

Formulation and Evaluation of Insulin Dry Powder for Pulmonary Delivery

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Abstract: Oral delivery is the most convenient and the most acceptable route. However, insulin by itself is degraded by intestinal enzymes and is not absorbed intact across the gastrointestinal mucosa. Now, a day gelatin, trypsin coated capsule, pill, pellets may be available. It has some disadvantages; a lot of the insulin will be wasted before it gets where it's going. Insulin taken as a pill is quickly broken down in the stomach, just like the food you eat. That makes it useless for lowering blood glucose levels. Intensified insulin therapy consists of basal insulin given in the form of either twice-daily injections of delayed action - lente or isophane (NPH) insulin or once- or twice- daily injections of longer acting ultralente Continuous subcutaneous Insulin infusion (CSII) from a portal pump is used for basal insulin. Prandial insulin is given by injection of short-acting insulin given 30 min before meal. **E.g.** Insulin syringe, pen, an insulin pump. The pulmonary route of administration offers several advantages. First, the lung has a large surface area for drug absorption, ranging from 100 to 140 m². In addition, the alveolar epithelium has permeability that allows for rapid absorption of solutes. Because the mucociliary clearance of the alveolar lung tissue is slower than that of the bronchiolar tissues, the alveoli provide a greater opportunity for the absorption of larger molecules (e.g., insulin).

Keywords: Diabetes Insipidus, antidiuretic hormone, Pulmonary Inhaler, Gel Electrophoresis

Introduction and Experimental: Diabetes Insipidus (DI): It is a disease of impaired renal conservation of water due to deficiency of antidiuretic hormone (ADH). Urine from patients with DI is tasteless, hence the name Insipidus^[1]. Diabetes Mellitus (DM): Is a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates and proteins and an increased risk of complications from cardiovascular disease. **Insulin preparations:** Short acting:

Plain (Regular), Insulin Zinc Suspension, Semi lente. Intermediate acting: Neutral Protamine Hagedorr, (NPH) Or Isophane insulin, Lente Long acting: Protamine Zinc Insulin (PZI), Extended Insulin Zinc suspension Or Ultralente^[2]

Pulmonary Inhaler Development

Inhaler Design:

Typical limitations of traditional inhalation devices include low efficiency, variable dosing, poor moisture

barriers, low drug content per inhalation, inapplicability to macromolecules, and sensitivity to the breathing maneuver. In comparison, the EXUBERA inhaler is a novel pulmonary delivery system developed by Nektar Therapeutics that solves many of these challenges. The pulmonary delivery system is a reusable dry powder inhaler that has been designed to deliver insulin to the small airways and alveoli for systemic insulin absorption^[1].

The inhaler was designed to provide reproducible powder extraction, deagglomeration, and dispersion, capable of aerosolizing relatively small amounts of cohesive powder (1–10) mg. It is solely mechanical, and requires only modest effort by the patient to operate. Unlike typical high-resistance inhaler systems, aerosolization of insulin powder is independent of patient inspiratory effort, providing opportunities for improved performance and product consistency^[1].

The inhaler consists of three subsystems: base, TransJector, and chamber/mouthpiece. The inhaler performs four key functions: puncture of the insulin powder-containing blister after loading into the device,

extraction of the powder from the blister, dispersal of the powder into the chamber, and facilitation of inhalation delivery of the powder cloud to the patient^[9].

Specifically, the base contains an air pump and valves that generate, store, and release compressed air. Upon actuation, an individual blister is punctured; and compressed air is released through a jet structure in the Trans-Jected and then vented into the chamber. The sudden sonic discharge of this mass of air through the small jets in the Trans-Jector results in extraction and dispersion of insulin powder^[9].

The rapidly moving air creates a vacuum in the Trans-Jector and blister, causing the insulin powder to be drawn into the chamber and dispersed into a cloud after the aerosol cloud has formed in the chamber, the patient rotates the mouthpiece 180° into the open position and inhales the dose. The emitted dose, particle size distribution, and fine particle dose of the aerosol are controlled primarily by characteristics of the insulin powder and the inhaler, and are relatively independent of variables that may be introduced by the patient. The inhaler was specifically designed such that patients can use the device with a simple, slow, deep inhalation^[11].

Powder Production

Insulin powders were made by dissolving bulk crystalline insulin in sodium citrate buffer containing excipient (mannitol, or raffinose, or none) to give final solids concentration of 7.5 mg/ml and a pH of 6.7±0.3. The spray dryer was operated with an inlet temperature between 110°C to 120°C and a liquid feed rate of 5 ml/min, resulting in an outlet temperature between 70°C and 80°C. The solutions were then filtered through a 0.22 µm filter and spray dried in a Buchi Spray Dryer to form a fine white amorphous powder. The resulting powders were stored in tightly capped containers in a dry environment (<10% RH)^[12].

2. Preparation of Insulin Powder Using a Spray – drying Technique:

Insulin suspensions and solutions were prepared by adding insulin with or without additives to distilled water. Insulin was suspended by simply adding to water. The decrease in pH of the insulin suspension.

- a. Insulin state in the stock solution.
 - b. Yield = amount of powder recovered/amount of ingredients in the sprayed solution.
- Below the isoelectric point (5.0–5.3) with a 1.0M HCl solution and the successive increase in the pH to 4 with a 1.0M NaOH solution gave an insulin solution. The addition of citric acid to an insulin Suspension resulted in the dissolution of insulin, while it was still suspended after the addition of bacitracin or Span 85.

The preparation of dry insulin powders by a spray drying technique was reported in our previous report. Briefly, the following standard operating conditions were used for spray-drying with an SD-1000 spray-drier an inlet

temperature of 90 °C, a drying air flow rate of 0.75ml/min, a solution feed rate of 5 ml/min, and an atomizing air pressure of 100 kPa. Operating under these conditions resulted in an outlet temperature 63–69 °C. The code names and compositions of the formulations are listed in Table 10.2. The dry powder INS SD was prepared from 1.0% insulin solution. The dry powders MI SD (susp) and MI SD were prepared by spray drying a 0.25% insulin suspension and 0.25% insulin solution, respectively, containing 5.0% mannitol. The dry powder MIC SD was manufactured with 0.25% insulin solution containing 0.20% citric acid and 5.0% mannitol. MIB SD and MIS SD were manufactured with 0.25% insulin suspension containing 10mM bacitracin and 1.0% Span 85, respectively, and 5.0% mannitol. The dry powder MI_ was prepared by spray drying a 0.5% insulin suspension containing 5.0% mannitol. MC was prepared by spray drying a 0.40% citric acid and 5.0% mannitol solution without insulin^[3].

In Vitro Powder Characterization: An HPMC capsule with 20 mg of insulin powder was loaded in an inhaler, the insulin powder was dispersed into an Andersen Cascade Impactor from the Jethaler for 10 s at an air flow rate of 28.3 ml/min. The amount of insulin powder deposited on each stage of the impactor was determined by measuring the difference in the weights of the plate before and after sampling. The insulin powder deposited on the other parts (device, throat, and cone) was dissolved with 0.01N HCl and assayed by an HPLC method. The output efficiency (OE) was determined as the percent of total powder mass exiting from the capsule and device. Respirable fraction (RF) of insulin powder was determined by dividing the powder mass recovered from the stages 2–7 <7.0 µm of the impactor by the OE value. A plot of the cumulative amount of powder deposited on each stage of the impactor on the probability scale axis against the logarithm of effective cut-off Diameter for that stage allowed calculation of the mass median aerodynamic diameter (MMAD) of the particles.

The particle size distribution was also measured with a laser micron size based on laser diffraction. We dispersed the dry powder into a laser beam directly from an apparatus used for intratracheal administration^[7].

Evaluation of Insulin Stability:

The dry insulin powders were placed in glass containers and stored at various temperatures in humidified chambers. Various relative humidities (RH) were achieved using the following saturated salt Solutions: NaI for 25% RH at 60 °C, NaBr for 50% RH at 60 °C, and NaCl for 75% RH at 60 °C. Desiccators with silica gel were used for the dry conditions at 40, 50, and 60 °C. The dry insulin powders were stored dry and at 75% RH at 60 °C for 0, 1, 2, 3, 5, and 10 days, while MIC SD and MIC Mix were stored under dry condition and 75% RH for 0,

4, 8, 12, and 24 h and 0, 2, 4, and 8 h, respectively. MI SD (suspension) powder was stored under dry condition at 40 °C for 0, 1, 2, 3, and 4 weeks.

The logarithm of insulin potency was plotted against time and the degradation rate constant was estimated from the slope assuming first-order degradation kinetics ^[2].

X-Ray Diffractometry (XRD):

X-ray X diffraction pattern analysis was performed with a RAD-II VC, using Cu K α radiation, over a range of (2 θ) 5–45° (Speed 0.02°/min) at room temperature ^[14].

Evaluation of Particle Size Distribution:

A scanning electron microscope (SEM) was used to observe the particle shape ^[15].

Moisture in the Insulin Dry Powder:

The moisture content in the dry insulin powders was analyzed by thermo gravimetric analysis (TGA) using Shimadzu DTG-60. Approximately 20 mg of the powder sample was heated from 25 to 150 °C at a rate of 5 °C/min ^[2].

Sodium Dodesyl Sulphate-Polyacrylamide Gel Electrophoresis:

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on a 15% SDS-polyacrylamide gel according to Laemmli's method with a constant current of 20mA for 120 min. Dry insulin powder and insulin standard were dissolved in Tris–HCl buffer (pH 6.8) containing 1% SDS and 1% 2-mercaptoethanol, 20% glycerol and 0.01% bromophenol blue. Samples of 30 μ g of insulin were loaded on each lane. Molecular weight standards used were ovalbumin (43,000), carbonic anhydrase (29,000), β -lactoglobulin (18,400), lysozyme (14,300), bovine trypsin inhibitor (6200), insulin (α and β chains) (3000). Proteins on the gel were stained using a silver stain kit according to the manufacturer's instructions ^[16].

Intratracheal Administration of Insulin Dry Powder in Rats:

Male Sprague–Dawley rats weighing 300–320 g were anesthetized with pentobarbital (50 mg/kg, i.p.) and secured on their backs on a board during the experiments. The trachea was exposed and a 3.5 cm length of PE-240 polyethylene tubing was inserted to a depth of 0.6 cm through an incision made between the fifth or sixth tracheal rings caudal to the thyroid cartilage. A PE-50 tubing cannula was placed in the carotid artery for blood sampling.

Insulin dry powder was administered as previously reported using an apparatus made with a disposable syringe, three-way stopcock, and disposable tip. The powder taken in the tip was dispersed in the rat lungs through the PE-240 tubing by releasing air compressed in the syringe by opening the three-way stopcock connecting the tip and the syringe. The amount of dry powder Administered was calculated by subtracting the tip weight after administration from that before administration ^[12].

Assay of Insulin:

Insulin was determined using a high performance liquid chromatography (HPLC) system using the raw insulin bulk drug as the standard (28.0 U/mg). The HPLC system was composed of a pump (LC-10ADvp), diode array detector (SPD-M10Avp), column oven (CTO-10ASvp), and LC work station (CLASS-LC10). The mobile phase was a 72:28 mixture of 0.1M ammonium sulfate buffer (pH 2.3) and acetonitrile at a flow rate of 1.2 ml/min. The column was a Shodex Asahipak ODP- 50 6D (4.6mm $\times$ 150 mm, 5 μ m) heated at 36 °C. Ultraviolet absorption was measured at 214 nm. The injection volume was 10 μ l. The potency of insulin in the powders was defined as the amount of insulin (unit) in 1mg of the product ^[2, 3, 7, 14, and 15].

Assay of Blood Glucose Level:

Blood samples (200 μ l) were collected before administration and every 30 min up to 360 min after insulin administration and centrifuged at 4 °C to separate plasma. The plasma glucose level was measured with a glucose assay kit, Glucose CII Test based on the mutarotase GOD method. The change in plasma glucose level (GLC) expressed in %/unit was calculated using the following equation:

$$\Delta \text{GLC} = \left(\frac{\text{GLC}_t - \text{GLC}_0}{\text{GLC}_0} \right) \text{ per dose} \times 100 \quad (1)$$

Where, GLC₀ and GLC_t are the plasma glucose concentrations at time 0 and at time *t*, respectively. The change in plasma glucose level was normalized by the dose because the doses were different for each administration. The area under the curve (AUC) for Δ GLC with respect to time from 0 until 360 min was calculated by the trapezoidal rule ^[7].

Statistical analysis:

Statistical differences in insulin absorption of insulin were examined using a one-way analysis of variance followed by least significant difference test. The significance level was set at $P < 0.05$ ^[3].

Results and Discussion

The Mass Median Aerodynamic Diameter:

The in vitro inhalation performance of spray-dried powders was evaluated using an Andersen Cascade. Impactor the mass median aerodynamic diameters of these powders estimated from the impactor data were less than 7.0 μm , suggesting that the spray-dry technique was successful in preparing powders suitable for reaching respiratory regions. INS SD powder had a superior inhalation performance (RF and OE) compared with the other powders^[5].

Potency of Insulin in the Dry Powder:

The effect of absorption enhancers on the stability of dry insulin powders stored at 60 °C and various humidities. Span 85 (MIS SD) and bacitracin (MIB SD) had no influence on the half-life of insulin at the 60 °C/dry condition. However, citric acid (MIC SD), which was a potent pulmonary Absorption enhancer for insulin powder, decreased the half-life of insulin.

MI SD (suspension), MI SD, INS SD, insulin bulk drug, and MIC SD were stored at various humidities. The half-life of insulin of these powders was decreased by the increase in relative humidities.

A desirable dry insulin powder for inhalation should be stable enough to allow storage at room temperature. The shelf life (T_{90}), which is defined as the period required for 10% potency loss in this study, of MIC SD at 25 and 15 °C was estimated from an Arrhenius plot using the degradation rate constants at 40, 50, 60 °C and dry condition. T_{90} was estimated^[6].

X-Ray Diffraction:

X-ray diffraction patterns of dry insulin powders are shown in above fig. The dry powder containing additives prepared from insulin solution with or without citric acid exhibited β polymorph of mannitol and decreased crystallinity compared with a physical mixture. On the other hand, MI SD (suspension) Prepared from insulin suspension exhibited β polymorph of mannitol. X-ray diffraction of INS SD prepared from insulin solution exhibited an intense peak at $2\theta = 31.8$. This X-ray diffraction pattern agreed with that of an insulin/NaCl physical mixture, which was a mixture of NaCl powder prepared by the spray-drying technique from pH 7.4 NaCl solutions and the insulin bulk drug^[5].

Morphological Change in Dry Insulin Powder:

The morphological change in MIC SD, MI SD (suspension), MI SD, INS SD, and insulin bulk drug was investigated. MI SD (suspension) was prepared from an insulin suspension, MI SD was prepared from an insulin solution, and INS SD and insulin bulk drug were

prepared without mannitol or absorption enhancers. MIC SD contained citric acid and was effective to increase insulin absorption from rat lungs. The morphology of these powders changed during storage at dry or 75% RH condition at 60°C (Fig. 6.4). The particle size should be not more than 7.0 μm for inhalation therapy in order to deliver the drug deep in the lung. The powder morphology and size did not change after 10-day storage at 60°C/dry. However, all powders except the insulin bulk drug changed their particle morphology and increased the size after 10-day storage at 60 °C/75% RH^[11].

Formulation of High Molecular Weight Protein in the Dry Insulin Powders:

Many different types of chemical and physical changes result in the formation of dimers or Aggregations of protein. As for chemical change, temperature, moisture, and formulation excipients affect the solid-state stability of proteins and peptides. The formation of high molecular weight.

Protein in the dry insulin powders was investigated using SDS–PAGE after the 10-day storage at 60 °C/dry and 3-day storage 60 °C/75% RH. High molecular weight insulin Aggregates were clearly observed for MIC SD to a greater extent than for the other powders examined. This indicates that citric acid tends to denature insulin, which agrees with the rapid decrease in the insulin potency determined by HPLC. The aggregates in the insulin powders prepared from insulin. Solutions (INS SD, MI SD) were less than those found in the insulin bulk drug stored at dry condition. Contrary to the dry condition, high molecular weight aggregates in the insulin bulk drug were less than those in the other dry insulin powders at 75% RH^[10].

Improvement of Insulin Stability with Citric Acid:

Although MIC SD was effective at increasing the pulmonary absorption of insulin, the stability should be improved for storage at room temperature. To improve the stability of insulin formulated with citric acid, we developed an MIC Mix powder, which is a combination of MI and MC. The 1:1 combination of MI and MC should theoretically have the same composition as MIC SD Fig. shows the stability of insulin in MIC Mix. The stability of MIC Mix was still inferior to that of MI SD; however, the half life of MIC Mix (8.5 ± 1.8 days (mean $\pm$ S.D.) at 60 °C/dry and 0.42 ± 0.05 days at 60 °C/75% RH) was significantly improved ($P < 0.05$, Student's t -test) and extended 6.5 and 2.6 times compared with MIC SD (1.3 ± 0.3 days at 60 °C/dry and 0.16 ± 0.05 days at 60 °C/75% RH), respectively^[8].

Studies on Pulmonary Insulin Absorption:

A rise in the plasma IRI and decrease in plasma glucose in normal and diabetic patients on administration of aerosolized insulin was reported. Recently insulin was

administered approximated 1.OU/kg body weight (without promoter) as an aerosol by oral inhalation non-smoking NIDDM patients. No side effects were reported following insulin or placebo aerosol inhalation. Additional studies are necessary to know the reproducibility of the glucose, the long-term effects of zinc and the preservative and the effective dose of aerosolized insulin required to normalize plasma glucose levels after a meal. A preservative - cresol was added^[5].

Insulin Absorption after Intratracheal Administration of MIC MIX:

The hypoglycemic effect of MIC Mix after pulmonary administration to the rat lung was examined and compared with those of INS SD, MI SD, MI SD (suspension), and MIC SD. MIC Mix showed a rapid onset and elongated hypoglycemic effect compared its INS SD, MI SD (suspension) and MI SD.

There was no significant difference observed between MIC SD and MIC Mix regarding the decrease in the plasma glucose level at each time point, suggesting the combination of insulin and citric acid powders was as effective as the dry insulin powder containing citric acid^[6].

Pharmacokinetic study:

A variety of short human studies have shown that regular insulin formulations are well absorbed by the lungs. All studies have used regular, soluble insulin.

Discussion

It is likely that the polymorphism of sugar affects insulin stability in the dry powder. Mannitol contents in the formulation would influence the insulin stability. Electrophoresis results showed that insulin in MIC SD was denatured and aggregated in a short time compared with the other powders. Insulin was highly unstable under acidic and basic conditions in solutions.

A combination of insulin and citric acid powders is a simple but effective method to improve the stability by physically decreasing the chance of interaction of insulin and citric acid^[4].

The morphological change in the particles would influence the inhalation performance such as drug emission from a capsule or device and drug deposition in the lung the hygroscopic of citric acid seems to influence the morphological change and moisture content in MIC SD.

The present study also revealed that the increase in water content resulted in the decrease in the stability. Dry insulin powders were successfully prepared for

inhalation. The therapeutic effect of insulin powders should be evaluated by in vivo absorption study results as well as in vitro powder Characterization study results^[7].

Future Aspects:

According to the World Health Organization (WHO), the global prevalence of diabetes is expected to reach 336.0 million by the year 2030. Diabetes incidence and prevalence are expected to increase by 21.0 per cent in the next 20 years. It is posing a great burden on the limited government health care budgets. It is estimated that EU spends 29.00 billion Euros towards diabetes care costs. The increasing costs due to diabetes and its complications are likely to increase at a geometric progression in the coming years unless measures are taken to control it^[17]. The current treatment options available for diabetes are inadequate to meet the needs of the diabetic patient. Disease awareness, lifestyle changes and more compliant treatment methods are the pressing need of the day. The European governments are taking initiatives to increase awareness and education programs. The importance of lifestyle modifications and strict treatment regimen is emphasized^[12].

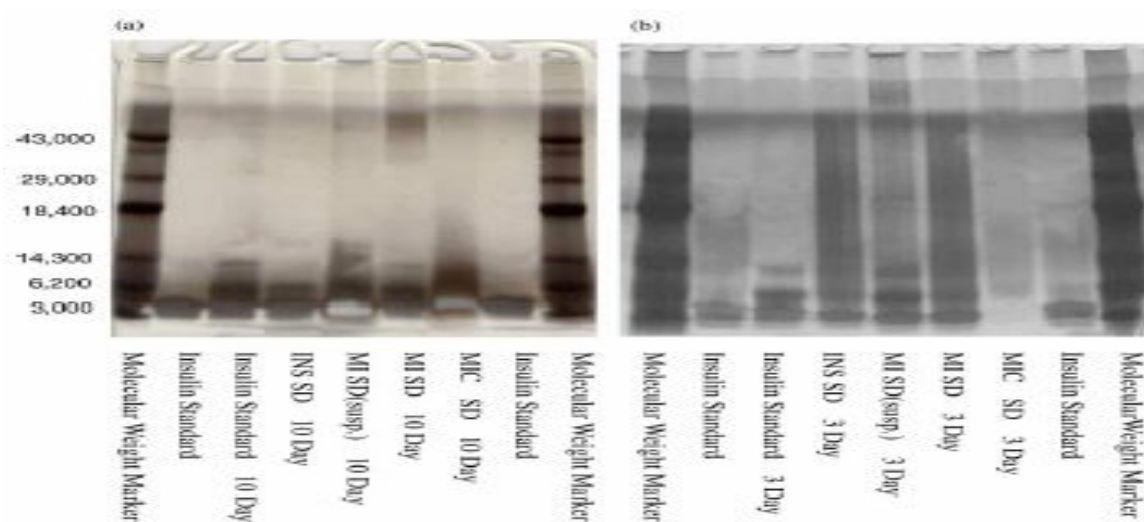
Attempts are being made to reduce the burden of the insulin taking diabetics. Various options for insulin delivery are currently under research to replace the vintage concept of subcutaneous insulin delivery. Oral insulin, Transdermal insulin and diabetes vaccines are in various phases of research. Nanotechnology is presently being utilized to enable better insulin delivery. This technology has tremendous potential to change the face of insulin delivery. The human genome project has opened up avenues for gene therapy. Gene therapy for diabetes is being given much importance owing to the detection of the diabetes gene. But the research is still in the initial stages of development. As diabetes is a multigene disorder, the approach is likely to consume more time to succeed^[13].

Conclusion

Although the hypoglycemic effect was greatly improved when the dry insulin powder with citric acid (MIC SD) was administered, insulin in the MIC SD was unstable compared with the other powders. We designed the dosage form to improve the insulin stability without loss of hypoglycemic activity. MIC Mix was formulated as a combination of insulin powder (MI) and citric acid powder (MC). MIC Mix showed hypoglycemic activity comparable to MIC SD and improved insulin stability. In this study, moisture affected the insulin stability and particle morphology. It was suggested that a package preventing moisture absorption was necessary for insulin powders prepared with citric acid.

Products under Development:

Dosage form	Trade name	Company	Development Stages
Solution	AERx iDMS	NOVO Nordisk, Aradigm	Phase 3 Clinical trial in progress
	Aerodose	Aerogen	Phase 2 clinical trial completed; development halted in JANUARY 2003.
Powder	Exubera	Pfizer, Aventis, Nektar	Phase 3 clinical trial completed; long term safety studies ongoing; marketing authorization application filed with European Medicine Evaluation Agency in MARCH 2004; new drug application filed with FDA in 2005.
	Promaxx	Epic Therapeutics	Preclinical studies
	Promaxx	Elililly, Alkermes	Phase 2 clinical trial in progress
	Spiros	Elililly, Dura pharmaceuticals	Discontinued after merger between Dura pharmaceuticals and Elan pharmaceuticals
	Technosphere insulin system	Mannkind Corporation	Late Phase 2 clinical trial ongoing. Phase 3 clinical trial initiated in Europe

**Figure Gel electrophoresis of dry insulin powders**

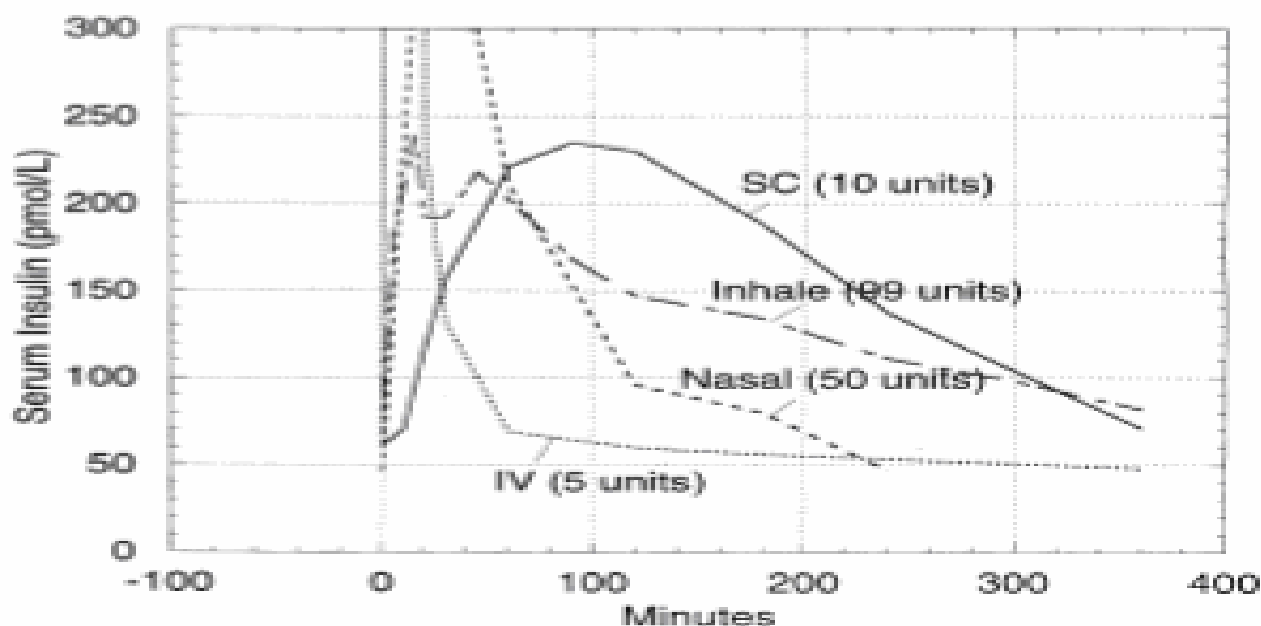


Figure: Comparison of insulin absorption from four different routes of delivery

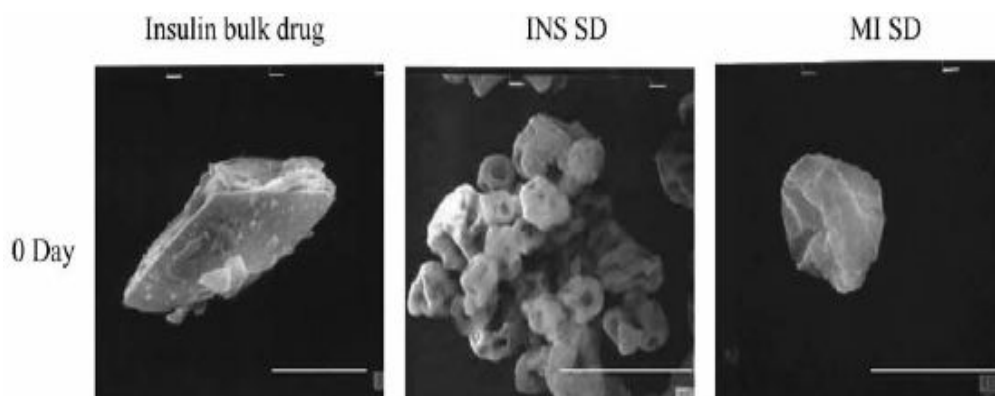


Fig. Scanning electron micrographs of dry insulin powders. Bar: 10 μ m.

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