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DEVELOPMENT AND EVALUATION OF ELASTIC NOISOMAL FORMULATION FOR MIGRAINE TREATMENT USING TRNA APPROACH

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ABSTRACT: The approach of site specific topical delivery of Rizatriptan Benzoate (RBZ), used for migraine using the elastic carrier at posterior cervical region for sustained action was attempted in the present study. RBZ-entrapped elastic niosomes were prepared using the modified mechanical stirring method. The formulation was optimized using 2^3 full factorial designs. The type of edge activator (EA), Tween: cholesterol: edge activator molar ratio (T:C:EA) and Total surfactant concentration (S) as independent and the percent entrapment efficiency (%EE) as dependent variables with visual stability of dispersion for two week as constrain factor for selection. The study revealed that interaction between EA and S was influencing factor for %EE. The optimized formulation EN3 having edge activator type, total surfactant concentration and molar ratio Tween 20: cholesterol: EA as Sodium cholate, 2% and 2:1:2 respectively, having high percent entrapment efficiency (92.22%), showed unilamellar smooth surface in SEM study. Zeta potential was found to be - 17.2 mV. *In-vitro* permeation from the elastic niosomal gel followed zero order model over a period of 12 h with non- Fickian diffusion mechanism. The biological anti-migraine test using acetic acid withering responses confirmed the sustained release of RBZ from Elastic niosome formulations.

INTRODUCTION: Migraine is a disabling neurovascular disorder characterized by mostly unilateral throbbing head pain and a host of neurological symptoms including hypersensitivity to light, sound and smell, nausea and variety of autonomic and cognitive, emotional and motor disturbances. Migraine pain is thought to be driven by activation and sensitization of peripheral neurons in the trigeminal ganglion that innervate the meninges (*i.e.*, meningeal nociceptors) and central trigeminovascular neurons in the upper cervical and medullary dorsal horn [1, 2].

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The most widely accepted theory regarding the physiological mechanism of migraine proposes that early in an attack, vasoactive peptides are released from the primary sensory nerve terminals that innervate meningeal blood vessels. These peptides activate perivascular trigeminal nerves and cause dilatation of arteries in the meninges as well as perivascular inflammation and extravasation of plasma proteins [3].

Several triptans are 5HT1B/1D receptor agonists commonly prescribed for migraine headache. Rizatriptan benzoate (RBZ) is a potent and selective serotonin (5-HT1B/1D) receptor agonist, it is a second-generation triptan and used in the acute treatment of migraine attacks with or without aura and cluster headaches [3, 4, 5, 6]. RBZ is metabolized primarily by monoamine oxidase A and excreted in the urine and bile. It is generally given by oral route which suffers from poor

bioavailability problem due to pre-systemic metabolism. It is also reported that the oral bioavailability of RZB is only 45% and half-life is 2-3 h, so require frequent administration [7, 8]. Again Migraine many times associated with nausea, vomiting and gastric stasis which make oral route unsuitable. It was investigated that topical administration may include increased efficiency by avoiding the first-pass effect of the liver, avoiding discomfort and risks of an intravenous treatment, avoiding side effects in the region of the gastrointestinal tract in the case of oral medication, and good patient acceptance. Absorption peaks involving the risk of systemic side effects may also be avoided [9, 10, 11].

Recently As per European patent no. 1435945 a concept of topical regional neuroaffetive (TRNA) therapy in which action of triptan at peripheral neural synapse take place after topical application at posterior cervical region *i.e.* back of the ear, preferably in close proximity to or on the area of skin above the brain stem had been proposed [12, 13]. In TRNA therapy drug need only transverse the stratum corneum of the skin to reach cutaneous free nerve endings for therapeutic effect. This alternate route of administration will promote drug localization at peripheral neural synapse and the therapeutic efficiency of RZB with sustained action and will also avoid cardiac side effects associated with RBZ.

Although targeted topical administration offers diverse advantages for non-invasive drug delivery, the drug must transit the lipid stratum corneum and cross the aqueous epidermal and dermal layers before reaching the target tissues. The main obstacle in topical drug delivery is crossing stratum corneum (SC) which can be overcome by using elastic niosomes (ENs), flexible vesicular drug delivery system. These second generation of elastic vesicles (EVs), mainly consisting of non-ionic surfactants (NISs), was introduced in 1999 by Van den Bergh, which found to deeply and easily penetrate through the skin. The elasticity of ENs is attributed to the use of edge activators (EAs). An edge activator is usually a single chain surfactant that causes the destabilization of the lipid bilayer of the vesicle and increases the vesicle-elasticity or fluidity by lowering its interfacial tension. ENs have been found to be more effective in enhancing drug transport as compared to rigid vesicles like conventional liposomes for number of drug molecules like Eprosartanmesylate [14], Eletriptan-hydrobromide [15], Clotrimazole [16], Tramadol [17], Ketoprofen [18], Naringenin [19] and Pentoxifylline [20]. In the literature, only few studies have been reported for sustained delivery of RBZ using microspheres and vesicular formulations as carrier system [4, 21, 22]. In the present study, an attempt has been made to develop and optimize novel surfactant-based ENs using different concentrations of several EAs. The *in-vitro* efficacy of this elastic system is examined, compared to free drug preparations.

MATERIALS AND METHODS:

Materials: The gift sample of Rizatriptan Benzoate (RBZ) was supplied by Cipla Ltd., Pune, India, Tween 20, cholesterol (CH), sodium cholate (SC) and sodium deoxycholate (SDC) purchased from Research Lab, Mumbai, India. Demineralized and double distilled water was used. Carbopol®934 was procured from SD Fines Chemical Ltd., Mumbai, India, Diethyl ether was obtained from Merck, Mumbai, India. All reagents used in this study were of analytical grade.

Analytical Method: RBZ is analyzed for % EE, percent cumulative release (% CDR) and percent diffusion study by UV double beam spectro-photometer (Shimadzu- 1800, Japan) in phosphate buffer pH 6.8 by generating standard curve for the range of 0 to 5 µg/ml at 225 nm [23, 24].

Formulation Development of RBZ Elastic Niosomes:

Preparation Method of ENs Formulations: Elastic noisome formulation was prepared with method of modified mechanical stirring. In this formulation a precisely weighed amount of Tween 20 and cholesterol in 2:1 molar ratio was added along with EA into diethyl ether (6 ml). RBZ (5mg/ml) was added into PBS pH 6.8 (44 mL). Then organic phase was added into aqueous phase placed on mechanical stirrer and stirring was carried out for 1 h at 60 ºC using 900 RPM. Vaporization of the ether leads to the formation of single layered vesicles. Total surfactant concentration 2% was used. ENs was formed spontaneously by this process. The EN dispersion was suitably diluted and put on a glass slide and

viewed with a binocular microscope with magnification of 10X to confirm the formation of intact vesicles.

Experimental Design: A 2^3 full factorial design was used in the present study. Several studies revealed that the edge activator concentration, total surfactant concentration and type of edge activator plays notable role in lamellar configuration, on entrapment of the drug and stability of the dispersion system [25, 26, 27, 28]. Here attempt was made to study the interactive effect of all these variables on encapsulation efficiency of ENs formulation. In this design three factors were evaluated, each at two levels, and experimental trials were carried out at all eight possible combinations. The design layouts and coded value of independent factor are shown in **Table 1**. One of independent factor selected was type of EA as sodium cholate and sodium deoxycholate. The other two numerical factors were S as 1.5% and 2%, different molar ratio of T: C: EA: as 2:1:0.2 and 2:1:0.3. Percent entrapment efficiency was selected as dependent variables. Design expert 10 trial version was used for the generation and evaluation of the statistical experimental design. The matrix of the design including response is shown in **Table 2**.

An ANOVA test was performed to evaluate the level of significance of the tested factors on the selected responses as well as the interactions between these factors. Application of regression analysis is important to check that our independent variables have a significant effect on our response or not. All data derived from the responses were evaluated statistically for their correlation by regression and ANOVA and visual stability of dispersion for two weeks was taken as constrain factor for optimize formulation selection.

TABLE 1: COMPOSITION OF DIFFERENT CODED VALUES IN 2^3 FULL (GENERAL) FACTORIAL DESIGNS OF RBZ ELASTIC NIOSOMES

Coded values		Independent variables (actual values)		
		X_1: A [type of EA]	X_2: B [Tween: Cholestrol: EA molar ratio]	X_3: C [total surfactant concentration (%)]
Levels	Low (1)	Sodium cholate	2:1:2	1.5
	High (2)	Sodium deoxycholate	2:1:3	2

Characterization of Niosomes:

%EE: ENs suspensions were cold centrifuged at 14,000 rpm for 90 min. After centrifugation, 1 ml of supernatant was diluted with the addition of 9 ml phosphate buffer (pH 6.8) and then the absorbance was measured using UV-Vis spectrophotometer by measuring absorbance at 225 nm. The drug entrapment efficiency was calculated as below,

$$EE = (WT - WF) / WT \times 100\%$$

Where, EE is the percent entrapment efficiency of drug, WT is the total amount of RBZ in ENs, WF is the free amount of RBZ that was in the supernatants.

Visual Stability: ENs dispersion stability was observed visually after storing it at 4 ºC for two weeks. Presence of layer formation, aggregates is indicates stability issue.

Characterization and Evaluation of Optimized Formulation:

Microscopy: Morphology of optimized EN formulation was studied using Scanning electron microscopy (SEM) by observing surface morphology, size, shape and the aggregation property of vesicles. Selected EN formulation was examined by scanning electron microscopy at different magnification powers at 10 KV.

Vesicle Size Distribution and Surface Charge: The vesicle size and size distribution of vesicles were determined by Malvern zeta sizer based on laser light scattering principle. Light scattering was monitored at 90º angle and at 25 ºC. The mean vesicle size was calculated from intensity, volume and bimodal distribution assuming spherical particles. The zeta potential is an indication of the stability of the colloidal systems and indicates charge present in the colloidal systems. Zeta potential of optimized formulations was determined using Malvern zeta sizer. Samples were placed in clear disposable zeta cells and results were recorded.

Stability Evaluation: The RBZ-loaded optimized EN dispersion was stored in a sealed glass vials and subjected to physical stability study in triplicate.

The vials were kept at two different storage conditions, *i.e.,* 4 ± 1 °C with ambient humidity and room temperature 25 ± 2 °C, and the samples were withdrawn periodically at an interval of 1 month for 3 months, suitably diluted with water and analyzed for EE. Samples were also observed for uniformity of dispersion.

Preparation of Gel: Gel base was prepared by dispersing 1% w/v Carbopol 934 in distilled water. The polymer was soaked in water for 2 h and then dispersed in distilled water using a magnetic stirrer so as to obtain a homogeneous gel adjusting the pH by using triethanolamine solution [29]. For preparation of EN gel, the EN suspension(s) was centrifuged at 14000 rpm for 20 min, and the pellets obtained were incorporated into the prepared gel base to get 5 mg RBZ per gram.

***In-vitro* Skin Permeation Studies:** The *in-vitro* skin permeation of RBZ from optimized EN suspension and gel, in comparison with plain drug solution were studied using Franz glass diffusion cell maintained at 32 ± 1 °C under non-occlusive conditions. The effective permeation area of the diffusion cell was 2.303 cm^2. The receptor compartment contained 48.00 ml phosphate buffered (pH, 6.8) and was constantly stirred at 100 rpm. Excised albino abdomen rat skin was mounted between the donor and the receptor compartment. EN formulation (1.0 ml) was applied to the epidermal surface of skin. The samples (1 ml) were withdrawn through the sampling port of the diffusion cell 1 h time interval for 12 h and analysed.

An equal volume of fresh phosphate buffer maintained at 32 ± 1 °C was replaced into the receptor compartment after each sampling. The samples were filtered through 0.45 µm nylon filter membrane and analyzed using spectrophotometer for drug content at 225 nm. The cumulative amount of drug permeated per unit area was plotted as a function of time. All investigations were performed as per the protocol approved by CSPCSE (CPCSEA; Letter no. SGRS/IAEC/8/2014-15).

Release Kinetic Studies - Release Pattern and Mechanism: The data of *in-vitro* RBZ release from EN dispersion and gel were evaluated kinetically using various mathematical models like zero-order, first-order, Higuchi and Koresmeyer - Peppas model equations to describe release patterns and mechanisms. The model dependent methods all rely upon a curve fitting procedure.

Biological Antimigraine Activity Evaluation: Antimigraine activity of RBZ was investigated with 0.3% acetic acid-induced abdominal constrictions in adult male mice [30]. Albino mice weighing 30 - 40 g, 12 - 14 weeks old were divided into four groups each comprising of three animals. The first group received topical treatment with PBS and served as control. The animals in the second, third, and fourth groups received topical application of RBZ at posterior cervical region, back of the ear in the form of solution in PBS, optimized EN suspension and EN Gel formulation respectively.

These formulations were applied topically 30 min before the intraperitoneal injection (IP injection) of 0.3% v/v acetic acid (10 ml/kg). The number of abdominal constrictions for 15 min after administration of IP injection of 0.3% acetic acid was counted at 1-, 6-, and 12-h time intervals for each group. The abdominal constrictions consist of constriction of abdominal muscles together with the stretching of hind limbs. Results were expressed as number of writhing responses with respect to control. All investigations were performed as per the protocol approved by CSPCSE (CPCSEA; Letter no. SGRS/IAEC/8/2014-15).

RESULTS AND DISCUSSION:

Elastic Niosomes Formation: ENs were spontaneously produced by using bilayer forming , hydrophilic surfactant Tween 20 and micelle forming surfactant (SC/SDC) along with addition of cholesterol. The incorporation of edge activator would increase the elasticity of the vesicles by destabilization of the bilayer. HLB value of Tween 20 is 16.5 which make it unable to form vesicles alone. The molar ratio Tween 20 and cholesterol 2:1 was found to form intact vesicles in the present study as shown in **Fig. 1**. The bile salts SC, SDC were selected as an edge activator because it is biocompatible, pharmaceutically acceptable, and provide optimum elasticity to vesicle membrane [31], also both the surfactants are recommended for systemic administration and have GRAS status by the USFDA.

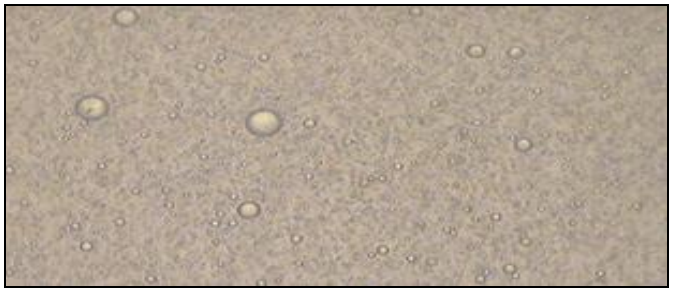

FIG. 1: PHOTOGRAPH OF ELASTIC NOISOME FORMULATION UNDER 10X

Optimization / Analysis of Factorial Design: The 2^3 full factorial was designed for optimization study. In this design effect of type of EA (A), molar concentration ratio of T:C:EA (B) and S (C) as formulating parameter and their interactions were analysed using a suitable statistical tool (Design expert 10 Trial version). % EE was selected as response parameters, as they play a decisive role in providing topical efficacy of formulations. The selection criterion for optimized formulation was based on the highest EE and visual stability of vesicles upon storage for a period of two weeks, especially in terms of aggregation/ irregularity or layer formation. The % EE of all vesicles investigated in experimental runs, with independent variables and the measured responses are shown in **Table 2** ranged from 72.14% to 92.22%.

TABLE 2: FULL FACTORIAL DESIGN MATRIX OF RBZ ENS AND EVALUATION FOR EE AND VISUAL STABILITY PARAMETERS

Formulation code	X_1:A [Type of EA]	X_2: B [Tween: Cholestrol: EA Molar ratio]	X_3: C [Total surfactant Concentration (%)]	*Y_1: %EE	Stability by visual means
EN1	SC	2:1:2	1.5	82.11 ± 1.31	Stable
EN2	SC	2:1:3	1.5	72.35 ± 1.54	Stable
EN3	SC	2:1:2	2	92.22 ± 1.01	Stable
EN4	SC	2:1:3	2	78.24 ± 0.94	Stable
EN5	SDC	2:1:2	1.5	76.13 ± 1.79	Stable
EN6	SDC	2:1:3	1.5	84.15 ± 2.03	Unstable
EN7	SDC	2:1:2	2	82.12 ± 1.91	Stable
EN8	SDC	2:1:3	2	88.05 ± 0.83	Unstable

*SD standard deviation from mean, n=3

The F-value of 20.36 observed with ANOVA data of the model for EE as response **Table 3**, indicated that the model is significant and the combined effect of the type and molar concentration of EA had a significant effect on the EE of RBZ vesicles and total surfactant concentration also showed significant effect (p = 0.0055, p = 0.0157, respectively).

TABLE 3: SUMMERY OF ANOVA FOR THE RESPONSE PARAMETER - %EE

Source	Sum of square	DF	Mean square	F-Value	P-Value
Model	277.16	4	69.29	20.36	0.0164
A-A	3.82	1	3.82	1.12	0.3670
B-B	11.98	1	11.98	3.52	0.1573
C-C	83.79	1	83.79	24.62	0.0157
A*B	0.1875	1	177.57	52.17	0.0055
A*C	0.0208	1	0.02083	0.27	0.612
B*C	0.0833	1	0.08333	1.06	0.315

A, B & C = main factors; A*B, A*C, & B*C = interactions between main factors; DF = degree of freedom

The Pareto chart also reflects same result as shown in **Fig. 2**.

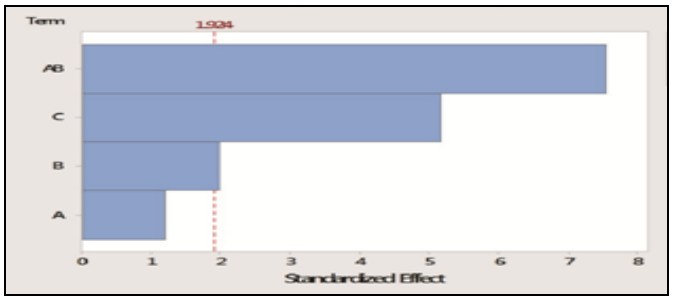

FIG. 2: PARETO CHART

The Final equation in terms of coded factors obtained was

$$EE = 81.92 + 0.61*A - 1.22*B + 4.71*AB$$

Where it can be noticed that among factors, total surfactant concentration have positive influence on % EE, and combined effect of type of edge activator and molar concentration of edge activator had more prominent positive effect on EE. By observing the effect of EA type and molar ratio on EE%, it was found that that the ratio of 2:1:2%

showed the highest EE% in case of SC, while a 2:1:3 ratio was optimum for drug entrapment in case of SDC. However, further increase in the EA's ratio in case of SC lead to a consequent decrease in EE% as when the concentration reaches a certain threshold, micelles or mixed micelles are starting to be formed, leading to a decrease in EE%. This was also previously reported in case of oestradiol ultadeformable liposomes with higher ratios of SC [32]. The EE also found to be influenced by type of edge activator. SC showed more EE than SDC.

The influences of factors on response Y1 here vesicle EE are further supported by surface plot study as shown in **Fig. 3** and **4**.

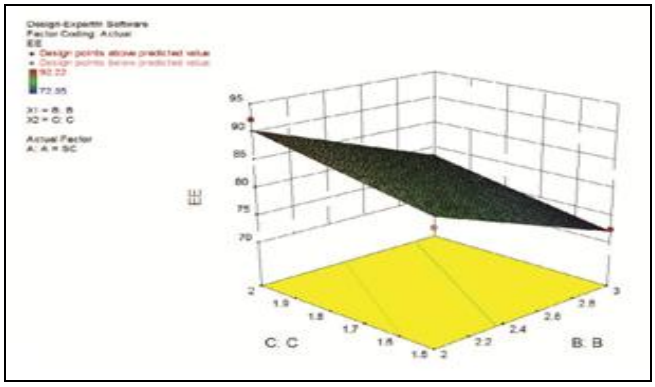

FIG. 3: SURFACE PLOT FOR SC

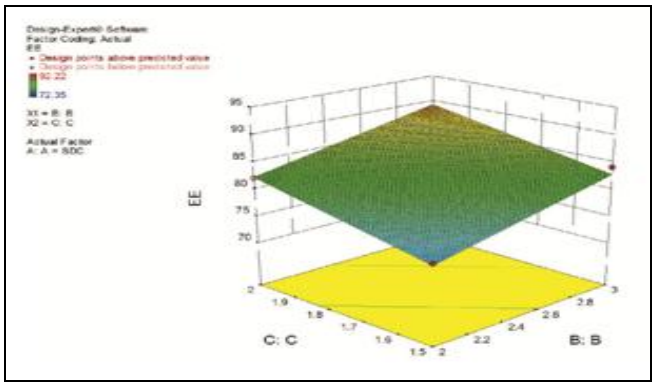

FIG. 4: SURFACE PLOT FOR SDC

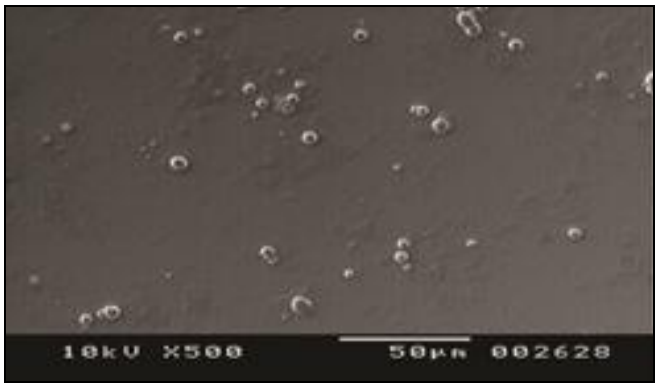

FIG. 5: SEM PHOTOGRAPH

The entrapment efficiency and stability of vesicular carrier are the parameters mostly influence the permeation of entrapped drug through skin. For this reason, formulation EN3, which is characterized by high entrapment efficiency (92.22%), was considered to be an optimal formulation and carried forward for further investigations. All the formulations under experimental run found to be stable as no signs of aggregates, layer separation were observed for two weeks of storage at 4 ºC except formulations containing SDC in 2:1:3 molar ratio at both surfactant level of 1.5 and 2%.

Evaluation of Optimized Formulation:

SEM Photomicrograph: It is seen from SEM photomicrograph **Fig. 5** that optimized EN vesicles are unilamellar , nano ranged with smooth surface and near spherical shape showing some deviation in terms of shape explaining the fluidizing effect of sodium cholate, a phenomenon observed earlier [17, 33]. The results showed that RBZ EN has a smooth surface.

Vesicle Size Distribution and Surface Charge: Polydispersity index (PDI) was determined as a measure of homogeneity of formulation [18, 34]. Dynamic Light Scattering (DLS) revealed small PI of optimized formulation as 0.312 **Fig. 6**. Small value of PDI less than 0.5 indicate a homogenous population of EN vesicles. The characterization of the developed formulations for their vesicles size demonstrated that the mean vesicles size was investigated showed 130.7 ± 0. 7 nm **Fig. 6**, representing that the prepared formulations had vesicles of small size which is necessary for their topical uses.

Zeta potential values were always negative (≥ -15 mV), indicative of a good stability against vesicle aggregation and fusion [35]. Zeta potential analysis revealed greater negative surface charge density, due to the incorporation of negatively charged bile salts into the niosomal membranes.

The zeta potential of selected EN dispersion was -17.2 mV which is sufficient to inhibit aggregation of EVs due to electric repulsion **Fig. 7**. This negative charge value of EN is attributed to the presence of SC.

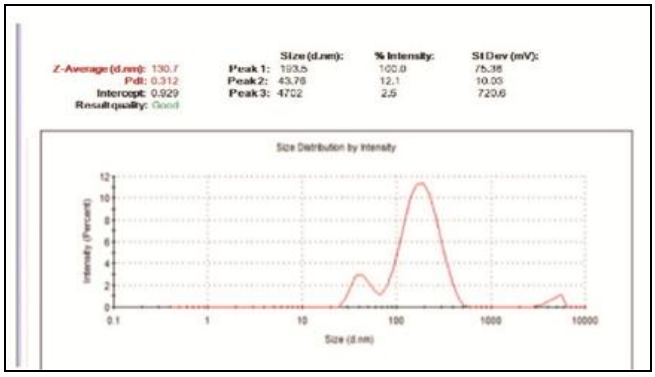

FIG. 6: PDI OF EN FORMULATION

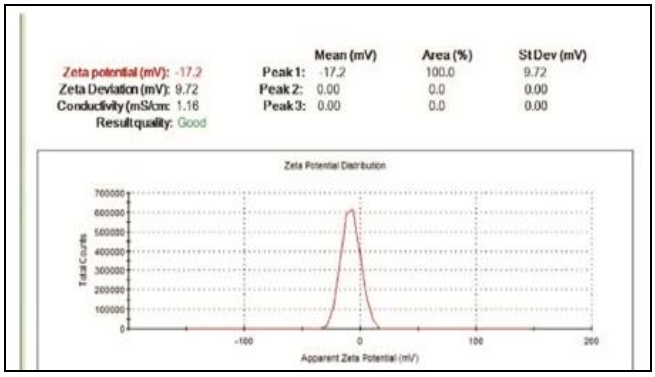

FIG. 7: ZETA POTENTIAL OF EN FORMULATION

Stability Evaluation Study: Physical stability study of optimized EN formulation was conducted at refrigeration temperature (4 ± 1 ºC) and at room temperature (25 ± 2 ºC) for a period of 3 months. No significant changes in EE were observed for the formulation stored at 4 ± 1 ºC but the average size of the vesicles in all formulations showed slight increase. While evaluating the stability of the vesicles low EE of EN were observed when stored at 25 ± 2 ºC **Table 4**.

At room temperature EN found to be stable .The percent RBZ retained by EN formulations after 90 days were 91.25 ± 1.52% and 75.46 ± 1.23% when stored at 4 ± 1 ºC and 25 ± 2 ºC respectively. The visual appearance observations revealed that the aqueous EN formulations stored at 4 ºC and 25 ºC showed no signs of separation or change in color up to 3 months. This reveals that the stability of RBZ EN is dependent on the storage temperature and age.

TABLE 4: STABILITY STUDY RBZ ELASTIC NIOSOMES AT DIFFERENT STORAGE CONDITIONS

Test time (months)	Physical stability of dispersion		Percent entrapment efficiency of RBZ in EN (% EE)	
	Storage at 4 ± 2 ºC	Storage at 25± 2 ºC	Storage at 4± 1 ºC	Storage at 25± 2 ºC
0	Uniform dispersion	Uniform dispersion	92.22± 1.12	92.02 ± 1.10
1	Uniform dispersion	Uniform dispersion	90.25± 0.59	87.27± 1.23
2	Uniform dispersion	Uniform dispersion	89.21 ± 0.79	80.14 ± 1.14
3	Uniform dispersion	Layer formation	87.15 ± 1.22	69.24± 1.15

***In-vitro* Skin Permeation Studies:** The investigations of efficient prolong release ability of EN formulation was measured using the abdominal portion of a rat and the method adapted was Franz diffusion cell measurement. The percent cumulative amount of RBZ permeated across excised rat skin over 8 h periods was plotted against the function of time and lag time was determined.

From the release profiles it was observed that after 3.5 h, maximum drug was permeated from the drug solution (99.1 ± 5.1%), this explains need for prolong release formulation development. Optimized EN RBZ formulation show significant reduction in *in-vitro* drug release ($p<0.001$) in 3.5 h compared with drug in solution. The release profile of RBZ from EN formulation and gel was biphasic.

The initial fast release of around 17.1% of the drug from the EN was observed in the first 1 h, which could probably be due to the portion of the drug that leaked out of EN and the unloaded drug. Initial rapid release up to 2 h followed by extended release up to 12 h with around percent cumulative release of around 93.4% was observed **Fig. 8**. The very low viscosity often exhibited by EN is not suitable for topical use. The viscosity can be increased by adding thickening agents, which also change the appearance of the system, usually

influencing drug release. As a vehicle for incorporation of EN for skin delivery, the EN formulation was loaded in 1% carbopol 934.

Among the various hydrogel bases, carbopol is used because of its high stability, compatibility and low toxicity [36]. *In-vitro* release data of EN gel revealed around 84.9% release of RBZ in 12 h in which around 12% release within 1 h which assumes to be sufficient as loading dose. The release of RBZ from the gel was characterized by an initial phase of high release *i.e.* burst effect and the remaining drug being released at a slower rate **Fig. 8**. This bi-phasic pattern release is a characteristic feature of matrix diffusion kinetics. The gel formulation showed controlled drug release due to the entrapment of drug in vesicles. Permeation study suggests that the gelling agent in the concentration used had little retarding effect on drug release and that the rate-limiting step was the diffusion of the drug out of the EN. The release experiments clearly indicated sustained release of RBZ from EN formulations.

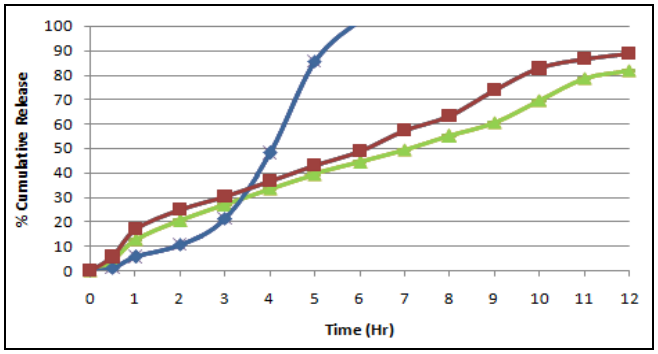

FIG. 8: GRAPH OF PERCENT CUMULATIVE AMOUNT OF RBZ FORMULATIONS THROUGH *IN - VITRO* RELEASE

Release Kinetic Studies: The results of the curve fitting into for mathematical models of release kinetics after comparing respective correlation coefficients indicate the *in-vitro* RBZ permeation behaviour of EN formulation and gel **Table 5** were followed the zero order kinetics. The 'n' values for solution and gel formulation were found to be 0.5984 and 0.77 respectively which are more than 0.5. This indicates that the release approximates non- Fickian diffusion mechanism [37].

TABLE 5: KINETIC MODELS OF FORMULATION

Formulation	Zero order	First order	Higuchi	Korsemeyer-Peppas	
	R^2	R^2	R^2	N	R^2
EN dispersion	0.9872	0.8774	0.9454	0.5984	0.9951
EN gel	0.9949	0.9254	0.9466	0.77	0.9963

Evaluation of Biological Antimigraine Activity: Studies on animals suggest that migraine pain may be caused by a neurogenic inflammatory process in the dura mater. Hyperalgesia induced by ip injection of a 0.3% acetic acid solution is a model in which the antihyperalgesic activity of some of antimigraine drugs had been proved previously [38, 39]. Results of writhing responses, abdominal constriction test are shown in **Fig. 9**.

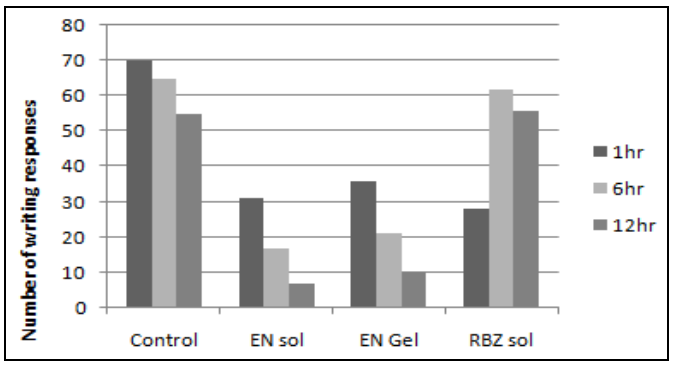

FIG. 9: BIOLOGICAL ANTIMIGRAINE ACTIVITY EVALUATION

The typical abdominal writhing response manifested as extension of the hind limb, contraction of the abdomen and rising of the croup. It is evident that topical application of RBZ solution and optimized EN solution and gelelicited a significant ($p<.05$) decrease in the number of abdominal constrictions in the acetic acid test as compared with mice treated with saline. The results indicated that RBZ solution decreased the number of abdominal constrictions to a greater extent as compared with RBZ EN GEL and EN dispersion within 1 h of topical application (28.3 ± 1.2,31.4 ± 1.5 and 36.2 ± 1.0 reduction, for RBZ solution ,EN solution and EN Gel respectively, at 1 h).

Furthermore, at 6 h post application, the decrease in number of constrictions was significantly higher ($p<.05$) with EN dispersion and gel as compared with that after application of RBZ solution (17.4 ± 1.2, 21.2 ± 2.0 and 62.2 ± 2.0, for EN solution, EN Gel and RBZ solution, respectively).

RBZ solution is found to be ineffective to reduce abdominal constrictions at 6 h of post treatment. EN solution and gel showed further reduction in abdominal construction at 12-hposttreatment. These results further strengthen the contention that the EN exhibited better skin penetration and deposition in the deeper layers of skin thus, sustaining the release of RBZ.

CONCLUSION: Stable elastic niosomes formulation containing RBZ was successfully formed using Tween 20, cholesterol and sodium cholate at particular molar ratio. Formulation attributes were shown to have marked impact with significant interaction effect on the average encapsulation efficiency of RBZ vesicles. In this study the optimum edge activator type, total surfactant concentration and molar ratio Tween 20: cholesterol: EA were found to be SC, 2% and 2:1:2 respectively. Interaction effect between type of EA and Molar ratio of EA was shown to have a major effect on the encapsulation efficiency of RBZ. The stability study suggests that EN dispersion were unstable at room temperature and stable at 4 ºC.

The *in-vitro* permeation study proved that the EN gel has potential to give sustained release for 12 h. The present study indicated that the developed EN gel formulation can be a good platform for topically delivering RBZ for site specific migraine treatment approach

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REFERENCES:

1. Goadsby PJ and Edvinsson L: The trigeminovascular system and migraine: Studies characterizing cerebro-vascular and neuropeptide changes seen in humans and cats. Ann Neurol 1993; 33: 48-56.
2. Burstein R, Jakubowski M and Rauch SD: The science of migraine. J Vestib Res Equilib Orientat 2011; 21: 305-14.
3. Jacobs B and Dussor G: Neurovascular contributions to migraine: Moving beyond vasodilation. Neuroscience 2016; 338: 130-44.
4. Sharma N, Kulkarni GT, Sharma A, Bhatnagar A and Kumar N: Natural mucoadhesive microspheres of *Abelmoschus esculentus* polysaccharide as a new carrier for nasal drug delivery. J Microencapsul 2013; 30: 589-98.
5. Nisha G, Maithil P and Charyulu R: Formulation and Development of Nasal in Situ Gels of Triptans. Int J Res Pharm Biomed Sci 2012; 3: 861-8.
6. Jagdale SC, Phule PS and Chavan GJ: Formulation and evaluation of modified pulsincap drug delivery system of rizatriptan benzoate. Int J Pharm Sci 2014; 6: 48-52.
7. Sharma N, Tyagi S, Gupta SK, Kulkarni GT, Bhatnagar A and Kumar N: Development and gamma-scintigraphy study of Hibiscus rosasinensis polysaccharide-based microspheres for nasal drug delivery. Drug Dev Ind Pharm 2016; 42: 1763-71.
8. Girotra P and Singh SK: Multivariate Optimization of Rizatriptan Benzoate-Loaded Solid Lipid Nanoparticles for Brain Targeting and Migraine Management. AAPS PharmSciTech 2017; 18: 517-28.
9. Pawar AP, Gholap AP, Kuchekar AB, Bothiraja C and Mali AJ: Formulation and Evaluation of Optimized Oxybenzone Microsponge Gel for Topical Delivery. J Drug Deliv 2015; 1-9.
10. Cosco D, Paolino D, Maiuolo J, Marzio L Di, Carafa M and Ventura CA: Ultradeformable liposomes as multidrug carrier of resveratrol and 5-fluorouracil for their topical delivery. Int J Pharm 2015; 489: 1-10.
11. Garg V, Singh H, Bhatia A, Raza K, Singh SK and Singh B: Systematic Development of Transethosomal Gel System of Piroxicam: Formulation Optimization, In Vitro Evaluation, and *ex-vivo* Assessment. AAPS PharmSciTech 2017; 18: 58-71.
12. Aung-din R: European patent 1 435 945 B1. 2008; 1: 1-17.
13. Aung-din R: United States Patent 8,329,734 B2. 2012; 2.
14. Ahad A, Al-Saleh AA, Al-Mohizea AM, Al-Jenoobi FI, Raish M and Yassin AEB: Formulation and characterization of novel soft nanovesicles for enhanced transdermal delivery of eprosartan mesylate. Saudi Pharm J 2017; 1-7.
15. Shalini MMHA and PKL: Formulation and evaluation of elastic niosomes of eletriptan hydrobromide. IJPSR 2016; 7: 1679-85.
16. Basha M, Hosam S, El-Alim A, Shamma RN and Awad GEA: Design and optimization of surfactant-based nanovesicles for ocular delivery of Clotrimazole. J Liposome Res 2013; 23: 1532–2394.
17. Singh S, Vardhan H, Kotla NG, Maddiboyina B, Sharma D and Webster TJ: The role of surfactants in the formulation of elastic liposomal gels containing a synthetic opioid analgesic. Int J Nanomedicine 2016; 11: 1475-82.
18. Uchino T, Lefeber F, Gooris G and Bouwstra J: Characterization and skin permeation of ketoprofen-loaded vesicular systems. Eur J Pharm Biophar 2014; 86: 156-66.
19. Tsai MJ, Huang Y Bin, Fang JW, Fu YS and Wu PC: Preparation and characterization of naringenin-loaded elastic liposomes for topical application. PLoS One 2015; 10: 1-12.
20. Al-Shuwaili AH, Rasool BKA and Abdulrasool AA: Optimization of elastic transfersomes formulations for transdermal delivery of pentoxifylline. Eur J Pharm Biopharm 2016; 102: 101-14.
21. Garg T, Jain S, Singh HP, Sharma A and AKT: Elastic Liposomal Formulation for Sustained Delivery of Antimigraine Drug : *in vitro* Characterization and Biological Evaluation. Drug Dev Ind Pharm 2008; 34: 1100-10.
22. Lahoti SR, Taur NG, Khan AR and Puranik PK: Enhanced Iontophoretic Permeation of Rizatriptan Benzoate from Thermo- reversible Gel Based System : Effect of Penetration Enhancers and Pulsed Current. Indian J Pharm Educ Res 2012; 46: 105-11.
23. Salehi S and Boddohi S: New formulation and approach for mucoadhesive buccal film of rizatriptan benzoate. Prog Biomater 2017; 6: 175-87.

24. Avachat AM, Gujar KN and Wagh K V: Development and evaluation of tamarind seed xyloglucan-based muco-adhesive buccal films of rizatriptan benzoate. Carbohydr Polym [Internet] 2013; 91: 537-42. Available from: http://dx.doi.org/10.1016/j.carbpol.2012.08.062
25. Mavaddati MA, Moztarzadeh F and Baghbani F: Effect of Formulation and Processing Variables on Dexamethasone Entrapment and Release of Niosomes. J Clust Sci 2015; 26: 2065-78.
26. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T and Praça FG: Development, characterization, and skin delivery studies of related ultradeformable vesicles: Transfersomes, ethosomes, and transethosomes. Int J Nanomedicine 2015; 10: 5837-51.
27. Nagalakshmi S, Sandeep T and Shanmuganathan S: Fabrication of Vesicular Drug Delivery of Niosomal Topical Formulation for the Effective Treatment of Osteoarthritis. Nano Hybrids Compos 2017; 12: 1-8.
28. Goindi S, Kumar G, Kumar N and Kaur A: Development of Novel Elastic Vesicle-Based Topical Formulation of Cetirizine Dihydrochloride for Treatment of Atopic Dermatitis. AAPS Pharm Sci Tech 2013; 14: 1284-93.
29. Ubaid M, Ilyas S, Mir S, Khan AK, Rashid R and Khan MZU: Formulation and *in-vitro* evaluation of carbopol 934-based modified clotrimazole gel for topical application. An Acad Bras Cienc 2016; 88: 2303-17.
30. Arulmozhi DK: Effect of *Sapindus trifoliatus* on hyperalgesic in vivo migraine models. 2005; 38: 469-75.
31. Marwah H, Garg T, Rath G and Goyal AK: Development of transferosomal gel for trans-dermal delivery of insulin using iodine complex. Drug Deliv 2016; 23.
32. El-Maghraby GMM, Williams AC and Barry BW: Skin delivery of oestradiol from lipid vesicles: Importance of liposome structure. Int J Pharm 2000; 204: 159-69.
33. Touitou E, Dayan N, Bergelson L, Godin B and Eliaz M: Ethosomes - Novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. J Control Release 2000; 65: 403-18.
34. Arzani G, Haeri A, Daeihamed M, Bakhtiari-Kaboutaraki H and Dadashzadeh S: Niosomal carriers enhance oral bioavailability of carvedilol: Effects of bile salt-enriched vesicles and carrier surface charge. Int J Nanomedicine 2015; 10: 4797-813.
35. Guo F, Wang J, Ma M, Tan F and Li N: Skin targeted lipid vesicles as novel nano-carrier of ketoconazole: characterization, *in-vitro* and *in-vivo* evaluation. J Mater Sci Mater Med 2015; 26: 175.
36. Zheng Y, Ouyang WQ, Wei YP, Syed SF, Hao CS and Wang BZ: Effects of Carbopol® 934 proportion on nanoemulsion gel for topical and transdermal drug delivery: A skin permeation study. Int J Nanomedicine 2016; 11: 5971-87.
37. Dash S, Murthy PN, Nath L and Chowdhury P: Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm 2010; 67: 217-23.
38. Manuscript A, Avenue L and Razzaque MS: Antihyperalgesic Effects of Anti-Serotonergic Compounds on Serotonin- and Capsaicin-evoked Thermal Hyper-algesia in the Rat. Neuroscience 2013; 5: 91-7.
39. Gawade S: Acetic acid induced painful endogenous infliction in writhing test on mice. J Pharmacol Pharmacother 2012; 3: 348.

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