ISSN: 2395-0536 Impact Factor- 3.50*

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

FORMULATION AND EVALUATION OF LEFLUNOMIDE LOADED TOPICAL ETHOSOMAL GEL

Ch. Buddeswara Rao*, Ch. Charishma Devi, D.Jasmine, G. Divya, G.Venkata Naga Devi, G. Jyothi Kamala
Department of Pharmaceutics, JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India.

ABSTRACT
The method described by Touitou et al., (2000) was employed with little modification for the preparation of various ethosomal formulations containing different concentration of ethanol (20 % to 40 %) with sonication . The techniques used were simple and reproducible. The prepared ethosomes were spherical and discrete in shape. However ethosomes prepared by sonication method were more uniform and small in size which is essential for skin penetration. While comparing the entrapment efficiency, ethosomes containing 40% w/w ethanol and prepared by soncation showed highest value respect to all other formulation; so it is concluded ethosomal prepared by sonication and containing 40 % w/w ethanol as the best formulation considering all other aspectsIncrease in thepolymerconcentrationledto increase in,%Drugentrapment efficiency, Particle size. The *invitro* drug releasedecreased with increase in thepolymer and copolymerconcentration.Among all formulations LE6 shows Maximum drug release upto 24hrs and shows maximum drug release.Analysis ofdrug release mechanism showedthatthedrug release fromthe formulations followed the Non fickian diffusion mechanism and follows 1storder kinectics.Based on the results of evaluation tests formulation code LE6 was concluded as best formulation.
Key Words: Ethosomes, Sonication, Transdermal,Entrapment, Stability

Author for correspondence
Ch. Buddeswara Rao,
Department of Pharmaceutics,
JITS College of Pharmacy, Kalgampudi, Andhra Pradesh, India.
Email id: eswar.eswar66@gmail.com

INTRODUCTION
Optimization of drug delivery through human skin is important in modern therapy. Recently, the transdermal route vied with oral treatment as the most successful innovative research area in drug delivery.Transdermal delivery is an important delivery route that delivers precise amount of drug through the skin for systemic action. Improved methods of drug delivery for biopharmaceuticals are important for two reasons; these drugs represent rapidly growing portion of new therapeutics, and are most often given by injection. Discovery of new medicinal agents and related innovation in drug delivery system have not been only enabled the successful implementation of novel pharmaceutical, but also permitted the development of new medical treatment with existing drugs. Throughout the past two decades, the transdermal patches has become a proven technology holding the promise that new compound could be delivered in a safe and convenient way through the skin. Since the first transdermal patch was approved in 1981 to prevent nausea and vomiting associated with motion sickness, the FDA has approved through the past 22 years more than 35 transdermal patch products spanning 13 molecules.Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism,

predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, and most importantly, it provides patient convince. But one of the major problems in transdermal drug delivery is the low penetration rate through the outer most layer of skin (1-3).Liposomes were discovered in the early 1960's by Bangham and colleagues and subsequently became the most extensively explored drug delivery system. In early 1960's a great knowledge of vesicle derivatives have been tested for their abilities. Most experiments, however, have centered on liposomes, since derivations only add to their basic properties. Vesicles are closed, spherical membrane that separate a solvent from the surrounding solvent. Possible use of liposomes in topical drug delivery vehicles for both water and lipid soluble drug has been investigated. While it has been suggested that the external envelop of a liposomes would allow it to pass through lipophilic skin, most researches show that liposomal vesicles become trapped within the top layer of the stratum corneum cells2. Generally liposomes are not expected to penetrate into viable skin, although occasional transport processes were reported. This behavior is useful both for local treatment of skin disorders and for cosmetic formulations. Specific drug accumulation at the site of action and decreased systemic drug absorption can impart increased efficiency as well as decreased side effect to a compound applied topically.Aim is to prepare and evaluate leflunomideethosomes containing different concentration of ethanol and phospholipids by sonication for size reduction of vesicles.

MATERIALS AND METHODS

PREPARATION OF LEFLUNOMIDE ETHOSOMES (BY COLD METHOD)

Preparation of Leflunomideethosomes was followed by method suggested by Touitou et al.,with little modification (5,6).The ethosomal system of Leflunomidecomprised of 2-4 % phospholipids, 20-40 % isopropyl alcohol, 10 % of propylene glycol, 0.005g of cholesterol and aqueous phase to 100 % w/w.Leflunomide 0.02 g was dissolved in IPA in a covered vessel at room temperature by vigorous stirring. Propylene glycol was added during stirring. Thismixture was heated to 30^0 in a separate vessel and was added to the mixture drop wise in the center of the vessel,which was stirred for 5min at 700rpm in a covered vessel the vesicle size of ethosomal formulation can be decreased to desire extend using sonicationorextrusion method. Finally, the formulation is stored under refrigeration. Ethosomes were formed spontaneously by the process (Table-1).

Table-1 Composition of different ethosomal formulations

Ethosomal formulation	Lecithin (Soya lecithin%)	IPA(%)	Propylene glycol (%)	Drug (g)	Cholesterol(g)	Water
LE_1	2ml	20ml	5ml	0.02g	0.005g	100ml
LE_2	3ml	20ml	5ml	0.02g	0.005g	100ml
LE_3	4ml	20ml	5ml	0.02g	0.005g	100ml
LE_4	2ml	30ml	5ml	0.02g	0.005g	100ml
LE_5	3ml	30ml	5ml	0.02g	0.005g	100ml
LE_6	4ml	30ml	5ml	0.02g	0.005g	100ml
LE_7	2ml	40ml	5ml	0.02g	0.005g	100ml
LE_8	3ml	40ml	5ml	0.02g	0.005g	100ml
LE_9	4ml	40ml	5ml	0.02g	0.005g	100ml

Preparation ofLeflunomideethosomal gel

The best achieved ethosomal vesicles suspension,was incorporated into carbopol gel(1%, 1.5%, 2% w/w).the specified amount of carbopol 934 powder was slowly added to ultrapure water and kept at 100^0c for 20min. triethanolamine was added to it dropwise.

Appropriate amount of formula G-2 containingLeflunomide (1.5% w/w was then incorporated into gel-base.Water q.s was added with other formulation ingredients with continuous stirring until homogenous formulation were achieved (G-1,G-2 and G-3).Gel containing freeLeflunomide was prepared by similar method using 1.5% carbopol(Table-2).

Table-2 Composition of different ethosomal gel formulation

Gel formulation	Leflunomideethosomal suspension(ml)	Carbopol934(%)	Triethanolamine (ml)	Phosphate buffer (pH 6.8)
G-1	20ml	1.0	0.5	q.s
G-2	20ml	1.5	0.5	q.s
G-3	20ml	2.0	0.5	q.s

***In-Vitro* Release Studies**

Drug Release Study from Dialysis Membrane

The skin permeation of Leflunomidefrom ethosomal formulation was studied using open ended diffusion cell specially designed in our laboratory according to the literates. The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm and 200 ml respectively. The temperature was maintained at 37 ± 0.5°C.The receptor compartment contained 200 ml of pH 6.8 buffer and was constantly stirred by magnetic stirrer at 100 rpm. Prepared dialysis was mounted between the donor and the receptor compartments. Ethosomal formulation was applied to the dialysis membrane and the content of diffusion cell was kept under constant stirring then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analysed by spectrometric method at246 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh pH 6.8 buffer. Triplicate experiments were conducted for drug release studies (7).

Stability Studies

Stability study was carried out for Leflunomide ethosomal preparation at two different temperature i.e. refrigeration temperature (4 ± 2° C) at room temperature (27 ± 2° C) for 8 weeks (as per ICH guidelines). The formulation was subjected to stability study and stored in borosilicate container to avoid any sort of interaction between the ethosomal preparation and glass of container, which may affect the observations. Stability of drug and stability of vesicles are the major determinant for the stability of formulation, studies were carried to evaluate total drug content at room temperature (27±2° C) and refrigeration temperature (4±2° C).samples was collected for every 2 weeks and absorbance was seen at 246nm in U.V spectrometer.

RESULTS AND DISCUSSION

FTIR studies were performed to understand the compatibilities between the drugs with different excipients. The figures above illustrate that the functional groups like N-H Stretch with the observation range of 3500-3400 has peaks at 3445.55 in pure drug and 3412.43 in optimized formulation. Similarly the functional group C-F has a peak range of 1400-1000 has peaks at 1237.27 in pure drug and 1261.50 in optimized formulation. Similarly the functional group C-O has a peak range of 1205-1124has peaks at 1124.11 in pure drug and 1145.45 in optimized formulation. The functional groups in both the pure drug and optimized formulation are found. Hence it can be concluded that the pure drug is compatible with the excipient used in the study.Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of ethosomal preparations (fig-1).

ISSN: 2395-0536 **Impact Factor- 3.50***

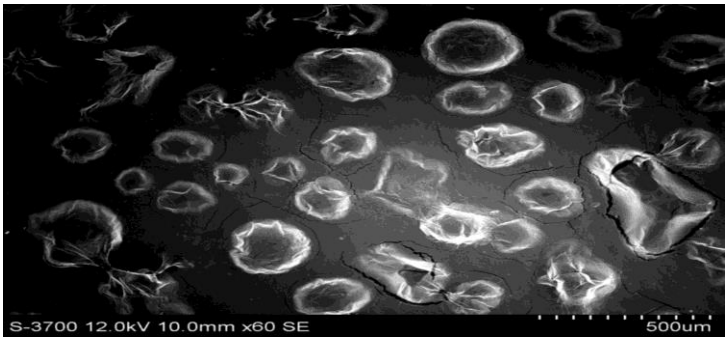

Fig-1 Scanning electron microscope image

Once the presence of bilayer vesicles was confirmed in the ethosomal system, the ability of vesicles for entrapment of drug was investigated by ultra centrifugation. Ultra-centrifugation was the method used to separate the ethosomal vesicles containing drug and un-entrapped or free drug, to find out the entrapment efficiency (Fig-2).

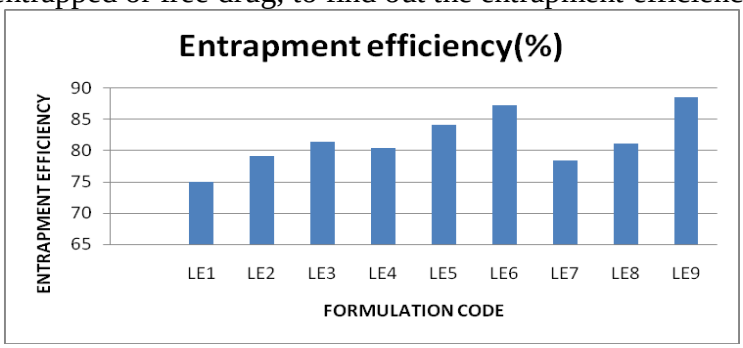

Fig-2Entrapment Efficiency for Le1-Le9 Ethosomal Formulations

From the fig-3,4 and 5, it was confirmed that the LE4, LE5, LE6, LE7, LE8, LE9 of ethosomal gel release theory up to 24 hrs. And also from the table, it was also confirmed that the formulation (LE6) showed maximum drug release up to 24hrs.

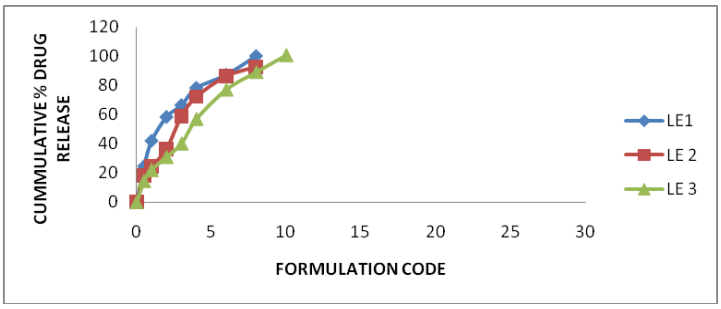

Fig-3 Graph showing dissolution profile for formulations LE1-LE3

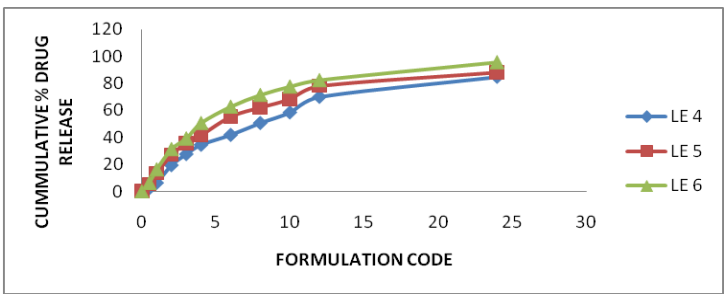

Fig- 4 Graph showing dissolution profile for formulations LE4-LE6

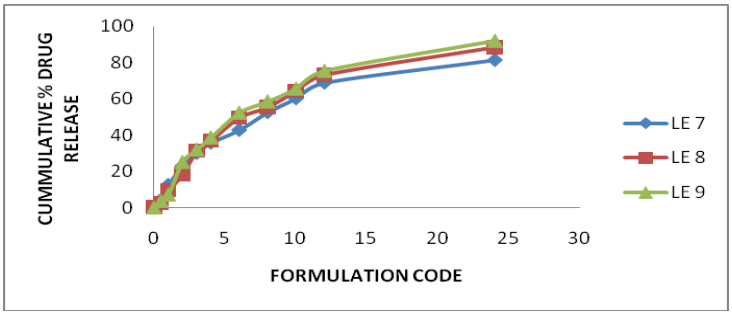

Fig-5 Graph showing dissolution profile for formulations LE7-LE9

The stability studies were carried out according to the procedure described in the section of chapter. The results are shown in the table-3.

Table-3 % Entrapment efficiency and % Drug content after stability studies

Number of Days	% Entrapment Efficiency at temperatures			% Drug Content at temperatures		
	4±2°C	25±2°C	37±2°C	4±2°C	25±2°C	37±2°C
15	87.3	87.28	87.26	98.4	98.27	98.18
30	87.2	87.11	86.81	98.28	98.16	98.6
45	87.14	86.79	86.43	97.18	98.12	98.5
90	86.27	86.38	85.17	97.13	98.7	98.2

CONCLUSION

The results of this investigation indicate that Ion gelation method can be successfully employed to fabricate Leflunomide ethosomal gels. FT-IR spectraof the physical mixture revealed thatthedrug is compatible with the polymers and copolymer used. Increase in thepolymerconcentrationledto increase in,%Drugentrapment efficiency, Particle size.The *invitro* drugreleasedecreased with increase in thepolymer and copolymerconcentration.Among all formulations LE6 shows Maximum drug release in 24hrs when compared with other formulations.Analysis ofdrug release mechanism showedthatthedrug release fromthe formulations followed the Non fickian diffusion mechanism and follows 1st order kinectics.Based on the results of evaluation tests form ulation coded LE6 was concluded as best formulation.

REFERENCES

1. Barry BW. Novel mechanism and devices to enable successful transdermal drug delivery. European J Pharm Sci 2004; 14: 101 - 114.
2. Jain N, Talegonkar S, Jain NK. New ways to enter the blood stream: Emerging strategies in transdermal drug delivery. The Pharma Review. Sep - Oct 2004; 41-60.
3. Jain NK. Advances in controlled and novel drug delivery. 1ed, New Delhi: st CBS publication; 2001; 428 - 451.
4. Jain S, Bhandra D, Jain S and Jain NK. Transfersomes- A novel carrier for effective transdermal drug delivery. Controlled and novel drug delivery 1 st Edition. CBS publishers and distributors new Delhi 1997: 426 - 451.
5. Vyas SP, Khar RK. Controlled drug delivery concepts and advances. Vallabhprakashan New Delhi. First Edition 2002: 173 - 243.
6. Tuitou E, Godin B, and Weiss C. Enhanced delivery into and across the skin by Ethosomal carriers. Drug Dev Research 2000; 50; 406 - 445.
7. Tauitou E, Dayan N, Bergelson L, Godin B and Eliaz M. Ethosomes – novel vesicular carrier for enhanced delivery: characterization and skin penetration properties. J Con Release 2000; 65: 403 - 418.