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Development and Evaluation of Nasal Mucoadhesive in Situ Gel Formulations of Carbamazepine Using In Vitro, Ex-Vivo and In-Vivo Methods

Sonali A. Nagare[1*], Jeevan R. Rajguru[2], Vikas G. Rajurkar[3], Mrunal K. Shirsat[4]
[1]Department of Pharmaceutical Chemistry, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Shirur, Pune, Maharashtra, India
[2]Department of Pharmaceutics, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Shirur, Pune, Maharashtra, India
[3]Department of Pharmaceutics, Vedprakash College of Pharmacy, Aurangabad, India
[4]Department of Pharmacognosy, Loknete Shri Dadapatil College of Pharmacy Mandavgan Pharata, Shirur, Pune, India
[*]Corresponding Author Email ID: sonalinagare93@gmail.com
Co-Author Email ID: jeevanrajguru97@gmail.com

Abstract*—The objective of the present research work was to develop and evaluate the nasal in situ gel formulations of Carbamazepine for better availability in the brain. Mucoadhesive in-situ gelling Carbamazepine formulation were successfully prepared by the spontaneous emulsification method (titration method) using Capmul MCM as the oil, Tween-80 as surfactant, and PEG-600 as co-surfactant phase, 0.5 % (W/W) mucoadhesive in-situ gelling polymer (Deacetylated Gellan gum) on the basis of solubility studies. Formulations were evaluated for gelation study, viscosity study, gel strength, mucoadhesion study, Drug content, permeation study through sheep nasal mucosa, histopathological evaluation of mucosa and pharmacodynamics study in rats, stability study. In-vitro and ex-vivo permeation studies showed an initial burst of drug release at 60 min and in-situ gelling mucoadhesive Carbamazepine formulation show diffusion of 94.30 ±0.01 % drug in 360 min, attributable to the presence of free drug entrapped in the in-situ gelling mucoadhesive layer. In vivo pharmacokinetic studies in rats showed that mucoadhesive in-situ gelling mucoadhesive Carbamazepine enhanced brain and plasma concentrations of Carbamazepine. Histopathological study did not show any damage to the nasal mucosa during permeation. The Anticonvulsants effect of Carbamazepine differed significantly by I.N and I.V routes compared to control. It can be concluded that Carbamazepine given by nasal route is more effective and show quick onset of action when compared to Intravenous administration of equivalent dose*.

***Keywords*—** *Mucoadhesive Nasal in situ gel, Gellan gum, Blood brain barrier*.

I. INTRODUCTION

Central nervous system disorders still remain as the world's leading cause of disability, although extensive research has been done to deliver therapeutics to the brain. Brain is the most important organ of human body and is protected by blood brain barrier (BBB) and blood cerebro spinal fluid barrier Treating CNS disorders and ailments in general which include Alzheimer's disease, Parkinson's, Schizophrenia, Stroke, Epilepsy, AIDS dementia complex, Brain tumor and Huntington disease is extremely challenging due to the presence of various obstacles that restrict passage of drug across the BBB for onward delivery to the brain. Status epilepsy is a serious neurological emergency characterized by severe bouts of seizures. It requires rapid termination of seizure activity because if the episode of epilepsy remains untreated, it may lead to a permanent damage to the brain. About 50 million people worldwide suffer from epilepsy and nearly two out of every three new cases are reported in developing countries. Epilepsy is more likely to occur in young children or people over the age of 65 years however it may occur at any age. Treatments of epilepsy include treatment with antiepileptic drugs or surgery. Carbamazepine is a major antiepileptic drug for the treatment of different form of seizures. Currently Carbamazepine is available only in the form of oral dosage forms including tablets, capsules, suspensions etc. The major limitation with Carbamazepine oral formulation is its slower and erratic absorption and thus a novel formulation of Carbamazepine to overcome the stated limitation is mandatory. Carbamazepine transnasal formulations are not available in market. Some researchers have worked on Carbamazepine nasal formulations but thorough characterization with pharmacodynamics studies has not been reported by the researchers as per the authors' knowledge. Carbamazepine aqueous solubility is very poor and enhancement is necessary for intranasal delivery of Carbamazepine as nasal delivery cannot permit administration of large volumes of liquids. Polymers can increase nasal absorption of drugs with several ways such as increasing the residence time of drug in the nasal cavity. Poloxamers or pluronics are triblock copolymers which form micelles at low concentration and clear, thermo reversible gel at high concentrations. The concentrated solutions (15-30%) are transformed from low viscosity transparent solutions at 50C to solid gel on heating to body temperature. By modulating the gelation temperature of different PF127 solutions, liquid bases for nasal use can be formulated that form a gel in nasal cavity at body temperature resulting in enhancement of residence time in the nasal cavity. Possible strategy used to decrease the mucociliary clearance by use of gel/ Mucoadhesive formulation to prolong nasal residence time. Nevertheless, the most prominent advantage of

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the in-situ gel over the silent gel is that it is fluid like prior to contact with nasal mucosa, a feature that offers the convenience of administration for patients since it can be easily instilled as a drop allowing accurate drug dosing.

II. MATERIALS AND METHOD

Carbamazepine was received as a gift sample from Macleods Pharmaceuticals Limited (Daman), Tween 80, polyethylene glycol 200, 400, 600 and 800 were purchased from LOBA Chem (Mumbai, India) and Capmul MCM EP oil was received as a gift sample from Abitec USA. Gellan Gum was received gift sample of CP Keloco, Pvt. ltd. Xanthan Gum, Alginate was received gift sample of Loba Chem, Mumbai. Eosin was received as a gift sample Dipa Chemical, Aurangabad. Hematoxylin was received as a gift sample Molychem lab, Mumbai.

Formulation and Development of Mucoadhesive In-Situ Gelling Carbamazepine Nanoemulsion

Selection of in-situ Gel forming polymer for formulation of mucoadhesive in-situ gelling Carbamazepine

TABLE 1. Gelling agent and condition require for gel forming.

Sr. No.	Gelling agent	Condition require for gel forming
1	Deacetylated gellan gum	Ion-Mediated gel forming
2	Xanthan gum	Ion-Mediated gel forming
3	Poloxamer-407	Temperature mediated gel forming (Gel form at 32-37 ^{0}C)
4	Poloxamer-188	Temperature mediated gel forming (Gel form at 32-37 ^{0}C)

Selection of *in-situ* gel forming polymer for formulation of mucoadhesive *in-situ* gel nanoemulsion done by on the basis of their condition require for gel forming, time require for gel forming and stability of gel. Poloxamer-407 and 188 not use for gelling polymer because it form gel by temperature mediated mechanism so if these polymer used as gelling agent so we require the formulation store in the cooler place. So poloxamer-407 and 188 rejected for the as gelling agent. Gellan gum and xanthan gum both gum formed gel by ion-mediated mechanism but xanthan gum did not show the long thermo stability so it rejected for as gelling agent. Only gellan gum show the stable gel for long time and gel form by contact with ion. Hence gellan gum used as *in-situ* gelling agent because formulation was given via intranasal route. Hence ion present in nasal mucosa fluid (Like Sodium, calcium, chloride) was sufficient for forming *in-situ* gel of nanoemulsion formulation after taken intranasal route.

Gelation Study:

Preparation of simulated nasal fluid:

TABLE 2. Preparation formula of simulated nasal fluid.

Sr.No.	Ingredients	Quantity (mg)
1	Sodium chloride	692
2	Potassium chloride	307.5
3	Calcium chloride	26.66
4	Sodium lactate	310
5	Water	q.s to 100 mL

Simulated nasal fluid prepared by simply mixing above ingredients in double distilled water.

Gelation study of deacetylated gellan gum:

The Simulated nasal fluid, SNF having the cationic composition of nasal secretions, was prepared according to the report. Gellan gum is a polymer which undergoes change from sol to gel in the presence of cations. Gelation is the process by which the liquid phase (sol) makes a transition into gel. Different DGG solution concentrations (0.1 %–1.0 %, 1.0mL) were mixed with 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 mL of simulated nasal fluid in vial. Vial placed on water bath at 32°C. After 20s, the vials were turned over. If the gels adhered to the vial instead of flowing or sliding down the side, the formulation showed gel formation. The coding for observation was given as below

-No Gelation
+Gelation occurred in few min and reminded for few h
++Gelation immediate, remained for h
+++ Gelation immediate and for extended period
++++very stiff gel

Determination of mucoadhesive strength:

The mucoadhesive strength was determined by using the modified method. The mucoadhesive potential of each gel ratio and formulation was determined by measuring a force required to detach the formulation from nasal mucosal tissue. A section of sheep nasal mucosa was fixed on each of two glass slides using thread. 50 mg of gel was placed on first slide and this slide placed below the height adjustable pan. While another slide with mucosal section was fixed in inverted position to the underside of the same pan. Both the slides with gel formulation between them held in contact with each other, for 2min to ensure intimate contact between them. Then weight was kept rising in second pan until slides get detached from each other. The mucoadhesive force expressed as the detachment stress in dynes/cm^2 was determined from the minimal weight that detached the mucosal tissue from surface of ratio and each formulation.

Mucoadhesive strength (dynes/cm^2) = mg/A,

Where, m = Weight required for detachment in gram, g= Acceleration due to gravity (980cm/s^2), A = Area of mucosa exposed.

Tests for nasal cilio toxicity of Mucoadhesive in-situ gel:

Freshly excised sheep nasal mucosa, except for the septum, will be collected from the slaughter house in saline phosphate buffer (pH 6.4). Three sheep nasal mucosa pieces (P1, P2, and P4) with uniform thickness were selected and mounted on Franz diffusion cells. P1 was treated with 0.5 mL of PBS (pH 6.4, negative control), P2 with 0.5 mL of isopropyl alcohol (Positive control), P4 was treated with mucoadhesive *in-situ* gelling carbamazepine formulation for 1 h. After 1 h, the mucosa were rinsed with PBS (pH 6.4) and subjected to histological studies by stained with using eosin-hematoxylin stain to evaluate the toxicities of nanoemulsion formulation and photographed by using digital microscopy (Motic Electronic, China).

Pharmacodynamics Activity:

Anticonvulsant activity:

The anticonvulsant activity of mucoadhesive *in-situ* gelling Carbamazepine formulation was studied against

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maximal electro-shock-induced convulsion in rats. Different types of epilepsies, i.e., grand mal, petit mal or psychomotor type, can be studied in laboratory animals. The maximal electro-shock (MES)-induced convulsions in animal represent grand mal type of epilepsy. In MES-convulsions electroshock is applied through the corneal electrodes. Through optic stimulation cortical excitation is produced. The MES-convulsions are divided in five phases such as a) tonic flexion, b) tonic extensor, c) clonic convulsions, d) stupor and d) recovery or death. A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES-convulsions.

In-vivo study (Nasal absorption and brain distribution studies):

Male Albino- Wistar rats weighing 250–280 g were obtained from the animal house of MES's College of Pharmacy, Sonai. Animal housing and handling were performed in accordance with good laboratory practice (GLP) mentioned in CPCSEA guidelines. All experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee prior to initiation of the experiment. The animals were housed in polypropylene cages (3 animal / cage) and placed in the experimental room where they were allowed to acclimatize for a week before experiment. A 10% air exhaust conditioning unit was maintained along with a relative humidity of 60±5 % and a temperature of 25±5 ^{0}C in the animal house facility. Alight/dark cycle of 10h / 14h was also regulated for the experimental animals. The animals were dived into 3 groups and kept in fasting condition over night before the experiment commenced. The 1st group was dosed with blank nanoemulsion, 2ndgroup with *in-situ* gelling Carbamazepine mucoadhesive formulation. For the intranasal administration, 0.30 mL of the nasal formulations was administered via a polyethylene 10 tube attached to a microliter syringe inserted 1cm into each nostril of rat at a dose of 5 mg/kg. The blood samples of 0.5 mL were withdrawn from each rat through retro-orbital sinus at 0, 1, 3, 6, and 7 h post-dose, collected into heparinized micro centrifuge tubes and centrifuged at 4000 rpm for 15 min. The animals were decapitated immediately after the blood collection and brain was excised at the above mentioned time points. Each brain tissue was quickly rinsed with saline and blotted up with filter paper to get rid of blood-taint and macroscopic blood vessels as much as possible. After weighing, the brain tissue samples were homogenized with 1 mL of saline at 10,000 rpm for 1 min over ice using a tissue homogenizer. The nasal mucosa was excised and homogenized in the similar process as that of brain samples. All the samples were kept frozen at prior to RP-HPLC analysis.

Estimation of drug content in the brain by HPLC method:

Male Albino- Wistar rats (n = 6) weighing between 200 ± 10 g were selected for the present study. The dose of CBZ nanoemulsion and selected nanoemulsion (0.1 mL) was administered at same dose of 1 mg/mL through the intranasal route with the help of 18/20 gauze cannula fitted in the 1 mL syringe and the rats were kept in upright position at an angle of 90◦, so that maximum drug concentration can reach to the brain. After 20 min of intranasal dosing the animals were sacrificed under ether anesthesia and the whole brain was excised, isolated and weighed. The brain tissue was then homogenized in 1% acetic acid using tissue homogenizer, and particulate matter removed by centrifugation and filtration. The clarified supernatant was analyzed for drug content in brain via HPLC method. The mobile phase consisted of Water: Methanol (50:50 v/v). The mobile phase was set at a flow rate of 1 mL/min. The run time of the sample was kept 10 min. The ultra-violet detection of Carbamazepine was performed at 285 nm. Pharmacokinetic parameters were evaluated using PK solver software (non-compartmental modeling). Pharmacokinetic parameters such as C_{max}, t_{max}, $t_{1/2}$, $AUC_{0\rightarrow 24}$, $AUC_{0\rightarrow\infty}$ and MRT were calculated for mucoadhesive *in-situ* gelling Carbamazepine formulation group.

Stability Study:

The shelf-life stability of mucoadhesive *in-situ* gelling Carbamazepine formulation as a function of time and storage temperature was routinely checked by visual inspection of the samples initially on a daily and later on a weekly basis. Stable systems were identified as those free of any physical change, such as phase separation, flocculation or precipitation and also check other parameter such as a percent transmittance, drug content, pH, Viscosity, conductivity, refractive index of *in-situ* gelling Carbamazepine mucoadhesive formulation at 15 day interval time period. Stability was monitored at, 37±3 ^{0}C.

Preparation of stock and standard dilutions of Carbamazepine for RP-HPLC analysis

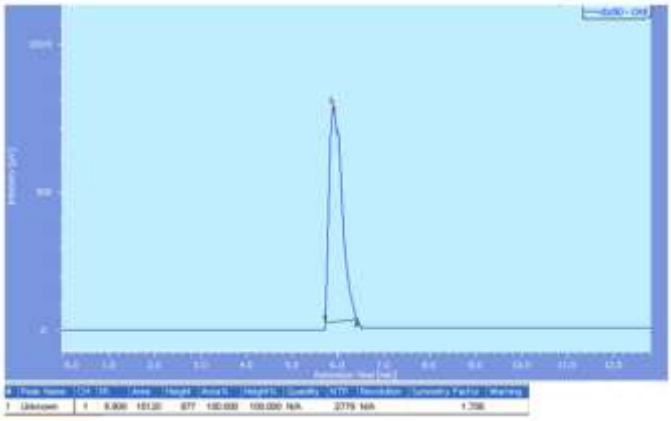

Fig. 1. RP-HPLC spectra of carbamazepine.

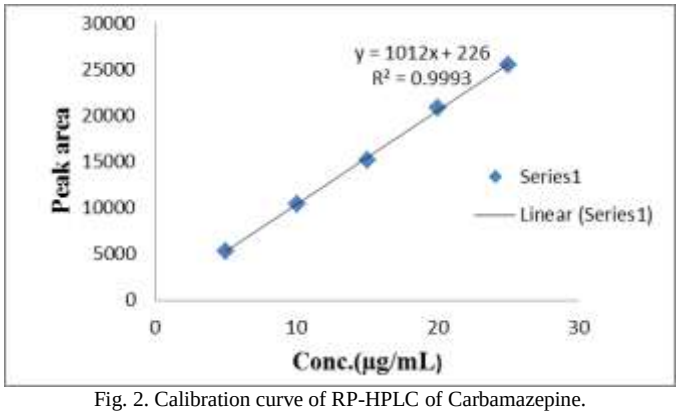

Fig. 2. Calibration curve of RP-HPLC of Carbamazepine.

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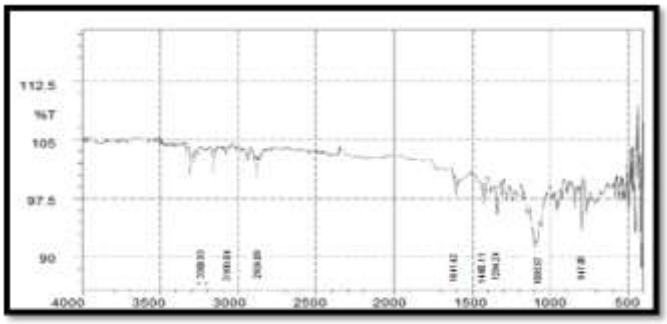

Fig. 3. FT-IR spectra of CBZ.

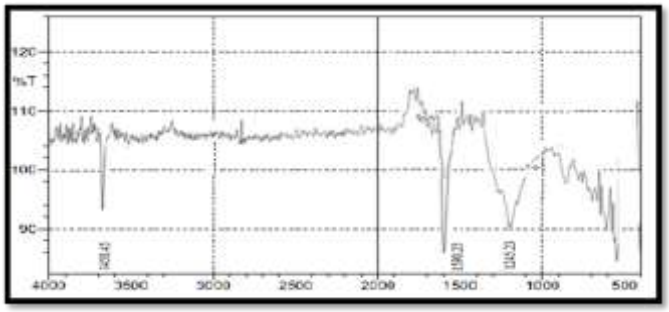

Fig. 4. FT-IR spectra of deacetylated gellan gum.

Dilution effect of the nasal fluid on the gelation of the in-situ gels

TABLE 3. Gelation reaction of DGG with artificial nasal fluids (n=3).

Sr.No.	Concentration of DGG (%)	Volume of SNF (mL)					
		0.05	0.10	0.15	0.20	0.25	0.30
1	0.1	-	-	-	-	+	+
2	0.2	-	-	-	-	++	++
3	0.3	-	-	-	+	++	+++
4	0.4	-	-	+	++	+++	++++
5	0.5	-	-	+	++	+++	++++
6	0.6	-	-	+	++	++++	++++
7	0.7	-	+	++	+++	++++	++++
8	0.8	-	+	+++	+++	++++	++++
9	0.9	+	++	+++	++++	++++	++++
10	1	+	++	+++	++++	++++	++++

-No gelation, +gelation occurred in few min and reminded for few hours, ++gelation immediate, remained for hours, +++ gelation immediate and for exended period, ++++very stiff gel

Mucoadhesive force measurements:

TABLE 4. Mucoadhesive force measurements of all concentration of DGG.

Sr.No.	Concentration of DGG (%)	Volume of SNF (mL)	Mucoadhesive force (dynes/cm^2)*
1	0.1	1	612.5±77.3
2	0.2	1	735±65.6
3	0.3	1	1102.5±40.33
4	0.4	1	1200±24.1
5	0.5	1	1225±85.64
6	0.6	1	1592±74.0
7	0.7	1	1862±65.2
8	0.8	1	2156±47.7
9	0.9	1	2303±87.2
10	1	1	2401±14.5

Gelation and mucoadhesive study of deacetylated gellan gum give in above Table 4 respectively. Increase concentration of deacetylated gellan gum so increase viscosity so we not use of high concentration of deacetylated gellan gum ratio in formulation of nanoemulsion. Hence 0.5% concentration of deacetylated gellan gum (DGG selected for formulation of mucoadhesive *in-situ* gelling formulation because it form stable gel with low amount of artificial nasal fluid and sufficient mucoadhesive strength. and slightly increase viscosity of formulation.

Characteristics of mucoadhesive in-situ gel Carbamazepine Formulation:

TABLE 5. Characteristics of mucoadhesive *in-situ* gel Carbamazepine formulation.

Batch code	Characteristics	Inference
NSG1	Appearance	Clear ,transparent ,yellowish liquid
	pH	6.14±0.24
	Refractive Index	1.383±0.0052
	Viscosity	142.13±4.53
	Percent Transmittance	98.99±0.11
	Assay	97.62±0.25
	Mucoadhesive force (dynes/cm^2)	1932.60±106.5

Ex-vivo drug release Study:

TABLE 6. Percent drug release of NSG1 CBZ mucoadhesive nanoemulsion formulation.

Sr.No.	Time (min)	% drug release of NSG1 formulation*	Sr.No.	Time(min)	% drug release of NSG1 formulation*
1	0	0	8	180	55.443±0.02
2	15	1.230±0.02	9	210	65.290±0.06
3	30	3.17±0.12	10	240	75.188±0.04
4	60	11.097±0.10	11	270	81.496±0.52
5	90	20.510±0.01	12	300	84.356±0.01
6	120	31.053±0.23	13	330	89.297±0.03
7	150	43.181±0.15	14	360	94.308±0.01

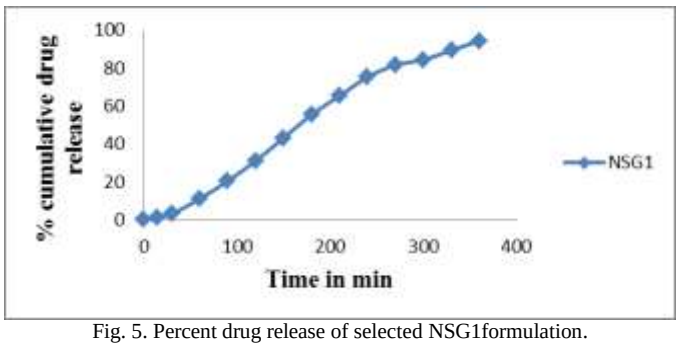

Fig. 5. Percent drug release of selected NSG1formulation.

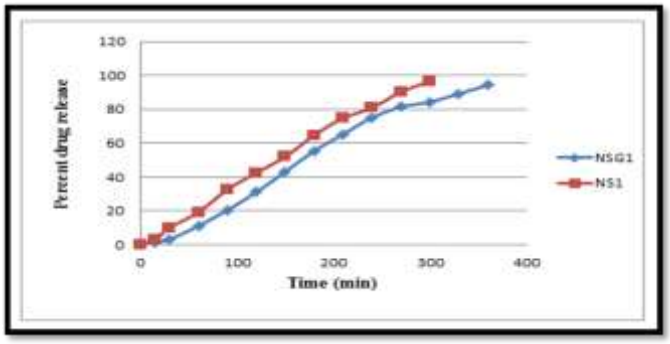

Fig. 6. Comparison of percent drug release of NSG1 Selected formulation.

Percent drug release study of NSG1 formulation given in Table 6. NSG1 formulation was *in-situ* gel formulation so

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drug release from the formulation in the control release manner due to mucoadhesive properties of *in-situ* gel nanoemulsion formulation due to increase the contact time with targeted site so drug release in long time and high percent release.

Tests for nasal cilio toxicity of selected nanoemulsion formulation

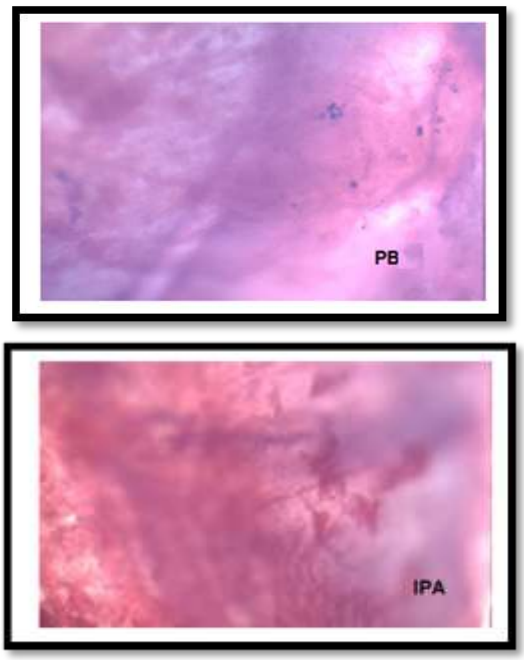

Fig. 7. Histological photomicrograph of eosin-hematoxyline stained sheep nasal mucosa of treated with phosphate buffer pH-6.4 and isopropyl alcohol.

Fig. 8. Histological photomicrograph of eosin-hematoxyline stained sheep nasal mucosa of treated with NSG1 in situ gelling formulation.

Nasal cilio-toxicity studies were carried out in an attempt to evaluate any potential toxic effects of excipients used in the formulation on the nasal mucosa. The nasal mucosa P1 treated with phosphate Buffer (pH 6.4, negative control) showed no nasociliary damage (Figure 7) and the nasal membrane remained intact, where as the second nasal mucosa P2 treated with isopropyl alcohol which an extensive damage to nasal mucosa (Figure 7) could be observed with positive control. However, the nasal mucosa NSG1 *in-situ* gelling nanoemulsion formulation treated P4 nasal mucosa with that show no damage to nasal mucosa and the nasal membrane remained intact could be observed (Figure 8), thus substantiating the safety of the excipients used in the formulation so excipient use in both nanoemulsion formulation was safe for inhalation.

In-Vivo Characterization:

Anticonvulsant activity

TABLE 7. Anticonvulsant activity of mucoadhesive *in-situ* gelling Carbamazepine formulation in rats.

Sr. No.	Body wt. (gm)	Treatment (Dose)	Time (sec) in various phases of convulsion				
			Flexion	Extensor	Clonus	Stupor	Recovery/ Death
1	208	Control	9	15	147	57	Recovery
2	207	Control	8	16	142	53	Recovery
3	206	Control	9	15	144	56	Recovery
Mean			**8.66**	**15.33**	**142**	**53.33**	
1	209	Standard	11	6	135	60	Recovery
2	205	Standard	14	7	151	62	Recovery
3	207	Standard	12	6	141	61	Recovery
Mean			**12.33**	**6.33**	**142.33**	**61**	
1	207	Test	7	4	152	41	Recovery
2	208	Test	12	5	154	44	Recovery
3	207	Test	11	6	152	43	Recovery
Mean			**10**	**5**	**152.66**	**42.66**	

The above Table 7 showed the results of anticonvulsant property of drugs. From the Table 7 it was observed that there is reduction in tonic extensor phase of rats treated with Phenytoin (Standard) and Carbamazepine formulation (Test). Both phenytoin and Carbamazepine showed reduction in tonic phase as compared to control; therefore we could say that the drug had anticonvulsant activity which was found out by maximal electro-shock-induced convulsions in rats.

In-vivo study (Nasal absorption and brain distribution studies):

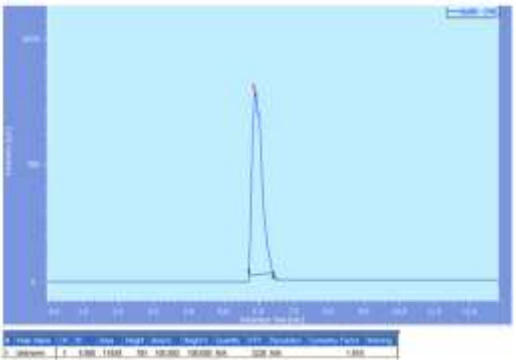

Fig. 9. Chromatograph of NSG1 mucoadhesive *in situ* gelling CBZ nanoemulsion formulation of rat brain extract after 60 min.

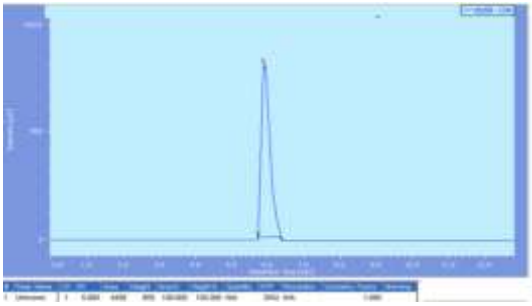

Fig. 10. Chromatograph of NSG1 mucoadhesive *in situ* gelling Carbamazepine formulation of rat plasma extract after 60 min.

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TABLE 8. Concentration of Carbamazepine in brain and plasma after intranasal administration of NS1 and NSG1 formulation in rats.

Sr.No.	Time (min)	Concentration (µg/mL) NSG1 Formulation	
		Brain*	plasma*
1	0	0	0
2	30	7.21±0.01	4.94±0.02
3	60	9.08±0.12	7.57±0.03
4	90	12.47±1.20	8.50±2.30
5	120	8.79±2.30	9.50±0.01
6	240	7.90±0.01	6.82±1.10
7	360	7.10±2.12	5.14±0.01
8	420	6.04±0.02	3.91±0.04

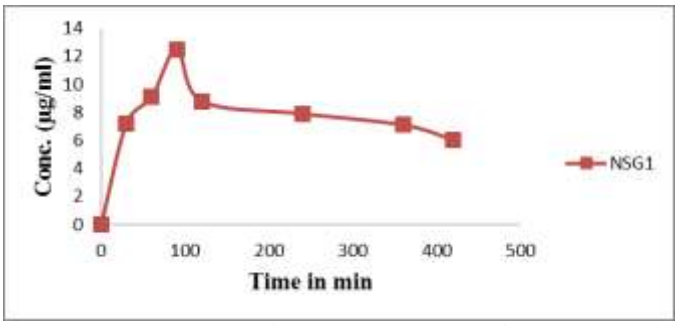

Fig. 11. Concentration of Carbamazepine in brain after intranasal administration of NSG1 formulation in rats.

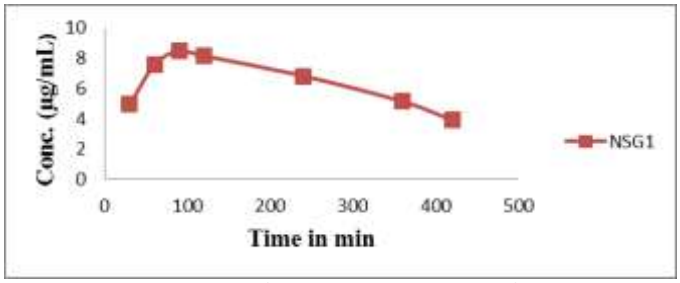

Fig. 12. Concentration of Carbamazepine in plasma after intranasal administration of NSG1 formulation in rats.

TABLE 9. Pharmacokinetic parameter of NSG1 formulation upon intranasal administration in rats.

Sr.No.	Pharmacokinetic parameter	NSG1 formulation	
		Brain*	Plasma*
1	C_{max} (µg/mL)	12.47±0.21	9.50±4.14
2	T_{max} (min)	90±5.15	180±3.04
3	AUC_{0-t} (µg/mL*min)	3158.55±0.25	2816.85±0.56
4	AUC_{0-inf} (µg/mL*min)	5497.69±1.90	5190.16±0.23
5	MRT (min)	487.94±15.23	517.38±6.23

The concentration of Carbamazepine in brain and plasma was determined after intranasal administration of selected NSG1 *(in-situ* gelling mucoadhesive formulation) and given above Table 9 respectively and Figure 11 and 12 of *in-vivo* study represents the mean brain tissue, and plasma concentration time profiles of Carbamazepine after intranasal administration of NSG1 formulations to rats at a dose of 1 mg/kg. Because so these indicate that drug directly pass through the via intranasal route through olfactory nerve and other pathway. NSG1 Carbamazepine formulation show control release activity because it form *in-situ* gel after intranasal administration so drug release in control manner form gel matrix. NSG1 was *in-situ* gelling mucoadhesive Carbamazepine formulation so increase targeted site retention time. So C_{max} (12.47±0.21µg/mL) of NSG1 *in-situ* gelling mucoadhesive Carbamazepine formulation is higher as compare to other formulation but due *in-situ* gelling property of NSG1 formulation so drug release form formulation in sustained manner so T_{max} (90±5.15 min) of NSG1 *in-situ* gelling mucoadhesive CBZ formulation was higher as compare to other formulation.

Stability study:

Fig. 13. Appearance study of NSG1 formulation before the stability study.

Fig. 13. Appearance study of NSG1 formulation after 3 month the stability study.

TABLE 10. Stability indicating parameter

Sr.No.	Time (Days)	Parameter			
		pH	Refractive index	Viscosity	Assay
1	0	6.40	193	1.388	98.60
2	15	6.33	191	1.387	98.57
3	30	6.30	188	1.386	98.16
4	60	6.16	186	1.383	98.60
5	90	6.03	185	1.381	97.84
6	120	6.01	181	1.379	97.06

During stability studies, appearance study, pH, viscosity, RI drug content, Percent transmittance and electrical conductivity were determined at 0, 30, 60, and 90 days. Stability data of NS1 and NSG1 Carbamazepine formulation given above table 10. The values of these parameters were slightly varied with respect to time. In stability study we found that the viscosity of NSG1 formulation was increased with respect to time as compare to other parameter. So according to above stability study NSG1 stable for long period time.

III. CONCLUSION

Carbamazepine *in-situ* gelling mucoadhesive formulation with 0.5% gellan gum is a promising nasal drug delivery system for the antiepileptic drug Carbamazepine, which would enhance nasal residence time owing to increased viscosity, mucoadhesive characteristics and permeation enhancing effect. Histopathological findings suggested that the formulation was safe for nasal administration. Pharmacodynamic studies revealed a quick onset of action by nasal route when compared to intravenous administration of

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equivalent dose. *In-vitro* and *ex-vivo* permeation studies showed an initial burst of drug release at 30 min and mucoadhesive property of *in-situ* gelling mucoadhesive Carbamazepine formulation, Carbamazepine rich in targeted site in high concentration as compare to other formulation. Hence C_{max} (11.26 ± 5.05 µg/mL) and T_{max} (90 ± 5.15 min) of *in-situ* gelling mucoadhesive Carbamazepine formulation (NSG1) was higher due to attributable to the presence of free drug entrapped in the *in-situ* gelling mucoadhesive layer. From *in-vitro* and *in-vivo* data it can be concluded that the developed gel formulation have great potential for intranasal to brain drug delivery.

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