

Development and Characterization of Clopidogrel-loaded Ethosomal Transdermal Patch for Antiplatelet Effect

Tripti Shukla¹, Aakanksha Verma¹, Neeraj Upmanyu¹, Subhendu S. Mishra², Satish Shilpi³

¹Department of Pharmacy, School of Pharmacy and Research, Peoples University Bhanpur, Bhopal, Madhya Pradesh, India, ²Department of Pharmaceutics, Gayatri College of Pharmacy, Gayatri Vihar, Jamadharpali, Sambalpur, Odisha, India, ³Department of Pharmaceutics, Ravishankar College of Pharmacy, Bhanpur, Bhopal, Madhya Pradesh, India

Abstract

Aim: Clopidogrel is an oral, thienopyridine-class antiplatelet agent used to inhibit blood clots in the condition of different artery and vascular disease and prevent the chances of myocardial infarction and stroke. Since oral bioavailability of clopidogrel is poor due to high first-pass metabolism (only 15% metabolite in active form), to increase the bioavailability, different matrix-type transdermal patches containing clopidogrel-loaded ethosomes were formulated in this work to release and absorb the drugs direct systemic circulation. **Materials and Methods:** Ethosomes prepared by hot method were characterized for size, surface charge, and entrapment efficiency. **Result and Discussion:** Ethosomes were characterized for size, surface charge, and entrapment efficiency which were 112.87 ± 1.90 nm, -17.4 ± 1.02 , and $71.48 \pm 4.23\%$, respectively. The polymers selected for preparation of ethosomes containing transdermal patches were polyvinyl alcohol, polyvinylpyrrolidone, and polyethylene glycol-4000 in different concentration ratios. The patches were evaluated for thickness, weight uniformity, drug content, moisture content, and uptake and found a significant result. Transmission electron microscopy analysis shows that the prepared ethosomes have the suitable diameter and spherical in shape. All the films were found to be stable at room temperature and accelerated temperature condition in respect to their physical parameters and drug content. *In vitro* drug release study after 72 h was found to be $57.39\% \pm 0.17\%$. **Conclusion:** This work may conclude that the prepared transdermal patches were stable and can be used to drug delivery application.

Key words: Clopidogrel, ethosome, transdermal patch

INTRODUCTION

The skin is one of the most extensive and readily accessible organs of the human body and provides a route of drug delivery which offers many advantages over conventional formulation including low plasma drug levels, avoidance of gastrointestinal disturbances and first-pass metabolism of the drugs, and high patient compliance. Transdermal drug delivery systems allow delivery of a drug into the systemic circulation via permeation through skin layers at a controlled rate. Along with these advantages, the skin poses a very good barrier to drug penetration and it is usually necessary to use enhancement techniques.^[1,2] Innovative research exploiting penetration-enhancing strategies such as iontophoresis, electroporation, microneedles,

and sonophoresis holds promise for the successful use of these drugs as consumer-friendly, transdermal dosage forms in clinical practice.^[1,3,4] Transdermal drug delivery has made a very important contribution to medical practice, but it has to fully achieve its potential as an alternative to oral delivery and hypodermic injections. Transdermal delivery shows an attractive alternative to oral delivery of drugs and provides an alternative to hypodermic injection.^[5]

Address for correspondence:

Subhendu S. Mishra, Department of Pharmaceutics, Gayatri College of Pharmacy, Gayatri Vihar, Jamadharpali, Sambalpur, Odisha - 768 200, India.
E-mail: sekhar.subhendu@gmail.com

Received: 02-06-2016

Revised: 18-09-2016

Accepted: 31-10-2016

One of the greatest disadvantages to transdermal drug delivery is the skin's low permeability that limits the number of drugs that can be delivered in this manner. The skin offers an excellent barrier to molecular transport as stratum corneum is the most formidable barrier to the passage of most of the drug.^[5-7]

Several researchers have developed novel elastic lipid vesicular systems to deeply and easily penetrate through the skin. Phospholipids, ethanol, bile salts, and many surfactants have been used to prepare these elastic vesicles. The high flexibility of vesicular membranes allows these elastic vesicles to squeeze themselves through the pores in stratum corneum, which are much smaller than their vesicular sizes.^[1,3,8,9] Ethosomes permeate by the skin layers more rapidly due to its ethanolic content interact with skin lipid followed by "ethosome effect," which includes interlipid penetration and permeation by the opening of new pathways and possesses significantly higher transdermal flux in comparison to conventional liposomes.^[6,10-13] Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from the immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration constant for longer periods of time.^[2,5,7,14-16]

Clopidogrel, an antiplatelet agent, is used to inhibit blood clots in a variety of conditions such as peripheral vascular disease, coronary artery disease, and cerebrovascular disease. The drug is an irreversible inhibitor of the P2Y₁₂ adenosine diphosphate receptor found on the membranes of platelet cells.^[11,17,18] Clopidogrel is approved for prevention of atherosclerotic events following recent myocardial infarction, stroke, or established peripheral arterial disease.^[19,20] It is also approved for use in acute coronary syndromes that are treated with either PCI or coronary artery bypass grafting. Clopidogrel use is associated with several serious adverse drug reactions such as severe neutropenia, various forms of hemorrhage, and cardiovascular edema. Clopidogrel generally available in tablet (75 mg, 300 mg) and on oral delivery leads to its metabolism by cyp-450 resulting in its active metabolite R-130964 and inactive metabolite. However, the percentage of inactive metabolite formed (85%) is more than that of its active metabolite (15%). Thus, to maintain proper loading dose, clopidogrel (75 mg) has to be taken frequently with 50% bioavailability. Orally clopidogrel bisulfate has a short elimination half-life (7-8 h).^[6,8,19,20]

To overcome the problem associated with drug, transdermal drug delivery system offers a potential tool which minimizes the adverse effect, minimizing the dosing frequency, and increases the bioavailability. In this work, clopidogrel-loaded ethosomes were prepared and then incorporated them in a transdermal patch and characterized for different parameter (Table 2). Transdermal patches work as a reservoir and sustained the release of drug-loaded ethosomes.^[11,17]

MATERIALS AND METHODS

Material

Clopidogrel bisulfate obtained as gift sample from Taj Pharmaceutical Ltd., Goregaon Mumbai. Phospholipids, propyleneglycol purchased from Himedia Laboratory, Mumbai. Ethanol, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), PEF-4000 purchased from CDH chemical Pvt. Ltd., New Delhi. Dialysis membrane of molecular weight cutoff 1200 was purchased from Himedia Laboratory, Mumbai. All other ingredients used were of analytical grade.

Method

The preparation of transdermal patches having clopidogrel bisulfate-loaded ethosomes was completed in two steps, first is the preparation of clopidogrel bisulfate-loaded ethosomes and second is the preparation of ethosomes containing transdermal patches.

Preparation of clopidogrel bisulfate-loaded ethosomes

Ethosomes were prepared by the method given by Touitou *et al.*, 2000, with slight modification in which drug was dissolved in methanol to give a concentration of 1.0% w/v of drug solution.^[15,16] From the above stock solution, 1 mL of were diluted with PBS buffer pH 7.4 up to 5 mL. The phospholipids were dispersed in water by heating in a water bath at 30°C until a colloidal solution was obtained. In a separate vessel, ethanol and propylene glycol were mixed and heated to 30°C. Once both mixtures reached a temperature of 30°C, the aqueous phase was added to the organic mixture with stirring at 700 rpm. Subsequent to mixing, stirring was continuous for a further 5 min. The temperature was maintained at 30°C during the entire process. The ethosomes prepared were subjected to sonication at 4°C in 3 cycles of 10 min with 5 min rest between the cycles. The obtained drug-loaded ethosomal formulation was stored at 4°C until further use.

Preparation of ethosomal transdermal patches

Ethosomes containing transdermal patch were prepared utilizing method given by Touitou *et al.*, 2001, with slight modification.^[16] The casting solution was prepared by dissolving weighed quantities of PVP (1-3% w/v) and PVA (0.5-1.5% w/v) in 10 mL of distilled water by heating on a water bath at 70°C. To the resulting solution, 2.5% w/v of polyethylene glycol (PEG)-4000 was added and then the solution was cooled. Then, required quantity of drug-loaded ethosomal formulation (equivalent to 0.75 % w/v) was added and mixed thoroughly to form a homogeneous mixture. The volume was made up to 6 mL with purified water. The casting solution was then poured into glass mold specially designed

to seize the contents. The glass mold containing the casting solution was dried at room temperature for 24 h in a vacuum oven. The patch was removed by peeling and cut into round shape of 1 cm². These patches were kept in desiccators for 2 days for further drying and enclosed in aluminum foil and then packed in self-sealing cover.

CHARACTERIZATION OF ETHOSOMES

Vesicle size

Microscopic analysis was performed to determine the average size of prepared ethosome. Ethosomal samples were diluted with distilled water to facilitate observance of the individual vesicle, and a drop of diluted suspension was taken on a glass slide covered with cover slip and examined under trinocular microscope ($\times 400$). The diameters of 150 vesicles were randomly determined using calibrated ocular and stage micrometer. The average diameter was calculated using the following formula.^[18]

$$\text{Average diameter} = \frac{\sum n \cdot d}{\sum n}$$

where, n = Number of vesicles; d = Diameter of the vesicles.

Surface charge

Surface charge measurement of the ethosomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowski from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with a field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50l S/cm.^[19]

Transmission electron microscopy (TEM)

The shape morphology of selected ethosomal formulation was evaluated by the TEM (TEM; Philips CM12 Electron Microscope, Eindhoven, Netherlands) from Punjab Technical University, Chandigarh, at an acceleration voltage of 20 kV being used to visualize ethosomes. A drop of the sample was placed onto a carbon-coated copper grid to leave a thin film. Before the film dried on the grid, it was negatively stained with 1% phosphotungstic acid. A drop of the staining solution was added onto the film, and the excess of the solution was drained off with a filter paper. The grid was allowed to air dry thoroughly, and samples were viewed in a TEM. TEM images of ethosomes were taken which are presented in Figure 1.^[17]

Entrapment efficiency

Entrapment efficiency of clopidogrel bisulfate in ethosomal formulation was determined using the Sephadex G-50

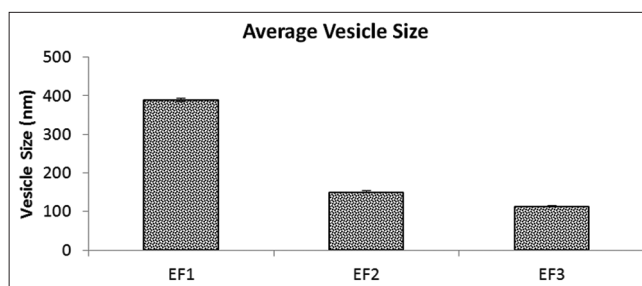


Figure 1: Average vesicle size (in negative value) of different ethosomal formulation

column. The weighed amount of Sephadex G-50 was properly mixed with sufficient amount of distilled water in a beaker and kept for 24 h for complete swelling. After complete swelling, Sephadex dispersion was placed in a 1 mL capacity of PVC syringe (Dispovan) packed with glass wool and a small piece of Whatman filter paper at the bottom end to provide stability for the Sephadex column at 3000 rpm. The entrapment efficiency of clopidogrel bisulfate in ethosomal vesicle was determined by ultracentrifugation, 10 mL of ethosomal formulation was passed from the column. The amount of drug not entrapped in the ethosomes was determined by passing the formulation from the Sephadex column, centrifuging at 3000 rpm, and collecting the elution using the equation given below. After removing the un-entrapped drugs, the ethosomes were collected and lysed using 1% Triton X100 and then centrifuged. The supernatant layer was separated, diluted with water suitably, and drug concentration was determined at 239 nm using UV spectrophotometer.^[20]

$$\% \text{ Entrapment efficiency} = \frac{\text{Drug content} - (\text{Theoretical} - \text{Practical})}{\text{Theoretical drug content}} \times 100$$

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter (Jyoti laboratories). Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7, and pH 9. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then, pH of selected formulation was measured, and readings shown on display were noted.

Characterization of ethosomal transdermal patch

Tensile strength

The tensile strength of the patch was evaluated using the tensiometer (Erection and Instrumentation, Ahmedabad). It consists of two load cell grips. The lower one was fixed, and upper one was movable. Film strips with dimensions of 2 cm \times 2 cm were fixed between these cell grips, and force was gradually applied till the film broke. The tensile strength was taken directly from the dial reading in kg.

Thickness

Patch thickness was measured using digital micrometer screw gauge at three different places, and the mean value was calculated.

Uniformity of weight

The weights of randomly selected patches prepared in batches were determined. The value reported must be the mean of three sets of experiments.

Percent moisture content

The films were weighed individually and kept in desiccators containing calcium chloride at room temperature for at least 24 h. Film was weighed again; the difference in weight (initial and final weight) gives moisture content.

$$\% \text{ Moisture content} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Percent moisture uptake

The films were weighed individually and kept in desiccator containing calcium chloride at room temperature for at least 24 h. The films were removed from desiccators and exposed to 4% relative humidity using a saturated solution of potassium chloride in another desiccator until a constant weight is achieved.

$$\% \text{ Moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Final weight}} \times 100$$

Percent drug entrapment study

To assess the total drug present in ethosomal patch and to find out drug entrapped in stored, vesicular system study was carried out for four weeks. Samples were withdrawn at an interval of 2 weeks and study was performed as mentioned earlier in the thesis under the drug entrapment efficiency by ultracentrifugation.

In vitro drug diffusion study

The *in vitro* diffusion study is carried out using Franz Diffusion Cell. Egg membrane is taken as a semi-permeable membrane for diffusion. The Franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 cm². The egg membrane is mounted between the donor and the receptor compartment. A 2 cm² size patch was taken and weighed, then placed on one side of the membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 37°C ± 0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon-coated magnetic bead which is placed in the diffusion cell.

During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each occasion. The samples withdrawn are analyzed spectrophotometrically at a wavelength of the drug.

Stability studies

Stability study was carried out for clopidogrel bisulfate ethosomal patch at two different temperatures, i.e., refrigeration temperature (4°C ± 2°C) and at room temperature (25-28°C ± 2°C), for 4 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any sort of interaction between the ethosomal patch and glass of container, which may affect the observations. The ethosomal patches were analyzed for any physical change such as color and appearance, entrapment study, and drug content.

RESULT AND DISCUSSIONS**Vesicle size and shape analysis**

Prepared formulations were optimized on basis vesicle size, shape, surface charge, and entrapment efficiency. Vesicle size of ethosomes was examined under trinocular microscope (×400) and found that average vesicle size of optimized formulation EF3 was 112.87±1.90 nm [Table 1 and Figure 1]. The vesicles size and size distribution were determined by dynamic light scattering method (Malvern Zetasizer, ZEM 5002, and UK).

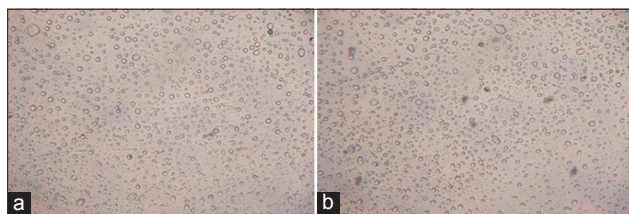
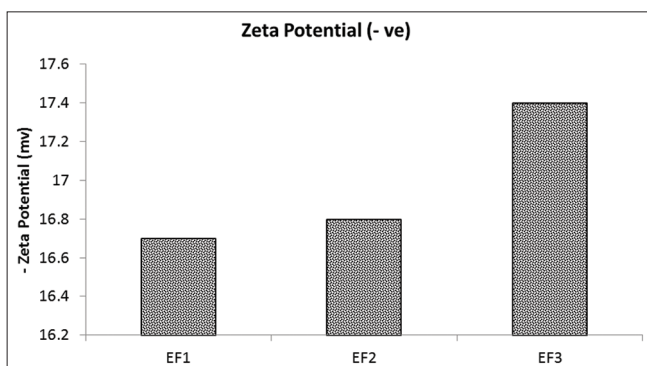
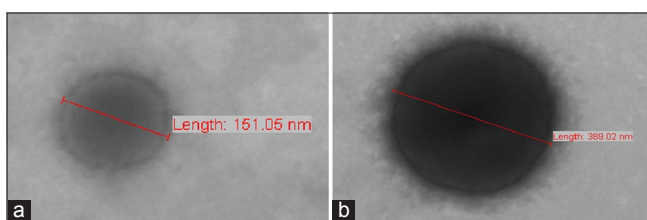
Formation of multi-lamellar vesicles was confirmed by examining the ethosomal dispersion under a trinocular microscope (×400). The examination of prepared formulations revealed the predominance of spherical-shaped vesicles. The vesicles were uniform in size and appeared to be multilayered. Visual observation of ethosomes confirmed the multi-lamellar vesicular structure; this confirms the existence of vesicular structure in ethosomes in spite of having high concentrations of ethanol. Photomicrographs were taken using video camera connected to the microscope with the help of computer installed with special software proved the multi-lamellar vesicular structure and spherical shape of ethosomes loaded with clopidogrel bisulfate [Figure 2a and b]. The clopidogrel bisulfate-loaded ethosomal formulation EF3 with 20% ethanol and 4% phospholipids is shown in Figure 2a and b has an average diameter of 112.87 ± 1.90 nm.^[15]

Surface charge

Surface charge ethosomal formulations were measured Malvern zetasizer (ZEM 5002, and UK) with a field strength of 20 V/cm on a large bore measures cell and surface charge of optimized formulation (EF3) was -17.4 mV and electrophoretic mobility mean was found to be -0.000135

Table 1: Preparation of ethosomes and their characterization

Formulation code	Phospholipid (% w/v)	Ethanol (% w/v)	Drug (% w/v)	Average particle size (nm)	% entrapment efficiency	Zeta potential (mV)
EF1	2	8	1	389.02±4.23	27.51±4.23	-16.7±0.53
EF2	3	7	1	151.05±3.12	51.03±4.23	-16.8±1.01
EF3	4	6	1	112.87±1.90	71.48±4.23	-17.4±1.02

**Figure 2:** (a and b) Optical microscopy of ethosomes using trinocular microscope at ×400**Figure 3:** Zeta potential (in negative value) of different ethosomal formulation**Figure 4:** Transmission electron microscopy image of ethosome (a) Without drug-loaded ethosome (b) Drug-loaded ethosome

cm² VS which revealed that formulation will be stable on administration to the body [Figure 3].

TEM

The results of TEM analysis show that the prepared ethosomes having spherical shape and the diameter of different ethosomal formulation (EF3) with and without drug were observed about 151.05 nm and 389.02 nm, respectively [Figure 4a and 4b]. TEM photomicrograph showing ethosomes were spherical in shape and in multi-layer vesicles form. This study result revealed that the ethosomal preparation can easily cross the

barriers of body ethanol facilitating the permeation of the ethosomes across the membranes.^[17,20]

Entrapment efficiency

Size and entrapment efficiency are always considered as parameters for optimizing vesicular formulations. Once the presence of bilayers of vesicle was confirmed in ethosomal system, the ability of vesicle for entrapment of drug was investigated using ultracentrifugation method. Using ultracentrifugation method, ethosomal vesicles containing drug and un-entrapped or free drug were separated to find out the entrapment efficiency. Results obtained are given in Table 1. From the data obtained, the maximum entrapment efficiency of ethosomal vesicle as determined by ultracentrifugation was 71.48% ± 0.89% for the EF3 formulation containing 20% ethanol concentration. This was much higher than ethosomal formulation EF2 with 15% ethanol. As the ethanol concentration increased from 10% to 20%, there was an improvement in entrapment efficiency [Figure 5]. The formulation EF1 with 10% ethanol concentration was having entrapment efficiency of 27.51% ± 0.67%, beyond 20% ethanol concentration; the entrapment efficiency was found to be at optimum level.^[15-17]

Evaluation of ethosomal patch

Physical evaluation

The evaluation of physical properties of prepared transdermal patches is given in Table 3. The thickness of the films was 0.67 ± 0.01, 0.80 ± 0.02, and 0.83 ± 0.01 mm for formulation EF3P1, EF3P2, and EF3P3, respectively, which revealed that no significant changes in thickness of patch. The thickness of prepared ethosomal patch was uniform throughout the distribution which confirms uniform dispersion of ethosomes in prepared patch and uniformity of thickness was also indicates the uniformity of weight of patches throughout its length. The folding endurance for formulation EF3P1, EF3P2, and EF3P3 was 192.2 ± 4.1, 198.4 ± 5.4, and 209.7 ± 6.7, respectively. The tensile strength of the patches was 3.4 ± 0.7 kg/cm, 3.7 ± 0.3 kg/cm, and 4.3 ± 0.5 kg/cm for EF3P1, EF3P2, and EF3P3, respectively. Good tensile strength and folding endurance revealed that patches would be stable and not break on stress and pressure condition and maintain their integrity during application. Data of percentage moisture absorption indicate that the formulation EF3P3 has shown maximum (18.7 ± 1.4%) absorption than

Table 2: Preparation of ethosomal transdermal patches

Formulation code	PVP (%w/v)	PVA (%w/v)	PEG-4000 (% w/v)	Drug/Ethosomes equivalent to
EF3P1	1	0.5	2.5	0.75
EF3P2	2	1	2.5	0.75
EF3P3	3	1.5	2.5	0.75

PEG: Polyethylene glycol, PVP: Polyvinylpyrrolidone, PVA: Polyvinyl alcohol

Table 3: Physical characterization of transdermal patches

Formulation code	Folding endurance	Tensile strength (kg/cm)	Moisture content (%)	% Moisture absorption	Thickness (mm)
EF3P1	192.2±4.1	3.4±0.7	1.3±0.2	11.8±1.5	0.67±0.01
EF3P2	198.4±5.4	3.7±0.3	1.8±0.3	15.6±1.3	0.80±0.02
EF3P3	209.7±6.7	4.3±0.5	2.1±0.6	18.7±1.4	0.83±0.01

Values are represented as mean±SD (n=3). SD: Standard deviation

the other formulations. Formulation EF3P3 has shown 18.7 ± 1.4 of moisture content.

Stability studies

Ethosomal patch preparations were observed for any change in appearance or color for the period of 4 weeks. There was no change in appearance in ethosomal formulation throughout the period of study. The stability of drug was further confirmed by spectral data, and there was no change observed.

In vitro drug release study

The objectives in the developments of *in vitro* diffusion study are to show the release rates and extent of drug release from the dosage form. The study was carried out for 72 h duration which was represented graphically in Figure 6. For the formulation EF3P3 with 3% w/v of PVP, the percentage drug release after 24 h was only 34.96% ± 2.04%, which was increased to 57.39% ± 0.17% after 72 h. The percentage drug release from EF3P1 and EF3P2 was 67.32±3.01% and 62.21% ± 2.92%, respectively, after 72 h. The reason of more sustained release of drug from EF3P3 in comparison of EF3P1 and EF3P2 was the increasing of concentration of PVP. The control sample in which film contained only plain drug (without ethosomes) shows more than 60 % drug release after 24 h which was approximately 50% of more drug release in comparison of EF3P3. The formulation EF3P1, EF3P2, and EF3P3 shows matrix diffusion Higuchi release kinetic model.

CONCLUSION

Thin, flexible, smooth, and transparent films were obtained with PVP, PVA, and PEG-4000 using propylene glycol as

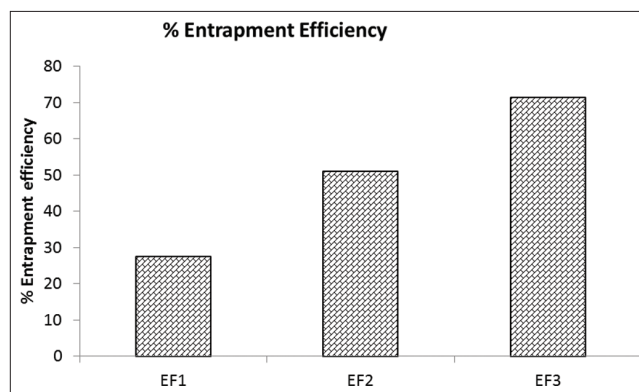


Figure 5: Percent drug entrapment efficiency of different ethosomal formulation. Values are represented as mean ± standard deviation (n=6)

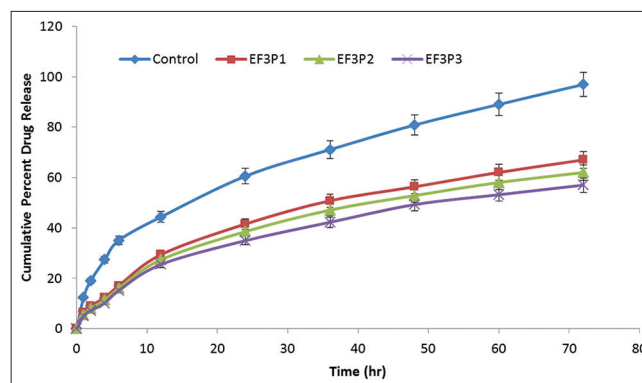


Figure 6: Cumulative percent drug release from the ethosomal patch. Values are represented as mean ± standard deviation (n=6)

plasticizers. The thickness of all the formulations remained uniform with low SD values. Ethosomal formulations shown significant drug encapsulation and obtained in suitable size and surface morphology as well as shows good surface charge which makes ethosomes for safe and effective delivery of selected antiplatelet drug. All the systems showed

good release pattern. The systems were found to be stable at different accelerated temperature condition. Formulation characterization studies have shown promising results and confirm that the ethosomal-based formulation can be used for the delivery of clopidogrel for antiplatelet effect and make possibility to further pharmacodynamics and pharmacokinetic evaluation.

ACKNOWLEDGMENTS

One of the authors want to acknowledge Taj Pharmaceutical Ltd to provide Clopidogrel bisulfate as gift sample.

REFERENCES

- Bhatia A, Kumar R, Katare OP. Tamoxifen in topical liposomes: Development, characterization and *in-vitro* evaluation. *J Pharm Pharm Sci* 2004;7:252-9.
- Chein YW. *Advances in transdermal systemic drug delivery*. New York: Marcel Dekker Inc.; 1988. p. 343-62.
- Chein YW. *Novel Drug Delivery Systems*. New York: Marcel Dekker Inc.; 1992. p. 301-80.
- Darr D, Dunston S, Faust H, Pinnell S. Effectiveness of antioxidants (vitamin C and E) with and without sunscreens as topical photoprotectants. *Acta Derm Venereol* 1996;76:264-8.
- Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: Ethosomes vs. Liposomes. *Biomaterials* 2000;21:1879-85.
- Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. *J Control Release* 2004;94:365-79.
- Jain NK. *Advances in Controlled and Novel Drug Delivery*. 1st ed. New Delhi: CBS Publication; 2001. p. 428-51.
- Jain N, Talegonkar S, Jain NK. New ways to enter the blood stream: Emerging strategies in transdermal drug delivery. *Pharm Rev* 2004:41-60.
- Jain S, Bhandra D, Jain S, Jain NK. Transfersomes - A novel carrier for effective transdermal drug delivery. *Controlled and Novel Drug Delivery*. 1st ed. New Delhi: CBS Publishers and Distributors; 1997. p. 426-51.
- Jain S, Maheshwari RB, Bhandra D, Jain NK. Ethosomes: A novel vesicular carrier for enhanced transdermal delivery of an anti HIV agent. *Indian J Pharm Sci* 2004;66:72-81.
- Khandare JN, Jiwandas BH, Uppal RR. Preparation and evaluation of nimesulide niosomes for topical delivery. *Indian Drug* 2001;38:197-202.
- Qvist MH, Hoeck U, Kreilgaard B, Madsen F, Hovgaard L, Frokjaer S. Application of confocal laser scanning microscopy in characterization of chemical enhancers in drug-in-adhesive transdermal patches. *AAPS Pharm Sci*, 2002;4:11-18.
- Pérez-Cullell N, Coderch L, de la Maza A, Parra JL, Estelrich J. Influence of the fluidity of liposome compositions on percutaneous absorption. *Drug Deliv* 2000;7:7-13.
- Swarbrick J, Boylan JC. *Encyclopedia of Pharmaceutical Technology*. Vol. 15. New York: Marcel Dekker Inc.; 1997. p. 253-92.
- 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.
- Touitou E, Godin B, Weirs C. Enhanced delivery into and across the skin by ethosomal carries. *Drug Dev Res* 2000;50:406-15.
- Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A, Levi-Schaffer F. Intracellular delivery mediated by an ethosomal carrier. *Biomaterials* 2001;22:3053-9.
- Touitou E. Composition of applying active substance to or through the skin, US patent, 5, 716, 638, 1996.
- Venkatararam S, Awni WM, Jordan K, Rahman YE. Pharmacokinetics of two alternative dosage forms for cyclosporine: Liposomes and intralipid. *J Pharm Sci* 1990;79:216-9.
- Vyas SP, Khar RK. *Controlled Drug Delivery Concepts and Advances*. 1st ed. New Delhi: Vallabh Prakashan; 2002. p. 173-243.

Source of Support: Nil. **Conflict of Interest:** The authors report no conflict of Interest. The authors alone are responsible for the content and writing of the paper.