

## Research Article



## Design, Development and Evaluation of Proniosomal Gel of an Antifungal Drug - Ketoconazole

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### ABSTRACT

Ketoconazole existing oral formulations suffer poor bioavailability since KTZ undergoes a marked first-pass effect and its absorption is dissolution rate-limited. In this study, a novel sustained release proniosomal system was designed using different non-ionic surfactants in which proniosomes were converted to niosomes upon skin water hydration following topical application under occlusive conditions. Different *in vitro* aspects (encapsulation efficiency, vesicle size and shape, *in vitro* release and stability) were studied leading to an optimized formula. All formulae exhibited high entrapment efficiencies, regardless of the surfactant HLB. Vesicle size analysis of promising formulation showed that all vesicles were in the quality range which favored efficient transdermal delivery. The entrapment efficiency of drug in optimized formulation (F3) containing Span 60 is high (94.93%) moreover, the extent of drug permeation through the membrane from the optimized formula was also quite high (93.52%) after 24 hrs. Concentration of cholesterol and lipid also plays an imp role in the entrapment efficiency of formed proniosomes. The above results indicate that the proniosomal gel of KTZ could be formulated for sustained release using optimum concentration of cholesterol, lipid and suitable surfactant to deliver a desired concentration of drug at site of action and overcoming the side effects of oral route.

**Keywords:** Proniosomes, Niosomes, Phospholipids, Ketoconazole, Transdermal delivery.

### INTRODUCTION

Since the first introduction of liposomes in the early 1960s, it was encouraging to observe the outbreak of both theoretical and practical applications, which this versatile drug delivery system has invoked. No doubt that drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have proved to possess distinct advantages over conventional dosage forms because the particles can act as drug reservoirs, can carry both hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning of these drugs into hydrophobic domains and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site.<sup>1</sup> Liposomes are unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids, assembled into bilayers. Because of their ability to carry a variety of drugs, liposomes have been extensively investigated for their potential application in pharmaceuticals, such as, drug delivery<sup>2-4</sup>, for drug targeting<sup>5</sup>, for controlled release<sup>6</sup>, or for increasing solubility.<sup>7</sup> However, there remain significant problems in the general application of liposomes for drug delivery. In a dispersed aqueous system, liposomes may suffer some chemical problems associated with degradation by hydrolysis or oxidation<sup>8</sup>, as well as physical problems as sedimentation, aggregation, or fusion of liposomes during storage.<sup>9</sup>

Two approaches were adopted in dealing with these problems; the first was to address the problems with the physical stability of aqueous suspensions of liposomes. In 1986, Payne<sup>10</sup> introduced 'proliposomes', a dry free-flowing granular product which could be hydrated

immediately before use. Proliposomes are composed of water-soluble porous powder as a carrier upon which one may load phospholipids and drugs dissolved in organic solvent. Proliposomes can be stored in a dry state and dispersed/dissolved to form an isotonic multilamellar liposomal suspension by the addition of water as needed. It was reported that amphotericin B proliposomes could be stored for 9 months without significant changes in distribution of vesicle size, and for at least 6 months without loss of pharmacological activity. Even though proliposomal formulations are an improvement over conventional liposome dispersions in terms of the physical stability of the preparation chemical instability is still present and therefore a vacuum or nitrogen atmosphere is recommended during preparation and storage to prevent the oxidation of phospholipids.<sup>11</sup> The second approach was to address the chemical problems by using alternatives to phospholipids in preparing vesicles.

One alternative involves the formation of 'niosomes' from hydrated mixtures of cholesterol and non-ionic surfactant. These 'niosomes' are quite stable, require no special conditions, such as low temperature or inert atmosphere for production or storage and the relatively low cost of the materials that form them make niosomes more attractive than liposomes for industrial manufacturing.<sup>12</sup>

However, even though niosomes exhibit good chemical stability during storage, aqueous suspensions of niosomes may exhibit problems of physical instability such as aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting their shelf life. The latest approach in the field of vesicular delivery is



to combine the two previously mentioned techniques by extending the pro-vesicular approach to niosomes through the formation of "proniosomes" which are converted to niosomes upon hydration. Proniosomes avoid many of the problems associated with aqueous niosome dispersions, and problems of physical stability (aggregation, fusion, leaking) could be minimized. The additional convenience of the transportation, distribution, storage, and dosing would make 'dry niosomes' a promising industrial product. Proniosomes offer a versatile vesicle delivery concept with potential for delivering drugs via transdermal route.

This is based on proniosomes ability to form niosomes upon hydration with water from skin following topical application under occlusive conditions.<sup>13</sup> Yet, and to the best of our knowledge, a very few number of research activities have been done to explore the full potential of these systems specially with transdermal delivery and published work have mainly used sorbitan esters (Spans) and polyoxyethylene sorbitan esters (Tweens) as non-ionic surfactants together with a limited amounts of lecithin and cholesterol.<sup>13-16</sup>

In this study, a novel approach was developed that utilizes non-ionic surfactants of different HLB number in formulating proniosomal systems.

Non-ionic surfactants having a polar head group and a non polar tail of different alkyl chain length. The stability and consequently the entrapment efficiency of the vesicle formed by using different non-ionic surfactants were highly affected by the intrinsic properties of the surfactant, such as HLB, chemical structure and phase transition temperature<sup>37</sup>.

They have HLB values from 1 to 16, and therefore they can be applied in many areas of pharmaceutical and cosmetically technology as emulsifiers, solubilizing agents, lubricants, penetrating enhancers and pore forming agents.<sup>17</sup> Possible future utilization of these surfactants in more developed systems is expected because they have low toxicity, biocompatibility, and excellent biodegradability.<sup>18</sup>

KTZ is very lipophilic which leads to accumulation in fatty tissues. KTZ is best absorbed at highly acidic levels, so antacids or other causes of decreased stomach acid levels will lower the drug's absorption when taken orally.

As with all azole antifungal agents, KTZ works principally by inhibition of an enzyme cytochrome P450 14-alpha-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that converts lanosterol to ergosterol. Since an acidic environment is required for the dissolution of KTZ, bioavailability is markedly depressed in patients taking H<sub>2</sub>-Histamine receptor blocking agents such as cimetidine or proton pump inhibitors.

After oral doses of 200, 400, and 800 mg, peak plasma concentrations of KTZ are approximately 4, 8 and 20 µg/ml. The half-life of the drug increases with dose, and it

may be as long as 7 to 8 hours when the dose is 800 mg. KTZ is metabolized extensively, and the inactive product appears in feces. Concentrations of active drug in urine are very low. In blood, 84% of KTZ is bound to plasma proteins, largely albumin; 15% is bound to erythrocytes; and 1% is free.

The concentration of KTZ in the CSF of patients with fungal meningitis is less than 1% of the total drug concentration in plasma. Based on these considerations and to overcome all these problems, the current work has aimed to develop a novel KTZ proniosomal controlled transdermal strategy, in which non-ionic surfactants were incorporated as permeation and absorption enhancers. In this way, smooth and continuous KTZ delivery through the skin into the bloodstream could be ensured thus avoiding its first pass metabolism and produce stable plasma concentrations over a long period.

The influence of HLB of different surfactants and their phase transition temperature on the in vitro characters of the formula (encapsulation efficiency, vesicle size and shape, drug release, drug permeation across skin and stability) were investigated.

## MATERIALS AND METHODS

Ketoconazole (KTZ) was kindly provided by Heliox Pharmaceutical Co. Ltd. Different grades of surfactants: Span 20, Span 40, Span 60, Span 80 were donated by Loba Chem Pvt Ltd, Mumbai and Tween 20, Tween 60 and Tween 80 were kindly donated by S.D Fine Chem Ltd. Mumbai.

Soya lecithin Phospholipon 90 H (stored at 2-5°C) was kindly donated by Lipoid (Lipoid, Germany). Cholesterol was donated by Sigma Aldrich, USA. Methanol was purchased from Ranbaxy Fine Chem Ltd, New Delhi. All other chemicals were of analytical reagent grade and were obtained from Merck Pharma Pvt. Ltd, S.D Fine Chem Ltd. and Loba Chem Pvt. Ltd, Mumbai.

## Preparation of Proniosomes

Proniosomal formulae were prepared by a method reported by Fang<sup>13</sup> using different types of non-ionic surfactants, lecithin and cholesterol and evaluated in a "one factor at a time" fashion. Appropriate amounts of proniosomal components together with KTZ were mixed with 125 ml absolute ethanol in a wide-mouth glass tube. The open end of the glass tube was covered with a lid and warmed in a water bath at 65 ± 3°C for 5 min. Eighty micro liters of pH 7.4 phosphate buffer was added and warming was continued on the water bath for about 2 min till a clear solution was observed. The mixture was allowed to cool down at room temperature till the dispersion was converted to proniosomal gel. The exact mechanism of conversion to the gel state is described in details by Vora<sup>14</sup> The effects of surfactant type, amount of surfactant, lecithin and cholesterol as well as drug load were studied. The composition of different formulations (F1–F15) is listed in Table 1.



**Table 1:** Composition of Proniosomes of Ketoconazole

Formulation Code	Drug (mg)	Span 20 (mg)	Span 40 (mg)	Span 60 (mg)	Span 80 (mg)	Tween 20 (mg)	Tween 60 (mg)	Tween 80 (mg)	Lecithin (mg)	Cholesterol (mg)	Ethanol ( $\mu$ l)
F1	7.37	90							90	10	125
F2	7.37		90						90	10	125
F3	7.37			90					90	10	125
F4	7.37				90				90	10	125
F5	7.37					90			90	10	125
F6	7.37						90		90	10	125
F7	7.37							90	90	10	125
F8	7.37			45					90	10	125
F9	7.37			135					90	10	125
F10	7.37			90					90	5	125
F11	7.37			90					90	15	125
F12	7.37			90					45	10	125
F13	7.37			90					135	10	125
F14	4.0			90					90	10	125
F15	10.0			90					90	10	125

**Table 2:** EE (%), Vesicle size and %CDR of various compositions

Formulation	% Entrapment Efficiency	Vesicle Size (nm)	% Cumulative drug release
F1	88.19 $\pm$ 2.1	2330 $\pm$ 1.1	87.17 $\pm$ 0.55
F2	93.62 $\pm$ 1.5	2470 $\pm$ 2.5	92.87 $\pm$ 0.88
F3	94.43 $\pm$ 2.8	3160 $\pm$ 1.6	93.52 $\pm$ 0.6
F4	84.90 $\pm$ 3.3	3660 $\pm$ 3.5	82.78 $\pm$ 0.92
F5	74.52 $\pm$ 3.0	3890 $\pm$ 2.6	73.91 $\pm$ 0.94
F6	78.41 $\pm$ 2.6	5780 $\pm$ 5.8	77.33 $\pm$ 1.3
F7	80.80 $\pm$ 2.1	5520 $\pm$ 4.9	79.11 $\pm$ 1.02
F8	93.00 $\pm$ 1.1	3200 $\pm$ 2.5	63.23 $\pm$ 0.97
F9	91.6 $\pm$ 2.0	4350 $\pm$ 1.6	71.07 $\pm$ 0.86
F10	89.66 $\pm$ 2.4	3740 $\pm$ 1.7	74.05 $\pm$ 1.04
F11	73.37 $\pm$ 2.4	1622 $\pm$ 1.2	65.68 $\pm$ 0.95
F12	80.27 $\pm$ 2.7	2450 $\pm$ 1.8	84.57 $\pm$ 0.91
F13	85.97 $\pm$ 3.0	7520 $\pm$ 2.0	64.72 $\pm$ 1.01
F14	85 $\pm$ 2.2	2910 $\pm$ 1.1	63.23 $\pm$ 0.88
F15	82.89 $\pm$ 2.5	4650 $\pm$ 1.9	71.07 $\pm$ 1.09

**Ketoconazole Entrapment Efficiency (% EE)**

Proniosomal gel (0.1 g) was reconstituted with 10 ml of pH 7.4 phosphate buffer in a glass tube. The aqueous suspension was sonicated in a sonicator bath (Rolex,INDIA) for 30 min.

The KTZ containing niosomes were separated from untrapped drug by centrifuging at 14,000 rpm at 4°C for 30 min (Ultra centrifuge 5417R, eppendorf, Hamburg, Germany). The supernatant was taken and diluted with methanol, and the KTZ concentration in the resulting solution was assayed by spectrophotometrically using UV-Vis Spectrophotometer (Shimadzu Corporation, Japan 1800).

The percentage of drug encapsulation was calculated by the following equation.<sup>13-15</sup>

$$EE (\%) = \left[ \left( \frac{C_t - C_f}{C_t} \right) \right] \times 100$$

Where,  $C_t$  is the concentration of total KTZ, and  $C_f$  is the concentration of free KTZ.

**Vesicle Size Analysis**

The vesicle size was determined using Master sizer S-laser diffractometer (Malvern Instruments, Malvern, Worcestershire, UK). In a glass tube, 0.2 g proniosome gel was diluted with 10 ml of pH 7.4 phosphate buffer. The vesicle measurements were done at temperature 25  $\pm$  0.5 °C using a lens (with a laser range of 300 mm that measure particle size range from 0.5 to 900  $\mu$ m and a beam length of 2.4 mm was attached to a measuring cell). The obscuration level was kept at 10% at a stable count rate. Three replicates were taken for each sample, and polystyrene beads were used as a standard to check

instrument performance.<sup>22</sup> The polydispersity index (PI) was determined as a measure of homogeneity. Small values of PI (<0.1) indicate a homogeneous population, while PI values >0.3 indicate high heterogeneity.<sup>23</sup>

## Microscopic Examination

### Optical Microscope

In a glass tube, 0.2 g proniosome gel of F3, F14 and F15 having different drug loads were diluted with 10 ml of pH 7.4 phosphate buffer; a few drops of the formed niosomal dispersion were spread on a glass slide and examined for the presence of insoluble drug crystals using ordinary light microscope with varied magnification powers (10 and 40). Photomicrographs were taken using a digital camera (Sony Cyber shot DSC-w55 7.2 megapixel, Tokyo, Japan).

### Scanning Electron Microscopy (SEM)

In an attempt to illustrate the role of cholesterol in vesicle formation, the morphological differences in shape and surface characteristics of the prepared proniosome-derived niosomes of formulae F3, F10 and F11 having different cholesterol contents were examined using a scanning electron microscope. In a glass tube, 0.2 g proniosome gel was diluted with 10 ml of pH 7.4 phosphate buffer; the dispersion was sprinkled and fixed on a SEM holder with double-sided adhesive tape and coated with a layer of gold of 150 Å for 2 min using a Sputter coater (Edwards, S-150A, England) working in a vacuum of ( $3 \times 10^{-1}$  atm) of Argon gas.

The samples were examined using a scanning electron microscope<sup>22</sup> (Jeol, JSM T20, Tokyo, Japan).

### In-vitro Release Test

*In-vitro* diffusion studies of proniosomal gel were carried out using egg membrane. Proniosomal gel equivalent 10 mg drug was taken. The membrane was mounted on a vertical Franz diffusion cell facing the receptor compartment. The donor side was charged with 10 mg equivalent drug formulation. The membrane surface area available for diffusion was 2.54 cm<sup>2</sup>.

The receptor compartment was filled with 5 ml of phosphate buffer. Temperature was maintained at  $37 \pm 0.5^\circ\text{C}$ . The receptor compartment was constantly stirred at 100 rpm. Samples from the receptor fluid (1ml) were withdrawn at various time intervals up to 24 hrs and replaced immediately by fresh buffer solution.

The samples were then assayed spectrophotometrically using UV-Vis Spectrophotometer (Shimadzu Corporation, Japan 1800) at 296 nm. At predetermined time intervals, namely 1, 2, 3, 4, 6, 8, 12, 24 and 48 h, samples of 1 ml were taken from the receptor compartment and the cell was refilled with an equivalent amount of fresh receptor solution. Samples were analyzed spectrophotometrically. Each experiment was replicated three times and from the concentration of ketoconazole in the receiving solution

the amount permeated through the membrane was calculated.

The cumulative amount of Ketoconazole permeated into the receptor compartment was plotted against time to obtain a percentage permeation profile.

### Stability

The optimized proniosomal formulae F3 was sealed in 30 ml clear glass vials and stored at refrigeration temperature at  $5 \pm 3^\circ\text{C}$ ;  $25 \pm 2^\circ\text{C}$  and  $60 \pm 5\% \text{RH}$ ;  $40 \pm 2^\circ\text{C}$  and  $75 \pm 5\% \text{RH}$ . After 90 days, hydration step was carried out and the entrapment efficiency as well as the mean particle size of each sample was determined and compared to the freshly prepared proniosomes-derived niosomes.

## RESULTS AND DISCUSSION

### Entrapment Efficiency% (EE %)

The entrapment efficiency is one of the important parameters in the design of vesicular formulations. Vesicle entrapment efficiency relies on the stability of the vesicle which is highly dependent on the type and amount of surfactant forming the bilayers, the amount of both cholesterol and lecithin and the drug load. Although it is important, the effect of drug load is usually missed in many publications.<sup>37</sup> The effects of these different parameters were discussed.

### Effect of Surfactant Type

Non-ionic surfactant is the main building block of niosomal vesicles. The stability and consequently the entrapment efficiency of the formed vesicles were highly affected by the intrinsic properties of the surfactant, such as, HLB, chemical structure and phase transition temperature.<sup>37</sup> The effect of using different traditional non-ionic surfactants on the entrapment efficiency of proniosomal formulations after hydration is shown in Table 2. Interestingly, formulations containing surfactants of either low or high HLB exhibited relative high entrapment efficiency, with some approaching 100% (Span 40, Span 60 and Tween 80). The vesicle formation ability of hydrophobic non-ionic surfactants could be understood as the molecule geometry fulfilled a proper critical packing parameter where the highly lipophilic drug is expected to be housed almost completely within the vesicles bilayer.<sup>13,15</sup> However, hydrophilic surfactants were also able to form vesicles due to the presence of sufficient amount of cholesterol.<sup>12</sup> These results indicated that not only the HLB but also the chemical structure of the surfactant which is related to the length of the alkyl chain was also governing the entrapment efficiency. Surfactants of longer saturated alkyl chains showed higher entrapment efficiency.<sup>38</sup> Another important factor that commonly helps to explain the vesicles entrapment patterns is the gel to liquid phase transition temperature ( $T_c$ ) of the surfactant forming the niosomal membrane which is directly proportional to the surfactant alkyl hydrocarbon chain length. Surfactants of higher  $T_c$  are



more likely in the ordered gel form forming less leaky bilayers, thus having higher entrapment efficiency, while surfactants of lower  $T_c$  are more likely in the less ordered liquid form.<sup>12,39</sup> On the basis of HLB, alkyl chain length and phase transition temperature, results in Table 2 could be explained as follows: Both Span 60 and Span 40 showed high EE% (94.43, 93.62 respectively) since both are hydrophobic surfactants with low HLB. Tween 60 and Tween 80 showed relative lower EE% (78.41, 80.80 respectively) due to their high HLB, yet Tween 80 revealed higher results due to its longer alkyl chain.

#### Effect of Surfactant Amount

Increasing the amount of surfactant, the main component responsible for vesicle formation significantly increased the EE%. This might be attributed to the increase in the number of formed niosomes and consequently the volume of the hydrophobic bilayer domain, the available housing for entrapment ketoconazole hydrophobic drug.<sup>40-41</sup>

#### Effect of Cholesterol Amount

Increasing the cholesterol content accompanied by a significant increase in EE% ( $p < 0.05$ ). However, further cholesterol increase resulted in EE% significant decrease ( $p < 0.05$ ). This pattern was reported previously by Mokhtar<sup>25</sup> and was suggested to take place due to the ability of cholesterol to abolish gel to liquid phase transition of niosomal systems resulting in less leaky vesicles.<sup>37</sup> Moreover, cholesterol molecules accommodate itself as "vesicular cement" in the molecular cavities formed when surfactant monomers are assembled into bilayers to form niosomal membranes.<sup>37-42</sup> This space filling action was responsible for the increased rigidity, decreased permeability of cholesterol-containing membranes compared to cholesterol-free membranes and the improved entrapment efficiency. In contrast, increasing cholesterol beyond a certain concentration may compete with the drug for the space within the bilayers, hence excluding the drug and can disrupt the regular linear structure of vesicular membranes.<sup>25</sup>

#### Effect of Lecithin Amount

Significant increase in EE% was observed with increasing lecithin content ( $p < 0.05$ ) which is commonly added to increase the system stability.<sup>43</sup> This result was substantiated by the high transition temperature of hydrogenated lecithin used in this study (48°C) and its unique advantage over unhydrogenated one in enhancing the rigidifying effect of cholesterol and formation of less leaky membrane bilayers.<sup>12-45</sup>

The presence of double bonds in the unhydrogenated phosphatidylcholine permit the chains to bend (undergo conformational rotations to give cis/trans conformations), causing the adjacent molecule not to be tightly close to the bent phosphatidylcholine molecule when they assembled to form the niosomal membrane; accordingly,

the membrane become more permeable.<sup>41</sup> The opposite held true upon saturation of this double bond, and the inclusion of hydrogenated lecithin forces the bilayer molecules to get in order resulting in more rigid and less permeable vesicle membrane.<sup>45</sup> Similar results were previously reported.<sup>13-27</sup>

#### Effect of Drug Load

Increasing the Ketoconazole drug load from 7.37 to 10 mg led to significant decrease in EE% ( $p < 0.05$ ). It should be pointed out that a fixed amount of vesicle components (surfactant, cholesterol and lecithin) produce a constant number of niosomes of definite entrapment efficiency<sup>41</sup> with precipitation of excess drug.<sup>25</sup>

#### Vesicle Size Analysis

All formulae were of small vesicle size ranging from 1622nm to 7520nm with low polydispersity index and unimodal size distribution (Table 2), which favors transdermal delivery of the drug.<sup>46</sup> Direct proportionality did exist between the vesicle size and both chain length and degree of hydrophilicity of the surfactants forming the vesicle bilayer.<sup>47,48</sup> The effect of chain length was illustrated by Israelachvili who proposed an equation by which the minimum vesicular radius (R) could be calculated:

$$R = \frac{l_c}{\left(1 - \frac{v}{a_o l_c}\right)}$$

where R = minimum vesicular radius that a vesicle can attain,  $l_c$  = hydrophobic chain length,  $v$  = hydrophobic chain volume,  $a_o$  = hydrophilic head group area.

While for the other factor, it was found that as the hydrophilicity of the niosome bilayers increased, the water intake of the bilayers of these vesicles increased too resulting in larger vesicle size.<sup>47</sup> Using these two factors, the differences in the mean vesicle size could be explained: being more hydrophobic, Span-based niosomes showed smaller vesicle size than Tween-based niosomes which are more hydrophilic. Yet, within each class, the surfactant of higher chain length (Span 80 and Tween 80) produced a larger vesicle than those of smaller chain length (Span 20 and Tween 20). Upon variation of amounts of proniosomal ingredients, the following effects on vesicle size were observed. Increasing surfactant/lipid ratio led to an increase in vesicle size which was substantiated by the increase in the overall degree of hydrophilicity. The opposite held true with increasing the cholesterol amount which was associated with a decrease in the hydrophilicity of bilayers, thus limiting the water intake to the vesicles core and resulted in a subsequent decrease in mean vesicle size. Also, one can accept the increase in mean vesicle size by increasing lecithin content if we considered the long hydrocarbon chain of lecithin molecules (18°C). Finally, the increase in mean vesicle size with increasing drug load was attributed to

the drug entrapped in the hydrophobic domain of the vesicle, causing the bilayer molecules to become apart from each other leading to an increase in vesicle size.<sup>49</sup> The optical microscopic picture of proniosome loaded with drug when converted in to niosomes when hydrated with phosphate buffer pH7.4 shown in Figure 1.

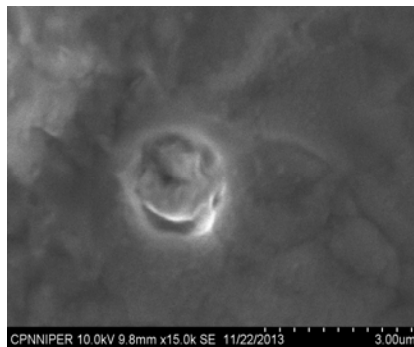
### Scanning Electron Microscopy

Scanning electron micrographs revealed the formation of well identified spherical niosomal vesicles with sharp boundaries after hydration of proniosomes. The surface characteristics of proniosomes-derived niosomes of F3, F10 and F11 containing 10mg, 5 mg and 15 mg of

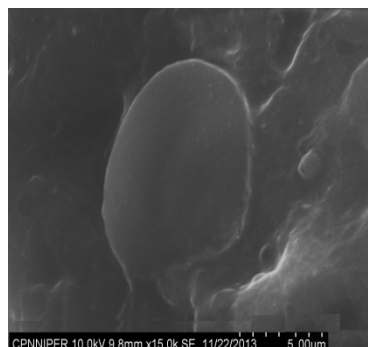
cholesterol, respectively, have shown an increased vesicle wall rigidity with increasing cholesterol content (Figure 2).



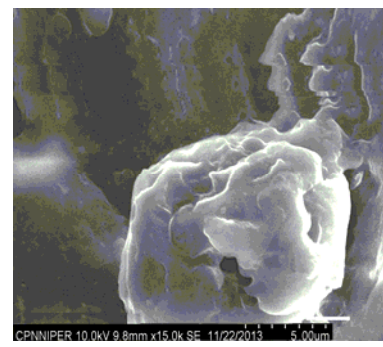
Figure 1: Optical Microscopy



(a) F3



(b) F10



(c) F11

Figure 2: SEM of Niosomes

### In vitro Release

Results of ketoconazole in vitro release from different formulae are illustrated in Table 1 and Figure 1 - 5. It was apparent that the incorporation of ketoconazole in all proniosomal formulations led to significant slower release profiles ( $p < 0.01$ ) compared to its conventional formulation in Carbopol where 96.66% was released in 6 h is illustrated in Table 2 and Figure 6. The kinetic analysis of all release profiles followed diffusion controlled mechanism with an initial relative fast release phase followed by a slower release one. The initial phase was due to the desorption of ketoconazole from the surface of niosomes while the drug release in the slower phase was regulated by diffusion through the swollen niosomal bilayers.<sup>52</sup> This profile could be advantageous if we considered the importance of epidermis saturation with initial fast drug released to achieve high concentration gradient required for successful drug delivery to the blood.<sup>17</sup> It is rather important to mention the inverse relation between the entrapment efficiency and the drug release. Entrapment efficiency is a measure of the vesicle ability to retain the drug; thus, the more the drug is retained in the vesicle, the slower the release profile will be.<sup>37</sup> Accordingly, factors that stabilize the vesicle membrane and increase the entrapment efficiency of a hydrophobic drug such as ketoconazole (namely, low HLB, long alkyl chain, high phase transition temperature) will slow down the release profile. The general features of the release profile of the proniosome derived niosomes prepared using conventional surfactants revealed

significant increase ( $p < 0.01$ ) in the percentage drug released with the increase in HLB since hydrophilic surfactants have higher solubilizing power on hydrophobic solutes in aqueous medium compared to hydrophobic surfactants.<sup>52</sup> Increasing the cholesterol amount from 5 to 15 mg and lecithin content from 45 to 135 mg resulted in a more intact lipid bilayer as a barrier for drug release which decreased its leakage and permeability, hindered the release of entrapped drug from the vesicles and led to a significant slow release profile ( $p < 0.05$ ).<sup>22,38</sup> In addition, the best release profile among different drug loads was observed upon using 7.37 mg ketoconazole. Therefore, based on in vitro characterizations, the optimized formula F3 based on Span 60 as traditional non-ionic surfactant was further progressed to Stability (Table 2). The amount of surfactant was set to 90 mg to ensure the highest dissolution rate and the highest entrapment efficiency as well, medium level of cholesterol and lecithin (10 mg and 90 mg respectively) were selected for optimal membrane stability and maximum release of entrapped drug.

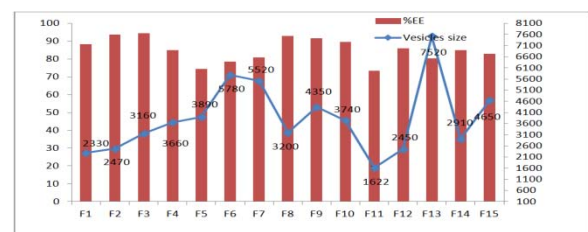
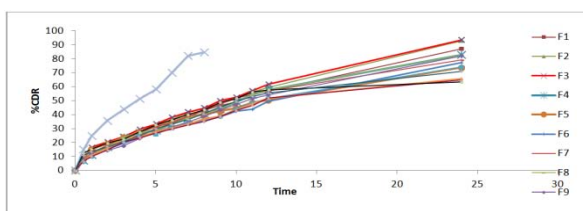


Figure 3: Comparison of %EE and Vesicle size of all formulations F1-F15.



**Figure 4:** *In-vitro* release profile of all formulation of 24 hours

### Stability Studies

Results presented in Table 3 revealed that after storage for 90 days the drug entrapment efficiency of F3 were not significantly different ( $p > 0.05$ ) from the same formulae when freshly prepared. The ability of proniosomes-derived niosomes to retain their entrapment efficiency was previously reported by Abd-Elbary.<sup>22</sup> Results showed that there were no significant changes in % drug entrapment at  $5 \pm 3^\circ\text{C}$ . On the other hand, significant changes was observed in the % drug entrapment after three months at  $25 \pm 3^\circ\text{C}$  and  $40 \pm 2^\circ\text{C}$  temperatures. The results of drug retention studies showed higher drug leakage at higher temperature. This may be due to the higher fluidity of lipid bilayers at higher temperature, resulting in higher drug leakage. Loss of drug from the vesicles stored at elevated temperature may be attributed to the effect of temperature on the gel to liquid transition of lipid bilayers together with the possible chemical degradation of the phospholipids, leading to defects in membrane packing. Acceleration in drug leakage at higher temperatures, as observed in storage stability studies, suggested keeping the proniosomal gel in the refrigeration condition.

**Table 3:** Stability Study of Proniosomal Formulation F3

Time (days)	$5 \pm 3^\circ\text{C}$	$25 \pm 3^\circ\text{C}, 60 \pm 5\%RH$	$40 \pm 2^\circ\text{C}, 75 \pm 5\%RH$
0	91.66	91.66	91.66
15	91.66	89.60	89.60
30	89.63	88.19	84.90
60	89.63	86.42	80.80
90	88.19	84.90	76.52

### CONCLUSION

Taking into consideration the high efficiency in systemic delivery together with lack of physical and chemical instability and excellent safety profile, Span 60 proniosomes could be considered as very promising candidates as absorption and penetration enhancer for delivering KTZ transdermally. From the stability study it can be concluded that  $5 \pm 3^\circ\text{C}$  is the most suitable temperature for the storage of the proniosomal gel formulation. Proniosomal gel system prepared with Span 60 (F3) exhibited optimum entrapment efficiency and has shown potential for delivery of antifungal drug KTZ.

### REFERENCES

1. Betageri G., Habib M., Liposomes as drug carriers, *Pharm. Eng.*, 14, 1994, 76–77.
2. Couvreur P., Fattal E., Andremont A., Liposomes and nanoparticles in the treatment of intracellular bacterial infections, *Pharm. Res.* 8, 1991, 1079–1086.
3. Antimisiaris S.G., Jayasekera P., Gregoriadis G., Liposomes as vaccine carriers. Incorporation of soluble and particulate antigens in giant vesicles, *J. Immunol. Methods*, 166(2), 1993, 271–280.
4. Kim S., Liposomes as carriers of cancer chemotherapy. Current status and future prospects, *Drugs*, 46(4), 1993, 618–638.
5. Booser D. J., Hortobagyi G.N., Anthracycline antibiotics in cancer therapy. Focus on drug resistance, *Drugs*, 47(2), 1994, 223–258.
6. Stenekes R.J., Loebis A.E., Fernandes C.M., Crommelin D.J., Hennink W.E., Controlled release of liposomes from biodegradable dextran microspheres: a novel delivery concept, *Pharm. Res.* 17(6), 2000, 690–695.
7. Gregoriadis G., Florence A.T., Liposomes in drug delivery. Clinical, diagnostic and ophthalmic potential, *Drugs*, 45(1), 1993, 15–28.
8. Hunt C., Tsang S., Alpha tocopherol retards auto-oxidation and prolongs the shelf-life of liposomes, *Int. J. Pharm.* 8, 1981, 101–110.
9. Wong M., Thompson T., Aggregation of dipalmitoylphosphatidylcholine vesicles, *Biochemistry*, 21, 1982, 4133–4139.
10. Payne N.I., Timmins P., Ambrose C.V., Ward M.D., Proliposomes: a novel solution to an old problem, *J. Pharm. Sci.* 75(4), 1986, 325–329.
11. Hu C., Rhodes D.G., Proniosomes: a novel drug carrier preparation, *Int. J. Pharm.* 185(1), 1999, 23–35.
12. Uchegbu I. F., Vyas S.P., Non-ionic surfactant based vesicles (niosomes) in drug delivery, *Int. J. Pharm.* 172, 1998, 33–70.
13. Fang J.Y., Yu S.Y., Wu P.C., Huang Y.B., Tsai Y.H., *In vitro* skin permeation of estradiol from various proniosome formulations, *Int. J. Pharm.* 215(1–2), 2001, 91–99.
14. Vora B., Khopade A.J., Jain N.K., Proniosome based transdermal delivery of levonorgestrel for effective contraception, *J. Control. Release*, 54(2), 1998, 149–165.
15. Alsarra I.A., Bosela A.A., Ahmed S.M., Mahrous G.M., Proniosomes as a drug carrier for transdermal delivery of ketorolac, *Eur. J. Pharm. Biopharm.* 59(3), 2005, 485–490.
16. Ibrahim M.M., Sammour O.A., Hammad M.A., Megrab N.A., *In vitro* evaluation of proniosomes as a drug carrier for flurbiprofen, *AAPS PharmSciTech*, 9(3), 2008, 782–790.
17. Csoka G., Marton S., Zelko R., Otomo N., Antal I., Application of sucrose fatty acid esters in transdermal therapeutic systems, *Eur. J. Pharm. Biopharm.* 65(2), 2007, 233–237.
18. Youan B.B., Hussain A., Nguyen N.T., Evaluation of sucrose esters as alternative surfactants in microencapsulation of proteins by the solvent evaporation method, *AAPS PharmSci*, 5(2), 2003, E22.
19. Feigin V.L., Doronin B.M., Popova T.F., Gribatcheva E.V., Tchervov D.V., Vinpocetine treatment in acute ischaemic stroke: a pilot single-blind randomized clinical trial, *Eur. J. Neurol.* 8(1), 2001, 81–85.
20. Miskolczi P., Kozma K., Polgar M., Vereczkey L., Pharmacokinetics of vinpocetine and its main metabolite apovincaminic acid before and after the chronic oral



- administration of vinpocetine to humans, *Eur. J. Drug Metab. Pharmacokinet.* 15(1), 1990, 1–5.
21. Hua L., Weisan P., Jiayu L., Ying Z., Preparation, evaluation, and NMR characterization of vinpocetine micro emulsion for transdermal delivery, *Drug Develop. Ind. Pharm.* 30(6), 2004, 657–666.
  22. Abd-Elbary A., El-laithy H. M., Tadros M.I., Sucrose stearate-based proniosome derived niosomes for the nebulisable delivery of cromolyn sodium, *Int. J. Pharm.* 357(1–2), 2008, 189–198.
  23. Sentjurs M., Vrhovnik K., Kristl J., Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method, *J. Control. Release*, 59(1), 1999, 87–97.
  24. Siewert M., Dressman J., Brown C.K., Shah V.P., FIP/AAPS guidelines to dissolution/*in vitro* release testing of novel/special dosage forms, *AAPS PharmSciTech*, 4(1), 2003, 177-187.
  25. Mokhtar M., Sammour O.A., Hammad M.A., Megrab N.A., Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes, *Int. J. Pharm.* 361(1–2), 2008, 104–111.
  26. Higuchi T., Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices, *J. Pharm. Sci.* 52, 1963, 1145–1149.
  27. Hwang B.Y., Jung B.H., Chung S.J., Lee M.H., Shim C.K., *In vitro* skin permeation of nicotine from proliposomes, *J. Control. Release* 49, 1997, 177–184.
  28. Williams A.C., Barry B.W., Penetration enhancers, *Adv. Drug Deliv. Rev.* 56(5), 2004, 603–618.
  29. Higashiyama M., Inada K., Ohtori A., Tojo K., Improvement of the ocular bioavailability of timolol by sorbic acid, *Int. J. Pharm.* 272(1–2), 2004, 91–98.
  30. El-Laithy H.M., Novel transdermal delivery of timolol maleate using sugar esters: preclinical and clinical studies, *Eur. J. Pharm. Biopharm.* 72(1), 2009, 239–245.
  31. Tang H., Mitragotri S., Blankschtein D., Langer R., Theoretical description of transdermal transport of hydrophilic permeants: application to low-frequency sonophoresis, *J. Pharm. Sci.* 90(5), 2001, 545–568.
  32. Lerk P.C., Sucker H., Application of sucrose laurate in topical preparations of cyclosporin A, *Int. J. Pharm.* 92, 1993, 203–210.
  33. Sintov A., Ze'evi A., Uzan R., Nyska A., Influence of pharmaceutical gel vehicles containing oleic acid/sodium oleate combinations on hairless mouse skin, a histological evaluation, *Eur. J. Pharm. Biopharm.* 47(3), 1999, 299–303.
  34. FDA, Guidance of Industry, Food Effect, Bioavailability and Bioequivalence Studies, 2002.
  35. Abd-Elbary A., Foda N., El-Gazayerly O., El-Khatib M., Reversed phase liquid chromatographic determination of vinpocetine in human plasma and its pharmacokinetic application, *Anal. Lett.* 35(6), 2002, 1041–1054.
  36. Hammes W., Weyhenmeyer R., Quantitative determination of vinpocetine in human plasma by capillary gas chromatography–mass spectrometry, *J. Chromatogr.* 413, 1987, 264–269.
  37. Uchegbu I.F., Florence A.T., Non-ionic surfactant vesicles (niosomes): physical and pharmaceutical chemistry, *Adv. Colloid Interface Sci.* 58, 1995, 1–55.
  38. Guinedi A.S., Mortada N.D., Mansour S., Hathout R.M., Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide, *Int. J. Pharm.* 306(1–2), 2005, 71–82.
  39. Mohammed A.R., Weston N., Coombes A.G.A., Fitzgerald M., Perrie Y., Liposome formulation of poorly water soluble drugs: optimisation of drug loading and ESEM analysis of stability, *Int. J. Pharm.* 285, 2004, 23–34.
  40. Van Hal D., Jeremiasse E., De Vringer T., Junginger H.E., Bouwstra J.A., Encapsulation of lidocaine base and hydrochloride into non-ionic surfactant vesicles (NSVs) and diffusion through human stratum comeum *in vitro*, *Eur. J. Pharm. Sci.* 4, 1996, 147–157.
  41. Hao Y., Zhao L., Li N., Yang Y., Li K., Studies on a high encapsulation of colchicine by a niosome system, *Int. J. Pharm.* 244, 2002, 73–80.
  42. Agarwal R., Katare O.P., Vyas S.P., Preparation and *in vitro* evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol, *Int. J. Pharm.* 228(1–2), 2001, 43–52.
  43. Varshosaz J., Pardakhty A., Baharanchi S.M., Sorbitan monopalmitate-based proniosomes for transdermal delivery of chlorpheniramine maleate, *Drug Deliv.* 12(2), 2005, 75–82.
  44. Coderch L., Fonollosa J., De Pera M., Estelrich J., De La Maza A., Parra J.L., Influence of cholesterol on liposome fluidity by EPR. Relationship with percutaneous absorption, *J. Control. Release* 68(1), 2000, 85–95.
  45. Subczynski W.K., Wisniewska. A, Physical properties of lipid bilayer membranes: relevance to membrane biological functions, *Acta Biochim. Pol.* 47(3), 2000, 613–625.
  46. Verma D.D., Verma S., Blumberg G., Fahr A., Particle size of liposomes influences dermal delivery of substances into skin, *Int. J. Pharm.* 258, 2003, 141–151.
  47. Balakrishnan P., Shanmugam S., Lee W.S., Lee W.M., Kim J.O., Oh D.H., Kim D.D., JKim J.S., Yoo B.K., Choi H.G., Woo J.S., Yong C.S., Formulation and *in vitro* assessment of minoxidil niosomes for enhanced skin delivery, *Int. J. Pharm.* (2009), doi:10.1016/j.ijpharm.2009.1004.1020.
  48. Israelachvili J.N., *Intermolecular and Surface Forces*, Academic Press, Sydney, 1985.
  49. Hathout R.M., Mansour S., Mortada N.D., Guinedi A.S., Liposomes as an ocular delivery system for acetazolamide: *in vitro* and *in vivo* studies, *AAPS PharmSciTech*, 8(1), 2007, 1.
  50. Szuts A., Pallagi E., Regdon Jr. G., Aigner Z., Szabo-Revesz P., Study of thermal behaviour of sugar esters, *Int. J. Pharm.* 336(2), 2007, 199–207.
  51. Cable C., *An Examination of the Effects of Surface Modifications on the Physicochemical and Biological Properties of Non-ionic Surfactant Vesicles*, Ph.D. Thesis, University of Strathclyde, Glasgow, UK, 1989.
  52. Pardakhty A., Varshosaz J., Rouholamini A., *In vitro* study of polyoxyethylene alkyl ether niosomes for delivery of insulin, *Int. J. Pharm.* 328(2), 2007, 130–141.

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