Nasal Insulin Gel as an Alternate to Parenteral Insulin: Formulation, Preclinical, and Clinical Studies

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ABSTRACT

The objective of the present study was to formulate insulin gel for intranasal administration and to evaluate with respect to in vitro release studies and hypoglycemic activity in animal model and healthy human volunteers. The insulin gel was formulated using the combination of carbopol and hydroxypropyl methylcellulose as gelling agent. The in vivo efficacy of insulin gel administered intranasally was assessed by measuring the blood glucose levels and serum insulin levels at specified time intervals in rats and humans. The use of bioadhesive nasal gel containing insulin not only promoted the prolonged contact between the drug and the absorptive sites in the nasal cavity but also facilitated direct absorption of medicament through the nasal mucosa. Absorption of the drug through the nasal mucosa was high in the first 0.5 to 1.5 hours of the study with a sharp decline in blood sugar and rise in insulin values corresponding to that decline in blood sugar. This study further demonstrates that administration of insulin intranasally in gel form is a pleasant and painless alternative to injectable insulin.

KEYWORDS: nasal, diabetes mellitus, insulin, gel, carbopol, preclinical, clinical

INTRODUCTION

Despite the significant developments made in insulin therapy over the past 60 years, noninvasive insulin delivery remains an elusive goal. Insulin is the only molecule to have attracted so much attention from drug delivery scientists, as witnessed by more than 2000 published articles and more than 100 granted patents on several aspects of insulin delivery.[1] Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Recently compiled data show that ~150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025.[2] There are 2 principal forms of diabetes: type 1 diabetes, in which the pancreas fails to produce the insulin, and type 2 diabetes, which results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common and is occurring at a younger age group in Indian population. Many patients seek relief with oral hypoglycemic agents due to local discomfort and disruption of perceived lifestyle owing to parenteral therapy, but many patients do require parenteral insulin therapy at a later age due to exhaustion of ß cell in the pancreas.[2,3]

Currently, insulin administration requires subcutaneous (sc) injection, which even in the simplest form (Nova-Pen system, Novo Nordisk, Bagsværd, Denmark) is cumbersome and unacceptable to many patients with diabetes. However it is every patient's dream to have access to insulin without the pain of injection. This desire has motivated the search for novel therapeutic approaches to replace the present parenteral insulin delivery. Ideally, an oral insulin dosage form would be preferred over the currently available parenteral route of administration, but this novel approach is confronted by common biological and physicochemical problems such as luminal degradation, particle aggregation, and polypeptide degradation in the absorptive area of the gastrointestinal tract. Several new and alternative routes including pulmonary, buccal, ocular, rectal, vaginal, transdermal, and others have also been explored for noninvasive delivery of insulin.[4] In a recent study, the development and evaluation of a novel composite microsphere delivery system composed of poly(acryloyl hydroxyethyl starch) (acryloyl derivatized HES; AcHES) (PLGA) and poly(D,L-lactide-co-glycolide) hydrogel using bovine insulin as a model therapeutic protein has been reported. The AcHES-PLGA composite microsphere system provides satisfactory in vitro and in vivo sustained release performance for a model protein, insulin, to achieve 10-day glucose suppression.[5]

Among these, nasal-pulmonary administration has frequently been proposed as the most feasible alternative to parenteral injections owing to the high permeability of the nasal epithelium, rapid drug absorption rate, and plasma drug profiles sometimes almost identical to those from intravenous injections.[6] Recently an attempt was made to achieve desired bioavailability after pulmonary administration of Levonorgestrel and to provide prolonged effective concentration of the drug in plasma and reduce reported side effects of orally administered drug.[7] In another approach, the

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effects of processing conditions on the characteristics of solid lipid microparticles of Compritol with a potential application as carriers for pulmonary administration was investigated.[8] In fact, nasal drug administration for systemic effects has been practiced since ancient times such as nasally administered psychotropic drugs by Native Americans, the use of tobacco snuffs, and the nasal administration of illicit drugs.[6] However, of late, evidence has suggested that this route may be useful for drugs hitherto only administered parentally. For example, the nasal administration of insulin to dogs resulted in a significant increase in blood immunoreactive insulin levels with a remarkable hypoglycemia. Many preclinical and clinical studies with other proteins, peptides, and DNA delivered through nasal route have been completed and demonstrated that efficacy can be achieved systemically. The number of potential drugs being tested by intranasal administration continues to increase, suggesting some promise of future success. Some of the nasal insulin formulations and technology (ie, dry powder inhalation by Vectura Ltd/ML Laboratories, Nasal Technology by West Pharmaceuticals Services, and Touch-spray by Odem Ltd, Bucks, UK/Pari GmbH, Müchen, Germany) have already been patented and are mostly under development.

Various bioadhesive polymers such as polyacrylic acids (eg, Carbopol 934P, polymethyl methacrylate) used in the gel forms prolong the residence time on oral mucosa.[6] The use of nasal bioadhesive gels blended with an appropriate chemical permeation enhancer might be used to provide an enhanced bioavailability compared with oral delivery. A very good example for such a system is EnerB (Nature's Bounty Inc, NY), a vitamin B-12 supplement available in gel form. The present study was designed to evaluate the feasibility of intranasal administration of insulin using a bioadhesive gel base. The absorption of insulin through nasal mucosa was estimated from reduction in blood glucose and increase in serum insulin levels in animal model (rats) and humans.

Materials and Methods

Materials

Bovine insulin injection was gifted by Knoll Pharma, Mumbai, India. Carbopol 934P and hydroxypropyl methylcellulose K4M (HPMC) were obtained from B.F. Goodrich Co (Charlotte, NC) and s. d. Fine Chemicals (Mumbai, India) respectively. Sodium deoxycholate was purchased from Sigma (St Louis, MO). All other chemicals, reagents, and ingredients used were of analytical grade.

Animals

Male Sprague-Dawley rats were obtained from Central Animal Housing, Kasturba Medical College, Manipal (an approved and registered facility under CPCSEA 1998: registration number 94/CPCSEA/1998). The experimental protocol for all in vivo studies was approved by the Institutional Animal Ethical Committee, Kasturba Medical College. The animals were maintained under controlled conditions of temperature and humidity in polypropylene cages filled with sterile paddy husk. They were fed with balanced diet (Lipton India Ltd, Mumbai, India) and water ad libitum.

Methods

Formulation of Insulin Gel

The gel consisted of Carbopol 934P (0.7%) and HPMC (1.3%). Required quantities of polymers were weighed and blended thoroughly. The polymers were then suspended in insulin solution and allowed to swell. Triethanolamine was added dropwise with gentle stirring till a clear, smooth, and translucent gel was obtained at a pH of 7.2 to 7.4. Sodium deoxycholate (1%) was added to the gel and mixed until a complete blend was obtained. Sodium metabisulphite (0.01%) and methylparaben (0.05%) were finally incorporated and mixed well to obtain a uniform gel. The insulin content of the gel was 100 IU/g.

In Vitro Drug Release Studies

A known quantity of the gel was weighed and filled in a dialysis tube to which a Sigma dialysis sac (Sigma Chemical Company, St Louis, MO) was attached to one end. The dialysis tube was suspended in phosphate-buffered saline (PBS) pH 7.4 and stirred with a magnetic stirrer; samples were withdrawn at specific time intervals. The samples were analyzed using Diode Array spectrophotometer 8453 (Hewlett Packard, Palo Alto, CA) at 269 nm.

Induction of Diabetes Mellitus and Hypoglycemic Activity in Diabetic Rats

Male Sprague-Dawley rats weighing 220 to 250 g were used for the study. Diabetes was induced in overnight fasted rats by injecting streptozotocin (50 mg/kg, intraperitonieally [ip]) dissolved in citrate buffer (3 mM, pH 4.5).[9] Seven days later, rats with blood glucose levels between 300 and 450 mg/dL were selected. Following an overnight fast, rats were divided into 2 groups (n = 6). Group 1 served as control and was injected with insulin (6 IU/kg, subcutaneously [sc]). Group 2 animals were administered intranasally with insulin gel (1.5 IU/kg) using a microsyringe attached to a blunt needle with a 0.5-inch polyethylene tube at the end. At time intervals of 2, 3, 6, 8, 10, 12 and 15 hours after treatment, blood was collected from orbital sinuses; blood glucose levels were determined using Accutrend Alpha Glucometer (Roche Diagnostics, Mannheim, Germany).

Clinical Studies

The study was conducted in 2 stages. In the first stage, the objective was to observe the absorption of insulin through nasal route. Hence the efficacy of insulin gel at doses of 2, 4, and 6 IU/kg was assessed to select the optimum dose. This effect was compared with that of a placebo gel. In the second stage, it was planned to have the most appropriate dose of first stage. The experimental protocol in human volunteers was approved by Hospital Ethical Committee, Kasturba Hospital, Kasturba Medical College.

Stage 1 (Phase 1, Placebo-controlled, Open-labeled Study)

In total, 24 volunteers were enrolled for stage 1 (6 each for placebo, 2, 4, and 6 IU/kg doses). The body weight of the volunteers was noted down the previous day. The gel, with and without insulin, was prepared and accurate volume was placed in 1 mL syringe depending on the dose of gel as per the body weight. The syringes were marked appropriately.

The volunteers underwent nasal examination to rule out local causes (eg, infection, allergy, nasal obstruction, inflammation) that may interfere with insulin absorption. General and systemic examinations including pulse and blood pressure were also performed. Volunteers were made to lie down with the head tilted slightly backwards to facilitate gel administration, and the appropriate volume of gel was applied to one of the nostrils using the applicator inserted 1-cm deep into the nostril. After gel application, the outer surface of the nose was gently rubbed for even distribution of the gel. The volunteers were asked not to breathe deeply or sniff for 5 minutes during and after the application of the gel. In addition they were asked not to pick their nose and to avoid sneezing for the next 10 minutes after the application.

Before the application of gel, blood was withdrawn for the determination of fasting blood sugar and fasting insulin. After insertion of the gel, sampling of blood was performed at 0.5, 1, 1.5, 2, 3, and 4 hours. During the 4-hour sampling period the volunteers were not allowed to take any food, but water was allowed ad libitum.

Stage 2 (Phase 1, Nonrandomized, Open-labeled Study)

In stage 2, 6 volunteers were enrolled. All 6 volunteers were administered with the same dose of insulin, which was determined from the previous study (6 IU/kg). The trial was continued as explained in the earlier section and blood sampling was done at 0.5, 1, 1.5, and 2 hours after gel application.

Stability Studies

Insulin gel was tested for stability under the actual conditions of storage (refrigeration condition) in clean, dry, airtight, moisture-proof collapsible tubes, stored away from light. The gel samples were withdrawn at 1, 2, 3, and 6 mo and evaluated for insulin content, viscosity, and pH. The viscosity of gel was determined using Brookefield synchro electric viscometer (Brookefield Engineering Ltd, Middleboro, MA). The TD bar spindle of LV-4 at 12-gear was employed. The pH of the gel was determined using digital pH meter (Digisun Technologies, Bangalore, India).

Analytical Method

Plasma was separated by centrifugation at 3000 rpm, and plasma glucose was determined by the glucose oxidase/peroxidase method.[8] Serum immunoreactive insulin was quantitated by a double-antibody radioimmunoassay (RIA) using RIA kit provided by Diagnostics Products Corporation (Los Angeles, CA).

Results and Discussion

Formulation Development

With nose drops and sprays, it is difficult to hold the medicament in the nasal cavity for a long period of time, hence is not useful for slow releasing preparations. An approach to improve nasal drug absorption is to increase the duration of formulation residence within the nasal cavity. This can be achieved by the use of mucoadhesive polymers. The formulation on application is wetted with the intranasal secretion and gradually swells and causes the released medicament to be absorbed through the nasal mucosa. Besides releasing the medicament slowly, this formulation also achieves safe administration of medicament because of the direct absorption of the drug through the mucosa of the nasal cavity. This process is promising in the case of a medicament such as insulin, which easily decomposes in the stomach and intestines and is not very effective by oral administration. Further, a sudden change in body condition, which may be caused by the administration of the medicament, can be dealt with easily by simply removing the pharmaceutical preparation from the nasal mucosa. In the present study, insulin gel for nasal administration has been formulated and evaluated. Antioxidants play an important role in maintaining the viscosity of the carbopol gel. Sodium metabisulphite was therefore used in the formulation as an antioxidant. The prepared aqueous gel could support the growth of fungi, hence methylparaben is incorporated as an antimicrobial agent as a precautionary measure. Sodium deoxycholate was used as permeation enhancer as it showed permeation enhancing property in many instances with lower toxicity.[4,10,11]

In Vitro Drug Release Studies

The results of in vitro drug release studies are shown in Figure 1. A continuous release of insulin was observed in

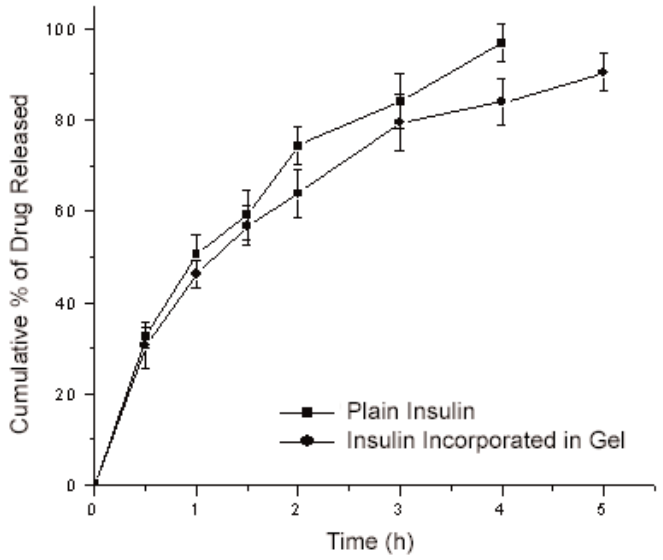

Figure 1. In vitro release profile of plain insulin and insulin incorporated in gel through dialysis membrane.

case of both plain insulin and insulin gel lasting for a period of 4 and 5 hours, respectively. At the end of 4 hours, the cumulative percentage of plain insulin released was 96.88% ± 4.23% and at the end of 5 hours, insulin released from gel formulation was 90.38% ± 4.15%. The slow release of insulin from the gel formulation in in vitro drug release studies may be due to the higher viscosity of the system. This shows that when mucoadhesive carbopol and HPMC are blended together to form an intimate mixture and then prepared as gel with insulin, a release of the incorporated drug can be prolonged in vitro.

Hypoglycemic Activity of Insulin Nasal Gel in Diabetic Rats

The results of hypoglycemic activity of nasal insulin gel in comparison with insulin sc injection (control group) in diabetic rats are presented in Table 1. In the control animals, treated with plain insulin injection (6 IU/kg, sc), a high hypoglycemic response (~70% decrease in blood sugar level) was seen at the first sampling point (2 hours) and steadily declined thereafter. However, in case of nasal insulin gel, a sustained action was noticed up to 10 hours, and the hypoglycemic effect lasted for 15 hours, which was the last sampling point. The hypoglycemic effect was almost ended at 8 hours with insulin injection; but with nasal insulin gel, the highest hypoglycemic effect was observed at 10 hours (~71% blood glucose reduction) and significant effect was observed even at the end of 15 hours (~25% blood glucose reduction).

The nasal gel in spite of its lower dose shows far better pharmacodynamic action when compared with the control group in rats. This is in accordance with previous reports stating that the kinetics of insulin absorption across the nasal mucosa resembles intravenous rather than subcutaneous or intramuscular

Table 1. Antidiabetic Activity of Insulin Gel in Diabetic Rats

Time (hours)	Percentage Reduction in Blood Glucose Levels	
	Plain Insulin (control)	Insulin Gel
0	0.00 ± 0.00	0.00 ± 0.00
2	70.00 ± 2.33	29.84 ± 4.99*
3	56.42 ± 5.28	54.82 ± 2.56
6	37.95 ± 2.88	69.57 ± 1.16†
8	20.12 ± 1.28	70.92 ± 3.26†
10	10.78 ± 3.00	71.04 ± 4.50†
12	6.00 ± 1.48	65.00 ± 5.21†
15	2.90 ± 1.91	25.00 ± 2.41*

*$P < .01$ compared with control.
† $P < .001$ compared with control

routes of administration.[10] The insulin gel also showed prolonged hypoglycemic action when compared with plain insulin. The use of bioadhesive nasal delivery system not only promotes the prolonged contact between the formulation and the absorptive sites in the nasal cavity but also facilitates direct absorption of medicament through the nasal mucosa owing to the relatively large surface area available for drug absorption. The specified ratio of the 2 polymers form the polymeric matrix and interact with the mucous covering of the biological tissues in such a way that the local residence time is prolonged, which helps in delaying the mucociliary clearance of the formulation.

Clinical Studies

The results of stage 1 clinical trials are shown in Figures 2 and 3. The blood glucose levels were reduced as the dose of insulin was increased from 2 IU/kg to 6 IU/kg. Maximum blood glucose reduction was achieved at 1, 1.5, and 1.5 hours from 2 IU/kg (85.50 ± 6.77 mg/dL), 4 IU/kg (73.00 ± 2.68 mg/dL), and 6 IU/kg (77.83 ± 5.03 mg/dL) doses, respectively (Figure 2). Likewise, the increase in the serum insulin was observed as the dose of insulin gel was increased. High serum insulin levels were observed with 6 IU/kg dose. Maximum blood glucose reduction was achieved at 0.5, 0.5, and 1.5 hours from 2 IU/kg (20.53 ± 1.90 μU/mL), 4 IU/kg (23.37 ± 3.17 μU/mL), and 6 IU/kg (24.95 ± 5.40 μU/mL) doses, respectively (Figure 3).

The results of stage 2 clinical trials are presented in Table 2. In the second phase of the study, 6 healthy volunteers were used with nasal insulin delivered at 6 IU/kg as this dose exhibited higher pharmacological effect. A high reduction in blood glucose level (74.33 ± 10.17 mg/dL) and high increase in serum insulin level (45.40 ± 11.08 μU/mL) was observed at 1 hour. There were proportionate changes observed with respect to blood glucose and serum insulin levels (Table 2).

The clinical data of stage 1 confirms that insulin delivered in a gel form is getting absorbed through the nasal mucosa. The

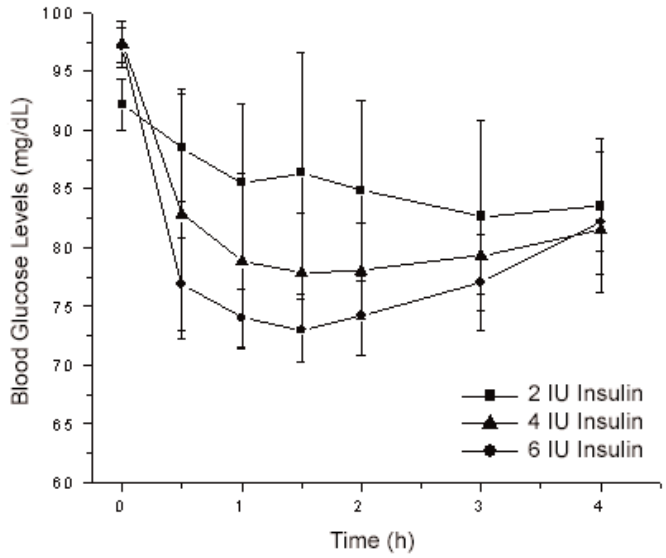

Figure 2. Effect of different doses of insulin gel on blood glucose levels in healthy volunteers (Stage 1). All values are expressed as mean ± SD; $n = 6$.

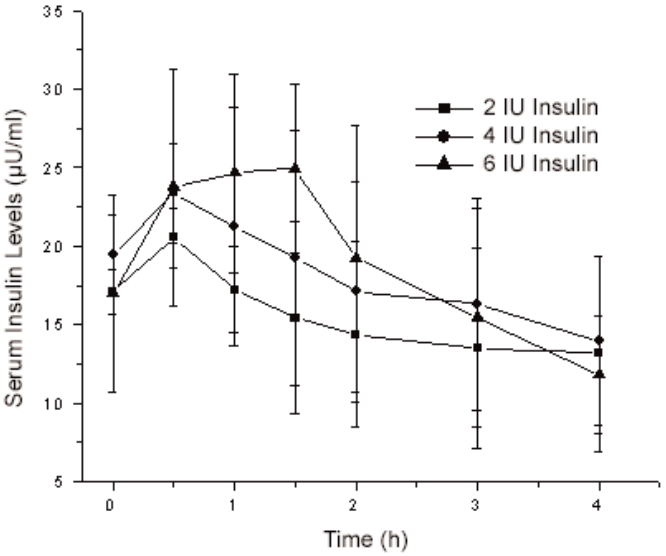

Figure 3. Effect of different doses of insulin gel on serum insulin concentrations in healthy volunteers (Stage 1). All values are expressed as mean ± SD; $n = 6$.

placebo group did not show any change in blood sugar, whereas the nasal insulin group showed a dip in sugars especially at a dose of 6 IU/kg body weight (Figure 2). Hence this dose was considered as the most appropriate dose for delivering insulin gel. Stage 1 of the study ruled out the remote possibility that sodium deoxycholate or the placebo might alone produce hypoglycemia and also helped to fix a dose for stage 2 of the study. Absorption was maximal in the first 0.5 to 1.5 hours of the study with a sharp decline in blood sugar and rise in insulin values corresponding to it. Hence, it was well established that insulin delivered in this form was absorbed quickly after application. The results of the first stage were confirmed during the second stage as maximum pharmacological effect was observed during the first 0.5 to 1.5 hours of the study.

Table 2. Blood Glucose Levels (mg/dL) and Serum Insulin Levels (µU/mL) in Different Volunteers (stage 2)*

Time (hours)	Blood Glucose Levels (mg/dL)	Serum Insulin Levels (µU/mL)
0.0	97.16 ± 10.94	25.80 ± 3.15
0.5	75.66 ± 10.80	34.69 ± 6.09
1.0	74.33 ± 10.17	45.40 ± 11.08
1.5	82.33 ± 6.21	29.67 ± 6.23
2.0	95.00 ± 4.33	21.47 ± 5.05

*All values are expressed as mean ± SD; $n = 6$.

In case of human volunteers, the maximum increase in serum insulin levels and reduction in blood glucose levels was noticed within 0.5 to 1.5 hours; whereas in rats, the maximum decrease in blood glucose levels was observed at 10 hours. This finding may be because the nasal clearance half-life in rats is 5 minutes, while in humans it is 15 minutes.[12] Hence, the hypoglycemic effect lasts longer in rats when compared with humans.

All the volunteers were monitored for adverse affects during stages 1 and 2. In total, 4 volunteers complained of a slight blocking sensation in the nose after application of gel. Hence, an attempt should be made to reduce the quantity of the gel needed to deliver similar doses of insulin. If these technical details can be taken care of, the nasal insulin delivered with gel could still remain a viable alternative to parenteral insulin.

Stability Studies

The United States Food and Drug Administration (FDA) accepts only real-time stability data for protein/peptide pharmaceuticals for the purpose of assessing shelf life, hence accelerated stability studies may only serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature.[13] The results of stability studies are shown in Table 3. The drug content results indicated that there was no significant change in the insulin content after 6 mo when compared with the initial value. One of the important parameters that have to be considered while formulating a gel formulation is the viscosity. In case of the nasal drug delivery, viscosity is also required to retain the formulation at the point of application. The results (Table 3) indicated that the formulation did not show any change in viscosity when stored under refrigeration conditions. Similarly no change in pH was observed during the stability testing period. Insulin (crystalline form and solution) available in the market has to be stored at 2°C to 8°C preferably in a refrigerator, but not in or near the freezing compartment. When insulin is exposed

Table 3. Stability Studies of Insulin Gel at Refrigeration Condition (0°C-4°C)*

Formulation	Time (months)	Morphology	Drug Content (%)	pH	Viscosity (cps)
Insulin gel	0	-	100.0 ± 0.00	7.40 ± 0.05	86000 ± 150
	1	-	99.90 ± 0.12	7.40 ± 0.05	86000 ± 150
	2	-	99.82 ± 0.11	7.32 ± 0.06	85900 ± 125
	3	-	99.71 ± 0.20	7.32 ± 0.02	85500 ± 125
	6	-	98.25 ± 0.21	7.25 ± 0.04	85200 ± 150

*Morphology scale: - no physical change.

to direct sunlight without refrigeration there is a loss in potency with the formation of a cloudy solution. Hence refrigeration condition is suitable to store insulin gel.

CONCLUSION

This study demonstrates that when insulin is administered in a gel form with a penetration enhancer, it traverses the nasal mucosa and rapidly passes into the systemic circulation. Further, insulin gel delivered via nasal mucosa is a pleasant and painless alternative to injectable insulin. However, as the absorption is quite quick, using this form of insulin delivery may not be feasible for chronic patients in the long run.

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