

Research Article

Perindopril Erbumine Loaded Ethosomes: Design and in Vitro Characterization

Prakash Goudanavar^{1*}, Manjunatha¹, Doddayya Hiremath¹, Santosh Fattepur²

¹Department of Pharmaceutics, N.E.T Pharmacy College, Raichur, Karnataka, India.

² Department of Pharmacology, Management and Science University Malaysia.

***Corresponding author**

Prakash Goudanavar

Email: pgoudanavar01@gmail.com

Abstract: The present work deals with the preparation of Perindopril erbumine ethosomes and study of effect of alcohol and phospholipid on transdermal delivery. Perindopril erbumine is ACE inhibitor which slowly inhibits the activity of the enzyme ACE, which decreases the production of angiotensin II. As a result, the blood vessels enlarge or dilate, and blood pressure is reduced. Perindopril erbumine loaded ethosomes were prepared by hot method by using different concentrations of Alcohol and Soya lecthin in different ratios and propylene glycol. The prepared ethosomal formulations were evaluated for Vesicle size analysis, Morphological studies, Entrapment efficiency, In vitro release, Stability studies, In vitro permeation study and kinetic data analysis. The vesicle size of ethosomes varied between $1.62 \pm 1.31 \mu\text{m}$ to $4.56 \pm 0.08 \mu\text{m}$. Entrapment efficiency between 54.81 ± 0.30 to $78.04 \pm 0.30\%$. FT-IR, DSC and Zeta potential studies revealed the integrity of the drug in the formulations. The cumulative percentage drug release from ethosomal formulations ETH₁ to ETH₆ was in the range of 71.52% to 85.75%. and for LPH it was $50.20 \pm 0.23\%$. The in vitro permeation across rat abdominal skin for the optimized formulations ETH₃ and ETH₆ after 24 hrs was found to be 75.46% and 80.24% respectively. Stability studies indicated that, the prepared ethosomes remained stable at refrigeration (4-8°C) and room (25±2°C) temperature. The prepared ethosomes showed promising results under in vitro conditions.

Keywords: Ethosomes, perindopril erbumine, in vitro permeation, stability studies.

INTRODUCTION

Hypertension (HTN) or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated above the normal value i.e. (140/90). The antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure). There are many classes of antihypertensives, which lower blood pressure by different means; among the most important and most widely used are the thiazide diuretics, the ACE inhibitors, the calcium channel blockers, the beta blockers, and the angiotensin II receptor antagonists [1].

Perindopril Erbumine (Perindopril tert-butylamine) is an ACE inhibitor, used in the treatment of hypertension and congestive heart failure, perindopril is converted in the body into active metabolite perindoprilate, Perindopril erbumine shows 65-75% bioavailability but presence of food reduces the conversion of perindopril to the perindoprilate. According to a previous research, the oxidation rate of Perindopril erbumine in dermal homogenate is significantly lower than the intestinal homogenate because the oxidative product of Perindopril erbumine a perindoprilate shows poor absorption from the intestine [2]. Perindopril erbumine when administered initially

causes hypotension, which can prove to be harmful in diuretic treated and congestive heart failure patients. Persistent hypotension may cause some trouble in myocardial infarction patients [3]. Therefore, the use of transdermal drug delivery system can reduce the side effects associated with Perindopril erbumine. Ethanolic Liposomal carriers, well known for their potential in topical drug delivery, have been used to transport perindopril erbumine molecule in the skin layer.

Skin acts as a major target as well as a principal barrier for topical/transdermal drug delivery. Despite the many advantages of this system, the major obstacle is the low diffusion rate of drugs across the stratum corneum. Several methods have been tried to increase the permeation rate of drugs temporarily. One simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers. Ethosomes have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier.

Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline; PC), ethanol at relatively high concentration and water. Ethosomes penetrate the skin

and allow enhanced delivery of various compounds to the deep strata of the skin or to the systemic circulation [4].

EXPERIMENTAL DETAILS

MATERIALS

Perindopril Erbumine obtained as gift sample from Glenmark Pharmaceuticals Pvt. Ltd, Goa. Cholesterol, Soya lecithin, propylene Glycol and Alcohol were procured from S.D Fine Chemicals Pvt Ltd, Mumbai.

METHODS

Preparation of Perindopril erubumine ethosomes:

The ethosomal system of perindopril erubumine prepared were comprised of 1.5-2.5 % phospholipids, 20-40 % ethanol, 0.4 % of perindopril erubumine and aqueous phase to 100 % w/w. Phospholipid and drug were dissolved in ethanol. In this solution double distilled water was added slowly in a fine stream with constant mixing at 700 rpm in a closed vessel. The temperature was kept 30°C throughout the experiment. The mixing was continued for addition of five minutes. The preparation was stored at 4°C [5, 6].

Preparation of Perindopril erubumine liposomes:

Liposomes were prepared by cast film method. Soya phospholipid (2 % w/w) and cholesterol (0.15 % w/w) were dissolved in minimum quantity of chloroform in a round bottom flask. The organic solvent was removed under reduced pressure to form a thin film on the wall of the flask. The deposited lipid film was hydrated with distilled water containing drug (25 ml) by mechanical shaker for 1hour at room temperature 4°C.

Characterization of ethosomes and liposomes:

Size and shape analysis:

Microscopic analysis was performed to determine the average size of ethosomes and liposome. A sample of ethosomes was suitably diluted with distilled water in order to observe individual vesicle and a drop of diluted suspension was put on a glass slide covered with cover slip and examined under microscope (magnification $15 \times 45 X$). The diameters of 150 vesicles were determined randomly using calibrated eyepiece micrometer with stage micrometer.

The average diameter was calculated using the formula,

$$\text{Average diameter (dav)} = \frac{\sum d}{n}$$

Where, n = number of vesicles
d = diameter of the vesicles

Further analyses of sonicated vesicles were done under a special microscope, which is connected with software, and photomicrographs were taken under 400 and 800 magnification. Selected photomicrographs were analyzed for size analysis by using special

software "particle size analysis" developed by BIOVIS. This special software works on images of photomicrographs with standard dimension [7].

Surface Morphological study:

The morphology of vesicles derived from ethosomal preparation was studied using Scanning Electron Microcopy. SEM revealed that the vesicles formed were spherical, smooth, and there was no formation of aggregates.

Entrapment efficiency:

The entrapment efficiency of Perindopril erbumine by ethosomal vesicle were determined by ultracentrifugation, 10ml of ethosomal and liposomal formulation were vortexed for 2 cycles of 5 min with 2 minutes rest between the cycles. 1.5ml of each vortexed sample and fresh untreated ethosomal formulations were taken into different centrifugal tubes. These samples were centrifuged at 20,000 rpm for 3 hours. The supernatant layer was separated, diluted with water suitably and drug concentration was determined at 206 nm in both vortexed and unvortexed samples.

The entrapment efficiency was calculated as follows,

$$\% \text{ Entrapment Efficiency} = \left[\frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \right] \times 100$$

Zeta potential:

Zeta potential is an important and useful indicator of particle surface charge, which can be used to predict and control the stability. In general, particles could be dispersed stably when the absolute value of zeta potential is upto 30mV due to the electric repulsion between particles [8].

In Vitro Release:

In vitro release studies on ethosomal preparation were performed using Franz-diffusion cell. The capacity of receptor compartment was 15 ml. The area of donor compartment exposed to receptor compartment was 1.43cm². The dialysis cellophane membrane (MMCO14KDC) was mounted between the donor and receptor compartment. A weighed amount of ethosomal preparation was placed on one side of the dialysis membrane. The receptor medium was phosphate saline buffer of pH 6.8. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1°C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically at 206 nm.

In vitro permeation studies:

The permeation of Perindopril erbumine from ethosomal formulations was determined by using Franz diffusion cell. The shaved abdominal skin of mice (0.5 ± 0.1 mm thickness and 3.17 cm^2 exposed surface areas) was mounted on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment. The receptor compartment was filled with 15 ml of pH 6.8 phosphate buffer maintained at 37.8°C and stirred by a magnetic bar at 600 rpm. One ml of ethosomal formulation was placed on the skin and the top of the diffusion cell was covered with paraffin paper. At appropriate time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h), 1 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain sink conditions samples withdrawn were analyzed spectrophotometrically at 206 nm [9].

Stability Studies:

The ability of vesicles to retain the drug (Drug Retention Behaviour) was assessed by keeping the ethosomal formulations at two different temperature conditions, i.e., Refrigeration Temperature ($4-8^\circ\text{C}$) & Room Temperature ($25 \pm 2^\circ\text{C}$). Throughout the study, ethosomal formulations were stored in aluminium foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of 8 weeks and were analysed for entrapment efficiency, drug content and *in vitro* release [10, 11].

RESULTS AND DISCUSSION

Vesicle size analysis:

Results of Vesicle size of perindopril erbumine ethosomal formulations are presented in Table 2, which indicated that Vesicle formed with 40% alcohol are smaller in size than vesicle formed with 20% alcohol this is due to increase in the alcohol content. It is indicated that increase in alcohol content as well as decreased in the concentration of the phospholipid content resulting in smaller vesicle size. Size of vesicle was reduced when dispersion was sonicated. The reason for this is attributed to increase in alcohol concentration reduces the strength of vesicular layer due to the perforation which results in breakage of larger vesicles to smaller vesicles. The size range was found to be $1.62 \pm 1.31 \mu\text{m}$ to $4.56 \pm 0.08 \mu\text{m}$. Vesicle size of liposomal formulation was found to be $5.21 \pm 0.02 \mu\text{m}$.

Alcohol used in ethosomes has a great effect on vesicle size. Vesicles formed from alcohols are of different size and they follow the order of $20\% > 30\% > 40\%$.

Surface morphological studies:

Surface morphological studies mainly done with the help of scanning electron microscopy (SEM) which indicated that vesicle formed in ethosomal formulation was spherical, rounded, smooth and there was no formation of any aggregates.

Zeta potential:

Zeta potential of optimised formulation obtained from Malvern Instruments Ltd. by using Zetasizer instrument was found to be 18.4mV. Thus the higher surface charge indicates there is no aggregation between the particles.

Entrapment efficiency (%):

Vesicle entrapment efficiency mainly dependent on the amount of phospholipid forming the bilayers and intrinsic properties of chemical structure, lipophilicity, phase transition temperature, alkyl chain length and alcohol concentration. It was found that phospholipid content which having higher lipophilicity, higher phase transition temperature and longer alkyl chain length shows higher entrapment. Thus depending upon these properties ethosomal formulations prepared with 2-3% of phospholipid and 30% alcohol shows higher entrapment efficiency than other formulations. The entrapment efficiency of formulations with 1-2% phospholipid and more than 30% of alcohol shows less than those of 2-3% of phospholipid and 30% of alcohol. This is due to reason that not uniform vesicle formation and more permeation of vesicle layer due to increased alcohol concentration. Values for entrapment efficiency were ranging from 54.81 ± 0.30 to 78.04 ± 0.30 (%) for different formulations.

In- vitro release study:

The perindopril erbumine ethosomal formulations were prepared by hot method incorporating phospholipid, alcohol and propylene glycol in different concentrations & in different ratios. In the later studies the effect of these phospholipid and alcohol on the *in vitro* release of the drug from different ethosomal formulations were carried out in phosphate buffer of pH 6.8 by using Franz diffusion cell.

The cumulative percentage drug release from ethosomal formulations ETH₁ to ETH₆ was in the range of 71.52% to 85.75%. and for LPH it was $50.20 \pm 0.23\%$. From the results obtained it was observed that ethosomes prepared with alcohol 30% and phospholipid 1.5-2.5% showed better release profile when compared to the ethosomes prepared with the same at different concentrations. This is due to fact that uniform vesicle formation with sufficient penetration through the skin.

In- vitro permeation study:

Permeation profile of perindopril erbumine from optimized ethosomal formulations ETH₃ and ETH₆ through the rat abdominal skin after 24 hrs is shown in (Fig.6). The value for drug permeation (release) for optimized formulation ETH₃ and ETH₆ through the rat abdominal skin after 24 hrs was found to be 75.46% and 80.24% which is significantly less as compared to drug permeated through cellophane membrane i.e. 80.97% and 85.75% respectively. The

reasons for this is that skin act as barrier for transport of drug across skin, fusion of ethosomal vesicle to surface of skin and interaction of ethosomal vesicle with surface of the skin.

Stability studies:

The stability studies were carried out for the optimized formulations (ETH₃, ETH₆ & LPH) at refrigeration temperature (4-8° C) and at room temperature (25±2° C) as per ICH guidelines. The optimized ethosomal formulation was evaluated for its appearance, entrapment efficiency (%), drug content study, *in vitro* drug release. No significant changes in the appearance, entrapment efficiency (%) and drug release study were observed during the stability study. The entrapment efficiency (%) of optimized formulations (ETH₃, ETH₆ & LPH) after stability studies

at refrigeration temperature and at room temperature was found to be 73.18±0.16%, 72.84±0.25%, 64.89±0.31%, 63.81±0.38% & 46.83±0.30%, 45.71±0.18% respectively. The *in vitro* drug release of optimised formulations (ETH₃, ETH₆ & LPH) after stability studies at refrigeration temperature and at room temperature was found to be 79.57±0.58, 78.65±0.23 & 84.61±0.31, 82.46±0.35 & 46.23±0.61, 45.22±0.48 respectively. Thus from the results it is found that no significant variations were observed in the entrapment efficiency (%) and *in vitro* release values when ethosomal formulations stored at refrigeration temperature than room temperature. This indicated that that ethosomal formulation remains fairly stable at refrigeration (4-8° C) temperature than room temperature (25±2° C).

Table 1: Composition of different unsonicated ethosomal and liposomal formulations

Formulation Code	Phospholipid (%w/w)	Ethanol (%w/w)	Propylene Glycol (%w/w)	Cholesterol (%w/w)	Drug (%w/w)	Distilled Water (%w/w)
ETH ₁	1.5	20	20	--	0.4	q.s
ETH ₂	2.5	20	20	--	0.4	q.s
ETH ₃	1.5	30	20	--	0.4	q.s
ETH ₄	2.5	30	20	--	0.4	q.s
ETH ₅	1.5	40	20	--	0.4	q.s
ETH ₆	2.5	40	20	--	0.4	q.s
LPH	2.0	--	20	0.15	0.4	q.s

Table 2: Physicochemical characterization of Perindopril erbumine ethosomal and liposomal formulations (ETH₁-ETH₆ & LPH)

Sr.No	Formulation Code	Vesicle Size(µm)	% Entrapment Efficiency
1	ETH ₁	2.86±0.04	62.45±0.24
2	ETH ₂	2.91±0.04	70.51±0.09
3	ETH ₃	2.44±0.02	73.67±0.30
4	ETH ₄	2.75±0.02	71.29±0.64
5	ETH ₅	1.75± 0.97	56.14±0.55
6	ETH ₆	2.06±0.02	65.26±0.26
7	LPH	5.21±0.02	49.07±1.56

Table 3: Size Distribution of Perindopril erbumine ethosomal formulation ETH₃

Size Range		Average Size(d)	No. of Vesicles (n)	% No. of Vesicles	n×d
Eye piece micrometer division	Size in micrometer				
0-1	0.00-3.00	1.50	106	70.66	159.00
1-2	3.00-6.00	4.50	41	27.33	184.50
2-3	6.00-9.00	7.50	03	2.00	22.50
3-4	9.00-12.00	11.50	00	00	00
4-5	12.00-15.00	13.50	00	00	00
Total			150		366.00

*Each value is an average of 3 replications.

nd

Average diameter (d_{av})= -----

n

= 2.84µm.

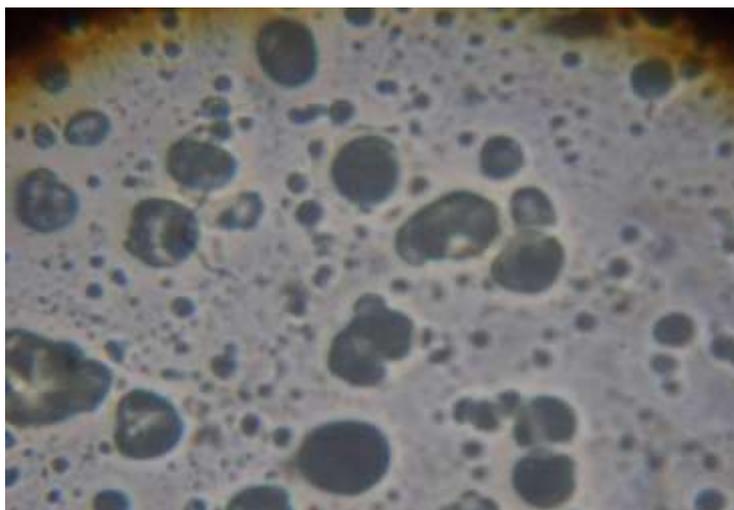


Fig- 1: Optical photomicrograph of optimized formulation of Perindopril erbumine ethomes (ETH₃)

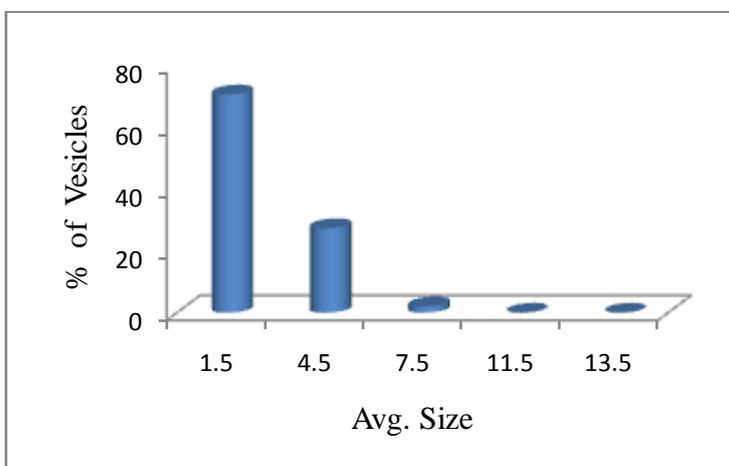


Fig- 2: Size distribution of ETH₃

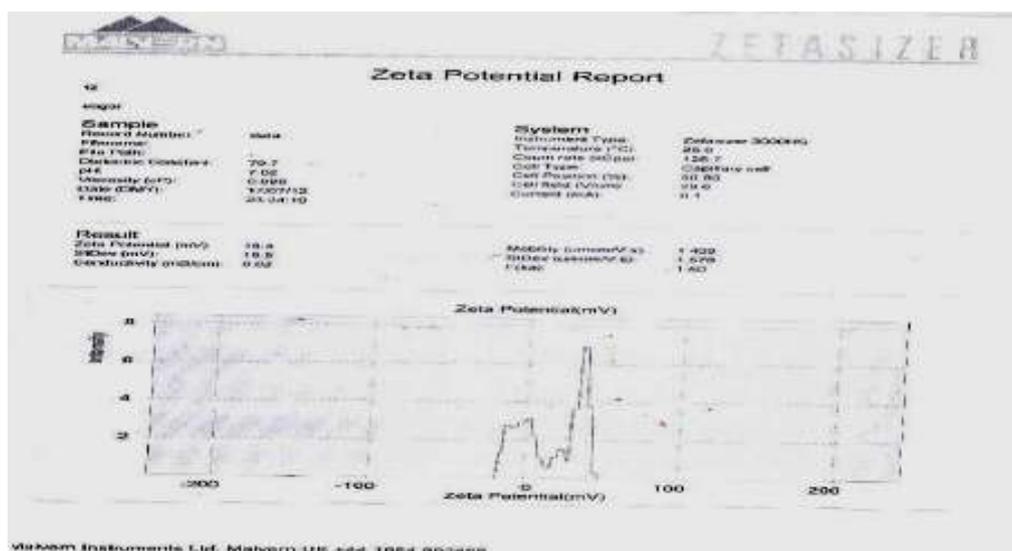


Fig-3: Zeta Potential of optimized formulation of Perindopril erbumine ethosomes. (ETH₃)

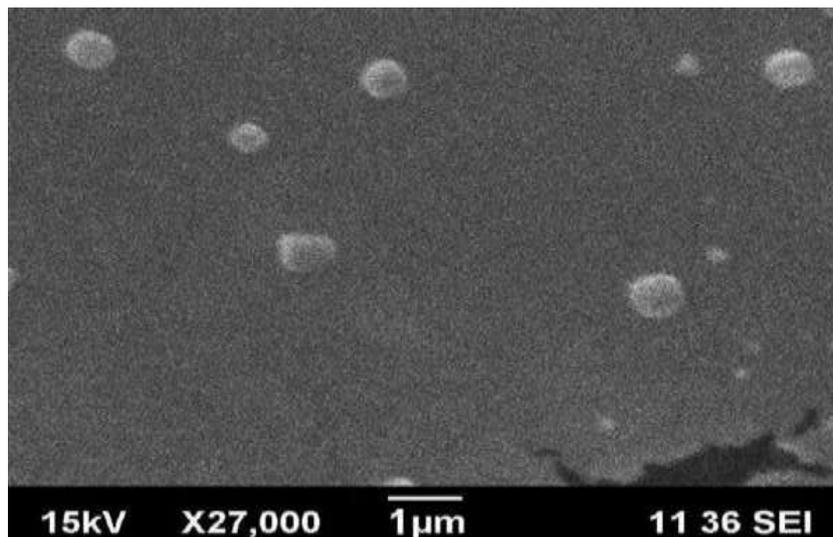


Fig-4. Scanning electron micrograph of optimized formulation of Perindopril erbumine ethosomes

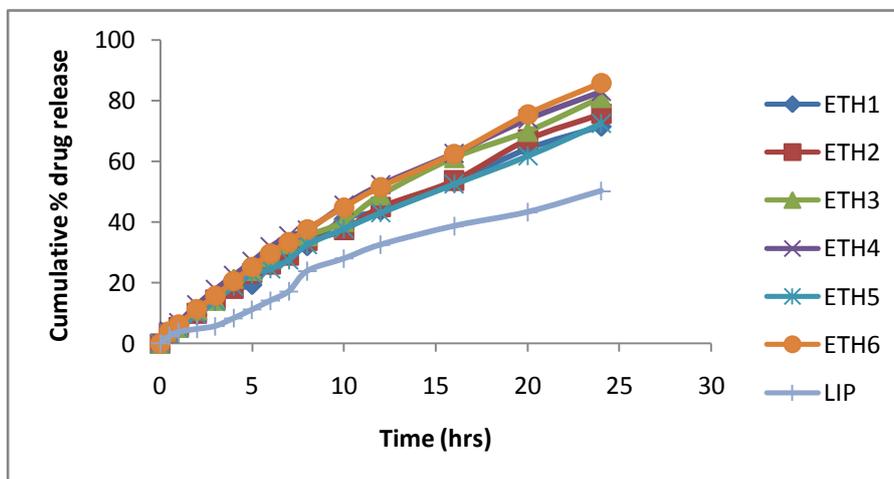


Fig-5: *In vitro* drug release of Perindopril erbumine from different ethosomal formulations.

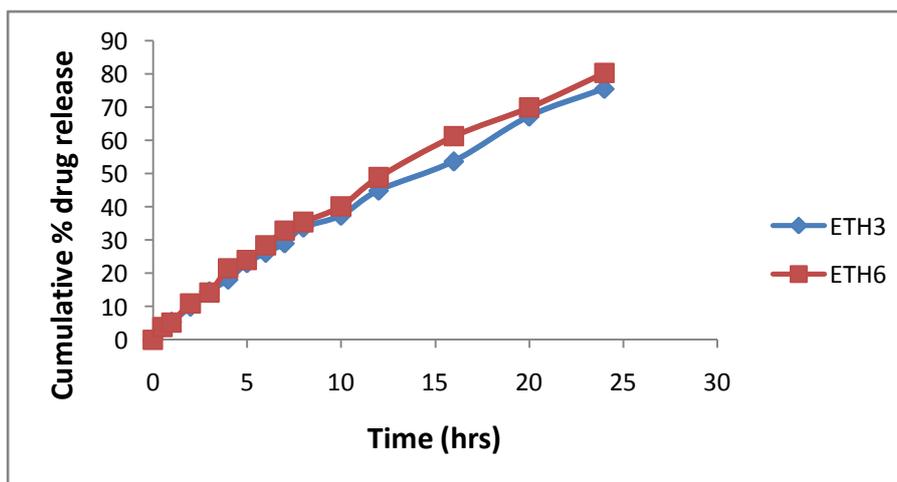


Fig-6: *In-vitro* skin permeation profile of perindopril erbumine from optimized ethosomal formulations. (ETH₃ & ETH₆)

CONCLUSION

Ethosomal formulations of Perindopril erbumine showed promising results under *in vitro* conditions and thus there exist a scope for

pharmacokinetic evaluation of the developed ethosomal formulations on suitable animal models.

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