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PREPARATION AND CHARACTERIZATION OF CHITOSAN NANOPARTICLES OF INSULIN FOR NASAL DELIVERY

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ABSTRACT

The aim of this study was to prepare and evaluate nanoparticles containing insulin in different polymer ratio by ionotropic gelation method. The average particle size was found to be 33.3±0.7-69.9±0.7nm. SEM indicates that nanoparticles have shown smooth and spherical shape. The zeta potential of optimized formulation was 35.5 mv which indicates moderate stability with no agglomeration. The *in vitro* drug release data was analyzed using zero order, first order, Higuchi, and Korsmeyer-Peppas models. It was observed the best fit model for nanoparticles was higuchi model. The developed formulation in situ polymeric gel is designed in such a way that the gel will load insulin in higher concentration and it will also contain penetration enhancer which will enhance the absorption of release drug from gel to systemic circulation.

Keywords: Insulin, Nanoparticle, *invitro* drug release study.

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INTRODUCTION

Controlled drug delivery system (CDDS) represents the developed technique of pharmaceutical sciences, it's includes various scientific approaches, managing for individual care. The drug development technology having abundant advantages than existing conventional type of dosage, it involves enhanced effectiveness, minimized poisoning, enhanced conformity of the consumer is ease [1]. As a kind of protein and peptide drug, insulin is widely accepted as an effective drug for eliminating the clinical symptoms of diabetes mellitus [2,3]. However, oral insulin has a very low bioavailability due to the destruction of gastric pH and gastrointestinal enzymes, as well as its low permeability across the intestinal epithelium [4,5]. Currently new and potent Protein/Peptide drugs are generally administered parenterally by IV, IM and SC routes, but needle phobia and stress of multiple daily injections and other associated disadvantages lead to development of new and significant approaches for their delivery. Insulin is given subcutaneously before meal to the patients suffering with insulin dependent diabetes mellitus because insulin is inactivated in gut lumen by its enzymes and acidic environment and of is having very limited GIT permeability as well as injection phobia, discomfortness and cause chances of infection, make necessitate to discover an alternate route which can safely deliver insulin systematically.

Hence, it is envisaged to design such delivery system and select route of administration, which will avoid insulin from its inactivation, absorbed effectively and enhance the bioavailable dose on administration. Nasal insulin delivery is the best way to deliver insulin safely without its degradation. Insulin directly enters into systemic circulation after absorption but low retention time of dosage form make nasal route is very challengeable. To overcome these problems, it is envisaged to formulate a delivery system i.e. in situ biodegradable polymeric gel, which can entrap insulin along with the penetration enhancers that will retain for longer period, protect and make it available to manage blood glucose level in the body.

In present study a novel drug delivery system i.e. in situ polymeric gel is designed in such a way that the gel will load insulin in higher concentration and it will also contain penetration enhancer which will enhance the absorption of release drug from gel to systemic circulation. Prepared formulation will remain in liquid form before administration but on administer nasal route then it will become gel due to its interaction with lachrymal fluid environment like pH, temperature and ions. Its gel form will retain for longer period of time and work as reservoir for insulin. The in situ gel will release the drug in very sustained and controlled manner as well as it also increases the retention and contact time thus increase the bioavailability of entrapped insulin by making it bioavailable by increase contact time for longer period of time. This novel in situ gel will overcome the disadvantages associated with conventional dosage form (drop and other) like low retention time and immediate absorption of drug that act only for short time. The in situ gel will protect insulin, increase its retention in ocular cavity and release it in very controlled manner for longer period of time which will reduce dose and manage blood glucose level effectively.

MATERIALS AND METHODS

Materials:

Insulin was purchase from himedia lab, India. Chitosan was was obtained as a gift sample from Torrent Pharmaceutical Pvt. Ltd (Mumbai, India). Poloxamer 407 was purchase from Sigma Aldrise, India. Carbopol 934 and other chemical purchase from himedia lab, India.

Methods:

Preparation of chitosan nanoparticles of Insulin

Nanoparticles (NP) will be prepared according to Calvo*et al.*, 1997 [6], using ionotropic gelation method with slight medication 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, surfactant concentration on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations.

Effect of chitosan quantity

The effect of chitosan quantity on the particle size was studied by varying one chitosan Chitosannanoparticles were prepared corresponding to varying concentrations of chitosan such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9% keeping the amount of Acetic acid (1% v/v), stirring time (3 hours) and stirring speed (600 rpm) constant. (Table 1)

Table 1: Composition of SLN by varying amount of lipid

Components	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Insulin	10	10	10	10	10	10	10	10	10
Chitosan	0.1%	0.2%	0.3%	0.4%	0.5%	0.6%	0.7%	0.8%	0.9%
Acetic acid	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Stirring speed (rpm)	600	600	600	600	600	600	600	600	600
Stirring time (hrs)	3	3	3	3	3	3	3	3	3

1.1.2.1 Effect of stirring time

Different batches of chitosan nanoparticles were prepared increasing of stirring time keeping the chitosan concentration (0.9%)and stirring speed (600 rpm) constant. (Table 2)

Table 2: Composition of chitosan nanoparticle by varying Stirring time

Components	Formulation code								
	F10	F11	F12	F13	F14	F15	F16	F17	F18
Insulin	10	10	10	10	10	10	10	10	10
Chitosan	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
Acetic acid	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Stirring speed (rpm)	600	600	600	600	600	600	600	600	600
Stirring time (hrs)	1	2	3	4	5	6	7	8	9

Effect of stirring speed

Four different batches of chitosan nanoparticles were prepared corresponding to 100, 200, 300, 400, 500, 600, 700, 800 and 900 rpm of stirring speed keeping the chitosan concentration (0.9%) and stirring time (4 Hour) constant. (Table 3)

Table 3: Composition of chitosan nanoparticle by varying Stirring speed

Components	Formulation code								
	F10	F11	F12	F13	F14	F15	F16	F17	F18
Insulin	10	10	10	10	10	10	10	10	10
Chitosan	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
Acetic acid	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Stirring speed (rpm)	100	200	300	400	500	600	700	800	900
Stirring time (hrs)	4	4	4	4	4	4	4	4	4

Evaluation of nanoparticles

Particle size analysis [6,7]

Particle size analysis The size distributions along the volume mean diameter of the nanoparticles. Particle size of all formulated nanoparticles was in the range between 130.4±3.4 to 155.5±6.4 and Zeta potential of all formulated nanoparticle was the range between were measured by laser scattering light using photon correlation spectroscopy using a Horiba Zetasizer Instruments. The obtained results are shown in Figure 3.

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. chitosan nanoparticles entrapped insulin were separated from the free drug by dialysis method. The above said formulations were filled into dialysis bags and the free insulin dialyzed for 24 hours into 50 ml of buffer pH 1.2. The absorbance of the dialysate was measured at 272.0 nm against blank buffer pH 1.2 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free insulin could be obtained from the absorbance difference based on standard curve. Standard curve was made by measuring the absorbance at 272.0.0 nm for known concentrations of insulin solution [7].

Drug content:

From the prepared chitosan nano formulation 1ml of suspension is dissolved in the 10 ml of 1.2 pH buffer. The amount of insulin was determined using UV spectrophotometer at 272nm.The placebo formulation prepared similarly to drug loaded nanoparticle was used as blank. The total drug content was calculated [8].

Shape and Surface Characterization of nanoparticle by Scanning Electron Microscopy (SEM):

From the formulated batches of chitosan nanoparticle optimized formulations which showed an appropriate balance between the percentage release were examined for surface morphology and shape using scanning electron microscope Jeol Japan 6000. Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 10 KV during scanning. Microphotographs were taken on different magnification and higher magnification (200X) was used for surface morphology.

***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 6.5. 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.

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.

1. Zero-order kinetics [9]: 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 initialamount of drug in the solution (most times, Q_o=0) and $K_o$is the zero order release constant (Bourne, 2002).

2. First-order kinetics [10]: 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.

3. Higuchi model [11]: 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.

4. Korsmeyer-Peppasmodel: 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 [12]. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism [13]. 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 I nstead 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 [13]. 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.

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}

RESULTS AND DISCUSSION

Particle size analysis

The size distributions along the volume mean diameter of the nanoparticles. Particle size of all formulated nanoparticles was in the range between 130.4±3.4 to 155.5±6.4. The zeta potential of optimized formulation was 35.5 mv which indicates moderate stability with no agglomeration. The obtained results are shown in table 4. Results showed that in formulation F-17 which contains higher level of chitosan and high stirring speed showed decrease in particle size and increase in entrapment efficiency, as level of chitosan decreased particle size of formulation increased.

Table 4: Evaluations of Nanoparticle formulations by OVAT

Formulation	Particle Size (nm)	Entrapment efficiency (%)	Drug content (%)
F1	55.2±0.4	71.2±0.5	69.98±0.45
F2	45.6±0.6	62.2±0.6	73.28±0.65
F3	60.1±0.8	65.4±0.4	70.56±0.85
F4	48.7±0.7	63.1±0.5	65.52±0.45
F5	55.6±1.2	61.2±0.8	66.92±0.65
F6	69.9±0.7	60.2±0.9	68.98±0.32
F7	65.5±0.5	59.9±0.1	71.12±0.25
F8	55.6±0.4	60.2±0.5	69.98±0.26
F9	65.4±0.6	58.8±0.3	73.12±0.21
F10	52.2±0.8	63.3±0.5	70.56±0.45
F11	41.2±0.9	60.2±0.4	69.98±0.36
F12	52.2±0.7	65.5±0.5	73.12±0.25
F13	45.5±0.8	68.9±0.6	75.45±0.45
F14	55.6±0.6	73.3±0.7	73.45±0.78
F15	60.2±0.6	75.4±0.6	78.98±0.65
F16	45.5±0.9	79.9±0.2	85.62±0.67
F17	33.3±0.7	82.5±0.3	89.98±0.45
F18	65.5±0.5	78.8±0.2	75.55±0.55

Particle size and zeta potential drug content of optimized formulation

Code	Entrapment efficiency (%)	Zeta potential (mv)	Particle size (nm)
F17	78.8±0.2	-35.5	33.3±0.7

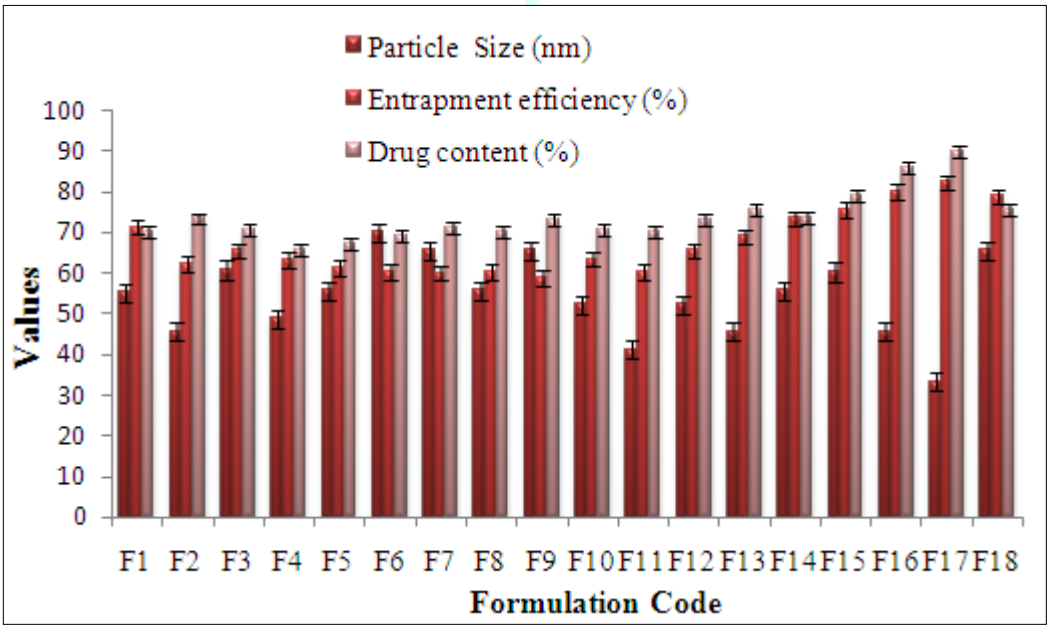


Figure 1: Evaluation of Nanoparticle formulations by OVAT

Shape and Surface morphology

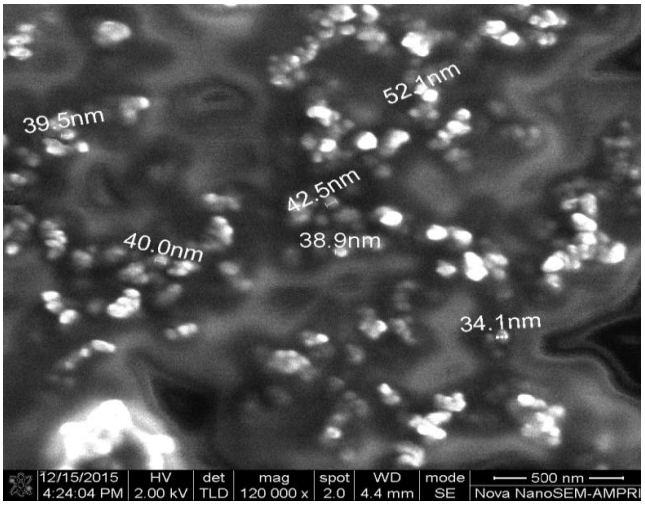
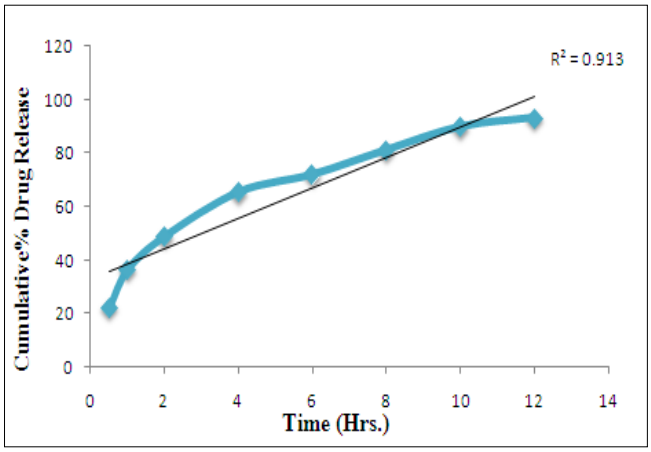


Figure 2: Scanning Electronic Microscopy Image of Optimized Formulation

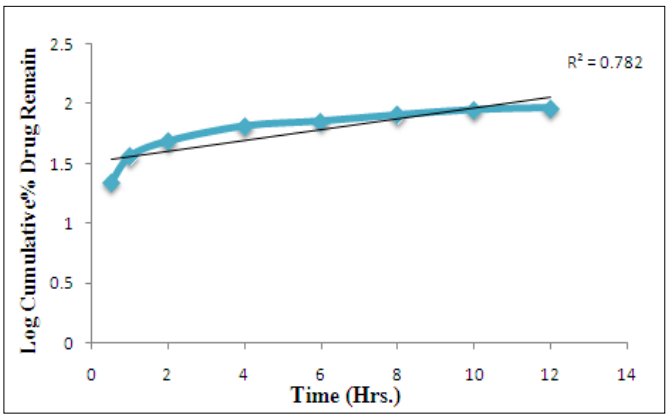
Shape and surface morphology of nanoparticles was studied by Scanning Electron Microscopy (SEM). SEM photographs of optimized formulations were shown in Figure 2. Insulin nanoparticles have shown smooth and spherical shape with different sizes depending on the ratios of the surfactant and polymer used.

***In Vitro* drug release Study**

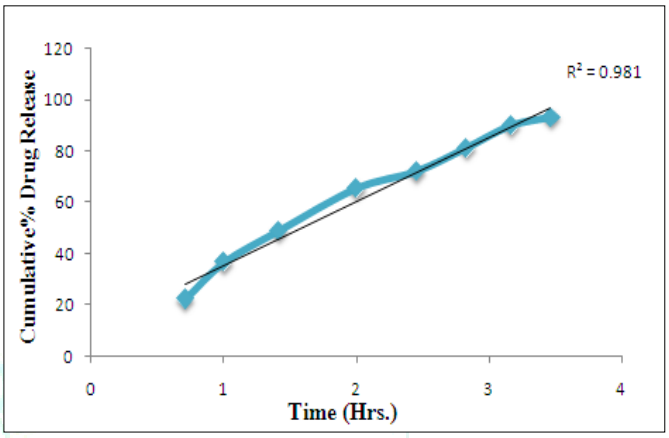
The *in vitro* release profile of optimized formulation is shown in fig.2. The optimized Insulin NPs showed initial burst release of 22.25 ± 1.3%, optimized Insulin NPs showed sustained drug release with maximum drug release of 93.32 ± 0.8% in 12 hours. Thus, it was clear that incorporation of Insulin nanoparticles could significantly sustain the release. The *in vitro* drug release data was analyzed using zero order, first order, Higuchi, and Korsmeyer-Peppas models. The graph for Higuchi model was plotted between log time and log percentage drug remaining and the correlation coefficient was found (*r*2) 0.981 for in vitro drug release, therefore the best fit model for nanoparticles was higuchi model.



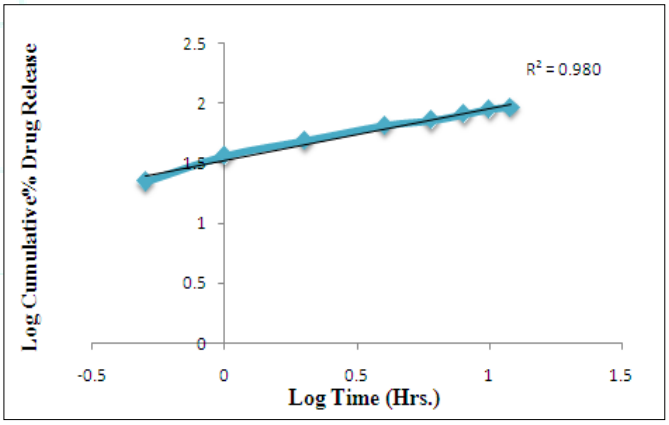
Zero order release Kinetics of optimized Formulation



First order release Kinetics of optimized Formulation



Higuchi release Kinetics of optimized Formulation



Graph of Peppas release Kinetics

Regression analysis data of optimized formulation

Batch	Zero Order	First Order	Higuchi	Korsmeyer-Peppas
	R^2	R^2	R^2	R^2
F9	0.913	0.782	0.981	0.980

When the regression coefficient values of were compared, it was observed that 'r' values of Korsmeyer-Peppas was maximum i.e. 0.980 hence indicating drug release from formulations was found to follow Korsmeyer-Peppas release kinetics.

CONCLUSION

Insulin loaded polymeric nanoparticle showed slow and constant release of insulin from nanoparticles maintain constant drug plasma concentration thereby increasing therapeutic efficacy. *In situ* polymeric gel is designed in such a way that the gel will load insulin in higher concentration and it will also contain penetration enhancer which will enhance the absorption of release drug from gel to systemic circulation. Prepared formulation will remain in liquid form before administration but on administer nasal route then it will become gel due to its interaction with lachrymal fluid environment like pH, temperature and ions. Its gel form will retain for longer period of time and work as reservoir for insulin.

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