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Original Research Article

Mucoadhesive carbamazepine gel for *in situ* olfactory delivery

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Abstract

Purpose: *To formulate mucoadhesive carbamazepine gel for delivery to the brain via the olfactory mucosa.*
Methods: *Carbamazepine transfersomes were formulated using Lipoid S 100 and sodium cholate. The transfersomes were evaluated for entrapment efficiency, in vitro release transmission electron microscopy, zeta potential, polydispersity index .The transfersomes were then incorporated into gellan gum gel, and the in situ gel formulation was evaluated for drug content, gel strength, in vitro release and mucoadhesive force. Transfersomes were also evaluated for bioanalytical study in rats.*
Result: *TEM analysis showed good regular spheres. The negative zeta potential ensures resistance to aggregation. The gel strength of the formulations was in the range of 0.6 to 7.4 g. In vitro diffusion study of transfersomal gel showed Fickian diffusion mechanism. Formulation F6 was optimized depending for gel strength (6.4 g) , drug content (99.47 ± 0.25 %), and good mucoadhesive force (50.24 ± 0.76 dyne/cm2).Bioanalytical study of F6 showed increased drug concentration in brain.*
Conclusion: *Mucoadhesive carbanmazepine gel can be used effectively to achieve increased concentration of drug in the brain via olfactory mucosal route.*

Keywords: *Olfactory delivery, Carbamazepine, In situ gel Bioanalytical study*

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INTRODUCTION

There has been a recent resurgence of interest in brain drug delivery for the treatment of epilepsy and other brain diseases. The principle reason for this is that the blood-brain barrier (BBB) renders an insuperable barrier for the great majority of drugs. This makes it very difficult for most of the drugs to pass in central nervous system (CNS) [1].

In recent years targeting to the brain via nasal olfactory route has been studied commonly [2]. Olfactory cells are in direct contact with the CNS, which provides a route of entry to the brain that circumvents the BBB [3].

Unfortunately, transnasal drug delivery offers certain limitations such as the mucociliary clearance, breakdown by nasal peptidase

enzymes and the poor nasal permeability [4]. These problems can be overcome by mucoadhesive formulations or vesicular drug delivery system [5,6].

Amongst all vesicular drug delivery systems transfersomes have shown most encouraging results with the possibility of achieving better fluidity and better penetration through small pores of the skin[7] and act as a drug carrier for non-invasive targeted drug delivery.

The term epilepsy refers to a chronic neurological disorder of the brain Rapid access of drugs to the brain is very important during an epileptic attack. In such a case intranasal drug delivery via transfersomes presents a promising approach.

Carbamazepine (CBZ) is an antiepileptic drug used in almost all types of seizures. Reportedly, CBZ is absorbed slowly and erratically after oral administration. Peak concentration in plasma is usually attained 4–6 h after oral ingestion but may be delayed by as much as 24 h. Therefore, an alternative dosage form and route of drug delivery is needed for the treatment of epilepsy [8].

It was therefore the aim of this study to develop rapid and reliable drug delivery system in the form of transfersomal *in situ* gel for CBZ.

EXPERIMENTAL

Materials

Carbamazepine was received as a gift sample from Ajanta pharma limited, Mumbai, India. Lipoid S100 was received as a gift sample from Lipoid KG, Germany. Gellan gum was supplied as a gift sample from C.P. Kelco, Denmark. Sodium cholate, sodium citrate, hydroxy propyl methyl cellulose K4M (HPMC K4M), mannitol and methyl paraben were procured from S. D. fine chemical limited, Mumbai, India. Dialysis membrane with a molecular weight cut off of 12000 – 14000 kDa was purchased from Himedia, Mumbai, India. All other chemicals were of analytical grade and obtained commercially.

Preparation of transfersomes

Transfersomes were prepared by a thin film method [9]. Lipoid S100 850 mg and sodium cholate 150 mg along with 100 mg of CBZ were dissolved in 10 mL of ethanol in a clean, dry round bottom flask. Ethanol was later removed by rotary evaporator (Eqitron rota evaporator, Medica instrument, India) with vacuum above the lipid transition temperature (50ºC) at 65 rpm. Lipid film was then hydrated with 10mLdeionised water at 65 rpm rotation (30 min) at room temperature [10].

Preparation of transfersomal gel

Different batches of transfersomal gel were prepared by dispersing varying concentrations of Gellan gum and 0.17% of sodium citrate in 10 mL of transfersomal stock solution (Table 1). Accurately measured quantities of mannitol (5%) and methyl paraben (0.033%) were added simultaneously. The final pH of the formulations was adjusted to 4.5-5 with the help of 0.1 N HCl. An optimized batch of transfersomal *in situ* gel was further modified with bioadhesive polymer HPMC K4M. Prepared gel was further evaluated [11].

Characterization of transfersomes

Prepared transfersomes were separated from unentrapped drug by ultracentrifugation method at 4ºC and 25,000*g* relative centrifugal force (rcf) for 30 min. The vesicles were further evaluated for entrapment efficiency and *in vitro* drug release [12].

Entrapment efficiency

Vesicles were dispersed in 5 mL ethanol and disruption was carried out by sonication (Pci Analytics JIJ 158) for 15 min. Obtained solution was then filtered and 1 mL of filtrate was further diluted with phosphate buffer of pH 6 up to 10 mL.

Table 1: Formulation of CBZ transfersomal gel

Formulation	F1	F2	F3	F4	F5	F6	F7
Transfersomal dispersion (mL) containing 0.1g of carbamazepine	10	10	10	10	10	10	10
Sodium Citrate (%w/v)	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Gellan Gum (%w/v)	0.2	0.4	0.6	0.8	0.4	0.4	0.4
HPMCK4M (%w/v)	--	--	--	--	0.3	0.4	0.5
Mannitol (%w/v)	5	5	5	5	5	5	5
Methyl paraben (%w/v)	0.033	0.033	0.033	0.033	0.033	0.033	0.033

Then the solution was further analysed spectrophotometrically for the drug content at a wavelength of 284 nm.

In vitro release study

CBZ release from transfersome was carried out at 37^{o}C using Franz diffusion cell with effective surface area 3.14 cm^{2}. Receptor medium was 15 mL phosphate buffer of pH 6 and donor medium was 1 mL transfersomes suspension. Samples of 1 mL were periodically withdrawn at specific time interval for 12 h and replaced immediately with an equal volume of the fresh phosphate buffer solution, and assayed by a spectrophotometer at 284 nm. These studies were carried out in triplicate.

Transmission electron microscopy

The surface morphology of transfersomes was examined using TEM (ModelJEM- 241 1230, JOEL, Tokyo, Japan). A drop of vesicle dispersion was applied on the carbon coated grid and left for 2 min, to allow its absorption in the carbon film. The excess of liquid was drawn off with filter paper and was stained with a 1% phosphotungstic acid. Then samples were examined and photographed by TEM at an accelerating voltage of 80 kV [9,13].

Zeta potential and polydispersity index

For the measurement of zeta potential and polydispersity index, 1 mL of vesicular suspension was diluted with 10 mL distilled water and was measured by dynamic light scattering using a Malvern Zetasizer (Ver. 6.32) MAL 1065515 [14,15].

Evaluation of transfersomal gel

Drug content

A sample of 1 mL *in situ* gel was dissolved with phosphate buffer of pH 6 and final volume was made to 100 mL. The solution was filtered through Whatmann filter paper and CBZ estimated spectrophotometrically at 284 nm [16]. All these studies were conducted in triplicate.

Gelation study

A sample of 5 mL of *in situ* gel was put in a 10 mL measuring cylinder . The formulation was gelled using 0.5 mL of simulated nasal fluid with the same cationic composition of nasal secretion (simulated nasal fluid used is an aqueous solution containing 8.77 mg/mL NaCl, 2.98 mg/mL KCl and 0.59 mg/mL $CaCl_2$). The consistency of formed gel was observed by visual examination and graded as follows [17]. – No gelation occurred; + Gelaion occurred within few minutes and remained for few hours; ++ gelation occurred immediately and remained for few hours; +++ gelation occurred immediately and remained for extended period; ++++ very stiff gel was formed.

Gel strength

A 50 g of *in situ* gel was placed in beaker and gelation was induced by simulated nasal fluid. The study was carried by *TA*-XT2 Texture analyzer (The experiments were con-ducted at Digital Scientific Equipments, RK Puram, New Delhi). The texture analyzer probe (7.6 cm) was inserted into the sample and analyzer was set to the `gelling strength test mode with speed of 1.0 mm/S, acquisition rate of 50 points/S and trigger force of 3 g. Gel strength was measured in terms of 'grams' [18].

In vitro diffusion study and kinetic analysis

After achieving the gelation of 0.1 mL of formulation using simulated nasal fluid, diffusion of CBZ from gel was studied by same method as described earlier under characterization of tranfersomes. The data obtained from *in vitro* drug release was further analysed for best fit linear regression by zero-order, first order, Higuchi square root and Korsmeyer Peppas power equation [11].

Mucoadhesive force

Mucoadhesive strength was determined using the digital mucoadhesion measuring device. A section of tissue specimen was taken from the sheep olfactory nasal cavity and was secured using a rubber band on the bottom of two glass vial with mucosal side facing upward. One vial was fixed to the digital mucoadhesive strength tester, while the second vial was hooked in an inverted position on the first vial. A sample of 50 mg was gelled and sandwiched between two vials. The vials were kept in this position for 2 min to ensure intimate contact and then the instrument was started. The mucoadhesive strength in dynes/cm^{2} was expressed as the minimum weight that detached the mucosal tissue from the surface of formulation and was calculated using following formula [19].

$$MS\ (dynes/cm^{2}) = W\ (dynes)/A \quad \text{.....(1)}$$

where MS is mucoadhesive strength; W is weight; A is area of the tissue exposed and it is equal to πr^{2}

Animal studies

The protocol for this study was as per Guide for the Care and Use of Laboratory Animals [20] and approved by the Institutional Animal Ethics Committee of Dr. L. H. Hiranandanani College of Pharmacy, Ulhasnagar, India (no. IAEC/PCEU-15/2014). Rats have been used widely for nasal drug delivery studies as it consist of large area of olfactory epithelium (50%) and in rats the olfactory area is spread throughout the whole cavity. A total of 54 Wister Albino rats (weighing between 200–250 g) were selected for the experiment. The animals were housed in cages filled with paddy husk. They were provided with balanced diet throughout the study and food was withdrawn 12 h before the study, whereas water was freely available during the study. All the animals were divided into two groups, having 27 animals in each. Intravenous injection was administered in group-I, whereas group-II received CBZ *in situ* gel.

Intravenous administration

A sterile solution (0.1 mg/mL) of CBZ in propylene glycol, distilled water and ethanol (5:3:2; v/v/v) was injected (0.2 mg/kg) into the tail vein with a slow IV bolus dose by a no. 7 needle in group-I.

Intranasal administration

Each rat of group-II was placed in supine position and 5 µL of CBZ *in situ* gel containing 0.05 mg of drug (0.2 mg/kg) was administered via micropipette (100 µL) fixed with low density polyethylene tube with 0.1 mm internal diameter at the depth of 5 mm in to one of the nostrils towards the roof of the nasal cavity and rats were kept in supine position for 2 min.

***In vivo* studies**

Upon administration of dose, three rats per time point were sacrificed with the cervical necrosis method at 15, 30, 60, 120, 240, 480, 720, 960 and 1200 min intervals for both groups and collected blood samples were centrifuged at 3000 rpm for 20 min. Separated plasma was immediately stored at − 20°C until analysed for CBZ. Brain was collected within 1 min following the blood collection and then brain samples were made free from blood taint and macroscopic blood vessels as much as possible by quick rinsing with saline. Brain sample were homogenized with 2 mL phosphate buffer (pH 6) in a tissue homogenizer and stored immediately at −20°C until analysis. Samples were extracted and analyzed by HPLC.

Bioanalytical HPLC method for CBZ

The method to assay CBZ and estimation of its concentration in plasma and brain earlier reported [21] was used with slight modification. A sample (1 mL) of rat plasma and brain homogenate were taken in separate stoppered glass tube of 15 mL capacity and each spiked with 2 and 8 µg of internal standard solution (phenacetin) respectively. The spiking was done by the addition of 25 µL of 80 µg/mL and 320 µg/mL methanolic solution of the drug respectively in plasma and brain homogenate. These spiked samples were vortexed for 1 min. An extraction solvent (ethyl acetate) was added (5 mL) to each tube and the tubes were shaken in an inclined position on a reciprocating shaker at 100 strokes per minutes for 30 min. The tubes were then centrifuged at 3000 rpm for 20 min at 4^{o}C. From each tube, 4 mL of organic layer was transferred to separate tubes and evaporated to dryness under a stream of nitrogen. The residues obtained upon evaporation to dryness were reconstituted with 250 µL of mobile phase (methanol : water 70:30) and drug content was determined using HPLC system (Agilent 1200 series)equipped with a Shodex C-18-4E (5 µm; 250 × 4.6 mm) column and UV detector at 284 nm. Flow rate of the mobile phase was set at 1.0 mL/min; the volume of the injected sample was 20 µL. Finally, graph of plasma or brain concentration verses time was generated by plotting the drug concentrations at each sampling point against time. The area under the curve (AUC) was calculated using the trapezoidal rule [22].

DTE (%) and DTP (%) were calculated as follows:

Drug targeting efficiency (DTE %) = (AUC brain / AUC blood) IN / (AUC brain / AUC blood) IV ×100…….. (2)

Wherein, IN express intranasal and IV express intravenous.

Nose to brain direct transport percentage (DTP %) = (Bi.n. – Bx) / Bi.n.× 100……(3)

where in Bx = (Bi.v. / Pi.v.)× Pi.n.

Bx is the brain AUC fraction contributed by systemic circulation through the BBB following intranasal administration. Pi.n. is the $AUC_{0–1200}$ min (plasma) following intranasal administration; Pi.v. is the $AUC_{0–1200\ min}$ (plasma) following intravenous administration; Bi.n. is the $AUC_{0\text{-}1200\ min}$ (brain) following intranasal administration;

Bi.v. is the $AUC_{0–1200min}$ (brain) following intravenous administration.

Statistical analysis

Data are presented as mean ± standard deviation (SD) and were analyzed by SPS software using t-test. Differences were considered significant at $p < 0.05$.

RESULTS

Entrapment efficiency and *in vitro* drug release

Transfersome formulation was prepared by using rotary evaporation method. Upon preparation, the vesicles were evaluated for preliminary evaluation. In a preliminary study, prepared transfersomes were evaluated initially for entrapment efficiency which was 96.13 ± 0.16 %. While *in vitro* drug release was 98.45 ± 1.36 % at the end of 12 h.

TEM results

TEM studies of the transfersomes reveal the presence of well defined, nearly perfect spheres as shown in Figure 1.

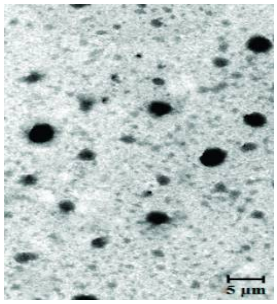

Figure 1: Transmission electron microscope (TEM) photomicrograph of transfersomes

Zeta potential and polydispersity index

The zeta potential value of final formulation was highly negative (-53.4 mV), ensuring resistance to aggregation process. Polydispersity index was also 0.7 confirming heterogeneous distributions.

Transfersomal gel characterization

Cevc G have compared hydro-gel and transfersome for diclofenac concentration, reported improved drug concentration with transfersomes than hydrogel [7]. However, transfersomes alone are difficult to deliver via the intranasal route because of mucocilliary clearance. Hence, in this study mucoadhesive transfersomal *in situ* gel was developed and evaluated for following parameters.

Drug content

Drug content ranged from 98.52 ± 0.63 to 99.53 ± 0.24 % w/v (Table 2).

Gelation

Gelation study was performed to know the integrity of formulation at the site of administration. From the results, it was revealed that formulation F1 formed weak gel while the formulation F4 formed a stiff gel. Reason could be attributed to minimum and high quantities of gellean gum. Whereas formulations F2 and F3 form instantaneous gelation. However, F2 with gelling capacity ++ was further selected for mucoadhesive formulation development, as the addition of HPMC K4 M had considerable effect on gelling capacity. Modified formulations F5 and F6 showed instantaneous gelation, while F7 showed stiff gelation (Table 2).

Gel strength

Gel strength was determined in order to allow easy administration as droplet and prevention of anterior leakage.

All formulations had gel strength between 0.6 g to 7.4g, which was affected by concentration of polymers. The data are given in Table 2.

Table 2: Evaluation of CBZ transfersomal gel

Formulation	Drug content (%w/v)	Gelation Study	Gel strength (g)	Mucoadhesive force (dyne/cm^2)
F1	98.52±0.63	+	0.6	11.58±1.28
F2	99.46±0.36	++	1.2	17.54±0.72
F3	99.35±0.73	+++	6.2	20.94±1.61
F4	99.53±0.24	++++	7.1	24.72±0.25
F5	98.57±0.79	+++	6.3	33.65±1.74
F6	99.47±0.25	+++	6.4	50.24±0.76
F7	99.15±0.46	++++	7.4	68.28±0.44

In vitro diffusion

The result is illustrated in Figure2. CBZ release from F1, F2, F3 and F4 is comparatively faster than F5, F6, and F7. This is attributed to the presence of HPMC K4 M in formulation F5, F6, and F7 which gave sustained release profile. The mechanism of drug release was further analysed. From the comparative release profile of the drug, it revealed a matrix kind of formulations with initial fast release and then slowly release. It indicates the CBZ release from gel follows zero order release. Higuchi's equation expresses best fitted *in vitro* release mechanism. The diffusion exponent 'n' is indicative of the mechanism of drug release from the formulation. Data from Korsmeyer Peppas model indicates the Fickian diffusion mechanism (Table 3).

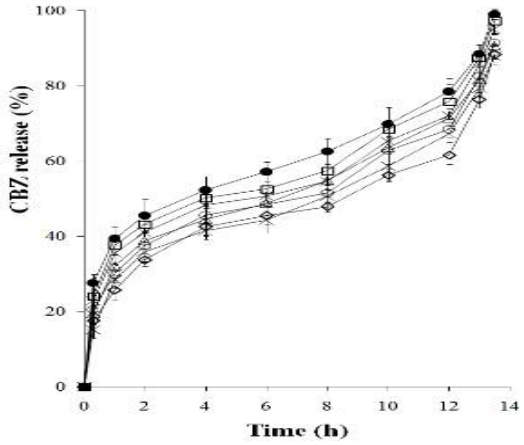

Figure 2: *In vitro* diffusion of CBZ from transfersomal gel formulations; F1 (●), F2 (□), F3 (Δ), F4 (×), F5 (ж), F6 (○) and F7 (◊)

Mucoadhesive force

The values are shown in Table 2. Mucoadhesive forces increased with increase in concentration of polymers. Mucoadhesive strength of F1, F2, F3 and F4 was lower as compared to *in situ* gels (F5, F6 and F7) containing mucoadhesive agent HPMC K4M, indicating the increase in mucoadhesion due to incorporation of mucoadhesive agent. Since formulation F6 showed instantaneous gelation (+++) which remained for extended period, acceptable gel strength (6.4 g), drug content (99.47±0.25%), and good mucoadhesive force (50.24±0.76 dyne/cm^2), it was considered as optimized batch.

In vivo CBZ chromatographic method validation

The standard calibration curve of CBZ for plasma (y =0.593x + 0.0358) was linear over the range of 0.25-5 µg/mL, while the calibration curve of CBZ for brain (y = 0.1318x + 0.0022) was linear over the range of 0.5-16 µg/mL. The typical chromatograms of CBZ for plasma and brain with phenacetin as internal standard are shown in Figure3 wherein peaks observed between RT of 2 min to 3.5 min are of plasma and blood constituents respectively whereas peak obtained at around 4.5 min RT is of phenacetin and peak obtained at around 5.7 min RT is of CBZ.

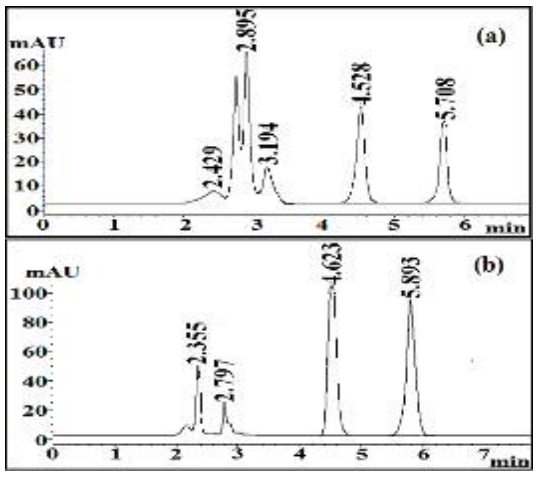

Figure 3: Chromatograms of CBZ for plasma(a) and brain(b) obtained from HPLC analysis

Bioavailability of CBZ

The mean concentration verses time profiles of CBZ in plasma and brain following intranasal and

Table 3: Kinetic data

Formulation	Correlation coefficient (R^2)				Korsmeyer-Peppas 'n' parameter
	Zero order	*First order*	*Higuchi*	*Korsmeyer-Peppas*	
F1	0.8746	0.6381	0.9354	0.936	0.2957
F2	0.8833	0.7202	0.9295	0.9264	0.3169
F3	0.9780	0.8442	0.9469	0.9490	0.3563
F4	0.9145	0.8463	0.9390	0.9477	0.4
F5	0.8843	0.7588	0.9245	0.9230	0.3235
F6	0.9015	0.8807	0.9333	0.9367	0.3499
F7	0.8961	0.7896	0.9215	0.9388	0.3732

intra-venous administration to rats are illustrated in Figure 4a and b. When AUC in the brain of these groups was compared, the intranasal route enhanced AUC (13310.5 ± 23.78 µg min/mL), when compared to the intravenous route (2912.17 ± 18.46 µg min/mL). Furthermore, after nasal *in situ* gel administration, maximum concentration (C_{max}) of CBZ in brain (13.5 ± 0.025 µg/mL) was achieved compared to intravenous injection (3.16 ± 0.037 µg/mL). Increase in AUC and C_{max} in the brain can be attributed to increase in transportation of drug through olfactory route. DTE (%) and DTP (%) were also calculated from the pharmacokinetics data. *In situ* gel showed DTE 341.47± 9.48% and DTP 70.71± 1.84 %.

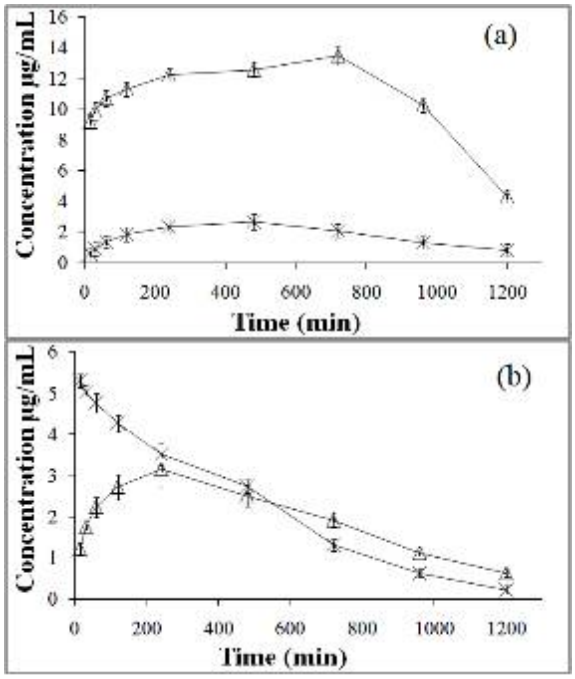

Figure 4: CBZ concentration in (×) plasma and (∆) brain (a) after intranasal administration, and (b) after intravenous administration

DISCUSSION

The main objective of this research work was a rapid onset of action and reliable brain delivery of CBZ. Transfersomes, owing to its good ability to squeeze themselves and achieve better targeting [23], were prepared using lipoid S 100 and sodium cholate and showed adequate entrapment efficiency as well as in vito drug release. Value of zeta potential was also found within acceptable limit, indicative of physical stability. In order to improve olfactory residence time, transfersome was incorporated into *in situ* gel. Ion sensitive *in situ* system was prepared with gellan gum, which upon contact with the nasal mucosa rapidly undergoes sol-gel transition and allow ease of administration.

Upon comparing different batches of *in situ* gel, it was observed that drug content was independent of polymer concentration. The two main prerequisites of gel system are optimum gelling capacity and gelling strength so as to have instantaneous sol-gel transition due to ionic interaction and preserving integrity. The observed increase in gelling strength with increase in concentration of gellan gum and HPMC K4M has been previously reported [24] and is attributed to increasing chain interaction with polymer concentration rising. Mucoadhesive force is an important parameter since it prolongs residence time of gel in nasal cavity. Fortification of the mucoadhesive forces in the nasal *in situ* gel is exhibited by gellan gum and HPMC K4M by interaction of secondary bond forming groups (hydroxy, ethoxy, and amine) with the glycoprotein chain in the mucous layers of epithelial cells of nasal cavity and this interaction occurs when the polymer swells by absorbing water from the mucous layer and adhesion binding occurs to form gel.

The key factors that play a major role in mucoadhesion are the physiological ions in the nasal cavity as well as the swelling capacity of the polymer which finally result in prolonged mucoadhesion and reduced mucocilliary clearance. Mucoadhesive force was found to increase with increase in concentration of polymers. Optimized formulation F6 was further evaluated for *in vivo* study.

CONCLUSION

Suitable *in situ* transfersomal gel has been successfully prepared and demonstrated to deliver CBZ to rat brain through olfactory mucosa. The transfersomal formulation enhances drug diffusion across the BBB. This might help in decreasing CBZ dose and frequency of dosing, and possibly enhance therapeutic index. Thus, direct delivery of drug to brain via olfactory mucosal route is promising approach that needs to be further investigated.

DECLARATIONS

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animals, and Professor Gregor Cevc, Advanced Treatment, Germany, for advice.

Conflict of interest

No conflict of interest is associated with this work. All authors hereby declare that this is an original, self-funded research work performed using the facilities available in our laboratories and authors do not have any financial, personal, employment related, stock ownership related, honoraria or paid expert testimony related, patent related, grants or other funding related or any other conflict with some people, organizations or consultancies that could inappropriately influence (bias) our work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dr. Sangeeta Sahasrabuddhe proposed this work Madhuri Gaikwad conducted all experimental work and prepared the draft. Dr Prashant Puranik has read and approved the draft.

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