CrossMark

Original Research

Thermoreversible nanoethosomal gel for the intranasal delivery of Eletriptan hydrobromide

Santosh Shelke[1] · Sadhana Shahi[2] · Kiran Jadhav[3] · Dinesh Dhamecha[3] · Roshan Tiwari[4] · Hemlata Patil[4]

Abstract The objective of the current study was to formulate and characterize thermoreversible gel of Eletriptan Hydrobromide for brain targeting via the intranasal route. Ethosomes were prepared by 3^2 factorial design with two independent variables (concentration of soya lecithin and ethanol) and two response variables [percent entrapment efficiency and vesicle size (nm)] using ethanol injection method. Formulated ethosomes were evaluated for preliminary microscopic examination followed by percent drug entrapment efficiency, vesicle size analysis, zeta potential, polydispersibility index and Transmission electron microscopy (TEM). TEM confirms spherical morphology of ethosomes, whereas Malvern zeta sizer confirms that the vesicle size was in the range of 191 ± 6.55–381.3 ± 61.0 nm. Ethosomes were incorporated in gel using poloxamer 407 and carbopol 934 as thermoreversible and mucoadhesive polymers, respectively. Ethosomal gels were evaluated for their pH, viscosity, mucoadhesive strength, in vitro drug release and ex vivo drug permeation through the sheep nasal mucosa. Mucoadhesive strength and pH was found to be 4400 ± 45 to 5500 ± 78.10 dynes/cm^2 and 6.0 ± 0.3 to 6.2 ± 0.1, respectively. In-vitro drug release from the optimized ethosomal gel formulation (G4) was found to be almost 100 % and ex vivo permeation of 4980 µg/ml with a permeability coefficient of $11.94 \pm 0.04 \times 10^{-5}$ cm/s after 24 h. Histopathological study of the nasal mucosa confirmed non-toxic nature of ethosomal gels. Formulated EH loaded ethosomal thermoreversible gel could serve as the better alternative for the brain targeting via the intranasal route which in turn could subsequently improve its bioavailability.

1 Introduction

Migraine is a disease characterized by severe throbbing pain over one or both halves of the scalp followed by nausea, vomiting, photophobia and/or phonophobia. Presently available treatment for migraine mainly includes triptans like Sumitriptan, Eletriptan, Zolmitriptan, Naratriptan etc., which acts by blocking serotonin receptors of the blood vessels in the brain to decrease the pain [1, 2].

Eletriptan hydrobromide (EH) which is chemically 3-{[(R)-1-methyl-2-pyrrolidinyl] methyl}-5-[2-(phenylsulfonyl) ethyl] indole hydrobromide acts as a potent antimigraine agent via its selective partial agonistic action at the 5-HT1B receptor [3–5]. The drug has an oral bioavailability of approximately 50 % with an elimination half-life ranging from about 4–7 h, metabolized mainly by cytochrome P450 (CYP3A4) hepatic enzyme system [6]. During migraine attack absorption of EH may be delayed by almost 50 %, which leads to decreased bioavailability. Low bioavailability of EH may also be due to an excessive first pass metabolism and poor aqueous solubility leading to increase in a dosing frequency to achieve the desired the

✉ Santosh Shelke
santoshshel@gmail.com

1 Department of Pharmaceutics, Yash Institute of Pharmacy, Bajaj Nagar, Aurangabad, Maharashtra 431134, India

2 Department of Pharmaceutics, Government College of Pharmacy, Osmanpura, Aurangabad, Maharashtra 431005, India

3 KLE University's College of Pharmacy, Nehru Nagar, Belgaum, Karnataka 590010, India

4 Department of Pharmaceutics and Drug Delivery, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

Springer

therapeutic index. Increased oral dosing of the available formulation of EH is associated with side effects like nausea, feelings of tingling/numbness, weakness, tiredness, drowsiness, or dizziness. It is also reported that EH causes a dose dependent increase in serotonin leading to a very serious condition known as serotonin syndrome/toxicity [7–9].

Nanotechnology is a potential area of current research due to its widespread applications in treating many diseases with targeted therapy. Nanoscale formulations could be successfully manipulated for targeted therapy of antimigraine drugs like EH. Moreover, EH owing to its lipophilic nature could easily penetrate through the brain tissues [6]. It is expected that an administration of nanocarriers like intranasal ethosomes should reach at the targeted receptors via nose to brain active targeting through the olfactory lobes. The present investigation was focused to develop a novel ethosomal formulation for active targeting of EH through the olfactory pathway to brain tissues. Till now very limited research has been carried on the novel formulations of EH to improve the targeting potential. However, most of the studies have shown that the intranasal drug delivery system could act as the innovative formulation to improve the drug targeting to the brain and thereby decrease in dosing frequency and thus a further reduction in the associated side effects [10]. To increase the rate of penetration and release in the intranasal region ethosomes acts as a better choice of vesicle when compared to other forms of liposomes [11].

The current literature reveals that thermoreversible gel preparations of Sumatriptan [12] and Domperidone [13] have been developed and found to have an increased permeation rate with prolonged nasal residence time and thereby improved nasal absorption [14]. Hence, intranasal ethosomal drug delivery system having advantages like biodegradability [15], targeting ability [16], biocompatibility [17], improved permeation profile [18] better stability than liposomes [19] and ease of usage could serve as a potential carrier for EH.

In the current study, the authors have formulated and characterized ethosomes using advanced analytical techniques like TEM, Malvern zetasizer etc. Thermoreversible gel was further formulated to incorporate EH loaded ethosomes into a finished dosage form. The gel was finally characterized for mucoadhesive strength, viscosity, in vitro and ex vivo drug release profile.

2 Materials

EH and Poloxamer 407 was obtained as a gift sample from Zydus Cadila Ltd., India and Shreya life sciences Pvt. Ltd., Aurangabad, India respectively. Other chemicals like Carbopol 934, cellophane membrane (12,000–14,000 MW) and Soya lecithin (30 %) were purchased from Hi-Media Lab Pvt. Ltd., Mumbai, India. All other reagents used in the study were of analytical grade.

3 Methodology

3.1 Compatibility studies

Compatibility studies were carried out at room temperature by Fourier transform infrared spectroscopy (FTIR) (Model-200, Thermo Electron, Shimadzu, Japan) to investigate any physical interactions between the drug and the excipients used in the formulation. The pure drug (EH) and polymers were subjected to FTIR studies alone and in combinations (1:1) and analyzed by KBr pellet technique.

3.2 Experimental design for formulation of ethosomes

A 3^2 factorial design consisting of two independent variables at three levels (high, medium and low) depending on preliminary studies was used for the formulation of ethosomes [20]. The independent variables selected for this study were X_1: Concentration of Soya lecithin and X_2: Concentration of ethanol. The response of independent variables on dependent variables that is Y_{EE}: Percent entrapment efficiency and Y_{VS}: Vesicle size (nm) is listed in Table 1. Statistical model, including polynomial terms was used to estimate the response shown by general binomial Eq. 1

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1X_1 + b_{22}X_2X_2. \qquad (1)$$

3.3 Preparation of ethosomes

The 3^2 factorial design was utilized (Table 1) by ethanol injection-sonication method [21]. Initially, EH (1 % w/v) was dissolved in ethanol and added to the mixture containing soya lecithin and propylene glycol (13 % v/v). The mixture was uniformly stirred using a magnetic stirrer (IKA India Private Limited, India) at 30 °C, speed 700 rpm for 30 min. During stirring, double-distilled water was added to the solution in the streamline using syringe at the flow rate of 200 μL/min to make up the volume up to 50 ml. The mixture was then subjected to probe sonication (Rivotek Ultrasonic sonicator, Mumbai, India) for a total of 45 min for 3 cycles of 15 min each (15 s on/off cycle). The formulation was then stored in refrigerator for further characterization.

Table 1 Observed responses from 3^2 factorial design of EH loaded ethosomal formulations

Formulation code	Independent variables		Dependent variables	
	X_1: Concentration of Soya lecithin	X_2: Concentration of ethanol	Y_{EE}: Entrapment efficiency (%)	Y_{VS}: Vesicle size (nm)
E1	−1	−1	30.3 ± 2.08	260 ± 5
E2	−1	0	35.33 ± 1.5	190 ± 3
E3	−1	+1	42.33 ± 2.5	180 ± 2
E4	0	−1	52.66 ± 2.08	320 ± 2.6
E5	0	0	61 ± 2.0	250 ± 3
E6[a]	0	+1	63.33 ± 2.30	191 ± 6.5
E7	+1	−1	64 ± 2	381 ± 3.6
E8	+1	0	65.33 ± 1.5	316 ± 3.6
E9	+1	+1	66 ± 2	270 ± 8.9

Coded values	Actual values	
	X_1 (% w/v)	X_2 (% v/v)
−1	2.5	30
0	3.0	35
+1	3.5	40

X_1 Concentration of Soya lecithin, X_2 concentration of ethanol, Y_{EE} percent entrapment efficiency, Y_{VS} vesicle size (nm)

[a] Indicates optimized formulation

3.4 Evaluation of ethosomes

3.4.1 Entrapment efficiency

Drug entrapment efficiency of ethosomes was determined by centrifugation method. EH loaded ethosomal nanodispersion was subjected to centrifugation at 50,000 rpm for 1 h at 4 °C using micro- ultracentriguge (Thermo scientific Sorvall MX 150 Micro-Ultracentriguge, India). Supernatant liquid was collected and analyzed for free EH by UV spectrophotometry (Shimadzu 1800, Japan) at 272 nm. The percent entrapment efficiency was calculated by using formula (Eq. 2)

Percent entrapment efficiency

$$= \frac{\text{total amount of drug loaded} - \text{free drug in supernatant}}{\text{total amount of drug used in the fomulation}} \times 100.$$

(2)

3.4.2 Vesicle size and zeta potential analysis

Ethosomal samples were diluted appropriately with deionized water and analyzed by Malvern Zeta Sizer (Nano ZS, Malvern Instruments Ltd, UK) to determine vesicle size, polydispersibility index (PDI) and zeta potential at a room temperature.

3.4.3 Morphological studies

Formation of vesicles was initially confirmed by examining ethosomes under optical trianocular microscope coupled with a camera (Metzer M, Optical Instruments Pvt. Ltd, India) at magnification of 10× and 40×. Transmission electron microscopy (TEM) (Tecnai G2 Spirit Bio Twin; FEI, Czech republic) coupled with a camera was used to visualize nanostructure of ethosomes. Samples of ethosomes formulation (10 μl) were retained onto copper grids until dry and then stained using 2 % w/v aqueous uranyl acetate and scanned to obtain images.

3.4.4 Determination of gelation temperature

Gelation temperature was determined by visual inspection method [22] to fix the concentration of poloxamer 407 (13–20 % w/v) for preparation of thermoreversible gel. Beaker containing 10 ml of poloxamer 407 solution in water was kept on digital stirrer with thermostat facility. The beaker was heated at constant heating rate (1 °C/min.) with continuous stirring and the temperature at which the

magnetic bead stopped moving due to increase in the viscosity was measured as gelation temperature.

3.5 Formulation of in situ nasal gel of ethosomal EH

Intranasal gels were prepared by cold technique [23], where EH loaded ethosomes were dispersed in water to get 2 % w/v drug content (Table 2). To the above mixture, carbopol 934 was slowly added, followed by addition of poloxamer 407 with continuous stirring. (Triethanolamine) (1–2 drops) were finally added to form a gel and kept in cool condition (4–8 °C) for further characterization.

3.6 Evaluation of in situ gel formulations

The formulated gels were evaluated for their physicochemical properties viz—pH, clarity and drug content. Prior to use, pH meter 510 (Eutech Instruments Pvt. Ltd., Singapore) was calibrated by standard buffer solutions of pH 4 and pH 7 (Thermo Fisher scientific standard buffers). The clarity was checked against standard white and black background apparatus. The drug content of the gel was determined by spectroscopic methods, where appropriate dilutions were made in the linear range and absorbance was measured at 272 nm against the blank. The viscosity of the developed formulations was determined by Brookfield viscometer (DV3T Rheometer, USA) at 32 ± 2 °C. The force required to detach the formulation from the nasal mucosal tissue was recorded as the mucoadhesive potential of gel formulations [24]. Sheep nasal tissue was obtained from a local slaughter house and the intact mucosal membrane was isolated within 1 h after killing the animal. The mucosa was separated from bone cartilage and tissues were cut into small portions. Two tissue portions approximately 20×20 mm^2 were tied to two different glass slides using thread. One glass slide was fixed on the underneath portion of a pan balance with two sided adhesive tape facing downside. The other slide was fixed on wooden board of balance in such a way that the tissue has been just beneath and facing upper tissue. 100 mg of gel was placed in between two mucosal tissues and held in contact for 2 min, dummy granules were then added slowly into the other pan until the tissues get separated. The nasal mucosal tissue was changed for each measurement. The

mucoadhesive strength of gel formulation was determined from minimal weight that detach the mucosal tissues from the surface of each formulation and was expressed as the detachment stress in dyne/cm^2 (Eq. 3)

$$\text{Mucoadhesive strength} \left(\frac{\text{dyne}}{\text{cm}^2} \right) = \frac{M \times G}{A} \times 100 \qquad (3)$$

where, M weight required for detachment in grams, G acceleration due to gravity (980 cm/s^2), A area of mucosa exposed

3.7 In-vitro drug release study from EH loaded thermoreversible intranasal gel

Drug release from gel was determined by using Franz diffusion six cell system (Thermo Fischer scientific, Haake S5P Newington, USA) using a cellophane dialysis membrane [25] (Molecular weight: 12,000–40,000 kDa). Prior to experimentation, pieces of cellophane membrane were soaked in receptor medium for 2 h. Membranes were then fixed on Franz diffusion cells having an effective permeation area of 2×2 cm^2. EH loaded ethosomal gel equivalent to 10 mg of EH was loaded into a donor compartment, whereas receptor compartment was filled with 12 ml of phosphate buffer pH 6.4. The study was conducted at the temperature of 34 ± 1 °C with standard stirring speed. An aliquots of 0.5 ml was withdrawn after every hour from the receptor compartment and replaced with fresh buffer until 8 h. The samples were diluted suitably and analyzed by a UV spectrophotometer at 272 nm.

3.8 Ex-vivo drug release study from EH loaded thermoreversible intranasal gel

Nasal cavity of sheep was obtained from the local slaughterhouse immediately after its sacrifice and stored in saline phosphate buffer pH 6.4. The intact nasal mucosa membrane was identified and separated from the nasal cavity, cleaned and stored in the buffer. The study was conducted using six cells Franz diffusion system with thermostat facility. Tissue samples were fixed on Franz diffusion cells having an effective permeation area of 2×2 cm^2. EH loaded ethosomal gel equivalent to 10 mg

Table 2 Composition of Eletriptan loaded ethosomal thermoreversible gel formulations

Formulation code	Drug (% w/v)	Poloxamer P 407 (% w/v)	Carbopol 934 (% w/v)
G1	2	18	0.1
G2	2	18	0.2
G3	2	18	0.3
G4	2	18	0.4
G5	2	18	0.5

of EH was loaded into a donor compartment, whereas receptor compartment was filled with 12 ml of phosphate buffer pH 6.4. The study was conducted at the temperature of 34 ± 1 °C with standard stirring speed. Aliquots of 0.5 ml was withdrawn after every 1 h from the receptor compartment and replaced with fresh buffer until 8 h. The samples were diluted suitably and analyzed by a UV spectrophotometer at 272 nm [26]. The effective permeability coefficient (cm/s) across the sheep nasal mucosa under steady state conditions was calculated according to the Eq. 4.

$$\text{Permeability coefficient} = (dc/dt) \left(\frac{dc}{dt} \right)_{ss} \times \frac{V}{AC_D} \qquad (4)$$

where $(dc/dt)_{ss}$ ($\mu g \ mL^{-1} \ s^{-1}$) is the change of concentration under steady-state; A (cm^2) is the permeation area; V (mL) is the volume of the receiver compartment; and C_D($\mu g \ mL^{-1}$) is the initial donor concentration.

3.9 Histopathological evaluation of mucosal tissue

Histopathological evaluation of tissue incubated in PBS (pH 6.4) was compared with post experiment tissue samples to determine the effect of formulation on nasal mucosa membrane. Both tissue samples were stored in buffered formalin to fix the tissue for histopathology studies. Standard haemotoxylin and eosin staining method was used and sections were then examined under a light microscope to detect any change in tissue structure during ex vivo drug permeation studies [27].

4 Results

4.1 Drug polymer compatibility studies

Drug polymer interaction studies by FTIR spectroscopy revealed that there was no major changes in peak positions (1977, 2358, 2542, 29,220, 2993, 3259 and 3475 cm^{-1}) seen in the FTIR spectra of drug (Fig. 1a) and in a physical mixture of EH with poloxamer 407 and carbopol 934 (Fig. 1b).

4.2 Experimental design for formulation of ethosomes

In the present investigation, the effect of concentration of soya lecithin (X_1) and concentration of ethanol (X_2) on percent entrapment efficiency (Y_{EE}) and vesicle size (Y_{VS}) was studied by using 3^2 factorial design are shown in Table 1. Results of ANOVA on measured responses are recorded in Table 3 and response surface curve and contour plots are shown in Fig. 2. To demonstrate the effect of

the X_1 and X_2, the response surface plots were generated for the dependent variables Y_{EE} and Y_{VS} using the Design-Expert® Software (Stat-Ease Inc., Minneapolis). The results showed that the optimized formulation of EH loaded ethosomes (E6) demonstrated desirable properties, i.e., >63 % entrapment efficiency and vesicle size of <200 nm when compared to other formulations.

4.3 Characterization of vesicular system

Drug loaded ethosomes were characterized for the vesicle size, percent entrapment efficiency, zeta potential, polydispersibility index and morphological characteristics. Ethosomes vesicle size (Fig. 3a) suggests that the formulation E6 prepared with 40 % v/v of ethanol and 3 % of soya lecithin showed acceptable vesicle size with 191 ± 6.5 nm. Percent drug entrapment efficiency of the EH ethosomal formulation is presented in Fig. 3b. Optimized ethosomal formulation (E6) showed entrapment efficiency of 63.33 ± 2.31 %. The zeta potential of optimized formulations was observed in the range of -9 ± 2 to -21 ± 2 (Fig. 3c) whereas the particle polydispersibility index was observed in the range of 0.28 ± 0.012 to 0.48 ± 0.034. The optimized formulation E6 has the Zeta potential of -20 ± 3 and PDI of 0.281 ± 0.012 suggesting desirable stability of the formulation. Preliminary morphological examination by Trianocular microscope coupled with a camera, suggested presence of spherical multilamelar vesicles, which was further confirmed by TEM micrograph which clearly depicts drug loaded vesicles with predominantly spherical morphology and smooth surface (Fig. 4).

#### 4.3.1 Determination of gelation temperature

In the present investigation, gelation temperature of formulation with different concentration of poloxamer 407 was studied and results are represented in Table 4. Results recorded clearly indicate that poloxamer 407 showed optimized gelation temperature of 33–34 °C at the concentration of 18 % w/v.

4.4 Formulation and evaluation of in situ nasal gel of ethosomal EH

Optimized concentration of poloxamer 407 (18 % w/v) was used for formulation of thermoreversible intranasal gel. Carbopol 934 in varying concentrations (0.1–0.5 % w/v) was used as a gelling agent as well as mucoadhesive polymer. The effect of a mixture of carbopol 934 and poloxamer 407 (18 % w/v) on gelling temperature, viscosity and mucoadhesive strength is as shown in Fig. 5. It was found that by addition of carbopol 934 in the

Fig. 1 FTIR sperctra of Eletriptan hydrobromide and physical mixture of Eletriptriptan and Excipients (Poloxamer 407 + Carbopol 934)
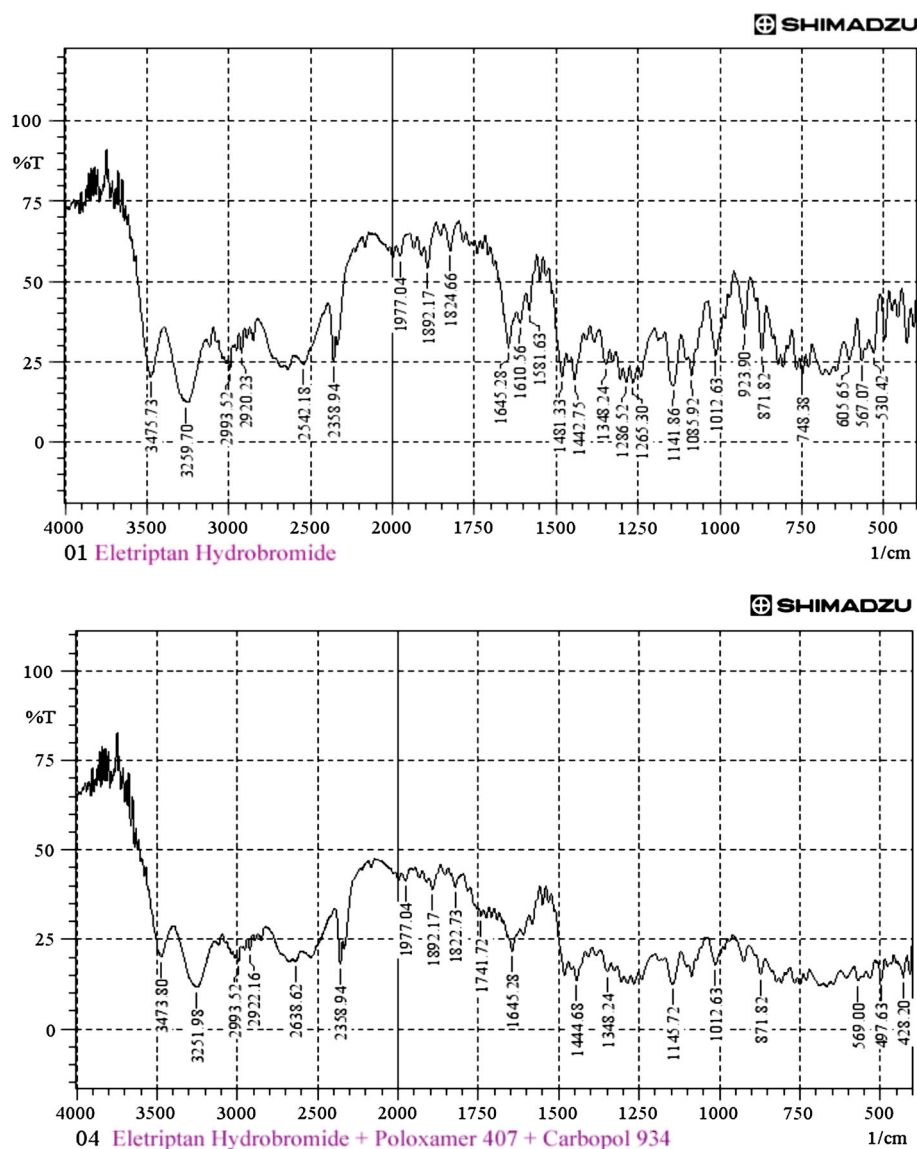

Table 3 Results of analysis of variance for measured response for Y_{EE} and Y_{VS}

Parameters	Degree of freedom	Sum square value	Mean square value	f value	P value
For Y_{EE}					
Regression	5	1543.28	308.61	92.91	0.0017
Residual	3	9.97	3.32		
Total	8	1553.54	–		
For Y_{VS}					
Regression	2	36997.58	7399.52	36.73	0.0068
Residual	6	604.42	201.47		
Total	8	37602.00	–		

Y_{EE}: Percent entrapment efficiency, Y_{VS} vesicle size (nm)

concentration range from 0.1 to 0.5 %, (Table 2) causes lowering of gelation temperature from 35 to 25 °C. As the physiological temperature of the nasal mucosa is in the range of 30–34 °C formulation G1 and G5 were not considered for further evaluation. G1 formulation showed a higher gelation temperature of 35 ± 0.5 °C whereas, G5
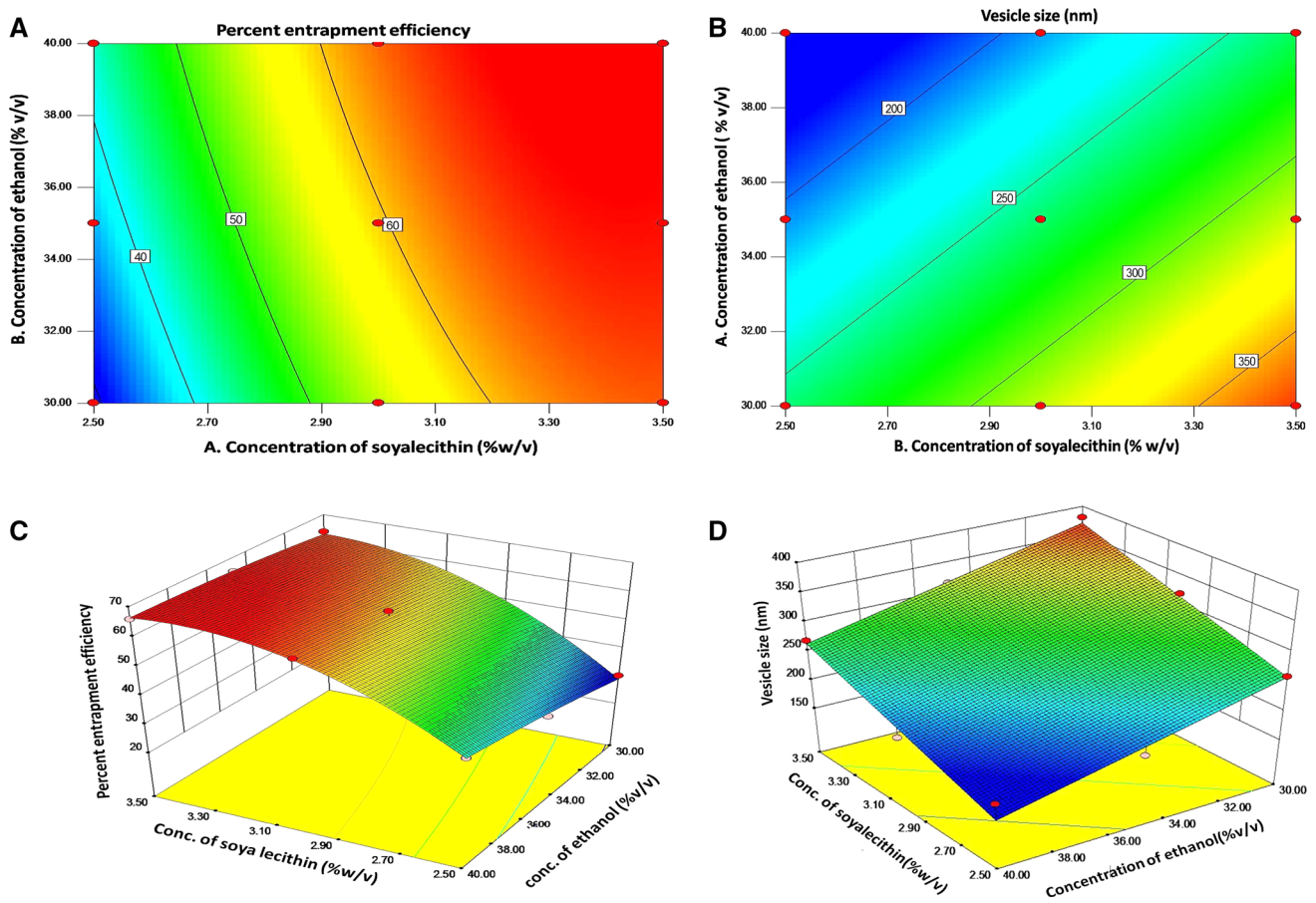
Fig. 2 Contour plot (**a**, **b**) and Response surface plot (**c**, **d**) showing effect of X_1 and X_2 on percent entrapment efficiency and vesicle size (nm). X_1 = A: Concentration of soya lecithin and X_2 = B: Concentration of ethanol

which is demonstrates lower gelation temperature of 25 ± 0.5 °C.

4.5 Evaluation of in situ gel formulations

Results of physicochemical evaluation of gel formulations are as shown in Fig. 5. The pH of all the formulations was found to be 6.1 ± 0.2 which is desirable and acceptable for nasal mucosal permeation studies. The drug content of the gel formulations was determined by a UV spectrophotometer after suitable dilutions at 272 nm and was found to be in the range of 98–99 %.

4.6 In-vitro drug release and Ex-vivo permeation from the EH loaded thermoreversible intranasal gel

Cumulative drug release from the EH loaded thermoreversible gel formulations were found to be in the range of 63 ± 1.90 to 71.67 ± 1.52 % after 12 h (Fig. 6), whereas

the cumulative release of almost 100 % was recorded for all the formulations where G4 showed the better release profile when compared to other formulations.

Results of ex vivo permeability studies (Fig. 7) suggest that the permeation kinetics was best in formulation G4 when compared to other formulations. Permeability co-efficient for gel formulations was calculated and found to be 7.52, 7.94 and 8.6 (10^{-5} cm/s) for G2, G3 and G4 formulations, respectively after 12 h and the average of 11.9 (10^{-5} cm/s) permeability was observed for all the formulations after 24 h.

4.7 Histopathological evaluation of mucosal tissue

Histopathology of the nasal mucosal tissue (Fig. 8) clearly indicated integrity of mucosa and the absence of any irritation or toxicity. Both mucosal membranes gel treated and untreated showed a similar microscopic tissue architecture with a slight detachment of epithelial cells in the treated mucosa.
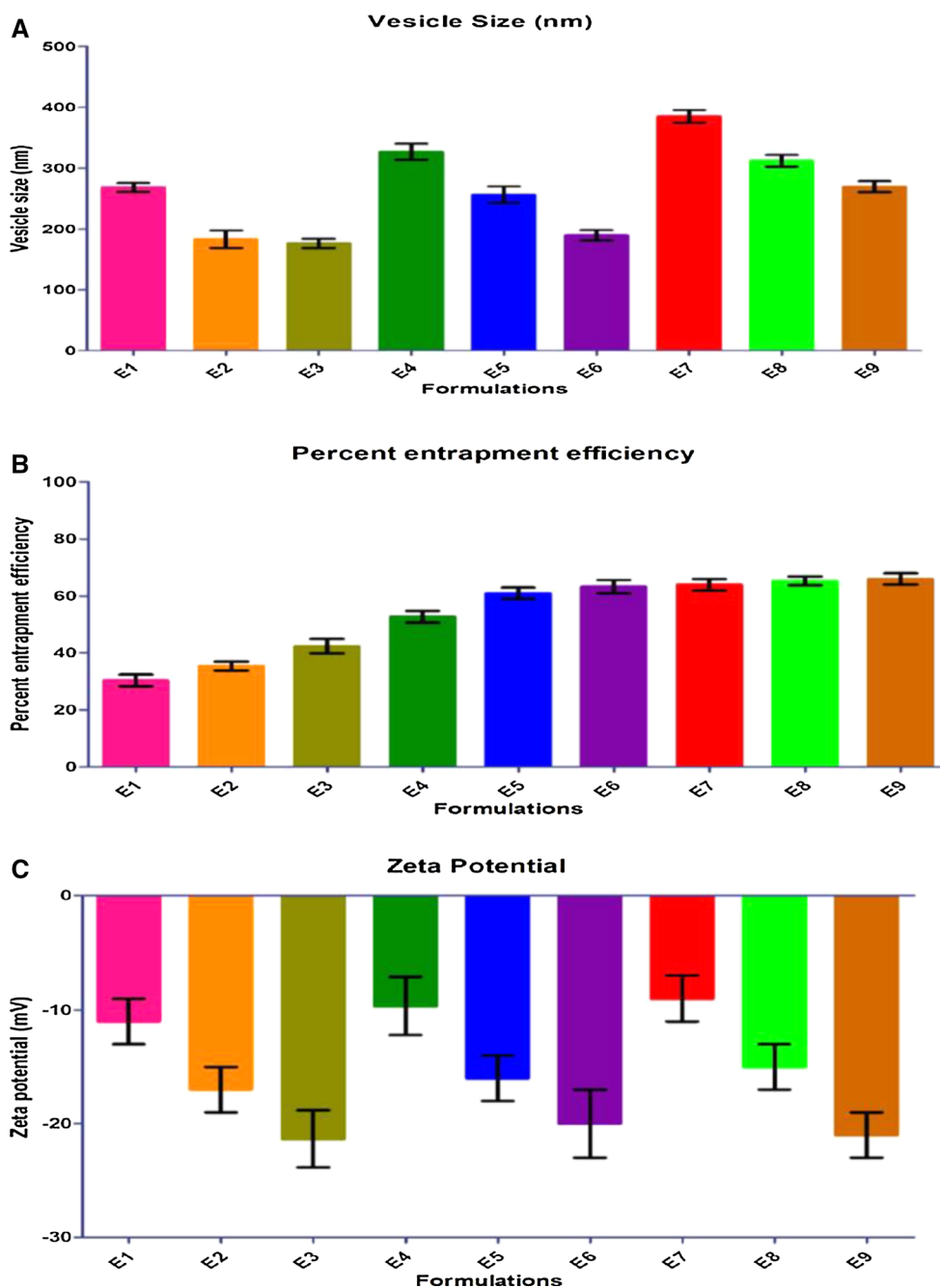
Fig. 3 Characterization of eletriptan loaded ethososmes, **a** vesicle size (nm), **b** percent entrapment efficiency and **c** zeta potencial (mV). All results are expressed in mean $\pm$ SD ($n = 3$)

5 Discussion

In the present investigation, EH loaded ethosomes were formulated by ethanol injection-sonication method and optimized by using a 3^2 factorial design consisting of two independent variables at three levels (high, medium and low) based on a preliminary study. Concentration of soya lecithin (X_1) and ethanol (X_2) was considered as an independent variable, whose response on dependent variables

such as percent entrapment efficiency (Y_{EE}) and vesicle size (Y_{VS}) was estimated. The prepared formulation was characterized using advanced analytical techniques such as TEM, Malvern zetasizer etc. Thermoreversible gel was further formulated to incorporate EH loaded ethosomes into a finished dosage form. The gel was finally characterized for effect of concentration of carbopol 934P on phase transition temperature, mucoadhesive strength, viscosity, in vitro and ex vivo drug release profile.
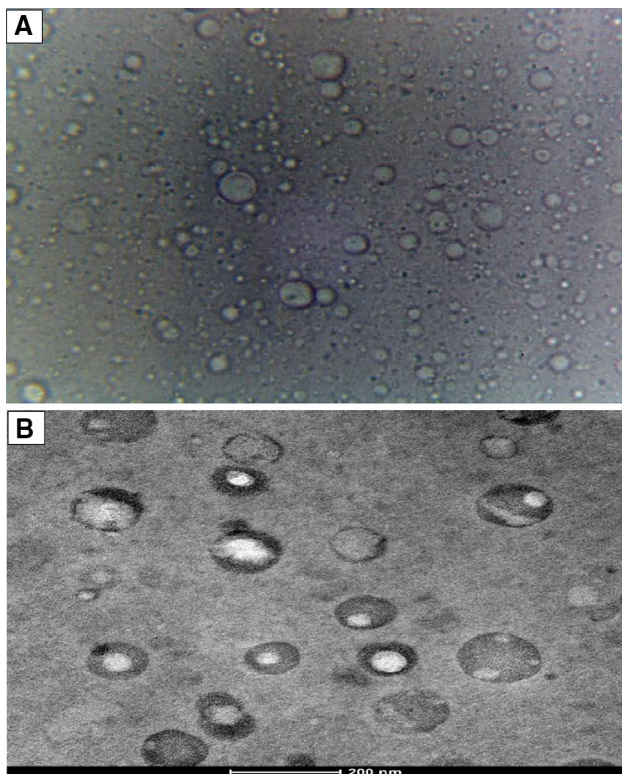
Fig. 4 **a** Preliminary microscopic image of eletriptan ethosomes at magnification of $40\times$ and **b** morphological study of ethosomes by TEM

Table 4 Results of gelation temperature

Sr. no.	Poloxamer 407 (% w/v)	Gelation temperature (°C)
1	15	No gelling until 41
2	16	No gelling until 40
3	17	Viscosity increased at 38
4	18	32–33
5	19	30–31
6	20	26–27

Prior to formulation of ethosomes, drug polymer compatibility study was performed by FTIR spectroscopy which showed no physical interaction between the drug, poloxamer and carbopol 934. FTIR spectra showed no major changes in peak positions of the drug (Fig. 1a) and in a physical mixture of EH with poloxamer 407 and carbopol 934 (Fig. 1b).

In the 3^2 factorial design used for formulation of optimized batches, the value of correlation coefficient indicates a good fit. Results of ANOVA on measured responses, and response surface curve and contour plots demonstrated the effect of X_1 and X_2. The response surface plots were generated for the dependent variables Y_{EE} and Y_{VS}

$$Y_{EE} = 59.52 + 14.56\ X_1 + 4.12\ X_2 - 2.51\ X_1X_2 - 8.45\ X_1^2 - 0.78\ X_2^2 \tag{5}$$

$$Y_{VS} = 243.00 + 56.17\ X_1 - 53.33\ X_2 - 7.75\ X_1X_2 + 12.50\ X_1^2 + 15.00\ X_2^2. \tag{6}$$

In the Eq. 5, b_1 and b_2 bearing positive sign indicates that percent entrapment efficiency is directly proportional to the independent variables (X_1 and X_2). In the Eq. 6, b_1 bearing positive sign indicates that the vesicle size is directly proportional to X_1, whereas negative sign of b_2 suggests the vesicle size is inversely proportional to X_2.

Drug loaded ethosomes were characterized for the vesicle size, percent entrapment efficiency, zeta potential, polydispersibility index and morphological characteristics. Vesicle size of ethosomes was directly proportional to concentration of ethanol and inversely proportional to the concentration of soya lecithin used in the formulation as demonstrated by formation E6. Optimized ethosomal formulation (E6) showed entrapment efficiency of 63.33 ± 2.31 % which is attributed to the high concentration of ethanol as ethanol helps to improve solubility of EH. Further, zeta potential is considered as the crucial factor determining the stability of colloidal dispersions. Balance between the attractive and repulsive forces within vesicles determines the zeta potential of the colloid. In the present formulation, both concentrations of soya lecithin and ethanol were the main element in managing the zeta potential of the optimized nanoethosomal formulation E6. Ethanol sustains the net negative charge, whereas the optimal concentration of soya lecithin retains desirable rigidity which circumvents aggregation and fusion of vesicles, which subsequently leads to thermodynamically stable colloid. In addition to it, hydroxyl group of propylene glycol in the prepared formulations induce transition in the charge of the vesicles to more negative value thus resulting in higher zeta potential and improved stability [28] when compared to other formulations.

Ethanol is known to cause change in the net surface charge of the system which subsequently results in some degree of stearic stabilization and leads to decrease in the mean vesicle size of formulation [29]. Increase in vesicle size was observed with increase in concentration of soya lecithin as it is used as a coating lipid for formation of ethosomes [30]. In addition, polypropylene glycol is categorized by a high hydrophobicity and show good solvent capability for EH due to its associated properties, such as partition coefficient, polarity, and ability to interpenetrate the lipids. It not only acts as humectant, but also acts as a penetration enhancer for ethosomal delivery through nasal mucosa [31].

Preliminary morphological examination by Trianocular microscope coupled with a camera suggested that spherical

Fig. 5 a Study of effect of concentration of carbopol 934P on phase transition temperature, **b** viscosity of gel formulations **c** Mucoadhesive strength of gel formulations (dynes/cm²). All determinations are expressed in mean ± SD (*n* = 3)
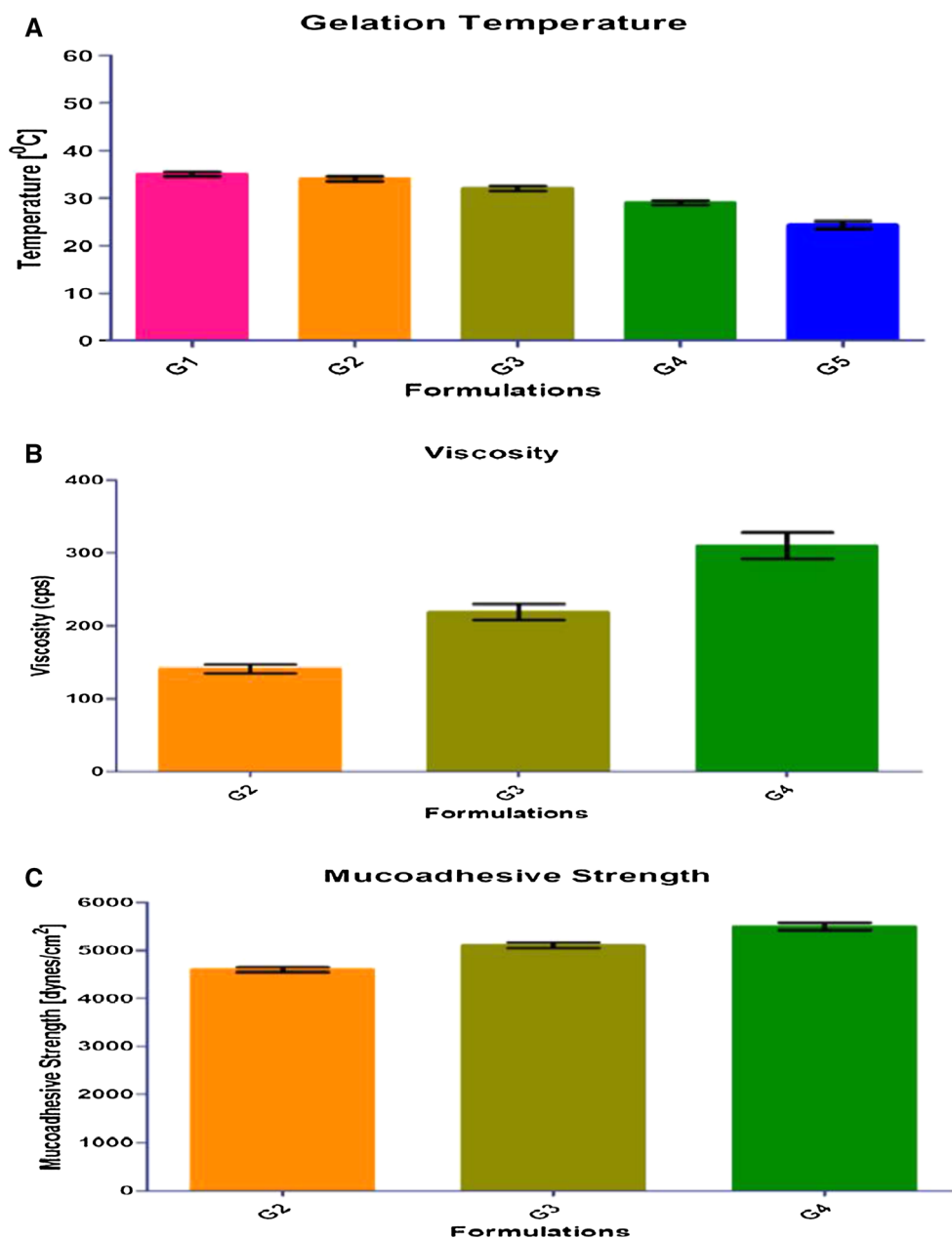

multilamelar vesicles have been formulated which was further studied by TEM. TEM image clearly depicts drug loaded vesicles with predominantly spherical morphology and smooth surface.

Nasal in situ gels are expected to increase the residence time of the dosage form at the local site without drainage or loss of drug content. As the temperature of the nasal cavity is 34 °C [32], present investigation was aimed at preparing thermoreversible liquid formulations using poloxamer 407 that may be transformed into gel below 34 °C. Lower gelation temperature (27 °C) of gels may lead to difficulty in the formulation and its intranasal administration. If the gelation temperature is >36 °C, formulation will exist in a

liquid form in nasal cavity resulting in drainage of dosage form [33]. In the present investigation, gelation temperature of formulation with different concentration of poloxamer was studied and results are represented in Table 4. An optimized gelation temperature of 33–34 °C at the concentration of 18 %w/v of poloxamer could be due to its thermoreversible property owning to a negative coefficient of solubility to block copolymer micelles. It is water soluble co-polymer of ethylene oxide and propylene oxide. Poloxamer 407 exhibits monomolecular micelles at low concentrations (up to 10 %) and multimolecular aggregates at the higher concentrations (above a critical gel concentration) results in the formation of gel [34]. The addition of

In-vitro drug release studies
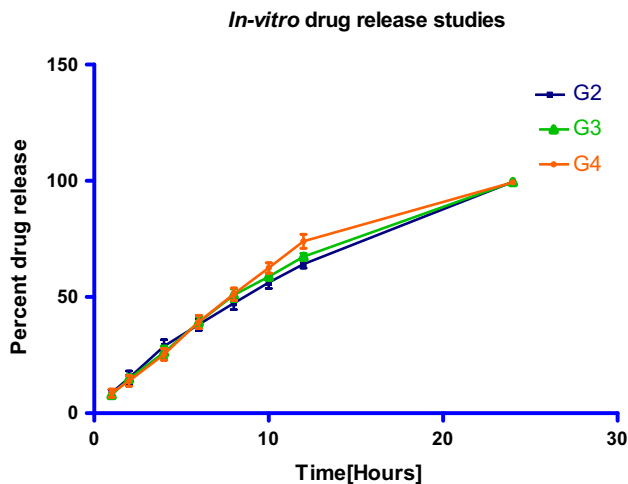
Fig. 6 In-vitro drug release studies of gel formulations through cellophane dialysis membrane. All determinations are expressed in mean ± SD ($n = 3$)

Ex-vivo drug permeation studies
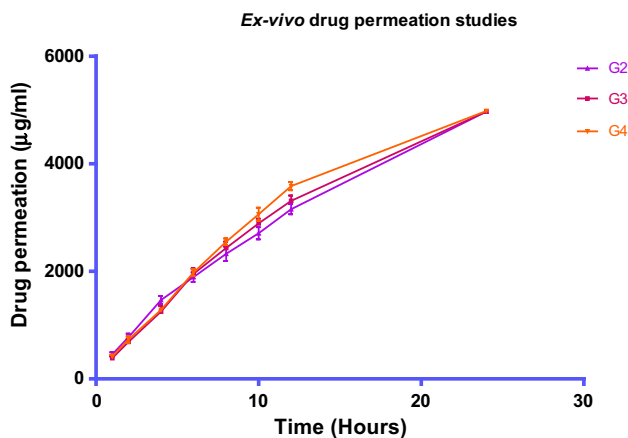
Fig. 7 Ex-vivo permeation studies of gel formulations through sheep nasal mucosa. All determinations are expresed in mean ± SD ($n = 3$)

poloxamer 407 might have interfered with soya lecithin molecules, causing voids in the region of phospholipid of the membrane bilayer, leading to relaxation of bilayer membrane and thereby improved drug permeation.

An optimized concentration of poloxamer 407 (18 % w/v) was used for the formulation of thermoreversible intranasal gel. Carbopol 934 in varying concentrations (0.1–0.5 % w/v) was used as a gelling agent as well as mucoadhesive polymer. Effect of a mixture of carbopol 934 and poloxamer 407 (18 % w/v) on gelling temperature, viscosity and mucoadhesive strength is as shown in Fig. 5. It was found that by addition of carbopol 934 in the concentration range from 0.1 to 0.5 %, (Table 2) causes lowering of gelation temperature from 35 to 25 °C. As the physiological temperature of the nasal mucosa is in the range of 30–34 °C formulation G1 and G5 were not

considered for further evaluation. G1 formulation having a higher gelation temperature 35 ± 0.5 °C can cause handling, administration problems leading to loss or drainage of the drug from the nasal cavity, whereas, G5 which is showing lower gelation temperature of 25 ± 0.5 °C could cause manufacturing and storage problems.

Physicochemical evaluation of gel for its pH, viscosity and mucoadhesive strength was performed. The pH of the optimized gel formulation 6.1 ± 0.2 was desirable and acceptable for nasal mucosal permeation studies. Viscosity and mucoadhesive strength of the gel formulations were found to be directly proportional to the concentration of carbopol used in the formulation. It could be due to the cross-linked nature of carbopol 934, chemically a polyacrylate polymer having abundant carboxylic groups which tends to form hydrogen bonding with sugar residues in oligosaccharide chains present in the mucus membrane. These chemical interactions lead to an association between polymer and mucus membrane leading to increase in mucoadhesive strength [35]. Higher mucoadhesive strength could prolong retention and subsequently increase the absorption across the nasal mucosal tissue.

Cumulative drug release from the EH loaded thermoreversible gel formulations and ex vivo drug permeation study of EH loaded ethosomal gels (G2, G3 and G4) were carried out on freshly excised sheep nasal tissue using the Franz diffusion cell system. The sinus anatomy in sheep is closely resembles to humans, suggesting a reasonable co-relation between in vitro and in vivo studies [36]. Results of ex vivo permeability studies (Fig. 7) suggest that the permeation kinetics was best in formulation G4 when compared to other formulations. Permeability co-efficient for optimized gel formulations was 8.6 (10^{-5} cm/s) after 12 h corroborates with the earlier studies which have indicated that the poloxamer 407 slightly decreases the rate of drug release due to enhanced micellar structure and gel network. Drug permeation through nasal mucosa was found to be directly proportional to the concentration of carbopol 934. This may be due to increase in the ionized carboxyl groups which might have caused conformational changes and swelling in the polymer chain and the gel network to become relaxed, resulting in initial delay in drug permeation for 4 h.

Histopathological images of nasal mucosal membranes treated with thermoreversible gel displayed slight disruption of epithelium cells whereas untreated mucosal membrane showed intact cellular integrity. The reason for this injury could be attributed to the physical impact caused by application of gel and pH shock. It could be due to the swelling nature of carbopol which resulted in a minor injury to epithelial cells with intact columnar cells. Absence of damage to mucus secreting glands, cell necrosis and columnar cells confirmed that these ethosomal
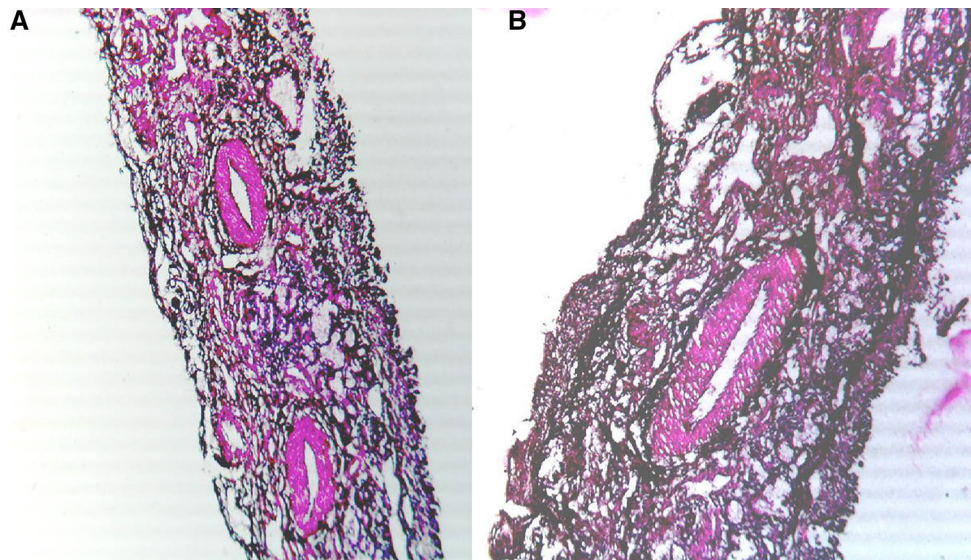
Fig. 8 Histopathological evaluation of sections of sheep nasal mucosa. **a** Mucosal tissue incubated in PBS (treated), **b** mucosal tissue incubated in diffusion chamber with G4 formulation (untreated)

thermoreversible gels were safe for nasal mucosa and could be administered by intranasal route to treat migraine.

6 Conclusion

In the present investigation, EH loaded ethosomal thermoreversible gel with favorable physico-chemical property for intranasal delivery to the brain was developed. Ethosomes were formulated by using a 3^2 factorial design which reveals that the concentration of soya lecithin and ethanol are the main determinants responsible for optimization of formulation. Optimized ethosomal formulation E6 having the concentration of soya lecithin (3 % w/v) and ethanol (40 % v/v) showed desirable entrapment efficiency (63.33 %) and vesicle size around 191 nm. Thermoreversible gels of EH loaded ethosomes were formulated by using poloxamer 407 (18 % w/v) and carbopol 934 (0.4 % w/v) as thermoreversible and mucoadhesive polymers, respectively. These thermoreversible gels showed suitable gelation temperature, viscosity, mucoadhesive strength and release kinetics. This route provides a needle free, non-invasive method for targeted drug delivery to the brain as a safe and effective drug delivery system for the treatment of migraine by passing the blood brain barrier and avoiding hepatic first pass metabolism.

References

1. Tfelt-Hansen P, Vries PD, Saxena PR. Triptans in migraine: a comparative review of pharmacology, pharmacokinetics and efficacy. Drugs. 2000;60(6):1259–87.
2. Brandes JL, Kudrow D, Stark SR, O'Carroll CP, Adelman JU, O'Donnell FJ, Alexander JW, Spruill SE, Barrett PS, Lener SE. Sumatriptan-naproxen for acute treatment of migraine: a randomized trial. JAMA. 2007;297:1443–54.
3. Takiya L, Lisa CP, Kamath V. Safety and efficacy of Eletriptan in the treatment of acute migraine. Pharmacotherapy. 2006;261:115–28.
4. McHarg AD, Napier CM, Stewart M, Melrose HL, Wallis RM. The functional activity of Eletriptan hydrobromide and other 5-HT1B/1D agonists at the human recombinant 5-HT1B and 5-HT1D receptor. Headache. 1999;39:369–70.
5. Hoskin KL, Lambert GA, Donaldson C, Zagami AS. The 5-hydroxytryptamine 1B/1D/1F receptor agonists eletriptan and naratriptan inhibit trigeminovascular input to the nucleus tractus solitarius in the cat. Brain Res. 2004;998:91–9.
6. Dirk D, Hanssens Y. Current and emerging second-generation triptans in acute migraine therapy: a comparative review. J Clin Pharmacol. 2000;40:687–700.
7. Pichard-Garcia L, Hyland R, Baulieu J, Fabre JM, Mlton A, Maurel P. Human hepatocytes in primary culture predict lack of cytochrome P-450 3A4 induction by Eletriptan vivo. Drug Metab Dispos. 2000;28:51–7.
8. Bardsley-EA Stuart N. Eletriptan. CNS drugs. 1999;12:325–33.
9. Milton KA, Allen MJ, Abel S, Jenkins VC, James GC, Rance DJ, Eve MD. The safety, tolerability, pharmacokinetics and pharmacodynamics of oral and intravenous eletriptan, a potent and selective "5-HT1D-like" receptor partial agonist. Cephalalgia. 1997;17:A414.
10. Wu H, Hu K, Jiang X. From nose to brain: understanding transport capacity and transport rate of drugs. Expert Opin Drug Deliv. 2008;5(10):1159–68.
11. Rattanapak T, Young K, Rades T, Hook S. Comparative study of liposomes, transfersomes, ethosomes and cubosomes for transcutaneous immunisation: characterisation and in vitro skin penetration. J Pharm Pharmacol. 2012;64:1560–9.
12. Agrawal V, Gupta V, Ramteke S, Trivedi P. Preparation and evaluation of tubular micelles of pluronic lecithin organogel for transdermal delivery of sumatriptan. AAPS Pharm Sci Tech. 2010;11:1718–25.
13. Singh RM, Kumar A, Pathak K. Mucoadhesive in situ nasal gelling drug delivery systems for modulated drug delivery. Expert Opin Drug Deliv. 2013;10:115–30.

14. Salama HA, Mahmoud AA, Kamel AO, Hady MA, Awad GA. Phospholipid based colloidal poloxamer 407-nanocubic vesicles for brain targeting via the nasal route. Colloid Surf B Biointerfaces. 2012;100:146–54.

15. Akhtar N. Vesicles: a recently developed novel carrier for enhanced topical drug delivery. Curr Drug Deliv. 2014;11:87–97.

16. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Control Release. 2000;65:403–18.

17. Choi MJ, Howard IM. Elastic vesicles as topical/transdermal drug delivery systems. Int J Cosmet Sci. 2005;27:211–21.

18. Bendas ER, Mina IT. Enhanced transdermal delivery of salbutamol sulfate via ethosomes. AAPS PharmSciTech. 2007;8:213–20.

19. Nava D, Toitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials. 2000;21:1879–85.

20. Acharya S, Patra S, Pani N. Optimization of HPMC and carbopol concentrations in non-effervescent floating tablet through factorial design. Carbohydr Polym. 2014;102:360–8.

21. Yang K, Delaney JT, Schubert US, Fahr A. Fast high-throughput screening of temoporfin-loaded liposomal formulations prepared by ethanol injection method. J Liposome Res. 2012;22:31–41.

22. Majithiya RJ, Ghosh PK, Umrethia ML, Murthy SR. Thermoreversible-mucoadhesive gel for nasal delivery of sumatriptan. AAPS PharmSciTech. 2006;7:E80–6.

23. Pisal SS, Paradkar AR, Mahadik KR, Kadam SS. Pluronic gels for nasal delivery of vitamin B 12. Part I: preformulation study. Int J Pharm. 2004;270:37–45.

24. Shah RA, Mehta MR, Patel DM. Design and optimization of mucoadhesive nasal in situ gel containing sodium cromoglycate using factorial design. Asian J Pharm. 2011;5:65–74.

25. Alsarra IA, Hamed AY, Mahrous GM, El Maghraby GM, Al-Robayan AA, Alanazi FK. Mucoadhesive polymeric hydrogels for nasal delivery of acyclovir. Drug Dev Ind Pharm. 2009;35:352–62.

26. Das MK, Abdul BA. Formulation and ex vivo evaluation of rofecoxib gel for topical application. Acta Pol Pharm. 2007;64:461–7.

27. Basu S, Bandyopadhyay A. Development and characterization of mucoadhesive in situ nasal gel of midazolam prepared with Ficus carica mucilage. AAPS PharmSciTech. 2010;11:1223–31.

28. Verma P, Pathak K. Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep fungal infections through topical gel formulation. Nanomed Nanotechnol Biol Med. 2012;8(4):489–96.

29. Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J control Release. 2007;123:148–54.

30. Maurya SD, Prajapati S, Gupta A, Saxena G, Dhakar RC. Formulation development and evaluation of ethosome of stavudine. Int J Pharm Edu Res. 2010;13:16.

31. Maura C, Carlo C, Dontella V, Manconi M, Chiara S, Fadda AM. Effect of penetration enhancer containing vesicles on the percutaneous delivery of quercetin through new born pig skin. Pharmaceutics. 2011;3:497–509.

32. Proctor DF, Andersen IB, Lundqvist GR. Human nasal mucosal function at controlled temperatures. Respir Physiol. 1977;30:109–24.

33. Choi HG, Oh YK, Kim CK. In situ gelling and mucoadhesive liquid suppository containing aetaminophen: enhanced bioavailability. Int J Pharm. 1998;165:23–32.

34. Escobar-Chavez JJ, Lopez-CM Naık A, Kalia D. Applications of thermoreversible Pluronic F-127 gels in pharmaceutical formulations. J Pharm Pharmaceut Sci. 2006;9:339–58.

35. Choi HG, Jae HJ, Jei-Man R, Sung-June Y, Yu-Kyoung O, Chong-Kook K. Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository. Int J Pharm. 1998;165:33–44.

36. Briner HR, Simmen D, Jones N, Manestar D, Manestar M, Lang A, Groscurth P. Evaluation of an anatomic model of the paranasal sinuses for endonasal surgical training. Rhinology. 2007;45:20.