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Research Article

Preparation and Characterization of Lipid Vesicles of Thiocolchicoside for Transdermal Drug Delivery System

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ABSTRACT

The aim of the current investigation is to evaluate the transdermal potential of novel vesicular carrier, liposomes, ethosomes, transferosomes, having thiocolchicoside, a potent, water soluble muscle relaxant drug with lesser transdermal permeation. Drug loaded liposomes, ethosomes, transferosomes had been prepared using phospholipid and ethanol, were optimized and characterized for entrapment efficiency, vesicular size, zeta potential, *invitro* skin permeation and stability. The ethosomal formulation having 10 mg of phospholipid and 10 ml of ethanol showing the greatest entrapment efficiency (23.16 ± 1%) with small particle size (502 ± 5nm) then liposomes, and transferosomes. The skin permeation studies were performed on ethosomal formulation, liposomal formulation, transferosomes formulation, aqueous drug solution. Among them, ethosomal formulation showed higher cumulative percentage of drug permeation (90 ± 5%) after 24 hours than the other formulations. Differential scanning colorimetery shows no intraction between lipid and drug. Zeta seizer revealed that the ethosomes has smaller vesicular size than the liposomes and transferosomes. FT-IR studies revealed no interaction between the drug and membrane components. The ethosomes, liposomes, transferosomes vesicles muscle relaxant efficiency was compared with the marketed thiocolchicoside gel. The pharmacodynamic studies showed that the muscle relaxant activity of ethosomes was more then liposomes, transferosomes suspension and less than the marketed gel formulation. Our results suggest that the ethosomes are an efficient carrier for dermal and transdermal delivery of thiocolchicoside.

INTRODUCTION

The skin covers a total surface area of approximately 1.8m^2 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.

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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 systems [1]. 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 drugs[2]. 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 ethosomes [2]. 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 *invivo* and *in vitro* skin delivery of various drugs. Contrary to liposomes, transferosomes, ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions[2]. Thiocolchicoside, acting as a GABA-A receptor antagonist, a phenyl acetic acid derivative, is a muscle relaxant agent, used for muscle relaxant and anti-inflammatory effects in the treatment of orthopedic, traumatic and rheumatologic disorders. Anti-inflammatory & Analgesic properties, used in combination with glafenine and meprobamate to tranquilize patients undergoing hysterosalpingography. In the treatment of painful muscle spasms. It is completely absorbed from the GI tract. However, the drug undergoes extensive first pass metabolism in the liver. Due to the extensive first pass metabolisms necessitate the need for investigating other route of drug delivery of thiocolchicoside. 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 thiocolchicoside lipid vesicles for transdermal delivery.

MATERIALS AND METHODS

Materials

Phospholipon 90 were received from Lipod grp and thiocolchicdoside were receieved as gifts from Aristopharma, Ethanol, chloroform and methanol purchased from Loba Chemical (India). Tween 80 from loba chemical (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.

Preparation of liposomes

Liposomes were prepared by using thin film hydration method. Accurately weighed 10 mg phosphatidylcholine (PC) were dissolve in 10 ml of ethanol. Film was prepared by slowly reducing the pressure from 500 to 1 mbar at 50°C using the rotator flask evaporator (IKA-RV 10). Obtained film was kept under vacuum for 2h at room temperature. Than after organic solvent was evaporated under a stream of nitrogen and solvent traces were removed by maintaining the deposited lipid films under vacuum overnight.

Drug solution of 1mg/ml was prepared in PBS (pH 7.4). The film was hydrated with drug solution for 75 min at 50°C. The hydration was done in rotator flask evaporator (IKA-RV 10) while vacuum is removed. The obtained formulation was then sonicated (15 min, duty cycle 50% 10W) with the probe sonicator (Remi). The sonication was done to get vesicles of uniform size and unilameller vesicles.

Preparation of ethosomes

Ethosomes can be prepared by cold method at 30°C. In this method 10 mg phospholipid, 10 mg drug were dissolved in 20 ml ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. This mixture was heated to 30°C in a water bath. The water heated to 30°C in a separate vessel is added to the mixture, which were then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation was stored under refrigeration[4.5].

Preparation of transferosomes

The mixture of vesicles forming ingredients, that is 10 mg phospholipids and 250 µg tween 80 were dissolved in chloroform: methanol (3:1). organic solvent evaporated above the lipid transition temperature (room temp. for pure PC vesicles, or 50°C for dipalmitoyl Phosphatidylcholine) using rotary evaporator. Final traces of solvent were removed under vacuum for overnight. The deposited lipid films were hydrated with buffer (pH 7.4) containing 1mg/ml solution of drug by rotation at 60 rpm min^{-1} for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature. To prepare small vesicles, resulting LMVs were sonicated at room temperature or 50°C for 30 min. using a B-12 FTZ bath sonicator or probe sonicated at 40°C for 30 min (titanium micro tip, Heat Systems W

380). The sonicated vesicles were homogenized by manual extrusion 10 times through a sandwich of 200 and 100 nm polycarbonate membranes.

Evaluation of vesicles

Entrapment Efficiency

The Entrapment efficiency of the vesicles was determined by ultracentrifugation method. 0.5 ml of the formulation was centrifuged at 4^0C at 19000 rpm for 1 hr. Supernatant containing the unentrapped drug was decanted. The vesicles was lyses using isopropyl alcohol (0.1%v/v)and after further dilution with phosphate buffer, and it was analyzed for drug content using UV Spectrophotometer (Shimadzu) at 259 nm. The entrapment efficiency was expressed as percentage of total drug entrapped using the following formula[5].

$$\text{Percentage entrapment} = \frac{C}{T} \times 100$$

Where T=theoretical amount of drug that was added, and C=amount of drug detected after dissolving the vesicles

Vesicles Size

Diameters were determined using photon correlation spectroscopy employing Zetasizer (Malvern Instruments, Malvern, UK). Samples used distilled water filtered through 0.2 μm membranes to minimize interference from particulate matter. Vesicles were suitably diluted with filtered solution of 1 Mm sodium chloride before taking observations. The particle size analysis was carried out of the final sample obtained by separation through sephadex column.

Zeta Potential

The zeta potential of the vesicles or complexes was determined by using the zetasizer nano series (Malvern Worcestershire, UK). Prior to the measurements, complexes were diluted in buffer (pH 7.4) and measurements were carried out at 25^0C.Each a sample was measured three times and the mean value was calculated.

***In vitro* permeation**

It was carried out by franz diffusion cell. To carry out this study fresh rat skin was cut and preserved in deep freezer. Before use the skin was hydrated in hydrating medium for period of one hour. Two compartments are there in the cell:

a) Donor compartment,

b) Receptor compartment

The volume of receptor compartment was found to be 19 ml and phosphate buffer filled up to mouth of the compartment and then skin was placed in direct contact to it. Continuous stirring was done with the help of magnetic stirrer and thermostat at 37^0C throughout study. The ethosomal formulation (0.5 ml) was applied on the skin in donor compartment, which was then covered with a paraffin to avoid any evaporation process. Samples (2 ml) were withdrawn through the sampling port of the diffusion cell at predetermined time intervals (1, 2, 3, 4, 5, 24 hrs) over 24 h and the receptor phase was immediately replenished with equal volume of fresh diffusion buffer. Then analyzed for drug content with the help of UV spectrophotometer (Shimadzu 1700), at 259 nm. Triplicate experiments were conducted for each study. Similar experiments were performed with liposomes, Transferosomes formulations and aqueous solution. Sink condition were maintained throughout all the experiment.

Fourier Transform Infra Red Spectroscopy (FTIR)

The interaction between vesicles membrane component phospolipon 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. Here FTIR spectroscopy can be used to investigated and predict any physiochemical interaction between different compounds in the formulation and therefore it can be applied for selection suitable chemically compatible excipient[6].

Differential Scanning calorimetery

Differential scanning calorimetery was used to evaluate the intraction between TCH and liposomes, ethosomes, Transferosomes with tween 80. PC was used for DSC measurements because its transistion temperature can easily measured. Phosphatidylcholine. TCH, and physical mixture were placed in a conventional aluminum pan and a scan in a range of 20^0C to 200^0C were recorded at the rate of 10^0C/min was employed. The weight of each sample was 10-12 mg. Each scan included a base line subtraction of a scan made with the reference sample (water or buffer).

Stability Studies

A stability test of the liposome or noisome dispersions was conducted by incubating the hydrolyzed vesicles with bidistilled water at 25°C. Samples were withdrawn after 48 h. Encapsulation efficiency of these dispersions were then determined as described above[4. 7-11].

Statistical Analysis

Data are expressed as means ± standard deviation (SD) of the mean and statistical analysis was carried out employing student's t test using the software PRISM (Graph- Pad). A value of P < 0.005 was considered statistically significant.

RESULTS

Vesicles Size

Liposomes and ethosomes and transferosome prepared in the current study were found to have mean vesicle size of 653 ± 6.4, 502 ± 5.4and 612 ± 6.7 nm. Diameters were determined using photon correlation spectroscopy employing Zetasizer (Malvern Instruments, Malvern, UK). Samples used distilled water filtered through 0.2 µm membranes to minimize interference from particulate matter. Vesicles were suitably diluted with filtered solution of 1 Mm sodium chloride before taking observations. The particle size analysis was carried out of the final sample obtained by separation through sephadex column. Particle size is important parameter studied and results for the particle size of formulations are as follows.

Table 1: vesicles size

Parameter	Liposome	Ethosomes	Transferosomes
Mean size (nm)	653±6.4	502±5.4	612±6.7

Zeta Potiential

The zeta potential of the vesicles or complexes was determined by using the zetasizer nano series (Malvern Worcestershire, UK). Prior to the measurements, complexes were diluted in buffer (pH 7.4) and measurements were carried out at 25^{0}C.Each a sample was measured three times and the mean value was calculated.

Table 2: zeta potential

Parameter	Liposome	Ethosomes	Transferosomes
Zeta potential (mv)	-23.4	-23.4	-12.7

A £ value > ± 30 mv is essential for effective stability and to inhibits aggregation. In the present study the £ potential for TCH loaded liposomes, Ethosomes, Transferosomes were found to be -23.4, -23.9, -12.7 mv respectively. It was observed that zeta potential of prepared vesicles has sufficient charge to inhibit aggregation of vesicles. Ethosomes have highest £ value of -23.9 mv, indicating most stable vesicles.

Entrapment efficiency

The percent drug entrapment in liposomes, Ethosomes, Transferosomes, was determined by centrifugation method and found to be 20 ± 0.8, 23.16 ± 1.0, 22.34 ± 0.8. The drug entrapment in case of Ethosomes prepared by hot method was found to be higher than liposomes, Transferosomes prepared by rotary evaporator method.

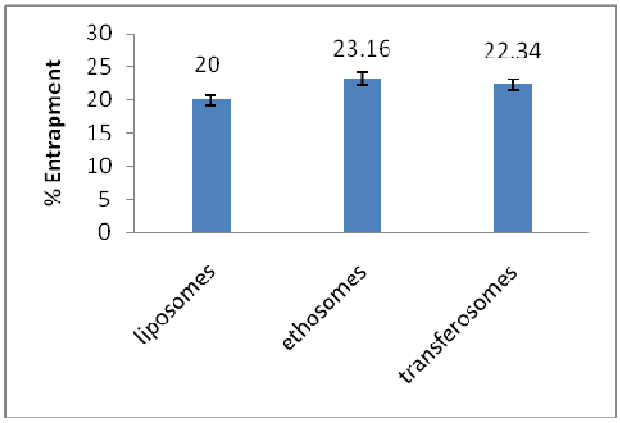

Figure 1: % Entrapment of different vesicles

***In vitro* skin permeation studies**

The percentage of drug release from, liposomal system, ethosomal system, Transferosomes, Drug solutions, Zyflex, were 57±3.8, 90±5.0, 78±4.0, 24±2.4, 90±4.0 respectively at the end of 24 hours study and the release profile are shown in figure.

Differential scanning calorimeter

Differential scanning calorimetery was used to evaluate the intraction between TCH and liposomes, ethosomes, Transferosomes with tween 80. PC was used for DSC measurements because its transistion temperature can easily measured. The DSC traces of PC liposomes and Ethosomes showed a peak transition at 50.7 ± 0.2^{o}C and an enthalpy of 24.7 ± 0.5 J/g. Incorporation of tween 80 into Transferosomes reduces the *T*m value to 43.6 ± 0.2 ^{0}C and an enthalpy to 22.7 ± 0.5 J/g.

Table 4: % drug permeate through rat skin in 24 hrs by the use of this lipid vesicles, drug solution and zyflex ointment.

Sl. No	Time (hrs)	Drug solution	Liposomes	Ethosomes	Transferosomes	Zyflex
1	0	0	0	0	0	0
2	1	0	15±1.5	42±2.4	34±2.0	34.14±2.0
3	2	0	22±2.1	48±3.8	41±2.3	44.87±2.3
4	3	15±1.2	26±3.4	55±3.7	44±2.0	55.60±2.4
5	4	17±2.0	32±3.0	59±4.3	48±3.0	64.56±3.0
6	5	20±2.2	36±3.4	64±5.0	55±3.5	73.45±3.7
7	24	24±2.4	57±3.8	90±5.0	78±4.0	90.23±4.0

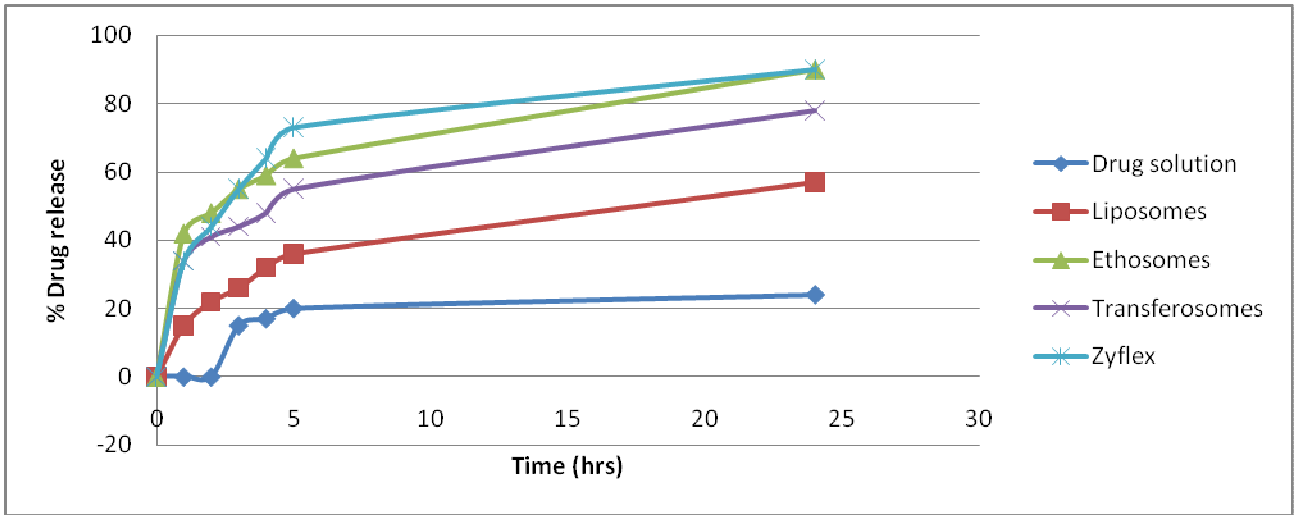

Figure 2: % Drug release of different vesicles, drug solution, and Zyflex ointment with time

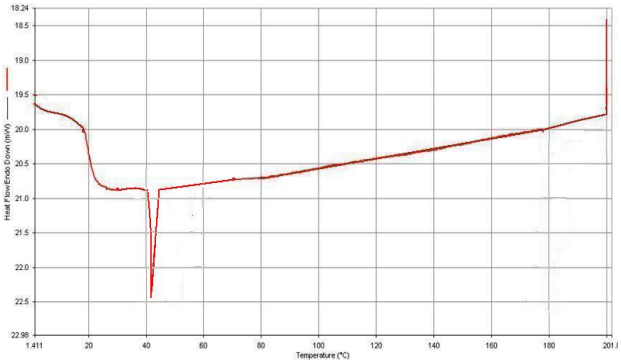

Figure 3: DSC thermogram of liposomes

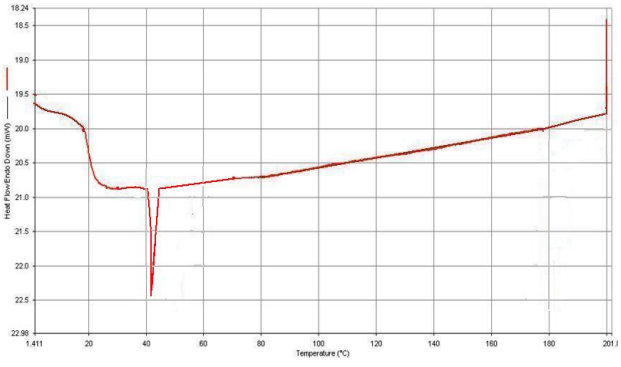

Figure 4: DSC thermogram of transferosomes

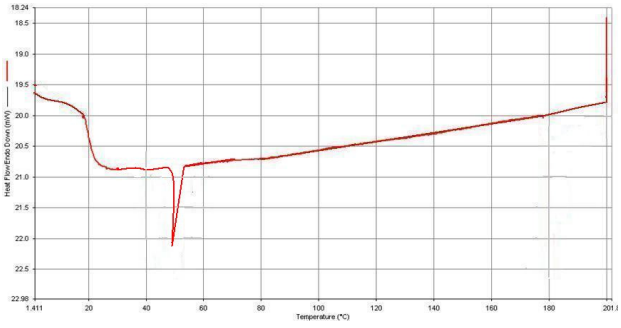

Figure 5: DSC thermogram of ethosomes

The decreases in *T*m value may indicate that the tween 80 perturbs the packing characteristic and, thus, fluidizes the lipid bilayer. The presence of TCH did not change either *T*m or enthalpy values, indicating that the molecule is entrapped in the hydrophilic core of the liposomes, Ethosomes, Transferosomes.

Stability studies

The encapsulated drug tends to leak out from the bilayer structure during storage. A significant loss in TCH encapsulation of PC vesicles was noted after incubation in suspension form for 48

h. Encapsulation loss was always associated with an increase in vesicle size, which is a thermodynamically more stable status as observed in TCH ethosomes stored in lyophilized form (Fang et al., 1997). Phospholipid loss, in the presence of water, from the liposome bilayers leads to the formation of pores and leakage. A higher susceptibility to lipid peroxidation for unsaturated fatty acid molecules as compared to saturated molecules was reported (Piraube et al., 1988; Vemuri and Rhodes, 1995). Span 80 and transferosomes showed good stability according to encapsulation after 48 h incubation as compared to liposomes. More is the zeta potiential more is the stability of the vesicles, Ethosomes showed higher zeta potiential and optimised ion-dipole intraction showed the higher entrapment efficiency after 48 h.

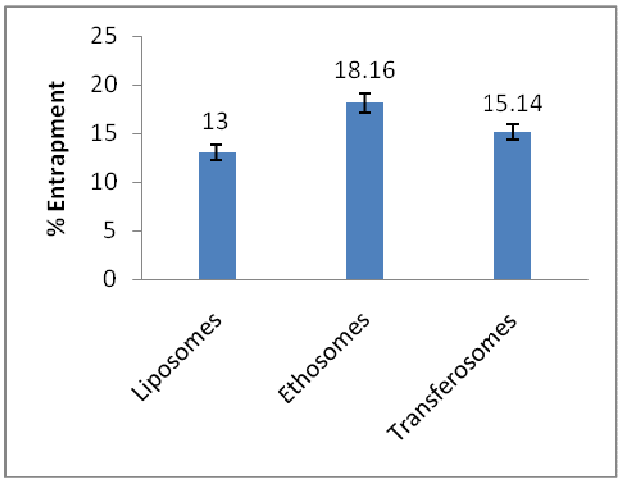

Figure 6: Entrapment of different vesicles

DISCUSSION

In the present study, we investigated the in vitro permeation .entrapment efficiency, particle size behavior of deformable liposomes, transferosomes prepared using Tween 80 as an edge activator, and ethosomes Based on study of different vesicles found that ethosome has showed higher entrapment efficiency than liposomes and transferosomes prepared using Tween 80.Main reason for the better penetration is due to its small size, ethosomes also have higher entrapment efficiency due to present of more ethanol as compare to other lipid vesicles. Higher is the ethanol content higher is the entrapment efficiency. Other several studies investigated possible mechanisms by which deformable vesicles could improve skin delivery of drugs. Two mechanisms were proposed. First, vesicles can act as drug carrier systems, whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin (mechanism 1). Second, vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify the intercellular lipid lamellae. This will facilitate penetration of free drug molecules into and across the stratum corneum (mechanism 2). The first mechanism was put forward by Cevc et al. for deformable liposomes.

The transport of the drug carried by deformable liposomes into the stratum corneum bypassing the main barrier for drug permeation will considerably improve skin delivery. This role may be of great effect in improving skin deposition. However, several factors might contribute to or contribute against this role in improving transdermal flux. Drug release from the vesicles in the stratum corneum is an important step that will affect transdermal flux.

For hydrophilic drugs, the penetration enhancing effects seem to play a more important role in the enhanced skin delivery than in case of lipophilic drugs. Since permeation of hydrophilic molecules tends to be relatively slower and hence more enhanceable. Results of the current study states that the penetration effect is more in ethosome than liposomes, Transferosomes, and drug solution.

Ethosome is a novel vesicular carrier, recently developed by Touitou et al., showing enhanced skin delivery. The ethosomal system is composed of phospholipid, ethanol and water. Although the exact process of drug delivery by ethosomes remains a matter of speculation, most likely, a combination of processes contribute to the enhancing effect. Ethanol is a well known permeation enhancer. However, previous studies that compared permeation enhancement of drugs from ethosomal systems versus hydroethanolic solutions showed that permeation enhancement from Ethosomes was much greater than would be expected from ethanol alone. Asynergistic mechanism was suggested between ethanol, vesicles and skin lipids. Ethanol may provide the vesicles with soft flexible characteristics which allow them to more easily penetrate into deeper layers of the skin. It was also proposed that phospholipid vesicles with ethanol may penetrate into the skin and influence the bilayer structure of the stratum corneum and this may lead to enhancement of drug penetration[11].

The DSC traces of PC liposomes and Ethosomes showed a peak transition at 50.7 ± 0.2°C and an

enthalpy of 24.7 ± 0.5 J/g. Incorporation of tween 80 into Transferosomes reduces the *T*m value to 43.6 ± 0.2 ^{0}C and an enthalpy to 22.7 ± 0.5 J/g. The decreases in *T*m value may indicate that the tween 80 perturbs the packing characteristic and, thus, fluidizes the lipid bilayer. The presence of TCH did not change either *T*m or enthalpy values, indicating that the molecule is entrapped in the hydrophilic core of the liposomes, Ethosomes, Transferosomes.

A £ value > ± 30 mv is essential for effective stability and to inhibits aggregation. In the present study the £ potential for TCH loaded liposomes, Ethosomes, Transferosomes were found to be -23.4, -23.9, -12.7 mv respectively. It was observed that zeta potential of prepared vesicles has sufficient charge to inhibit aggregation of vesicles. Ethosomes have highest £ value of -23.9 mv, indicating most stable vesicles.

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