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Original Article

A COMPARATIVE STUDY OF TERBINAFINE ETHOSOMAL FORMULATIONS : A NOVEL APPROACH

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Abstract :
The present research work aimed at the impact of reduced vesicular size on the characteristics of ethosomes by comparing with the regular vesicular size of ethosomes as topical drug delivery vehicle to achieve optimal localized drug concentration and reduced dose frequency of the Terbinafine hydrochloride (TH), an antifungal drug. Oral use of TH contraindicated resulting from sever side effect, thus topical administration is recommended. Commercially available TH creams, lotions and sprays, have limitation of relatively short residual period at target site. The entrapment of drug in vesicles improves localization, solubility and availability of drug at the site; resulting in reduction of the dose. Ethosomes containing drug were prepared by employing higher concentration of alcohol in the form of hydroalcoholic or hydroglycolic phospholipid. Sonicated and unsonicated ethosomes were investigated for shape, particle size, and entrapment efficiency. Electronic microscope investigation not only revealed, vital evidence for presence of phospholipid vesicles in TH ethosomal systems but also displayed greater uniformity in size and shape of sonicated ethosomes than unsonicated ethosomes. Furthermore, the Comparative investigation was carried out for *ex vivo* skin permeation, *ex vivo* drug release and entrapment efficiency studies. Drug release followed zero order release rate kinetics. Drug accumulation study showed more than 19.01 % of drug was deposited into skin by sonicated ethosomal formulation as compared to 2.57 % by unsonicated ethosomal formulation. Sonicated and unsonicated ethosomes were found stable at refrigeration and room temperature conditions during stability studies. Drug accumulation studies in deep skin strata was found to be comparatively greater in sonicated ethosomes, which indicates higher localized drug and that in turn reduces dose frequency.

Keywords : Sonicated Ethosomes; unsonicated ethosomes; Terbinafine hydrochloride; *ex vivo* characterization.

Introduction :

Terbinafine hydrochloride (TH) is the allyl amine available for systemic use in the treatment of dermatophytes (Trichophyton, Epidermophyton and Microspora) including tinea infections. TH topical administration is usually recommended because commercial conventional terbinafine hydrochloride tablets are considered to be administered for a longer duration of time to achieve higher systemic absorption of drug resulting in systemic adverse side effects; since one tablet daily for 12 weeks achieves a 90% cure which is a lengthy and expensive duration of therapy. Conventional topical drug delivery systems, such as creams, lotions and spray, are known to have limitations like inadequate localization of drug within the skin to enhance the local effect or increase the penetration through the stratum corneum and viable epidermis for systemic effects[1].

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A novel approach has been recently developed by Touitou et al., to address the limitations of conventional topical drug delivery systems; in the form of Ethosome which is predominantly a lipid carrier. The importance of lipids has especially increased after realizing the utility of phospholipids which is a natural bio-friendly molecule and when collaborated with water can form diverse types of supermolecular structures[1,2]. Further the research has

proved that entrapment of drug in vesicles may help to localize delivery of drug and enhance solubility and availability of drug at the site for systemic action which intern may reduce dose and systemic side effects[1,2,3]. There are enough research articles displaying the rational use for preferring ethosomal over liposomal drug delivery system.

Hence, the present research is aimed to investigate the effect of modified vesicular size on the properties of ethosomes by comparing with the regular vesicular size of ethosomes. The modification of vesicular size is achieved by subjecting the ethosomes formulated by HOT technique to sonication using Sonicator vibra cell instrument. Both, the sonicated and unsonicated ethosomes were studies for their ability to effectively deliver drug molecules to and through the skin to the systemic circulation; this property was observed by using fluorescent probes in ex vivo permeation experiment.

Material and Method :

Soya Phospholipid purchased from Himedia Laboratories Pvt. Ltd. Mumbai; Distilled Ethanol from Samsung distillery; Rhodamine Base dye from Genuine Chemical, Mumbai; Triton X – 100 purchased from Loba Chemie Pvt. Ltd., Mumbai; Cholesterol purchased from Nice Chemicals Pvt. Ltd., Kochi; Sonicator Vibra cell, Sonics and Materials Inc., CT, USA and all the other ingredients used were of analytical grade.

1. Methods:

1.1. Preparation of unsonicated terbinafine hydrochloride ethosomes

Terbinafine hydrochloride 1 %w/v was accurately weighed then mixed with ethanol and propylene glycol and heated to 40 °C. In a separate vessel soya phospholipid was dispersed in distilled water by heating on water bath at 40 °C until a colloidal solution was obtained. Once both mixtures reached 40 °C, the organic phase was added to the aqueous phase under constant stirring at 700 RPM, on completion of adding, mixing was further continued for 5 min at 40 °C. Total volume prepared was 25 ml[4,5] (Table 1).

1.2. Preparation of sonicated ethosomes

Ethosomes prepared by the above procedure were subjected to sonication at 4 °C using probe Sonicator in 3 cycles of 5 min with 5 min rest between the cycles[4,5] (Table 2).

1.3. Preparation of rhodamine B stained ethosomes

Accurately 4 mg of rhodamine was weighed and to it ethanol (30 %w/v) and propylene glycol (10 %w/v) are mixed and heated to 40 °C. In a separate vessel soya phospholipid (0.5 %w/v) was dispersed in distilled water by heating on a water bath at 40 °C until a colloidal solution was obtained. Once both the mixtures reached at 40 °C, the organic phase was added to the aqueous phase with stirring at 700 RPM. After adding, mixing was continued for another 5 min. Temperature was maintained at 40 °C for the entire process. Total volume of the preparation was 25 ml[4,5]. The formula for the different batches is given in the Table 1 and 2. The same method was adopted for sonicated ethosomes also.

2. Characterization of unsonicated and sonicated ethosomes

2.1. Shape analysis

Ethosomes were examined by negative stain. A drop of the vesicular system was applied to a film coated copper grid. Phosphotungstic acid (PTA) solution was dropped onto the grid. The stained sample was examined in a Philips Tecnai 20 transmission electron microscope (Philips, Holland) accelerated at 200 kV[6].

2.2. Size analysis

vesicle size of ethosomal formulations both sonicated and unsonicated was determined by Nano Zeta Sizer (Malvern Instruments Ltd., USA)[7,8].

2.3 Entrapment efficiency

The entrapment efficiency of terbinafine hydrochloride by ethosomal formulations was determined by ultracentrifugation. Ethosomal formulations of 10 ml was mixed with 1 ml of 1 % triton X-100 solution. Each sample was vortexed for 2 cycles of 5 min with 2 min rest between the cycles. Each vortexed sample of 1.5 ml and fresh

untreated formulations were taken into different centrifugal tubes. These samples were centrifuged at 20,000 rpm for 3 h. The supernatant layer was separated, diluted with 40 % v/v hydroethanolic solution and drug concentration was analyzed at 283.5 nm in both vortexed and unvortexed samples using UV spectrophotometer (UV – 1600/1700 series)[9].

The entrapment efficiency was calculated as follows

$$\% \text{ Entrapment Efficincy } \frac{(T-C)}{T} 100$$

Where;

'T' = The total amount of drug that detected from supernatant of vortexed sample.

'C' = The amount of drug unentraped and detected from supernatant of unvortexed sample.

2.4 Ex vivo characterization

(a) Preparation of porcine skin

Abdominal porcine skin obtained from the local slaughterhouse (Kankanady Market, Mangalore. Karnataka) was incised and freed from fats for study. The skin was then cut into pieces of suitable size with thickness ranging from 2 mm to 3 mm and stored under frozen condition[5,10,11].

(b) Drug release study from porcine skin

The ex vivo release of terbinafine hydrochloride from ethosomal formulations was studied separately using diffusion cell specially designed in our laboratory as per literature. The effective permeation area of the diffusion cell and receptor cell volume was 2.23 cm^2 and 100 ml respectively. The temperature was maintained at 37 ± 1 °C. The receptor compartment contained 100 ml of 40 %v/v hydroethanolic solution and was constantly stirred by magnetic stirrer at 600 RPM during 6 h. Prepared porcine skin was mounted between the donor and receptor compartments. Ethosomal formulations 0.5 ml was applied to the skin surface and the content of diffusion cell was kept under constant stirring, then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analysed by using UV spectrophotometer (UV – 1600/1700 series) at 283.5 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh 40 % v/v hydroethanolic solution[12].

(c) Penetration study from porcine skin

The ex vivo penetration of rhodamine base stained ethosomal formulations was studied separately using diffusion cell specially designed in our laboratory as per literates. The effective permeation area of the diffusion cell and receptor cell volume was 2.23 cm^2 and 100 ml respectively. The temperature was maintained at 37 ± 1 °C. The receptor compartment contained 100 ml of 40 % v/v hydroethanolic solution and was constantly stirred by magnetic stirrer at 600 RPM during 6 h. Prepared porcine skin was mounted between the donor and receptor compartments. Rhodamine base stained ethosomal formulations of 0.5 ml was applied to the skin surface and the content of diffusion cell was kept under constant stirring, then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals. The receptor phase was immediately replenished with equal volume of fresh 40 % v/v hydroethanolic solution[12,13]. After ex vivo experiments, the skin was removed (2.23 cm^2) and carefully cleaned with distilled water. 10 µm slices by vertical cutting were obtained by cryomicrotome. Slices were observed under a confocal laser scanning microscope (CLSM).

(d) Drug accumulation in the skin

After ex vivo release study, the treated porcine skin (2.23 cm^2) was cleaned on both sides by distilled water and cut into small pieces, ultracentrifuged in 20 ml 40 % v/v hydroethanolic solution for 3 h at 20,000 RPM. The supernatant layer was separated, diluted with 40 %v/v hydroethanolic solution and drug concentration was determined using UV spectrophotometer (UV – 1600/1700 series) at 283.5 nm[13,14].

2.5. Drug release kinetic

The drug release data from the formulations was treated according to Higuchi's equation by plotting a graph of cumulative percentage of drug released vs square root of time and calculating the correlation coefficient of

regression (R^2)[13,14,15].

2.6. Stability studies

Stability study was carried out for sonicated and unsonicated terbinafine hydrochloride ethosomal formulations, especially for the size, shape and entrapment efficiency of the vesicles, as they are the major determinant factors in the present investigation. Two different temperature conditions were selected namely refrigeration temperature (4±2 °C) and room temperature (27±2 °C) for 4 weeks. Elevated temperature conditions above room temperature was not used to perform stability studies, as phospholipids constitute major component of the present ethosomal formulation which gets deteriorated at higher temperature. The formulations subjected for stability study were stored in borosilicate container to avoid any sort of interaction between the formulations and glass of container, which may affect the observation. The formulations were analyzed for any physical changes such as color, appearance and entrapment efficiency[3,15].

Result and discussion:

2.1. Shape and size analysis

The average size of unsonicated ethosomes was found out to be 332.7 nm while that of sonicated ethosomes was 76.11 nm. The shape of the unsonicated and sonicated ethosomes was spherical, but comparatively sonicated ethosomes had smaller and more uniform in vesicular size and shape than unsonicated ethosomes.

2.2. Entrapment efficiency

The maximum entrapment efficiency in unsonicated and sonicated ethosomal vesicles was are determined by ultracentrifugation method. In unsonicated ethosomes the maximum entrapment efficiency was found to be 61.23 % in ET2 formulation containing 30 % ethanol as compared to 54.89 % and 57.043 % in ET1 and ET3 containing 20 % and 40 % ethanol respectively (Table 3). The sonicated ethosomal vesicles showed maximum entrapment efficiency of 76.43 % in ET7 containing 30 % ethanol when compared to 63.16 % and 58.93 % in ET6 and ET8 containing 20 % and 40 % ethanol respectively (Table 4). In both, unsonicated and sonicated ethosomes, it is evident that as the ethanol concentration increased from 20 % to 30 % w/v, the entrapment efficiency also increases; But with further increase in the ethanol concentration (>30 % w/v) the vesicle membrane becomes more permeable that led to decrease in the entrapment efficiency. When the result of entrapment efficiency between sonicated and unsonicated ethosomes was compared, sonicated ethosomes showed significantly high values. This proves that as the vesicle size is reduced the entrapment efficiency is increased.

2.3. Ex vivo characterization

(a) Drug release study from porcine skin

The Ex vivo studies were conducted only on those formulations, which have showed high entrapment efficiency. Ex vivo drug release study was conducted by diffusion method to verify the release rate and extent of the drug release from the dosage form. A perusal of Figure 1, at the end of 6 h, the sonicated ethosomes (ET7), drug release was found to be 41.16%, which is greater when compared to 12.68 % of the unsonicated ethosomes (ET2).

(b) Penetration study from porcine skin

Unsonicated ethosomes and sonicated ethosomes were compared with the aim to study the penetration depth of fluorescent probe and the relative intensity of fluorescence into skin layers. Remarkable differences were observed 6 h after the application. The penetration depth of the fluorescent label was higher for sonicated than unsonicated ethosomal formulation. The sonicated ethosomal formulation displayed high fluorescence intensity in the stratum corneum as well as in viable epidermis than unsonicated ethosomal formulation (Figure 2). It is indicated that the sonicated ethosomal formulation can facilitate the drug to reach the deeper skin structures, such as pilosebaceous follicle.

© Drug accumulation into skin

Drug accumulation into skin is useful parameter if the drug is intended to give subdermal action other than transdermal effect as in the case of dermal fungal infection. After the completion of the ex vivo diffusion studies, skin

was extracted and amount of drug was analyzed. Drug accumulation study showed 19.01 % of drug deposition into skin by sonicated ethosomes as compared to 2.57 % drug deposition by unsonicated ethosomes (Table 5).

2.4. Drug release kinetic

Terbinafine hydrochloride released was found to be linear and proportional to square root of time in the sonicated ethosomes. The correlation coefficient of regression showed 0.9709 for sonicated ethosomal formulation whereas for sonicated ethosomal formulation it was 0.8916 (Table 6).

2.5. Stability studies

The stability studies performed over a period of 4 weeks at refrigeration temperature and room temperature storage conditions proved that there was no change in morphological properties with respect to size and shape. Entrapment efficiency of all the sonicated and unsonicated formulations did not show any deviation from the initial drug content. Hence, all the ethosomal formulations were found to be stable.

Conclusion:

The results of the fore said investigation conclusively demonstrate the encroachment of the reduced vesicular size results in enhanced characteristics of ethosomal formulation when compared with the regular vesicular size of ethosomal formulation as topical drug delivery. Optimal localized drug concentration and higher drug entrapment efficiency was attained by reducing the vesicular size. It provides better remission from the disease and reduces the duration of therapy. However, this formulation can find a place in clinical use after clinical evaluation.

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Table 1: Formulation of unsonicated ethosomes

Formulation Code	Drug (%w/v)	Phospholipid (%w/v)	Ethanol (%w/v)	Propylene glycol (%w/v)
ET1	1.0	0.5	20	10
ET2	1.0	0.5	30	10
Et3	1.0	0.5	40	10

Table 2: Formulation of sonicated ethosomes

Formulation Code	Drug (%w/v)	Phospholipid (%w/v)	Ethanol (%w/v)	Propylene glycol (%w/v)
ET6	1.0	0.5	20	10
ET7	1.0	0.5	30	10
ET8	1.0	0.5	40	10

Table 3. Drug entrapment efficiency of unsonicated ethosomes.

Sample		Absorbance*	Concentration C (µg/ml)	Amount of drug C ? DF (µg)	Entrapped drug E=TU/T	% Drug Entrapped %E= E ?100
Et1	Total drug (T)	0.4115	20.1753	2017.53	0.5489	54.89
	Free drug (U)	0.1856	9.1011	910.11		
Et2	Total drug (T)	0.4416	21.6482	2164.82	0.6123	61.23
	Free drug (U)	0.1712	8.3930	839.30		
Et3	Total drug (T)	0.4466	21.8934	2189.34	0.5704	57.043
	Free drug (U)	0.1918	9.4055	940.55		

*Each value is an average of 3 replications.
DF= Dilution factor (100)

Table 4. Drug entrapment efficiency of sonicated ethosomes.

Sample		Absorbance*	Concentration C (µg/ml)	Amount of drug C ? DF (µg)	Entrapped drug E=TU/T	% Drug Entrapped %E= E ?100
ET6	Total drug (T)	0.4989	24.4571	2445.71	0.6316	63.16
	Free drug (U)	0.1838	9.0100	901.00		
ET7	Total drug (T)	0.5074	24.8763	2487.63	0.7643	76.43
	Free drug (U)	0.1196	5.8634	586.34		
ET8	Total drug (T)	0.4875	23.8976	2389.76	0.5893	58.93
	Free drug (U)	0.2002	9.8148	981.48		

*Each value is an average of 3 replications.
DF= Dilution factor (100)

Table 5. Results of drug accumulation in the skin from unsonicated ethosomes (ET2) and sonicated ethosomes (ET7)

Formulation	Absorbance*	Amount of drug accumulated	
		µg	Percentage (%)
ET2	0.1309	6.424	2.571
ET7	0.9695	7.52	9.01

*Each value is an average of 3 replications.

Table 6. Drug release kinetics from unsonicated ethosomes (ET2) and sonicated ethosomes (ET7)

Formulation	Higuchi's Equation (R^2)
ET2	0.8916
ET7	0.9709

Figure 1. Ex vivo drug release profile of unsonicated ethosomes (ET2) and sonicated ethosomes (ET7).

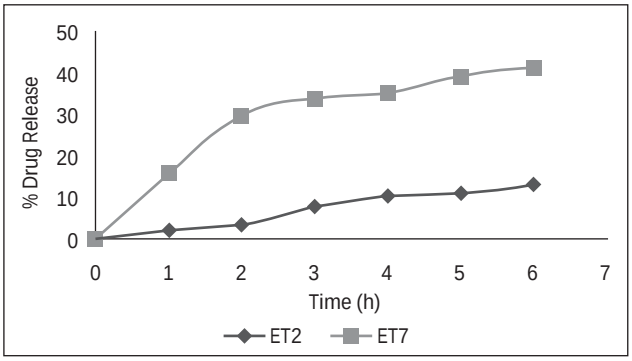
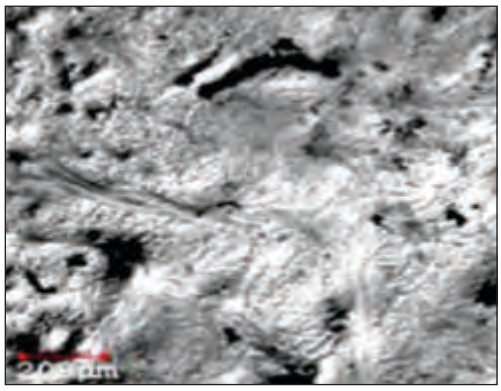
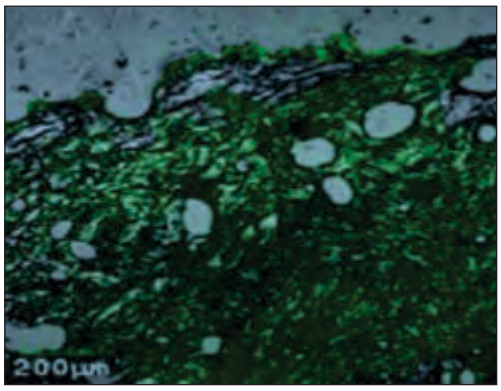


Figure 2. Skin optimal section showing rhodamine base stained unsonicated and sonicated ethosomes into porcine skin.

(A) Unsonicated Ethosomes (ET2)



(B) Sonicated Ethosomes (ET7)



Reference:
1. Kumar R, Katare OP. Lecithin organogels as a potential phospholipid structured for topical drug delivery: A review. AAPS PharmSciTech. 2005; 6(4):56-61.
2. Schmid MH, Korting HC. Liposomes: A drug carrier system for topical treatment in dermatology. Crit. Rev. Ther. Drug Carrier Syst. 1994; 11:97-118.
3. Gelone S, O'Donnell JA. Anti-Infectives. Remington: The science and practice of pharmacy. 21st edn. 2006; pp.1673-75, Lippincott Williams and Wilkins, Philadelphia.
4. Touitou E, Godin B, Weiss C. Enhanced delivery into and across the skin by ethosomal carriers. Drug Dev. Res. 2000; 50:406-445.
5. Jain S, Mishra D, Kuksal A, Tiwary AK, Jain NK. Vesicular approach for drug delivery into or across the skin, current status and future prospects. Int. J. Pharm. 2005; 8:2-32.
6. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl hydrochloride: Ethosomes vs liposomes. J. Biomaterials. 2000; 21:1879-1885.
7. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 2001; 14:101-114.
8. Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Int. J. Pharm. 2007; 332:1-16.
9. Jain N, Talegankar S, Jam NK. New ways to enter the blood stream. Emerging strategies in transdermal drug delivery. The pharma review. 2004; 17:41-66.
10. Heeremans JLM, Gerristen HR, Meusen SD, Mijnheer FW, Ganagaram RS, Pandye G. The preparation of tissue type plasmigen activator (t-PA) containing liposomes: entrapment efficacy and ultracentrifugation damage. J. Drug Target. 1995; 3:301-310.

11. E. G. De Jalon, Blanco-Prieto MJ, Ygartua P, Santoyo S. PLGA microparticles: Possible vehicles for topical drug delivery. Int. J. Pharm. 2001; 226:181-184.
12. Aibinder D, Touitou E. Testosterone ethosomes for enhanced transdermal delivery. Drug Delivery. 2005; 12:297-303.
13. Kaplun-Frischott Y, Touitou E. Testosterone skin permeation enhancement by method through formation of eutectic with drug and interaction with skin lipids. J. Pharm. Sci. 1997; 86:1394-1399.
14. Jain S, Umamaheshwari RB, Bhadra D, Jain NK. Ethosomes: A novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent. Indian J. Pharm. Sci. 2004; 66(1):72-81.
15. Raghuram Reddy K, Mutalik S, Reddy S. Once-daily sustained release matrix tablets of nicorandil: Formulation and *in-vitro* evaluation. AAPS PharmSciTech. 2003 ;4(4):4.