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Development & characterization and *in vivo* assessment of insulin loaded thermo sensitive *in situ* nano gel for nasal delivery

Subhendu Sekhar Mishra and Ashish Kumar Sharma

Abstract
The mucoadhesive gel formulations are helpful to prolong the residence time at the nasal absorption site and thereby facilitate the uptake of drug. The objective of the present study was to develop a thermo sensitive *in Situ* nanogel system based on chitosan and tripolyphosphate for nasal delivery of insulin. Nanogel containing insulin was prepared through an ionic gelation method. The concentration of the components was optimized during formulation development and then characterized in terms of Drug content, mucoadhesive strength, pH, Spreadability, and stability study and drug release behavior. The drug release results were fitted on five mathematical models to choose the model best describing the phenomenon. The *in vitro* release of insulin from gel network was observed spectrophotometrically which was good enough to maintain blood glucose level for 14 hour. Then the nano-formulation and insulin Sc injection as control were administered in the nasal cavity for rats and after 2, 4, 6, 8 and 10 hrs their blood glucose levels, serum insulin level analyzed for antidiabetic activity. The observed *in vitro* and *in vivo* results indicate that the proposed thermo sensitive *in Situ* gelling system has substantial potential as nasal delivery system for insulin.

Keywords: Mucoadhesive, insulin, *in Situ* Nano gel, ionic gelation method, antidiabetic activity

Introduction
Diabetes mellitus represents the most prevalent metabolic disorder nowadays, with 345 million people affected worldwide [1]. Furthermore, it is believed that in 2030 the number of patients will raise up to 552 million, which can be considered a threat for public health [2]. A major concern is that life expectancy is reduced by many years in patients with type 1 or 2 diabetes. The therapy involves different approaches, including diet, physical exercise and hypoglycemic drugs. For Type 1 diabetes patients, due to insufficient insulin production, exogenous hormone is needed [3]. The peptide insulin is the most effective drug for diabetes treatment with high specificity and activity [4]. Over the past few decades, there has been a considerable interest in the development of effective drug delivery systems for proteins and investigation of a large number of recombinant proteins for therapeutic applications [5]. Insulin, a 51-amino acid protein, is the most important regulatory hormone in the control of glucose homeostasis. Insulin-dependent diabetics require single or multiple subcutaneous insulin injections per day. These self-injections however cause pain and discomfort resulting in low patient compliance. Additionally, subcutaneous insulin injections do not really mimic the pulsatile pattern of endogenous insulin secretion in no diabetics [6]. Because of these disadvantageous injections, alternative ways for insulin delivery are intensively investigated. Besides pulmonary insulin administration, nasal insulin delivery seems to be one of the most promising routes. The large surface area and the high vascularity of the nasal mucosa favor a fast absorption of nasally administered compounds. Furthermore, insulin blood concentrations after nasal insulin application would much more mimic the postprandial insulin pattern in no diabetics. Substances like propranolol given nasally lead to a bioavailability of 100% [7]. Substances of comparatively higher molecular weight and lower lipophilicity like insulin, in contrast, are more poorly absorbed. To increase the nasal absorption of insulin, permeation enhancers can be added to the delivery system. Another possibility is the use of mucoadhesive polymers that are able to prolong the residence time of a drug delivery system at the nasal mucosa [8]. Chitosan represents a multifunctional polymer, featuring both mucoadhesive [9] and permeation-enhancing properties [10]. Chitosan is therefore a promising vehicle for nasal insulin delivery. Research efforts into the employment of Mucoadhesive viscoelastic nanogels

in nasal drug delivery are rationalised in terms of their potential for prolonging the residence time of the active on the mucosal surface. Such systems lend themselves to administration as sprays or drops and may be designed such that they undergo a sol–gel transition at the temperature of the site of deposition [11,12], with the implication that the increased viscosity and rheological synergy of the resulting mucus/mucoadhesive system effects prolonged residence at the site of action [13-17]. Nanogel is broadly a Nano particulate system incorporated uniformly within a hydrogel or organogel matrix. The nanoparticles are located within the gel matrix itself or exterior Nano particulate systems such as incorporating Nano emulsion, liposome or Nano suspension into a gel matrix [18, 19]. Nanogel can provide a homogenous distribution of nanoparticles with an enhanced thermodynamic activity of drug within gel formulation, with an ability to form aqueous solution with higher colloidal stability, an ability to accommodate macromolecules such as peptide and proteins, capability to load a higher drug quantity with no chemical reaction and a sustained drug delivery for prolonged time [20-22]. The object of present study was to formulated and evaluated insulin loaded thermo sensitive *in situ* nanogel for nasal delivery.

Materials and Methods

Materials

Insulin from bovine powder purchase from Sigma-Aldrich, USA. Chitosan was purchased from Himedia, Mumbai. Sodium tripolyphosphate was purchased from Loba Chem. Pvt. Ltd. (Mumbai, India). Glacial acetic acids were purchased from Merck Specialities Pvt. Ltd., Mumbai. Poloxamer-188 and Carbopol 934 were purchased from S.D. Fine Chem. Ltd. Mumbai. Hydroxy propyl methyl cellulose, Propylene Glycol, Benzalkonium Chloride, Triethanolamine was purchased from Himedia Chem. Lab, Mumbai. All other ingredients used were of analytical grade. Triple distilled water was generated in house.

Methods

Development of insulin-loaded nanoparticles

Nanoparticles (NP) will be prepared according to Calvo *et al.,* 1997 [23], using ionotropic gelation method with slight modification in which chitosan (0.4% w/v) will be dissolved in aqueous acetic acid solutions (1% v/v) (pH 6.1), while TPP (0.1% w/v) will be dissolved in deionized water. Insulin solution will be premixed with chitosan solution before the addition of the TPP solution drop wise into the chitosan solution under magnetic stirring (600 rpm) at ambient temperature for 2-4 hr. The obtained nanoparticles formulation will be lyophilized and stored in 4-8° C until it will further use.

Optimization of process variable

The effect of formulation process variables such as stirring time, stirring speed on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations. Different insulin incorporated nanoparticles (F1-F18) were prepared using effect of chitosan quantity, stirring time and stirring speed. The prepared nanoparticle was further evaluated for entrapment efficiency, particle size, zeta potential and *In Vitro* drug release study. In all prepared formulations, F17 was found to be more suitable, was further incorporated into *In Situ* gel.

Preparation of Insulin *in Situ* Nanogel

Accurately weighed quantity of the drug was dissolved in distilled water. The solutions of Poloxamer-188 and Carbopol-934 were prepared using cold method. A certain volume of distilled water was cooled down to 4°C. Poloxamer-188 and Carbopol 934 was sprinkled over deionized cold water separately and was allowed to hydrate for 12 hours to produce a clear solution. Then both the polymer solutions were mix properly with continuous stirring. The Benzalkonium chloride was added to the above polymer dispersion. Than stored in the refrigerator. The dispersions were then stored in a refrigerator until clear solutions were obtained and polymer dispersion was slowly added to the drug solution under aseptic condition. The formulation was aseptically transferred to previously sterilized glass bottles and sealed [24]. The composition of formulations was given in Table 1, 2.

Table 1: Formulation development of *In Situ* nanogel (F1-F-9)

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Nanoparticles	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Poloxamer-188	14	16	20	14	16	20	14	16	20
Carbopol	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.3
HPMC	-	-	-	-	-	-	-	-	-
Propylene Glycol	1	1	1	1	1	1	1	1	1
Benzalkonium Chloride (% w/v)	1	1	1	1	1	1	1	1	1
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified water (ml)	100	100	100	100	100	100	100	100	100

Table 2: Formulation development of *In-situ* nanogel (F10-F-18)

Formulation	F10	F11	F12	F13	F14	F15	F16	F17	F18
Nanoparticles	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Poloxamer-188	14	16	20	14	16	20	14	16	20
Carbopol	0.1	0.1	0.1	0.2	0.2	0.2	0.	0.3	0.3
HPMC	0.1	0.1	0.1	0.2	0.2	0.2	0.	0.3	0.3
Propylene Glycol	1	1	1	1	1	1	1	1	1
Benzalkonium Chloride (% w/v	1	1	1	1	1	1	1	1	1
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified water (ml)	100	100	100	100	100	100	100	100	100

Evaluation of Nanogel of Insulin

Drug content

From the prepared *In Situ* gel formulation equivalent to 1mg of Insulin was dissolved in the 10 ml of SNF (Simulated nasal fluid). The amount of insulin was determined using UV spectrophotometer at 272nm. The total drug content was calculated using calibration curve method [25].

Determination of pH

Weighed 50 gm of each gel formulation were transferred in 10 ml of beaker and measured it by using the digital pH meter. pH of the *In Situ* nasal gel formulation should be between 3-9 suitable for nasal delivery [26].

Measurement of viscosity

The viscosity of gels was determined by using a Brook Field viscometer DV-II model. T-Bar spindles in combination with a helipath stand were used to measure the viscosity and have accurate readings [27]. The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size etc. which affect the viscosity were maintained during the process. The helipath T-

bar spindle was moved up and down giving viscosities at number of points along the path. The torque reading was always greater than 10%. Five readings taken over a period of 60 sec. were averaged to obtain the viscosity.

Mucoadhesives strength
Detachment Stress is the force required to detach the two surfaces of mucosa when a formulation/gel is placed in between them. The detachment stress was measured by using a modified analytical balance.
Force of adhesion (N) = (bio adhesive strength/1000) × 9.81
Bond strength (N/m2) = force of adhesion (N)/surface area of disk (m2)

***In-vitro* diffusion study**
An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. *In-situ* gel equivalent to 100 mg of Insulin was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 5.5. The diffusion cells were maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer [28].

Mathematical treatment of *in-vitro* release data
The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used.

Zero-order kinetics
The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model:

$$Q_t = Q_o + K_o t$$

Where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initial amount of drug in the solution (most times, Q_o=0) and $K_o$is the zero order release constant [29].

First-order kinetics
The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

Where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initialamount of drug in the solution and $K_1$is the zero order release constant. In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish.

Higuchi Model
Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The simplified Higuchi model is expressed as:

$$Q = K_H . t^{1/2}$$

Where Q is the amount of drug released in time t and K_H is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drugs [30].

Korsmeyer-Peppas model
Korsmeyer *et al.* used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\ t^n$$

Where $M_t/M_\infty$is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log M_t/M_∞ versus log time curve [31]. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of n =0.5 for fickian diffusion and higher values of n, between 0.5 and 1.0, or n =1.0, for mass transfer following a non-fickian model (Table 3). In case of a cylinder n =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent n the portion of the release curve where $M_t/M_\infty < 0.6$should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (*l*) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\ (t - l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and bvalue would be zero and only $at^n$is used. This mathematical model, also known as *Power Law*, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms [32].

Table 3: Interpretation of diffusional release mechanisms

Release exponent (*n*)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5<*n*<1.0	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

Stability Studies

Optimized formulations of *In-situ* gel were subjected to accelerated stability testing under storage condition at 4±1°C and at room temperature (28±1°C). Both formulations were stored in screw capped, amber colored small glass bottles at 4±1°C and 28±1°C. Analysis of the samples were characterized for vesicle size and drug content after a period of 7, 14, 21 and 28 days.

Effect of Storage Temperature on Viscosity

Subsequent change in vesicle size of the formulations stored at 4±1°C and 28±1°C was determined using a Brook field viscometer after a period of 7, 14, 21 and 28 days.

Effect of storage temperature on drug content

After storage for a specified period of time of 7, 14, 21 and 28 days, the drug content of the formulations was determined. Drug content in *In Situ* gel was determined spectrophotometric ally to indirectly estimate the amount of drug content.

Anti-Diabetic Activity

Animals

All ethical and handling guidelines were followed as set by Indian Legislation and approved by Institutional Animal Ethics Committee. Male & female Wistar albino rats (140-200g) were provided by Technocrats institute of technology (Pharmacy), Bhopal, Madhya Pradesh, India. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44-56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during experiment. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Technocrats institute of technology (Pharmacy), Bhopal, (M.P). (Reg No. 831/bc/04/CPCSEA). Protocol approval reference no. TIT/IAEC/831/P`col/2016/56.

Grouping of animals

Animals were divided in four groups with six animals in each group.

Group I: Normal Control Group (0.9% saline; 5 ml/kg body weight orally for 21 days.

Group II: Diabetic Group (Alloxen i.p.50 mg/kg) in addition with 5% (w/v) glucose solution in feeding bottles for next 24 hrs.

Group III: Control with insulin (6 IU/kg, subcutaneously).

Group IV: administered intranasally with insulin nanogel (1.5 IU/kg) using a micro syringe attached to a blunt needle with a 0.5-inch polyethylene tube at the end.

Induction of diabetes in experimental animals

Alloxan monohydrate was dissolved in saline and administered intraperitonieally [ip] into fasted rats at a dose of 50 mg/kg body wt. The solution should be fresh and prepared just prior to the administration. The rats were given 5% (w/v) glucose solution in feeding orally to prevent hypoglycaemia after alloxan injection. After 72 h rats with BGL greater than 200 mg/dl and less than 400 mg/dl were selected and observed for consistent hyperglycaemia (fasting blood glucose level greater than 200 mg/dl and lesser then 400 mg/dl) upto 7 days. Following an overnight fast.

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). Body weight of all animals was measured on the 2hr, 6hr, 8hr, 10hr and 15hr after treatment with the nanogel. The percentage change of body weight was calculated from its initial weight. Alloxan may cause severe ketoacidosis and may lead to death of animal. In view of this the mortality rate was monitored throughout the study.

Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean± standard error of mean (SEM). The results are analyzed for statistical significance using one-way ANOVA followed by Dunnett's test $P< 0.05$ was considered significant.

Results and Discussion

Drug content analysis

The Drug content of the formulations was found to be close to 100% and was in the range of 93.34 ±0.024 to 97.56±0.018, as shown in Table 4. In all formulations the maximum drug content was found in formulation F12 (97.56±0.018), the results of percentage assay was found slight vary due to the difference in concentration of polymers like carbopol, HPMC and Polaxomer 188.

Determination of pH

The pH of the formulations was found to be satisfactory and was in the range of 6.8±0.039 -7.4±0.053, as shown in Table 4.The formulations were liquid at room temperature and Terminal sterilization by autoclaving had no effect on the pH.

Measurement of viscosity

The viscosity of gels was determined by using a Brookfield viscometer DV-II model. The results (Table 4) show that the viscosity of the gels increased with an increase in polymer concentration. The increase in viscosity with the polymer concentration may be due to increase in bonds between the polymer molecules which lead to formation of a hard and dense compact mass. This may also be due to less amount of liquid in gels with high polymer concentration as compared to gels of low polymer concentration or in other words it can be said the higher the polymer concentration more shear stress if required to produce a specified rate of shear.

Mucoadhesive Strength

The result of mucoadhesive strength was show in table 4. The mucoadhesive strength of all formulations was varies from 2398±0.0004to 4945±0.0002 dynes/cm^2.

Table 4: Results of Insulin Nasal *In Situ* gel formulations

Code	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)	Mucoadhesive Strength (Dynes/cm^2)	Drug content (%)
F1	6.9±0.021	11.75±0.075	6540.06±1.70	2489±0.0007	93.34 ±0.024
F2	7.2±0.040	11.08±0.042	9467.03±0.86	3492±0.0004	94.74±0.020
F3	7.3±0.060	10.75±0.059	9746.37±1.90	3495±0.0005	95.18 ±0.021
F4	7.3±0.039	11.57±0.053	7594.68±1.90	2554±0.0004	94.33 ±0.024
F5	7.4±0.053	10.83±0.058	8948.86±0.89	2564±0.0006	95.74±0.020
F6	7.2±0.038	11.53±0.046	9684.11±0.74	3674±0.0004	93.18 ±0.021
F7	6.9±0.059	10.29±0.046	7737.49±1.86	3812±0.0002	95.30 ±0.024
F8	7.1±0.048	11.89±0.051	9837.37±0.85	2821±0.0003	96.13±0.020
F9	6.9±0.052	10.92±0.061	6948.13±1.59	4845±0.0002	95.12 ±0.021
F10	7.2±0.029	11.63±0.076	9165.15±0.74	4945±0.0002	96.58±0.015
F11	7.3±0.039	12.03±0.063	8794.57±1.23	3965±0.0003	95.56±0.011
F12	7.3±0.042	11.52±0.053	9663.65±1.73	2985±0.0005	95.89±0.015
F13	7.1±0.057	11.06±0.039	9683.64±1.53	2125±0.0006	97.56±0.018
F14	6.8±0.039	12.31±0.061	9217.74±1.83	4145±0.0004	96.74±0.022
F15	6.9±0.022	11.92±0.058	8769.74±1.38	4125±0.0006	94.18 ±0.021
F16	7.3±0.034	10.82±0.048	7865.68±0.87	4134±0.0004	94.30 ±0.021
F17	7.1±0.041	11.49±0.036	8742.19±1.46	3378±0.0003	93.13±0.025
F18	7.2±0.053	12.29±0.059	8764.91±1.82	2398±0.0004	95.12 ±0.023

In-vitro drug release study

In-vitro diffusion study of the *in Situ* gel (F1-18) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffer pH 6.5 for a period of 14 hours. The data obtained from diffusion studies are summarized in Table 5& 6 and Figure 1. The release rate of Insulin from *in Situ* formulation over dialysis membrane was significantly higher than its transport across skin, indicating the barrier properties of skin for drugs. The *In vitro* release data were fitted into different kinetic models *viz* Zero-order, First order, Higuchi model and Korsmeyer Peppas equation (Table 7). The zero-order plots were found to be fairly linear. In order to determine the exact mechanism of drug release from Insulin gel the *In vitro* release data were fitted to Korsmeyer Peppas equation and the 'n' values were calculated. 'n' values were found to be in the range of $0.5<n<1.0$, which suggests that the drug release mechanism from the gel followed non-Fickian diffusion mechanism (Anamolous transport). Nasal *in Situ* gel released drug in controlled release manner in 14 hour.

Table 5: *In-vitro* drug release data for formulation F1-F9

Time (Hr)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	8.229	9.6	9.143	9.143	9.668	9.853	10.569	10.338	11.658
2	15.08	22.857	15.92	15.92	17.638	18.346	21.986	19.659	15.92
3	26.97	32	28.8	28.8	29.647	30.763	31.548	30.037	32.563
4	34.28	43.429	37.029	37.029	38.564	41.673	41.569	39.769	39.029
6	44.80	50.286	48.457	48.457	51.385	53.761	49.657	51.659	53.457
8	52.80	69.6	63.84	63.84	68.964	69.753	58.765	63.876	68.84
12	64.32	80.16	76.32	76.32	82.748	83.856	76.16	79.91	74.32
14	76.80	84.08	87.84	87.84	92.674	94.748	86.08	88.19	85.84

Table 6: *In-vitro* drug release data for formulation F9-F18

Time (Hr)	F10	F11	F12	F13	F14	F15	F16	F17	F18
1	9.528	11.398	12.658	7.579	10.569	13.769	15.468	13.658	12.278
2	18.21	21.569	23.659	11.749	14.986	19.659	22.468	28.658	22.768
3	29.186	29.647	30.763	25.673	31.548	34.769	36.358	33.679	31.547
4	37.58	38.564	41.673	35.6559	41.569	43.768	46.769	43.873	41.456
6	49.369	51.385	52.498	47.548	47.65	51.678	53.674	52.673	51.657
8	66.649	68.964	70.65	56.587	58.765	62.658	75.768	71.659	68.769
12	79.649	82.748	86.856	71.659	76.16	76.32	81.769	79.769	78.876
14	89.37	92.674	93.659	82.769	86.08	79.84	89.876	87.879	86.763

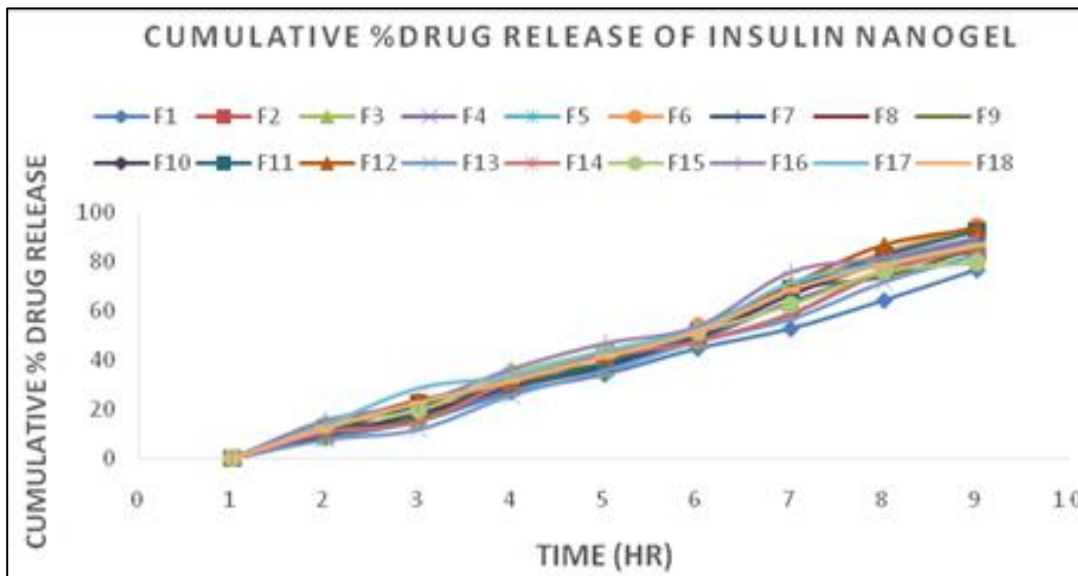

Fig 1: Graph of release study of formulation F1-F18

Table 7: Regression analysis data of nasal *in Situ* gel formulation

Batch	Zero Order		First Order		Higuchi's Model		Korsmeyers Peppas Equation	
	K_0 (mg.h^{-1})	R^2	K_1 (h^{-1})	R^2	K_h (mg.h$^{-1/2}$)	R^2	n	R^2
F1	5.0852	0.937	0.0376	0.986	23.645	0.988	0.595	0.975
F2	6.322	0.940	0.0605	0.985	29.299	0.985	0.863	0.967
F3	6.249	0.957	0.0545	0.994	28.784	0.990	0.699	0.955
F4	6.508	0.949	0.061	0.994	30.093	0.99	0.598	0.964
F5	6.813	0.964	0.0668	0.992	31.273	0.991	0.688	0.949
F6	6.846	0.957	0.069	0.995	31.562	0.992	0.791	0.959
F7	5.660	0.957	0.0501	0.990	26.101	0.993	0.593	0.969
F8	6.305	0.979	0.0589	0.998	29.017	0.997	0.675	0.963
F9	6.070	0.911	0.053	0.961	28.293	0.965	0.653	0.950
F10	6.490	0.964	0.060	0.993	29.805	0.992	0.546	0.952
F11	6.593	0.970	0.065	0.991	30.177	0.992	0.839	0.945
F12	6.776	0.976	0.0745	0.982	30.936	0.992	0.816	0.942
F13	5.916	0.948	0.0475	0.994	27.522	0.986	0.654	0.956
F14	5.908	0.944	0.0514	0.986	27.256	0.980	0.878	0.952
F15	5.664	0.938	0.051	0.992	26.274	0.984	0.978	0.967
F16	6.27	0.922	0.0645	0.962	29.089	0.967	0.672	0.947
F17	5.941	0.937	0.586	0.978	27.514	0.980	0.731	0.961
F18	6.124	0.950	0.578	0.989	28.278	0.998	0.824	0.961

Stability Study

Stability studies for optimized formulations were carried out at 4.0±0.5°C and 28 ±0.5°C for a period of four weeks. There was no significant variation found in physical appearance, viscosity, % drug content and cumulative % drug release of the optimized formulation (F12) of the *in Situ* nanogel, No visible changes in the appearance of the gel formulation were observed at the end of the storage period as shown in Table 8.

Table 8: Characteristics of *In Situ* nanogel of insulin after stability study

Time (Days)	Drug Content		Spreadability (gm. cm/sec.)		pH		Cumulative % Drug Release (%)	
	4.0 ±1°C	28 ± 1°C	4.0 ±1°C	28 ± 1°C	4.0 ±1°C	28 ± 1°C	4.0 ±0.5°C	28 ± 0.5°C
0	98.37±0.053	98.37±0.053	11.52±0.076	11.52±0.053	7.3±0.042	7.3±0.042	93.659±0.76	93.659±0.76
7	97.91± 0.57	96.24±0.048	11.02±0.049	10.76±0.046	7.3±0.083	7.2±0.046	93.256±0.76	92.458±0.46
14	97.26 ±0.27	94.86±0.023	10.86±0.057	10.26±0.043	7.2±0.062	7.2±0.059	92.986±0.95	89.27±0.27
21	97.11 ±0.26	91.45±0.042	10.59±0.081	9.83±0.046	7.2±0.072	7.1±0.018	91.245±0.42	87.14±0.76
28	97.08 ±0.88	88.46±0.071	10.26±0.029	9.45±0.084	7.1±0.053	7.0±0.087	89.124±0.63	85.15±0.14

Effects of induction of diabetes mellitus and hypoglycemic activity 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 9. In the control animals, treated with plain insulin injection (6IU/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 for15 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 15hours (~25% blood glucose reduction). The nasal gel in spite of its lower dose shows far better pharmacodynamics 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 routes of administration. The insulin gel also showed prolonged hypoglycemic action when compared with plain insulin. The use of bio adhesive 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.

Table 9: Antidiabetic activity of insulin gel in diabetic rats

Time (Hr)	Blood Glucose Levels (mg/dL)	Serum Insulin Level (µU/mL)
0	00 ±00.00	0.00 ± 0.00
2	60.00 ±1.54	24.73 ± 4.99*
3	53.49 ±2.47	52.72 ± 2.56
6	33.23 ±1.48	61.28 ± 2.26†
8	20.73 ±5.27	65.48 ± 2.35†
10	12.74 ±2.85	72.26 ± 3.53†
12	5.39 ±1.76	63.57 ± 3.93†
15	2.33 ±1.73	21.49 ± 2.26*

All values are mean ± SEM, n = 6. *$p<0.01$, †$p<0.001$

Conclusion

In the present study was to develop a thermosensitive *in Situ* nanogel system based on chitosan and tripolyphosphate for nasal delivery of insulin was prepared through an ionic gelation method, thus, the prepared *in Situ* nanogel proved to be suitable for administration of insulin through nasal route. Hence, this can be viewed as a viable alternative to conventional nasal drops and painful injection by virtue of its ability to enhance nasal residence time and thereby intranasal bioavailability. The ease of administration coupled with less frequent administration, thus enhancing patient compliance. Additionally, the *in vivo* results clearly indicated that the insulin loaded nanogel could effectively reduce the blood glucose level in a diabetic rat model.

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