIGURE 11 | PLGA NPs were cationically modified with chitosan/polyethyleneimine to promote intestinal adhesion. Moreover, their surface was coated with a pH-responsive methyl methacrylate trimethylamine ethyl chloride copolymer for gastric protection. This figure has been adapted from Ref. [84]. Permission from Elsevier, copyright (2025).

NPs, which showed admirable drug protection and rapid mucus penetration owing to the high stability of the PEG coating in jejunal fluid, while in the jejunal epithelium, PEG was rapidly cleaved, converting the NPs' surface from hydrophilic to hydrophobic, thereby facilitating internalization into cells. The intrajejunal administration of the PLGA-Hyd-PEG NPs decreased blood glucose levels in both normal and diabetic rats relative to control NPs. Furthermore, they reduced blood glucose by 35% for up to 10 h after oral administration to diabetic rats [81].

Cashew gum is a biopolymer that can be modified with phthalic groups and that stood out as a promising biopolymer due to its distinctive structural and physicochemical characteristics. Consequently, Bezerra et al. [82] produced phthalated cashew gum and chitosan NPs via the polyelectrolyte complexation technique for oral insulin administration. The nanosystems exhibited a PS, a PDI, a ZP, and an EE% of 390 nm, 0.04, +15.4 mV, and 77.5%, respectively. Insulin release in the simulated gastric and intestinal environments maintained sustained

release, with about 20% of insulin released at pH 1.2 after 2 h and 80% released at basic conditions (pH 6.8). The result can be attributed to the fact that, under acidic conditions, carboxylic groups exist in the nonionized form, whereas in basic media they remain ionized and hydrophilic. Furthermore, cell viability after 48 h of treatment in murine fibroblasts (L-929) demonstrated that the produced nanosystem exhibited relatively low cytotoxicity [82].

Novel colon-targeted NPs were developed by incorporating insulin into pectin-chitosan NPs. The modified trimethyl chitosan was soluble at physiological pH, coated with a colon-degradable polymer, and prepared by ionic gelation. The resulting NPs had a PS of 379.40 ± 40.26 nm, a PDI of 24.10 ± 1.03 , a ZP of $+17.20 \pm 0.52$ mV, and an EE% of $83.21 \pm 1.23\%$. Compared to uncoated NPs, insulin-loaded pectin-trimethyl chitosan NPs decreased insulin loss in simulated gastrointestinal fluid by approximately $67.23 \pm 0.97\%$. They also showed controlled insulin release in simulated colonic fluid. In vitro bioactivity tests on the HT29 cell line confirmed the NPs' safety, with a cell

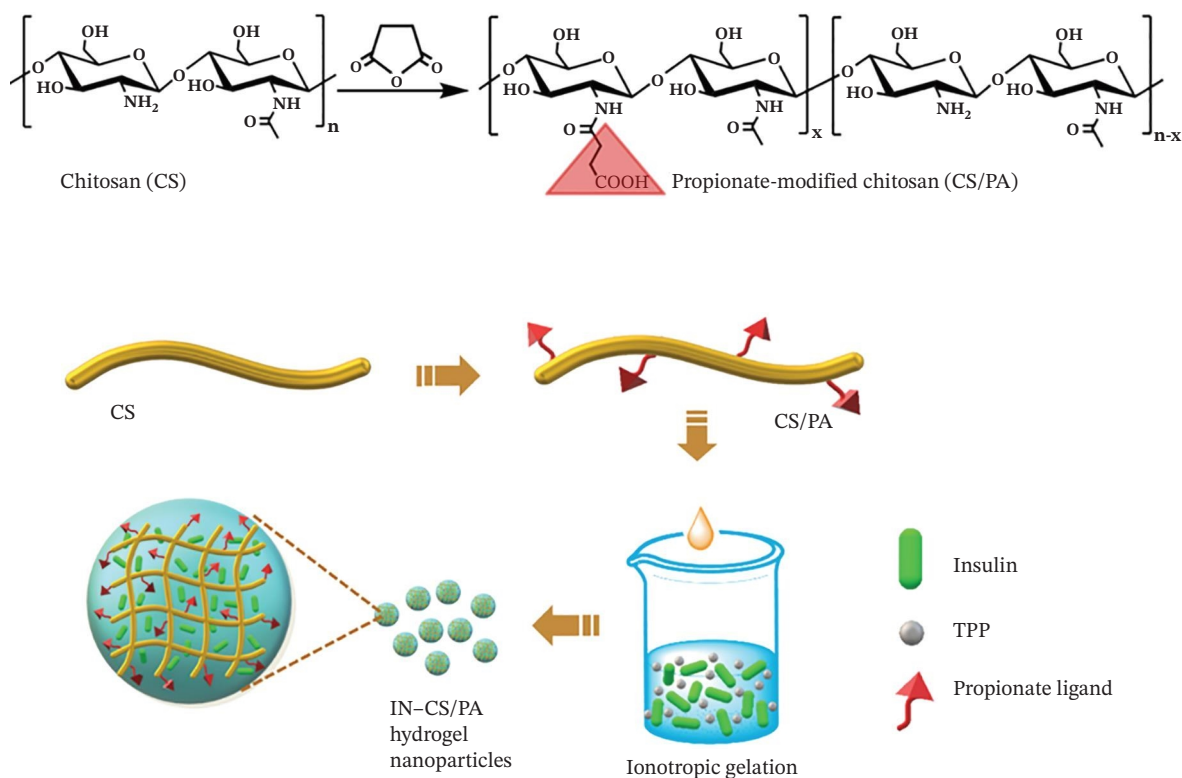


FIGURE 12 | Schematic illustration of the design of IN-CS/PA HNPs. The chitosan polymer was modified with a propionate ligand, which was mixed with insulin and tripolyphosphate (TPP) via ionotropic gelation to obtain the final NPs. This figure has been adapted from Ref. [86]. Permission from Elsevier, copyright (2025).

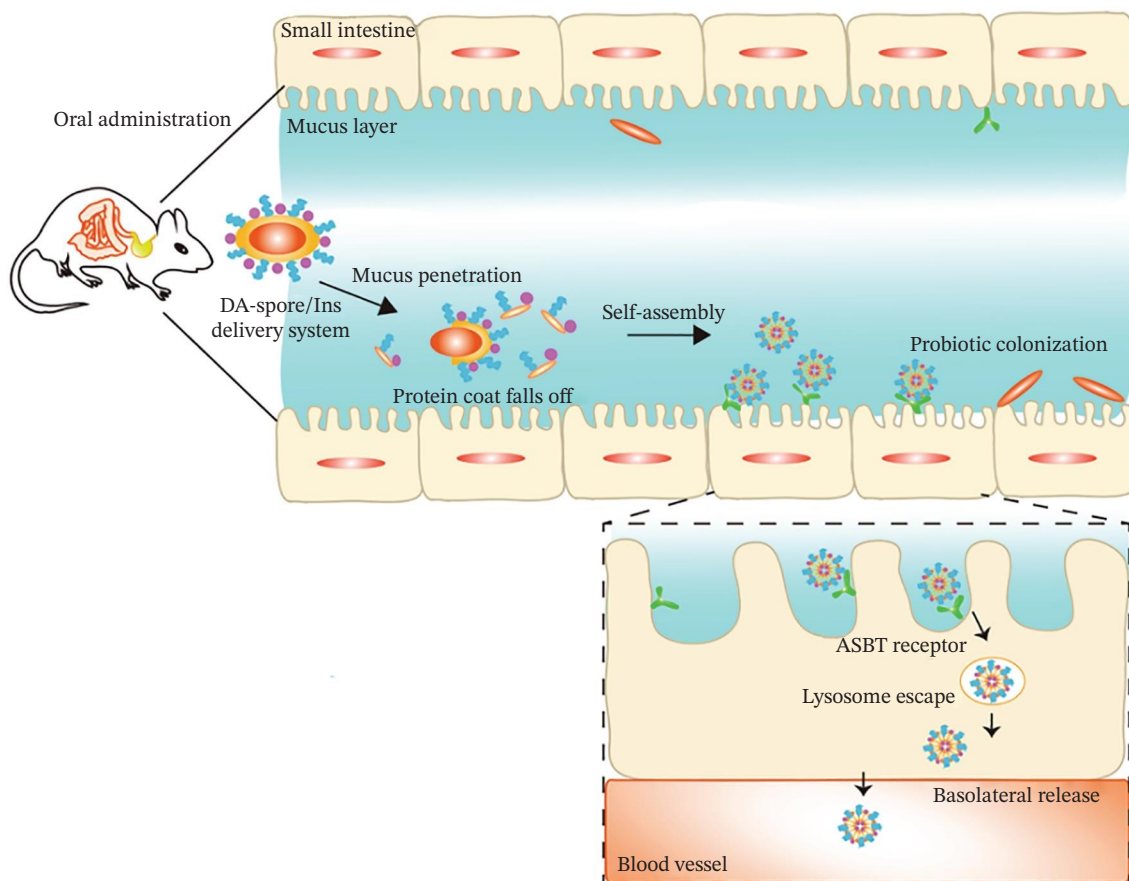


FIGURE 13 | Following oral administration, DA-Spore/Ins germinate in the intestine, shedding their amphipathic protein coat, which self-assembles with insulin to form biogenic NPs. The spore surface harbors probiotics that slightly degrade mucus, facilitating NP crossing. The attached DA helps the NPs enter intestinal cells via the bile acid transporter pathway (ASBT). Once inside the cells, the NPs escape lysosomal degradation and gradually release insulin into the bloodstream. This figure has been adapted from Ref. [87]. Permission from Elsevier, copyright (2025).

viability of $91.12 \pm 0.91\%$ after 24 h of treatment, and demonstrated high cellular uptake, with a fluorescence intensity of 37.80 ± 2.40 after 4 h of incubation. In addition, the *in vivo* study revealed a sustained decrease in blood glucose levels following oral administration in diabetic male Sprague-Dawley rats, reaching a peak at 8 h with a reduction of $87 \pm 1.03\%$ [83].

Wang et al. [44] developed simple, inexpensive, and safe dual β -cyclodextrin/dialdehyde glucan-coated keratin NPs (β -CD-K-IN-DG) for effective oral insulin delivery in diabetic patients (Figure 10). The β -CD-K-IN-DG NPs had a PS, PDI, and ZP of 258.1 ± 6.74 nm, 0.51 ± 0.02 , and $+13.8 \pm 0.7$ mV, respectively, and an ultra-high insulin loading of 98.52%. The NPs protected insulin from acid and enzymatic degradation, demonstrated a notable hypoglycemic effect with nearly 80% insulin release after 4 h of incubation under hyperglycemic conditions, and demonstrated high mucus-penetration ability during *ex vivo* testing. The Caco-2 cells and LO2 hepatic cells exhibited minimal cytotoxicity at 0.05 mg/mL, with cell viability exceeding 94%. Transepithelial transport and uptake analyses indicated that β -CD-K-IN-DG entered intestinal epithelial cells primarily via bypass pathways and endocytosis, resulting in an increased insulin bioavailability of 12.27% [78].

Another trial involved encapsulating oral insulin in PLGA nanocapsules to regulate blood sugar levels. The oral NPs had a PS of 140 ± 13.43 nm, a PDI of 0.167 ± 0.05 , a ZP of -38.73

± 2.10 , an insulin EE% of 54.3%, and a DLC% of 2.2%. Following encapsulation, PLGA NPs were cationically modified with chitosan/polyethyleneimine to promote adhesion and permeation through the intestinal mucus layer. Moreover, the NPs' surface was coated with a pH-responsive copolymer of methyl methacrylate and trimethylamine ethyl chloride, which, in turn, provided 100% gastric protection (Figure 11). The resulting NPs demonstrated pleasant physicochemical stability, sustained-release properties, and enhanced *in vitro* acid resistance. The rat blood glucose analysis revealed that subcutaneous injection in the control group caused a rapid decrease in glucose levels within 1 h, returning to baseline within 4 h. In contrast, the oral NPs gradually lowered blood glucose to 51.3% of the initial value and sustained this reduction for up to 10 h [84].

Another recent study encapsulated insulin into dioleoylglycerophosphate-diethylenediamine (DOP-DEDA) lipid NP and coated it with PEG. DOP-DEDA is a pH-responsive and charge-reversible lipid for cytosolic cargo delivery that exhibits a positive charge at gastrointestinal pH levels, which enhances stomach stability and facilitates small intestine absorption, while remaining noncationic at physiological pH, indicating low toxicity. The PEGylated NPs had a PS of 125.4 nm, a PDI of 0.047, a ZP of -4.1 mV, and an EE% of 57.2%. They retained 89%, 51%, and 44% of insulin after 60 min of incubation in simulated gastrointestinal and physiological fluids at pH 1.2, 6.8, and 7.4,

respectively. Additionally, *in vivo* studies on diabetic mice showed a potent and sustained hypoglycemic effect after oral administration, with an average reduction of 40% in blood glucose levels over more than 10 h, indicating an optimal pharmacodynamic profile [85].

The microbial metabolite propionate was used to decorate chitosan for the development of the orally administered insulin-loaded propionate-modified chitosan hydrogel NPs (IN-CS/PA HNPs) via ionotropic gelation (Figure 12). IN-CS/PA HNPs demonstrated a PS of 245.9 ± 28.3 nm, a PDI of 0.227 ± 0.036 nm, a ZP of -24.8 ± 0.4 mV, a high EE%, and DLC% for insulin of $> 95\%$ and $\sim 10\%$, respectively. Furthermore, the propionate functional group was identified and transported via the monocarboxylate transporter protein 1, enabling targeted delivery of IN-CS/PA HNPs, which subsequently led to significantly enhanced permeability and endocytic uptake of insulin by intestinal epithelium in both Caco-2 cells and ligated intestinal loops of rats. In Type 1

diabetic rats, oral delivery of IN-CS/PA HNPs with 60 IU/kg insulin resulted in a more stable and prolonged hypoglycemic effect than a 5 IU/kg dose of subcutaneously injected insulin, attaining 2.29-fold and 11.88-fold greater relative oral bio-availability compared to empty IN-CS HNPs and free insulin, respectively [86].

Inspired by the natural physiological properties of *Heyndrickxia coagulans* spores, new deoxycholic acid-modified spores encapsulating insulin (DA-Spore/Ins) were developed as generators of autonomous bio-based NPs. The NPs exhibited a PS of 193 ± 2.5 nm, a ZP of -15.2 ± 0.2 mV, an EE% of $86.3 \pm 2.7\%$, and a DLC% of $12.6 \pm 0.3\%$. After administration, DA-Spore/Ins can withstand the harsh conditions of the GIT. They germinate in the intestine, shedding their amphipathic protein coat, which self-assembles with insulin into biogenic NPs. The spore surface harbors probiotics that degrade mucus slightly, facilitating NP crossing. Conversely, the attached DA facilitates NPs' entry into intestinal cells via the bile acid transporter pathway (ASBT).

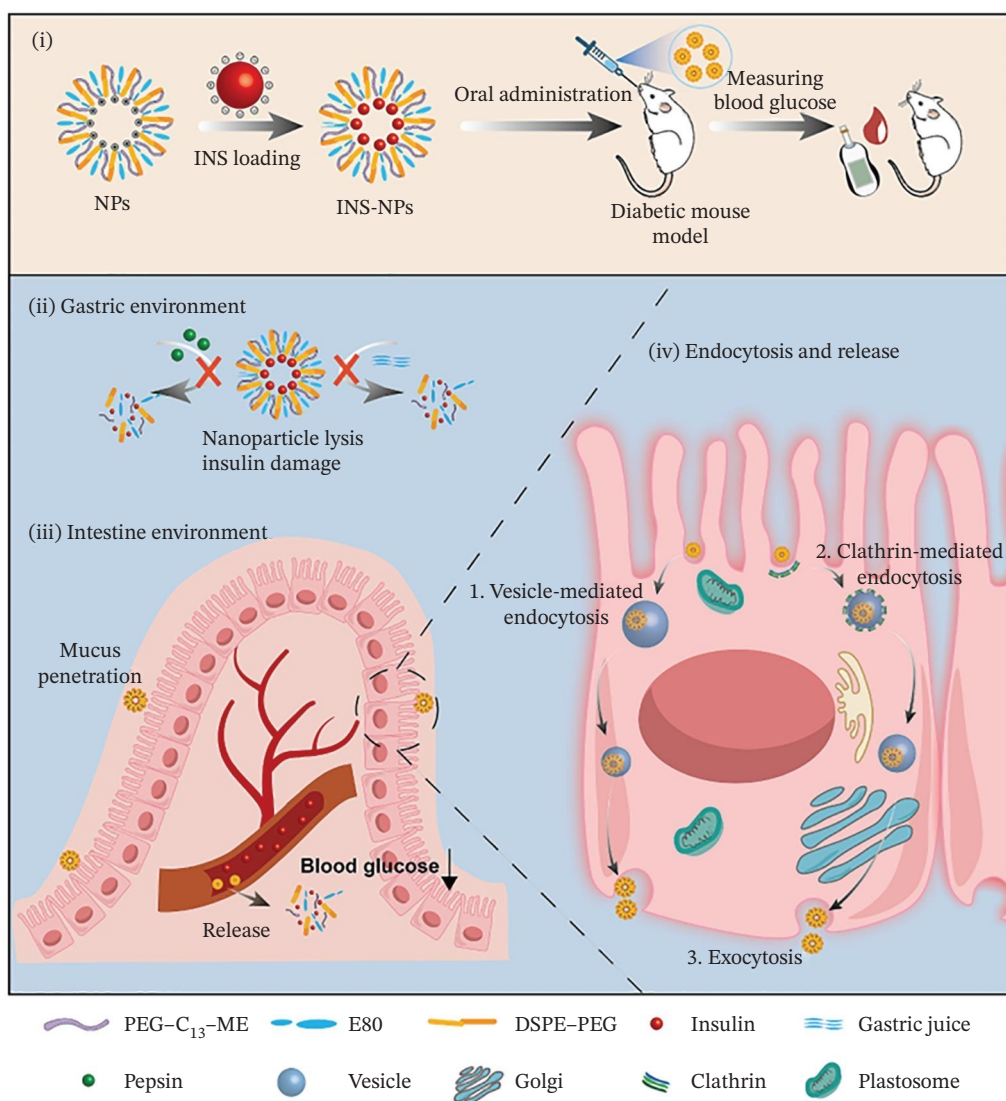


FIGURE 14 | Schematic illustration of the synthesis of oral insulin NPs and their assessment in diabetic mouse models. NPs protect their cargo from degradation by the harsh gastric environment. Intestinally, insulin uptake is an energy-dependent process that involves multiple endocytic pathways, primarily caveolae-mediated and clathrin-dependent mechanisms. This figure has been adapted from Ref. [88]. Permission from Elsevier, copyright (2025).

TABLE 6 | Overview of nanoformulations administered orally, detailing nanocarrier type, physicochemical characteristics (size, PDI, ZP, EE%, and DLC%), cell lines, intended use, and reported therapeutic advantages.

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
PEG-coated zein NPs	270	0.16	−39.2	81	—	HT29-MTX and Caco-2 cells.	Diabetes	PEG coating significantly increased oral insulin bioavailability and enhanced hypoglycemic effects, likely because PEG can penetrate the mucus layer and reach enterocytes, while mucoadhesive zein NPs tend to stay trapped in the mucus. This was supported by their notable hypoglycemic activity, with PEG NPs showing an oral bioavailability of 10.2% and coated NPs at 4.2% in diabetic rat models.	[80]
PLGA-Hyd-PEG NPs	139.6 ± 1.10	0.169 ± 0.02	−32.8 ± 0.72	48.03 ± 3.30	4.65 ± 0.30	HT29-MTX-E12 and Caco-2 cell lines.	Diabetes	The intrajejunal administration of the PLGA-Hyd-PEG NPs decreased blood glucose levels in both normal and diabetic rats relative to control NPs. They reduced blood glucose by 35% for up to 10 h after oral administration to diabetic rats.	[81]
Cashew gum-chitosan NPs	390	0.04	+15.4	77.5	—	L-929 cell line.	Diabetes	Insulin release in the simulated gastric and intestinal environments maintained sustained release, with about 20% of insulin released at pH 1.2 after 2 h and 80% released at basic conditions (pH 6.8).	[82]

(Continues)

TABLE 6 | (Continued)

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
Pectin-trimethyl chitosan NPs	379.40 ± 40.26	24.10 ± 1.03	+17.20 ± 0.52	83.21 ± 1.23	—	HT29 cell line.	Diabetes	The NPs decreased insulin loss in simulated gastrointestinal fluid by approximately 67.23 ± 0.97%. In vitro, they demonstrated high cellular uptake, with a fluorescence intensity of 37.80 ± 2.40 after 4 h of incubation. In vivo study revealed a sustained decrease in blood glucose levels, reaching a peak at 8 h with a reduction of 87 ± 1.03%.	[83]
β-CD-K-IN-DG NPs	258.1 ± 6.74	0.51 ± 0.02	+13.8 ± 0.7	98.52	—	Caco-2 and LO2 cell lines.	Diabetes	In ex vivo testing, β-CD-K-IN-DG NPs protected insulin against acid and enzymatic destruction, released over 80% of its insulin after 4 h of incubation under hyperglycemic circumstances, and showed a considerable hypoglycemic impact.	[78]
PLGA NPs	140 ± 13.43	0.167 ± 0.05	−38.73 ± 2.10	54.3	2.2	—	Diabetes	The rat blood glucose analysis revealed that subcutaneous injection in the control group caused a rapid decrease in glucose levels within 1 h, returning to baseline within 4 h. In contrast, the oral NPs gradually lowered blood glucose to 51.3% of the initial value and sustained this reduction for up to 10 h.	[84]

(Continues)

TABLE 6 | (Continued)

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
PEG-lipid NPs	125.4	0.047	-4.1	57.2	—	Tested in vivo on streptozocin-induced diabetic mice	Diabetes	PEG NPs retained 89%, 51%, and 44% of insulin after 60 min of incubation in simulated gastrointestinal and physiological fluids at pH 1.2, 6.8, and 7.4, respectively. In vivo studies on diabetic mice showed a potent and sustained hypoglycemic effect after oral administration, with an average reduction of 40% in blood glucose levels over more than 10 h, indicating an optimal pharmacodynamic profile.	[85]
Propionate-modified chitosan hydrogel NPs	0.227 ± 0.036	0.227 ± 0.036	-24.8 ± 0.4	> 95	~10	Caco-2 cells.	Diabetes	Insulin in IN-CS/PA HNPs at a dose of 60 IU/kg had a 2.29-fold greater relative oral bioavailability compared to empty IN-CS HNPs and an 11.88-fold higher relative oral bioavailability compared to free insulin in Type 1 diabetic rats, resulting in a more stable and long-lasting hypoglycemic effect.	[86]

(Continues)

TABLE 6 | (Continued)

Type of nanocarrier	Size (nm)	PDI	ZP (mV)	EE%	DLC%	Cell line	Intended use	Advantages	Reference
DA-Spore NPs	193 ± 2.5	—	−15.2 ± 0.2	86.3 ± 2.7	12.6 ± 0.3	Caco-2 and human intestinal epithelial cell line (HIEC).	Diabetes	DA-Spore/Ins NPs demonstrated 9.2-fold higher mucus diffusion, 21.6-fold greater cellular uptake, and an apparent permeability coefficient of 12.1×10^{-6} cm/sec. In vivo, they resulted in a relative bioavailability of 15.1% and a more pronounced hypoglycemic effect, with blood glucose levels reaching 7.8 ± 1.0 mmol/L at 4 h and 8.5 ± 0.8 mmol/L at 8 h.	[87]
PEG-C ₁₃ -ME NPs	218	0.198	−2.7	44 ± 1.8	16.3 ± 0.6	Caco-2 and CT26 cell lines.	Diabetes	The NPs enhanced cellular uptake, with an internalization rate of 62% in Caco-2 cells and 40.2% in CT26 cells. Additionally, insulin NPs effectively reduced blood glucose levels in diabetic mice, reducing blood glucose levels to ~3.1 mmol/L and maintaining normal levels of around 4.5 mmol/L for up to 10 h.	[88]

Once inside the cells, the NPs escape lysosomal degradation and gradually release insulin into the bloodstream (Figure 13). DA-Spore/Ins NPs demonstrated 9.2-fold higher mucus diffusion, 21.6-fold greater cellular uptake, and an apparent permeability coefficient of 12.1×10^{-6} cm/sec, which is 11.2 times higher than free insulin. In vivo studies in Type I diabetic rats showed that oral administration of the DA-Spore/Ins delivery system resulted in a relative bioavailability of 15.1% and a more pronounced hypoglycemic effect, with blood glucose levels reaching 7.8 ± 1.0 mmol/L at 4 h and 8.5 ± 0.8 mmol/L at 8 h [87].

Another recent study reported the synthesis of a novel amphiphilic triblock copolymer, PEG–tridecanedioic acid–metformin (PEG–C13–ME), to develop oral NPs for insulin protection and intestinal delivery. Metformin imparts a positive charge to the NPs' hydrophobic core, facilitating efficient insulin encapsulation and markedly decreasing its susceptibility to degradation. The thin-film hydration method produced NPs with a PS of 218 nm, a PDI of 0.198, a ZP of -2.7 mV, an EE% of $44 \pm 1.8\%$, and a DLC% of $16.3 \pm 0.6\%$. Cytotoxicity assays on Caco-2 and CT26 (murine colon carcinoma) cells showed that the synthesized NPs exhibited minimal toxicity and favorable biocompatibility. CLSM and flow cytometry confirmed that the NPs enhanced cellular uptake, with internalization rates of 62% in Caco-2 cells and 40.2% in CT26 cells, demonstrating a significant enhancement over the free insulin group, which exhibited uptake rates of 46.7% and 11.5%, respectively. Additionally, insulin NPs effectively reduced blood glucose levels in diabetic mice, lowering them to ~ 3.1 mmol/L and maintaining normal levels around 4.5 mmol/L for up to 10 h [88] (Figure 14).

Hence, developing various oral nanoplateforms aimed at protecting insulin against harsh conditions and enhancing permeation across the intestinal membrane. It has extended residence time, improved target specificity, and enhanced controlled-release kinetics, thereby substantially augmenting the oral bioavailability of insulin and offering patients a more convenient, user-friendly alternative to conventional insulin administration methods. However, the design of oral insulin formulations requires enhanced accuracy to release insulin at the right time and in the right amount to achieve optimal glycemic regulation.

All data regarding the type of nanocarrier, size, PDI, ZP, EE%, DLC%, cell lines, intended use, and the advantages of the oral nanovehicles are summarized in Table 6.

In summary, each of the discussed platforms demonstrates tremendous potential for insulin delivery, owing to their unique characteristics and distinct advantages that enhance drug stability, absorption, and overall therapeutic efficacy. For instance, the nasal route is considered a leading candidate for immediate clinical applications due to its excellent biological performance and favorable bioavailability. At the same time, the oral route is considered the most promising platform for long-term treatment applications because it more closely follows the natural biological pathway and offers better patient compliance than other routes, as evidenced by the recently achieved high bioavailability using biogenic NP generators. Meanwhile, the inhalation route is considered beneficial for immediate treatment applications because it can achieve significantly higher systemic absorption rates than conventional insulin solutions.

8 | Conclusion and Future Directions: Roadmap for Clinical Translation

Insulin is a vital peptide hormone indispensable for maintaining glucose homeostasis, with diverse pharmacological properties. However, its routine injection application is often accompanied by pain, needle phobia, phlebitis, tissue necrosis due to routine injection, and rapid elimination, which limits therapeutic efficacy, high cost, and low patient compliance. This review presents the latest state-of-the-art studies on recent advances in nanoformulations for transmucosal insulin delivery, describing how organic nanocarriers, whether lipid- or polymer-based, can address these shortcomings. The rapid evolution of nanotechnology has brought forward a wide range of nano delivery systems that have significantly improved transmucosal administration and transformed the paradigm of transmucosal insulin delivery. Several studies have reported the use of various nanodelivery systems to escort therapeutic agents, owing to their unique ability to prolong the circulation time of their cargo, thereby enhancing bioavailability and water solubility.

Nevertheless, current knowledge remains limited. Despite this progress, multiple challenges and hurdles stand in the way of these nanoformulations transitioning from bench to clinic. Looking ahead, integrating nanomedicine with precision therapeutics will expedite the translation of preclinical promise into clinical reality. First of all, there is an urgent need to progress beyond in vitro characterization and move toward robust in vivo models and early-phase clinical trials. In addition, pharmacokinetic studies, including absorption, distribution, metabolism, and elimination pathways, as well as potential immunological responses of nanocarrier-based systems, remain unaddressed in most recent clinical studies. Moreover, the scalability and reproducibility of synthetic methods should be prioritized, along with thorough bio-distribution studies in relevant animal models and systematic evaluation of biosafety and long-term toxicity. Combining chemistry, immunology, and nanomedicine will be the ultimate interdisciplinary strategy for developing insulin-loaded nanocarriers toward therapeutic use.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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