ISSN 22846808

Open Access Journal

Letters in Applied NanoBioScience

Volume 3, Issue 1, 151-157, 2014

www.NanoBioLetters.com

DEVELOPMENT AND CHARACTARIZATION OF PERINDOPRIL ERBUMINE LOADED ETHANOLIC LIPOSOMES

Prakash Goudanavar[1*], Manjunatha[1], Doddayya Hiremath[1]

[1]*Department of Pharmaceutics, N.E.T. Pharmacy College, Raichur, Karnataka – 584103, India*

Article info

Received: 19.03.2014
Accepted: 29.03.2014
Published: 30.03.2014

Abstract

The present work describes the preparation of Perindopril erbumine ethosomes and study of effect of alcohol and phospholipid on transdermal delivery. Perindopril erbumine is an ACE inhibitor which slowly inhibits the activity of the enzyme ACE, which decreases the production of angiotensin II, is being involved in the blood pressure regulation. Perindopril erbumine loaded ethanolic Liposomes were prepared by an hot - cold method using different concentrations of Alcohol and Soya lecithin in different ratios and propylene glycol. The prepared ethosomal formulations were subjected to 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 1.96±0.003 to 4.56±0.008 µm (Without sonication) and from 1.62±1.31 to 1.99±1.02 µm (With sonication), Entrapment efficiency between 43.91±0.57 to 78.04±0.30%. FT-IR, DSC and Zetapotential studies revealed the integrity of the drug in the formulations. In vitro release profiles indicated that the highest % of drug release is 95.22±0.35 over period of 24 hrs with 30% alcohol & 2% phospholipid (ETH8) compared to other formulations. The in vitro permeation across rat abdominal skin for the optimized formulations ETH3 and ETH8 after 24 hrs was found to be 79.63% and 85.33% 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*

*Corresponding author e-mail address: **pgoudanavar01@gmail.com**

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. The antihypertensive are a class of drugs that are used to treat hypertension. There are many classes of antihypertensive, 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 perindroprilate. 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]. When administered initially Perindopril erbumine 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 corneum stratum. 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].

Experiment Details

Materials. Perindopril Erbumine was obtained as gift sample from GlenmarkPharmaceuticals 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 the prepared perndopril erubumine comprised of 1-4 % phospholipids, 20-40 % ethanol, 0.4 % of perndopril 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 to 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 Perndopril 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.

Table 1: *Composition of different unsonicated ethosomal and liposomal formulations*

Formulation Code	Phospholipid (%w/w)	Ethonal (%w/w)	Propylene Glycol (%w/w)	Cholesterol (%w/w)	Drug (%w/w)	Distilled Water (%w/w)
ETH_1	1.0	20	20	--	0.4	q.s
ETH_2	2.0	20	20	--	0.4	q.s
ETH_3	3.0	20	20	--	0.4	q.s
ETH_4	1.0	30	20	--	0.4	q.s
ETH_5	2.0	30	20	--	0.4	q.s
ETH_6	3.0	30	20	--	0.4	q.s
ETH_7	1.0	40	20	--	0.4	q.s
ETH_8	2.0	40	20	--	0.4	q.s
ETH_9	3.0	40	20	--	0.4	q.s
LPH	2.0	--	20	0.15	0.4	q.s

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 (magnification15 × 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: Average diameter (dav)= nd/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 800x magnification. Selected photomicrographs were analysed 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 efficency**. The entrapment efficiency of Perindopril erbumine by ethosomal vesicle were determined by ultracentrifugation, 10ml of ethosomal and liposomal formulation were vortexed for 2 cycle 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 centrifuge tubes. These samples were centrifuged at 20,000 rpm for 3 hours. The supernatant layer was separated, diluted with water and drug concentration was determined at 206 nm in both vortexed and unvortexed samples. The entrapment efficiency was calculated as follows, % Entrapment Efficiency= [Total drug- Free drug / Total drug)] x 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 up to 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±1oC. 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 [9]. ***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 cm2 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. 8C 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 and were analyzed spectrophotometrically at 206 nm[10]. **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 (4-80C) & Room Temperature (25±20C).

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 effiency, drug content and in vitro release [11-12].

Results and Discussions

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 and this is due to increase in the alcohol content.

Table 2: *Physicochemical charaterization of Perindopril erbumine ethosomal and liposomal formulations (ETH_1-ETH_9 & LPH)*

Sr.No	Formulation Code	Vesicle Size(µm)	% Entrapment Efficiency
1	**ETH_1**	2.54±0.03	54.81±0.30
2	**ETH_2**	3.37±0.10	74.52±0.14
3	**ETH_3**	4.56±0.08	69.24±0.20
4	**ETH_4**	1.96±0.03	67.03±0.61
5	**ETH_5**	2.84±0.04	78.04±0.30
6	**ETH_6**	3.31±0.01	69.61±1.16
7	**ETH_7**	1.62± 1.31	68.95±0.57
8	**ETH_8**	1.99± 1.02	62.01±0.83
9	**ETH_9**	2.14±0.03	67.79±0.37
10	**LPH**	5.21±0.02	49.07±1.56

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 vesicles was reduced when the dispersion was sonicated. We explain this by the fact that the 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± 1.31 µm to 4.56±0.08µm.

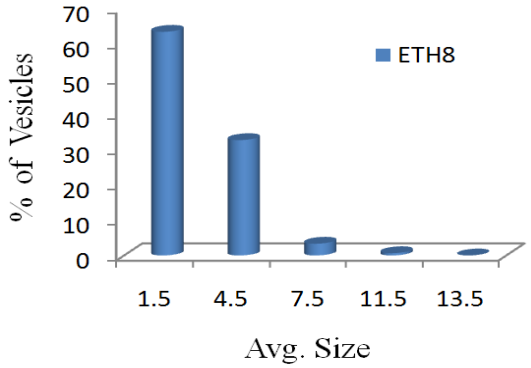

Figure 1: *Size distribution of ETH_5*

Vesicle size of liposomal formulation was found to be 5.21±0.02 µm. Alcohol used in ethosomes has a great effect on vesicle size. Vesicles formed from different alcohols are of different size and they follow the order Ethanol 20%> 30% > 40% of alcohol.

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

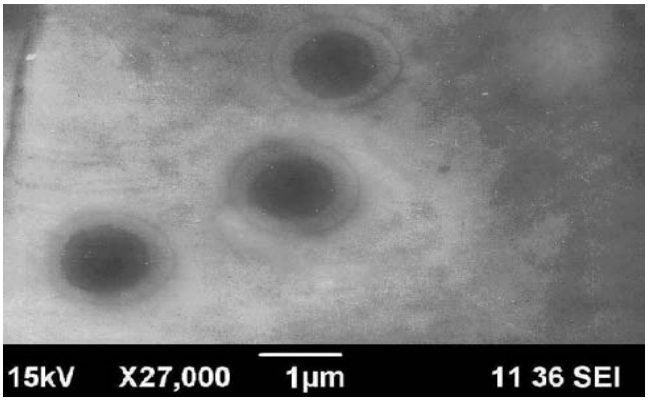

Figure 2: *Scanning electron micrograph of optimized formulation of Perindopril erbumine ethosomes.*

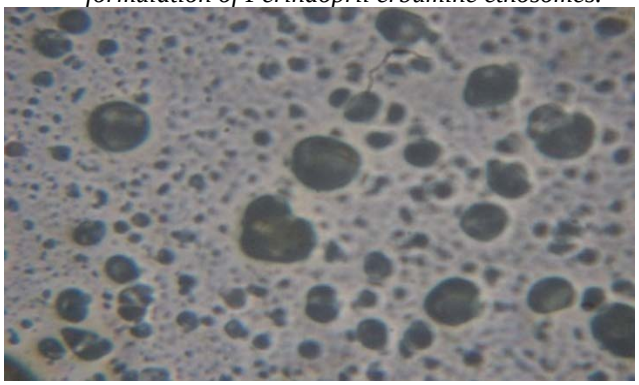

Figure 3: *Optical photomicrograph of optimized formulation of Perindopril erbumine ethomes (ETH8)*

Zeta potential. Zeta potential of optimised formulation obtaind 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.

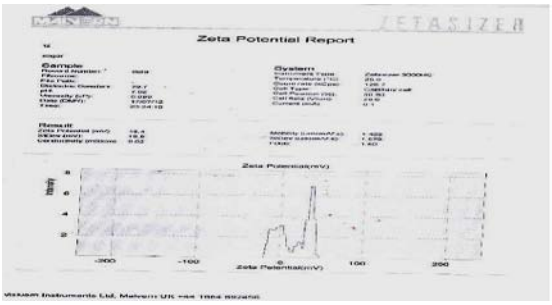

Figure 4: *Zeta Potential of optimized formulation of Perindopril erbumine ethosomes.* ***(ETH_8)***

Entrapment efficiency (%). Vesicle entrapment efficiency was mainly dependent on the amount of phospholipids, forming the bilayers and intrinsic properties of chemical structure, liphophilicity, phase transition temperature, alkyl chain length and alcohol concentration. It was found that phospholipid content having a higher liphophilicity, higher phase transition temperature and longer alkyl chain length shows higher entrapment. Thus depending upon these properties ethosomal formulations prepared with 2-3% phospholipids 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 phsopholipid, alcohol and propylene glycol in different concentrations and in different ratios. In other 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 [9]. The cumulative percentage drug release from ethoosomal formulations ETH1 to ETH9 was in the range of 67.45% to 95.22%. and for LPH it was 50.20±0.23%. The results demonstrate that the ethosomes prepared with alcohol 30% and phospholipid 2% showed the highest release profile when compared to the ethosomes prepared with different concentrations of the same compounds.

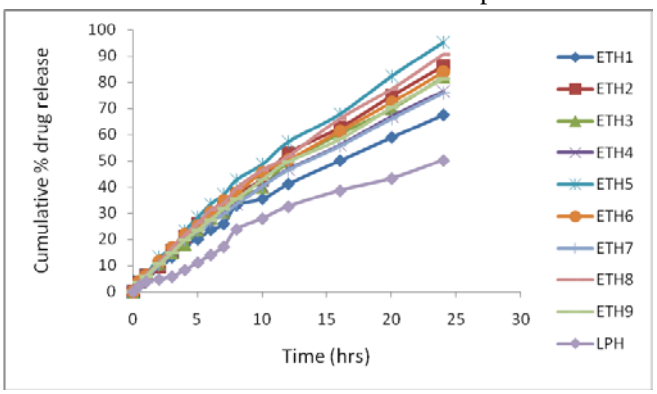

Figure 5: *In vitro drug release of Perindopril erbumine from different ethosomal formulations.*

This is due to the fact that uniform vesicle formation has sufficient penetration through the skin.

In vitro **permeation study**. Permeation profile of perindopril erbumine from optimized ethosomal formulations ETH3 and ETH8 through the rat abdominal skin after 24 hrs is shown in Fig. 6.

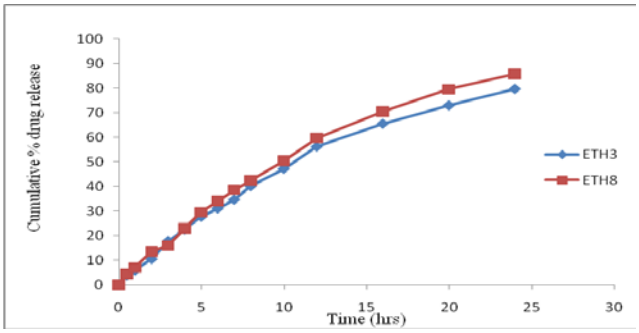

Figure 6: *In vitro skin permeation profile of perindopril erbumine from optimized ethosomal formulations. (ETH3 & ETH8)*

The value for drug permeation (release) for optimized formulation ETH3 and ETH8 through the rat abdominal skin after 24 hrs was found to be 79.63% and 85.20% which is significantly less as compared to drug permeated through cellophane membrane i.e. 86.20% and 95.22% respectively. The reasons for this result is that skin act as a barrier for the transport of the 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, ETH8 & LPH) at refrigeration temperature (4-8o C) and at room

temperature (25±2o C) as per ICH guidelines. The optimized ethosomal formulation was evaluated for its appearance, entrapment efficiency (%), drug content study, and 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, ETH8 & LPH) after stability studies at refrigeration temperature and at room temperature was found to be 72.18±0.16%,71.84±0.25% 74.89±0.31%, 73.81±0.38% & 46.83±0.30%, 45.71±0.18% respectively. The in vitro drug release of optimised formulations (ETH3 ETH8 & LPH) after stability studies at refrigeration temperature and at room temperature was found to be 83.57±0.58, 82.65±0.23 & 92.61±0.31, 91.46±0.35 & 46.23±0.61, 45.22±0.48 respectively. Similarly in drug content study of optimized formulations (ETH3 ETH8 & LPH) after stability studies at refrigeration temperature and at room temperature was found to be 98.83±0.12%, 98.16±0.08% & 98.67±0.14%, 97.93±0.11% & 98.47±0.12%, 97.53±0.16% respectively. Thus from the results it can be observed that no significant variations appear in the entrapment efficiency (%) and in vitro release values when ethosomal formulations were stored at room temperature or refrigerator. This indicates that that ethosomal formulation remains fairly stable at refrigeration (4-80 C) temperature and room temperature (25±20C).

Table 3: *Size Distribution of Perindopril erbumine ethosomal formulation* ***ETH_5***

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	95	63.33	142.50
1-2	**3.00-6.00**	4.50	49	32.66	220.50
2-3	**6.00-9.00**	7.50	05	03.33	37.50
3-4	**9.00-12.00**	11.50	01	00.66	11.50
4-5	**12.00-15.00**	13.50	00	00	00
Total			150		427.00

*Each value is an average of 3 replications; Average diameter (dav)= nd/n = 2.84 µm.

Conclusions

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.

Acknowledgements

We are grateful to Glenmark Pharmaceuticals Pvt. Ltd, Goa for gift sample of Perindopril Erbumine and Management of N.E.T Pharmacy College, Raichur for providing excellent facility to carry out this work.

References

[1] K.D Tripathi, Essentials of Medical Pharmacology, New Delhi, India, Jaypee Brothers. 449-454, **2003**

[2] Martindale, The complete drug reference. 34th Ed. Pharmaceutical Press, Great Britain, 980-81, **2005**

[3] X.H Zhou, P.A Li Wan, Stability and in-vitro absorption of captopril, enalapril and lisinopril across the rat intestine. *Biochem Pharmacol.*47, 1121-1126, **1994**

[4] E Touitou, B Godin, C Weirs, Enhanced Delivery into and across the skin by Ethosomal carries. *Drug Dev. Research*, 50, 406-415,**2000**

[5] D.D Verma, A Fahr, Synergistic penetration effect of ethanol and phospholipids on the topical delivery of Cyclosporin. *A, J. Control Release*, 97,55-66, **2004**

[6] E Touitou, Composition of applying active substance to or through the skin, *US patent*. 5,540,934, **1998**

[7] S Jain, R.B Umamaheshwari, P Tripathi, N.K Jain, Ultradeformable liposomes: A recent tool for effective transdermal drug delivery, *Ind J Pharm Sci*,65, 223-231, **2003**

[8] N Dayan, Touitou, Carrier for skin delivery of trihexyphenidyl HCl: Ethosomes vs. liposomes E. *Biomaterials*, 21,1879-1885, **2000**

[9] M. K. Chourasia, L Kang, S Y Chan, Nanosized ethosomes bearing ketoprofen for improved transdermal delivery, *Results in Pharma Sciences*, 1(1), 60–67, **2011**

[10] I.A Alsarra, A.A Bosela, S.M Ahmed, G M Mahrous,Proniosomes as a drug carrier for transdermal delivery of ketorolac, *Eur J Pharm and Bio*, 59, 485–490, **2005**

[11] V Dubey, D Dinesh Mishra, N.K Jain, T Dutta, M Nahar, D.K Saraf, Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes, *J. Control. Release*, 123, 148-154, **2007**

[12] S Jain, A.K Tiwary, B Sapra, N.K Jain, Formulation and evaluation of ethosomes for transdermal delivery of lamivudine, *AAPS Pharm.Sci.Tech*, 8(4), E1-E9, **2007**