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Creation, Characterization, and In vivo Evaluation of GLP-1 Agent-Loaded Thermosensitive *Insitu* Nanogel for Nasal Administration (Dulaglutide)

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ABSTRACT

Mucoadhesive gel formulations help to increase the length of time a drug is present at the nasal absorption site, which helps to promote drug uptake. The goal of this work was to create a tripolyphosphate and chitosan-based thermosensitive in situ nanogel technology for the nasal delivery of dulaglutide, a GLP-1 drug. An ionic gelation method was used to make the nanogel containing dulaglutide. During formulation development, the components' concentrations were tuned, and the drug's loading, morphology, size, zeta potential, stability studies, and release behavior were all examined. Five mathematical models were fitted with the drug release data in order to determine which model best captured the phenomenon. Spectrophotometric analysis revealed that the in vitro release of dulaglutide from the gel network was sufficient to sustain blood glucose levels for a duration of 14 hours. Following the administration of the nano-formulation and the dulaglutide Sc injection as a control, rats' blood glucose levels and serum insulin levels were measured for antidiabetic action after 2, 4, 6, 8, and 10 hours. The findings from both in vitro and in vivo experiments suggest that the thermosensitive in situ gelling system that has been suggested has significant promise for use as a nasal delivery method for dulaglutide.

Keywords: Mucoadhesive, dulaglutide, *In situ* Nanogel, Ionic gelation method, Antidiabetic activity

Introduction

With 345 million cases worldwide, diabetes mellitus is currently the most common metabolic disease [1]. In addition, it is projected that by 2030, there would be up to 552 million patients, posing a risk to public health [2]. One big worry is that people with type 1 or type 2 diabetes have a significantly shorter life expectancy—many years shorter. There are several ways used in the therapy, such as hypoglycemic medications, food, and exercise. Patients with Type 1 diabetes require exogenous hormone therapy because their bodies do not produce enough insulin [3]. With their high specificity and activity, GLP-1 agents are the most effective medication for treating diabetes [4]. In recent years, there has been a notable focus on the creation of efficient protein drug delivery systems and the exploration of numerous recombinant proteins for potential medicinal uses [5]. When taken in conjunction with a diet and exercise regimen, dulaglutide injection helps regulate blood sugar levels in adults and children with type 2 diabetes who are 10 years of age or older. However, the pain and suffering associated with these self-injections lead to limited patient compliance. Furthermore, the pulsatile pattern of endogenous insulin secretion in non-diabetics is not accurately simulated by subcutaneous injections [6]. Due to these unfavorable injections, there is a lot of research being done on alternate medication delivery methods. One of the most promising delivery methods appears to be nasal administration, aside from pulmonary insulin administration. Nasally given chemicals are quickly absorbed due to the nasal mucosa's vast surface area and strong vascularity. Additionally, in non-diabetics, insulin blood concentrations following nasal administration would more closely resemble the postprandial insulin pattern. When administered nasally, drugs such as propranolol have a 100% bioavailability [7]. In contrast, substances like insulin that have a lower lipophilicity and a somewhat higher molecular weight are more poorly absorbed. It is possible to incorporate permeation enhancers into the delivery system to boost the drug's nasal absorption. Using mucoadhesive polymers, which can extend a drug delivery system's residence time at the nasal mucosa, is an additional option [8]. Chitosan is a polymer with many functions, possessing the ability to enhance permeability [9] and act as a mucoadhesive [10]. Therefore, chitosan presents a viable delivery system for insulin through the nose. The rationale for investigating the use of mucoadhesive viscoelastic nanogels for nasal drug delivery stems from their capacity to extend the duration of the active's residency on the mucosal surface. These systems can be applied as sprays or drops and can be made to go through a sol-gel transition at the temperature of the deposition site [11,12]. This means that the resulting mucus/mucoadhesive system will have a longer residence time at the site of action due to its increased viscosity and rheological synergy [13–17]. In general, nanogel is a system of uniformly integrated nanoparticulates within a hydrogel or organogel matrix. The nanoparticles can be found inside the gel matrix itself or outside of it, for example, when a gel matrix is combined with a liposome, nanoemulsion, or nanosuspension [18, 19]. A homogeneous distribution of nanoparticles with improved thermodynamic activity of the drug within the gel formulation can be obtained with nanogel. It can also form an aqueous solution with higher colloidal stability, accommodate macromolecules like proteins and peptides, load a higher quantity of drug without causing a chemical reaction,

and provide sustained drug delivery for an extended period of time [20–22]. The purpose of this study was to develop and assess a thermosensitive insitu nanogel filled with dulaglutide for nasal administration.

Materials and methods

Materials

We purchased dulaglutide from Novo Nordisk. We bought the chitosan from Qualigens in Mumbai. The supplier of sodium tripolyphosphate was Loba Chem. Pvt. Ltd. (Mumbai, India). We bought glacial acetic acids from Merck Specialities Pvt. Ltd. in Mumbai. We bought Carbopol 934 and Poloxamer-188 from S.D. Fine Chem. Ltd. in Mumbai. Himedia Chem. Lab, Mumbai is the source of hydroxy propyl methyl cellulose, propylene glycol, benzoalkonium chloride, and triethanolamine. Analytical grade substances were employed in all other cases. In-house production of triple-distilled water was done.

Methods

Development of insulin-loaded nanoparticles

With a small modification, ionotropic gelation will be used to generate nanoparticles (NP) in accordance with Calvo et al., 1997 [23]. TPP (0.1% w/v) will be dissolved in deionized water, and chitosan (0.4% w/v) will be dissolved in aqueous acetic acid solutions (1% v/v) (pH 6.1). Prior to adding the TPP solution dropwise into the chitosan solution while magnetic stirring (600 rpm) is occurring at room temperature for two to four hours, the dulaglutide solution will be premixed with the chitosan solution. The formulation of the resulting nanoparticles will be lyophilized and kept between 4 and 8° C until needed.

Optimization of process Variable

It was investigated how the length of time and speed of the stirring operation affected the size of the particles during the formulation process. Based on the results, the ideal level of each variable was determined and maintained for the ensuing assessments. Different dulaglutide-incorporated nanoparticles (F1–F18) were made by varying the amount of chitosan, the speed and duration of stirring, and other factors. The produced nanoparticle underwent additional testing for In Vitro drug release research, particle size, zeta potential, and entrapment efficiency. F17 was determined to be more appropriate in all prepared formulations and was added to the *in-situ* gel.

Preparation of Dulaglutide *In Situ* Nanogel

A precisely measured amount of the medication was dissolved in distilled water. The polymer solutions of Poloxamer-188 and Carbopol-934 were made by cold method; a specific volume of distilled water was cooled to 4°C. The polymer solutions of Poloxamer-188 and Carbopol 934 were then sprinkled over deionized cold water separately and allowed to hydrate for 12 hours to produce a clear solution. Both polymer solutions were then properly mixed with continuous stirring. Then, the polymer dispersion was added to the above polymer dispersion, and the mixture was refrigerated until clear solutions were obtained. The polymer dispersions were then maintained in a refrigerator until clear solutions were obtained, and the polymer dispersion was gradually added to the drug solution while maintaining aseptic conditions. The formulation was

then aseptically transferred to previously sterilized glass bottles. 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	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Poloxamer-188	16	18	22	16	18	22	16	18	22
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	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Poloxamer-188	16	18	22	16	18	22	16	18	22
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 dulaglutide

Drug content

Ten milliliters of SNF (simulated nasal fluid) were mixed with 1 milligram of dulaglutide that was dissolved from the prepared In situ gel formulation. The UV spectrophotometer was used to measure the drug's concentration at 265nm. The calibration curve approach was used to determine the overall amount of drug content [25].

Determination of pH

50 grams of each gel formulation were weighed, then placed in a 10-milliliter beaker and the pH was determined using a digital meter. For nasal distribution, the pH of the In situ nasal gel formulation should be between 3 and 9 [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.

Mucoadhesive 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**

A modified Franz diffusion cell was used for an in-vitro drug release study. The donor and receptor compartments were separated by a dialysis membrane (Hi Media, Molecular weight 5000 Daltons), and the receptor compartment was filled with phosphate buffer (pH 5.5) and an in-situ gel containing 100 mg of drug. The diffusion cells were kept at 37±0.5°C with 50 rpm stirring throughout the experiment. Five milliliters of aliquots were removed from the receiver compartment through a side tube at various intervals, and their drug content was determined using a UV Visible spectrophotometer [28].

Mathematical treatment of *in-vitro* release data

Using mathematical formulas that express the dissolution results as a function of certain of the dosage forms properties facilitates the quantitative study of the values obtained in dissolution/release experiments.

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:

$$\mathbf{Q_t = Q_o + K_o t}$$

Where $Q_t$is the amount of drug dissolved in time t, $Q_o$is the initialamount 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 spectrophotometrically to indirectly estimate the amount of drug content.

Anti Diabetic Activity

Animals

All handling and ethical standards as stipulated by Indian law and authorized by the Institutional Animal Ethics Committee were adhered to. Technocrats Institute of Technology (Pharmacy), located in Bhopal, Madhya Pradesh, India, supplied male and female Wistar albino rats weighing between 140 and 200 grams. During the trial, the animals were given a regular pallet meal and were allowed unlimited access to water in a cross-ventilated animal housing with a temperature of 25±2°C and a relative humidity of 44-56%. The Technocrats Institute of Technology (Pharmacy), Bhopal, (M.P.) Institutional Animal Ethics Committee (IAEC) accepted the use of animals in experiments. Regulation Number: TIT/IAEC/831/P'col/2017/05. Reference number for protocol approval: 831/BC/05/CPCSEA.

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 dulaglutide (6 IU/kg, subcutaneously).

Group IV: Intra nasally with dulaglutide gel (1.5 IU/kg)

Induction of diabetes in experimental animals

Alloxan monohydrate was dissolved in saline and administered intravenously 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 bottles for next 24 h in their cages 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. The treatment was continued for the next 24hour and blood samples were collected on 2, 3, 6, 8, 10, 12 and 15 hour after treatment. Blood glucose level (BGL) was estimated with the help of UV spectrophotometer by ERBA diagnostic Glucose kit. 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 keto acidosis and may lead to death of animal. In view of this the mortality rate was monitored throughout the study. The % of mortality was calculated at the end of each hour of treatment.

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 - 7.3±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±0.0004 to 4945±0.0002dynes/cm^2.

Table 4 Results of Dulaglutide Nasal *In Situ* Gel Formulations

Code	pH	Spreadability (gm.cm/sec.)	Viscosity (cps)	Mucoadhesive Strength (Dynes/cm^2)	Drug content (%)
F1	6.8±0.022	11.75±0.075	6540.06±1.70	2489±0.0007	93.34 ±0.024
F2	7.3±0.040	11.08±0.042	9467.03±0.86	3492±0.0004	94.74±0.020
F3	7.2±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	7.3±0.053	10.83±0.058	8948.86±0.89	2564±0.0006	95.74±0.020
F6	7.3±0.038	11.53±0.046	9684.11±0.74	3674±0.0004	93.18 ±0.021
F7	6.8±0.059	10.29±0.046	7737.49±1.86	3812±0.0002	95.30 ±0.024
F8	7.2±0.048	11.89±0.051	9837.37±0.85	2821±0.0003	96.13±0.020
F9	6.8±0.052	10.92±0.061	6948.13±1.59	4845±0.0002	95.12 ±0.021
F10	7.3±0.029	11.63±0.076	9165.15±0.74	4945±0.0002	96.58±0.015
F11	7.2±0.039	12.03±0.063	8794.57±1.23	3965±0.0003	95.56±0.011
F12	7.2±0.042	11.52±0.053	9663.65±1.73	2985±0.0005	97.56±0.018
F13	7.1±0.057	11.06±0.039	9683.64±1.53	2125±0.0006	95.89±0.015
F14	6.9±0.039	12.31±0.061	9217.74±1.83	4145±0.0004	96.74±0.022
F15	6.8±0.022	11.92±0.058	8769.74±1.38	4125±0.0006	94.18 ±0.021
F16	7.1±0.034	10.82±0.048	7865.68±0.87	4134±0.0004	94.30 ±0.021
F17	7.2±0.041	11.49±0.036	8742.19±1.46	3378±0.0003	93.13±0.025
F18	7.3±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 dulaglutide 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	10.569	11.658

2	15.08	22.857	15.92	15.92	17.638	18.346	21.986	21.986	15.92
3	26.97	32	28.8	28.8	29.647	30.763	31.548	31.54	32.563
4	34.28	43.429	37.029	37.029	38.564	41.673	41.569	41.569	39.029
6	44.80	50.286	48.457	48.457	51.385	53.761	47.65	47.65	53.457
8	52.80	69.6	63.84	63.84	68.964	69.753	58.765	58.765	68.84
12	64.32	80.16	76.32	76.32	82.748	83.856	76.16	76.16	74.32
14	76.80	84.08	87.84	87.84	92.674	94.748	86.08	86.08	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

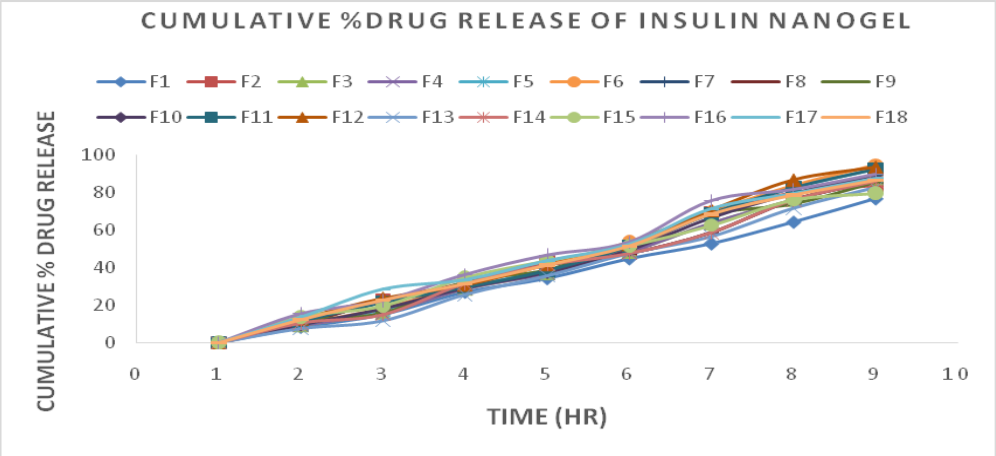

Fig.1 Graph of release study of formulation F1-F18

Table 7 Release kinetics of optimized formulation F12

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	12.658±0.379	1.102	87.342	1.941
2	1.414	0.301	23.659±0.875	1.374	76.341	1.883
3	1.732	0.477	30.763±0.459	1.488	69.237	1.840

4	2.000	0.602	41.673±0.626	1.620	58.327	1.766
6	2.449	0.778	52.498±0.579	1.720	47.502	1.677
8	2.828	0.903	70.650±0.895	1.849	29.35	1.468
12	3.464	1.079	86.856±0.653	1.939	13.144	1.119
14	3.742	1.146	93.659±0.764	1.972	6.341	0.802

*Average of three readings

Stability Study

Stability studies for optimized formulations were carried out at 4.0±0.5°C and 28 ±0.5ºC for a period of four weeks. There was no significant variation found in physical appearance, average particle size and % drug content of the *in situ* nanogel F12, No visible changes in the appearance of the gel formulation were observed at the end of the storage period as shown in Table 8 to Table 11.

Table 8 Effect of storage temperature on the Particle size of optimized formulation F12

Time (Days)	**Average Particle size (nm)**	
	4.0 ± 0.5°C	**28 ± 0.5ºC**
0	52.2±0.73	52.2±2.73
7	51.93±0.36	52.09± 1.75
14	51.73±2.37	51.86±3.62
21	51.67±1.63	51.73±4.74
28	51.62±3.53	51.59±3.17

*Average of 03 readings

Table 9 Effect of storage temperature on the % entrapment efficiency of optimized formulation F12

Time (Days)	**Drug Content (%)**	
	4.0 ±1°C	**28 ± 1ºC**
0	73.12±0.25	73.12±0.25
7	73.06± 0.57	73.03±0.48
14	72.86± 0.72	72.81± 0.37
21	71.35± 0.47	71.27± 0.74
28	71.20± 0.62	71.17± 0.52

*Average of 03 readings

Table 10 Effect of storage temperature on the drug content of optimized formulation F12

Time (Days)	**Drug Content (%)**	
	4.0 ±0.5°C	**28 ± 0.5ºC**
0	99.120 ± 0.022	99.120 ± 0.022
7	98.917± 0.576	99.083± 0.159

14	98.265 ±0.280	98.692 ± 0.574
21	98.119 ± 0.266	98.096 ± 0.876
28	98.086 ± 0.888	98.852 ± 0.746

*Average of 03 readings

Table 11 Effect of storage temperature on the *in-vitro* release of optimized formulation F12

Time (Days)	Cumulative % Drug Release (%)	
	4.0 ±0.5°C	**28 ± 0.5°C**
0	98.120 ± 0.021	98.120 ± 0.021
7	97.917± 0.575	98.083± 0.158
14	97.265 ±0.279	97.692 ± 0.573
21	97.119 ± 0.265	97.096 ± 0.875
28	97.086 ± 0.887	96.852 ± 0.745

Effects of induction of diabetes mellitus and hypoglycemic activity in diabetic rats

The results of hypoglycemic activity of nasal dulaglutide gel in comparison with dulaglutide Sc injection (control group) in diabetic rats are presented in Table 12. In the control animals, treated with plain dulaglutide 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 dulaglutide 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 dulaglutide injection; but with nasal dulaglutide 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 dulaglutide gel also showed prolonged hypoglycemic action when compared with plain dulagluitde. 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.

Table 12 Antidiabetic activity of dulaglutide 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	62.00 ±1.56	26.84 ± 4.99*
3	55.49 ±2.49	54.83 ± 2.57
6	35.23 ±1.50	63.39 ± 2.27†
8	22.73 ±5.29	67.59 ± 2.36†
10	14.74 ±2.87	74.37 ± 3.54†

12	7.40 ±1.78	65.68 ± 3.94†
15	4.34 ±1.75	23.50 ± 2.27*

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

Conclusion

The goal of the current study was to create a thermosensitive in situ nanogel system based on chitosan and tripolyphosphate for the nasal delivery of GLP-1 agents (dulaglutide). The in situ nanogel that was created using an ionic gelation method turned out to be appropriate for drug administration via the nasal route. Because it can increase nasal residence length and intranasal bioavailability, this can be seen as a good substitute for unpleasant injections and traditional nasal drops. Patient compliance is improved by the simplicity of administration combined with less frequent administration. Furthermore, the in vivo findings demonstrated unequivocally that the dulaglutide-loaded nanogel could successfully lower blood glucose levels in a model of diabetic rats.

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