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Formulation and evaluation of carbamazepine loaded ethosomal nasal in-situ gel for brain targeted drug delivery

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ABSTRACT

Objective: This study aimed to develop a novel intranasal drug delivery system for carbamazepine, an antiepileptic drug, to enhance its therapeutic efficacy through targeted and sustained delivery. The goal was to evaluate the suitability of various polymers and excipients for formulating an effective and mucoadhesive nasal gel.
Methods: Ethosomes were prepared using the cold method and evaluated for particle size, zeta potential, entrapment efficiency, and drug release. Gels were formulated using poloxamer 407 and carbopol 934 and characterized for their physicochemical properties. The optimized ethosomal gel was further assessed for mucoadhesive properties and *in vitro* drug release. Nasal in-situ gels were also prepared using carbopol and HPMC k 100, and their spreadability and drug release profiles were compared.
Results: The optimized ethosomal batch (ET3) exhibited a particle size of 200.7 nm, a zeta potential of −54.8 mV, and a high drug entrapment efficiency of 93%. The *in vitro* drug release from ET3 was 88.64%. Among the nasal in-situ gels, the carbopol-based batches demonstrated better spreadability compared to HPMC k 100. The optimized in-situ gel batch (G2) showed a gelation temperature of 33.7 °C and an *in vitro* drug release of 94.05%.
Conclusion: The developed ethosomal gel and in-situ gel formulations demonstrated sustained drug release, enhanced mucoadhesion, and targeted delivery, making them promising alternatives for the treatment of epilepsy. This intranasal delivery system could improve patient compliance and therapeutic outcomes by providing a non-invasive and effective route for carbamazepine administration.

1. Introduction

Epilepsy is a neurological disorder that affects approximately 50 million people worldwide, and 80% of these people affected come from developing countries. According to the Epilepsy Centre in New Delhi, India, the prevalence of epilepsy is 500–1000 cases per 100,000 people, resulting in approximately 200,000–500,000 new cases in India each year. Epilepsy is caused by electrochemical disturbances in the brain, which is characterized by recurrent seizure episodes. Seizures are time-bound episodes that can cause abnormal motor activity, loss of senses, and unconsciousness. Often, seizures originate in a small part of the brain and spread to other regions of the brain. Epilepsy can be diagnosed by investigating electrical signals in the brain using electroencephalography (EEG). For treating mild and complicated partial seizures, carbamazepine is the first-choice antiepileptic medication. It is a tricyclic lipophilic molecule.[1–3] Just a little amount of it is eliminated in urine in its original form after being nearly entirely metabolised by the body. Drugs can be delivered to the deep skin layers and/or the systemic circulation via ethosomes, which are non-invasive carriers. Phospholipids like phosphatidylserine, phosphatidylcholine, water, and ethanol (at relatively high concentrations) make up the majority of these soft, flexible vesicles. Ethosomes are highly intelligent, secure, and readily constructed systems. Intranasal administration is one approach to ethosomal drug delivery that shows promise.[4–6] Bypassing the blood-brain barrier (BBB), this technique uses the trigeminal and olfactory nerve pathways to deliver medications straight to the brain. The nasal cavity is innervated by both the trigeminal and olfactory nerves, which offer a direct link to the central nervous system. The olfactory pathway was once thought to be responsible for the direct transfer of medications from the nose to the brain. Drug delivery via enzymatic chelation has been investigated for a number of conditions, including Parkinson's and Alzheimer's disease. The BBB is a barrier that prevents some chemicals from entering the brain from the circulation.

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Lipid-based nanoparticles called ethosomes have the ability to interact with the BBB and promote the transport of carbamazepine. Carbamazepine may be delivered to the brain more effectively since the in-situ gel formulation kept the medication in the nasal canal, extending its duration of contact with the nasal mucosa and facilitating absorption.[7–10] The use of carbamazepine in an ethosomal in-situ nanogel offers several advantages for brain targeting, including enhanced delivery, improved bioavailability, sustained release, and reduced side effects. This approach has the potential to improve treatment of epilepsy and other brain disorders.

2. Materials and methods

2.1. Preparation of carbamazepine loaded ethosomes

2.1.1. Materials

Carbamazepine was purchased from CTX Life Science Pvt Ltd, Gujrat. Soya lecithin, Cholesterol, Propylene glycol, Ethanol, and Distilled water was provided by Research Lab Fine Chem Industries, Mumbai. Poloxamer 407, HPMC k 100, and Carbopol 934 was provided by Research Lab Fine Chem Industries Pvt Ltd, Mumbai.

2.1.2. Preformulation study

The interaction between drug and polymer was tested by pre-formulation parameters including physical appearance, Fourier Transfer Infra-red Spectroscopy (FTIR), Differential scanning colorimetry (DSC).[11,12]

2.1.3. Preparation method of carbamazepine loaded ethosomes

When preparing ethosomal material, this is the approach that is most frequently utilised. Using vigorous stirring, dissolve the phospholipids, medication, and other lipid components in ethanol in a covered container at room temperature. A water bath is used to bring the mixture to a temperature of 30 °C. The water is brought to a temperature of 30 °C in a different pot, then added to the aforementioned combination. Stir the mixture for 5 min under a closed pot. To make the ethosomal formulation last longer, one can use sonication or extrusion to reduce the vesicle size. Last but not least, the mixture needs to be kept in the right way in the refrigerator[11,13–16] (Table 1).

2.2. Characterization of carbamazepine loaded ethosomes

2.2.1. Size and charge

Size and zeta were determined by dynamic light scattering (DLS) using a computerized inspection system (HORIBA SZ-100, Japan).

2.2.2. Entrapment efficiency

The percent entrapment was calculated using the formula:

$$\%\ \text{entrapment} = \frac{\text{amount of carbamazepine drug in sediment}}{\text{amount of carbamazepine drug added}} \times 100$$

Table 1
Carbamazepine loaded ethosome batches.

Chemicals	Batch-ET1	Batch-ET2	Batch-ET3
Carbamazepine (mg)	0.10	0.10	0.10
Soya lecithin (mg)	0.10	0.20	0.30
Cholesterol (mg)	0.02	0.02	0.02
Propylene glycol (mL)	_	5	5
Ethanol (mL)	20	30	40
Distilled water (mL)	Quantity sufficient (QS)	QS	QS

Table 2
Formulation of carbamazepine loaded ethosomal in-situ gel batches.

Chemicals	Quantity						
	G1	G2	G3	G4	G5	G6	G7
ET3 Ethosomal dispersion (mL)	10	10	10	10	10	10	10
Poloxamer 407 (mg)	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Carbopol 934 (mg)	0.1	0.2	0.3	_	_	_	0.2
HPMC k 100 (mg)	_	_	_	0.1	0.2	0.3	0.2
Distilled water QS (mL)	20	20	20	20	20	20	20

2.2.3. In vitro drug release study

The *in vitro* permeation study was carried out by using modified Franz diffusion cells with egg membranes.

2.2.4. Stability study

Optimized ethosomal formulations were selected for stability study. Formulations were stored at 4 ± 2 °C, 8 °C, and at room temperature.

2.3. Preparation method of carbamazepine loaded ethosomal in-situ gel

Poloxamer 407 was distributed in 80 mL of cold distilled water and allowed full hydration overnight. The poloxamer dispersion was then gradually supplemented with carbopol or HPMC powder. Concurrently, the carbamazepine-loaded ethosomal dispersion was added to this mixture on a magnetic stirrer, and distilled water was added to bring the volume up to the desired level.[1,2] (Table 2).

2.4. Evaluation of carbamazepine loaded ethosomal in-situ gel

2.4.1. Appearance and pH

The pH is recognised as one of the most essential parameters for in-situ gel formation. The pH of the nasal gel should be comparable to the pH of your nose to avoid inflammation. The pH of gels was evaluated using a digital pH meter. Visual inspection was performed to determine homogeneity, and presence or absence of aggregation in its physical appearance.[1,2]

2.4.2. Morphology by scanning electron microscopy (SEM)

The shape of the ethosomal in-situ gel was investigated using SEM after a drop of the BE formulation was diluted in 100 mL of double-distilled water (DW) and allowed to air dry on a sample holder. The sample was next examined under vacuum using a variety of magnifications and accelerating voltages, up to 15,000 V.[4–6]

2.4.3. Spreadability

Measure the amount of ethosomal in-situ gel required on a slide. Placed another slide on top of the gel-coated slide. Loaded the gel by placing a 100 g weight on top of the slide; this distributed the gel evenly over the entire slide without leaving any residue. The pulley was fixed to the upper slide and had a fixed weight attached to it. Recorded the time it took for the top slide to travel the distance between the two slides, starting at zero seconds. Calculated spreadability using the following formula: Spreadability (S), mass (M), length (L) and time (T) are the variables that determine whether the slide can slide over time.[17]

2.4.4. Determination of gelation temperature

Thermostatically controlled digital magnetic stirring was used to raise the temperature of the beaker containing 10 mL of carbamazepine ethosomal in-situ gel at a rate of 10 °C/min. Shake the beaker to see whether it has gelled. When the beaker is 90° tilted and the gel is not flowing, note the temperature at which the gelation temperature is reached.[4–6]

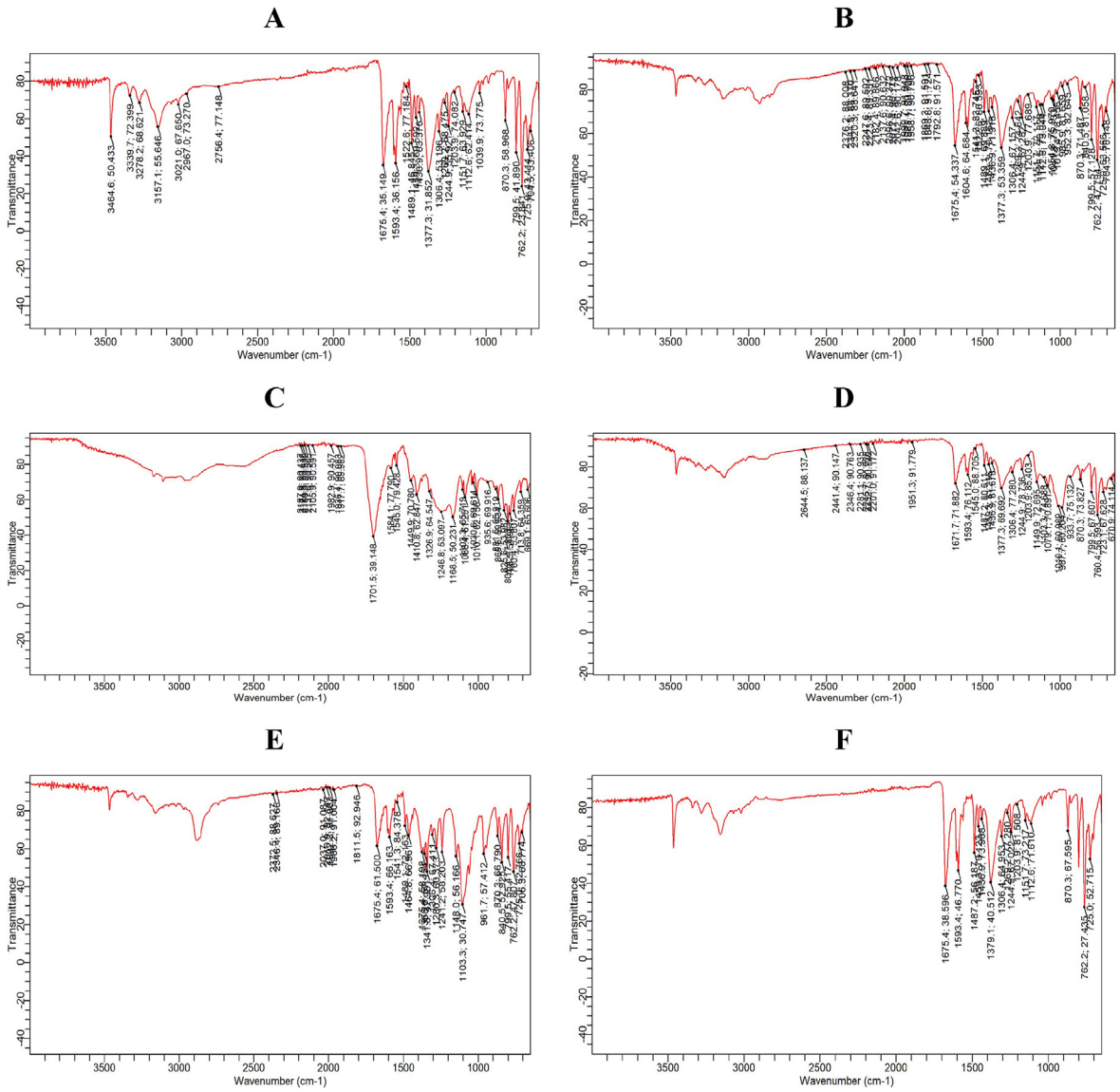

Fig. 1. FTIR spectra. A: Carbamazepine; B: Carbamazepine + Cholesterol; C: Carbamazepine + Carbopol 934; D: Carbamazepine + HPMC k 100; E: Carbamazepine + Soya lecithin; F: Carbamazepine + Physical mixture of excipients blend.

2.4.5. Stability of carbamazepine ethosomes in in-situ gel

Stability of ethosomes in terms of particle size and zeta potential was evaluated.

2.4.6. Release of optimized carbamazepine loaded ethosomal batch

In vitro release studies were performed on optimized ethosomal batch ET3.

2.4.7. Release of optimized carbamazepine loaded ethosomal in-situ gel batch (G2)

Franz diffusion cells were used in the previous studies to examine how medicines diffuse in various formulations. Diffusion membranes having a molecular weight cut-off of 12,000 × 14,000 kDa were used for dialysis. Before commencing the test, the filter was put in a phosphate buffer solution (pH 6.8) for 24 h. The dialysis membrane was attached to the diffusion cell, which was then filled with 21 mL of pH 6.8 phosphate buffer solution. In the donor room, gel having a dosage of 2.5 mg of medication was used. The temperature range for the water bath was 32–34 °C. At various periods (0, 1, 2, up to 8 h), 1 mL samples were withdrawn and then replaced with an equivalent volume of fresh solution. A UV–visible spectrophotometer calibrated to detect the present solution at a wavelength of 284 nm[4-6,18].

3. Results

3.1. Pre-formulation study

From the pre-formulation study, it was found that the physicochemical parameters of carbamazepine were as per the standards in the official books and the colour was found to be white, the odour was odourless, and the nature was crystalline.

3.2. FTIR of carbamazepine

From the FTIR study it was observed that there was no significant interaction between drug and excipient.

The FTIR spectra of carbamazepine showed a characteristic peak at 3462 cm^{-1} (–NH valence vibration), 1676 cm^{-1} (–CO–R vibration), 1598 cm^{-1} (–C=C– and –C=O vibration), and 1384 cm^{-1} (–NH deformation) (Fig. 1A). It was also observed from Fig. 1B–F that the characteristics of bands for carbamazepine were clearly present in all the excipients as well as physical mixture. The FTIR spectra showed that

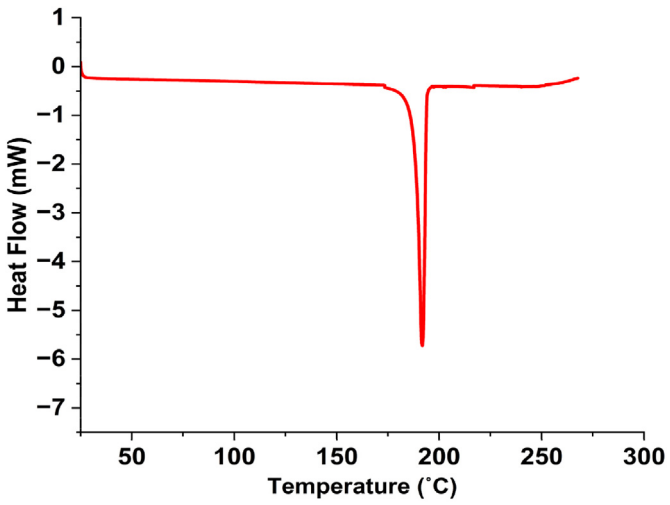

Fig. 2. DSC of carbamazepine. DSC: Differential scanning colorimetry.

there was no significant alteration in the bands of carbamazepine although the excipients exhibited characteristic peaks at their respective values, but they do not change the parent pattern of pure drug absorption and transmittance bands of carbamazepine.

3.3. DSC of carbamazepine

From DCS study, it was observed that the drug was having a sharp endomethacin picture at 193 °C which lay in conformity to the reported melting point of 193 °C for carbamazepine as per the standards in official monograph (Fig. 2).

3.4. Characterization and evaluation of carbamazepine loaded ethosomes and their in-situ gel

3.4.1. Particle size and zeta potential

Particle size of carbamazepine loaded ethosomes of batch ET3 was 200.7 nm and Zeta potential was −54.8 mV (Fig. 3A–B).

3.4.2. Visualization of ethosome vesicles by using SEM

From the SEM study, it was observed that the morphological characteristic exhibited spherical particles of ethosomes embedded in gel (Fig. 4).

3.4.3. Entrapment efficiency of carbamazepine loaded ethosomes

Entrapment efficiency of carbamazepine loaded ethosomes of batch ET1 was 79.83% ± 2.38%, batch ET2 was 86.58% ± 0.94%, and batch ET3 was 93.00% ± 4.07%.

3.4.4. In vitro drug release of carbamazepine loaded ethosomes

In vitro drug releases of carbamazepine loaded ethosomes were as follows: Batch ET1 was 79.25%, batch ET2 was 83.10%, and batch ET3 was 88.64% (Fig. 5).

3.4.5. Evaluation of carbamazepine loaded ethosomal in-situ gel

The visual appearances for batches G1-G7 were all milky. The pH, gelation temperature, spreadability, and drug content of all these batches were shown in Table 3.

3.4.6. Stability of carbamazepine ethosomes in in-situ gel

It was observed that the particle size and zeta potential of carbamazepine loaded ethosomes in in-situ gel were 198.5 nm and −55.2 mV, indicating that the size and zeta were almost same as in prepared ethosomes which predicted the stability in gel formulation (Fig. 6A–B).

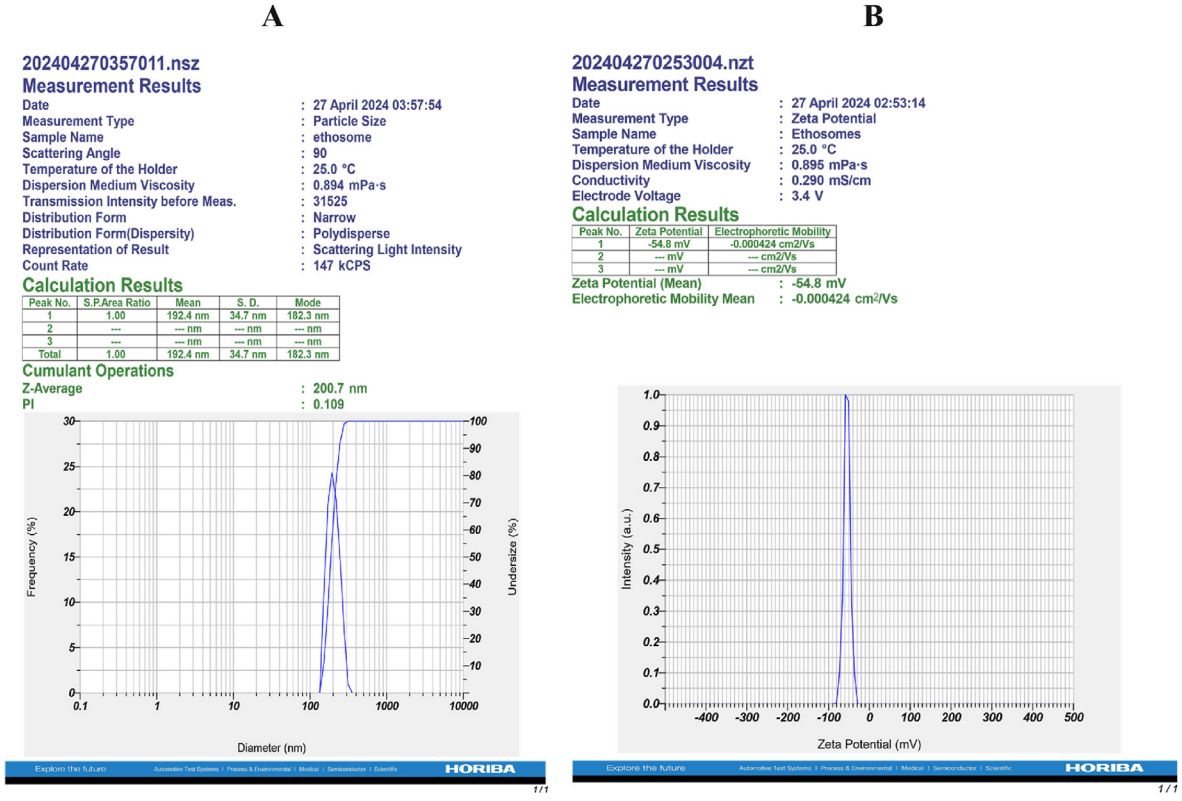

Fig. 3. Particle size (A) and Zeta potential graph (B) of carbamazepine loaded ethosomes.

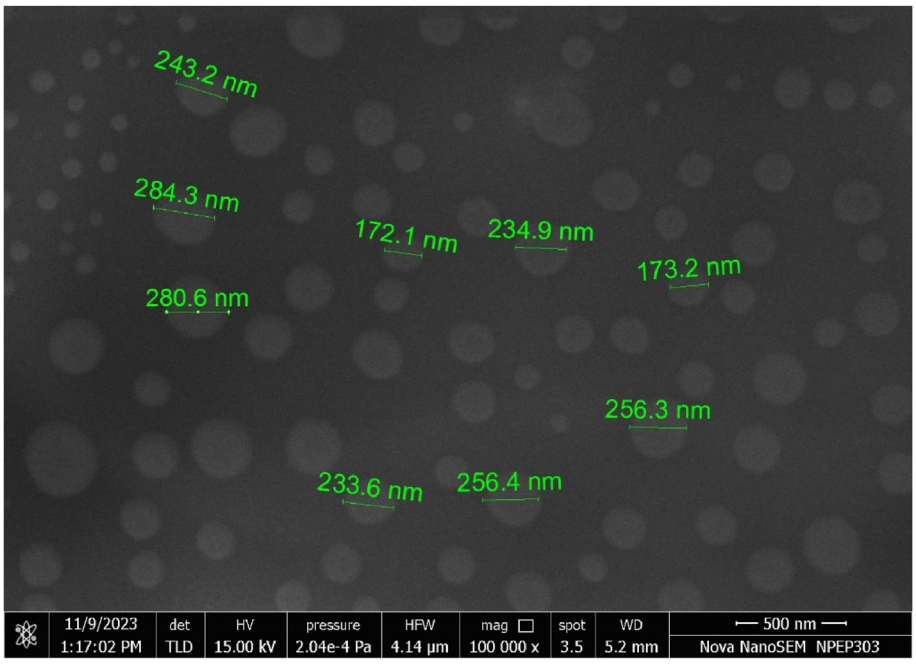

Fig. 4. SEM image of carbamazepine loaded ethosome vesicles. SEM: scanning electron microscopy.

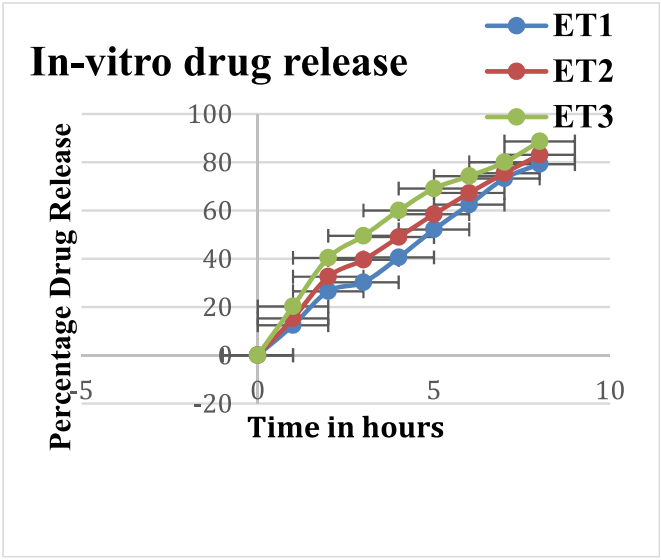

Fig. 5. *In vitro* drug release of batches ET1-ET3 of carbamazepine loaded ethosomes.

Table 3
Evaluation of carbamazepine loaded ethosomal in-situ gel.

Batch code	pH	Gelation temperature (°C)	Spreadability	Drug content (%)
G1	5.8 ± 0.01	33.9 ± 0.20	9.68 ± 1.31	87.20 ± 1.21
G2	5.3 ± 0.03	33.7 ± 0.43	12.84 ± 1.43	96.05 ± 1.96
G3	5.1 ± 0.01	31.9 ± 0.10	6.57 ± 1.27	84.16 ± 1.16
G4	5.4 ± 0.02	34.2 ± 0.26	7.64 ± 0.98	89.28 ± 1.43
G5	6.0 ± 0.01	30.1 ± 1.00	6.82 ± 1.34	91.40 ± 1.90
G6	6.2 ± 0.02	28.9 ± 1.30	5.37 ± 1.29	90.85 ± 1.59
G7	6.5 ± 0.01	35.6 ± 1.21	7.81 ± 1.17	93.72 ± 1.23

3.4.7. In vitro drug release of carbamazepine loaded ethosomal in-situ gel

From *in vitro* drug release studies of batches G1–G7 of carbamazepine loaded ethosomal in-situ gel, it was found that drug release from batch G2 was found to be high as it contains a combination of poloxamer 407, carbopol 934, and HPMC k l00 with respect to all other batches (Fig. 7).

3.4.8. In vitro drug release of optimized batch G2 of carbamazepine loaded ethosomal in-situ gel

The optimized batch G2 was compared with the marketed drug and it was found that the release from the G2 formulation was superior in terms of steady drug delivery for carbamazepine (Fig. 8).

4. Discussion

This study aimed to develop an intranasal drug delivery system that targets the brain to treat epilepsy. As the in-situ gels are thermosensitive, they are liquid at room temperature and change phase at nasal physiological temperature (30–34 °C). The design of formulating the drug in the form of ethosomes and loading it into the gel was highlighted in this research, which was supposed to deliver the drug in the brain to avoid repetitive dosing and to improve patient compliance. Considering this approach, various polymers (thermoreversible and mucoadhesive agents) and excipients were identified from the literature and were considered for pre-formulation and compatibility studies. The compatibility studies were confirmed with the help of DSC and FTIR. Individual samples of the drug and excipients and a combination of physical mixtures of the drug and excipients were prepared for the analysis. All prepared samples were stored according to ICH guidelines and analyzed for any interactions between the drug and excipients. Weak interactions between the drug and excipients were shown by the thermographs of DSC and FTIR spectra. The thermoreversible polymer, poloxomer 407, and the mucoadhesive polymer, carbopol 934, were used to make the gels. The gelation temperature, gel strength, mucoadhesive strength, viscosity, *in vitro* drug release, and *ex vivo* permeation studies using sheep nasal mucosa were all used to characterize the produced gels. Research focused on creating an ethosomal thermoreversible mucoadhesive in-situ intranasal gel after the in-situ gel of carbamazepine was created.

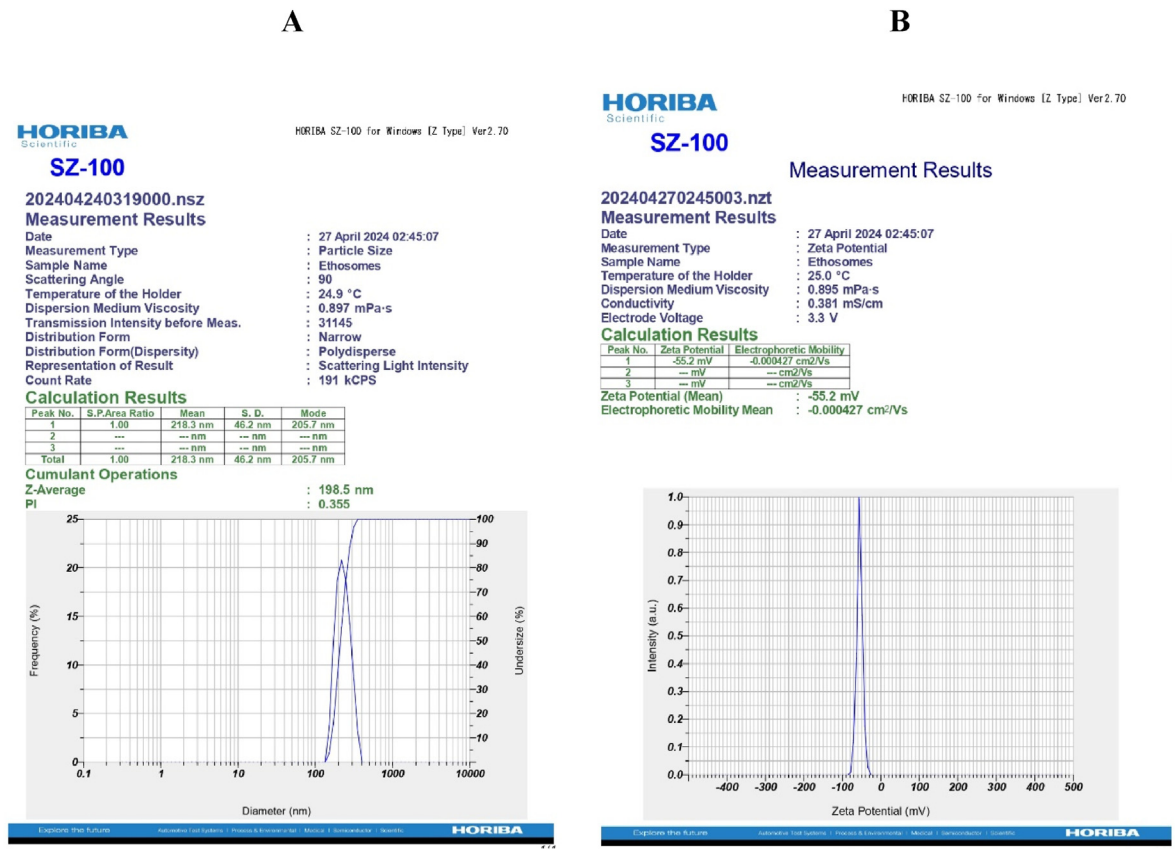

Fig. 6. Particle size (A) and zeta potential (B) graph in the formulation of carbamazepine loaded ethosomal in-situ gel.

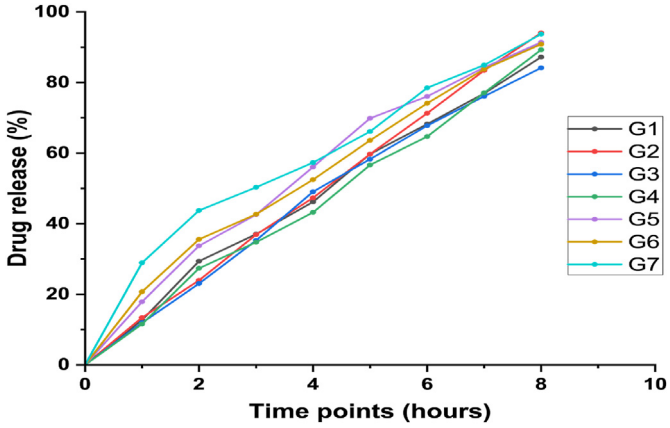

Fig. 7. *In vitro* drug release graph of batches G1–G7 of carbamazepine loaded ethosomal in-situ gel.

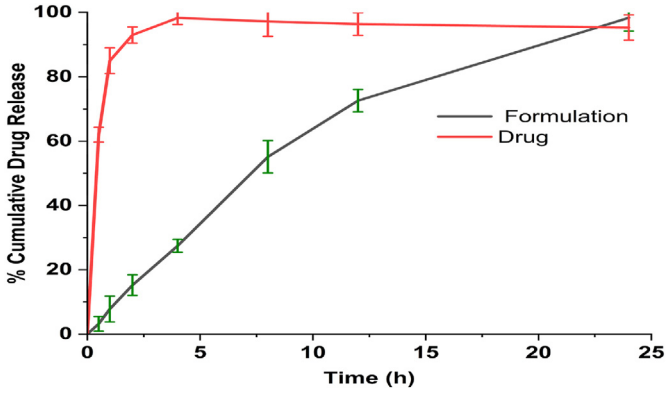

Fig. 8. *In vitro* drug release graph of optimized batch G2 of carbamazepine loaded ethosomal in-situ gel compared with the marketed drug.

Therefore, the creation and characterization of a thermoreversible ethosomal in-situ gel for intranasal brain targeting was the main goal of the current investigation. The cold technique was used to prepare the ethosomes. Ethanol and soya lecithin concentration and two response variables (vesicle size (nm) and % entrapment efficiency) were used. The formulated ethosomes were assessed for zeta potential, vesicle size analysis, and percent entrapment efficiency. The spherical morphology of the ethosomes was confirmed by SEM, while the HORIBA zeta sizer verified the vesicle size. According to the report, formulation ET3 was optimized, with an average vesicle size of 200.7 nm, and the entrapment efficiency of the carbamazepine-loaded ethosomes was 93.00% ± 4.07%.[19–21] The ethosomes were incorporated in gel form using thermoreversible polymer (poloxamer 407) and mucoadhesive agent (carbopol 934 and HPMC k 100) at different concentrations. The pH, viscosity, and *in vitro* drug release were assessed. The results showed that the pH and mucoadhesive strength were 5.1 and 6.5, respectively. *In vitro* drug release from the improved carbamazepine-loaded ethosomal in-situ gel formulation was determined to be 94.05%. It was intended to do more research to create and characterize the thermoreversible ethosomal gel of carbamazepine for intranasal delivery to the brain. A number of criteria were used to assess and describe the created ethosomes. The surface shape and particle size of ethosomes were investigated using a SEM and a HORIBA zeta sizer. The observed vesicle size ranged from 200.7 nm to 345.6 nm, while the percent entrapment effectiveness ranged from 79% to 93%. In order to verify the produced ethosomes' stability in nasal physiology, they were put into the gel and assessed for the relevant characteristics.[1–6] The findings showed that the formulation

had a temperature range of 28.9–35.6 °C (gelation temperature) and a pH range of 5.1–6.5. 94.05% of the medication was released *in vitro* from the optimized ethosomal gel formulation (G2). The ratio of ethanol to PG plays a crucial role in reducing the size of the carbamazepine-loaded ethosome gel formula's particles. In the meanwhile, it has been shown that ethosomes with low phospholipid concentrations have favorable entrapment efficiency. In gel formulation, carbopol outperformed HPMC in terms of spreadability. The carbamazepine-loaded binary ethosome gel was created as a potentially effective non-invasive drug delivery method for treating epilepsy, based on the improved bioavailability and prolonged drug release. The nasal in-situ gel assessment resulted in ethosomes of optimized batch ET3 particle size 200.7 nm, zeta potential −54.8 mv, superior drug percentage drug entrapment 93.00%, and *in vitro* drug release 88.64%. Spreadability is superior for Carbopol batches compared to HPMC k 100. A 33.7 °C in-situ gel-optimized batch G2 was discovered, and 94.05% drug release was observed *in vitro* (as reported in Fig. 7). The stability of ethosomes in gel formulation was evaluated based on particle size and zeta potential, and it was observed that the particle size was 198.5 nm and zeta potential was −55.2 mV in gel formulation, which showed no significant difference between plain ethosomes and ethosomes embedded in gel matrix in final formulation subjected to intranasal delivery of carbamazepine loaded ethosomes via in-situ nasal gel formulation.

The biosafety of carbamazepine-loaded ethosomal nasal in-situ gel is primarily dependent on the safety profile of carbamazepine, the excipients used in the formulation, and the potential for any adverse effects associated with nasal administration needing further investigation. Carbamazepine itself is a well-established anti-epileptic drug with a known safety profile. The excipients used in the formulation, such as phospholipids, cholesterol, and gelling agents, are generally considered safe for topical application. However, as with any drug delivery system, potential adverse effects such as local irritation, allergic reactions, and systemic side effects cannot be entirely ruled out. Therefore, thorough preclinical and clinical studies are essential to assess the safety and efficacy of this formulation.

5. Conclusion

This study successfully developed a thermoreversible ethosomal in-situ gel for the intranasal delivery of carbamazepine, a drug used to treat epilepsy. The ethosomal formulation enhanced drug entrapment efficiency and controlled drug release. The in-situ gel, composed of poloxamer 407 and carbopol 934, provided sustained drug release and mucoadhesive properties. The optimized formulation demonstrated promising potential for targeted drug delivery to the brain, offering a non-invasive alternative for epilepsy treatment. Further research is warranted to evaluate the *in vivo* efficacy and safety of this novel drug delivery system.

CRediT authorship contribution statement

Arjun Bilapatte: Methodology. **Anjali More:** Writing – original draft. **Kranti Satpute:** Writing – review & editing. **Shoaeb Mohammad Syed:** Supervision, Conceptualization.

Conflict of interest

None.

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