

## Ethosome for Enhanced Transdermal Drug Delivery of Aceclofenac

Vivek Dave<sup>1\*</sup>, Dharendra Kumar<sup>1</sup>, Shaila Lewis<sup>1</sup>, Sarvesh Paliwal<sup>2</sup>

### \*Corresponding author:

Vivek Dave

<sup>1</sup> Manipal College of Pharmaceutical Sciences, Manipal-576104, Karnataka, India

Tel: +91 0819829581

E-mail:

[vivekdave1984@yahoo.com](mailto:vivekdave1984@yahoo.com)

<sup>2</sup>Banasthali University Dept. of Pharmaceutical Chemistry Banasthali, Tonk-304022, Rajasthan, India

### Abstract

The aim of the current investigation is to evaluate the transdermal potential of novel vesicular carrier, ethosomes, bearing aceclofenac, Non-steroidal anti-inflammatory drugs (NSAIDs) agents having limited transdermal permeation. Aceclofenac loaded ethosomal carriers were prepared, optimized and characterized for vesicular shape and surface morphology, (SEM) scanning electronic microscopy, vesicular size, entrapment efficiency, stability, in-vitro release study. The formulation (Etho5) having 3% phospholipids content and 40% ethanol showing the greater entrapment (93.3%) and optimal average vesicle size of formulation (Etho5) determined by Malvern Zetamaster ZEM & 0.696 $\mu$ m and zeta potential of formulation was -6.74 mV. The formulation (Etho12) having 3% phospholipids content and 40% isopropyl alcohol showing the greater entrapment (95.7%). stability profile of prepared system assessed for 45 days. The vesicular suspension was kept in sealed vials (10ml) at 4  $\pm$  2°C and at room temperature for 45 days no change is shown in the entrapment efficiency. The optimized ethosomal formulation showed transdermal flux (226.1  $\mu$ g/cm<sup>2</sup>/hr) for ethanolic drug solution which is greater than that of isopropyl alcohol solution (159.0  $\mu$ g/cm<sup>2</sup>/hr). The result advocates the potential of ethosome formulation to treat rheumatic disease where facilitated penetration of the drug into muscle and synovial fluid is desirable. In light of the data obtained from experimental work we can expect the ethosome formulation to be safe and very efficient as a drug carrier for systemic as well as topical delivery of drug, holding future in effective transdermal delivery.

**Keywords:** Ethosome; Aceclofenac; Phospholipid; Transdermal

### Introduction

Ethosomes are interesting and innovative vesicular systems that have appeared in the field of pharmaceutical technology and drug delivery in recent years. This carrier presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformability. Ethosomes are soft, malleable vesicles tailored for enhanced

delivery of active agents. It has been shown that the physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the stratum corneum into the deeper layers of the skin than conventional liposomes. This aspect is of great importance for the design of carriers to be applied topically both for

topical and systemic drug administration [1]. The skin acts as a major target as well as a principle barrier for topical/transdermal (TT) drug delivery. The stratum corneum plays a crucial role in barrier function for TT drug delivery. Despite major research and development efforts in TT systems and the advantages of these routes, low stratum corneum permeability limits the usefulness of topical drug delivery. To overcome this, methods have been assessed to increase permeation. One controversial method is the use of vesicular systems, such as liposomes, ethosomes and niosomes, whose effectiveness depends on their physicochemical properties.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation. Aceclofenac, an NSAID, has been recommended orally for the treatment of rheumatoid arthritis and osteoarthritis. It also has anti-inflammatory, antipyretic, and analgesic activities. The short biological half-life (about 4 h) and a higher dosing frequency make aceclofenac an ideal candidate for sustained release. The oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding with chronic use. Because of gastrointestinal bleeding, it also causes anemia. Using the transdermal route eliminates these side effects, increases patient compliance, avoids first-pass metabolism, and maintains the plasma drug level for a longer period of time. Therefore, an improved aceclofenac formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of supporting structures of the body.

Touitou discovered lipid vesicular systems ethosomes embodying ethanol in relatively high concentration. Ethosomes contain phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. Unlike classical liposomes, ethosomes were shown to permeate through the stratum corneum barrier and were reported to possess significantly higher transdermal flux in comparison to liposomes. The exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. However, synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayer. The use of lipid vesicles as drug delivery systems for

skin treatment has attracted increasing attention in recent years. However, it is generally accepted that conventional ethosomes are of little value for this purpose. Ethosomes remain confined to the upper layer of stratum corneum (SC) and hence, are suitable for topical drug delivery. Only specially designed vesicles were shown to deliver drugs across the skin layers [1]. Hence the aim of present study was to develop sustained topical ethosomal gel of aceclofenac and to evaluate with respect to various *in vitro* evaluation tests. Presently no scientific reports are available on the formulation of topical drug delivery system of ethosomal gel of aceclofenac. Hence, in this study an attempt has been made to prepare and evaluate a topical drug delivery system of ethosomal gel of aceclofenac.

#### **Material and methods**

Aceclofenac was received as a gift sample from Lupin Research Park, Pune, Soya phosphatidyl choline were purchased from Sigma-Aldrich Chemie, Germany, ethanol, propylene glycol, methanol, chloroform, isopropyl alcohol from Qualigens, Mumbai Carbopol 934k from S d fine-chemical limited Mumbai, All other chemicals used were of analytical grade.

#### **Method of preparation**

Aceclofenac ethosome was prepared as described by Touitou *et al.* The ethosomal system of aceclofenac was comprised of 0.5 – 4 % phospholipids, 10 – 50 %w/w of ethanol, 1 %w/w aceclofenac, 5 % propylene glycol and water upto 100 %w/w. Aceclofenac ethosomes were prepared using different concentrations of lecithin, ethanol, isopropyl alcohol, propylene glycol and aceclofenac as given in the (table 3.4 and 3.5). Phospholipids and drug were dissolved in ethanol and propylene glycol. The mixture was heated to 30° C in water bath. In this solution distilled water was added slowly in a fine stream with a constant mixing (Mechanical stirrer, Remi equipment, Mumbai) at 700 rpm in a closed vessel. The temperature was maintained at 30° C during the experiment. The mixing was continued for 5 minutes. The preparation was stored at 4° C. Ethosome prepared by the above procedure were subjected to sonication at 4°c using probe sonicator in 3 cycles of 5 minutes with 5 minutes rest between the cycles [2].

### Incorporation into Gel

Carbopol 934P 0.75% w/v was soaked in minimum amount of water for an hour. Ethosomal suspensions 20 ml containing aceclofenac (200mg) was added to the swollen polymer under stirring. Stirring was contained were then incorporated in gel with continuous stirring at 700 rpm in a closed vessel and maintained at temperature 30°C until homogeneous ethosomal gels were achieved. The pH was then adjusted to neutral using (TEM) triethanol amine and stirred slowly till a gel was obtained. pH measurement of the formulations were carried out using pH meter by dipping the glass electrode completely into the semisolid formulation so as to cover the electrode [3, 4].

### Fourier Transform Infrared Spectroscopy

Infrared spectroscopy was conducted using a Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded in the region of 4000 to 400  $\text{cm}^{-1}$ . The procedure consisted of dispersing a sample (drug and drug-excipient mixture, 1:1 ratio) in KBr (200-400 mg) and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained. All spectra were collected as an average of three scans at a resolution of 2  $\text{cm}^{-1}$ .

**Table 1. Composition and characteristics of ethosomal gel containing ethanol.**

Composition in	Etho1	Etho2	Etho3	Etho4	Etho5	Etho6	Etho7	Etho8	Etho9	Etho10
% w/w										
Drug	1	1	1	1	1	1	1	1	1	1
Phospholipid	2	2	2	2	3	3	3	3	4	3
Ethanol	40	35	30	25	40	35	30	25	40	50
Propylene glycol	1	1	1	1	1	1	1	1	1	1
Carbopol 934k	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Water	q.s									
Characterization										
%Entrapment efficiency	83.0± 3	79.2± 6	77.8± 5	73.8± 6	93.2± 6	91.0± 8	89.5± 1	83.5± 1	90.6± 6	81.6± 9
Vesicle size	-	-	-	-	0.696	-	1.140	-	-	-
pH	6.6	-	-	-	5.7	-	6.2	-	-	-
Gel strength	++	++	++	++	++	++	++	++	++	++
J (Flux)	198.8	-	-	-	226.1	-	201.8	-	-	-
R (Regression coefficient)	0.9886	-	-	-	0.9464	-	0.991	-	-	-

### Differential Scanning Calorimetry

Differential scanning calorimetry was performed by using DSC-60. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), Thermal analyzer (TA 60) and operating software TA 60 from (Shimadzu Corporation, Japan.) The samples were placed in aluminium pans and were crimped,

followed by heating under nitrogen flow (30 ml/min) at a scanning rate of 5°C/min from 25°C to 200°C. Aluminium pan containing same quantity of indium was used as reference. The heat flow as a function of temperature was measured for both the drug and drug-excipient mixture.

### Optical Microscope Observation

The ethosomal dispersion was spread on the glass slide using a glass rod. Formation of multilamellar vesicles was confirmed by examining the ethosomal suspension under an optical microscope with the magnification power of 100 x. (Olympus CX41, Philippines) Photographs of vesicles were taken using Olympus camera.

**Table 2. Composition and characteristics of ethosomal gel containing IPA**

Composition	Etho11	Etho12	Etho13	Etho14
<b>in % w/w</b>				
Drug	1	1	1	1
Phospholipid	2	3	3	3
Isopropyl alcohol	40	40	30	(20IPA+ 20Ethanol)
Propylene glycol	1	1	1	1
Carbopol 934k	0.75	0.75	0.75	0.75
Water	q.s	q.s	q.s	q.s
Characterization				
%Entrapment	88.01	95.73	84.04	83.82
Vesicle size	-	-	-	-
pH	-	7.2	7.1	7.4
Gel strength	++	++	++	++
J (Flux)	-	159	-	146
R(Regression)	-	-	-	-

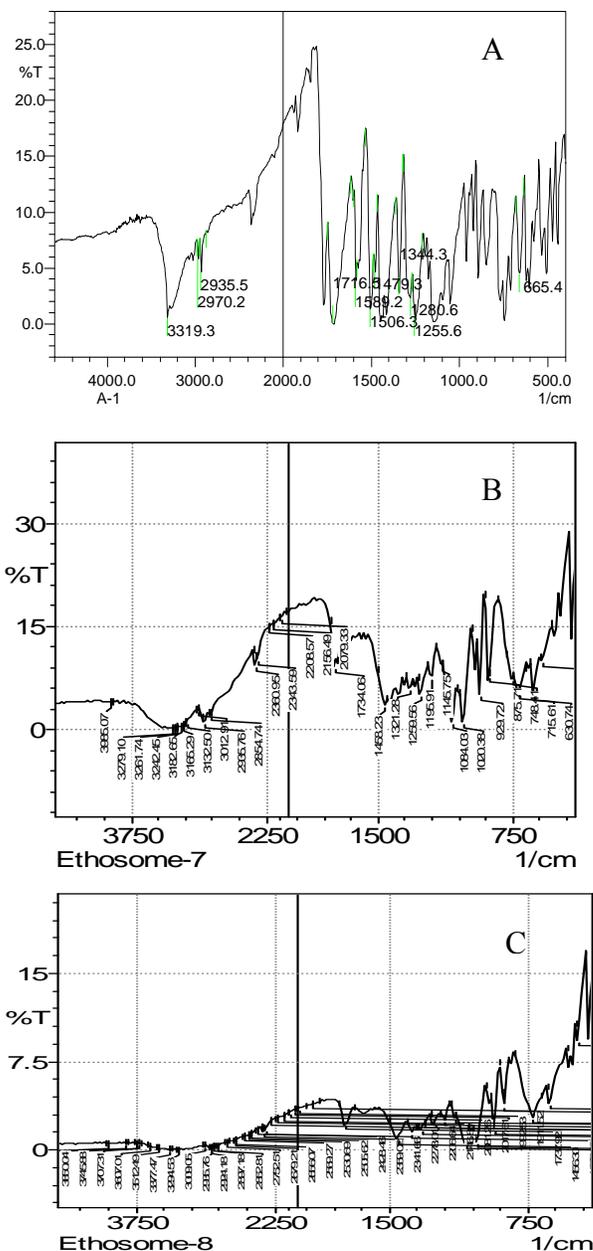
### Vesicular characterization

#### (a) Vesicle size and size distribution

The vesicle size, size distribution and zeta potential of optimized ethosomes formulation were determine by the dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster (ZEM 500962, Malvern, UK). The electric potential of ethosome, including its stern layer (ZETA POTENTIAL) was determine by injecting the diluted system into a zeta potential measurement cell.

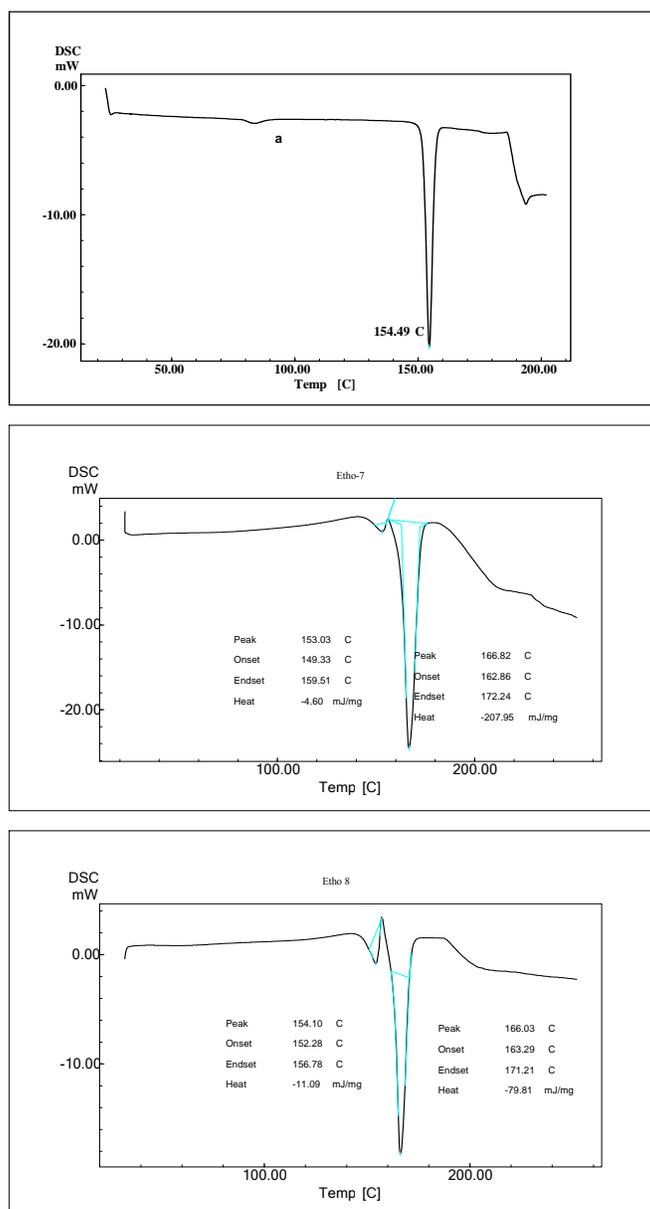
#### (b) Vesicular shape and surface morphology

Scanning electronic microscopy (SEM) was also conducted to characterize the surface morphology of the ethosome vesicles were analyzed by scanning electron microscopy (SEM). Prior to analysis, the ethosome were mounted onto double-sided tape that has previously been secured on copper stubs and coated with platinum, then analyzed at different magnifications.



**Figure 1. IR spectra: A) aceclofenac, B) ethosome 7, C) ethosome 8**

Ethosome vesicles were visualized using a Philips TEM (transmission electron microscope) CM 12 electron microscope (TEM, Eindhoven, The Netherlands), with an accelerating voltage of 100 kV. Samples were negatively stained with a 1% aqueous solution of PTA (phosphotungstic acid). Ethosomal solution (10  $\mu$ l) was dried on a microscopic carbon-coated grid for staining. The excess solution was removed by blotting. After drying, the specimen was viewed under the microscope at a 10–100 k fold enlargement.



**Figure 2. DSC thermogram of A) aceclofenac, B)Etho7, C)Etho8.**

### Entrapment efficiency

The entrapment capacity of aceclofenac ethosomes was measured by the ultracentrifuge method. Vesicular preparations containing 1% aceclofenac were kept overnight at 4°C and centrifuged in a ultracentrifuge (Remi) equipped with TLA-45 rotor at 4°C, at 30 000 rpm for 2 h. aceclofenac was assayed both in the sediment and in the supernatant. The entrapment capacity of aceclofenac was calculated from the relationship  $[(T-C)/T] \times 100$ , where T is the total amount of aceclofenac that is detected both in the supernatant and sediment, and C is the amount of aceclofenac detected only in the supernatant [5].

### In vitro permeation studies

The in-vitro skin permeation of aceclofenac from ethosomal formulation was studied using locally fabricated diffusion cell. The in-vitro diffusion of the drug through semipermeable membrane was performed. The semi permeable membrane soaked in a buffer for 6-8 hours. It was clamped carefully to one end of the hollow glass tube of 17 mm (area 2.011 cm<sup>2</sup>) (dialysis cell). This acted as donor compartment. 50 ml of PBS 6.4 was taken in a beaker which was used as a receptor compartment. The known quantity was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at  $37 \pm 0.1$  °C. The solutions of the receptor side were stirred by externally driven Teflon-coated magnetic bars. At predetermined time intervals, sample was withdrawn and replaced by 3 ml of PBS. The drug concentrations in the aliquot were determined at 275 nm against appropriate blank. This experiment was done in triplicate and average value was reported. In-vitro skin permeation studies were conducted for different formulation and effect of variation in composition on permeation rate. Ethanol and phospholipid skin permeation from ethosomal systems or isopropyl alcohol and phospholipids skin permeation from ethosomal system was also studied.

### Permeation enhancers

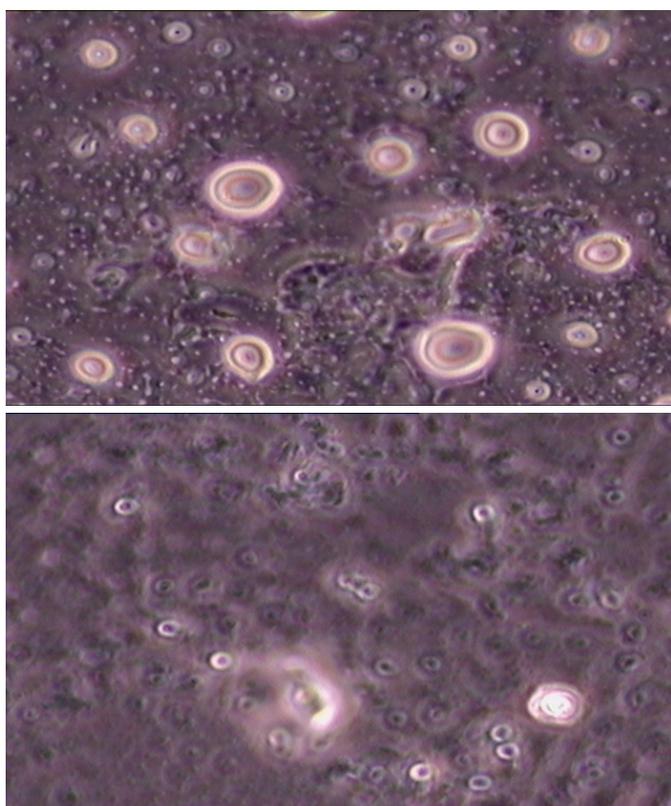
These are compound, which promote skin permeability by altering the skin as a barrier to the flux of a desired penetrant.

The flux, J, of drug across the skin can be written as:

$$J = D \frac{dc}{dx}$$

Where D is the diffusion coefficient and is a function of the size, shape and flexibility of the diffusing molecule as well as the membrane resistance, C is the concentration of the diffusing species, X is the spatial coordinate.

The *in vitro* skin permeation of aceclofenac from ethosomal formulation was studied using diffusion cell specially designed in our laboratory according to the literates. The effective permeation area of the diffusion cell is 2.011 cm<sup>2</sup>. The temperature was maintained at 37 ± 0.5°C [6].



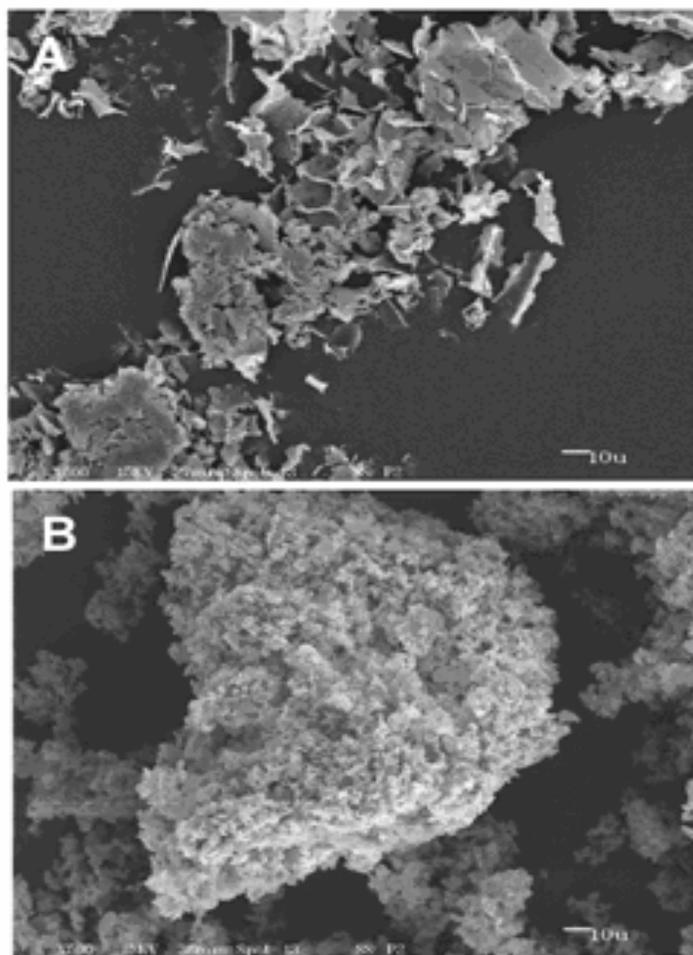
**Figure 3. Microscopic images of aceclofenac ethosome (Etho 5)**

#### pH Measurements

The pH measurements of the formulations were carried out using a pH meter by dipping the glass electrode completely into the semisolid formulation as to cover the electrode showed in (table 1 and 2).

#### Stability study

All regulatory bodies accept only real time data for any drug or pharmaceutical for all purpose of assessing the shelf life and only accelerated stability studies may serve as a tool for formulation screening and stability issues related to shipping or storage at room temperature. The accelerated stability studies were carried out in accordance with the ICH guidelines. The ability of vesicles to retain the drug was assessed by keeping the ethosomal suspension at different temperature. Optimized ethosome formulation was selected for stability studies of vesicles. The vesicular suspension was kept in sealed vials (10ml) at 4 ± 2°C and at room temperature for 45 days. Percent entrapment was determined at different time intervals. It was observed that the ethosomal system was more stable at 4 ± 2°C.



**Figure 4. SEM of ethosome powder formulation Etho 5 after lyophilization.**

## Result and discussion

### IR, DSC, SEM studies

The possible interaction between the drug and the excipients were studied by IR spectroscopy and DSC. IR spectra of pure aceclofenac, its physical mixture with phospholipids and the prepared formulation are shown in Fig 1. Pure aceclofenac showed major peaks at 3317.3, 2970.2, 2935.5, 1716.5, 1589.2, 1506.3, 1479.3, 1344.3, 1280.6, 1255.6, and 665.4  $\text{cm}^{-1}$ . The result revealed no considerable change in the IR peaks of aceclofenac in the physical mixture or in the prepared crystals when compared to pure drug there by indicating the absence of any interaction.

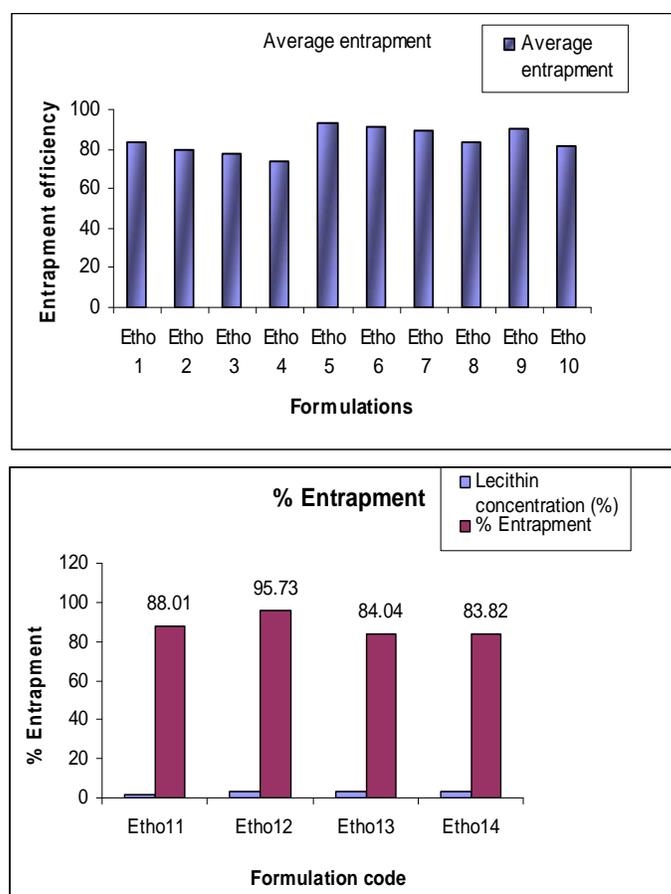
The results of DSC studies are given in fig.2. Pure aceclofenac showed a sharp endotherm at 154.49°C corresponding to its melting point/transition temperature. There was no appreciable change in the melting endotherms of the physical mixture (aceclofenac + polymer) compared to pure drug. This observation further supports the IR spectroscopy results, which indicated the absence of any interactions between drug and additives used in the preparation. However there was slight decrease in the melting point of the drug. It was also observed that there was a noticeable reduction in the enthalpy of the formulation with compare to aceclofenac formulation (Etho7) showed -4.6  $\text{J/mg}$ . The lowest melting point was 153°C. Surface morphology and three-dimensional nature of ethosomes were further confirmed by SEM, justifying the vesicular characteristics possessed by this novel carrier (Fig.4).

Ethosomal carriers are a system containing soft vesicles and are composed mainly of phospholipids (phosphatidylcholine PC), ethanol, isopropyl alcohol in relative higher concentration and water. The compositions of different ethosomal formulation are shown in the table1 and 2. Ethosome are soft, phospholipids nanovesicles (tiny, bubble-like, lipid spheres) which due to their structure, are able to overcome the natural dermal barrier, delivering drugs through the skin layers. The ethosome delivery system can be modulated not only for enhanced skin penetration but localizes the drug at the site of action, enable drugs to reach the deep skin layers.

### Microscope Observation

The ethosomal dispersion was spread on the glass slide using a glass rod. Formation of multilamellar vesicles was confirmed by examining the ethosomal suspension

under an optical microscope with the magnification power of 100 x. (Olympus CX41, Philippines) Photographs of vesicles were taken using Olympus camera. Vesicular size and entrapment efficiency are the basic parameter on the basis of which the formulations were optimized. The effect of phospholipids and ethanol concentration on the size distribution of ethosome vesicles was investigated using dynamic light scattering (DLS) method. Formulation Etho 5 and Etho 7 have same concentration of lecithin 3% but ethanol concentration is varied. In formulation Etho 5 ethanol concentration is 40% and in formulation Etho 7 the ethanol concentration is 30%.

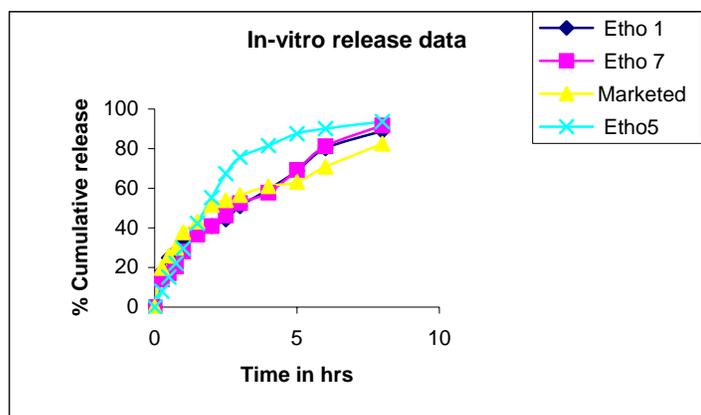


**Figure 5. Percent entrapment of A) Ethanol concentration B) Isopropyl alcohol concentration**

For ethosome formulation with 40% ethanol and 3% phospholipids, the data shows narrow particle size distribution with an average vesicles size of 0.696  $\mu\text{m}$ . In the ethanol concentration range of 20%-45%, the size of the vesicles increase with decreasing the ethanol

concentration. It was observed that preparation containing 30% ethanol formulation Etho 7 the vesicular size is 1.140  $\mu\text{m}$  and the preparation containing 40% ethanol formulation Etho 5 the vesicular size is 0.696  $\mu\text{m}$ . The size of the vesicles was decreased with increasing ethanol concentration. The size analysis was done for formulation Etho 5 and Etho 7 which contain same concentration of phospholipids. Hence the effect of phospholipids concentration on size could not be explained. The zeta seizer report showed that there was less difference in vesicles size. Zeta potential of formulation was  $-6.74\text{s mv}$  which showed that the higher concentration of ethanol make the negative charge on vesicles surface.

These result further supported by the observation of methotrexate (MTX) loaded ethosome made by Dubey *et al* [7, 8]. Though with increase in ethanol concentration the vesicular size decreased, a phenomenon observed by number of scientific groups. This indicates that at higher ethanol concentration the membrane thickness is reduced considerably, probably due to the formation of a phase with interpenetrating hydrocarbon chain. Further, an interesting possible mechanism given by Lasic *et al.*; 1998, which state that ethanol causes a modification of the net charge of the system and confers it some degree of steric stabilization that may finally lead to a decrease in the mean particle size, could also be considered.

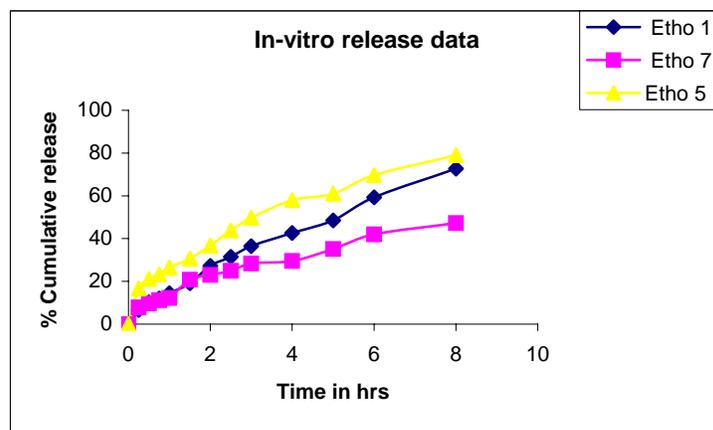


**Figure 6. *In vitro* drug release of aceclofenac from Etho 1, 5, 7 and Marketed formulations**

#### Entrapment efficiency

The entrapment efficiency of ethosomes was determined for all formulation. Effect of ethanol

concentration was observed on percent entrapment of ethosomes. The entrapment efficiency was maximum for formulation Etho 5 (93.3%) and minimum for formulation Etho 4 (74.02%). The entrapment was found to increase with increase in ethanol concentration, however percent entrapment decreased when ethanol concentration exceeded 40 %. Solubility of aceclofenac also increased when ethanol was used in higher concentration. Therefore, the drug also entrapped in the core of the vesicles. But as the concentration of ethanol increased above 40 % there was leakage of the drug from bilayer of the vesicles. The entrapment efficiency increased with an increase in concentration of lecithin but, above 3% of lecithin concentration there was no significant increase in percent entrapment. In formulations Etho 1 to Etho 4, 2% lecithin was used and the concentration of ethanol was varied. Formulation Etho 1 showed maximum entrapment efficiency (83%). Formulation Etho4 showed minimum entrapment efficiency (73.84%) as shown in the (Table 1). and bar graph (Fig 5). The results showed that as the concentration of ethanol increased from 25% to 40% the entrapment efficiency increased. In formulations Etho 5 to Etho 8, 3% lecithin was used and the concentration of ethanol varied. Formulation Etho 5 showed the maximum entrapment efficiency (93%). Formulation Etho 8 showed the minimum entrapment efficiency (83.56%) as shown in the (Table 1). and bar graph (Fig 5). The results show that as the concentration of ethanol increased from 25% to 40% the entrapment efficiency increased.



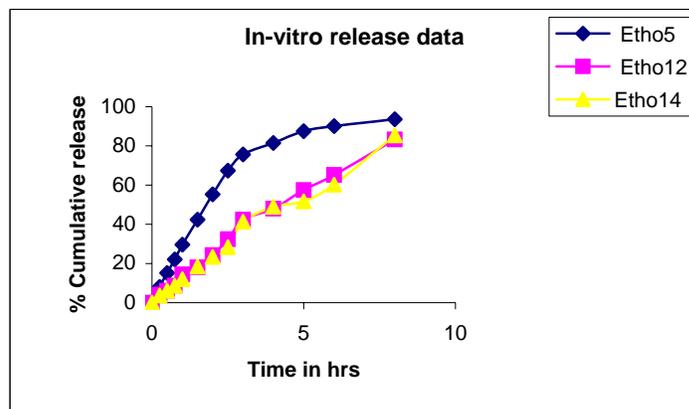
**Figure 7. *In vitro* release of aceclofenac from mouse skin**

However when we compare formulation Etho 1 and formulation Etho 5 both have similar ethanol concentration 40% but differ in lecithin concentration. In formulation Etho 1 the lecithin concentration was 2% and in formulation Etho 5 the lecithin concentration was 3%. It is shown that increase in the lecithin concentration increases entrapment. In formulation Etho 1 the entrapment was (83%) and in formulation Etho 5 the entrapment was (93.2%).

The entrapment efficiency in formulation Etho 5 to Etho 8 was much greater compared to formulation Etho1 to Etho4. Formulation Etho 5-8 have 3% lecithin where as formulation Etho 1-4 have 2% lecithin. This indicates that lecithin play an important role in percent entrapment. This was shown in the bar graph. The entrapment efficiency increased with an increase in the concentration of lecithin but, above 3% lecithin concentration there was no significant increase in the entrapment efficiency. This is evident from the entrapment efficiency of formulation Etho 9 (90%) in which 4% lecithin was used. In formulation Etho10 the ethanol concentration was 50% but there was no increase in the entrapment. Formulation Etho 5 and Etho 10 have same composition but differ in the ethanol concentration. Increasing the ethanol to 40% w/w, increased in the entrapment efficiency and further increased in the ethanol concentration (> 40%w/w) the vesicle membrane become more permeable that leads to decrease in the entrapment efficiency of ethosome formulation. In formulation Etho 5 the entrapment was (93.3%) and where as in formulation Etho 10 the entrapment was low (81.6%).

Another attempt was made with the help of isopropyl alcohol. As such there is no work reporting the effect of isopropyl alcohol on entrapment and penetration. Formulations of ethosomes were made to compare the effect of ethanol and isopropyl alcohol on entrapment and permeation. The entrapment efficiency of ethosomes was determined for all formulation. Effect of isopropyl alcohol concentration was observed on percent entrapment of ethosomes. The entrapment efficiency was maximum for Etho 12 (95%) and minimum for Etho 14 (83.8%). In formulation Etho 14 we use 20% of ethanol and 20% of isopropyl alcohol. Where as formulation Etho 12 contain only isopropyl alcohol 40%. The entrapment efficiency increased with an increase in the concentration of lecithin. In formulation Etho 11, 2% lecithin was used the entrapment was (88%) and in formulation Etho 12, 3%

lecithin was used the entrapment was (95.7%) but isopropyl alcohol concentration is same 40%. shown in the (Table 1). and bar graph (Fig 5). When we compare the Etho 12, Etho 13 and Etho 14 the alcohol concentration is varied but the lecithin concentration is same. The formulation Etho 12 showed maximum entrapment 95% compare to Etho 13 and Etho 14, which showed 84% and 83.3% entrapment efficiency. This indicates that isopropyl alcohol has better entrapping efficiency than ethanol.



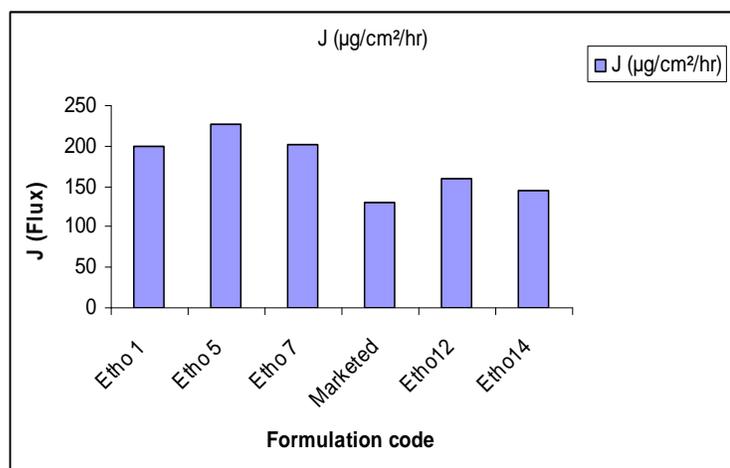
**Figure 8. In-vitro drug release of aceclofenac from Etho 5, Etho12 and Etho14 formulations**

#### ***In vitro* permeation study**

The composition of optimized formulation was as follow (1) Aceclofenac 1% (2) soya lecithin 3% (3) ethanol 40% (4) Propylene glycol 5% (5) water upto 100%. Release study of different formulations was undertaken in a modified diffusion apparatus containing sigma membrane mouse skin. All the formulations had propylene glycol as a skin penetration enhancer. The drug incorporated was 200 mg; lecithin concentration is 2 % and ethanol concentration 40 %. The formulation Etho 1 showed 88.88% drug release within 8 h.

The *in vitro* drug releases of various formulations are shown in (graph 6) The cumulative release of the ethosome gel (Etho5) was 93.6% in 8 hrs, which is greater than that of marketed gel (Zynac gel), which showed 82.8% in 8 hrs. There was increase in cumulative amount of drug permeated at higher ethanol concentration upto 40 % then there was reduction in permeation. This was due to the disruption of lipid bilayer at higher ethanol concentration. Ethanol provides the vesicles with soft flexible characteristics,

which allow them to more easily penetrate into deeper layers of the skin. When lecithin concentration was increased, there was increase in drug permeation. Use of propylene glycol also influenced the amount of drug permeation. Propylene glycol also acts as permeation enhancer, which increases permeability of vesicle through biological membrane due to synergistic effect with ethanol on bilayer of the vesicles.<sup>(121-124)</sup> Formulations (Etho 7) containing 3 % lecithin and 30 % ethanol showed 91 % drug release within 8 h. The release study was also carried out using mouse skin. The *in vitro* drug release of various formulations is shown in (graph 7). Formulation (Etho 1) containing 2 % lecithin and 40 % ethanol showed 72.7% drug release within 8 h. Formulation (Etho 5) containing 3 % lecithin and 40 % ethanol showed 79 % drug release within 8 h. Formulation (Etho 7) containing 3% lecithin and 30% ethanol result showed 47.2% drug release within 8 h. This observation indicates that alcohol is important for penetration compared to lecithin concentration. Such an observation was not seen in commercial sigma membrane. Since the mouse skin is a biological membrane consisting of lipid layers, penetration requires enhancers.



**Figure 9. Permeation parameter of aceclofenac via different formulation**

Ethanol provides the vesicles with soft flexible characteristic, which allow them to more easily penetrate into deeper layers of the skin. When ethosomal carriers, which contain ethanol, and soft small vesicles are applied to the skin a number of concomitant processes may take place, involving the

stratum corneum and pilosebaceous pathway. Evidence of existence of the follicular transport pathway taken by the lipid vesicles have been reported [7-17]. first, ethanol disturbs the organization of the stratum corneum lipid bilayer and enhances its lipid fluidity. The flexible ethosome vesicles can then penetrate the disturbed SC bilayers and even forge a pathway through the skin by virtue of the particulate nature. The release of the drug in deep layer of the skin and its transdermal absorption could then be the result of fusion of ethosome with skin lipid and drug release at various points along the penetration pathway [9].

The *in vitro* drug release of various formulations is shown in (graph 8) Formulation (Etho 12) containing 3 % lecithin and 40 % isopropyl alcohol showed 83.2% drug release in 8 h. whereas formulation (Etho 14) containing 3 % lecithin, 20 % ethanol and 20 % isopropyl alcohol showed 85.4 % drug release within 8 h. When we compare formulation Etho 5,12 and 14. Formulation Etho 5 showed higher release than that of Etho 12 and Etho 14. When we consider the amount of drug released from formulation Etho 12, 14, formulation Etho 14 released higher (85.4%) as compared to formulation Etho 12 (83.2%). This indicates that isopropyl alcohol does not promote better release as ethyl alcohol. The incorporation of ethyl alcohol in formulation Etho 14 (50%) slightly increased the release. This is further supported by comparison of formulation Etho 5 and Etho 12. Formulation Etho 5 contains ethyl alcohol and formulation Etho 12 contains isopropyl alcohol. The release of Etho 5 is 93% and Etho 12 is 83.2%. Hence we can conclude that isopropyl alcohol has poor influence on release.

The values of different ethosomal formulation were observed between 116.5 µg/cm²/hr to 226.1 µg/cm²/hr. Formulation (Etho 5) shows maximum J value 226.1 µg/cm²/hr as compared to marketed one 131.1 µg/cm²/hr. The data also suggested that the value of flux also depends on the ethanol concentration. As the concentration of ethanol increased, flux value of aceclofenac increased. Because in formulation Etho 5 ethanol concentration is 40 % and in formulation Etho 7 ethanol concentration is 30 %.

Formulation (Etho 12) shows J value 159.0 µg/cm²/hr. Formulation (Etho 14) which is the combination of (20% IPA+ 20% Ethanol) shows J value 146.0 µg/cm²/hr. All the formulation shows higher J value

compared to marketed one The transdermal flux of aceclofenac was also carried out using mouse skin. Formulation Etho 5 (171.0  $\mu\text{g}/\text{cm}^2/\text{hr}$ ) shows higher J value then the marketed one (131.1  $\mu\text{g}/\text{cm}^2/\text{hr}$ ).The data also suggested that the value of flux also depends on the ethanol concentration. As the concentration of ethanol increased, flux value of aceclofenac increased. Formulation Etho 5 and Etho 1 containing same percent of ethanol 40% whereas compare to formulation Etho 7 containing 30% ethanol. These result further supported by the observation of zidovudine-loaded ethosome made by Jain *et al* (2004). The data suggested transdermal flux of zidovudine increased with increase in ethanol concentration. The effect on ethanol on stratum corneum lipids and on vesicle fluidity as well as a dynamic interaction between ethosomes and the stratum corneum all may contribute to the superior skin penetration ability of ethosomes [9-12].

**Table 3. Stability studies date of aceclofenac**

Time ( days)	Percent entrapment ( $4 \pm 2^\circ\text{C}$ )	Percent entrapment ( RT)
1	91.1 $\pm$ 0.8	91.1 $\pm$ 0.8
15	91.1 $\pm$ 0.5	90.8 $\pm$ 0.2
30	90.4 $\pm$ 0.6	89.2 $\pm$ 0.4
45	89.9 $\pm$ 0.6	88.9 $\pm$ 0.8

Mean  $\pm$  SD, n = 3, RT = Room temperature

### Analysis of drug release mechanism

When the data was plotted as cumulative percentage of drug permeated versus time, the profile show that the gel releases the drug rapidly in the initial hours followed by the slow release in the next hours. The graph show that the profiles did not match zero order release parttern. Hence the data was subjected to first order equation and the line were not linear ( $R^2 = 0.671 - 0.9047$ ). further to find out whether diffusion is involved in the drug release, the data was subjected to Higuchi's equation the line obtained comparatively more linear ( $R^2 = 0.9464 - 0.991$ ). To confirm further release mechanism of drug, the data was subjected to Korsmeyer's equation. ( $R^2 = 0.9638 - 0.989$ ). As done in the previous experiment, the drug permeation data was plotted according to first order, Higuchi's and Korsmeyer's equation to know the release mechanisms. The formulation was not shown fair linearity with respect to first order ( $R^2 = 0.4554 - 0.6396$ ) and

Higuchi's equation ( $R^2 = 0.9684 - 0.9933$ ) and Korsmeyer's equation ( $R^2 = 0.4717 - 0.5629$ ) these data showed that the previous results showed linear line compare to skin release. The observation confirmed that diffusion is dominant mechanism for drug release.

### Stability study

The ability of vesicles to retain the drug was assessed by keeping the ethosomal suspension at different temperature. Optimized ethosome formulation (Etho 5) was selected for stability studies of vesicles. The vesicular suspension was kept in sealed vials (10ml) at  $4 \pm 2^\circ\text{C}$  and at room temperature for 45 days. Percent entrapment was determined at different time intervals. It was observed that the ethosomal system was more stable at  $4 \pm 2^\circ\text{C}$  showed in (table 3).

### Conclusion

The aim of the current investigation is to evaluate the transdermal potential of novel vesicular carrier, ethosomes, bearing aceclofenac, Non-steroidal anti-inflammatory drugs (NSAIDs) agents having limited transdermal permeation. The result advocates the potential of ethosome formulation to treat rheumatic disease where facilitated penetration of the drug into muscle and synovial fluid is desirable. In light of the data obtained from experimental work we can expect the ethosome formulation to be safe and very efficient as a drug carrier for systemic as well as topical delivery of drug, holding future in effective transdermal delivery. Ethosome has proven to be superior even to the marketed gel for the topical administration. Further, these finding may help the industry for development and scaling up a new formulation.

### References

1. Touitou E, Merdan VM. Vesicular carriers for topical delivery. *Acta Technologiae et legis Medicament* ; 1998; 91: 1-6
2. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J. Control. Release*, 2000; 65:403
3. Touitou, E. Composition of applying active substance to or through the skin., US patent, 1996,5,716,638,

4. Touitou, E. Composition of applying active substance to or through the skin., US patent, 1998,5,540,934,
5. Dubey V, Jain NK. Melatonin loaded liposomes: physicochemical characterization and enhanced transdermal delivery. *Euro J Pharm* 2007; 67: 398-405.
6. Mishra AN, Jain NK. Transdermal drug delivery system, controlled and novel drug delivery system, Edited by Jain N. K. (2007)
7. Klecker JT RW, Collitus JM. *Clin. Pharmacol* 1987; 41: 407.
8. Horwitz E, Pisanty S, Czerniski R, Touitou E. *Oral Surg. b Oral Med. Oral radiol.* 1999; 87: 700.
9. Jain S, Umamaheshwari RB, Bhadra D, Jain NK. *Ind. J. Pharm. Sci.* 2004; 66: 72-81.
10. Jain S, Jain S, Jain NK. Transfersomes for enhanced delivery of dexamethasone, Proceeding of 28th Conference of CRS, USA, 2001, 5207.
11. Jain S, Jain S, Jain NK. *Drug Delivery Tech.* 2002; 2: 70-71.
12. Jain S, Jain P, Jain NK. *Drug Dev. Ind. Pharm.* 2003; 29: 1013-1026.
13. Jain S, Jain N, Bhadra D, Tiwary AK, Jain NK. Sustained and targeted delivery of an analgesic agent using ultradeformable lipid vesicles. Proceeding of the 54rd Indian Pharmaceutical conference, Pune (India), 2002: A-67
14. Jain S, Jain N, Bhadra D, Tiwary AK, Jain NK. *Curr Drug Del* 2004; 2: 222-233
15. Jain S, Tiwary AK, Jain NK. Sustained and targeted transdermal delivery of an anti-HIV agent using elastic liposomal formulation: Mechanism of action. *Curr Drug Del* 2005; 3: 157-164
16. Touitou E, Godin B, Weiss C. Enhanced delivery of drugs into and across the skin by ethosomal carriers. *Drug Develop Res* 2000;50: 406 - 415
17. Touitou E, Godin B, Dayan N, Weiss C, Piliponsky A. Levi-Schaffer F.; Intracellular delivery mediated by an ethosomal carrier. *Biomaterials* 2001; 22: 3053-3059
18. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. *Biomaterials* 2000; 21: 1879-1885
19. Touitou E. Drug delivery across the skin. *Biol. Therm.* 2002; 2: 723-733