

ORIGINAL RESEARCH ARTICLE

## Preparation and Characterization of Repaglinide Loaded Ethosomal Gel for the Treatment of NIDDM

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### ABSTRACT

The main objective of current investigation is to evaluate the transdermal sustained release delivery systems potential of self penetrating liposomes– ‘ethosomes’. Repaglinide entrapped ethosomal carriers were prepared, optimized and characterized for vesicular shape, vesicular size, entrapment efficiency, stability, *in-vitro* drug release and *ex-vivo* drug release study. Effect of different concentration of lipid and ethanol on different properties of ethosomal formulations (EFC-1 to EFC-9 and EFH-1 to EFH-9) were studied. The size of the vesicles was found to have increased with increasing lipid concentration. Also, it was observed that the size of the vesicles decreased with increasing ethanol concentration. EFC-6 ethosomes with 20mg lipids and 15 ml ethanol formulated with cold method were found to show highest release ( $73.23 \pm 2.32$ ). In final phase of formulation development, EFC-6 ethosomes were converted to gel (EFC-6) using three different carbopol concentrations (1, 1.5, 2.0% w/w). Repaglinide encapsulated in EFC-6 ethosomes in 1.0% gel was found to have shown maximum *in-vitro* drug release ( $89.67 \pm 2.35$ ) as compared to other carbopol concentrations and free drug gel. Stability studies were performed for EFC-6 ethosomes to study effect of different temperature conditions on percent entrapment and optimized gel formulation to study content uniformity and physical appearance for 3 months. Finally, in light of the current data, it can be concluded that ethosomes were a promising candidate for transdermal delivery of repaglinide and the results advocate the potential of ethosomes of being a safe and very efficient drug carrier for transdermal delivery of drug.

**Purpose:** The present study concerns the production and characterization of ethosome as drug delivery systems for repaglinide, taken as model anti-diabetic drug.

**Methods:** Dispersions were produced by cold and hot method. Morphology and dimensional distribution of the disperse phase have been characterized by scanning electron microscopy and photon correlation spectroscopy, respectively. An *in-vitro* diffusion study was conducted by Franz cell associated to stratum corneum epidermis membrane on ethosome dispersions viscosized by carbopol 934.

**Key words:** Transdermal sustained release delivery systems, liposomes, Repaglinide, ethosomes, shape, vesicular size, entrapment efficiency, stability, *in-vitro* and *ex-vivo* drug release study.

### INTRODUCTION

Oral and parenteral delivery is the most common therapy used in treatment of diabetes. Controlled drug delivery into a body is one of the novel methods in pharmaceutical research. The path way through the skin, for example is a predestined route for the drugs, which undergo degradation and metabolism in the gastrointestinal tract or the liver after oral application.<sup>[1]</sup>

Vesicular approaches, such as liposomes, niosomes, transferosomes and ethosomes also

have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier.<sup>[2]</sup>

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. Ethosomes are soft, malleable vesicles composed mainly of phospholipids (phosphatidylcholine, phosphatidylserine, and phosphatidic acid), ethanol (relatively high concentration) and water.

These “soft vesicles” represents novel vesicular carrier for enhanced delivery to/through skin. The soft, malleable vesicles tailored for enhanced delivery of active agents. The size of Ethosomes can be modulated to range anywhere from 30nm to a few microns.<sup>[3]</sup>

The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also, because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum lipids.<sup>[4]</sup>

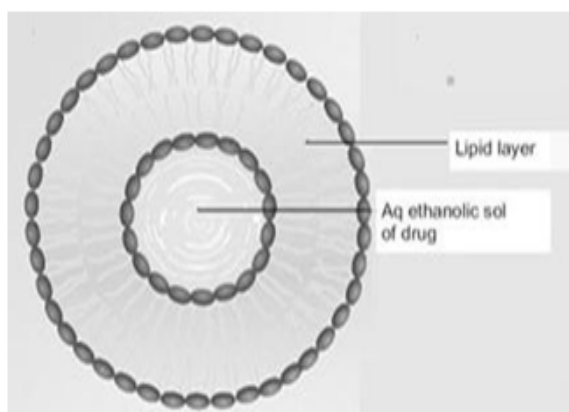


Fig 1: Structure of Ethosome

A possible mechanism for this interaction has been proposed. The drug absorption probably occurs in following two phases:

- **Ethanol effect:** Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.
- **Ethosomes effect:** Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin<sup>[5]</sup>.

The Ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, Ethosomes may contain phospholipids

with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC, 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.<sup>[6]</sup>

Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in Ethosomes. However, due to the interdigitation effect of ethanol on lipid bilayer, it was commonly believed that vesicles could not coexist with high concentration of ethanol discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them Ethosomes. The synergistic effect of combination of relatively high concentration of ethanol (20-50%) in vesicular form in Ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in Ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration the Ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids.<sup>[7]</sup>

## MATERIALS AND METHODS

### Materials:

The drug Repaglinidewas a giftsample from JublientPvt. Ltd. (Nodia). Soya lecithin, Ethanol and Phosphate buffer saline (PBS) was purchased from Sigma Aldrich (St.Louis, MO, USA). All chemicals used in thestudy were of analytical grade and used without furtherpurification.

### Methods:

Ethosomeswere prepared by two different methods

#### Cold Method:

This is the most common and widely used method for the ethosomal preparation. Phospholipid, drug and other lipid materials were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. The mixture was heated at 30 °C in a water bath. Water was heated upto 30 °C in a

separate vessel and was added to the mixture and then stirred for 5 min. The vesicle size of ethosomal formulation was decreased to desire extent using sonication. Finally, the formulation was properly stored.

#### Hot Method:

According to this method, phospholipid was dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. Ethanol, propylene glycol and drug was mixed in a separate vessel and heated upto 40°C. Organic phase was added to aqueous phase and stirred for 5 min. The vesicle size of ethosomal formulation was decreased to desire extent using sonication. Finally, the formulation was properly stored.<sup>[8]</sup>

**Table 1: Composition of Various Ethosomal Formulations**

Batch Code		Concentration	
Cold Method	Hot Method	Lipid (mg)	Ethanol(ml)
EFC-1	EFH-1	10	10
EFC-2	EFH-2	15	10
EFC-3	EFH-3	20	10
EFC-4	EFH-4	10	15
EFC-5	EFH-5	15	15
EFC-6	EFH-6	20	15
EFC-7	EFH-7	10	20
EFC-8	EFH-8	15	20
EFC-9	EFH-9	20	20

### CHARACTERIZATION OF VESICLES:

#### Vesicle Shape:

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. Samples were prepared by mounting the dispersion to silver studs with carbon conductive double-sided tape. The samples were coated with a goldfilm under vacuum for 2 minute. The specimens were transferred to an ISI ABT SX-40A scanning electron microscope and digital images were captured.

#### Particle Size Distribution Measurement:

The particle size of the ethosome was determined through dynamic light scattering. DLS sometimes referred to as photon correlation spectroscopy is a well established technique for measuring the size of molecules and particles in the submicron range.

Analysis was performed using zetasizer 3000 PCS equipped with 5 mW helium neon laser with a wave length output of 633 nm. Samples of filtered dispersions were diluted 1:9v/v with demineralized water. Measurements were made at 25°C at an angle of 90 with a run time of at least 180sec. data was interpreted using the method of cumulates.

#### Determination of Drug Entrapment Efficiency:

With the aim to quantify repaglinide content encapsulated in ethosomes after production, 0.5 ml of ethosomes containing repaglinide was added into the reservoir of centrifuge (Remi Instrument Ltd, Mumbai). After centrifuging the ethosome dispersion at 15,000 rpm for 90 min, the filtrate which contained free drug was removed. The vesicles were lysed using Triton-X 100 (0.1% v/v) and after further dilutions with ethanol, repaglinide was analyzed for drug content using UV Spectrophotometer (Shimadzu) at 243 nm. The entrapment efficiency was expressed as percentage of total drug entrapped using the following formula.

$$\text{Percentage Entrapment} = C/T \times 100$$

Where, T = theoretical amount of drug that was added, and

C = amount of drug detected after dissolving the vesicles.

#### Zetapotential determination:

Zeta potential of the vesicles was determined using Zetasizer (Nano-ZS, Malvern, U.K.). The measurements were made in triplicate.

#### Preparation of ethosomal and free drug gel:

The ethosomal vesicles showing highest *in-vitro* release profile (EFC-6) was further incorporated in to carbopol gel. The specified amount of carbopol (1%, 2.0%, 2.5% w/w) was slowly added in minimum amount of buffer and kept for an hour. Appropriate amount of optimized Repaglinideethosomes (EFC-6) was incorporated to the swollen polymer with continuous stirring and maintained at temperature 30°C until homogeneous ethosomal gels were achieved. The pH was then adjusted to neutral using Triethanolamine and stirred slowly. A non ethosomal Repaglinide gel formulation was also prepared by similar method using 1.0% carbopol.

**Table 2: Composition of various tretinoin and resorcinol gel formulations (%w/w)**

Gel Formulation Code	Drug (in vesicles)	Carbopol (%w/w)	Triethanolamine (%w/w)	Phosphate Buffer
Cb-G1	0.05	1	0.5	Qs
Cb-G2	0.05	2	0.5	Qs
Cb-G3	0.05	2.5	0.5	Qs
G*-4	0.05	2	0.5	Qs

\*contains free drug

### Evaluation of Repaglinide entrapped Ethosomal vesicles and Ethosomal gel: *In vitro* drug release studies

Franz Diffusion Cell with a diffusion area of 9.42 cm<sup>2</sup> and a receiver volume of 14 mL was used in *in-vitro* release studies and all experiments were conducted in triplicate. The acceptor compartment with acceptor fluid (phosphate buffer, pH 5.5) with ethanol at the concentration of 30% was used as the acceptor medium. A suitable size of egg membrane was cut and mounted in between donor cell and acceptor cell of the Franz diffusion cell. The donor compartment was filled with 1mL of vesicular formulation and 1.0 g of gel formulation. The acceptor compartment was maintained at 37 ± 1°C. Samples (1 mL) were withdrawn through sampling port of the Franz diffusion cell at predetermined time intervals over 24 h and immediately replenished with equal volume of fresh phosphate buffer. Samples were analyzed by UV spectrophotometer at  $\lambda_{max}$  243nm. Sink condition was maintained throughout the experiment.

## RESULTS AND DISCUSSIONS

### Characterization of Vesicles:

#### Vesicle shape

Scanning electron microscopy was used for visualizing optimized ethosomes vesicles (EFC-6). Microscopic evaluation revealed that ethosomes were spherical and somewhat irregular in shape and drug is entrapped within the lipid bilayer.

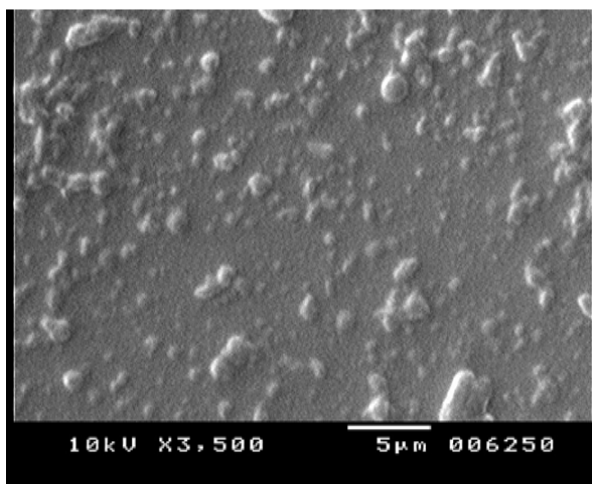


Figure 2: Scanning electron microscope pictures of Repaglinide loaded optimized ethosome vesicles (EFC-6)

#### Entrapment Efficiency:

Drug entrapment within a vesicular carrier is one of the important features to evaluate the potential of the vesicular drug delivery system. For this reason, the entrapment efficiency of Repaglinide

within the Ethosomal vesicles was determined for all formulations. Effect of ethosome composition i.e. lipid and ethanol concentration on Repaglinide loading capacity was also investigated. Different concentration of lipid and ethanol, used for ethosomes preparation has a marked influence on the entrapment efficiency in ethosomes vesicles. The entrapment efficiency of ethosomes was calculated as percent total drug entrapped within the vesicles. The entrapment efficiency was found to vary with the varying concentration of lipid and ethanol. The entrapment was found to be maximum in vesicles of EFC-6 (81.5% ± 0.64%).

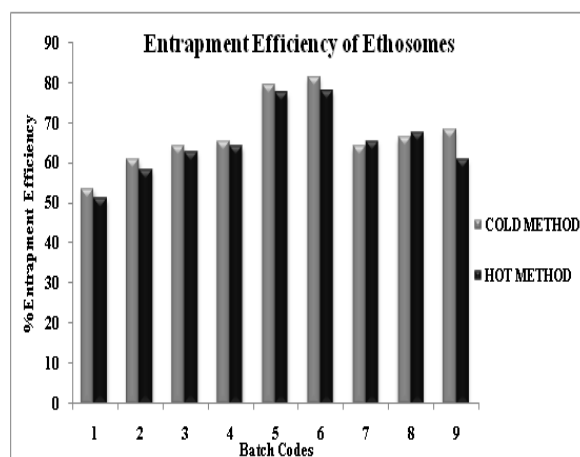


Figure 3: Variation in entrapment efficiencies of repaglinide ethosomal vesicles

#### Zeta potential determination:

Zeta potential is an important parameter that affects the aggregation of vesicles and depicts the physical stability of vesicular systems. Zeta potential of optimized formulation EFC-6 was found to be high (-15.5mV). High zeta potential prevents the aggregation between vesicles and hence, enhances its physical stability. It has been investigated that high zeta potential in ethosomes increase the interbilayer distance owing to electrostatic repulsion. In current investigation, it was observed that there was not much difference in zeta potential of different ethosome formulation. This signifies that there is no major influence of lipid and ethanol on zeta potential of ethosomes.

#### Stability studies of ethosomal gel

##### Physical appearance:

It was observed that optimized gel kept for 1, 2 and 3 months under 4°C ± 2°C as well as 25°C ± 2°C temperature conditions showed no change in their physical appearance.

##### Content uniformity:

Optimized ethosomal gel kept for 1, 2 and 3 months under 5°C ± 2°C as well as 25°C ± 2°C

temperature conditions were studied for uniformity of content. The results showed no significant changes in content uniformity at  $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$  after 3 months. At  $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$  content uniformity was found to show approximately 3.2% decrease ( $81.5 \pm 0.64\%$  to  $78.3 \pm 0.382\%$ ) and at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  content uniformity decreased from ( $81.5 \pm 0.64\%$  to  $65.8 \pm 1.127\%$ ).

### Evaluation of Repaglinide Entrapped Ethosomal Vesicles and Ethosomal Gel:

#### *In vitro* drug release studies of ethosomal vesicles

Repaglinide ethosomal gels were prepared with different concentrations of Carbopol® 934. On comparing the release profile from various ethosomal gels it was evident that EFC-6 shows maximum release. Though there was not much difference in release between (EFC-6 and EFC-5). But it was found consistency of EFC-5 was less as compared to EFC-6. Now since, EFC-6 was found to have shown maximum release and good consistency, it was considered favorable for the final development of the formulation.

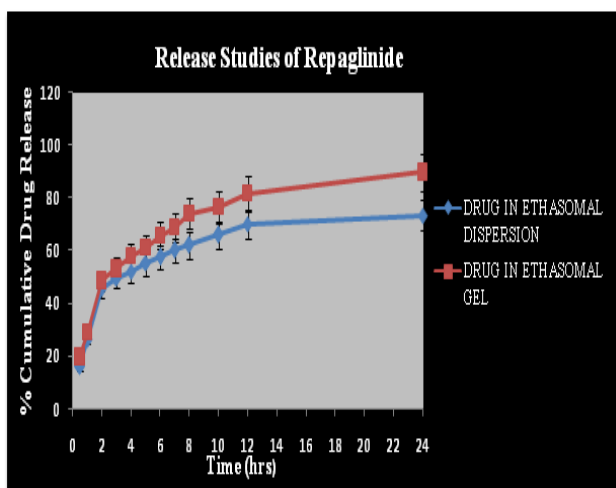


Figure 4: *In vitro* drug release studies

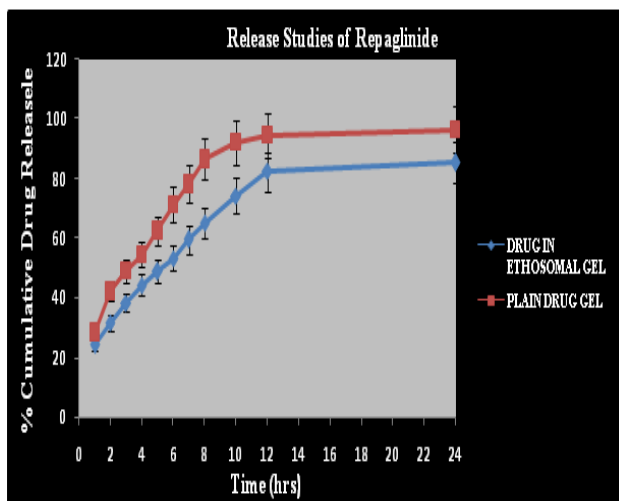


Figure 5: *Ex- Vivo* drug release studies

### CONCLUSION

The present study demonstrated that ethosomes formulation developed for transdermal delivery of repaglinide can overcome problems associated with the oral delivery of repaglinide. It possessed better skin permeation potential. It leads to the improvement in bioavailability of drug, reduction of dose and dosing frequency.

Introduction of ethosomes has initiated a new area in vesicular research for transdermal delivery. Different reports show a promising future of ethosomes for TDDS. Further research in this area will allow better skin deposition of drug in deeper layers of skin and permeation of drug across the skin making the therapy more effective. Ethosomes offer a good opportunity for the non invasive delivery of drug molecules. Thus it can be concluded that ethosomes formulation possesses promising future in effective dermal/transdermal delivery of bioactive agents.

The potential of ultradeformable lipid vesicles for transdermal delivery of repaglinide can be investigated further by performing *in vivo* and pharmacodynamic study and establish it as a promising carrier for the transdermal delivery for the treatment of diabetes which would lead to the increased patient compliance.

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