

Nano-Encapsulated Inhalable Insulin Dry Powder for Rapid Glycemic Control in Type 1 and Type 2 Diabetes Mellitus: A Comprehensive Review

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Abstract

Diabetes mellitus remains a major global health burden and estimates have shown that over 537 million people across the globe are infected with the disease. Such an epidemiological scale highlights the urgency to find new treatment approaches that can enhance glycemic control levels within a population. Although the conventional methods of administering insulin by injection into the subcutaneous tissue are foundational in the management of diabetes, several limitations associated with the therapy make them prone to compromise the treatment cost. Suffering, needle phobia, and fluctuating kinetics of absorption often lead to poor glycemic activities and decreased compliance in patients. The pulmonary route turns out to be attractive as a non-invasive method, which relies on the large surface area of the alveoli (about 140 m²), the extremely thin lining of the wall and the rich vascular system, which also allows the rapid uptake of the delivered drugs into the system. These physiological and anatomical properties of the respiratory tract qualify it as an attractive route of administration of biologics like insulin. Biodegradable polymers have been considered in nano-encapsulation of insulin such as poly(lactic-co-glycolic acid) (PLGA) and chitosan to reduce enzymatic breakdown and improve pulmonary deposition. The further conversion of these nanoparticles into inhalable dry powders through the application of techniques like spray drying or freeze drying is a complex procedure that will enhance the pulmonary bioavailability without causing drug degradation. This review of the literature will explore the science behind pulmonary insulin delivery, outline the pathophysiology of diabetes mellitus, and briefly review the progress of nano-encapsulation technologies. In addition, we specify the formulation strategies of dry powder inhaler (DPI) and the critical parameters of characterization, which define the performance of a product such as fine particle fraction (FPF), mass median aerodynamic diameter (MMAD) and drug entrapment efficiency (DEE). The pharmacodynamic performance in vitro and in vivo is critically evaluated to give an insight into the translational nature of these technologies. The clinical evidence of the currently marketed inhalable insulin products, that is, Exubera and Afrezza, are considered in the context of the developing research on the nanoparticle-based DPI systems. It is followed by the discussion of the aspect of stability, the issues of regulations and the perspectives of the translation of nano-encapsulated inhalable insulin to the routine clinical practice. We synectically summarize evidence that nano-encapsulated inhalable insulin dry powders have a high potential of providing rapid and reproducible glycemic control and improving patient adherence among Type 1 and Type 2 diabetes mellitus patients.

Keywords: Nano-encapsulation; Inhalable insulin; Dry powder inhaler; Pulmonary drug delivery; PLGA nanoparticles; Chitosan; Glycemic control

1. Introduction

Diabetes mellitus (DM) is a long term metabolic disorder that is marked by sustained hyperglycaemia which is caused by insulin dysregulation, impaired insulin action or both. The International Diabetes Federation (IDF) states that in 2021, there were 537 million adults aged 20-79 years with diabetes, and this will reach over 783 million by 2045 [1]. The disease has been divided into Type 1 diabetes mellitus (T1DM), which is an autoimmune disease that involves the destruction of pancreatic b-cells, and Type 2 diabetes mellitus (T2DM), a progressive disease that is caused by insulin resistance and subsequent relative insulin deficiency [2]. Both forms also require exogenous insulin therapy to achieve euglycaemia, avoid acute conditions like diabetic ketoacidosis, decrease the risks in the long-term of nephropathy, retinopathy, neuropathy and cardiovascular disease [3].

Subcutaneous (SC) route has been the most common clinical route of administering insulin since more than a century. Although there have been improvements in the use of insulin analogues and insulin delivery devices, subcutaneous injection is associated with several limitations such as injection-site pain, lipodystrophy, needle phobia,

hypoglycaemia, and delayed onset of the pharmacokinetic effect as opposed to endogenous secretion of insulin through the first-pass hepatic metabolism [4]. The problem of post-prandial glycaemic spike is especially hard to manage when taking SC because of the delays in insulin absorption in the depot. These restrictions all influence the compliance and glycaemic control of patients in the long-term and the necessity to find alternative, non-invasive approaches to insulin delivery is acute [5].

Inhaled route of delivery has been developed as the most clinically feasible non-invasive systemic insulin delivery route. The human lung provides a surface area of about 140 m², a thickness of the epithelial layer under 0.1 µm in the alveolar cavity, and a highly endowed blood supply, which allows rapidly and effectively taking up macromolecular drugs into the system circulation [6]. Dry powder formulations are inhalable, which is an added benefit over nebulised solutions and pressurised metered-dose inhalers (pMDIs), such as an enhanced chemical and physical stability, the absence of cold-chain storage needs and the potential to operate the device breath-actuated and portable [7].

The technology of nano-encapsulation has also enhanced the future of inhalable insulin by overcoming the challenge posed by the enzyme degradation in the lung lumen, mucociliary clearance, and macrophage phagocytosis. Nanoparticles made of biodegradable excipients, like PLGA, and chitosan shield insulin against proteolytic enzymes, increase its retention period within the absorption site and enhance transcytosis through the alveolar epithelium [8,9]. In converting nano-encapsulated insulin dispersions into dry powder, through controlled drying conditions and designed to an optimum range of aerodynamic diameter of 1-5 µm, the resulting formulations result in fine particle fractions of the highest quality and reproducible deep lung deposition [10].

The review critically assesses the scientific basis, formulation approaches, characterisation approaches, preclinical and clinical data, stability and future research on nano-encapsulated inhalable nano insulin dry powder formulations as rapid glycaemic control in T1DM and T2DM.

2. Pathophysiology of Diabetes Mellitus and the Need for Rapid Insulin Action

Insulin is a 51 amino acid peptide hormone that consists of two polypeptide chains (A: 21 amino acids; B: 30 amino acids) that are connected by two disulphide bonds and has a molecular weight of about 5,808 Da. It is released in a biphasic response by pancreatic β-cells: an acute phase of secretion, the first phase, which takes place in 1-3 minutes of glucose stimulation, and a second phase of a sustained secretion [11]. Autoimmune destruction of β-cells in T1DM totally removes the endogenous secretion of insulin and results in full replacement that has to be exogenous. Progressive β-cell failure on top of peripheral insulin resistance contributes to insufficiency in insulin secretion in relation to demand, especially during the post-prandial condition in T2DM [1,2].

In both T1DM and T2DM, post-prandial hyperglycaemia is one of the leading causes of glycaemic fluctuation, oxidative stress, and vascular inflammation. The discrepancies between the pharmacokinetic characteristics of conventional administrated SC rapid-action insulin analogues (onset: 10-20 minutes; peak: 1-3 hours) and the physiological kinetics of insulin secretion (onset: < 2 minutes) cause insufficient prandial glycaemic coverage [12]. Conversely, inhaled insulin reaches peak plasma concentrations 10-20 minutes after inhalation and closely resembles the first-phase insulin secretory pattern and offers better post-prandial glycaemic control [13]. The need to fill in this prandial gap is one of the main reasons why fast-acting inhalable insulin preparations are developed.

3. The Pulmonary Route for Systemic Insulin Delivery

3.1 Anatomical and Physiological Advantages

The respiratory system comprises of conducting airways (trachea, bronchi and bronchioles) and respiratory zone (respiratory bronchioles, ducts of alveoli and sacs of alveoli). The deposition of drug particles in the alveolar region is extremely efficient because of the thinness of the alveolar-capillary membrane (0.1-0.5 µm), the high area of surface (~140 m²), and the duality of the blood supply through the pulmonary and bronchial circulations. Type I pneumocytes (constituting most of the alveolar surface, approximately 95 percent of the alveolar surface area) are the primary cell type that lines the alveolar surface, and which provide little resistance to transcellular drug passage [6,14]. Besides, the cardiac output is delivered entirely to the lungs (~5 L/min), so absorbed insulin is distributed to the systemic circulation without first-passing the liver, which is a pharmacokinetic advantage that is not present with SC delivery [15].

3.2 Barriers to Pulmonary Insulin Absorption

Although pulmonary delivery of insulin has anatomical advantages, it has a number of formidable obstacles. Protein-based drugs are destroyed by proteolytic enzymes found in the airway lining fluid such as serine proteins, metalloproteins, and lysosomal enzymes before and during absorption [8]. Physically mucociliary clearance occurs in the conducting airways and prevents the inhaled particles in the conducting airways before they can reach the alveoli. Alveolar macrophages can internalize particles a few minutes after they are deposited, especially the ones whose diameter ranges are 1-3 μm . The difference in the mode of inhalation, alteration in the geometry of the airways when exposed to the disease, in diabetic patients with co-occurring respiratory disease and hygroscopic growth of particles in the humid respiratory tract make the delivery of the dose more difficult to be consistent [9,16]. Nano-encapsulation is a logical way of engineering around most of these obstacles.

4. Nano-Encapsulation Strategies for Inhalable Insulin

4.1 Poly(lactic-co-glycolic acid) (PLGA) Nanoparticles

The most researched carrier in insulin nano-encapsulation is called PLA, which is biodegradable and biocompatible FDA-approved copolymer of lactic acid and glycolic acid, it is referred to as PLGA. The fabrication of PLGA nanoparticles is usually done using the two-emulsion solvent evaporation (w/o/w) process, nanoprecipitation, or supercritical fluid processing. The insulin is dissolved in an aqueous inner phase and emulsified by an organic solution of PLGA and this primary emulsion is in turn re-emulsified in an outer aqueous phase with a stabiliser like polyvinyl alcohol (PVA). The use of solvent removal produces insulin loaded PLGA nanoparticles that have encapsulation efficiencies of 40 to 80 percent [17].

Razavi Rohani et al. (2014) utilized mannitol as carrier in the form of aerodynamic microspheres to systematically assess spray-dried powders of insulin that was to be delivered to the lungs incorporating PLGA microspheres, which displayed the highest fine particle fraction (FPF) of $18.3 \pm 1.65\%$ with respirable aerodynamic diameters (1-5 μm) [16]. There is evidence that insulin is protected against degradation by proteases in the bronchoalveolar lavage fluid and the insulin is released during the 24-48 hour time frame in the presence of PLGA nanoparticles, and the burst release part addresses the acute post-prandial requirement [17,18].

4.2 Chitosan Nanoparticles

Chitosan is a natural cationic polysaccharide derived as a by-product of chitin, which has gained significant attention as a delivery excipient of pulmonary insulin due to its mucoadhesive characteristics, the temporary ability to penetrate tight junctions between epithelial cells by interacting with ZO-1 and occludin proteins, and biocompatibility and biodegradability [5,9]. Chitosan nanoparticles loaded with insulin are usually prepared by ionic gelation tripolyphosphate (TPP) at particles that are of 200-600 nm diameter. Shen et al. (2016) have shown that Insulin encapsulation efficiency and permeation across simulated pulmonary epithelial membranes are increased by N-trimethyl chitosan (TMC), a quaternised derivative with enhanced aqueous solubility, and reveals an absolute bioavailability of up to 30% in rodent inhalation studies [19].

4.3 Lipid-Based Nanoparticles

A new category of insulin nanocarrier-based on lipid delivery into the lungs is solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). SLNs are fabricated using physiological lipids like glyceryl trimyristate, Compritol, and glyceryl monooleate which have good biocompatibility, oxidative and hydrolytic degradation protection of insulin and regulated release profiles. Spray drying can be used to produce lipid nanoparticles in the presence of cryoprotectants like trehalose or mannitol to produce inhalable dry powders [7,20]. It has also been shown that lipid nanoparticles increase the transport of insulin through the lipid-raft transcytosis pathway across the alveolar epithelium leading to a faster rate of action as compared to PLGA-based systems [20].

5. Formulation of Nano-Encapsulated Inhalable Insulin Dry Powder

5.1 Spray Drying

The most commonly used method of converting the nanoparticle dispersions to inhalable dry powders is the spray drying method. The aqueous nanoparticle suspension, which is usually enriched with cryoprotectant and bulking aids

(mannitol, trehalose, leucine), is atomised by a 2 fluid or rotary atomiser into a hot drying zone. The resulting powder is made of composite nano-in-micro particles, where nano-particles are incorporated into a matrix of dissolvable or dispersible excipients, which are released under deposition into the lung resulting in individual nano-particles to contact the epithelium directly [8,10]. Cao et al. (2024) surveyed the progress in these concepts of a nano-in-micro such DPI systems and mentions that leucine additions at 10-20% w/w of the feed greatly increased powder dispersibility by lowering inter-particle cohesive forces and obtained MMAD values under 3.5 μm [10].

5.2 Freeze Drying (Lyophilisation)

Freeze drying is another powder formation method that is more appropriate with biomolecules that are sensitive to heat like insulin. The nanoparticle suspension is initially frozen at -80°C and primary drying is then done under a vacuum to eliminate ice through sublimation and secondary drying is done to eliminate any residual bound water to moisture content usually less than 3 percent w/w. Freeze-drying Trehalose, sucrose, and mannitol at 5-10 percent levels are necessary to prevent aggregation of nanoparticles and maintain insulin biological activities throughout the freeze- drying process [7,11]. Omidian (2025) has examined the use of lyophilised DPI formulations of synthetic biomolecules, which ensured that suitable cryoprotection provided reconstitution-ready dry powders with retained aerodynamic characteristics that made them available to inhalation delivery [7].

5.3 Role of Excipients in DPI Performance

The choice of excipients is extremely relevant to the aerodynamic performance, stability and patient tolerability of inhalable insulin dry powders. The most commonly used DPI carrier is lactose monohydrate (1-10 μm) which offers physical stability, and is also capable of enhancing flow characteristics due to the ordered mixing with active fine particles [15]. Sub-1 percent of magnesium stearate as a lubricant can inhibit inter-particle adhesion and enhance de-agglomeration at inhalation. The L-leucine (hydrophobic amino acid) in the surface of the particle decreases the hygroscopic tendency and increases aerolisation efficiency. Pulmonary surfactant, which is mainly composed of dipalmitoylphosphatidylcholine (DPPC) has been incorporated in DPI preparations to recreate the natural lung environment and suppress mucosal irritation [8,16,18].

Table 1: Comparison of Nanoparticle Carrier Systems for Inhalable Insulin Dry Powder Formulations

Nanoparticle System	Key Materials	Particle Size (nm)	Encapsulation Efficiency (%)	Key Advantages	Limitations
PLGA Nanoparticles	PLGA, PVA, Mannitol	200–600	40–80	FDA-approved, biodegradable, sustained release, protects insulin from proteolysis	Acidic microenvironment during degradation may denature insulin; high cost
Chitosan Nanoparticles	Chitosan/TMC, TPP, Trehalose	200–500	60–85	Mucoadhesive, opens tight junctions, enhances epithelial permeability, natural origin	pH-dependent solubility; chloride ions reduce gelation efficiency
Solid Lipid Nanoparticles	Glyceryl trimyristate, Compritol, Tween 80	100–400	55–75	Physiological lipids, rapid release profile, excellent biocompatibility	Polymorphic transitions during storage; low drug loading capacity
Lipid-Polymer	PLGA core, lipid shell,	150–450	65–88	Combines advantages of both systems; stealth effect	Complex manufacturing; scale-up

Hybrid NPs	PEG coating			reduces uptake	macrophage	challenges; higher cost
Liposomes	DPPC, DPPE, Cholesterol	100–300	30–60	Biomimetic, surfactant low toxicity	lung-composition;	Physical instability; aggregation on spray drying; low encapsulation efficiency

PLGA: poly(lactic-co-glycolic acid); PVA: polyvinyl alcohol; TMC: N-trimethyl chitosan; TPP: tripolyphosphate; DPPC: dipalmitoylphosphatidylcholine; DPPE: dipalmitoylphosphatidylethanolamine; PEG: polyethylene glycol.

6. Characterisation of Nano-Encapsulated Inhalable Insulin Dry Powders

To determine the quality, safety and efficacy of inhalable insulin dry powder formulations, comprehensive characterisation of the physico-chemical and aerodynamic properties is needed. The parameters that are regularly assessed are as per pharmacopoeial guidelines (USP, Ph.Eur.) as well as regulatory guidelines provided by the US FDA and EMA in relation to inhalation products [14,18].

6.1 Nanoparticle Characterisation Parameters

Dynamic light scattering (DLS) measures the size and size distribution of particles and the data is reported as Z-average diameter and polydispersity index (PDI). The optimal size of nanoparticles to be delivered to the lungs is 200–600 nm, which bypasses uptake in the macrophage but undergoes transcytosis across the alveolar epithelium [9]. The light scattering of electrophotometry measures the zeta potential, which is an indicator of the colloidal stability; a zeta potential above ± 30 mV gives sufficient information about the extent to which the molecules are repelled by electrostatic repulsions. Drug entrapment efficiency, (DEE), is calculated by ultracentrifugation or dialysis and HPLC or UV spectrophotometric determination of the insulin that was not encapsulated in the supernatant. The visual confirmation of the morphology of the spherical particles and the nanoparticle surface architecture is offered by morphological characterisation by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) [16,17].

6.2 Aerodynamic Performance Parameters

Dry powder formulations are characterized in terms of aerodynamic performance, in terms of a Next Generation Impactor (NGI) or a Twin Impinger at the airflow rate measured by the DPI device (usually 60–90 L/min). The most important parameters are the following: (i) Mass Median Aerodynamic Diameter (MMAD): the radius of aerodynamic size distribution of particles; (ii) Fine Particle Fraction (FPF): the proportion of particles with aerodynamic diameter less than the nominal value; (iii) Geometric Standard Deviation (GSD): a measure of the size distribution of the aerodynamic size distribution of particles; (iv) Emitted Dose (ED): the proportion of the nominal dose that is expelled out of the inhaler mouthpiece per actuation [10,14]

6.3 Powder Flow Properties

The Carr Index (CI = (tapped density - bulk density)/tapped density x 100) and Hausner Ratio (HR = tapped density/bulk density) have been used to measure powder flowability. Values that are less than 15 percent signify excellent flow and values greater than 25 percent signify poor flow that could result in inconsistency in doses. The angle of repose (< 25deg: excellent flow; > 40deg: poor flow) also gives more information on the behaviour of the powder during filling and actuation of the device. The moisture content, which is usually determined using Karl Fischer titrimetry, must not exceed 3 per cent to ensure that the protein will not be diverted into aggregation, insulin will not be destroyed, and the amorphous excipients will not be crystallised too soon [8,11].

Table 2: Critical Characterisation Parameters for Inhalable Insulin Dry Powder Formulations and Acceptable Ranges

Parameter	Method / Instrument	Acceptable Range / Target	Significance
Particle size (Z-average)	Dynamic Light Scattering (DLS)	200–600 nm	Alveolar deposition; macrophage evasion
Polydispersity Index (PDI)	DLS	< 0.3	Uniform size distribution; batch reproducibility
Zeta Potential	Electrophoretic Light Scattering	> ± 30 mV	Colloidal stability; aggregation prevention
Drug Entrapment Efficiency	HPLC / UV Spectrophotometry	> 60%	Insulin loading and process efficiency
MMAD	Next Generation Impactor (NGI)	1–5 μ m	Deep lung deposition in alveolar zone
Fine Particle Fraction (FPF)	NGI / Twin Impinger	> 35–40%	Proportion of dose reaching lower airways
Geometric Std. Deviation (GSD)	Cascade Impaction	< 2.5	Monodisperse aerosol for predictable deposition
Moisture Content	Karl Fischer Titrimetry	< 3% w/w	Stability; prevention of aggregation and degradation
Carr Index / Hausner Ratio	Bulk/Tapped Density Measurement	CI < 15%; HR < 1.2	Powder flowability; dose uniformity
Angle of Repose	Fixed Funnel Method	< 30°	Powder flowability assessment
In vitro Drug Release	Dialysis Membrane / Franz Cell	> 80% within 4–8 h	Predicts in vivo pharmacokinetic behaviour
Insulin Structural Integrity	FTIR / Circular Dichroism	Retention of native α -helical conformation	Confirms biological activity post-processing
Aerosol Morphology	SEM / TEM	Spherical; smooth surface	Flow and dispersion characteristics

MMAD: mass median aerodynamic diameter; GSD: geometric standard deviation; HPLC: high-performance liquid chromatography; SEM: scanning electron microscopy; TEM: transmission electron microscopy; FTIR: Fourier-transform infrared spectroscopy.

7. In Vitro and In Vivo Evaluation

7.1 In Vitro Drug Release and Permeation Studies

In Drug release of nano-encapsulated insulin dry powders in vitro is traditionally evaluated by dialysis membrane method in simulated lung fluid (Gamble solution, pH 7.4) of phosphate-buffered saline, 37degC, agitation, physiologically relevant. A biphasic release profile, which is composed of an initial burst release of surface-adsorbed insulin (20-30% in the first 1-2 hours), and a sustained release of insulin controlled by matrix-diffusion (12-48 hours) is found to be ideal both to meet the post-prandial insulin demand and the basal replacement need [17,18]. Transepithelial electrical resistance (TEER) results recorded upon permselectivity across Calu-3 bronchial epithelial

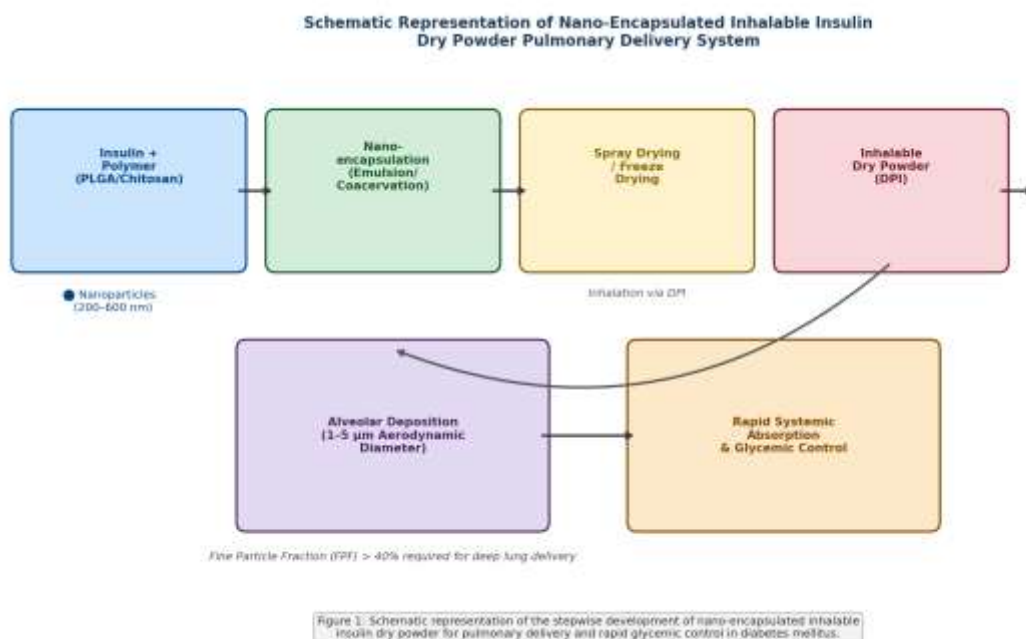
cell monolayers or A549 alveolar epithelial monolayers give mechanistic information regarding the absorption-enhancing properties of chitosan and other formulation ingredients [5,19].

7.2 In Vivo Pharmacodynamic Studies

The in vivo hypoglycaemic testing of streptozotocin (STZ)-induced diabetic rat and rabbit models has been highly adamant in showing how nano-encapsulated inhalable insulin dry powders have the best pharmacodynamic performance as compared to plain insulin dry powders and SC insulin controls. Maximum glucose reduction (E_{max}) of 55-75% of baseline levels during 30-45 minutes of insufflation of the PLGA-based dry powders in STZ-diabetic rats was detected in controlling and maintaining a hypoglycaemic effect of 24-48 hours- a profile that was significantly extended over plain inhaled insulin (4-6 hours) [13,17]. Karmakar et al. (2023) made an extensive review of the new insulin delivery systems, such as inhalable systems and confirmed that the nanoparticle encapsulation decreased the effective dose of insulin to achieve the same glycaemic control by about 40-60% compared to the plain formulations, which demonstrates an increase in pulmonary bioavailability [3].

The pharmacokinetic analyses indicate that the pulmonary bio availability of nano-encapsulated insulin varies between 25% and 40% of the dose given depending on the parameters of formulation, design of the device, and inhalation manoeuvre. This is a 2-3 fold enhancement over insulin powders that are sprayed dry. Time of peak plasma insulin levels (T_{max}) in response to optimised nano-encapsulated dry powder formulations vary between 10 and 20 minutes, which is closely related to the physiological kinetics of insulin secretion during the first phase, and has significant prandial glycaemic coverage [12,13,16].

8. Schematic of Pulmonary Delivery System



9. Clinical Perspective: Marketed Inhalable Insulin Products

Two approved products have realised the clinical translation of inhalable insulin and provide crucial evidence of proof-of-concept. The first inhalable insulin product, Exubera(r) (Pfizer/Nektar Therapeutics), (2006), used regular human insulin in the form of a spray-dried powder containing mannitol and glycine to produce 3.5 μm MMAD. Although proven to be effective, Exubera(r) was discontinued in 2007 because of the less than good ergonomics of the device, pulmonary function concerns, and lack of commercial adoption [15].

The only FDA-approved inhalable insulin is Afrezza(r) (MannKind Corporation), which is based on the Technosphere(r) delivery platform, which consists of fumaryl diketopiperazine (FDKP) microparticles with a mean diameter of 2-2.5 μm , which adsorb rapid-acting insulin and dissolve in the neutral pH of alveolar lining fluid to

provide peak plasma insulin concentrations within 12-15 minutes of inhalation. Clinical trials Phase III randomized controlled clinical trials showed that post-prandial glucose excursions in both T1DM and T2DM patients were reduced by Afrezza(r) similar to SC insulin with a much lower rate of hypoglycaemia [12,13]. Nonetheless, Afrezza(r) should not be used in patients with chronic obstructive pulmonary disease (COPD) and asthma, which implies the need to evaluate the pulmonary safety of new inhalable insulin preparations. These commercialized products are direct sources of lessons applicable in designing next generation nano-encapsulated inhalable insulin dry powders, especially on particle engineering, integration into a device, and regulatory strategy [15].

10. Stability Considerations

Stability of nano-encapsulated inhalable insulin dry powders includes physical stability (resistance to aggregation, caking and hygroscopic growth), chemical (resistance to insulin oxidation, deamidation and disulphide exchange), and biological (preservation of the three-dimensional structure of insulin and its receptor-binding activity) stability. Insulin is especially prone to denaturation at air-water interfaces typically found in spray drying and to acid-induced degradation of degrading PLGA nanoparticles because lactic and glycolic acid are accumulated in the nanoparticles [8,11]. The stability-enhancing interventions are: (i) supplementation with sugars (trehalose, sucrose) that create amorphous glassy matrix around insulin, which inhibits molecular mobility and crystallisation; (ii) surface modification of nanoparticles with PEG to reduce the water absorption; (iii) incorporation of antioxidants (methionine or ascorbic acid); and (iv) keeping the moisture content to less than 3% during the shelf life [7,20].

The ICH Q1A(R2) guidelines provide that stability test under accelerated (40degC/75% relative humidity) and long-term (25degC/60% RH) stability studies are necessary to make regulatory filing. Recent stability trials of nano-encapsulated insulin DPI formulations reported by Imenshahidi et al. (2025) showed that formulations which included trehalose (5% w/w) and L-leucine (15% w/w) retained over 95% of original insulin activity after 6 months at accelerated storage conditions and the MMAD and FPF values were within $\pm 10\%$ of original values, indicating that the studies had sufficiently robust formulations to be practicable in storage and distribution [6].

11. Challenges and Future Directions

Although there has been a significant advancement in the science as discussed in this paper, there are some obstacles to be overcome before nano-encapsulated inhalable insulin dry powder can be widely used in the clinical setting. Patient-specific airway geometry, disease condition, inhalation method, and device design, which result in the variability of bioavailability of inhaled insulin that makes safe and predictable results in glycaemic control vital, require highly reproducible formulation and device performance [4,14]. The dose titration is possibly one of the most difficult, because the dependency between inhaled nominal dose and absorbed systemic dose is non-linear and inter-patient variability of 25-50% exists [13].

A major issue is long-term pulmonary safety. Although it has already been established that there is a cumulative biocompatibility profile of both PLGA and chitosan, long-term inhalation of nanoparticles leaves unanswered the issues of cumulative pulmonary toxicity, and inflammatory possibilities as well as effects of nanoparticles on mucociliary clearance and alveolar macrophage activity with years of daily exposure. Non-human primates Two-year inhalation toxicology studies must be conducted comprehensively before regulatory approval [9,14]. The review by Feng et al. (2024) of the opportunities and challenges of inhalable nanomedicines in respiratory diseases highlights that the toxicological database of inhalation of chronic nanoparticles is not complete and that standardised techniques of measurement of the pulmonary nanotoxicity urgently require [4].

Future directions in research involve: (i) fabrication of glucose-sensitive "smart" inhalable nanoformulations which release insulin in response to ambient glucose concentration, by using boronic acid-phenylacrylamide or glucose oxidase-based biosensing; (ii) surface functionalisation of nanoparticles with PEGylated targeting ligand (transferrin, RGD peptide) to direct insulin to alveolar type II pneumocytes and enhance transcytotic transfer; (iii) combining inhalable insulin systems with digital inhaler monitoring technologies. This should raise the possibilities of decreasing the costs of raw materials and ensuring a wider access to inhalable insulin treatments by low- and middle-income countries due to the increasing availability of the biosimilar insulins.

12. Conclusion

Nano-encapsulated inhalable insulin dry powder is a scientifically sound and a clinically compelling method of overcoming the pharmacokinetic and compliance barriers of current subcutaneous insulin treatment. With optimisation of spray-drying techniques and choice of intelligent excipients, the overlap of more advanced nano-encapsulation technology based on biodegradable polymers (PLGA, chitosan), lipid-based carriers, and optimisation of MMAD in the 1-5 μm respirable range, FPF of over 40 and drug entrapment efficiencies of over 60-80, inhalable dry powders with MMAD can be produced. In vivo and preclinical in vitro experiments indicate that it exhibited rapid onset of hypoglycaemic effect (10-20 minutes Tmax), great pulmonic bioavailability (25-40%), and increased glycaemic control (24-48 hours) compared to plain inhaled insulin. Clinical effectiveness of Afrezza(r) confirms the pulmonary route as a viable method of systemic insulin delivery, and the enhanced protective properties as well as bioavailability of nanoparticle encapsulation has a potential in next-generation products which have higher performance and wider applicability to patients. The remaining challenges of bioavailability reproducibility, long-term pulmonary safety, scale-up manufacturing and regulatory characterisation will also be important in ensuring that this promising technology, which is tested in the laboratory, is translated to clinical reality that can ultimately benefit the 537 million people with diabetes across the globe.

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