



FORMULATION AND EVALUATION OF DICLOFANAC POTASSIUM ETHOSOMES

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ABSTRACT

The aim of the current investigation is to evaluate the transdermal potential of novel vesicular carrier, ethosomes, having diclofenac potassium, a potent, water soluble non-steroidal anti-inflammatory drug with lesser transdermal permeation. Drug loaded ethosomes had been prepared using phospholipid and ethanol, were optimized and characterized for entrapment efficiency, vesicular size, shape, invitro skin permeation, skin retention, drug-membrane component interaction and stability. The ethosomal formulation having 4%w/v of phospholipid and 40%v/v of ethanol (F_{16}) showing the greatest entrapment efficiency (72.91±0.64%) with small particle size (251±23nm) was selected for further skin permeation studies. The skin permeation and skin retention studies were performed on ethosomal formulation, liposomal formulation (4%w/v of phospholipid without alcohol), hydroethanolic drug solution and phosphate buffer saline (pH7.4) drug solution. Among them, ethosomal formulation showed higher cumulative percentage of drug permeation (60.37±5%) and more skin retention (619.60±18µg/cm²) after 12 hours than the other formulations. Scanning electron microscopy confirmed the three dimensional nature of ethosomes. Dynamic light scattering technique proved that the ethosomes has smaller vesicular size than the liposomes prepared without alcohol. FT-IR studies revealed no interaction between the drug and membrane components. The ethosomal vesicles were incorporated in carbopol gel base and its anti-inflammatory efficiency was compared with the marketed diclofenac gel. The pharmacodynamic studies showed the enhanced anti-inflammatory activity of ethosomal gel than the marketed gel formulation. Our results suggest that the ethosomes are an efficient carrier for dermal and transdermal delivery of diclofenac potassium.

Keywords: Transdermal, ethosomes, phospholipid, nanomeric, liposomes.

INTRODUCTION

The skin covers a total surface area of approximately 1.8m² and provides the contact between the human body and the external environment. Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases and other inflammatory conditions. This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic side effects. Transdermal drug delivery uses the skin as an alternative route for the delivery of systemically acting drugs.

The structure of stratum corneum is often compared with a brick wall, with the corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae. Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin. One of the most controversial methods is the use of vehicle formulations as skin delivery systems¹

Even though, some authors suggested that the conventional liposomes as suitable carriers for transdermal delivery of some drugs, it became recently evident that in most cases, classic liposomes are of little or no values as carriers for transdermal drug delivery as they do not deeply penetrate skin but rather remain confined to upper layers of the stratum corneum. Confocal microscopy studies showed that the intact liposomes were not able to penetrate the granular layers of the epidermis.

Ethosomes are novel lipid carrier developed by Touitou et al showing enhanced skin delivery of drugs². The ethosomal system is composed of phospholipid, ethanol and water. Although liposomal formulations containing up to 10% ethanol and up to 15% poly propylene glycol were previously described by Foldvary et al (1993)³, the use of high ethanol content was first described by Touitou et al (1997) for ethosomes². Due to the interdigitation effect of ethanol on lipid bilayers, it was believed that the high concentrations of ethanol are detrimental to liposomal formulations. However, ethosomes which are novel permeation enhancing lipid vesicles embodying high concentration (20-45% v/v) of ethanol were developed and investigated.

Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles as well as by the presence of ethanol in ethosomes which allows for better

solubility of many drugs. Ethosomes were reported to improve *in vivo* and *in vitro* skin delivery of various drugs. Contrary to deformable liposomes, ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions².

Diclofenac potassium, a phenyl acetic acid derivative, is a prototypical non-steroidal anti-inflammatory agent, used for anti-inflammatory and analgesic effects in the symptomatic treatment of acute and chronic rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. It is completely absorbed from the GI tract. However, the drug undergoes extensive first pass metabolism in the liver. Oral dose of diclofenac potassium causes an increased risk of serious gastrointestinal adverse events including bleeding, ulceration and perforation of the stomach or the intestines which could be fatal. Due to the presence of these oral adverse effects necessitate the need for investigating other route of drug delivery of diclofenac potassium. Transdermal delivery of the drug can improve its bioactivity with reduction of the side effects and enhance the therapeutic efficacy. The objective of the present study is to design, characterize and evaluate the diclofenac potassium ethosomes for enhanced anti-inflammatory activity.

MATERIALS AND METHODS

Materials

Phospholipon 90 and diclofenac potassium were received as gifts from Natterman GMBH (Germany) and Apex laboratories (Chennai, India) respectively. Ethanol, chloroform and methanol were purchased from Ranchem (India). Potassium di hydrogen phosphate and disodium hydrogen ortho phosphate were purchased from Nice chemicals (India). Sodium chloride was purchased from Central drug house (India). All the materials used in this study were of analytical and pharmaceutical grade.

Methods

Preparation of ethosomes^{4,5}

The diclofenac potassium ethosomal formulations were prepared by Classic mechanical dispersion containing phospholipon 90(1-4%) and ethanol (10 to 40%). The drug concentration was fixed as 10mg/ml or 1% w/v.

Accurately weighed quantity of phospholipon 90 was dissolved in chloroform: methanol (3:1) mixture and the organic solvents were removed in the rotary flash evaporator above the lipid transition

temperature (55°C) (at 60rpm) to form a thin lipid film in the flask. The traces of organic solvents mixture were further removed by maintaining the temperature under reduced pressure for additional 30 minutes after the thin film was formed. Then the lipid film was hydrated with different concentration of hydroethanolic mixture containing diclofenac potassium (1% w/v) in the rotary flash evaporator at 60rpm for 1hour in the room temperature. The preparation was vortexed followed by sonication at 4°C in an ice bath using probe sonicator at 40W in three cycles of five minutes with five minutes rest in between the cycles. After sonication the ethosomal formulation was stored in refrigerator (4°C) for further studies.

Liposomes were prepared by the previously described procedure, but the resulting film was hydrated with 1%w/v of drug in distilled water instead of ethanol². The composition of various ethosomal formulations and liposomal formulation were represented in table 1.

Evaluation of ethosomes

Visualization by scanning electron microscopy (SEM)^{4,6&7}

The size and shape of the vesicles were observed in the scanning electron microscopy. One drop of ethosomal suspension (F₁₆) was mounted on a clear glass stub. It was then air dried and gold coated using sodium auro thiomalate to visualize under scanning electron microscope at 10,000 magnifications.

Determination of entrapment efficiency⁵⁻¹⁰

Entrapment efficiency of diclofenac potassium ethosomal vesicles was determined by centrifugation method. The vesicles were separated in a high speed cooling centrifuge at 20,000rpm for 90 minutes in the temperature maintained at 4°C. The sediment and supernatant liquids were separated; amount of drug in the sediment was determined by lysing the vesicles using methanol. From this, the entrapment efficiency was determined by the following equation,

$$\text{Entrapment efficiency} = D_E / D_T \times 100 \text{ Where,}$$

D_E – Amount of drug in the ethosomal sediment

D_T – Theoretical amount of drug used to prepare the formulation (equal to amount of drug in supernatant liquid and in the sediment)

Vesicular size and size distribution^{4,5,8&9}

Dynamic light scattering technique was used to determine the vesicular size and size distribution. One drop of ethosomal formulation was diluted to 10ml with hydroethanolic mixture used in the formulation and the measurements were taken. The size distribution of the liposome formulation was also determined after diluted with distilled water.

Comparison of *in vitro* skin permeation of drug form various formulations^{4-7&11}

In vitro skin permeation of diclofenac potassium in ethosomes, liposomes, hydroethanolic solution (1%w/v) and in phosphate buffer saline pH 7.4 (1%w/v) were studied using Franz diffusion cell with an effective permeation area of 2.54cm². The ethosomal formulation was selected for the *in vitro* skin permeation on the basis of high entrapment efficiency and smaller vesicular size.

Rats (male albino) 6 to 8 weeks old, weighing 120 to 150g were sacrificed for abdominal skin. After removing the hair, the abdominal skin was separated from the underlying connective tissue with scalpel. The excised skin was placed on aluminum foil and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue. The skin was checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or crevices in the portion that is used for transdermal permeation studies. The *in vitro* study was approved by the institutional ethical committee.

The skin was mounted between donor and receptor compartment with the stratum corneum side facing upward into the donor compartment. Phosphate buffer saline pH 7.4 was taken in the receptor compartment. The formulation was applied on the skin in donor compartment which was then covered with aluminum foil to avoid any evaporation process. Samples were withdrawn at predetermined time intervals over 12 hours, and suitably diluted with phosphate buffer saline pH 7.4 to analyze the drug content in UV-Visible spectrophotometer at 276nm using phosphate buffer saline pH 7.4 as blank. The receptor medium was immediately replenished with equal volume of fresh medium to maintain the sink conditions throughout the experiment. The percentage of drug release was plotted against time to find the drug release pattern.

Skin retention studies⁷

The amount of diclofenac potassium retained in the skin was determined at the end of the 12 hours *invitro* permeation studies. The formulation remain in the *invitro* permeation experiment was removed by washing with distilled water. The receptor content was replaced by 50% v/v ethanol and kept for further 12 hours with stirring and the drug content was estimated spectrophotometrically at 276nm. This receiver solution diffused through the skin, disrupting any liposome and ethosome structure and extracting deposited drug from the skin.

FT-IR studies⁶

The interaction between ethosomal membrane component phospholipon 90 and drug was observed from IR-Spectral studies by observing any shift in peaks of drug in the spectrum of physical mixture of drug and phosphatidylcholine.

Stability studies^{4,7&11}

Stability studies were carried out by storing the ethosomal formulations at two different temperatures 4°C and 25±2°C. The drug content was estimated for every 15 days to identify any change in the entrapment efficiency of ethosomal formulation.

Preparation of ethosomal gel¹²

The ethosomal formulation (F₁₆) having high entrapment efficiency and smaller vesicular size was centrifuged in the temperature 4°C at 20,000rpm for 90 minutes to separate the ethosomal vesicles. The Ethosomal sediment which contains only the entrapped drug was collected and dispersed in the carbopol 980(2%) gel base with gentle stirring to obtain the total drug equivalent to 1%w/w of diclofenac potassium.

In vivo anti inflammatory activity¹³

The anti inflammatory activity was carried out by carrageenan induced paw oedema method to compare the activity of marketed product and the formulated gel. After getting ethical committee clearance male albino rats of Wister strain (150-200) were used for anti-inflammatory activity. The rats were fed with standard food and water. Food was withdrawn 12 hours before and during the experimental studies.

The animals were divided into four groups having three animals in each group. First and second group served as normal control receiving normal saline and gel base without drug respectively. Third group received diclofenac potassium ethosomal gel and fourth group received plain diclofenac gel (marketed).

RESULTS AND DISCUSSION

Visualization of vesicles, size and entrapment efficiency¹⁴⁻¹⁸

Ethosomal formulations were prepared by classic mechanical dispersion method were translucent and had uniform dispersion of ethosomal vesicles. The three dimensional nature of formulated ethosomal vesicles were confirmed by scanning electron microscopy shown in figure 1.

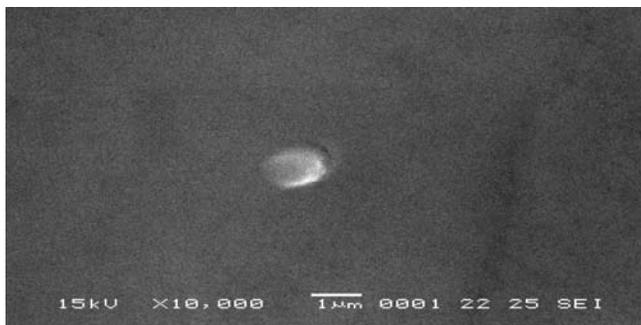


Fig. 1: visualization by scanning electron microscopy

The ethosomal vesicles of selected formulations were evaluated for vesicular size and the results listed in table 1. The vesicular size of the ethosomes decreased with increase in ethanol concentration and increased with increase in phospholipid concentration. At the same time, the content of ethanol and phospholipid had significantly positive effect on the entrapment efficiency of the ethosomal carriers. Increase in the contents of ethanol and phospholipid caused increase in entrapment efficiency. The greater entrapment of diclofenac potassium in ethosomes than the conventional liposomes could be attributed due to the presence of ethanol in ethosomal core³.

The range of entrapment efficiency of sixteen ethosomal formulations was about 18.74% to 72.91%. The maximum

entrapment efficiency was observed in the formulation containing 4%w/v of phospholipids and 40%v/v of ethanol. The mean vesicle size and the entrapment efficiency of liposomes prepared from 4%w/v of phospholipid without alcohol were 422±38nm and 42.46±1.01% respectively. The entrapment efficiency of liposomal formulation was comparatively inferior to the ethosomes and the particle size was also high. The results are shown in Table No. 1. The entrapment efficiency and average particle size of the carrier are important indexes affecting the medicine efficacy. Because of the small particle size and high entrapment efficiency, the ethosomal formulation F₁₆ was chosen for the skin permeation *in vitro* and *in vivo* anti-inflammatory experiment.

Table 1: composition, entrapment efficiency and average particles size of ethosomal and liposomal formulations

S.no	Formulation	Phospholipon 90 (%w/v)	Ethanol (%v/v)	Entrapment efficiency ± S.D* (%)*n=3	Average vesicular size ± S.D (nm)
1.	F1	1	10	18.74±1.11	125±20
2.	F2	1	20	27.17±1.24	-
3.	F3	1	30	30.94±0.73	-
4.	F4	1	40	34.42±0.50	-
5.	F5	2	10	27.77±0.74	137±32
6.	F6	2	20	31.04±1.58	-
7.	F7	2	30	37.07±1.09	-
8.	F8	2	40	40.37±1.85	-
9.	F9	3	10	39.18±0.98	272±21
10.	F10	3	20	41.86±0.14	-
11.	F11	3	30	55.33±2.02	-
12.	F12	3	40	63.69±1.28	-
13.	F13	4	10	55.35±1.21	325±37
14.	F14	4	20	60.41±0.83	301±32
15.	F15	4	30	64.47±0.50	265±18
16.	F16	4	40	72.91±0.64	251±23
17.	LIPOSOMES	4	-	42.46±1.01	422±38

* Drug concentration used in each formulation kept as constant 10mg/ml or 1% w/v.

In-vitro skin permeation and skin retention studies^{4-7& 11}

The cumulative percentage of drug release from ethosomal system, liposomal system, hydroethanolic and phosphate buffer saline pH

7.4 drug solutions were 60.37%, 15.95%, 49.90% and 11.65% respectively at the end of 12 hours study and the release profile are shown in figure 2.

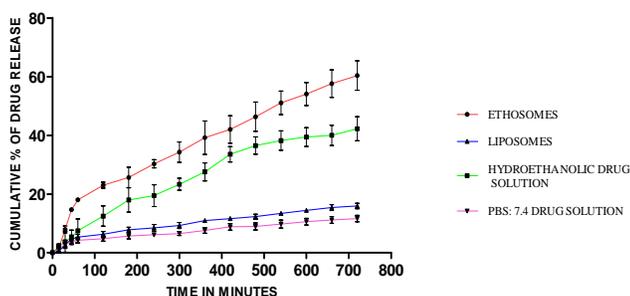


Fig. 2: Comparison of Invitro skin permeation of drug from various formulations in phosphate buffer saline pH: 7.4 n=3

From this study it has been observed that the ethosomal system showed higher skin permeation of drug than the liposomes (4 times) and phosphate buffer saline pH 7.4 drug solution (5 times). Further the permeation enhancement of drug from ethosomes was also greater than the hydroethanolic solution

The ethosomal formulation, liposomal formulation, hydroethanolic solution and phosphate buffer saline pH 7.4 drug solutions showed the skin deposition $619.6 \pm 18.3 \mu\text{g}/\text{cm}^2$, $363.16 \pm 7 \mu\text{g}/\text{cm}^2$,

$220.86 \pm 14.2 \mu\text{g}/\text{cm}^2$ and $136.46 \pm 5.7 \mu\text{g}/\text{cm}^2$ respectively at the end of 12 hour experiment shown in figure 3. The skin deposition of ethosomal formulation was approximately 2, 3 times and 5 times greater than the hydroethanolic solution, liposomal formulation and phosphate buffer saline pH 7.4 drug solutions respectively. The ethosomes showed significantly higher skin deposition possibly due to combined effect of ethanol and phospholipid thus providing a mode for dermal and transdermal delivery of diclofenac potassium.

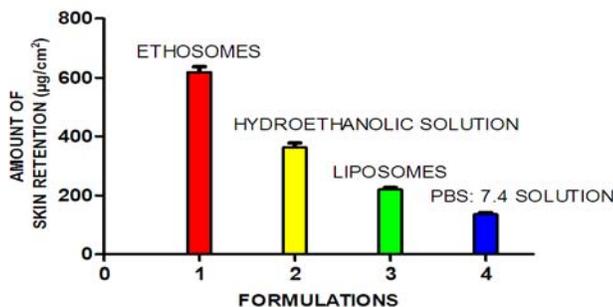


Fig. 3: Skin Retention of drug various formulations n=3

Stability and interaction analysis^{4, 6, 7&11}

The results revealed that the drug retention capacity (entrapment efficiency) was more with ethosomal formulation stored at 4°C than at 25±2°C. Hence increase in temperature decreased the drug

retention capacity of ethosomes as shown in Table 2. The decrease in entrapment efficiency may be due to drug leakage from the ethosomes at higher temperature. The IR spectral studies showed no shift in peaks indicated no interaction exists between the drug and other lipid components show in figure 4.

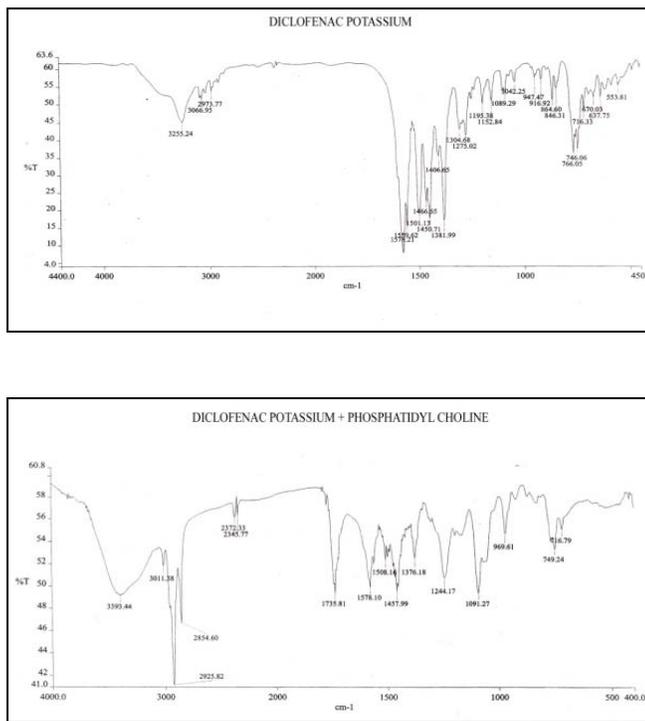


Fig. 4: IR spectrum of the drug and physical mixture

Table 2: Stability testing of ethosomal formulation (f_{16}) (Percentage entrapment efficiency)

	Storage at 4°C	Storage at 25°C
Immediately after preparation	72.91%	
After 15 days	71.73%	71.43%
After 30 days	71.13%	70.24%

In vivo anti-inflammatory activity¹³

From the anti-inflammatory studies it was observed that, after 4 hours, ethosomal gel formulation showed marked percentage inhibition of inflammation (44.44%) when compared to marketed

formulation (35.80%) with lesser time for onset of action. This may be due to the efficient skin permeation of ethosomes. Hence, this ethosomal formulation may be considered as a good choice to improve absorption of the anti-inflammatory drug. The results of the anti-inflammatory study are shown in figure 5.

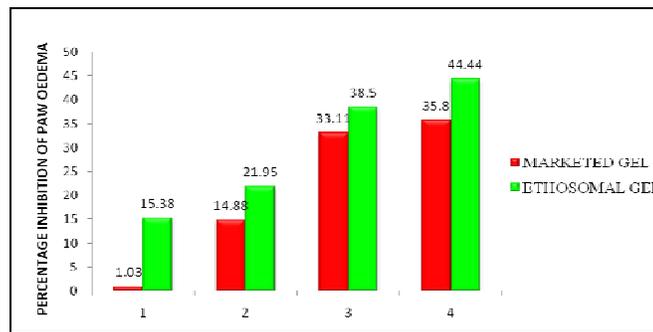


Fig. 5: Percentage inhibition of paw odema

CONCLUSION

Ethosomes have been considered as a possible vesicular carrier for transdermal delivery of diclofenac potassium an analgesic anti-inflammatory agent. The study confirmed that ethosomes are very promising carrier for the transdermal delivery of diclofenac potassium revealed from higher entrapment efficiency, better stability profile and faster anti-inflammatory efficiency. The enhanced accumulation of diclofenac potassium via ethosomal carrier within the skin might help to optimize targeting of this drug to the epidermal and dermal sites, thus creating new opportunities for modern topical application of diclofenac potassium in the inflammatory conditions.

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