

RESEARCH ARTICLE

FORMULATION AND EVALUATION OF ETHOSOME FOR ECONAZOLE NITRATE AS A MODEL DRUG TO ENHANCED TRANSDERMAL DELIVERY

*Praveen Kumar, Anil K. Patel, Raj K. Prasad, Singh S. Gautam

*Shambhunath Institute of Pharmacy, Jhalwa, Allahabad, U. P.-211012

Date Received: 10th March 2016; Date Accepted: 24th March 2016
Date published: 28th March 2016

Email: anilanilpt538@gmail.com

Abstract: Ethosome are soft malleable vesicle tailored for enhanced delivery of active agent. The application of medicines to the skin surface serves the purpose of delivering the active ingredient into the skin (dermal delivery) or through the skin and into the systemic blood circulation (transdermal delivery). The objective of present study was to prepare and evaluate the ethosomes of Econazole nitrate as a model drug to increase its depth of penetration to through skin for enhanced dermal/transdermal delivery. Novel penetration enhancer, ethosomes of Econazole nitrate were prepared successfully using mechanical- dispersion method for prolonged as well as controlled release. The prepared formulation were characterized for various evolutionary parameters like vesicle size, entrapment efficiency, vesicle elasticity, in-vitro drug deposition study and rate of transdermal flux across stratum corneum and prepared formulation were also characterized for in-vitro release study by using cellophane membrane. From in-vitro drug release studies and the biological activity study suggests that the ethosomal formulation shows good release rate in agar plate model that

indicate that ethosomes would probably shows good release in humans.

Key words: Ethosome, Econazole nitrate, transdermal delivery and dispersion method.

INTRODUCTION:

The application of medicines to the skin surface serves the purpose of delivering the active ingredient into the skin (dermal delivery) or through the skin and into the systemic blood circulation (transdermal delivery). The objective of present study was to prepare and evaluate the ethosomes of Econazole nitrate as a model drug to increase its depth of penetration to through skin for enhanced dermal/ transdermal delivery ¹⁻³.

Ethosomes are an interesting and innovative vesicular system that has appeared in the field of pharmaceutical technology and drug delivery in recent years. Ethosome are soft malleable vesicle tailored for enhanced delivery of active agent. It has been shown that the physiochemical characteristics of ethosomes allow the vesicular carrier to transport active substance more efficaciously through stratum corneum into deeper of skin than conventional liposome ⁴⁻⁶. This aspect is off great importance for the design of carrier to be applied topically for both topical and systemic drug administration. Furthermore, the ethosomal carrier is also able to provide an effective intracellular delivery of both hydrophilic and lipophilic molecule and also the preparation of an antibiotic peptide i.e. bacitracin, with fibroblast cells was facilitated ⁷⁻⁹. The ethosomes are vesicular carrier comprise of hydro alcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, ethosomes may contain phospholipids, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. Econazole Nitrate, Fig. 1, is an antifungal agent that prevents fungal organisms from producing vital substances required for growth and function. This medication is effective only for infections caused by fungal organisms ¹⁰⁻¹⁵.

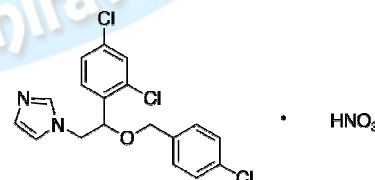


Fig. 1. Chemical Structure of Econazole Nitrate

Material and Methods¹⁶⁻¹⁸

The drug sample (15 gm) was gifted by Innova Cat Cab pharmaceuticals, Baddi, Himanchal Pradesh, India. In Pre-formulation we have studied the melting point using capillary method, The Solubility study of drug was performed in different solvent like alcohol, distilled water, methanol, ether, chloroform, PBS pH- 7.4., The partition coefficient of drug (econazole nitrate) was determined in solvent system: octanol/water and octanol/ phosphate buffer pH- 7.4. FT-IR Spectroscopy for compatibility between drug and polymers and the absorbance of the solutions was measured at $\lambda_{\max}$ 212nm in buffer solution pH-7.4 using UV-Vis spectrophotometer.

PREPARATION OF FORMULATION

The ethosomal system was prepared by dissolving phospholipids and drug/fluorescent probe in ethanol (10-50%). Then drug is dissolved in water then added slowly up to 100% in a fine stream with a constant mixing at 700 rpm with a magnetic stirrer in a well closed container (to avoid the evaporation on any traces of solvents i.e. ethanol) to obtain the ethosomes colloidal suspensions. Mixing was continued for additional 5 minutes. Ethosomes suspension was finally sonicated for 15 minutes by using ultra sonicator. The system was kept at 30°C throughout preparation. The final milky solution of ethosomes was left to cool at room temperature, Table 1. The ethosomal suspension was centrifuged and pellets obtained were incorporated into the prepared gel base with constant mixing at 800rpm using rami mixture to make a gel of 1% w/w of econazole nitrate¹⁹⁻²¹.

Characterization of Formulation

Ethosomes vesicle was visualized by using transmission electron microscope, with an accelerating voltage of 80 kV. The effect of phospholipid and ethanol concentration on the size and distribution of ethosomes vesicle was investigated using Mavem zetasizer.

Drug Entrapment efficacy

Econazole nitrate entrapped within ethosomes was estimated after removing the un entrapped drug. The untrapped drug was separated from ethosomes by subjecting the dispersion to centrifugation in a cooling centrifuge (Remi equipment, Mumbai) at 22000 rpm at a temperature of 40°C for 45 minutes, the combined supernatant was analyzed for the drug content after suitable dilution with phosphate buffer solution by measuring absorbance at 212 nm using shimadzu 1700 UV spectrophotometer. The elasticity of ethosomes vesicle was measured using extrusion method²²⁻²³.

In-Vitro Drug release study

The synthetic semi-permeable membrane or the skin of

the new born mice was mounted between the donor and receptor compartments. The ethosomal formulation was applied to the membrane. Sample were withdrawn through sample port of the diffusion cell at predetermined time interval over 24 hrs and analyzed by UV spectrophotometer. The receptor phase was immediately replenished with equal volume of phosphate buffer solution of pH 7.4 sink condition was maintained throughout the experiment²⁴⁻²⁵. Triplicate experiments were conducted for each study.

Determination of Antifungal Activity

All of the ethosomal formulation was evaluated for antifungal activity against *Candida albicans* (MTCC-227) by using Econazole as standard drug. Cup plate method was used for the determination of antifungal activity. Growth medium were used as culture medium and petri plates were incubated at 25°C for 48 hr. Econazole showed MIC at concentration of 100µg/ml. The test compounds were used at concentrations 100µg/ml.

Result and Discussion

Melting point of pure drug was found to be 165-166 °C. Cilnidipine was freely soluble in methanol and buffer solution pH 7.4. $\lambda_{\max}$ of econazole was found to be 212nm and draw the calibration curve, Fig. 2 and 3.

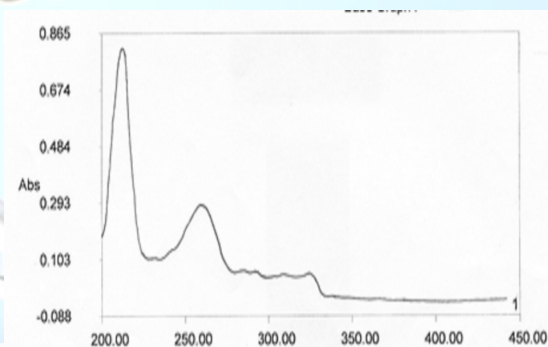


Fig. 2: UV spectra of Econazole Nitrate in buffer solution (pH 7.4)

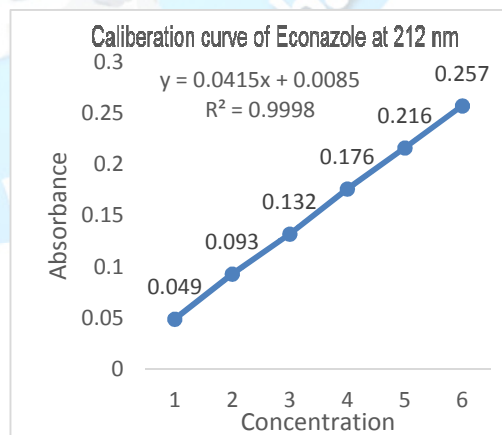


Fig. 3: Calibration curve of econazole Nitrate.

Partition coefficient was found in octanol/ water and octanol/ phosphate buffer pH- 7.4 is 1.18 and 1.324 respectively. Result of FTIR analysis of the mixture show the no

compatibility of drug with polymer, Fig. 4. Results of Visualization of selected Ethosomes vesicle was reported in Fig 5.

Table 1: Batch specification of different formulation.

Formulation Code	ET-1	ET-2	ET-3	ET-4	ET-5	ET-6	ET-7	ET-8
PL (mg)	40	40	40	40	50	50	60	60
Ethanol (ml)	10	20	30	40	30	40	30	40
Drug (mg)	10	10	10	10	10	10	10	10

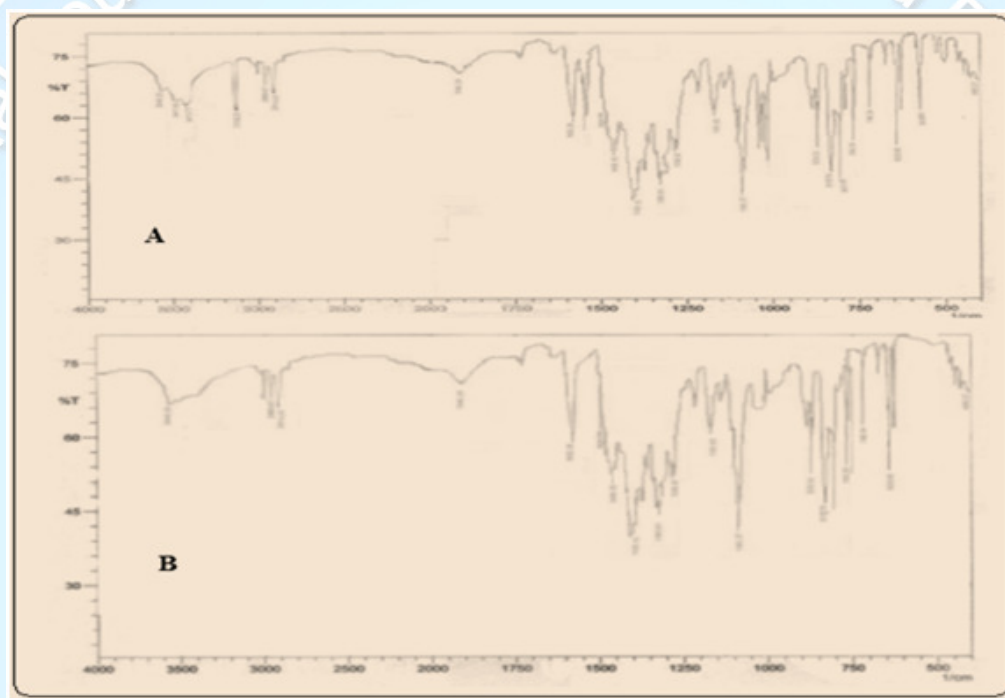


Fig. 4: FTIR spectrum of (A) econazole nitrate (B) Econazole nitrate with polymer

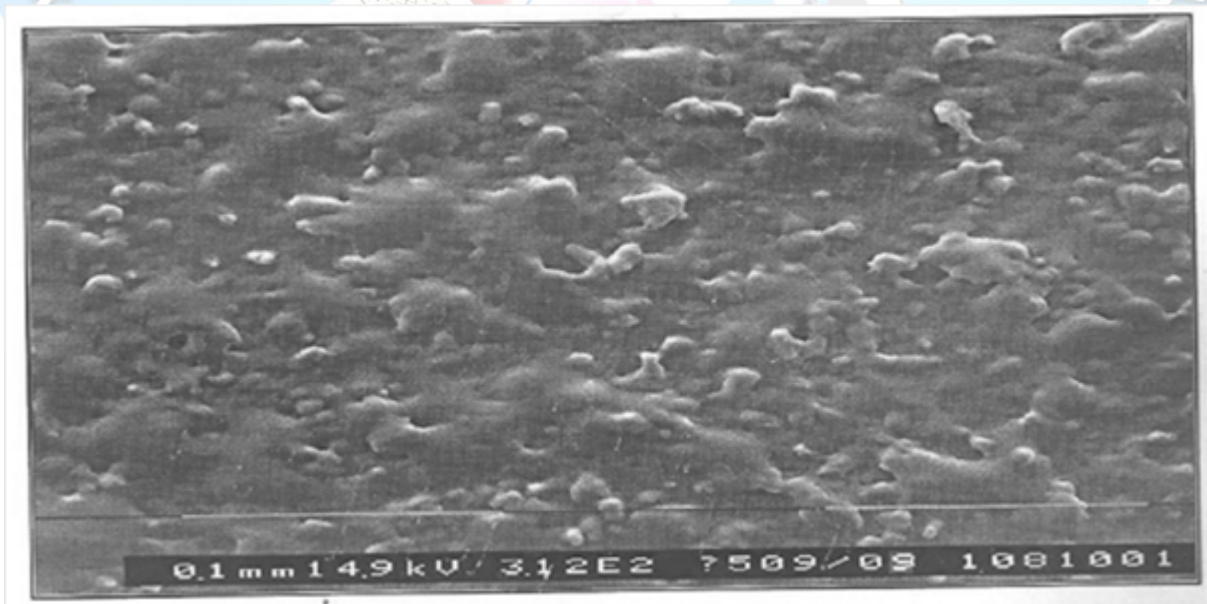


Fig. 5: SEM of ethosomes loaded with econazole nitrate**Table 2. % Entrapment efficacy of different formulation.**

S. No.	Formulation code	Vesicle size(nm)	Zeta potential(mV)	%Entrapment efficiency
1.	ET-1	674.89	-14.8	44.75
2.	ET-2	609.36	-19.8	46.75
3.	ET-3	599.13	-21.3	49.0
4.	ET-4	538.32	-22.0	51.25
5.	ET-5	614.33	-13.2	55.0
6.	ET-6	602.72	-16.4	55.75
7.	ET-7	790.98	-13.8	46.0
8.	ET-8	714.41	-14.7	49.0

Table 3: Vesicle elasticity measurement

Formulation Code	ET-1	ET-2	ET-3	ET-4	ET-5	ET-6	ET-7	ET-8
Vesicle elasticity (E)	22.6±2.1	27.4±2.3	33.8±1.9	38.6±2.2	26.8±2.8	22.4±2.5	25.3±3.1	20.8±2.6

Table 4: % Cumulative release of drug ($\mu\text{g}/\text{cm}^2$) from Ethosome

S. No	Time /hrs	ET-1	ET-2	ET-3	ET-4	ET-5	ET-6	ET-7	ET-8
1	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1	0.42±0.83	0.65±0.67	1.34±0.46	1.47±0.39	1.12±0.46	1.66±0.67	1.80±0.76	2.00±0.68
3	2	1.00±0.37	1.20±0.78	2.89±0.56	3.23±0.68	2.38±0.78	3.68±0.72	3.40±0.89	3.92±0.67
4	4	2.73±0.56	3.20±0.45	5.56±0.78	6.26±0.48	5.67±0.98	8.40±0.34	6.36±0.46	8.50±0.96
5	6	4.85±0.98	6.08±0.049	8.28±0.85	9.00±0.38	7.91±0.45	11.49±0.56	9.90±0.12	11.75±0.37
6	8	7.44±0.78	9.09±0.67	10.98±0.78	12.08±0.56	10.69±0.35	15.28±0.70	13.0±0.56	15.38±0.71
7	10	10.23±0.59	11.67±0.78	14.17±0.46	15.24±0.59	13.65±0.67	19.18±0.59	16.33±0.23	19.72±0.12
8	12	12.52±0.45	13.43±0.67	17.34±0.23	18.66±0.89	16.5±0.28	24.25±0.12	19.4±0.59	23.5±0.34
9	14	14.93±0.89	15.93±0.23	20.60±0.89	21.87±0.48	19.0±0.56	27.53±0.45	21.14±0.78	27.68±0.63
10	16	17.45±0.83	18.85±0.79	23.40±0.59	25.08±0.67	20.75±0.46	30.52±0.56	24.7±0.67	30.48±0.89
11	18	19.49±0.079	21.0±0.78	25.80±0.78	28.11±0.78	24.29±0.49	32.92±0.56	26.60±0.34	33.0±0.67
12	20	21.28±0.90	23.51±0.69	27.59±0.29	30.11±0.56	26.51±0.51	34.0±0.98	28.0±0.46	35.01±0.91
13	22	21.84±0.46	24.40±0.68	28.57±0.90	31.49±0.67	27.98±0.91	34.48±0.46	29.15±0.58	35.60±0.86
14	24	22.26±0.68	24.96±0.46	28.69±0.97	32.00±0.46	28.40±0.46	34.68±0.67	29.49±0.89	36.00±0.67

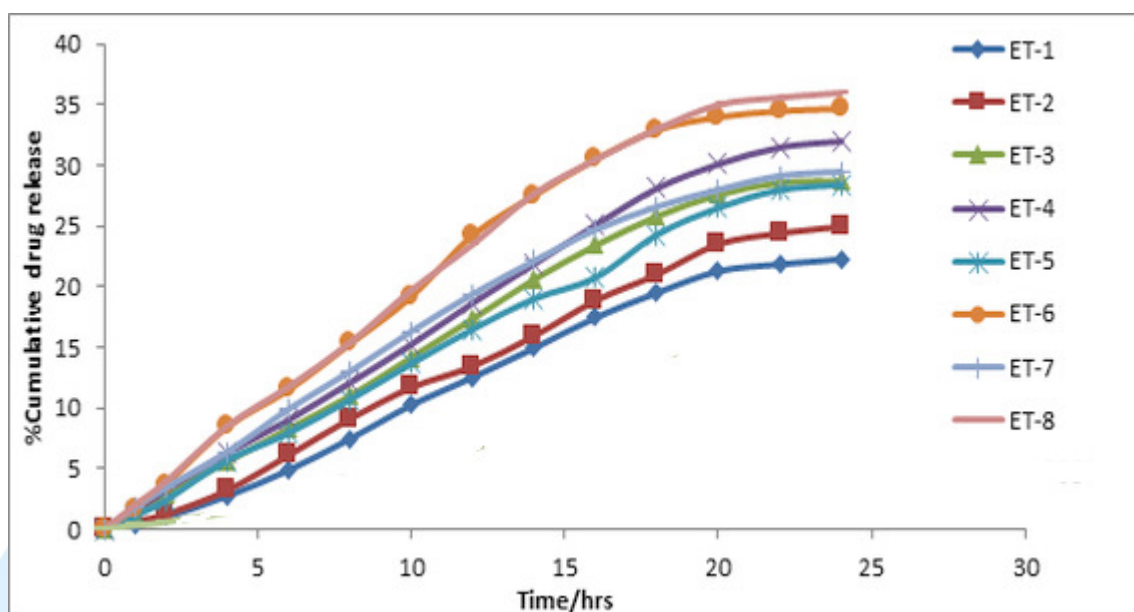


Fig. 8: % Cumulative release profile of drug ($\mu\text{g}/\text{cm}^2$) from Ethosome

The particle size study results showed that as the ethanol concentration increase, the size of vesicles decreases, so increase in alcohol concentration leads to increase in skin delivery of drug. Results of zeta sizer reported in Table 2. Ethosome vesicles have a negative zeta potential this will indicate greater stability of ethosomes vesicles. This higher charge induced on vesicles just because of addition of alcohol to ethosomal formulation which will impart greater stability to ethosomal formulation. In terms of entrapment efficacy, the ethosomal formulation ET-7 (60 mg lecithin and 30 ml alcohol) showed the highest entrapment thus justifying, itself as the optimized formulation with highest entrapment efficacy (56%) and optimum size (790.98.nm), Table 2 and Fig. 6. In term of vesicle size indicated that two fold increase in PL concentration from 40mg to 50 mg results in increase the size from 599.13nm to 614.33nm, means ethosomes size exhibit limited dependence of phospholipids concentration, Table 2.

Results of elasticity of ethosomes vesicle is reported in Table 3 and Fig. 7 and *In-vitro* % cumulative release of econazole nitrate from ethosomal formulation and release profile reported in Table 4 and Fig. 8 respectively. This Data indicate that phospholipids, ethanol at relatively high (up to 30-40ml) concentration and water forms vesicular systems enhanced skin permeation properties. Results of evaluated antifungal activity against *Candida albicans* (MTCC-227) of selected formulation ET5, ET6, ET7 ET8 using Econazole as standard drug reported in Table 5. The results of microbiological Study studies are shown in Fig. 9. Maximum zone of inhibition was found in ET-7 and ET-8 that shows highly active formulation.

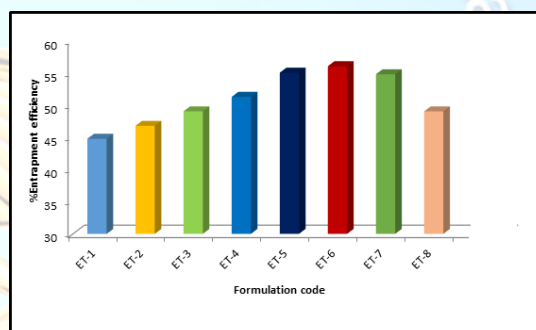


Fig 6: Entrapment efficiency of different formulations

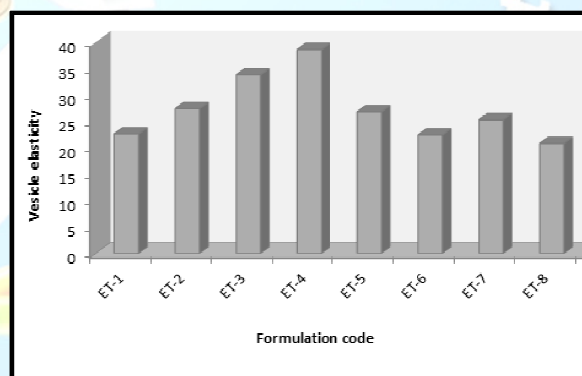
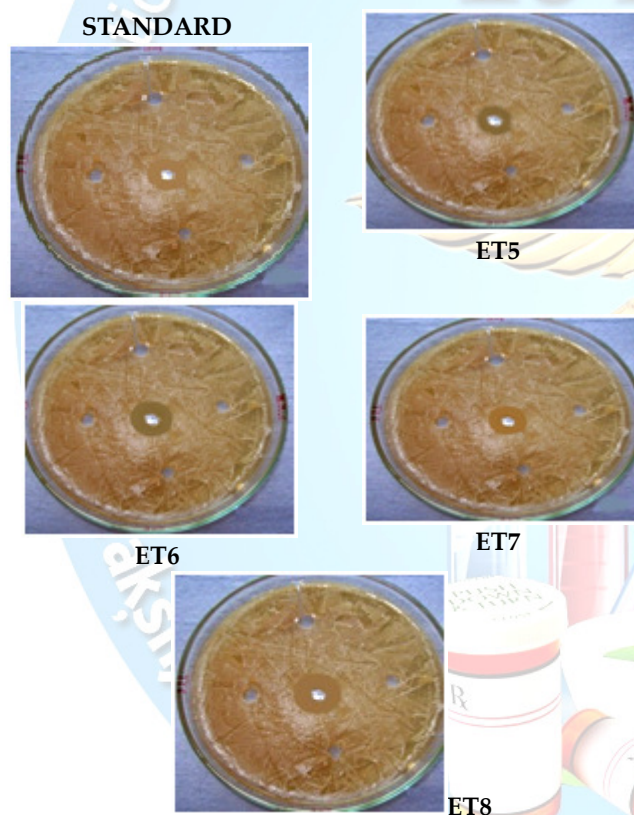


Fig. 7: Vesicle elasticity of different formulations.

Table 5: Antifungal activity of optimized compound

Compound code	<i>Candida albicans</i> (MTCC-227)
ET-5	+
ET-6	++
ET-7	+++
ET-8	+++
STANDARD	++
Key Symbols: Highly active = +++ (inhibition zone >12 mm) Moderately active = ++ (inhibition zone 6-12 mm) Slightly active = + (inhibition zone < 6 mm)	

**Fig.9: Antifungal activity of formulations against *Candida albicans*****Conclusion**

Novel penetration enhancer, ethosomes were prepared successfully by mechanical- dispersion method for prolonged as well as controlled release of econazole nitrate across SC. From in-vitro drug release studies, it is concluded that by changing the ratio of PL and ethanol, econazole release can be controlled for a prolonged period of time by reducing possible side effects occurred dur-

ing conventional therapy. Ethosomes of different vesicle size and drug content could be obtained by varying ratio of PL and ethanol. The biological activity study suggests that the ethosomal formulation shows good release rate in agar plate model that indicate that ethosomes would probably shows good release in humans. The result of study shows that the ethosomal system are much better penetration enhancer than the other transdermal formulation are more versatile in nature.

References:

1. Yie W. Chien and Senshang Lin; Drug Delivery: Controlled Release from Encyclopedia of Pharmaceutical Technology; 2007;1082-95
2. Patel S S, Patel M.S. Ethosomes: A promising tools for transdermal delivery of drug. Pharmainfo.net 2007; 4:1-9.
3. Touitou N, Godin B, Intracellular delivery mediated by an ethosomal carrier. Biomaterials 2001; 22:3053-3059.
4. Robinson R joseph, h. Lee I Vincent." Controlled drug delivery fundamental and application". 2nd edition revised and expanded, 1987;7-9.
5. C. Kochhar, G. Imanidis. In vitro transdermal iontophoretic delivery of leuprolide - Mechanisms under constant voltage application. J. Pharm. Sci., 2003; 92, 84-96.
6. Wertz, W.P.; Miethke, M.C.; Long, S.A.; The composition of the ceramides from human stratum corneum and from comedones. J. Int. Dermatol. 1985; 84, 410-412.
7. Touito E, Dayan N, Godin B, Ethosomes- novel vesicular carriers for enhanced delivery: J. Control release: 2000; 65, 403-418.
8. Jain S., Misra D, Kuksal A and Jain N.K. Vesicular approach for drug delivery into or across the skin: current status and future prospects. Pharmainfo.net. 2007; 4, 1-31.
9. Lopez-Pinto JM, Gonzalez- Rodriguez ML, Rabasco AM, Effect of cholesterol and ethanol on dermal delivery from dppc liposomes. Int j pharm. 2005; 298, 1-12.
10. Young J.Y., Huang Y. L., Fang C.L. Enhancement of the transdermal delivery of catechins by liposomes incorporating anionic surfactant and ethanol. Int j pharm. 2006; 310, 131-138.
11. Gupta PN, Dubey P, Jain S. Non-invasive vaccine delivery in transferosomes, niosomes and liposomes: A comparative study. Int J Pharm.2005; 293, 73-82.
12. Ebtessam A, Essa Michael C, Bonner, Brian W.Barry. Electroporation and Ultradeformable liposomes: human skin barrier repaired by phospholipid. J Control Release, 2003; 92, 163-172.

13. Ylva Y. Grams, Joke A. Boustra. Penetration and distribution of three lipophilic probes in-vitro in human skin focusing on their hair follicle. *J. Control Release*, 2002; 83:253-262.
14. Gopinath D, Ravi D, Rao BR, Apte, SS, Renuka, Rambhau D. Ascrboyl palmitate vesicle (apsosome): formation, characterization and application. *Int J Pharm.*2004; 273, 95-113.
15. Jain S. K, Transdermal delivery and anti-inflammatory effect in murine model, *J control release*, 2003; 93, 377-387.
16. Honeywell-Nguyen ; Enhancement of follicular delivery of finasteride by liposomes and niosomes 1. In-vitro permeation and in-vivo deposition studies using hamste flank an ear models. *Int J Pharm.* 2006; 323, 1-10.
17. Gupta PN, Mishra V, Rawat A, Dubey P, Mahor S, Jain S, Chatterji DP, Vyas SP, Non-invasive vaccine delivery in transferosomes, niosomes and liposomes: a comparative study. *Int J Pharm*, 2005, 293,73-82.
18. Ebtessam A, Essa, Michael C, Bonner, Brian W, Barry, Electroporation and ultradeformable liposomes: human skin barrier repair by phospholipids. *J control release*. 2003; 92,163-172.
19. Ainbinder D and Touitou E. Testosterone ethosomes for enhanced transdermal delivery. *Drug Delivery*, 2005; 12:297-303.
20. Fang C.L. Huang Y. L., Enhancement of the transdermal delivery of catechins by liposomes incorporating anionic surfactant and ethanol. *Int j pharm.* 2006; 310, 131-138.
21. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosome for skin delivery of ammonium glycyrrhizinate: In-Vitro percutaneous permeation through human skin and in-vivo anti-inflammatory activity on human volunteers. *J control Release*. 2005; 106:99-110.
22. Sreenivasa Reddy M, Mutalic S and Veerabhadra G Rao. Preparation and evaluation of minoxidilzgrld gels for topical application in alopecia. *Ind J Pharm Sci.* 2006; 68(4), 432-436.
23. Jain S, Tiwary AK, Sapra B, Jain NK, Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. *AAPS Pharma SciTech.* 2007; 12(21): E1-E9.
24. Ming Thau Sheu, An-Bang Wu, Keng-Ping Lin, Chao-Hui Shen, Hsiuo Ho. Effect of tocophery polyethylene glycol succinate on percutaneous penetration of minoxidil gel from water/Ethanol/Polyethylene glycol 400 solution. *Drug develo and Indust Pharmacy.* 2007; 32:595-607.
25. Elsayed MMA, Abdallah OY, Naggar VF, Halafallah NMK. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery, *Int J Pharm.* 2005; 322, 60-66.

