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Transdermal permeation enhancement of Tolterodine Tartrate through invasomes and iontophoresis

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ABSTRACT

The purpose of this work was to study the comparative and combination enhancement of an almost ideal transdermal drug through physical and chemical penetration enhancement methods. Tolterodine tartrate loaded invasomes were prepared using soya lecithin, ethanol and three different terpenes viz. limonene, fenchone and anethole. The FT-IR results showed the compatibility of these excipients with the drug. The obtained invasomes were of a sufficiently small vesicle size (1.3µm) with polydispersity index of 0.188. The vesicles were stable with no significant changes in the entrapment efficiency for a period of two months and there was no observed erythema which was proved by skin irritation studies on rabbits. Ex- vivo skin penetration data revealed that the invasome dispersion showed a significantly enhanced penetration of the drug through the skin compared to vesicles without terpenes, ethanolic drug solution and drug solution. Invasome formulation containing limonene showed high penetration because of its lipophilicity and low boiling point. The results of iontophoretic drug transport showed that the permeability of tolterodine tartrate released from invasomes was higher compared with that of free drug proving the additive effect of invasomes and iontophoresis. Hence successful transdermal penetration was obtained in combination with both physical and chemical penetration techniques.

Keywords: Tolterodine tartrate, Invasomes, Iontophoresis, Transdermal permeation enhancement techniques.

INTRODUCTION

Tolterodine tartrate is the drug which has ideal characteristics for transdermal drug delivery, which include extensive liver metabolism, low dose < 10mg/day, aqueous solubility >1mg/ml, partition coefficient between 1 to 3, molecular weight < 500 Daltons, half life <10 h. Apart from these the oral formulation of tolterodine tartrate has reported to have dose-related adverse side effects, including dry mouth, tachycardia, dizziness, and gastrointestinal obstructive disorder. In order to overcome these adverse effect transdermal formulations are highly preferred by patients as it bypasses first-pass metabolism (which is especially important in patients with liver disease), minimizes the gastrointestinal side effects, increases patient compliance, maintains a constant drug level in plasma and makes it possible to interrupt or terminate treatment when necessary.

An essential prerequisite for the development of a transdermal drug delivery system is that the drug must be capable of passing through the skin at a sufficiently high rate to achieve therapeutic plasma concentrations. However, the outermost layer of skin, the stratum corneum (SC), forms a major barrier to most exogenous substances, including drugs. One popular approach to deliver an effective dose of drug through skin is to reversibly reduce the barrier function of the skin with the aid of physical or chemical penetration enhancement techniques.

Hence in the present study, tolterodine tartrate was formulated as invasomes which are novel vesicles incorporating terpenes with enhanced penetration compared to the conventional liposomes. These are soft liposomal vesicles with very high membrane fluidity, containing terpenes, which are playing the role of penetration enhancement [1, 2]. The

presence of terpenes and ethanol makes invasomes unique. These vesicles have shown to possess the combined advantages of liposomes which are potential carriers and penetration enhancement of the terpenes which are having the ability to modify the order of stratum corneum packing thus promoting skin delivery [3, 4].

The effect of iontophoresis on the drug solution and was studied. The work was further extended by studying the combination effect of invasomes and iontophoresis of penetration enhancement.

MATERIALS AND METHODS

Materials

Tolterodine tartrate was obtained as a gift sample from R A Chem, Soya Phosphotidylcholine 70 was purchased from Sonic-Biochem extractions, Methanol and ethanol from S.D. Fine Chemicals Limited, limonene, fenchone, and anethole from Alfa Aesar. Male Wistar rats and white rabbits were used with grant from the ethics committee.

Preparation of invasomes by thin film hydration method [5, 6]:

Phospholipids are dissolved in chloroform and is dried to a thin film by slowly reducing the pressure from 500 to 1 mbar at lipid transition temperature using the rotary flash evaporator. The film was kept under vacuum (1 mbar) for 2 h at room temperature. Deposited lipid film was hydrated for 30 min at lipid transition temperature with a mixture of phosphate buffer (pH 7.4; SPB) containing drug, 10% (v/v) ethanol, 1% (v/v) terpenes in order to obtain invasomes. The obtained vesicles were ultrasonicated. The invasomes TTL1- TTL4 were prepared using limonene and different concentrations of soya lecithin 1, 3, 5& 7% , similarly TTF1-4 were prepared using fenchone and TTA1-4 were prepared using anethole keeping the concentration of ethanol common to all the preparations.

Characterization of prepared invasomes:

Invasomes prepared with different concentrations of phospholipids in combination with different terpenes were analyzed for % entrapment efficiency study, vesicle size analysis, surface morphology, ex vivo permeation study, skin deposition study, skin irritation study and stability study.

Entrapment efficiency study

1ml of invasome formulation was transferred into an ephendroff tube, and was centrifuged at 15,000 rpm at 4˚C for 15 minutes in two cycles to separate tolterodine tartrate- containing invasomes from un-entrapped drug. The clear fraction was used for the determination of free drug.

Vesicle size analysis

The vesicle size was measured by Delsa™ Nano. The polydispersity index (PDI) was used as a parameter of the size distribution. The formulation diluted with de-ionized water before the size measurements. The particle sizes were measured at 25˚C.

Surface morphology

The surface morphology (roundness, smoothness and formation of aggregates) of invasomes was studied by Scanning Electron Microscopy.

Ex vivo permeation and skin deposition study

Preparation of skin membranes

Male wistar rats of about 200gm were euthanized. The fur on the ventral side of the rat was removed by a depilatory, and then the skins were excised and kept at -20°C until used. Immediately before the experiment, the skins were taken out and left to thaw at room temperature. The skin was cleaned of adhering fat deposits and was carefully mounted on top of the diffusion cells and left to hydrate for 1 hour before the application of the formulation.

General Procedure

The permeation of invasome formulations was determined by using Franz (vertical) diffusion cell. The effective diffusion area of the cell was 2.0 cm^2 and had a receptor volume of 20ml. The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The top of the diffusion cell was covered with lid. The donor compartment was applied with the invasome formulation. 20 ml of 7.4 pH saline phosphate buffer was used as receptor medium which was stirred at 700 rpm and thermo stated at 37±0.5°C. Under these conditions the temperature at the skin surface was 32±0.5°C. At appropriate intervals, 2 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed by UV spectro photometric method. *Ex vivo* permeation rate studies such as % drug release, steady state transdermal flux (SSTF) and enhancement ratio for transport of tolterodine tartrate across rat skin were estimated for different formulations. The ANOVA for these parameters was carried out using Graph Pad.

Iontophoretic studies

***Preparation of electrodes* [7]**

Iontophoresis experiments were conducted using silver/silver chloride electrodes. The silver chloride electrodes were prepared as follows: silver wires (0.5 mm diameter) were immersed in 0.1N HCl solution and connected to the anode and cathode of an electric current source (12 V). A gray silver chloride layer was gradually coated on the anodal silver wires, and after 24 hours these wires were ready for use as iontophoresis cathodal electrodes.

***General Procedure* [7]**

Similar procedure was followed for the preparation of the cells as described earlier. A silver wire representing the anode was placed in the donor compartment, and a silver chloride cathode was placed in the receptor compartment for the drug solution and ethanolic drug solution and vice-versa in case of invasome formulation and for vesicles without terpenes. Both electrodes were attached to a constant current power supply of 12 V. Current density of $0.5mA/cm^2$ with a pulse on/off interval of 1: 1 ratio [8] was applied to stimulate the permeation tolterodine tartrate.

At predetermined times 2ml of samples were withdrawn from the receptor compartment and were immediately replaced by the same volume of the fresh buffer solution to maintain a constant volume. The samples were analyzed by UV spectrophotometric method in order to determine the amount of tolterodine tartrate permeated.

Skin irritation study

Skin irritation study was performed by using control, standard skin irritant, placebo and test which were applied on the left and right dorsal surface of rabbit skin and rabbits were examined for 24 hrs and erythema and edema was evaluated and the score was given according to the Primary Dermal Irritation Index classification (PDDI).

Stability studies

The optimized formulation was evaluated for physical stability testing to investigate the leaching of drug from the vesicles. The invasome samples were sealed in 10 ml glass vials and stored at refrigeration temperature (4˚C - 8˚C) and at 30˚C for two months. The EE of all the samples was determined for every month in the same manner as prescribed previously for every month.

RESULTS AND DISCUSSION

Drug-excipient compatibility study by FTIR:

Tolterodine tartrate compatibility with excipient was studied by FTIR. The FTIR spectra of formulations with terpenes reveal no interaction between drug and excipient; both the drug and excipient peaks were identified and interpreted in the spectra. The spectra confirmed the absence of any chemical interaction between the drug, terpene and phospholipids.

Preparation, evaluation and optimization of invasomes with different terpenes

In recent years several reviews were published on the use of vesicular system for dermal and transdermal system. Elastic vesicles were more efficient in enhancing the transport of drugs than rigid vesicles and no increase in drug transport was obtained when the drugs were not associated with the vesicles. Invasomes are the novel elastic vesicles which have generated interest as a topical formulation with enhanced skin permeation.

Invasomes were prepared by thin film hydration technique. Mechanical dispersion method was also a proposed method of preparation for the invasomes where the preparation has to be carried out in a closed environment in order to avoid the evaporation of ethanol and terpene, as this was tedious film hydration method was selected. Concentration of ethanol was selected based on the previous literature and different concentrations of soya lecithin were selected in order to check for its effect on the entrapment efficiency and penetration enhancement. Previous studies demonstrated that the increase in the concentration of terpenes (> 1%) increased the size of the vesicles and the decrease in the concentration of terpene i.e < 1% had less penetration enhancement effect hence 1% was taken as the optimum concentration of the terpene and was used in the study.

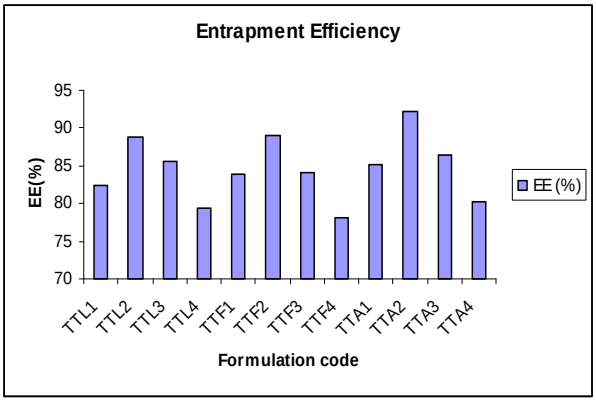
Characterization of prepared invasomal formulations:

Entrapment efficiency of prepared invasomes:

Drug entrapment within a vesicular carrier is an important parameter to be defined to really evaluate the delivery potentiality of the system. For this reason, the entrapment of tolterodine tartrate within the formulations was evaluated in an attempt to investigate the influence of invasome composition, i.e., the quantity of lecithin and type of terpene, on the drug loading capacity. As shown in figure 1, lecithin amounts, used for invasome preparation, influenced the entrapment efficiency of the colloidal carrier. Namely, the higher the amount of lecithin, the greater

the tolterodine tartrate entrapment within the invasomes up to 3% lecithin but further increase in the concentration decreased the entrapment efficiency .

Figure 1: Entrapment efficiency of invasomes prepared with different concentrations of soya lecithin using different terpenes.



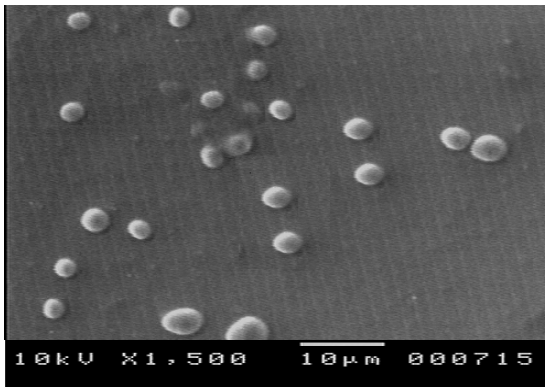
Vesicle Size Analysis

The vesicles obtained had a diameter of 1.3µm and the polydispersity index < 0.2, indicating that all of them were highly homogeneous suspensions.

Surface morphology

Surface morphology and the three-dimensional nature of the invasomes studied through SEM, confirmed the preparation as smooth-surfaced nanocarriers possessing vesicular characteristics.

Figure 2: SEM photograph of TTL2 invasome formulation



Ex- Vivo skin permeation studies

The formulations which had highest entrapment efficiency i.e. those which contained 3% of soyalecithin were evaluated for ex –vivo studies.. The release profile of these formulations was compared with that of drug solution (TT), ethanolic drug solution (TTE) and vesicles without terpenes (TTNT).

Table 1: *Ex vivo* release data, steady state transdermal flux (SSFT) and enhancement ratio (ER) for optimized invasomes with different terpenes in comparison with TT, TTE, TTNT.

Formulation Code	Q_{24} (%)	SSTF (µg/cm^2/hr)	ER	% Skin deposition
TTL2	64.13	0.0086	3.44	33.42
TTF2	56.5	0.0079	3.16	26.61
TTA2	60.07	0.0082	3.28	29.03
TTNT	49.09	0.0065	2.6	31.57
TTE	42.56	0.0060	2.4	28.6
TT	19.82	0.0025	-	16.26

Invasomes where prepared by using different terpenes, the lipophilicity of the terpenes was taken into consideration during their selection. The log P values of the terpenes limonene, fenchone and anethole were 4.8, 2.13, and 3.39 respectively. Invasomes containing limonene as permeation enhancer showed better permeation than the other

invasome formulations. The order of permeation was limonene > anethole > fenchone. Terpene enhancers had significant effect on the percutaneous permeation of the drug; limonene (highest lipophilicity) provided the highest increase in the flux of the evaluated drug. The lowest increase in the flux was observed with fenchone. The reason for increased penetration enhancement of tolterodine tartrate a slightly lipophillic drug may be due to the lipophilicity of the terpene limonene. Researches have shown that lipophilic terpenes have caused enhanced permeation of both hydrophilic [9] and lipophilic drugs [10]. Hence studies are to be carried out with different model drugs and with different terpenes in order to further investigate the exact role of terpenes lipophilicity in drugs' permeation. The higher enhancement of limonene may also be attributed to its low boiling point The low boiling points of terpenes indicate the weak cohesiveness or self-association of the molecules and therefore they may more easily associate or interact with lipid components of stratum corneum and alter the barrier property [11].
Invasomes containing 1% terpenes revealed a significantly enhanced penetration and accumulation of tolterodine tartrate in the skin compared to the vesicles without terpenes. Namely, the amount of tolterodine tartrate delivered by invasomes containing 1% terpenes was 3.4 fold ($p< 0.0001$) higher than the amount delivered by drug solution. Further the influence of the vesicles on the penetration of tolterodine tartrate was observed by comparing ethanolic drug solution with that of vesicles containing only ethanol (no terpenes). The vesicles with 10% ethanol were compared with the ethanolic drug solution and drug solution. The results revealed a 2.6 ($p< 0.0001$) and 1.08 ($p> 0.05$) fold higher penetration of tolterodine tartrate into the skin indicating the positive effect of ethanol contained in the vesicles on the penetration of tolterodine tartrate compared to drug solution and ethanolic drug solution. In comparison to vesicles without terpenes, invasomes with 1% terpenes showed a 1.3 fold higher deposition of tolterodine tartrate in the skin.

On the basis of these results an additive penetration enhancement mechanism of phospholipids, ethanol and terpenes was proposed. These conclusions are in accordance with results found by other authors who proposed that ethanol and phospholipids [12] as well as terpenes, phospholipids and ethanol, applied together have an additive effect on fluidizing the intercellular SC lipids, which results in an enhanced penetration of substances. Upon applying invasomes to the skin a number of concomitant processes may take place. Ethanol in the invasome dispersion fluidizes the intercellular SC lipids, disturbing the organization of the bilayer structure of the intercellular lipid matrix of SC [13]. Since, we propose that one part of the vesicles is fragmented during their penetration into the upper skin layers, the released terpenes, as well as phospholipids, act also as penetration enhancers fluidizing the intercellular lipids [14].

In addition, an increased flexibility, i.e. deformability of invasomes induced by the presence of ethanol and especially terpenes, was proposed, which was shown by cryo-electronmicroscopy [6]. These phenomena, i.e. disturbed organization of SC lipids, high deformability of invasomes and the presence of the transepidermal osmotic gradient might facilitate the penetration of some small intact invasomes, which did not disintegrate, into the SC, where they can release their incorporated drug. The release of the drug in the deeper layers of the SC could be a result of fusion of vesicles with skin lipids and drug release along the penetration pathway. According to some researchers some of the small invasomes may have reached either deeper SC layers intact or passed through the SC following small hydrophilic channels present in the intercellular space of the SC or the invasomes may have followed the follicular transport pathway [6]. The penetration of intact vesicles was also proposed by other authors. Cevc and Blume [15] claimed that intact vesicles could pass into and even through SC under the influence of the transepidermal osmotic gradient. Also, Honeywell-Nguyen et al. [16] demonstrated the partitioning of intact vesicles into the deeper layers of SC. An interesting point in their investigation was the difference in size between elastic vesicles at the skin surface and in the channel-like regions in the deeper SC, showing smaller vesicles in the channels in the deeper SC. This finding suggested that only smaller vesicles can partition into the SC through the channel-like regions. On the other hand, some authors [17] support the theory that vesicles disintegrate at the skin surface and that vesicle components penetrate molecularly dispersed into the intercellular lipid matrix, where they mix with the SC lipids modifying the lipid lamellae and enhancing the drug penetration. On the basis of the results from this study and results found by the aforementioned groups [16, 17] the following theory is supported: some of the invasomes were fragmented during their penetration through the SC, while some of the small and deformable invasomes could have penetrated to the deeper SC layers intact. However, the mechanism of the penetration enhancing ability of invasomes should be further investigated.

Iontophoresis

The present work is directed to a method to study the penetration of the drug solution and the drug encapsulated within a lipid vesicle, by iontophoresis. The combination of vesicular delivery with iontophoresis is unexpectedly beneficial. The iontophoresis device comprises an active electrode which is placed in the donor compartment. Neutral compounds are usually iontophorised under the anode to obtain the benefit of electroosmotic flow that is the bulk fluid flow in the direction of counter ion movement carries drug molecules with it. This mechanism was suggested to be the main driving force for uncharged species during iontophoresis [18] However, as invasomes were

negatively charged according to Nina Dragicevic-Curic [19] they were driven under the cathode. The results of iontophoretic drug transport showed that the permeability of tolterodine tartrate released from invasomes was higher compared with that of free drug.

Since current can easily be controlled by the use of electronics, it is a convenient mean to control delivery of drugs to the body. However, a large increase beyond the permissible limits causes irritation and can damage the skin. In general, 0.5mA/cm^2 is often stated to be the maximum iontophoretic current which should be used on human beings [20].

The persistent use of direct current (DC), proportional to time, can reduce the iontophoretic flux because of its polarization effect on the skin. Which was overcome by the use of pulsed DC which is a direct current delivered in a periodic manner. The stratum corneum of the skin is composed of keratinocytes, which is a poor conductor because it contains high intercellular lipids and its water content is only approximately 20% When continuous DC is applied on the skin, this layer of the skin may act as resistance and thus result in magnetic polarization currents. This polarization operates against the applied electrical field and greatly decreases the magnitude of effective current across the skin and the efficiency of transdermal delivery of drugs by iontophoresis. To avoid this polarization, the current should be applied in a periodic manner (pulsed current). With pulsed current, the electrical field is switched on and off periodically. In the "on" state, drugs are delivered by the iontophoretic diffusion process into the skin channels, and they could be blocked and accumulated in the skin reservoir. In the "off" state, electrical field is removed to permit the skin to depolarize and the drugs slowly diffused out of the skin and the current is discharged. Therefore, every new cycle could start with no residual polarization remaining in the skin from the previous cycle. In our experiment on/off interval of 1:1 was chosen, as this ratio efficient delivery than other ratios according to Liu Wei et. al [8] , when the on/off interval ratio was too low, like 1:5 or 1:3, the skin had long depolarization time and the effective iontophoresis time was short; when the on/off interval ratio was high, like 3:1 or 5:1, the skin was depolarized. Therefore, in the iontophoretic process, suitable on/off interval ratio of the current should be selected to enhance the iontophoretic efficiency.

Table 2: *Ex vivo* release data of drug solution, optimized formulation and vesicles without terpene by iontophoresis

Formulation Code	Q_8 (%)	SSTF (µg/cm^2/hr)	ER
TTL2i	99.54	0.0387	15.48
TTNTi	72.50	0.0276	11.04
TTEi	62.58	0.0223	8.92
TTi	40.46	0.0135	5.4

Table 2 shows that the iontophoretic permeation of tolterodine tartrate across rat skin was increased when encapsulated in invasomes. This phenomenon can be well explained by the following inferences and findings: the addition of phosphatidylcholine to dermal dosage forms has been reported to enhance transdermal absorption. The invasome may be adsorbed by and fuse with the surfaces of the skin, which can alter the lipid barrier and result in a more permeable structure. Moreover, the observed fusion of phospholipids on the skin surface may facilitate accumulation of water in the SC which subsequently contributes to the increase of conductivity in skin during iontophoresis and enhancement of drug permeation

The results also showed that invasomes were superior to iontophoresis. However, the use of invasomes in combination with iontophoresis resulted in an additive effect on the flux of tolterodine tartrate. The flux increased from 0.0086 and 0.0135 when invasomes with limonene or iontophoresis were used alone to 0.0387µg/hr/cm^2 ($p< 0.0001$) when both were combined.

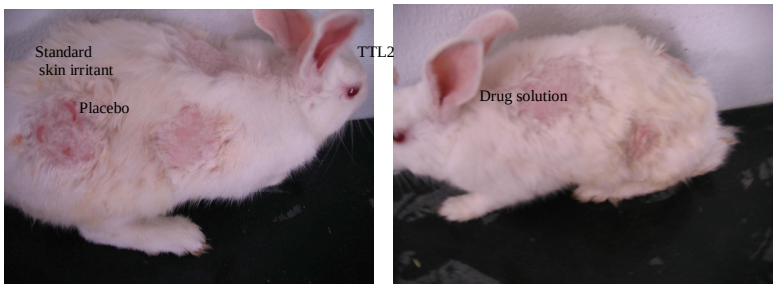
It has been shown that the activity of terpene enhancers depends upon the physicochemical properties of the drug as well as the properties of the enhancer itself. Lipophilic terpenes were shown to be more effective in enhancing the permeation of lipophilic. Hence limonene with greater lipophilicity might have increased the permeation of tolterodine tartrate a slightly lipophilic drug.

In conclusion, invasomes were more effective than iontophoresis alone in enhancing tolterodine tartrate transdermal delivery across hairless rat skin. However, the combination of terpenes and iontophoresis generally resulted in an additional increase in tolterodine tartrate flux.

Skin irritation studies

The optimized formulations TTL2 (limonene), placebo and drug solution showed irritation potential of '0', thus proving to be non-irritant. The '0' value in an irritancy test indicates that the applied formulations are generally non irritant to human skin. No obvious erythema and edema was observed on rabbit skin after 24 hr of application of the optimized invasomal formulations.

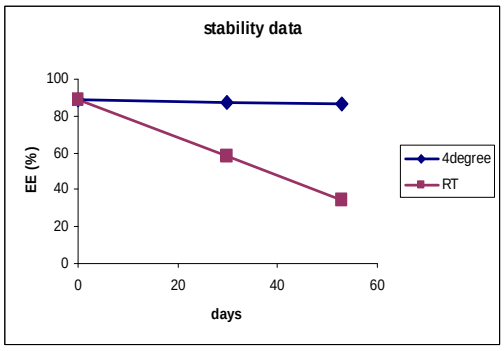
Figure 3: Skin irritation study on rabbit after application of standard skin irritant, placebo, and invasomal formulation.



Stability studies of optimized invasomal formulation

The invasomal formulation was sealed in 10 ml glass vials and stored at refrigeration temperature (4˚C - 8˚C) and at 30˚C for two months. The EE of optimized samples were determined in the same manner as prescribed previously after two months and the results were obtained.

Figure 4: Stability study data TTL2 formulation



The percentage of drug retained in the vesicles after a period of two months at 4˚C and room temperature were found to be 86.51% and 34.67% respectively for TTL2 formulation. The results indicate that more than 90% of the drug was retained in the invasomal formulation for a period of two months at 4˚C. The decrease in the entrapment efficiency was may be due to fusion or aggregation of vesicles. The stability profile of tolterodine tartrate loaded invasomal formulations evaluated for substantial loss of drug at various temperatures suggested the storage of the novel formulation at refrigerated temperature (4±2 °C), as at elevated temperatures greater drug loss from the system was observed (Figure 4), that might be ascribed to the effect of temperature on the gel-to-liquid transition of lipid bilayers together with possible chemical degradation of the phospholipids, leading to defects in the membrane packing [21].From this it can be concluded that invasomal formulations were stable to store under refrigeration temperature with least leakage.

CONCLUSION

Promising results were obtained with invasome formulation containing limonene because of the lipophilicity of the terpene and its low boiling point. Findings from this study demonstrate that transdermal delivery of invasomes encapsulating drug molecules in combination with iontophoresis may be applicable to various drugs in order to increase the permeation through the skin; however the enhancement achieved will depend to a great extent on the concentration of phospholipid and type of the terpene. In conclusion, results of this study demonstrate that it is possible to achieve additive enhancement by combination of physical and chemical penetration enhancements techniques.

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