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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF ION AND PH DUAL ACTIVATED NASAL *IN-SITU* GEL CONTAINING LEVODOPA AND CARBIDOPA FOR THE TREATMENT OF PARKINSON'S DISEASE

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Keywords:

Levodopa, Carbidopa, Nasal *in-situ* gel, Nose to brain drug delivery system, Targeted drug delivery, Prolonged drug release

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ABSTRACT: Levodopa suffers from low oral bioavailability and less than 1% of the drug reaches the brain because of its peripheral degradation. This study aimed to prepare a nasal *in-situ* gel of levodopa and carbidopa to provide prolonged drug release, improve bioavailability, and increase drug uptake by the brain. Preliminary studies were carried out using different polymers. The concentration of polymer to be employed for the development of the formulations was deduced by carrying out placebo studies (F1-F12). All the batches were assessed for various parameters such as pH, viscosity, gelation time, and mucoadhesive strength. Out of 12 placebos, F11 which is pH and ion dual activated *in-situ* formulation showed satisfactory results. Therefore, F11 formulation was selectedto prepare levodopa and carbidopa nasal *in-situ* gel. Drug incorporated formulation was found to have a viscosity of 2870.13 cps, assay of 95.7% of levodopa and 100.83% of carbidopa, and gelation time of 35 s, and the *in-vitro* drug release of levodopa and carbidopa after 8 h is 95.7% and 99.38%, respectively. *Ex-vivo* drug release is 97.9% and 99.2% within 5 hours for levodopa and carbidopa respectively. The histopathological study revealed that there was no cell necrosis and no loss of the epithelium. These results indicate that the *in-situ* gel was able to maintain its viscosity over time and was able to release the drug at a prolonged rate. These results suggest that the developed Levodopa and carbidopa nasal *in-situ* gel may be the promising drug delivery for the treatment of Parkinson's disease.

INTRODUCTION: Parkinson's disease is a chronic neurodegenerative disorder of the brain that results in involuntary or uncontrolled movements, including tremors, rigidity, and impaired balance and coordination. This condition is characterized by the depletion of melanin-containing neurons that are responsible for pigmentation in the midbrain. This results in the degeneration of dopaminergic neurons located in the substantia nigra, leading to a deficiency of dopamine [1]. The first symptoms of Parkinson's disease (PD), such as tremors and bradykinesia, appear when the amount of dopamine decreases below 20% of its initial level [2] People over the age of 50 are more likely to be affected.

The occurrence of the disease is higher in males than females due to the neuroprotective nature of estrogens [3]. The treatment of Parkinson's disease includes the administration of the drugs orally, parenterally or the use of device-based therapies such as deep brain stimulation (DBS), levodopa-carbidopa intestinal gel infusion (LCIG), and

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subcutaneous infusion of the dopamine agonist apomorphine, surgeries and rehabilitation. Several of these methods are considered hazardous, intrusive, and have limited efficacy. They may also result in damage of neural tissue [4].

Currently, levodopa is one of the most successful treatments for Parkinson's disease. It is a dopamine precursor. Dopamine lacks the ability to the blood-brain barrier whereas L-Dopa is capable of crossing the blood-brain barrier with the help of large neutral amino acid transporter (LAT1) and converts to dopamine. Levodopa has low oral bioavailability and brain uptake due to its extensive metabolism by the L-aromatic amino acid decarboxylase enzyme in the peripheral circulation. The oral bioavailability of L-dopa is 5-15%, and less than 1% of the drug reaches the brain. Hence, it is co-administered with carbidopa, a peripheral amino acid decarboxylase inhibitor, which prevents the peripheral degradation of levodopa resulting in a 3-fold increase in the amount of levodopa in the systemic circulation [5].

Levodopa and carbidopa achieve better systemic bioavailability the *via* nasal route compared to oral and parenteral administration. The nasal route is advantageous due to Rapid and easy absorption, and quick onset of action because of the existence of a rich vasculature and a highly permeable structure in the nasal mucosa. It avoids first-pass metabolism. Through the intranasal drug delivery system drugs can directly reach the CNS by bypassing the blood-brain barrier. The nose-to-brain drug delivery involves the olfactory or trigeminal nerves as a direct pathway for transporting drugs to the central nervous system (CNS) [6].

One of the efficient strategies for delivering drugs to the brain *via* intranasal administration is mucoadhesive *in-situ* gel. *In-situ* gel formulations are in sol form before administration in the body, after administration, undergo sol-to-gel transition at physiological conditions. The gelation can be induced by external stimuli such as temperature, pH or ion change.

The main objective of the current study was to develop a nasal *in-situ* gel to stabilize levodopa, increase its bioavailability and improve brain uptake through nose-to-brain delivery. Preliminary studies were carried out using poloxomer, polycarbophil and xanthan gum at different concentrations. Developed formulations were optimized with respect to their sensitivity (thermal, pH or ionization) and gelation time.

MATERIALS AND METHODS:

Materials: Levodopa and carbidopa were procured from Divi's Laboratories Limited. Poloxomer 188 was procured from Sigma-Aldrich. Noveon AA-1 Polycarbophil was procured from Lubrizol advanced materials. Xanthan Gum was procured from Zoetis Pharmaceutical Research Pvt. Ltd. HPMC was procured from Dupont Nutrition and Biosciences. All other chemicals and solvents were purchased from domestic suppliers.

Methods:

Preparation of Placebo: Placebo studies were carried out using different concentrations of poloxomer, xanthan gum, and polycarbophil. The concentrations of polymer to be employed for the development of the formulations were deduced by carrying out gelation studies using simulated nasal fluid. Formulations were prepared by cold technique. Different concentrations of polymers were added into each beaker and continuous stirring using a magnetic stirrer until a clear solution is formed. After a clear solution is formed, other excipients were added and volume was made up to 100ml using distilled water. pH was adjusted using 0.5M sodium hydroxide and gelation was checked at 37°C using SNF. Composition of placebos are shown in **Table 1.**

TABLE 1: PLACEBO FORMULATION COMPOSITIONS

S. no.		Poloxomer 188	Polycarbophil	Xanthan gum	HPMC K100M	PG:PEG	Benzyl alcohol	Ascorbic acid	water
F1	Temperature	16	0.2	-	-	10%	0.03%	2%	Q.S
F2	and pH sensitive	17	0.2	-	-	10%	0.03%	2%	Q.S
F3	*in-situ* gel	18	0.2	-	-	10%	0.03%	2%	Q.S
F4	Ion sensitive *in-*	-	-	0.1	0.2	10%	0.03%	2%	Q.S
F5	*situ* gel	-	-	0.2	0.2	10%	0.03%	2%	Q.S
F6		-	-	0.2	0.4	10%	0.03%	2%	Q.S

F7	pH sensitive *in-*	-	0.4	-	0.2	10%	0.03%	2%	Q.S
F8	*situ* gel	-	0.5	-	0.2	10%	0.03%	2%	Q.S
F9		-	0.5	-	0.4	10%	0.03%	2%	Q.S
F10	Ion and pH	-	0.1	0.2	-	10%	0.03%	2%	Q.S
F11	sensitive *in-situ*	-	0.3	0.2	-	10%	0.03%	2%	Q.S
F12	gel	-	0.4	0.2	-	10%	0.03%	2%	Q.S

Evaluation of Placebo:

Clarity and Texture: The clarity and texture of various formulations was determined by visual inspection under the black and white background [7].

pH: Digital pH was calibrated by using pH buffers of 4 and 7. 15 ml of each formulation was taken in a beaker and a glass electrode was sufficiently dipped into the samples. Then, the pH of the solution was determined [8].

Viscosity: A Brookfield viscometer was used to measure the viscosity. Using spindle number LV-3, the viscosity of the solution and the gel was measured at 10 rpm for 30 seconds [8].

Gelation Time: The gelation time was measured for each experiment by placing 1 drop of the prepared formulation into a vial containing 2 ml of freshly prepared SNF solution. Gelation was assessed visually and noted the time for the gelation. [9]

Mucoadhesive Strength: An analytical balance was used to measure the detachment stress. The bottom side of the right pan glass slide was attached and goat nasal mucosa of 3 cm^2 was tied to the glass slide. The formulations which are in solution form are converted to gel by adding SNF. A flat plate was placed under the right pan and a thin layer of gel was evenly spread on it. Due to the weight of the glass slide the right pan was lowered. A beaker was placed on the left pan and water was gradually added to the beaker using a dropper, which led to the gradual separation of the nasal mucosa from the gel. The minimum weight of water required to break the mucosal adhesion was measured [9].

Preparation of Drug Incorporated Ion- and pH-Dual Induced Nasal *In-situ* Gel: Polycarbophil was dissolved in water. Once a clear solution was obtained xanthan gum was added and stirred until a transparent solution is formed. This solution was kept for hydration overnight. Then this polymer solution was gradually incorporated into the solution containing levodopa and carbidopa. Next, the other excipients were added and the volume was made up. **Table 2** shows composition of drug incorporated Ion- and pH-dual induced nasal *in-situ* gel.

TABLE 2: COMPOSITION OF OPTIMIZED FORMULATION

Ingredients	Use	F (%W/V)
Levodopa	API	4
Carbidopa	API	1.8
Polycarbophil	pH sensitive polymer	0.1
Xanthan gum	Ion sensitive polymer	0.2
PG:PEG	Co solvent	10
Benzyl alcohol	Preservative	0.03
Ascorbic acid	Antioxidant	2
Water	Solvent	100

Evaluation of Drug Incorporated Ion- and pH-Dual Induced Nasal *In-situ* Gel:

Assay: Drug content assay of levodopa and carbidopa was determined using the reverse phase HPLC method using C18, 150 × 4.6mm, 5µm column. The mobile phase consists of gradient elution using a mixture of 0.04M Potassium dihydrogen phosphate buffer and methanol in the ratio of 980:20 v/v as mobile phase A and 400:600 v/v of buffer and methanol as mobile phase B.

Related Substance: The related substance analytical study was performed using the reversed-phase HPLC method using C18, 250 mm ×4.6 mm; 3 µm column. The mobile phase consists of gradient elution using sodium dihydrogen phosphate anhydrous buffer as mobile phase A and Sodium dihydrogen phosphate anhydrous buffer, methanol, and Isopropyl alcohol in the ratio of 600:300:100 v/v as mobile phase B.

Cumulative *In-vitro* Drug release Studies: *In-vitro* drug diffusion study was performed using Franz diffusion apparatus. The cellulose nitrate membrane was used as a diffusion membrane. Prior to the experiment the dialysis membrane was soaked in phosphate buffer pH 6.8 for 12 h. phosphate buffer pH 6.8 was used as receptor medium. RPM was set to 500 and temperature was

34±2. The samples withdrawn for every 1 h up to 8 h and samples were filtered and used for analysis. Chromatographic conditions were same as drug content assay.

***Ex-vivo* Drug Release Study:** Materials required were procured from local slaughterhouse. Nasal mucosa from the olfactory region of goat was carefully extracted. Prior to the experiment the nasal mucosa was soaked in a phosphate buffer pH 6.8 for 6 h. The samples withdrawn for every 1 h up to 5 h and samples were filtered and analysed.

Drug Toxicity Studies: Extent of drug irritation through the nose is studied using histopathological studies on goat's nasal mucosa. The tissue which was used in *ex-vivo* study is placed on the glass slide. The tissue was stained using eosin and it was covered with cover slip. The tissue sections were subjected to light microscopy analysis to identify any tissue damage. Isopropyl alcohol was used as a positive control, while phosphate buffer pH 6.8 was used as the negative control.

Stability Study: The short term stability study was conducted at refrigerated condition, room temperature and elevated temperature.

The formulations were packed in 5 different VP 7 multi dose spray pump bottles. The stability was assessed at 0,1,2,4 weeks. The formulations were assessed in terms of appearance, pH, viscosity and drug content.

RESULTS AND DISCUSSION: In this project nasal *in-situ* gels of levodopa and carbidopa was successfully formulated. The results obtained on evaluation of formulations are shown below

Evaluation of Placebos:

Clarity, Texture, Viscosity and Mucoadhesive Strenght: The placebos F1 to F12 were evaluated for clarity and texture. The results of clarity, texture, viscosity and mucoadhesive strength are shown in the **Table 3.**

TABLE 3: EVALUATION DATA OF CLARITY, TEXTURE, VISCOSITY, MUCOADHESIVE STRENGTH OF PLACEBOS F1 TO F12

Formulation	Clarity	Texture	Viscosity(cps)		Mucoadhesive strengths (g)
			Before gelation	After gelation	
F1	Clear	Soft gel	189 ± 0.5	2520 ± 0.5	29 ± 0.5
F2	Clear	Viscous gel	209 ± 0.5	3214 ± 0.5	36 ± 0.5
F3	Clear	Viscous gel	429 ± 0.5	4571 ± 0.5	50 ± 0.5
F4	Clear	Loose watery matrix	135 ± 0.5	2221 ± 0.5	21 ± 0.5
F5	Clear	Loose watery matrix	171 ± 0.5	2377 ± 0.5	29 ± 0.5
F6	Clear	soft gel	324 ± 0.5	2434 ± 0.5	40 ± 0.5
F7	Clear	Loose watery matrix	193 ± 0.5	2042 ± 0.5	23 ± 0.5
F8	Clear	Loose watery matrix	240 ± 0.5	2192 ± 0.5	20 ± 0.5
F9	Clear	Soft gel	290 ± 0.5	2558 ± 0.5	32 ± 0.5
F10	Clear	Loose watery matrix	224 ± 0.5	2210 ± 0.5	20± 0.5
F11	Clear	Soft and firm gel	263 ± 0.5	2780 ± 0.5	42 ± 0.5
F12	Clear	Viscous gel	340 ± 0.5	3980 ± 0.5	54 ± 0.5

Formulation F1, F2, F3 are temperature and pH sensitive *in-situ* gels and are prepared using poloxomer and polycarbophil. With increase in the concentration of the poloxomer from 16% to 18% the viscosity of the gel improved. F3 which contains 18% polymer had highest viscosity of 4571 cps which correlates with its increased mucoadhesive strength of 50 ± 0.5 g. The texture of the gel F3 is hard and firm. F1 and F2 the gels were less viscous and lacked sufficient mucoadhesive strength. Formulations F4, F5, F6 are ion sensitive gels and are prepared using xanthan gum and HPMC. With the increase in the concentration of HPMC from 0.2% to 0.4% the viscosity of the gel improved. F6 which contains 0.2% xanthan gum and 0.4% HPMC had highest viscosity of 2434 cps which correlates with its increased mucoadhesive strength of 40g. The texture of the gel F6 is soft but lacked firmness. F4 and F5 the gels were less viscous and lacked sufficient mucoadhesive strength. Formulation F7, F8, F9 are pH sensitive polymers and are prepared using polycarbophil and HPMC. With increase in concentration of HPMC from 0.2% to 0.4% the viscosity of the gel improved. F9 which contains 0.5% polycarbophil and 0.4% HPMC has viscosity of 2558 cps which

correlates with its increased mucoadhesive strength of 32g. F7 and F8 the gels were less viscous and lacked sufficient mucoadhesive strength. F10, F11, F12 are pH and ion sensitive *in-situ* gels and are prepared using polycarbophil and xanthan gum. With the increase in concentration of polycarbophil from 0.1% to 0.4% the viscosity of the gel improved. F11 which contains 0.3% polycarbophil and 0.2% xanthan gum has viscosity 2780 cps which correlates with its mucoadhesive strength of 42g. F10 the gel lacked consistency and F12 the gel formed very hard and firm gel. Out of all the placebos compositions, the concentration of polymers incorporated in F11 yielded a gel which is firm and possessed good mucoadhesive strength.

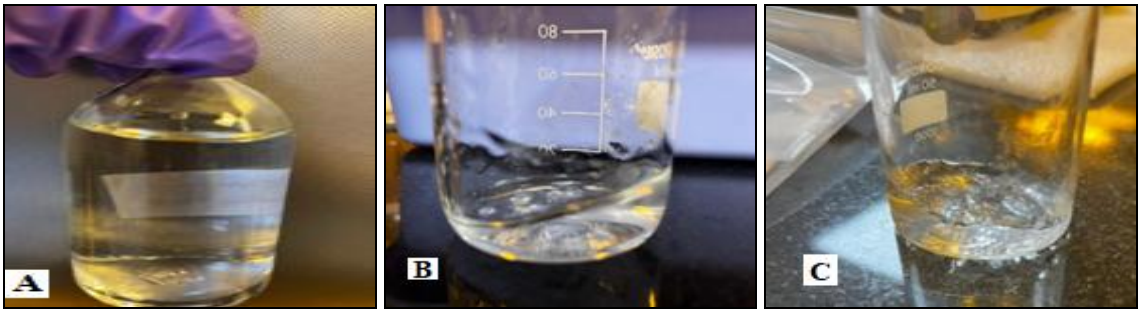

FIG. 1: A- SOLUTION OF FORMULATION F11 BEFORE CONVERTING INTO GEL, B- SOFT AND FIRM GEL FORMED IN FORMULATION F11, C- HARD GEL FORMED IN FORMULATION F3

pH: The placebos F1 to F12 were evaluated for pH using digital pH meter. pH obtained shown in the **Table 4.** The pH of all formulations were adjusted to 5 using 0.5M sodium hydroxide. The range of results is found to be within the nasal pH range, i.e 5 to 6.5.

TABLE 4: PH OF F1 TO F12

Formulation	pH before gelation	After gelation
F1	5.2	5.23
F2	5.4	5.38
F3	5.29	5.3
F4	5.3	5.3
F5	5.13	5.2
F6	5.32	5.29
F7	5.2	5.24
F8	5.14	5.2
F9	5.31	5.3
F10	5.1	5.16
F11	5.29	5.3
F12	5.24	5.3

Gelation Time: The gelation time was measured for each formulation by placing 1 drop of the prepared formulation into stimulated nasal fluid. The results are shown in **Table 5.** Literature indicated the gelation time in the range of 25 to 50 seconds to be appropriate for *in-situ* nasal gel and if it is more than 50 s they might demonstrate lack of structural integrity leading to quick mucociliary clearance [7]. Formulations F4, F5, F7, F8 and F10 showed gelation time above 120 s as they lack consistency, Formulations F1, F6, F9 showed gelation time between 90-110 s and formed soft gels. Formulations F2, F3, F12 showed gelation time between 50-80 s and formed hard gels. But formulation F11 showed optimum gelation time of 32 s and formed soft and firm gel which is satisfactory.

TABLE 5: GELATION TIME OF THE PLACEBOS F1-F12

Formulation	Gelation time(s)
F1	110± 0.5
F2	80± 0.5
F3	60± 0.5
F4	165± 0.5
F5	124± 0.5
F6	98± 0.5
F7	135± 0.5
F8	120± 0.5
F9	90± 0.5
F10	122± 0.5
F11	32± 0.5
F12	53± 0.5

Evaluation of Levodopa and Carbidopa *In-situ* Gel: The placebos F1 to F12 were evaluated for clarity, texture, pH, gelation time, viscosity and, mucoadhesive strength. Out of 12 placebos formulation F11 showed satisfactory results. Therefore, F11 formulation was selected for preparation of levodopa and carbidopa nasal in situ gel. The formulated gel was evaluated for various parameters and the results are shown in the below **Table 6.**

TABLE 6: EVALUATION DATA OF LEVODOPA AND CARBIDOPA *IN-SITU* GEL

Parameters	Results
Clarity	Clear
Texture	Soft and firm
pH	5.29
Gelation time(s)	33
Viscosity (cps)	Viscosity of solution – 270 Viscosity of gel – 2498
Mucoadhesive strength(g)	44

The results correlates with that of F11.

Assay: Assay of Levodopa and Carbidopa was determined by a reverse phase HPLC method using C18 column. 6 injections of standard solution containing 0.075g of levodopa and 0.050g of carbidopa were injected. The chromatograms of standard and sample are shown in figure2 and standard and sample chromatograms data is shown in **Table 7** and **8** respectively.

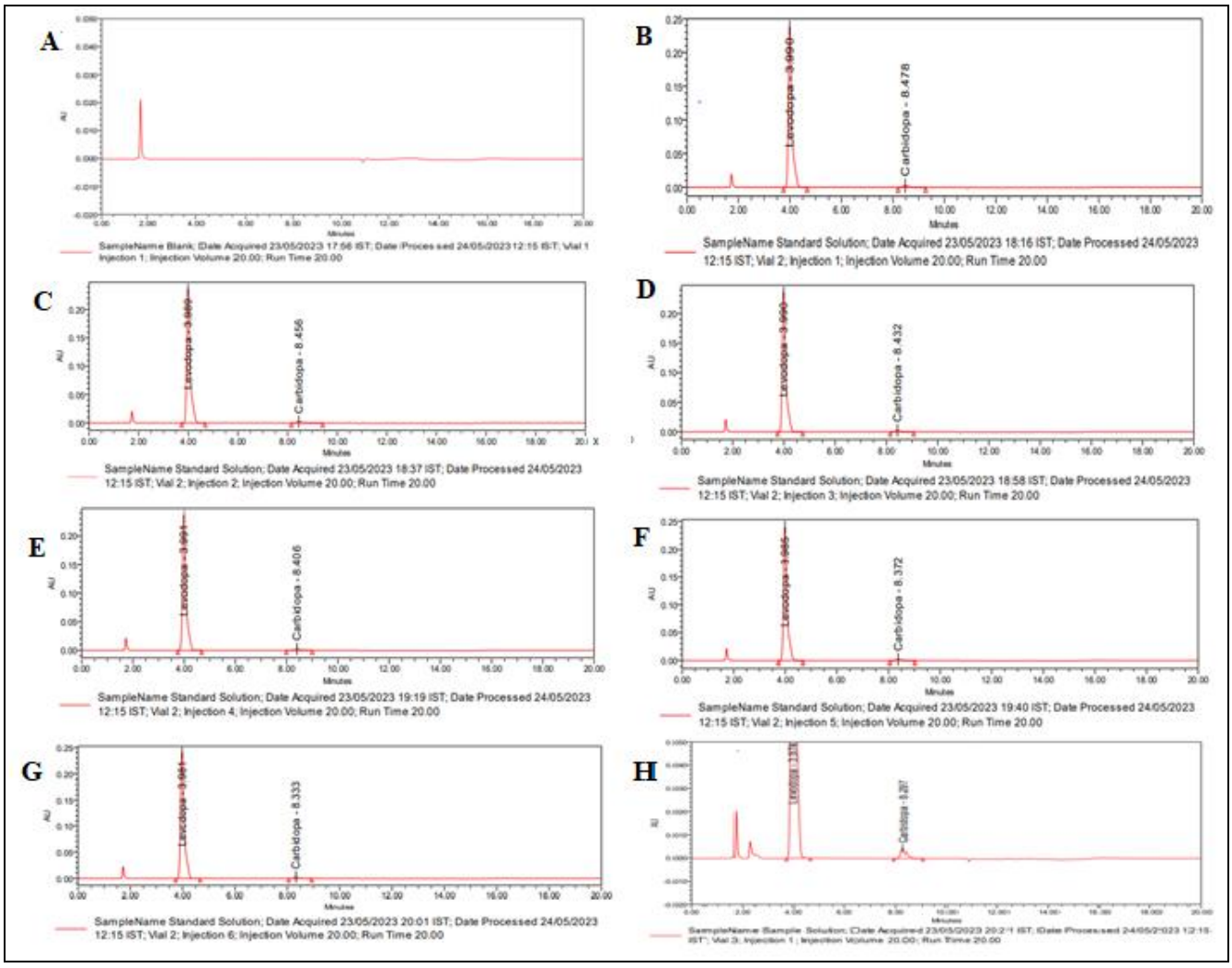

FIG. 2: ASSAY CHROMATOGRAMS: (A) BLANK; (B)STANDARD INJECTION 1; (C) STANDARD INJECTION 2; (D) STANDARD INJECTION 3; (E) STANDARD INJECTION 4; (F) STANDARD INJECTION 5; (G) STANDARD INJECTION 6; (H) SAMPLE

TABLE 7: CHROMATOGRAM DATA OF STANDARD

Standard										
Drug	**Levodopa**					**Carbidopa**				
No of injection	**Area**	**Rt**	**Usp tailing**	**Usp plate count**	**Height**	**Area**	**Rt**	**Usp tailing**	**Usp plate count**	**Height**
1	2262282	3.990	1.7	4932	239415	39454	8.478	1.6	8438	2575
2	2287008	3.989	1.7	4707	236968	40316	8.456	1.6	8192	2575
3	2269055	3.990	1.7	4761	237214	38960	8.432	1.5	8445	2597
4	2267697	3.991	1.6	4707	237931	39292	8.406	1.5	8437	2620
5	2284794	3.985	1.6	4741	240190	39370	8.372	1.5	8517	2664
6	2281693	3.981	1.6	4739	240190	39404	8.333	1.5	8602	2690
Mean	2275422					8.413				
Std. Dev.	0.004					0.054				
RSD	0.10					0.64				

TABLE 8 CHROMATOGRAM DATA OF SAMPLE

S. no.	Drug	RT	Area	%Area
1	Levodopa	3.974	2454023	97.48
2	Carbidopa	8.297	63549	14.9

Assay was calculated using formula:

Drug content = Test peak area / Standard peak area × Standard wt. (g) / Standard dilution × Test dilution / Test wt. (g) × Purity of Std. / Lable claim of formulation (mg

The assay of levodopa and carbidopa was found to be 101.0 and 99.16 respectively. Which is within the acceptable range (80% to 120%).

Related Substance: Related substance study is used to identify levodopa and carbidopa's known and unknown impurities. Chromatograms are shown in **Fig. 3** and evaluation data is shown in **Table 9.**

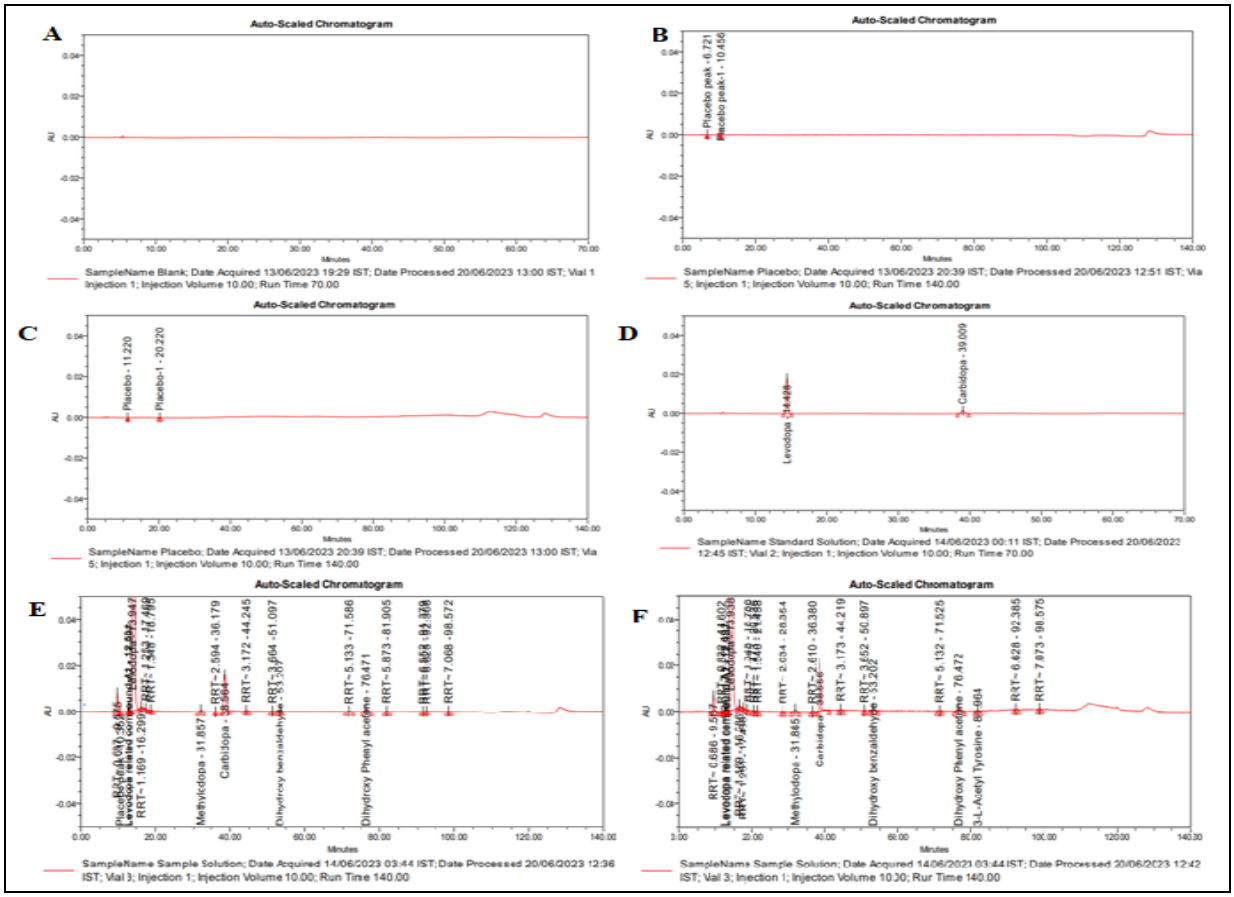

FIG. 3: RELATED SUBSTANCE CHROMATOGRAMS: (A) BLANK; (B) PLACEBO AT 280 NM; (C) PLACEBO AT 235 NM; (D) STANDARD SOLUTION; (E) SAMPLE SOLUTION AT 280 NM; (F) SAMPLE SOLUTION AT 235 NM

TABLE 9: IMPURITIES OF LEVODOPA AND CARBIDOPA

Impurities	Carbidopa	
	235nm	280nm
Methyldopa	0.1	0.1
Dihydroxy benzaldehyde	0.1	0.1
Dihydroxy Phenyl acetone	0.2	0.2
RRT~5.873	0	0.1
RRT~6.623	0.1	0
Total Impurities	0.5	0.5
% Purity	99.5	

Impurities	Levodopa	
	235nm	280nm
RRT~0.686	0.3	0.4
Levodopa related compound A	0.1	0.1
Levodopa related compound B	0.1	0.1
RRT~1.169	0.1	0.1
3-L-Acetyl Tyrosine	0.1	0
Total Impurities	0.7	0.7
% Purity	99.3	

The chromatogram of blank and standard solution is shown in **Fig. 4A** & **D**. It is observed from the figure that the chromatogram of the standard solution and diluents did not show any extra peaks. Thus, formulations were further subjected to the described methodology. The sample's chromatogram shows that the impurities were well separated, and the peak purity data shows that there were no co-eluting peaks or impurity interference during the retention period. The total impurities of levodopa and carbidopa are 0.7 and 0.5 respectively. The Indian Pharma-copoeia states that the related substance should be ≤2% for major analytes and 5-10% for low level impurities. Therefore, the total impurities are within the acceptable range.

Cumulative *In-vitro* Drug Release: The *in-vitro* release was carried out for the formulated *in-situ* gel using phosphate buffer pH 6.8 as receptor medium. The chromatogram data of Levodopa and Carbidopa is shown in table 10 and 11. Percentage drug release of levodopa and carbidopa is shown in **Fig. 4** data is shown in **Table 12.**

TABLE 10: LEVODOPA CHROMATOGRAM DATA

Levodopa Cumulative *in-vitro* drug release results								
Standards		**Cell Orifice Dia. (cm)**	**0.9**		**Orifice Area**		**0.636**	
Injection	**Area**	**Total Volume Removed**	**1.000**		**Standard Conc. (mg/mL)**		**1.00000**	
1	3477433							
2	3479949							
3	3480953		Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
4	3477351	Cell volume	10.000	10.000	10.000	10.000	10.000	10.000
5	3485198	Time (min)				Peak Area		
6	3475425	60	405493	413662	410167	419973	419832	410632
		120	556492	583206	534810	669186	677181	563676
		180	789606	758444	756924	839220	840391	838391
Average	3479385	240	862211	862211	845524	898742	948233	923229
RSD	0.10	300	879915	804671	851541	945995	1051378	977701
		360	926857	944478	834249	976049	1139126	972672
		420	965414	1030501	895489	1001992	1141494	1008794
		480	1321223	1131468	966740	1815226	1128003	1250903

TABLE 11: CARBIDOPA CHROMATOGRAM DATA

Carbidopa Cumulative in-vitro drug release results								
Standards		**Cell Orifice Dia. (cm)**	**0.9**		**Orifice Area**		**0.636**	
Injection	**Area**	**Total Volume Removed**	**10.000**		**Standard Conc. (mg/mL)**		**0.10000**	
1	183536							
2	182869		Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	Cell 6
3	182856	Cell Volume	10.000	10.000	10.000	10.000	10.000	10.000
4	183037							
5	183150	Time (min)				Peak Area		
6	182262	60	10259	10610	10998	10935	10896	11281
		120	14198	15273	14087	14087	14980	14855
		180	18935	18214	19542	21109	21323	20605
Average	182952	240	20810	17476	21628	22259	24213	23383
RSD	0.23	300	20579	19377	21212	23049	26310	24222
		360	21198	21959	20044	22790	28350	23460
		420	22279	24938	22026	23903	28538	24280
		480	32410	27493	23693	47906	27844	31275

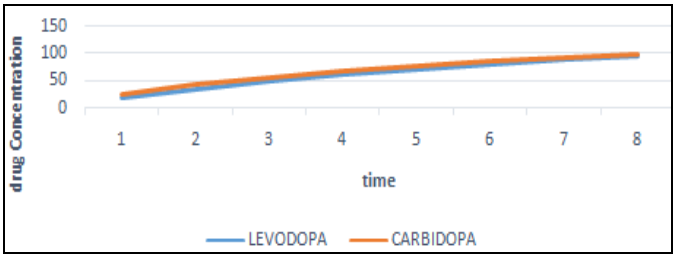

FIG. 4: CUMULATIVE *IN-VITRO* DRUG RELEASE

TABLE 12: PERCENTAGE DRUG RELEASE OF LEVODOPA AND CARBIDOPA

Time	% drug Release of Levodopa	% drug Release of Carbidopa
0	0	0
1	18.65	25.2
2	34.04	42.5
3	49.37	55.62
4	63.3	68.64
5	72.34	77.92
6	80.2	87.71
7	88.12	94.43
8	95.7	99.38

The cumulative drug release of levodopa and carbidopa are 95.7 and 99.38% respectively. Formulation is able to release the drug at a prolonged rate for 8 hours.

***Ex-vivo* Drug Release:** *Ex-vivo* permeation was observed for the optimised formulation F11 by using goat nasal epithelial membrane. The *ex-vivo* drug release data of Levodopa and Carbidopa is shown in **Fig. 5** and **Table 13.**

TABLE 13: *EX-VIVO* DRUG RELEASE OF LEVODOPA AND CARBIDOPA

Time	% drug Release of Levodopa	%drug Release of Carbidopa
0	0	0
1	20.45	19.2
2	42.9	32.2
3	58.2	46.8
4	65	59.3
5	74.6	69.9
6	89.1	83.2
7	97.9	99.2

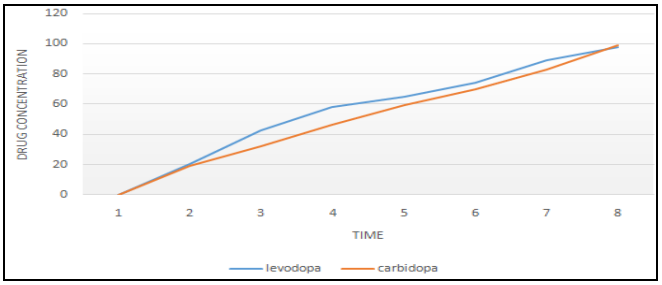

FIG. 5: *EX-VIVO* DRUG RELEASE OF LEVODOPA AND CARBIDOPA

The drug release of levodopa and carbidopa is 97.9 % and 99.2% within 7 hours which is satisfactory.

Drug Toxicity Studies: Drug toxicity studies were performed using light microscope. Histopatho-logical results of goat nasal mucosa are shown in **Fig. 6**. Isopropyl alcohol was used as a positive control, while phosphate buffer pH 6.8 was used as the negative control**.**

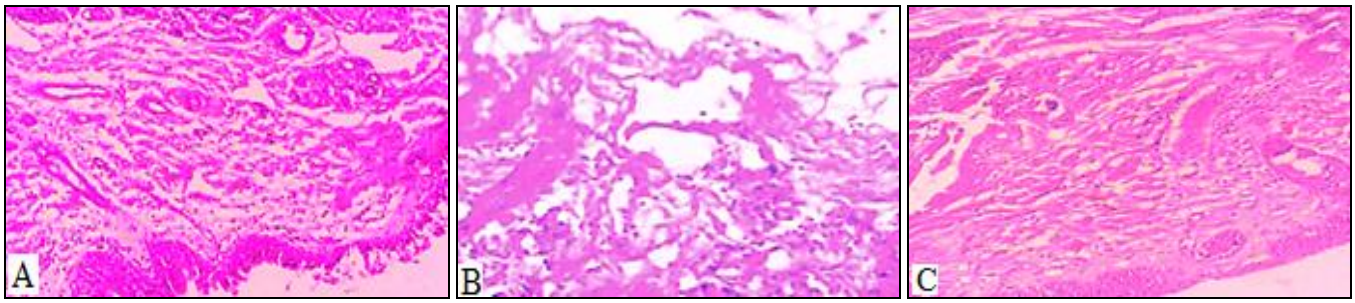

FIG. 6: (A) HISTOPATHOLOGY CONDITION OF GOAT NASAL MUCOSA AFTER TREATMENT WITH PHOSPHATE BUFFER; (B) HISTOPATHOLOGY CONDITION OF GOAT NASAL MUCOSA AFTER TREATMENT WITH PROPYL ALCOHOL; (C) HISTOPATHOLOGY CONDITION OF GOAT NASAL MUCOSA AFTER TREATMENT WITH LEVODOPA CARBIDOPA *IN-SITU* GEL

The microscopic study reveals that the formulation has did not inflict any structural damage to the nasal mucosa, as shown in **Fig. 6A** & **C**. The mucosa when treated with isopropyl alcohol, separation of cilia, cell necrosis, and expansion of epithelial cell was observed, which Indicates severe mucosal injury as shown in **Fig. 6B**. therefore from the above results it is clear that the prepared formulation is non-irritant and did not show any structural damage.

Stability Studies: Accelerated short term stability studies were assessed for 4 weeks. The samples were examined for physical state, pH, viscosity and assay. **Table 14** depicts the stability data.

TABLE 14: STABILITY STUDY DATA

			Assay	
Time period	**Description**	**pH**	**Levodopa**	**Carbidopa**
Initial				
	Clear	5.2	99.16	101.0
15 days				
4 °C ± 1 °C	Clear	5.1	100.708	95.3626
RT (25 °C ± 2 °C)	Clear	5.2	97.4901	92.0573
40 °C	Slightly yellow	5.6	87.5937	88.1639
30 days				
4 °C ± 1 °C	clear	5.1	101.42	92.8464
RT (25 °C ± 2 °C)	Slightly yellow	5.3	96.7579	90.5051

It is observed from the results that the assay of the formulation at 40ºC is decreasing and the colour of the formulation turned slightly yellowish. Which indicates that the formulation at 40ºC is degrading. Formulation stored at room temperature has no significant change in assay but after 30 days the formulation turned slightly yellow in colour. There is no significant change in the description, pH and assay when the formulation is stored in 4°C.

CONCLUSION: The aim of the research work was to formulate and evaluate nasal *in-situ* gel containing levodopa and carbidopa.

The concentration of polymer to be employed for the development of the formulations was deduced by carrying out placebo studies (F1-F12). Placebos from F1 to F12 were assessed for various parameters such as pH, viscosity, and gelation time, mucoadhesive strength. Levodopa and carbidopa nasal *in-situ* gel produced soft and firm gel at pH 5.29 with gelation time 33 s and viscosity of the solution was 270cps and viscosity of gel is 2498 cps and mucoadhesive strength was found to be 44g. Assay of formulation F11 was found to be 95.7% of levodopa and 100.83% of carbidopa levodopa and carbidopa's known and unknown impurities are identified using related substance study. The total impurities of levodopa and carbidopa are 0.7 and 0.5 respectively. The *in-vitro* release was carried out using a Franz diffusion cell. The drug release of Levodopa and Carbidopa after 8 h is 95.7% and 99.38%, respectively. *Ex-vivo* drug release is 97.9 % and 99.2% within 7 hours for levodopa and carbidopa respectively. The drug toxicity study on nasal mucosa revealed that there was no cell necrosis and no loss of the epithelium. The stability data showed that the formulation is stable at 4°C. These findings showed that the *in-situ* gel was able to maintain its viscosity over time and was able to release the drug at a prolonged rate. These results suggest that the developed Levodopa and carbidopa nasal *in-situ* gel may be the promising drug delivery for the treatment of Parkinson's disease.

The objective of this research work has meet as a stable nonirritant levodopa and carbidopa nasal *in-situ*gel was formulated. This study lead to development of successful nasal *in-situ* gel. In order to make a major contribution to the treatment of Parkinson's disease, the focus of research in the future should be on *in-vivo* profiling of levodopa and carbidopa nasal *in-situ* gels.

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CONFLICTS OF INTEREST: Nil

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