doi.org/10.36721/PJPS.2024.37.3.REG.515-525.1

Formulation and development of frovatriptan succinate in situ gel for nasal drug delivery: *In vitro* and *ex vivo* evaluation

Mohammed Layth Hamzah[1]*, Hanan Jalal Kassab[1] and Sultan M. Alshahrani[2]
[1]Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq
[2]College of Pharmacy, King Khalid University, Abha, Kingdom of Saudi Arab

Abstract: Magrain is a depleting disease that sometimes requires extensive treatment, ideally with medication that targets the brain, with minimized systemic adverse effects, preferably with a single daily medication; these properties are offered partially by the current dosage form of Frovatriptan. formulation of Frovatriptan binary ethosome into mucoadhesive nasal in situ gel to extend the drug's residence time. The particle size was 154.1±4.38 nm of t he Frovatriptan binary ethosome. In situ, gel formulas were prepared to utilize the cold technique, using 18%w/v poloxamer 407 with different concentrations of Carbopol 934 and the clarity, pH, Frovatriptan content spreadability, mucoadhesive force, *in vitro* diffusion via nasal mucosa and the optimal formula underwent further investigations. In-situ gel F2 (0.2% Carbopol) demonstrated the best spreadability of 12.88±0.186 cm^2/min, 99% drug content mucoadhesive strength of 645.32±0.054 dynes/cm^2, percent release of 98.56±0.041 after 24 hours and permeability increased by around 3.68-fold compared to the pure drug and histopathologically showed favorable outcomes. Mucoadhesive Frovatriptan-binary ethosome-loaded nasal in situ gel is an effective method of treating migraines.

Keywords: Carbopol, frovatriptan, poloxamer, intra-nasal, in situ gel.

INTRODUCTION

Migraine headaches affect more than one billion individuals each year across the world. It is the second most prevalent cause of job absenteeism, second only to flu (Amiri *et al.*, 2021). Migraine might be treated with either preventative or acute alleviative medications such as serotonin (5-HT) receptor agonists (commonly known as triptans), which primarily target the 5-HT 1B/1D receptor, which prevents the abnormal vasodilation of blood vessels, a common migraine symptom.

Seven triptans are on the market, given orally, intranasally and subcutaneously. While Frovatriptan succinate (FVT) parenteral formulation has a very high absolute bioavailability (96 percent), it causes painful administration. Nonetheless, despite its long half-life (26 h) and potency, the medication is classified as a low-efficacy triptan because of its delayed onset of action and it is formulated as film-coated tablets or fast-dissolving films (Al-anbagi *et al.*, 2018). However, current dosage formulations have several drawbacks, including a slow onset of action, inadequate bioavailability (10-30%) because of its low solubility and degradation in the gastrointestinal tract (GIT) (Anitha *et al.*, 2021) and undesirable side effects such as coronary vasospasm, pain, tightness in the chest and finger numbness. One of the drawbacks of film-coated tablets is that they need repeated doses to be administered orally. FVT's inability to cross the blood-brain barrier may be because it is a hydrophilic drug (Deepika *et al.*, 2019).

While intranasal (IN) drug delivery is an attractive option (Younis and Abd Alhammid, 2023), the challenge for the formulator is great when attempting to find a method to infiltrate the mucus lining of the nasal canal without inflicting irreversible tissue damage. The main disadvantages are small nasal cavity capacity (200 µL), mucociliary clearance and anterior leakage, which might impair the effectiveness of the nasal medication delivery system (Sherafudeen and Vasantha, 2015, Sulaiman *et al.*, 2018, Salih and Ghareeb, 2021, Yousif and Khalil, 2009, Adnan *et al.*, 2022). In situ gels provide several advantages over typical gels, such as being simpler to produce and longer retention in the nasal mucosa time owing to gelling and mucoadhesion (Nief *et al.*, 2019, Alabdly and Kassab, 2022).

This study developed new in situ-gel formulations of FVT for intranasal application to extend the drug's residence time in the nasal cavity and enhance its absorption across the nasaomucosal barrier.

MATERIALS AND METHODS

Materials

Frovatriptan succinate (CAS Number: 158930-17-7) was purchased from Pharmaffiliates (Catalogue number: PA 06 83000, India), Poloxamer 407 was purchased from Sigma-Aldrich® Solutions, Germany (CAS Number: 9003-11-6, ID: 16758), Carbopol 934 was purchased from HiMedia Laboratories LLC, USA (stock keeping unit: GRM6761) and Dialysis Membrane-135 was purchased from HiMedia Laboratories LLC, USA (stock keeping unit: LA398), phospholipon ® 90H [Phospholipids, hydrogenated, with 70% phosphatidylcholine] was purchased from Pharma Excipients ™ (CAS-No. 97281-

*Corresponding author: e-mail: uobaghdad.edu.iq

48-6), the rest of the materials were procured from local vendors.

Methods

Characterization of FVT-loaded binary ethosome dispersion formula Assessment of particle vesicle size, polydispersity and ζ- potential binary ethosome

Particle size using Zetasizer Malvern Ultra Red, Worcestershire, U.K.) (Muslim and Maraie, 2022); all measurements were run in triplet and the particle size was 154.1±4.38 nm ,as illustrated in fig.

Formulation of in situ nasal gel of FVT

The artificial nasal fluid was synthesized by mixing potassium chloride (745 mg), calcium chloride (145 mg) and sodium chloride (2,1925 g) to a total volume of 250 ml D.W. (Mahajan *et al.*, 2009). FVT as binary ethosome (200mg/40mL) was prepared previously (5ml used in each formulation, which contains 5mg per ml FVT); later, it was incorporated into In situ nasal gels by mixing Poloxamer 407 (18% w/v) with a cold method (Kempwade and Taranalli, 2014), the in situ gel was hydrated overnight at (4-8°C). Binary ethosome dispersion of FVT was mixed with different concentrations of Carbopol 934 with a preservative (table 1) and this dispersion was added to the poloxamer dispersion.

Evaluation of in-situ gel

Several evaluation steps, including the particle size, clarity, pH, FVT content homogeneity, spreadability, *in vitro* diffusion via nasal mucosa, gel strength, stability study, mucoadhesive force, histopathology investigation, *ex vivo* permeation research, were performed to assess the investigated formulations (Rajput and Butani, 2019, Silva *et al.*, 2018).

Visual Appearance and Clarity (refractive index) Measurement

The naked eye inspected the prepared formulas. Using an Abbes refractometer (Heathrow Scientific, USA), it was possible to assess the clarity of sols or formulations regarding their refractive indexes before gelling. Calibration was done using D.W. as a standard for the refractometer (Galgatte *et al.*, 2014b). The telescope's cross wire was accurately positioned on the boundary separating bright and dark areas by modifying the refractometer scale (Gupta and Sharma, 2009). Repeating the process for each formulation F1 through F6 allowed for comparing results to the refractive index of water (1.33) (Galgatte *et al.*, 2014b).

pH measurement

The electronic pH meter was calibrated using 4 and 7 pH buffer solutions (Equip-Tronics EQ-610, India). The glass electrodes were dipped into each formula collected in a beaker. Following that, the pH of the solution was checked three times (Nisha *et al.*, 2012).

Spreadability

A slip-and-drag method was used to measure spreadability. A precise quantity of FVT in situ gel was carefully measured and put onto a transparent glass plate. A second transparent slide was positioned above the gel-coated slide affixed to a wooden block. To ensure uniform distribution of the gel, a weight of 100 grams was positioned on the upper slide for a while, causing the gel to spread evenly between the slides without leaving any residue on the sides. The weight was removed and placed on the wooden block (Swati *et al.*, 2017). The duration required for the upper plate to fully detach from the bottom plate. The concept of spreadability is quantified as follows:

$$S = mx \frac{L}{t}$$

m: upper plate mass (g), S: spreading area mm^2, L: glass plates length (cm), depending on mass, t: time taken to separate (s).

Drug content

The drug content of formulations was evaluated in triplicate with a double-beam ultraviolet (U.V.) visible spectrophotometer (Shimadzu UV-1800). One ml of the formula was transferred into a 10-ml volumetric flask and the remaining 9 milliliters were double-distilled water. A 1 ml sample was prepared by diluting the original solution with 10 mL of double-distilled water. As a last step, a UV-visible spectrophotometer (Gaikwad, 2010) was used to determine the absorbance of the solution after it was created.

Viscosity

The viscosity analyses used the Brookfield viscometer RVDV-IIþ pro model (Brookfield Engineering Laboratories, USA). The viscosity tests used a small-volume adaptor (Mahajan *et al.*, 2009). Prepared compositions' apparent viscosity values were tested and set to spindle 3 and 100 rpm and the measurement temperature was taken between 32-34ºC.

Mucoadhesive strength

The formulation's mucoadhesive force and detachment stress were determined using a modified version of the mucoadhesive force measuring instrument—the experimental procedure involved utilizing a modified balance approach, employing two glass vials and sheep nasal mucosa. The vials were subjected to a temperature range of 32-34ºC for 10 minutes. A vial was affixed to a single side of the balance. At the same time, a 0.5 ml quantity of gel specimen was positioned between the two mucosal membranes connected to the lower portion of the vials. The researchers measured the lowest water weight necessary to disrupt the mucosal adhesion (Gaikwad, 2010, Kumar *et al.*, 2010).

$$\text{Mucoadhesive strength (dyne/cm2)} = \frac{M.G}{A}$$

where M = the weight needed to separate tissues in g, G = gravity's acceleration (980 cm/s^2) and A = mucosal exposed area.

Gelling temperature and time

It was determined using Miller and Donovan's method. A volume of 2 mL from each formulation was extracted as an aliquot and placed into a test tube, which was subsequently immersed in a water bath. One °C systematically raised the temperature of the water bath at a time, then left it to stabilize for five minutes at each consecutive temperature increment. The specimens were examined to determine if gelation had occurred, indicated by the cessation of meniscus flow when tilting the samples at a 90° angle. The gelling time was measured as the duration from the initial detection of gelation (Miller and Donovan, 1982).

Scanning electron microscopy (SEM)

Using SEM, the morphology of F2 and F3 was examined (SEM, Hitachi, Tokyo, Japan). A drop of in situ gel formulation of F.T. was diluted in double-distilled water (D.W.) (1 in 100) and then air-dried on a sample holder. A range of magnifications and accelerating voltages, up to 15,000 volts, were then used to examine the sample. To get clear pictures, researchers used a high vacuum (Eltahir *et al.*, 2022).

In-vitro drug release study

The researchers studied the diffusion of drugs in different formulations using a Franz diffusion cell (Gowda *et al.*, 2011). A diffusion membrane (molecular weight of 12,000-14,000 Da) was employed for dialysis. The dialysis membrane was immersed in a phosphate buffer solution with a pH of 6.4 for 24 hours before the commencement of the test. The diffusion cell was filled with 21 mL of phosphate buffer solution at a pH of 6.8 and a dialysis membrane was affixed to the cell. The gel, which contained a medication corresponding to a dosage of 2.5 mg, was applied to the donor chamber. A circulating water bath regulated the 32-34°C temperature. 1 ml specimens were collected at different time points (0.5, 1, 3, 6, 12 and 24 hours) and subsequently substituted with an equal volume of the new solution. Subsequently, the specimens underwent filtration and the amount of the pharmaceutical product was quantified by employing a UV-visible spectrophotometer configured to operate at a wavelength of 255 NM.

Compatibility studies

Fourier transform infrared spectroscopy was used at room temperature to examine excipient and medication compatibility (Model-200, Thermo Electron, Shimadzu, Japan). Pure FVT and polymers (both by themselves and in different combinations) were analyzed by Fourier transfer infrared (FTIR) spectroscopy (1:1). The KBr pellet method (Pappas *et al.*, 2003) was used for the analysis of the samples.

Differential scanning calorimetry (DSC)

DSC was used for testing purposes (Netzsch, Hanau, Germany). The samples were heated between 50 and 300 degrees Celsius at 10 degrees Celsius per minute. The empty aluminum pan served as a control in this study of the material; the DSC profiles of FVT, Carbopol 934P and in situ gel (dry power of gel obtained using lyophilization method). A common thermal analysis technique to learn about the energy and physical features of substances and mixtures is the DSC.

Ex-vivo drug permeation study and nasal mucosa preparation

The euthanasia procedure involved the intravenous administration of pentobarbital sodium (100 mg/kg) to three healthy lambs at the age of 6 months. Following the demise of the subjects, the lambs underwent decapitation. Subsequently, the distinct components of the nasal cavity, namely the nasal concha and septum, were submerged in a solution consisting of PBS medium supplemented with 5% double antibody and 1% amphotericin and gentamicin. Subsequently, the distinct variations of the nasal cavity were carefully placed into a 50 ml centrifuge tube pre-filled with PBS and antibiotics. Following the agitation of the container, it was subjected to a series of washing procedures, repeated 3 to 4 times. Each wash cycle was conducted for a duration of 5 to 10 minutes. After the completion of the process, the washed nasal tissue was carefully transferred onto a sterile plate. The nasal mucosa tissue was carefully removed using ophthalmic scissors and tweezers.

Furthermore, a segment of the nasal mucosa is extracted and carefully positioned on a fresh, clean plate, ensuring that the surface is upward. Afterward, the nasal mucosa is treated, resulting in uniformly shaped round tissue fragments of 8 mm in width, using a tissue sampler. The mucosal membrane was pretreated and then placed in a 12-hole transwell equipment, with the mucosal surface facing upwards. The upper compartment filter membrane has a pore diameter of 0.4 μM. Approximately 600 μL of culture media was introduced into the complete transwell device, with a thickness of only half that of the mucosal tissue. The tissue was cultured at 37°C and in an atmosphere containing 5% carbon dioxide. The culture medium was refreshed regularly (Alfi *et al.*, 2020, Zheng *et al.*, 2023).

At 34 degrees Celsius, 12 milliliters of phosphate buffer saline (pH 6.8) were added to the acceptor chamber and agitated with a magnetic stirrer (pure drug solution (2.5 mg/100 dispersion) and optimal formula (F2) were used). After incubating the donor part for 30 minutes, thermoreversible gel compositions were added. 1ml aliquots of samples were taken out at regular intervals up to 24 hours and replaced with phosphate buffer saline pH 6.8. As the dust settled, a spectrophotometer set to 255

nm was used to examine the samples. The drug formulations' permeability coefficients (in cm/s) were determined with the use of the following formula,

$$\text{Premeability coefficient} = (\frac{d_c}{d_c})_{ss} \times \frac{V}{AC_d}$$

Where V (mL) is the volume of the receiver compartment; A (cm^2) is the permeation area; C_d (µg mL^{-1}) is the initial donor concentration and $(dc/dt)_{ss}$ (µg mL^{-1} s^{-1}) change of concentration under steady-state (Shelke *et al.*, 2015).

Histopathological assessment

After being treated in PBS (pH 6.8) or a diffusion chamber with the gel formulation, tissue samples were histo-pathologically examined. A 10% buffered formalin solution (pH 6.8) was used to fix the tissue. After being broken into smaller pieces and put on glass slides, paraffin blocks were stained with hematoxylin and eosin. A pathologist unaware of the study's objectives examined tissue slices under a light microscope for indications of drug penetration outside the body (Majithiya *et al.*, 2006).

ETHICAL APPROVAL

The University of Baghdad approved the study – College of Pharmacy, Research Ethical Committee (approval name: RECAUBCP532022G, approval date: 5-2-2022).

STATISTICAL ANALYSIS

GraphPad Prism 10 was used to make the statistical analysis. One-way ANOVA was used to analyze the difference in mean and post hoc Tukey U test to assess the pair-wise comparison. Alpha level was ≤0.05 (significant level).

RESULTS

Visual Appearance Refractive index (ARI)

The refraction index was near the value of 1.33, which is the value for water; F1 showed the highest ARI compared to other formulas (F2 to F6, with no significant difference between them), table 3.

Assessment of pH

The pH for every formula was determined and found to be within the suitable range for the nasal passages (between 5.3 and 7.0 (LEE *et al.*, 2009)). F2 and F6 (no significant difference) showed significantly higher pH values than the other formulas, table 3.

Drug content

According to table 3, the average percentage of active pharmaceutical ingredients across all formulations was between 92.27±0.21% and 99.72±0.02%, with no significant difference among various formulas, table 2.

Viscosity

The Brookfield viscometer readings for each formulation are illustrated in table 4; F2 showed the lowest viscosity and was significantly lower than the other formulas.

Gelling temperature and time

Mucociliary clearance represents a significant constraint in the context of nasal medication delivery. Consequently, utilizing thermoreversible gels is a potentially advantageous preparation strategy for addressing this challenge. The current study involved the development of thermoreversible gel formulations using poloxamer and carbopol to improve the drug's bioavailability (table 4). The optimal temperature range for nasal administration has been documented to be between 25 and 37 °C (Cho *et al.*, 2011); therefore, the gelling temperature of the product must exceed 25°C to mitigate challenges encountered throughout the mixture's production, handling and administration processes (Cho *et al.*, 2008), the temperature of the nasal cavity is typically approximately 34°C (Türker *et al.*, 2004).

Therefore, we aimed to develop a thermoreversible gel using P407 that would exhibit a gel-like form within the 25 to 34°C temperature range and transition to a liquid state below 25 °C. In the current study, F2 showed the lowest gelation time (8.23±0.32 sec), with a gelation time range for all formulas between 8.23±0.32 to 12.43±0.41 sec, the same time gelation temperature range was 29.32±0.25 to 36.38±0.32°C, indicating all formulas exhibit acceptable gelation temperature. The process of immediate gelling increases the duration of drug residence and improves the extent to which the drug is absorbed and available for use in the body (Pathak *et al.*, 2019). All the formulas become gel quickly. Thus, F2 showed the best value in these regards.

Adding the mucoadhesive ingredient carbopol 934 P to the formula reduces the gelling temperature because it can attach to the poly(ethylene) oxide chains in the poloxamer 407 molecule. As a result, this attachment causes dehydration and enhances the entanglement of neighboring molecules. Micellar interaction is facilitated by the considerable intermolecular hydrogen bonding (Majithiya *et al.*, 2006).

Mucoadhesion

Mucoadhesion data for the F2 and F3 formulation series (645.32±0.054 and 753.24±0.062, respectively) are shown in table 4. In situ gelling nasal formulations benefit greatly from a high mucoadhesion force since this increases the amount of time the gel spends in the nasal cavity before being cleared out without causing any leaks (Galgatte *et al.*, 2014a). More importantly, a gel's excessively high mucoadhesive force can damage the nasal mucosal membrane [7]; thus, F2 is ideal.

Spreadability

Table 3 illustrates the spreadability profile of various formulas; the F2 formulation of the in-situ gel had the greatest spreading ability (12.88±0.186).

In-vitro cumulative release study

By analyzing how quickly the drugs were released from F2 and F3, we may deduce that the Carbopol's chemical composition is critical for facilitating drug release. A quick initial release was followed by a delayed maintenance release of the FVT-loaded in situ gel in F2. In the first 30 minutes, around 22.3% of the medication is released, whereas after 24 hours, about 98.56% is released (fig. 2).

Surface morphology - scanning electron microscopy

Shape optimization, segregation, uniformity and monodispersity all point to a stable nanoformulation (Fig. 3). The nanoparticles (N.P.) could stay separated because of the surface charge existing on them, as shown by their monodisperse distribution.

Compatibility studies

The FTIR spectra of the drug in different polymer mixes were compared to the FTIR spectrum of the pure drug. FVT spectra showed a clear peak at 3120.94 and 3497.06 cm^{-1}, confirming the presence of the primary amide group in monohydrate form. Spectral bands at 827 and 1100 cm^{-1} indicate the presence of an aromatic group, whereas the peak at 1666.48 cm^{-1} is characteristic of the presence of a C=O bond. The drug is compatible with the gelling agent's poloxamer 407 and Carbopol 934, as shown by FTIR spectroscopy investigations of drug-polymer interactions, revealing weak physical contact. Figure 3 shows the FTIR spectra of the medication and a physical combination of FVT with poloxamer 407 and Carbopol 934. The peaks at 1690, 1810, 2530, 2900 and 3300 cm^{-1} were all present, but the strength of the peak at 3500 cm^{-1} was reduced, demonstrating the weak physical interaction, as illustrated by fig. 4.

DSC studies

The DSC thermogram of FVT-loaded in situ gel, optimized binary ethosome formula and pure FVT drug are seen in fig. 5. The FVT pure drug showed an endothermic peak at 152.98°C, indicating that FVT is pure and crystalline, associated with its melting point and breakdown. The optimized formula FVT binary ethosome showed an endothermic peak of the drug at 102.75°C. The FVT-loaded in situ gel (F2) showed an endothermic peak of the drug at 104.92°C. The decrease in the heat-absorbing capacity of the drug might be attributed to the combination of the drug and other substances, which decreased the purity of each component in the final product. However, this does not necessarily imply probable incompatibility (Rajendra *et al.*, 2016).

Ex vivo permeation study

Ex vivo studies on goat nasal mucosa examined the drug penetration characteristics of in situ gel loaded with FVT. After 48 hours, those exposed to the F2 absorbed 496.38±2.21 mg/cm^2, but those exposed to the FVT solution absorbed just 148.32±1.64mg/cm^2. The steady-state flow rate of the F2 was 6.985 $mg/cm^2/h$, whereas that of the FVT solution was only 1.896 0.14 $mg/cm^2/h$. When FVT was encapsulated in situ gel, the medication's permeability increased by around 3.68-fold compared to the drug solution, indicating that the therapy would diffuse through the nasal mucosa (fig. 6).

Histopathological evaluation of nasal mucosa

Microscopic investigation (as shown in fig. 7) reveals that the new and better formulation did not cause any appreciable changes to the mucosal microstructure. No cell necrosis or epithelium loss was seen in the nasal mucosa while using the formulation and buffer at pH 6.8.

DISCUSSION

Ethosomal systems are lipid vesicles that serve as carriers with a comparatively elevated ethanol concentration. These nanocarriers are precisely designed to efficiently carry therapeutic drugs with various physicochemical characteristics (Abdulbaqi *et al.*, 2016). Binary ethosomes were introduced by Zhou (Zhou *et al.*, 2010). Essentially, the development process involved the incorporation of an additional kind of alcohol into the traditional ethosomes. Propylene glycol (P.G.) is the predominant alcohol in binary ethosomes (Li *et al.*, 2012, Zhang *et al.*, 2012, Akhtar and Pathak, 2012). Phospholipids derived from various sources have been utilized to develop ethosomal systems. The choice of phospholipid type and concentration is crucial in forming an ethosomal system as it directly affects the size, entrapment efficacy, ζ-potential, stability and penetrating properties of the vesicles (Abdulbaqi *et al.*, 2016).

The main factor in ethosomal preparations is the intrinsic characteristics or physicochemical qualities of the drug/agent that will be incorporated because the drug/agent can potentially affect the structure of the ethosomal systems. Generally, ethosomal vesicles demonstrate a negative charge (Abdulbaqi *et al.*, 2016), which aligns with the results of the current investigation. The user's text is empty.

Ethosomal gels show compatibility with ethosomal systems, displaying the required viscosity and bioadhesive properties (Patel *et al.*, 2012, Bhana *et al.*, 2013). Thus the current work directed to formulate a noval binary ethosome nanocarrier as in situ gel formulation by enhancing the characteristic of the final formulation; this work was in agreement with previous work done on two other triptans, namely, Zolmitriptan (Shelke *et al.*, 2016b) and Eletriptan (Shelke *et al.*, 2016a).

Table 1: Composition of in situ nasal gel of FVT formulations

Drug/Excipients	F1	F2	F3	F4	F5	F6
Binary ethosome dispersion (containing 5 mg of FVT / mL)	5 mL	5 mL	5 mL	5 mL	5 mL	5 mL
Poloxamer 407 (%w/v)	18	18	18	18	18	18
Carbopol 934 (%w/v)	0.1	0.2	0.3	0.4	0.5	0.6
Methyl paraben (%w/v)	0.02	0.02	0.02	0.02	0.02	0.02
SNF q. s.	10 mL	10 mL	10 mL	10 mL	10 mL	10 mL

SNF: Sulphonated Naphthalene Formaldehyde, q.s.: sufficient quantity

Table 2: Physical characterization of FVT loaded in situ gel formulations.

Batch code	Visual Appearance	Refractive index	pH	% Drug contents
F1	Milky white	1.465±0.02[a]	5.49±0.023[a]	92.27±0.21
F2	Milky white	1.339±0.01[b]	6.35±0.164[b]	99.72±0.02
F3	Milky white	1.349±0.01[b]	5.46±0.052[a]	95.43±0.14
F4	Milky white	1.385±0.02[b]	5.39±0.027[a]	98.34±0.37
F5	Milky white	1.341±0.03[b]	5.84±0.126[a]	98.21±0.28
F6	Milky white	1.351±0.01[b]	6.02±0.135[b]	96.56±0.11
p-value	-	0.003	0.001	0.948

A column with a similar letter indicates no significant difference (p-value>0.05)

Table 3: Physical parameters of FVT loaded in situ gel formulations.

Batch code	Mucoadhesive strength (dyne/cm^2)	Viscosity (cps) (100 rpm)	Cumulative Drug release (%) After 24 hours	Spreadability (cm^2/min)	Gelation temperature (□)	Gelation time (Sec)
F1	896.45±0.025[a]	1235.2±1.253[a]	75.38±0.025	9.68±1.31[ab]	36.38±0.32[a]	9.29±0.26[a]
F2	645.32±0.054[b]	658.92±0.649[b]	98.56±0.041	12.88±0.19[ab]	34.14±0.45[b]	8.23±0.32[a]
F3	753.24±0.062[c]	1564.2±1.283[c]	83.21±0.329	10.57±1.27[ab]	32.15±0.32[c]	10.65±0.17[b]
F4	986.41±0.342[d]	1127.3±0.462[d]	90.68±0.162	8.64±0.98[ab]	30.34±0.52[d]	11.74±0.45[b]
F5	1234.52±0.049[e]	1059.4±1.385[e]	88.03±0.318	7.82±1.34[ab]	31.42±0.32[d]	12.43±0.41[b]
F6	1568.92±0.021[f]	984.32±1.426[f]	75.34±0.214	6.37±1.29[b]	29.32±0.25[e]	10.56±0.32[a]
p-value	<0.001	<0.001		0.023	<0.001	<0.001

A column with a similar letter indicates no significant difference (p-value>0.05)

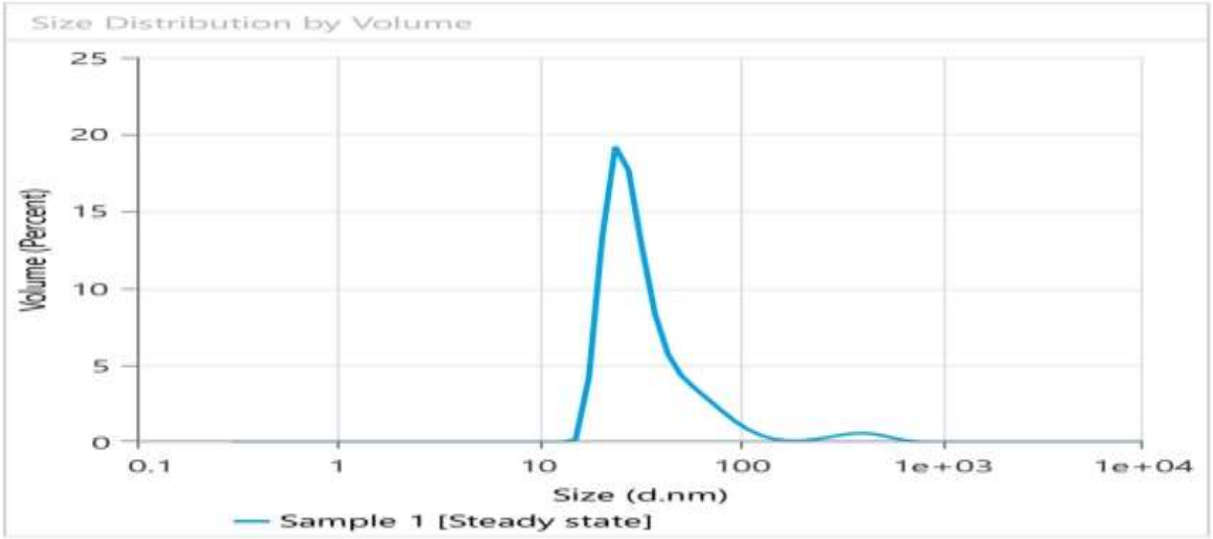

Fig. 1: particle size of FVT-loaded binary ethosome dispersion formula

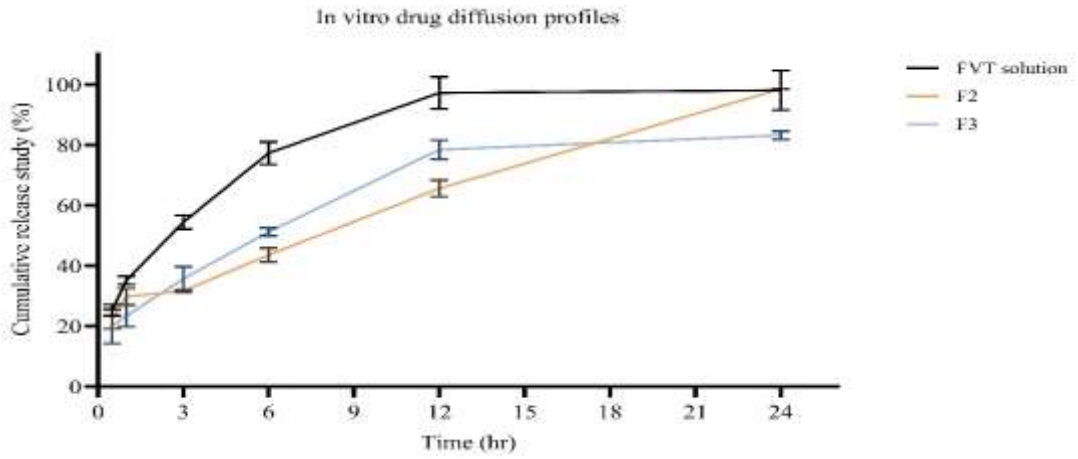

Fig. 2: *In vitro* drug diffusion profiles of FVT solution, F2 and F3 in situ gel formulation.

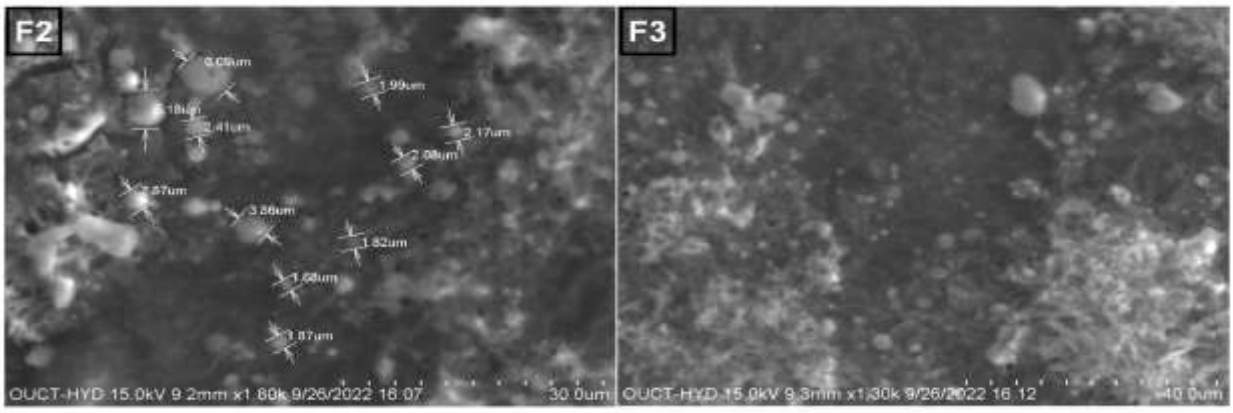

Fig. 3: SEM image of an optimized batch of F2 and F3

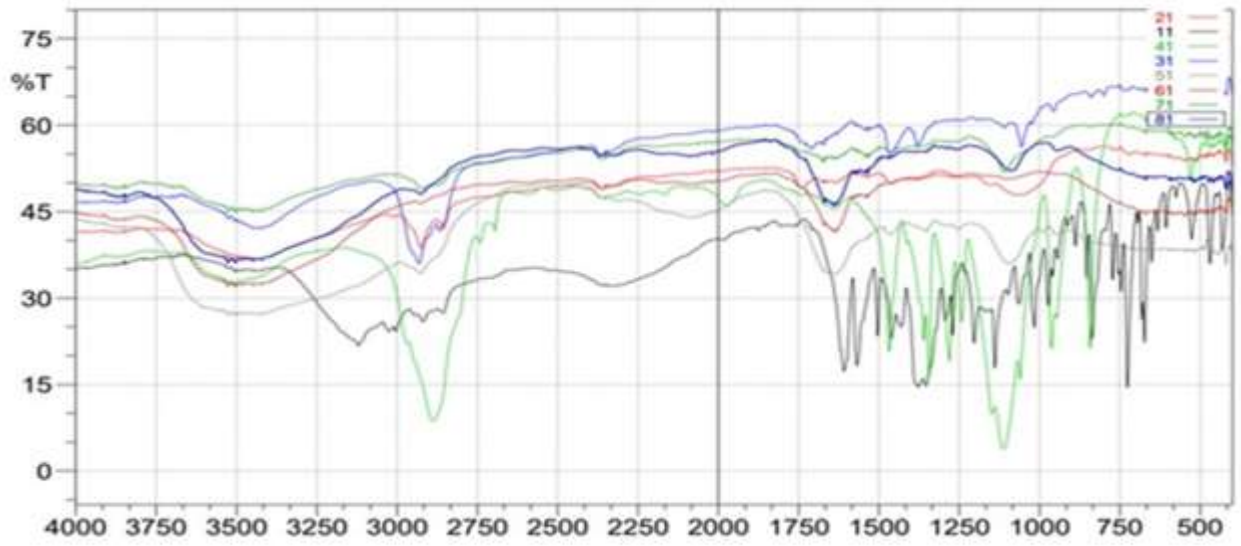

Fig. 4: FTIR spectral studies of 1) Pure Drug (black line; line-11), 2) phospholipon 90H (red line; line-21), 3) Ethanol (white, blue; line-31), 4) propylene glycol (light green; line-41), 5) Poloxamer 407 (white gray; line-51), 6) F9 (dark red; line-61), 7) Carbopol 934 (dark green; line-71), 8) G2 (dark blue; line 81)

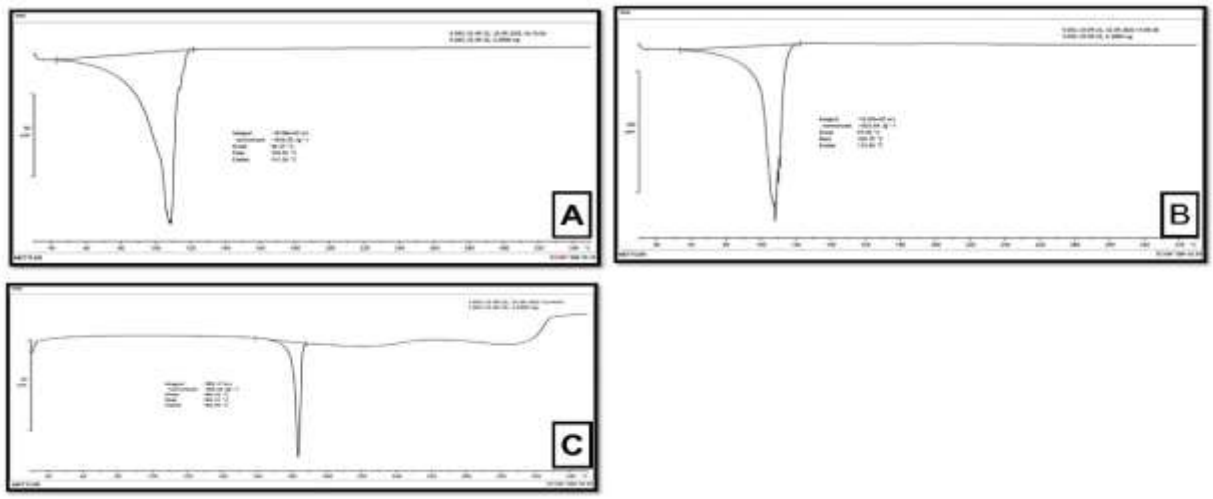

Fig. 5: DSC Thermograms of A) optimized formula FVT binary ethosome, B) FVT loaded in situ gel (F2), C) Pure Drug

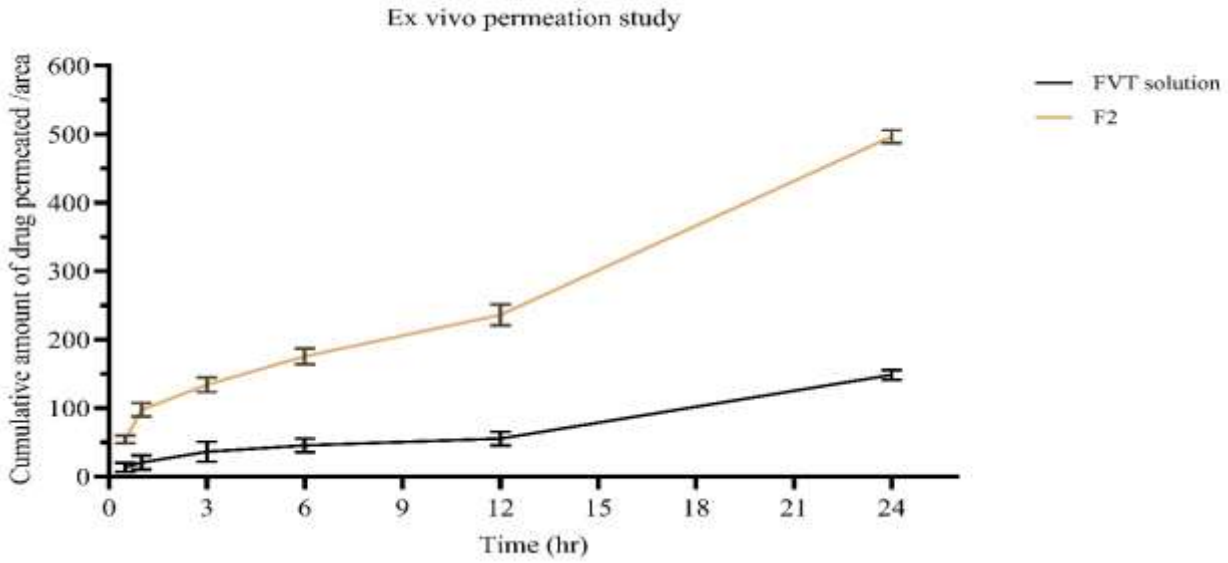

Fig. 6: *Ex vivo* permeation study at nasal pH 6.4

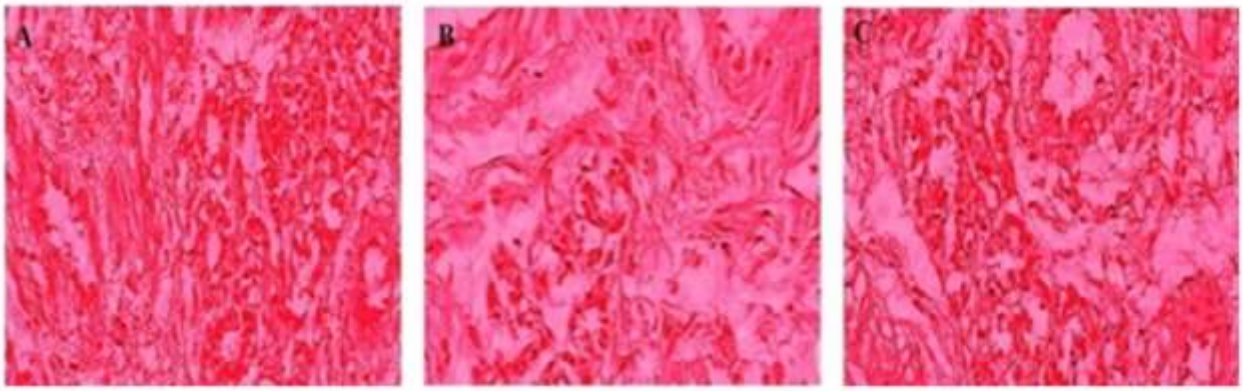

Fig. 7: Histopathological studies of A) Normal, B) Positive control and C) F2.

Zolmitriptan is a partial 5HT571B/1D receptor agonist employed to treat acute migraines (Gooriah *et al.*, 2015). It is commercially available in orally disintegrating tablets and nasal sprays (Dowson *et al.*, 2002, Belvís *et al.*, 2009, Yates *et al.*, 2002). The main challenges of traditional distribution include first-pass metabolism, low bioavailability, multiple administration and significant systemic adverse effects (Solomon *et al.*, 1997). To address this issue, Shelke *et al*. (2016) incorporated Zolmitriptan into nanoethosomes and developed a thermosensitive mucoadhesive in situ gel containing the drug-loaded N.P. The N.P. was formulated using the ethanol injection method. The refined composition was integrated into a thermoresponsive in situ gel based on P407, with Hydroxypropyl Methyl Cellulose K100 and carbopol 934 as the mucoadhesive agent. The formulation exhibited gelation at temperatures ranging from 32 to 34°C. The gel charecterization exhibited a positive correlation with the quantity of the mucoadhesive agent. The pH was measured to be 6.1, which indicates that it is within a range that the nasal mucosa can tolerate well. The drug release in a controlled environment and the drug penetration through biological tissues outside the body demonstrated a continuous and gradual release of the drug through the process of diffusion. The incorporation of the gelling agent and mucoadhesive polymer resulted in a deceleration of the medication release rate. Furthermore, the penetration coefficient was determined to be 5.9 $\mu g/cm^2$. Additionally, the histological analysis verifies the formulation's lack of toxicity. Overall, the current technique provides a superior approach for managing migraines (Shelke *et al.*, 2016b).

Comparably, Shelke *et al*. 2016 replicated a similar approach with another triptan drug (eletriptan hydrobromide); eletriptan hydrobromide functions as an agonist for the 5HT1B receptor (Takiya *et al.*, 2006, Slassi, 2002, Hoskin *et al.*, 2004) due to its limited oral bioavailability of 50%, the drug's therapeutic effectiveness is reduced. In addition, the administration of the medication orally results in a range of unfavorable side effects, such as nausea, numbness, dizziness, sleepiness, weakness and serotonin poisoning that is dependent on the dosage (Pichard-Garcia *et al.*, 2000). To overcome the constraints and create a highly efficient system for delivering eletriptan hydrobromide to the brain, Shelke *et al*. devised and enhanced the drug-loaded ethosomal formulation using the method previously outlined. In addition, the ethosome that was created was included in a thermoreversible in situ gel. The formulation exhibited a sol-gel transition temperature ranging from 32 to 34°C, which is suitable for nasal delivery. The formulation's pH is also within an appropriate range; chemical linking between the mucoadhesive polymer and the mucus membrane led to a robust mucoadhesive property of the gel (Wang *et al.*, 2013). In addition, it revealed sustained release properties *in vitro* release. In addition, no indication of local toxicity was observed in the *ex vivo* permeation analysis and histological assessment. The IN in situ gel demonstrated efficacy and safety in delivering anti-migraine drugs to the brain (Shelke *et al.*, 2016a).

CONCLUSION

The thermoreversible polymer and carbopol in In Situ Gel F2 provided superior gelation viscosity, gel strength, drug release capabilities and mucoadhesive strength. A thermoreversible, mucoadhesive in situ intranasal gel containing frovatriptan succinate was created. Gel's mucoadhesive quality extends its stay in the nasal cavity and its thermoreversible nature makes it convenient to handle and administer. Carbopol 934 at a w/v concentration of 0.2% is optimal regarding mucoadhesive strength and the required phase transition temperature. The drug's efficacy is boosted by the carbopol, which also facilitates better penetration. This formulation did not significantly harm the nasal mucosa.

The present study's findings support using Frovatriptan succinate as a novel mucoadhesive in situ intranasal gel for treating migraines. We recommend using this formulation instead of the available markered oral dosage form, which has poor systemic bioavailability and permeability to the brain since it enhances Frovatriptan succinate bioavailability and permeability to the brain.

ACKNOWLEDGMENT

We want to thank the Department of Pharmaceutics College of Pharmacy University of Baghdad for their help completing this work.

REFERENCES

Abdulbaqi IM, Darwis Y, Khan NA, Assi RA and Khan AA (2016). Ethosomal nanocarriers: the impact of constituents and formulation techniques on ethosomal properties, *in vivo* studies and clinical trials. *Int. J. Nanomedicine*, **11**: 2279-304.

Adnan M, Nief R, Abd-Al Hameed SN and Kharaba HA (2022). Preparation and *in-vitro* evaluation of floating oral in-situ gel of montelukast sodium (Conference Paper). *Iraqi J. Pharm. Sci.*, **31**(Suppl.): 162-167.

Akhtar N and Pathak K (2012). Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: Development and comparison with ethosomal gel. *AAPS PharmSciTech.*, **13**(1): 344-55.

Al-anbagi MS, Rajab NA and Khalil YI (2018). Preparation and characterization of timed drug delivery system of sumatriptan using natural polymers. *Iraqi J. Pharm. Sci.*, **27**(1): 89-99.

Alabdly A and Kassab HJ (2022). Formulation variables effect on gelation temperature of nefopam

hydrochloride intranasal in situ gel (Conference Paper). *Iraqi J. Pharm. Sci.,* **31**(Suppl.): 32-44.

Alfi O, From I, Yakirevitch A, Drendel M, Wolf M, Meir K, Zakay-Rones Z, Nevo Y, Elgavish S, Ilan O, Weisblum Y, Tayeb S, Gross M, Jonas W, Ives J, Oberbaum M, Panet A and Wolf DG (2020). Human nasal turbinate tissues in organ culture as a model for human cytomegalovirus infection at the mucosal entry site. *J. Virol,* **94**(14): 1-16.

Amiri P, Kazeminasab S, Nejadghaderi SA, Mohammadinasab R, Pourfathi H, Araj-Khodaei M, Sullman MJM, Kolahi AA and Safiri S (2021). Migraine: A review on its history, global epidemiology, risk factors and comorbidities. *Front. Neurol.,* **12**: 800605.

Anitha M, Sravani B, Swathi G and Divyasree P (2021). Development and evalauation of frovatriptan bioadhesive films. *Int. J. Adv. Res. Innov. Ideas Educ.*, **7**: 888-98.

Belvís R, Pagonabarraga J and Kulisevsky J (2009). Individual triptan selection in migraine attack therapy. *Recent Pat. CNS Drug Discov,* **4**(1): 70-81.

Bhana R, Verma A and Jain S (2013). Development and characterization of ethosomes bearing losartan potassium for transdermal drug delivery. *Int. J. Pharm. Pharm. Sci.*, **5**(1): 35-40.

Cho E, Gwak H and Chun I (2008). Formulation and evaluation of ondansetron nasal delivery systems. *Int. J. Pharm.,* **349**(1-2): 101-107.

Cho HJ, Balakrishnan P, Park EK, Song KW, Hong SS, Jang TY, Kim KS, Chung SJ, Shim CK and Kim DD (2011). Poloxamer/cyclodextrin/chitosan-based thermo-reversible gel for intranasal delivery of fexofenadine hydrochloride. *J. Pharm. Sci.,* **100**(2): 681-91.

Deepika D, Dewangan HK, Maurya L and Singh S (2019). Intranasal drug delivery of frovatriptan succinate-loaded polymeric nanoparticles for brain targeting. *J. Pharm. Sci.,* **108**: 851-859.

Dowson AJ, MacGregor EA, Purdy RA, Becker WJ, Green J and Levy SL (2002). Zolmitriptan orally disintegrating tablet is effective in the acute treatment of migraine. *Cephalalgia,* **22**(2): 101-6.

Eltahir S, Al homsi R, Jagal J, Ahmed IS and Haider M (2022). Graphene oxide/chitosan injectable composite hydrogel for controlled release of doxorubicin: An approach for enhanced intratumoral delivery. *Nanomaterials,* **12**(23): 4261.

Gaikwad V (2010). Formulation and evaluation of in-situ gel of metoprolol tartrate for nasal delivery. *J. Pharm. Res.,* **3**(4): 788-793.

Galgatte UC, Kumbhar AB and Chaudhari PD (2014a). Development of in situ gel for nasal delivery: Design, optimization, *in vitro* and *in vivo* evaluation. *Drug Delivery,* **21**(1): 62-73.

Gooriah R, Buture A and Ahmed F (2015). Evidence-based treatments for cluster headache. *Ther Clin Risk Manag,* **11**: 1687-96.

Gowda D, Tanuja D, Mohammed SK and Desai JS (2011). Formulation and evaluation of In-Situ gel of Diltiazem hydrochloride for nasal delivery. *Der Pharmacia Lettre,* **3(1)**: 271-81.

Gupta H and Sharma A (2009). Ion activated bioadhesive in situ gel of clindamycin for vaginal application. *Int. J. Drug Deliv. Technol.,* **1**: 32-40.

Hoskin KL, Lambert GA, Donaldson C and Zagami AS (2004). The 5-hydroxytryptamine1B/1D/1F receptor agonists eletriptan and naratriptan inhibit trigeminovascular input to the nucleus tractus solitarius in the cat. *Brain Res.,* **998**(1): 91-9.

Kempwade A and Taranalli A (2014). Formulation and evaluation of thermoreversible, mucoadhesive in situ intranasal gel of rizatriptan benzoate. *J. Sol-Gel Sci. Technol.,* **72**(1): 43-48.

Kumar JR, Jayachandran E and Srinivas GY (2010). Formulation and evalution of pH-induced povidone iodine in situ gel for oralthrush. *J. Pharm. Sci. Res.,* **2**(5): 294-301.

Lee HJ, Choi JC, Yoon JH, Joe NS, Kim CH and Kim JY (2009). The study of pH in nasal secretion in normal and chronic rhinosinusitis. *J. Rhinol.,* **16**(2): 105-109.

Li G, Fan Y, Fan C, Li X, Wang X, Li M and Liu Y (2012). Tacrolimus-loaded ethosomes: Physico-chemical characterization and *in vivo* evaluation. *Eur. J. Pharm. Biopharm.,* **82**(1): 49-57.

Mahajan H, Shaikh H, Gattani S and Nerkar P (2009). In-situ gelling system based on thiolated gellan gum as new carrier for nasal administration of dimenhydrinate. *Int. J. Pharm. Sci. Nanotechnol.,* **2**(2): 544-550.

Majithiya RJ, Ghosh PK, Umrethia ML and Murthy RS (2006). Thermoreversible-mucoadhesive gel for nasal delivery of sumatriptan. *AAPS PharmSciTech,* **7**(3): 67.

Miller SC and Donovan MD (1982). Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. *Int. J. Pharm.,* **12**(2-3): 147-152.

Muslim RK and Maraie NK (2022). Preparation and evaluation of nano-binary ethosomal dispersion for flufenamic acid. *Materials Today: Proceedings,* **57**(Part 2): 354-361.

Nief RA, Tamer MA and Abd Alhammid SN (2019). Mucoadhesive oral in situ gel of itraconazole using pH-sensitive polymers: Preparation and *in vitro* characterization, release and rheology study. *Drug Invent. Today,* **11**(2): 1450-1455.

Nisha G, Maithil P and Charyulu R (2012). Formulation and development of nasal in situ gels of triptans for anti migraine activity. *Int. J. Res. Pharm.* **3**(2): 861-8.

Pappas CS, Tarantilis PA, Harizanis PC and Polissiou MG (2003). New method for pollen identification by FT-IR spectroscopy. *Appl. Spectrosc.,* **57**(1): 23-7.

Patel KK, Kumar P and Thakkar HP (2012). Formulation of niosomal gel for enhanced transdermal lopinavir

delivery and its comparative evaluation with ethosomal gel. *AAPS PharmSciTech.,* **13**(4): 1502-10.

Pathak K, Kumar A & Yadav E (2019). Development and correlation between *in vitro* drug release and *in vitro* permeation of thermally triggered mucoadhesive in situ nasal gel of repaglinide PVP K30 complex. *Int. J. Pharm. Sci. Drug. Res.*, **11**(1): 22-30.

Pichard-Garcia L, Hyland R, Baulieu J, Fabre JM, Milton, A and Maurel P (2000). Human hepatocytes in primary culture predict lack of cytochrome P-450 3A4 induction by eletriptan *in vivo*. *Drug Metab Dispos,* **28**(1): 51-7.

Rajendra KJ, Ramesh B and Velmurugan S (2016). Development of a new single unit dosage form of propranolol HCl extended release non-effervescent floating matrix tablets: *In vitro* and *in vivo* evaluation. *J. Appl. Pharm. Sci.*, **6**(5): 112-118.

Rajput AP and Butani SB (2019). Resveratrol anchored nanostructured lipid carrier loaded in situ gel via nasal route: Formulation, optimization and *in vivo* characterization. *J Drug Deliv Sci Technol*, **51**(2): 214-223.

Salih OS and Ghareeb MM (2021). Formulation and *in-vitro* evaluation of thermosensitive ciprofloxacin hcl in-situ gel for local nasal infection. *IJDDT,* **11**(4): 1295-1301

Shelke S, Shahi S, Jadhav K, Dhamecha D, Tiwari R and Patil H (2016a). Thermoreversible nanoethosomal gel for the intranasal delivery of Eletriptan hydrobromide. *J. Mater. Sci. Mater. Med.*, **27**(6): 103.

Shelke S, Shahi S, Jalalpure S and Dhamecha D (2016b). Poloxamer 407-based intranasal thermoreversible gel of zolmitriptan-loaded nanoethosomes: Formulation, optimization, evaluation and permeation studies. *J. Liposome Res.*, **26**(4): 313-323.

Shelke S, Shahi S, Jalalpure S, Dhamecha D and Shengule S (2015). Formulation and evaluation of thermoreversible mucoadhesive in situ gel for intranasal delivery of Naratriptan hydrochloride. *Drug Deliv. Sci. Technol.*, **29**: 238-244.

Sherafudeen SP and Vasantha PV (2015). Development and evaluation of in situ nasal gel formulations of loratadine. *Res. Pharm. Sci.*, **10**(6): 466-476.

Silva BG, Gouveia THN, da Silva MAP, Ambrosano G MB, Aguiar FHB and Lima D (2018). Evaluation of home bleaching gel modified by different thickeners on the physical properties of enamel: An in situ study. *Eur. J. Dent.*, **12**(4): 523-527.

Slassi A (2002). Recent advances in 5-HT1B/1D receptor antagonists and agonists and their potential therapeutic applications. *Curr. Top. Med. Chem.*, **2**(6): 559-574.

Solomon GD, Cady RK, Klapper JA, Earl NL, Saper JR and Ramadan NM (1997). Clinical efficacy and tolerability of 2.5 mg zolmitriptan for the acute treatment of migraine. The 042 Clinical Trial Study Group. *Neurology*, **49**(5): 1219-1225.

Sulaiman HT, Jabir SA and Al-Kinani KK (2018). Investigating the effect of different grades and concentrations of ph-sensitive polymer on preparation and characterization of lidocaine hydrochloride as in situ gel buccal spray. *Asian J. Pharm. Clin. Res.*, **11**(11): 401-7.

Swati T, Preetam N, Braja G, Chaitali D, Ashwini J and Anil J (2017). A research article on nanogel as topical promising drug delivery for diclofenac sodium. *Indian J. Pharm. Educ. Res.*, **51**(4S): S580-S587.

Takiya L, Piccininni LC and Kamath V (2006). Safety and efficacy of eletriptan in the treatment of acute migraine. *Pharmacotherapy,* **26**: 115-128.

Türker S, Onur E and Ozer Y (2004). Nasal route and drug delivery systems. *Pharm. World Sci.,* **26**: 137-142.

Wang LL, Zheng WS, Chen SH and Fang XQ (2013). Development of in situ gelling and bio adhesive 5-Fluorouracil enema. *PLoS One,* **8**: e71037.

Yates R, Nairn K, Dixon R and Seaber E (2002). Preliminary studies of the pharmacokinetics and tolerability of zolmitriptan nasal spray in healthy volunteers. *J. Clin. Pharmacol.*, **42**: 1237-1243.

Younis YK and Abd Alhammid SN (2023). Intranasal Oleic acid-based nanoemulsion of Diazepam: Design, formulation and *in-vitro* evaluation. *J. Res. Pharm.*, **27**(2): 529-543.

Yousif HS and Khalil YI (2009). In situ gelling formulation of Naproxen for oral sustained delivery system. *Iraqi J. Pharm. Sci.,* **18**: 13-20.

Zhang JP, Wei YH, Zhou Y, Li YQ and Wu XA (2012). Ethosomes, binary ethosomes and transfersomes of terbinafine hydrochloride: A comparative study. *Arch. Pharm. Res.*, **35**: 109-117.

Zheng J, Lin J, Ma Y, Yang C, Zhong Q, Li Y and Yang Q (2023). Establishment of sheep nasal mucosa explant model and its application in antiviral research. *Front. Microbiol.*, **14**: 1124936.

Zhou Y, Wei Y, Liu H, Zhang G and Wu X (2010). Preparation and *in vitro* evaluation of ethosomal total alkaloids of Sophora alopecuroides loaded by a transmembrane pH-gradient method. *AAPS Pharm Sci Tech.*, **11**: 1350-1358.