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FORMULATION AND EVALUATION OF CHITOSAN NANOPARTICLE BASED *IN-SITU* NASAL GEL FOR PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease is chronic progressive neurodegenerative disease. As the disease affects the central nervous system drug delivery has to cross blood brain barrier. Intranasal delivery to brain via olfactory region is best way to treat CNS diseases. Here we formulated chitosan nanoparticles by ionic gelation technique which then incorporated into the *in-situ* nasal gel. Poloxamer 407 at the 20% concentration is best for thermosensitive gelling mechanism. Batch F8 of nanoparticles were optimized by considering the Particle size (**109.9±1.30 nm**), PDI (**0.363±0.013**), Zeta potential (**26.36±2.58 mV**), %Entrapment efficiency of Levodopa (**91.03±3.02**) and % Entrapment efficiency of Selegiline Hydrochloride (**55.01±1.97**). Levodopa-selegiline hydrochloride chitosan (LD-SLG-CS) nanoparticle based *in-situ* nasal gel was formulated by taking the batch F8 and it was found to be clear and had pH of **6.2**, gelling temperature of **29 ± 0.6 ^{0}C**, drug content of Levodopa in formulation was **90 ± 0.84%** while of selegiline hydrochloride was found **85 ± 0.45 %.** *In-vitro* release of LD-SLG-CS nanoparticle based in-situ nasal gel for drug Levodopa followed Higuchi kinetics and selegiline HCl followed zero order kinetics. Ex-*vivo* permeation study for 7 hours showed that Levodopa and selegiline HCl had 26.339 and 69.041% cumulative release respectively. *In-vivo* pharmacodynamic study on female wistar rats were performed; in that locomotion activity by open field test, grip strength and akinesia

test had significant results which indicates that formulation had dynamic effect on the Parkinsons disease.

KEYWORDS: Parkinson's disease, Chitosan nanoparticles, *in-situ* nasal gel, Levodopa, Selegiline hydrochloride.

INTRODUCTION

Dr. James Parkinson in 1817 first described the Parkinsons disease as a "shaking palsy." It is a chronic, progressive neurodegenerative disease characterized by both motor and nonmotor features. This disease significantly affect on the patient and his family through the degenerating effects of disease on patients movement daily activity and normal routine behavior. Parkinsonism is not related to single characteristic of disease; it is the set of some complex symptoms which include the muscle rigidity, bradykinesia and resting tremors. In Asian Countries the prevalence rate of the disease is 15 per 100,000 to 328 per 100,000.[1] 1-methyl-4phenyl-1,2,3,6-tetrahydro-pyridine (MPTP), pesticides, α-Synuclin are some important risk factors for this disease.[2,3,4,5] It is belived that caffeine, ciggarate smoking and statins mitigate the risk of Parkinson's disease.[6]

Conventional preparations like solution, suspensions and emulsions have various limitations like high dose low bioavailability, first pass metabolism, instability etc. to overcome these limitations its need to use novel drug delivery systems like nanoparticles.[7] Parkinson's disease requires the direct delivery of drug to brain hence intranasal route is preferred as the olfactory region of the nasal epithelium and the trigeminal neural region are two direct nose to brain pathways.[8,9] *In-situ* nasal gel is best approach for effective delivery of drug to brain.[10]

MATERIAL AND METHODS

API: Levodopa received as a gift sample, Selegiline Hydrochloride purchased from Himedia. Polymer : Chitosan (LMW); Cross Linking Agent: Sodium Tripolyphosphate purchased from the Sigma Aldrich, Gelling agent: Poloxamer 407 also purchased from Sigma Aldrich; Other Agents: Acetic Acid, Potassium Dihydrogen Phosphate, Sodium Hydroxide, etc. received from department store, dialysis membrane purchased from Himedia laboratory.

Preparation of Levodopa Selegiline loaded Chitosan nanoparticles (LD-SLG-CS-NP)

Ionotropic gelation Technique

The method for the preparation of chitosan nanoparticle is Ionic gelation technique which was first reported by Calvo *et al.*, 1997 and has been widely examined and developed. Here firstly Chitosan dissolved in 1% v/v acetic acid. To the chitosan solution 0.5% v/v Tween 80 was added. The pH of the above solution was adjusted to 5.5 by using 5N NaOH. Solution of Sodium tripolyphosphate (STPP) 0.1% w/v also prepared simultaneously. Levodopa 42 mg and Selegeline Hydrochloride 8 mg were added to chitosan solution. Both the drugs were allowed to dissolve completely in this solution. Chitosan-Levodopa-Selegiline (CS-LD-SLG) solution taken in a beaker and kept for stirring on magnetic stirrer. The solution of STPP was added to the Chitosan solution dropwise at a speed of 2 ml/min by needle. The stirring was allowed for 30 minutes, followed by homogenization by High Shear Homogenizer (Model No.T18 D). The nanoparticles so formed centrifuged at 4^0C at 12000 rpm for 30 min in REMI CENTRIFUGE (C-24PLUS). Pellets formed at bottom were removed and washed with mannitol 2% w/v solution and kept for lyophilization in Freeze dryer (SCIENTECH SE-1/2-B). Finally free flowing lyophilized powder obtained.[11,12]

Preparation of Thermosensitive in-*situ* nasal gel

Formulation of *in-situ* nasal gel was prepared by Cold method. Initially in a very small quantity of water, poloxamer dissolved separately in a concentration of 20% w/v at cold condition. Then HPMC dissolved in the quantity as specified in the composition table. Later APIs, PEG 400 and methyl paraben were incorporated and stirred until clear solution was obtained. Finally made the volume up to 100 ml with distilled water and kept it overnight at freezing conditions (4 - 10°C).[13,14]

Characterization of nanoparticles

1. FT-IR studies

FTIR spectra of Levodopa (LD), Selegiline Hydrochloride (SLG) and Chitosan (CS) obtained by using a FTIR spectrometer-430 (Shimadzu 8400S, Japan). IR Spectrum of LD, SLG and taken individually and LD, SLG and CS mixture with potassium bromide (KBr) of IR grade in the ratio of 1:100 and compressed using motorized pellet press. The pellets were then scanned using FT-IR spectrophotometer and compared with the reference standard IR spectrums of the drugs.[15,16]

2. DSC studies

The melting temperature of pure drug was determined by differential scanning calorimetry (DSC). The drug was hermetically sealed in perforated aluminium pans and heated at constant rate of 5˚C/min over the temperature ranges of 30-300˚C. Thermogram of LD, SLG, Chitosan and mixture of LD, SLG, Chitosan and Sodium tripolyphosphate (STPP) was obtained using DSC (SHIMANDZU, USA).

3. Average Particle Size and Polydispersity index

Particle size and Polydispersity index (PDI) of the drug loaded chitosan nanodispersion were measured by the Malvern Zetasizer (Version 6.32). Probe sonicated nanodispersion used for the measurement of average particle size.

4. Zeta potential

Zeta Potential of the drug loaded chitosan nanodispersion was measured by the Nanoplus Particulate system (Version 5.22 / 3.00). Probe sonicated nanodispersion used for the measurement of average particle size.

5. Drug% EE

Nanoparticles were separated from dispersion by centrifugation at 15,000 rpm for 30 min. The supernatant obtained after centrifugation was suitably diluted and analyzed for free Levodopa and Selegiline Hydrochloride by UV–Visible spectrophotometer (Model No. UV1800 240V, UV–visible spectrophotometer, Shimadzu, India) at 280 and 257 nm respectively. The percentage entrapment efficiency was calculated as:

$$\% \text{ Entrapment efficiency} = \frac{[\text{Drug}]_{\text{total}} - [\text{Drug}]_{\text{supernant}}}{[\text{Drug}]_{\text{total}}} \times 100$$

6. Surface Morphology: SEM photomicrographs of batch F8 nanoparticles were taken by JEOL Model JSM - 6390LV which showed the morphology of nanoparticles.

Evaluation of *in-situ* nasal gel

1. Clarity

The developed formulations were inspected visually for clarity, colour in sol and gel form against white background and for any particulate matter if present.[13,17]

2. pH of gel

pH of each formulation was measured using pH meter which was previously calibrated using standard buffers of pH 4, pH 7 and pH 9.[13,17]

3. Measurement of Gelation Temperature

It was determined by using modified Miller and Donovan technique. A 2 ml aliquot of gel was taken into the test tubes which were placed in water bath at 4ºC inside an insulating chamber. The temperature of water bath was increased in the increment of 1ºC. The samples were examined for gelation, which was said to have occurred when the meniscus would follow non Newtonian flow upon tilting.[13,17]

4. Drug Content Estimation

1 ml formulation was taken in 10 ml volumetric flask and then diluted using distilled water upto 10 ml. Again 1 ml quantity from this solution was taken and diluted with 10 ml of distilled water. Finally the absorbance of prepared solution was measured at 280 and 257 nm against blank using UV visible spectrophotometer (Shimadzu UV1800). Finally concentration of the drug present in formulation was computed with the help of calibration curve.[13,17]

5. Gel Strength

A sample of 50 g of nasal gel was taken in 100 ml graduated cylinder and gelled in thermostatically controlled water bath at 37ºC. Weight of 35 g was placed onto the gelled solution. The gel strength, which is an indication for the viscosity of the nasal gel at physiological temperature, was determined as time in seconds required by the weight to penetrate 5 cm into gel.[13,17]

6. Viscosity

The rheological studies were carried out using the Brookfield viscometer. The gel formulation under study was placed in the sample holder and then suitable spindle was selected and inserted perpendicular into the sample.[13,17]

7. *In-Vitro* diffusion study

In vitro release profile of *in-situ* nasal gel of plane drug and nanoparticle loaded *in-situ* gel was carried out for 8 hrs using Franz diffusion cell resembling the conditions of nasal mucosa at 34±0.5°C maintaining sink conditions. This Franz diffusion cell has 2 cm diameter and 20

ml capacity. Dialysis membrane having cut off molecular weight 12000 to 14000 KDa was used as membrane. The formulation of 1 ml was placed in each cell. Receptor medium 1 ml was withdrawn from the receptor chamber at the predetermined time interval, and immediately replaced by the fresh saline phosphate buffer maintained at 34±0.5˚C. The Levodopa and Selegiline Hydrochloride in sample was determined by the UV Spectroscopic method.[18]

8. *Ex-vivo* permeation study

Nasal cavity of goat was obtained from local slaughter house. It was safely transported to laboratory by keeping it in the saline phosphate buffer (pH 6.4). The intact nasal mucosa was separated, cleaned and stored in the saline phosphate buffer. The study was conducted using a Franz diffusion system. The nasal mucosa was fixed on the Franz diffusion cell having effective permeation area of 3.14 cm^2. After 30 min of incubation time, the optimized formulation 1ml was placed in the donor compartment. The temperature of the chamber was maintained at 34±0.5˚C. The saline phosphate buffer (pH 6.4, 20 ml) was used as receptor medium. Receptor medium 1 ml was withdrawn from the receptor chamber at the predetermined time interval, and immediately replaced by the fresh saline phosphate buffer. The Levodopa and Selegiline Hydrochloride in sample was determined by the UV Spectroscopic method.[19]

9. Histopathological Examination of Nasal Mucosa

The effect of LD-SL-CSNP based *in-situ* nasal gel on the structural integrity of nasal mucosa was investigated by histopathological examination of goat nasal mucosa previously obtained from local slaughterhouse. Freshly isolated goat nasal mucosa was sectioned in four pieces. The first piece was positive control which was treated with isopropyl alcohol (nasal mucociliary toxicity agent used as a positive control) for 2 hours; other three were negative control treated with PBS (pH 6.4), Nanosuspension of LD-SG and *in-situ* nasal gel of LD-SL-CSNP respectively. All four pieces were fixed in 10% formalin kept in individual sample bottle. These pieces after 24 hour embedded in paraffin. Sections (5 µm) were cut and de-paraffinized using xylene and ethanol, followed by staining with hematoxylin and eosin. The sections were inspected for tissue damage using optical microcopy at 10× magnification.[20,21,22]

10. *In*-vivo study

Behavioural animal study was performed on female wistar rats having average weight of 200 gm with prior permission of Institutional Animal Ethics Committee with registration number IAEC/UDPS/2018/34. In this study the Parkinsons disease was induced by the reserpine by administering 1 mg/ kg.[23]

Experimental Design

Animals were randomly assigned into 3 groups. Each group contained 3 animals.

Group 1: Vehicle group (Naive Group) labeled as N1, N2 and N3

Group 2: Disease control group to which Reserpine 1 mg/ kg was given and labeled as H, B and T.

Group 3: Test group to which nanoparticle loaded *in-situ* nasal gel 50 micro-liters + Reserpine 1 mg/kg was given labeled as U1, U2 and U3.[24]

Induction of Parkinson's disease in rat

Reserpine was dissolved in glacial acetic acid (GAA) and then volume adjusted by using distilled water. Reserpine was administered to rats (1 mg/kg, s.c.) by each alternate day for 3 days i.e. on 1st, 3rd and 5th day and formulation was administered daily for 5 days. The induction of PD was observed by performing various behavioral tests.[23]

Behavioral studies

All behavioral tests were performed on day 1st before the administration of drug and induction of Parkinson's disease. The tests were performed sequentially namely grip strength, akinesia and OFT. The experiments in behavioral studies were carried out in a silent and dimly lit room.[25]

a. Open field test

To analyze possible changes in spontaneous locomotor and exploratory activity caused by treatment with reserpine, the animals were placed individually in the center of a circular open field arena divided into nine parts. The effect of drugs on behavior was examined after reserpine and treatment (on day 5). The number of rearings and the number of line crossings were measured over 5 min.[26]

b. Grip strength

Grip strength tests are highly specific, in that they attempt to measure a single, well-defined aspect of behavior. Grip strength has been measured by testing the ability of the mouse to remain clinging to an inverted surface such as a cage lid for a period of time, usually up to 1 minute; by testing the ability of the mouse to hang on a wire with its forepaws until its grip fails.[27]

c. Akinesia: It was determined by holding the tail of animal and putting the front paws on the platform and let the animal to walk while holding (number of steps taken with forelimbs of animal were counted for 1 min.

11. Stability Study

Stability studies were conducted to test the physical and chemical stability of the developed in situ nasal gel. A sufficient quantity of in situ gel, in screw capped vials was stored at different temperature condition as 5 ± 3 °C for 3 month.[28] The physical stability, including appearance, color, pH, Tsol-gel temperature, viscosity, and drug content was studied.[18]

RESULT AND DISCUSSION

Characterization of Naoparticles

1. FTIR study

FT-IR spectrum of Levodopa and Selegiline HCl were taken and it was observed that both drugs having similar spectrum as that of their standard spectrum so it conclude that both drugs were pure. FT-IR spectrum of lyophilized LD-SLG-CS nanoparticles didn't show the peaks which were present in the of Levodopa and Selegiline hydrochloride. From this we can conclude that drugs get entrapped in the chitosan-tripolyphosphate (CS-TPP) complex. FTIR spectrum of pure drugs and nanoparticles are shown in figure 1(a, b & c).

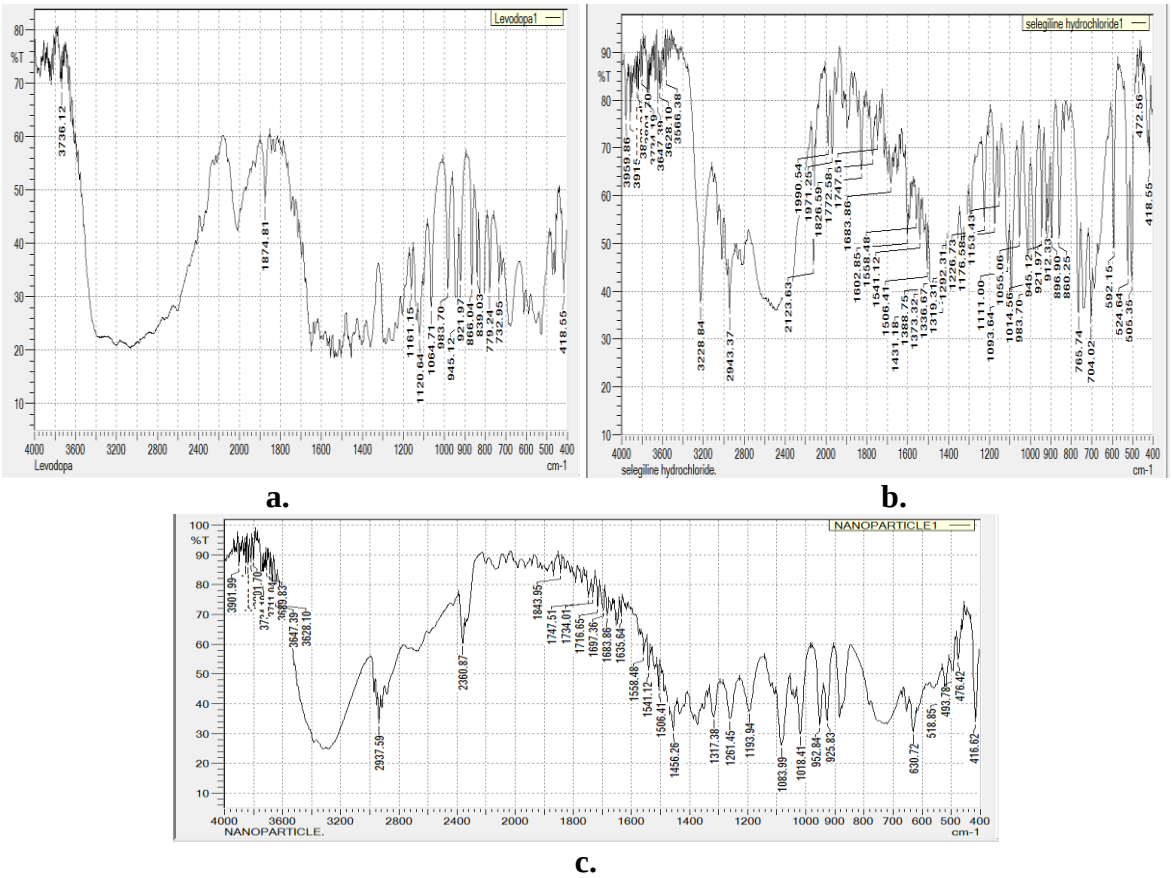


Figure 1: a) FT-IR spectrum of Levodopa: b) FT-IR spectrum of Selegiline Hydrochloride; c) FTIR Spectrum of Lyophilized LD-SLG-CS nanoparticles.

2. DSC Study

DSC thermogram of Levodopa and Selegiline hydrochloride shows the single endothermic peaks at 283.35^{0}C and 143.94 ^{0}C respectively, corresponding to their melting points which confirmed that both drugs were pure. DSC thermogram of lyophilized nanoparticles were taken which gave the intense endothermic peak which is corresponding to the melting point of the mannitol which was used for lyophilization , we can conclude that mannitol might be adsorbed on the nanoparticles that's why gave only one endothermic peak. DSC thermogram of Levodopa, Selegiline HCl and lyophilized LD-SLG-CS nanoparticles are shown in figure 2(a, b & c).

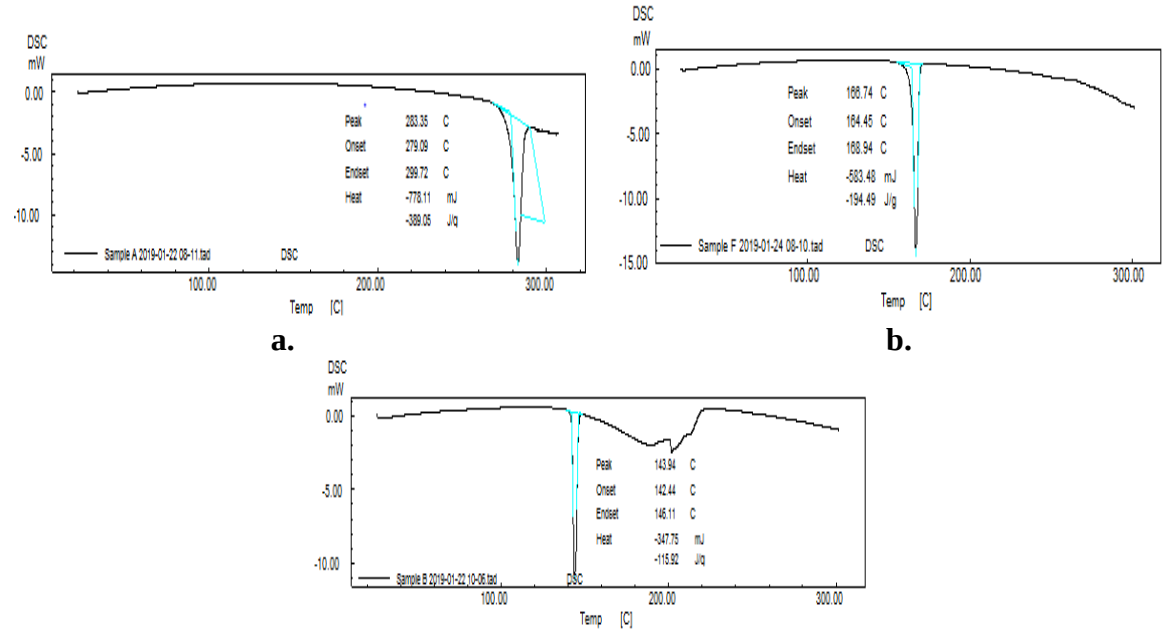


Figure 2: a) DSC Thermogram of Levodopa; b) DSC Thermogram of Selegeline Hydrochloride; c) DSC thermogram of Lyophilized LD-SLG loaded Chitosan nanoparticles.

3. Average particle size and Polydispersity Index

The formulation batches were evaluated for average particle size, PDI and entrapment efficiency. The formulation batch F8 showing average particle size, PDI and high entrapment efficiency was selected. The results obtained in particle size, PDI and entrapment efficiency of batches are given in Table 1. The particle size of optimized batch is shown in figure 3.

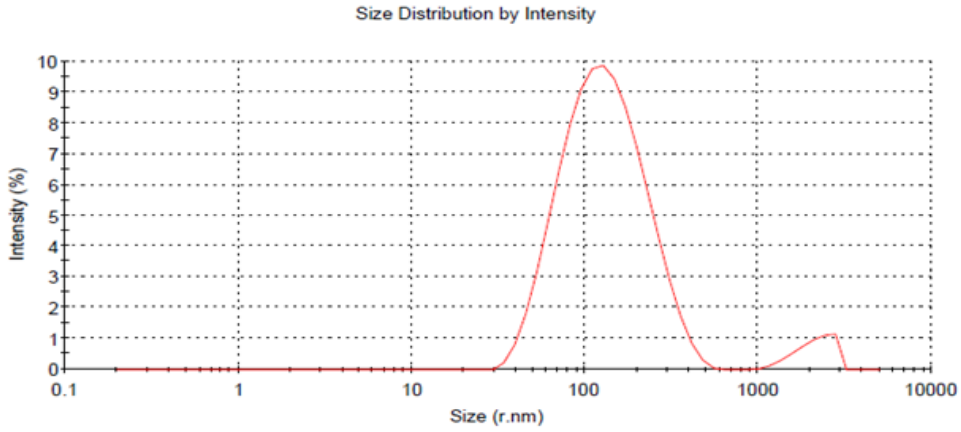


Figure 3: Average Particle Size of optimized batch F8.

4. Zeta Potential

Zeta potential of all batches estimated and it was found in the range of 23.81 to 31.20 mV and the optimized F8 batch having the zeta potential **26.36 ± 2.58** mV. Zeta potential of optimized batch is shown in figure 4.

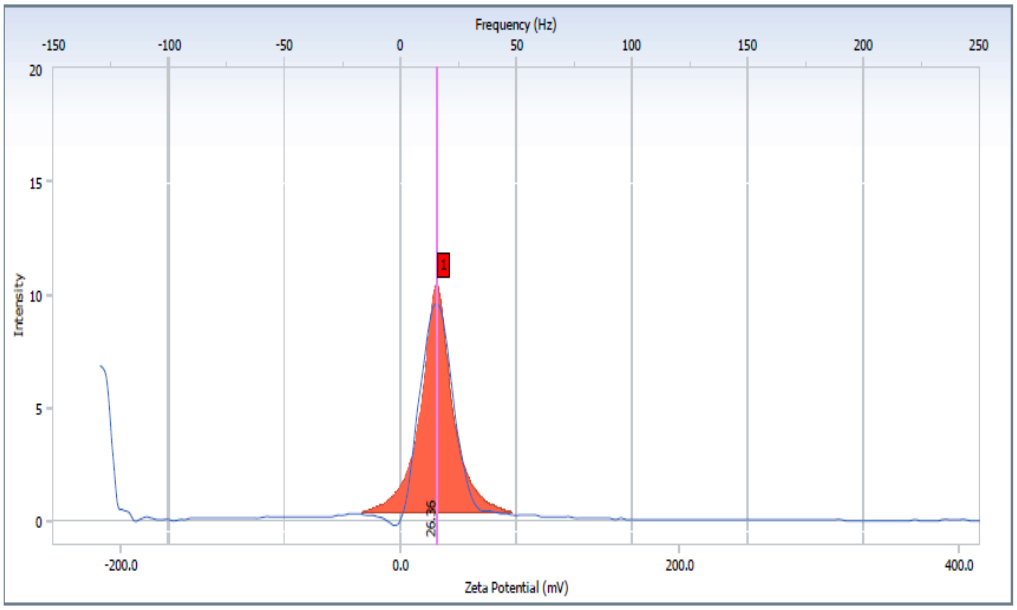
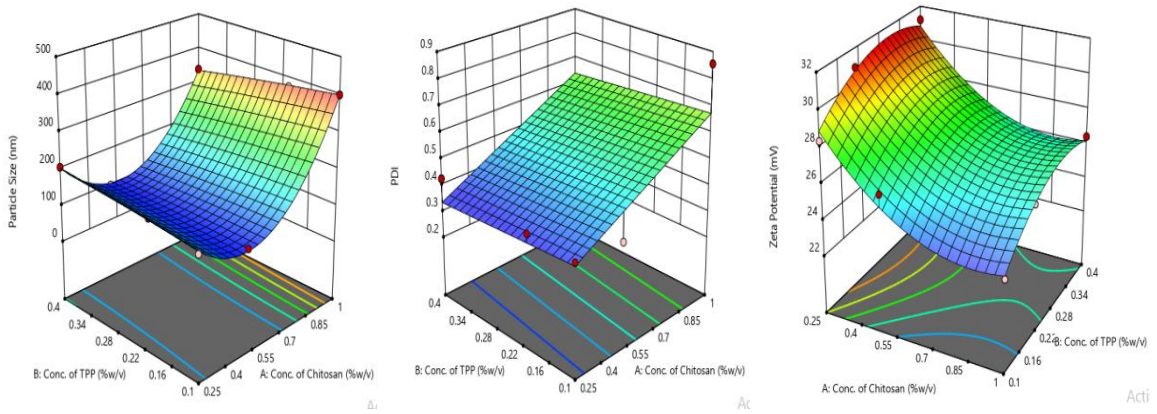


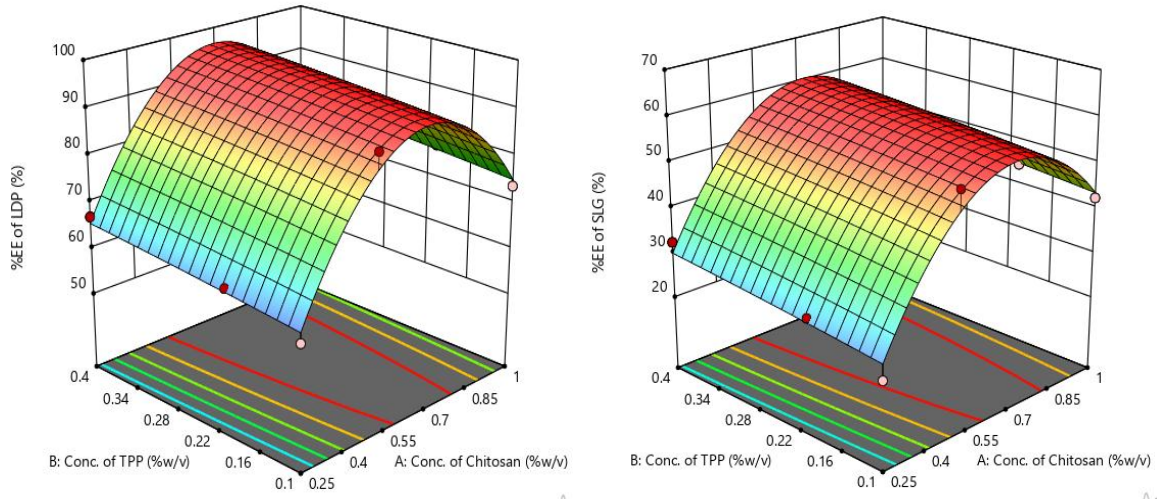
Figure 4: Zeta Potential of optimized batch F8.

5. Percent Entrapment efficiency

% Entrapment efficiency of all batches was calculated and showed in table 1. Batch F8 has highest entrapment for Levodopa and Selegiline HCl i.e. 91.03±3.02 and 55.01±1.97 respectively.



a **b** **c**



d e

Figure 5: 3D Response surface plots of a) Particle size; b) PDI; c) Zeta potential d) % EE of Levodopa e) %EE of Selegiline HCl.

Response surface plot

3D Surface response plots are given in figure 5. The 3 D response surface plots of factors vs responses were obtained from the Design of Expert software.

3D response surface plot of particle size indicates that as the concentration of chitosan increased from 0.25% to 0.5% the particle size gets reduced firstly and when concentration of chitosan increased from 0.5% to 1.0% particle size gets increased. In case of TPP at 0.1 % concentration maximum particle size is 400 nm and at 0.4 % concentration maximum particle size is 388 nm; this indicates that there is small decrease in particle size by increase in concentration of TPP.

PDI increased with increase in concentration of chitosan and slightly decreased with increase in concentration of TPP. Zeta potential get reduced with increase in concentration of chitosan while gets increased with increase in concentration of TPP. At the concentration of 0.5% of chitosan and 0.1% TPP optimum zeta potential is obtained.

As the concentration of chitosan increased from 0.25 % to 0.5 % the % entrapment efficiency of Levodopa and Selegiline HCl also increased. As the concentration of chitosan increased from 0.5% to 1% the % entrapment efficiency get reduced. By considering the all responses F8 is selected as optimum batch having particle size **109.9±1.30** nm, polydispersity index

0.363±0.013, zeta potential **26.36±2.58** and % entrapment efficiency for Levodopa **91.03±3.02** and for selegiline HCl **55.01±1.97.**

6. Surface Morphology

The SEM photomicrograph of LD-SLG-CS-NP is given in below figure 6 shows nanoparticles of dispersed size.

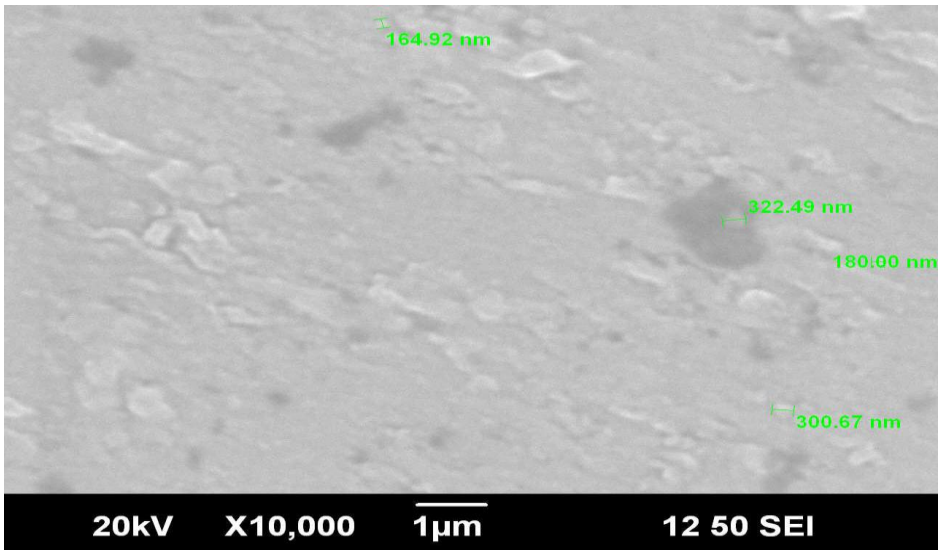


Figure 6: SEM photomicrograph of LD-SLG-CS-Nanoparticles.

Table 1: Average particle size, polydispersity index and entrapment efficiency of formulation batches with standard deviation (SD).

Batch	Factor 1 A:Conc. of Chitosan %w/v	Factor 2 B:Conc. of TPP %w/v	Response 1 Particle Size±SD nm	Response 2 PDI±SD	Response 3 Zeta Potential±SD mV	Response 4 %EE of LD±SD %	Response 5 %EE of SLG±SD %
F1	0.25	0.4	205.9±1.20	0.427±0.023	31.2±1.6	66.88±2.31	32.4±2.36
F2	1	0.1	400.2±1.35	0.856±0.063	23.81±1.33	73.56±3.36	42.35±2.66
F3	0.5	0.2	105.1±2.02	0.389±0.012	27.45±1.64	87.19±2.56	51.32±4.29
F4	0.5	0.4	99.3±1.32	0.412±00.39	27.11±1.88	89.23±1.03	50.42±2.69
F5	1	0.4	338.2±1.75	0.612±0.054	26.43±2.03	79.46±1.65	48.88±2.57
F6	1	0.2	376.5±1.23	0.574±0.041	25.92±2.55	77.34±2.36	44.63±2.77
F7	0.25	0.2	188.4±0.96	0.393±0.055	30.94±1.96	63.99±2.68	28.45±2.88
F8	**0.5**	**0.1**	**109.9±1.30**	**0.363±0.013**	**26.36±2.58**	**91.03±3.02**	**55.01±1.97**
F9	0.25	0.1	162.7±2.6	0.385±0.038	28.37±2.94	59.66±2.09	22.48±2.65

Characterization of *in-situ* nasal gel

1. Clarity

The developed formulation was inspected visually for clarity against the white background and none particles seen in the sol as well as gel form of formulation.

2. pH of gel

pH of sol form of formulation was measured by using the calibrated pH meter pH was found to be 6.2 which were compatible with the nasal secretion and nasal mucosa.

3. Gelation temperature

The gelation temperature was found 29 ± 0.6 ^{0}C.

4. Drug content estimation:

The drug content was estimated by using the standard calibration curve of both the drugs; concentration drugs in the formulation was found to be –

Table 2: Drug content estimation in nanoparticle loaded *in-situ* nasal gel.

Sr. No.	Drug	Drug content in formulation
1.	Levodopa	90 ± 0.84 %
2.	Selegiline Hydrochloride	85 ± 0.45 %

5. Gel strength

The gel strength of the gelled formulation was estimated and it was found be 30 seconds.

6. Viscosity

Viscosity of formulation in both forms i.e. in sol and gel form was estimated by Brookfield viscometer by using spindle no. 5.

Table 3: Viscosity of *in-situ* nasal gel.

Viscosity of sol (cps)	Viscosity of gel (cps)
600 ± 10.2	4000 ± 60.53

7. *In-Vitro* study

In-vitro release profile of *in-situ* nasal gel was carried out for 8 hrs using Franz diffusion cell resembling the conditions of nasal mucosa at 37±0.5°C maintaining sink conditions. The % drug release is given in the Table 4 given below.

Table 4: *In-vitro* diffusion study of plane drug loaded in-*situ* nasal gel and nanoparticle loaded *in-situ* nasal gel.

Sr. No.	Time in Hours	% Cumulative drug release (plane drug loaded in situ nasal gel)		% Cumulative drug release (nanoparticle loaded in-situ nasal gel)	
		LD	SLG HCl	LD	SLG
1	½	0.000	5.371	0.000	0.783
2	1	16.115	22.288	0.611	6.343
3	2	25.964	45.945	5.009	11.229
4	3	35.920	61.063	8.695	19.771
5	4	57.613	71.455	11.358	25.857
6	5	66.325	80.075	15.078	38.486
7	6	79.453	88.963	16.787	48.257
8	7	85.633	99.463	18.313	56.657

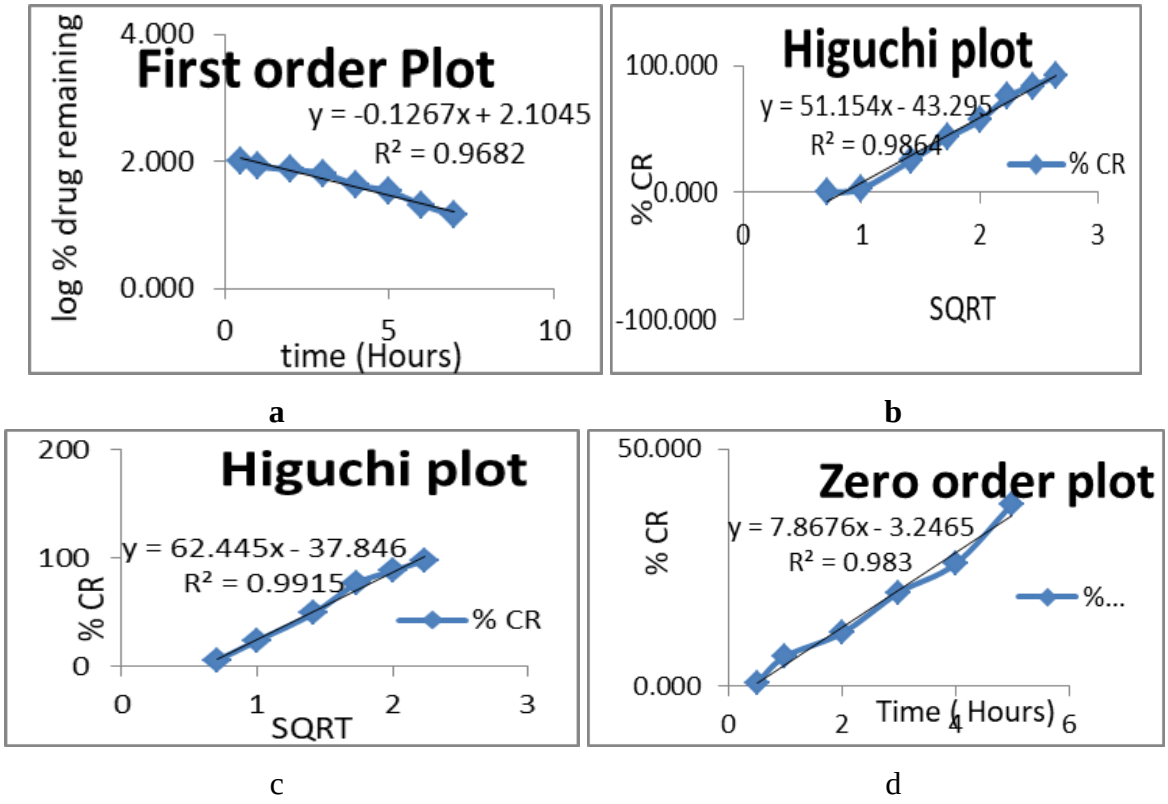


Figure 7: *In-vitro* release kinetics of a) Plane in-situ nasal gel for drug Levodopa; b) Plane in-situ nasal gel for drug Selegeline HCl; c) LD-SLG-CS nanoparticle based in-situ nasal gel for drug Levodopa d) LDSLG-CS nanoparticle based in-situ nasal gel for drug Selegiline HCl.

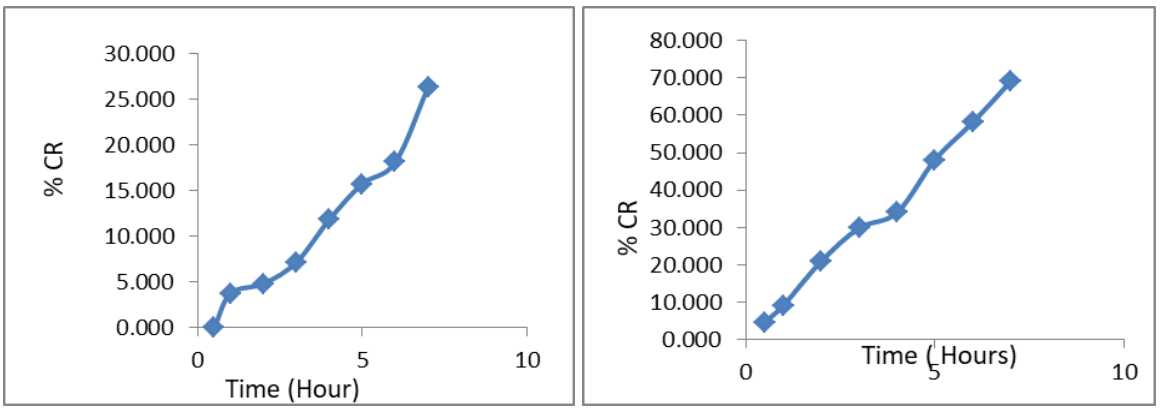
Release Kinetic study

The *in-vitro* drug release for plane *in-situ* nasal gel and LD-SLG-CS nanoparticle loaded in-situ nasal gel was performed. Various release kinetics observed for both formulations (Figure 7). The release of Levodopa from plane *in-situ* nasal gel follows first order kinetics while in

nanoparticle loaded *in-situ* nasal gel it follows Higuchi kinetics. The release of Selegiline HCl from plane *in-situ* nasal gel follows Higuchi kinetics while in nanoparticle loaded *in-situ* nasal gel it follows zero order kinetics.

8. *Ex-Vivo* permeation study

The *ex-vivo* drug LD-SLG-CS nanoparticle loaded in situ nasal gel was performed. The graphs plotted as Time vs % Cumulative drug Release (% CR). Levodopa showed that 26.339 % CR in 7 hours, while selegiline hydrochloride showed 69.041 % CR in 7 hours (Figure 8).



a b

Figure 8: *Ex-vivo* release kinetics of LDSLG-CS nanoparticle based *in-situ* nasal gel for: a) Levodopa b) Selegiline HCl.

9. Histopathological Study: The histopathological study was performed in an attempt to reveal the toxic effects of excipients used in formulation. The nasal mucosa which was treated with isopropyl alcohol showed that extensive damage of nasal membrane (Figure 9a). Phosphate buffer solution (PBS, 6.4 pH) treated mucosa showed intact nasal mucosa which was considered as negative control, (Figure 9b). Nanosuspension and nanoparticle loaded in situ nasal treated nasal mucosa also showed intact nasal mucosa (Figure 9c and 9d respectively). This histopathological study shows that there is no any toxic effect of nanosuspension as well as nanoparticle loaded *in-situ* nasal gel.

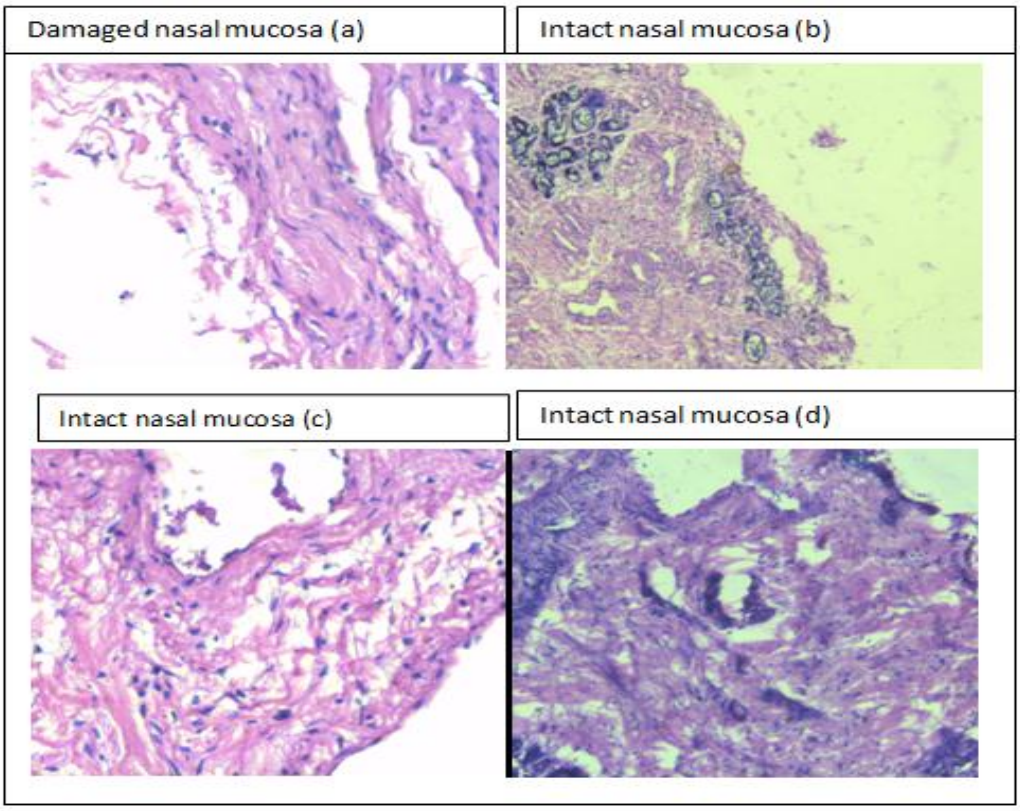


Figure 9: Histopathological study showing the effect of a) Isopropyl alcohol b) Phosphate buffer.

c) Nanosuspension and d) Nanoparticle loaded in-situ nasal gel; on Goat nasal mucosa

10. In vivo study

In vivo study of LD-SLG-CS nanoparticle based *in-situ* nasal gel was performed by using female wistar rats of average weight 200 grams. Locomotion activity (open field test), Grip strength and Akinesia test were performed on naive group, test group and disease controlled group.

a. Open field test

In this test number of ambulations, grooming, wall climbing and rearing by animals were recorded and it was found that most of the parameters were significant (Table 5 & Figure 10).

Table 5: Locomotion activity by open field test.

at	No. of ambulations	Grooming	Wall climbing	Rearing
N1	36	32	07	06
N2	30	25	17	13
N3	26	30	03	04
H	20	30	10	02

B	22	28	06	00
T	18	23	05	00
U1	14	10	02	00
U2	07	16	01	00
U3	04	08	00	00

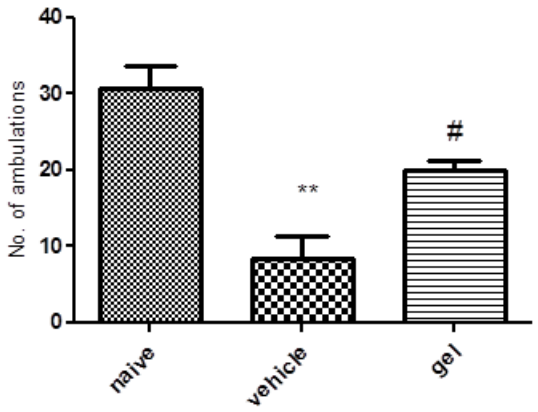


Figure 10: Graphical comparison between naive, vehicle and *in-situ* nasal gel treated rats for number of ambulations.

**P<0.01 vehicle vs naive

# p< 0.05 vehicle vs gel

b. Grip strength and akinesia

Grip strength and akinesia test performed for all three groups and their values are given in the table 6 below and it was found that all the parameters were significant (Table 6 and Figure 11 & 12).

Table 6: Grip strength and Akinesia.

Rat	Grip strength(sec.)	Akinesia
N1	60	46
N2	60	49
N3	60	55
H	40	35
B	48	30
T	52	20
U1	13	24
U2	27	25
U3	10	18

$ p>0.05 gel vs vehicle indicates non-significant difference between gel and vehicle treated animals. This is unexpected result for akinesia test; reason behind this was unknown but might be due to the experimental conditions, laboratory conditions or any other unknown causes.

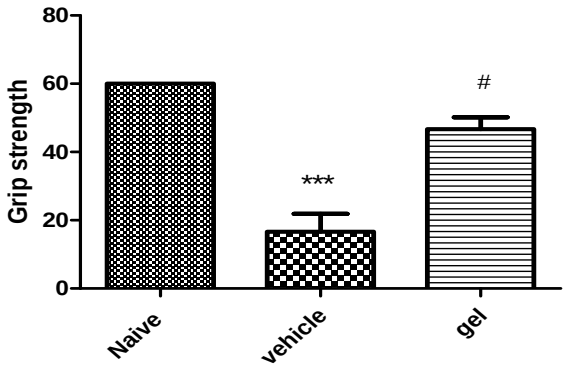


Figure 11: Graphical comparison between naive, vehicle and *in-situ* nasal gel treated rats for grip strength.

***p < 0.001 vehicle vs naive

# p < 0.01 gel vs vehicle

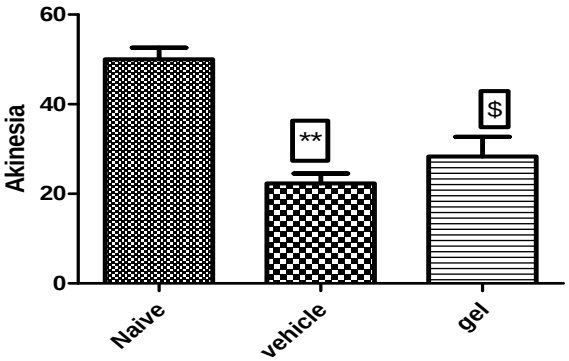


Figure 12: Graphical comparison between naive, vehicle and *in-situ* nasal gel treated rats for akinesia test.

** p< 0.01 vehicle vs naïve.

$ p>0.05 gel vs vehicle

9. Stability Study

The LD-SLG-CS nanoparticle based in situ nasal gel was evaluated for stability studies stored in refrigerator at 5 ± 3 ^{o}C temperature for 3 month. The formulation shown good stability with no remarkable changes in viscosity, drug content, and gelation temperature which are shown in Table 7.

Table 7: Stability study testing.

Sr. no.	Parameters		Storage period 3 months at 5 ± 3°C temp.			
			Initial	1 month	2 months	3 months
1	Viscosity (Cps)	sol	600 ± 10.2	650 ±12.05	630±14.23	626±14.09
		gel	4000 ±60.53	4213 ±45.12	4022±60.42	4043±12.03
2	Drug content (%)	LD	90±0.84	89.23±0.65	89.20±0.26	89.16±0.44
		SLG	85±0.45	83.78±0.82	83.12±0.53	83.03±023
3	Gelation Temperature		29 ± 0.6 ^{0}C	28.60 ±0.3^{0}C	28.90±0.4 ^{0}C	29.5±0.6 ^{0}C

CONCLUSION

From the above study we can conclude that the Levodopa and selegiline combination may be the rational combination as no any evidence of their physical interaction were found also this combination omits the carbidopa as which is adjuvant in oral Levodopa therapy. Nanoparticles may reduce the dosing frequency. Ionotropic gelation technique is best suitable technique for the preparation of chitosan nanoparticles to deliver the antiparkinsonian drugs like Levodopa and Selegiline. As the gelation temperature of this formulation is 29 ^{0}C it will be suitable for human nasal administration and lowers the chances of drug wastage. Use of selegiline in combination with Levodopa may prove beneficent. *In-vivo* animal study also shown that the significant effect of formulation on Parkinson's disease induced female wistar rat.

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