Preparation and *in vitro* evaluation of liposomal/niosomal delivery systems for antifungal drug clotrimazole

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Clotrimazole, an imidazole derivative antifungal agent is widely used for the treatment of mycotic infections of the gastrointestinal tract. In order to develop alternative formulation for the vaginal administration of clotrimazole to provide sustained and controlled release of appropriate drug for local vaginal therapy, liposomes/niosomes were evaluated as delivery vehicles. To optimize the preparation of liposomes/niosomes with regards to size and entrapment efficiency, multilamellar liposomes/niosomes containing drug were prepared by lipid hydration method. The ability of the systems to deliver clotrimazole into and through the mucosa was evaluated *in vitro* using rabbit vaginal mucosa with vertical Franz diffusion cells. The *in vitro* permeation data showed that the liposome/niosome system increased the clotrimazole total penetration through the vaginal mucosa by 1.6, 1.5-fold, the accumulation of clotrimazole into the mucosa was increased by 3.1, 2.3-fold, respectively, as compared with control during 24 hr. These results suggest that the studied liposomes/niosomes systems may be appropriate vesicles for the vaginal mucosa delivery of clotrimazole for local vaginal therapy.

**Keywords:** Clotrimazole, Liposomes, Niosomes, Vaginal drug delivery

Vulvovaginal candidiasis is the infection with *Candida albicans*1. Approximately 75% of women have a vaginal infection with a candida strain during their life and about 40 to 50% of them suffer a second one, and a small percentage show a chronic course2. Clotrimazole, which is an imidazole derivative is widely and effectively used for the treatment of vulvovaginal candidiasis3. Unfortunately, oral use of clotrimazole is unacceptable due to the severe side effects. The plasma half-life of clotrimazole is 3-6 hr, suggesting that frequent dosing is needed. Thus, topical administration of clotrimazole is recommended. However, it is limited by its very low water solubility requiring it to be incorporated into a suitable vehicle. Commercial conventional clotrimazole vaginal delivery systems, such as creams, foams, and gells, are considered to reside for a relatively short period of time at the targeted site and have higher systemic absorption of the drug resulting in systemic side effects. The entrapment of drug in vesicles may help in the localized delivery of the drug and an improved solubility and availability of the drug at the site may reduce the dose and systemic side effect.

Liposomes are being widely investigated in topical applications for skin4-6, oral7 and vaginal diseases8-9. Liposomes have been shown to enhance the penetration of vesicle-bound-drugs into the skin, after topical application, and act as “drug localizers”, with low systemic absorption of the drugs, as compared to other galenical formulation, resulting in less drug side-effect and sustained drug releasing8. Analogous to liposomes, niosomes are formed from the self-assembly of nonionic amphiphiles in aqueous media resulting in closed bilayer structures. They need some forms of energy to form the vesicles, and offer several advantages over liposomes like, higher chemical, stability, intrinsic skin penetration enhancing properties and lower costs10. Therefore, niosomes have also been widely studied as drug carriers for controlled and targeting delivery11-12. Preliminary studies indicate that niosomes behave *in vivo* like liposomes, prolonging the circulation of entrapped drug to alter its organ distribution and metabolic stability, or prolonging the contact time of drug with the applied tissues in topical application13-17, which demonstrated that niosomes could improve drug skin...
penetration and increase its accumulation in the superficial skin strata. However, little work has been carried out on the application of drug-loaded niosomes in vaginal therapy investigation.

The long-term goal of this work is to develop vaginal antifungal drug formulations for treatment of vulvovaginal candidiasis. In the current study it was to investigate the feasibility of liposomes and niosomes to formulate the vaginal mucosa administration of model drug clotrimazole. Multilamellar vesicles (MLV) formed using conventional lipid film evaporation method. Formulations composed of egg lecithin and nonionic surfactant compositions have been characterized by particle morphology, and encapsulation efficiency. Furthermore, we are investigating the optimized liposomes/niosomes in vitro the accumulation and diffusion of clotrimazole throughout the mucosa. Previous histology studies suggest that the rabbit may be a good model for studying mucosal absorption. The vaginal mucosa of rabbit is similar to that of human. Like the human, the rabbit vaginal mucosa consists of many layers of stratified squamous cells, beneath which are basal cells and the mucosal and muscularis mucosa layers. Since the rabbit lacks an estrous cycle, its vaginal epithelium is not subject to the time-dependent histology changes seen in other laboratory animals. Therefore, the rabbit model has been chosen for studying the clotrimazole loaded liposomes/niosomes vaginal mucosa permeation studies as the membrane permeability remains fairly constant.

The present investigation has been undertaken to study the feasibility of liposomes and niosomes in developing a vaginal antifungal drug formulation for treatment of vulvovaginal candidiasis.

Materials and Methods

Materials—Clotrimazole and egg phospholipids (EP) (>98%) were the generous gifts by Dr Fu and Cheng (Xi’an Libang Liposomes Pharmaceutical Company). Sorbitan monoesters (Span40; HLB=6.7), dicetylphosphate (DCP) cholesterol (CH) were brought from Sigma. Cellulose nitrate membrane filters (0.22 μm, Whatman, Maidstone, UK); Sephadex G-75 (medium) was purchased from Sigma. Buffer PBS (pH 7.4) was made of 8 g NaCl, 0.2 g KCl, 0.025 g NaH2PO4·2H2O and 0.050 g NaH2PO4·2H2O per 1L. All other reagents used in the study were of analytical grade.

Preparation of liposomes/niosomes—To study the effect of composition of the vesicles containing clotrimazole, a series of formulations containing different compositions with EP, sorbitan ester (Span™) and cholesterol were designed (Table I). Conventional Multilamellar vesicles (MLV) were prepared by thin lipid evaporation method. The formulations containing phospholipid or nonionic surfactants, cholesterol and DCP were resolved in ethanol, the desired volumes were added to a 100 ml round-bottom flask. The flask was attached to a rotary evaporator (BÜchi Rotavapor R 110, Switzerland), lowered into a 30°C water bath (BÜchi 461 water bath, Switzerland), and the organic solvents were evaporated under reduced pressure at 150 rpm to form a thin, dry film on the wall of the flask. Any excess organic solvents were removed by leaving the flask in a desiccator under vacuum, overnight. The dried lipid film was hydrated when required with buffer phosphate buffered saline, pH 7.4, followed by vigorous shaking in an incubator at 30°C (for liposomes) or 60°C (for niosomes) for about 60 min to form large multilamellar, and probe sonicating (Probe sonicator, Taijing Autoscience company, China) for 2 min to form the blank liposomes/niosomes. Conventional, drug-containing liposomes/niosomes were prepared by adding drug (clotrimazole was dissolved in ethanol previously) to the surfactant mixture prior to evaporating the organic solvent.

Morphology of liposome/niosome particles—Multilamellar vesicles after dilution with 5% mannitol were viewed under optical microscope (Olympus BHA, Japan) to observe the shape and lamellar nature of vesicles. Photomicrographs were prepared by a camera attached to the optical microscope in 10×100 magnifications.

Particle size of liposomes/niosomes—The size distribution of the resultant dispersion was characterized using a laser particle size analyzer based on laser diffraction, using the Malvern Mastersizer (Malvern, Model S, ver.2.15, UK). The apparatus consisted of He-Ne laser (5 mW) and a small volume sample-holding cell. Routine analysis was done at 90° using both unimodal (cumulant) fit that yields mean particle size and standard deviation in μm and size distribution processor analysis that resolves the component of polydispersed samples. The dispersions were diluted to an appropriate concentration so that the intensity of the transmitted laser beam was within
the limits required by the instrument for measurement. The sample was stirred using a magnetic stirrer bead to keep and maintain the sample in suspension. Each formulation was measured three times.

Entrapment efficiency of drug in the liposomes/niosomes—The drug/lipid ratio represents the drug encapsulating capacity of lipid. Separation of free from liposomal/niosomal drug was performed by centrifugation and gel chromatography.

Briefly, the liposomes/niosomes dispersion was centrifuged at 7000 rpm, and 4°C for 30 min in order to separate the incorporated clotrimazole from free clotrimazole molecules. The vesicles were reconstituted in buffer at pH 7.4 to obtain a constant clotrimazole concentration before entrapment efficiency determination and in vitro permeation study. For chromatographic separation the entrapment of liposomes/niosomes was separated by Sephadex G-75, at dilution rate 1 mL min⁻¹ which was controlled by pump (LKB, Peristaltic Pump). An aliquot (1 ml) of clotrimazole-containing liposomes/niosome preparation was loaded onto a Sephadex G-75 column (1.6×30 cm) and eluted using buffer solution, pH 7.4, at ambient temperature. Liposomes/niosome and free drug were separated well and collected respectively.

Separated clotrimazole-loaded vesicles were added with chloroform-methanol (2:1) to disrupt the vesicles, and the supernatant was centrifuged, through 0.22 μm polycarbonate membrane filters. The filtering solution was analyzed for clotrimazole content by HPLC analysis.

The HPLC system consisted of Gold Nouveau software workstation, a Beckman 126 NM solvent delivery system, Beckman 508 autosampler with a 100-μL loop, and Beckman 168 NM PDA detector. The column used was Beckman C18 dp 5 μm, 4.6 mm × 25 cm (Beckman, USA). The mobile phase consisted of methanol and 0.1 M phosphate buffer (pH 3) (27:73). Prior to use the buffer was filtered through 0.22 μm membrane filter (Millipore Corp., Milford, MA, USA). The flow rate was 1 ml/min. The chromatogram was monitored at a wavelength of 254 nm wavelength. This method was validated in terms of specificity, linearity and reproducibility. The limit of quantification was 0.1 μg/ml. The exact amounts of clotrimazole were determined using a calibration curve.

Recovery of drug, determined for all samples, was between 90.1-94.2 % of the amount taken into preparation.

Stability of vesicles at storage condition and in simulated vaginal fluid—The clotrimazole phospholipids liposomes formulation code No. 5 (CPL5) and clotrimazole Span 40 niosomes formulation code No.4 (CSN4) were stored in glass vials after purging with nitrogen and kept at 4°±1°C (refrigerator), 25°±2°C (room temperature), and 37°±1°C for 3 months. The samples from liposomes/niosomes were withdrawn at definite time intervals; the residual amount of drug in vesicles was determined as described above. Further, in order to investigate the formulations stability in simulated physiological condition, the E% (percent of original entrapped drug amount) change of liposomes/niosomes was tested in acetic acid buffer (pH 4.5) at 37°±1°C.

Solubility study—The solubility study of clotrimazole was carried out in thermostatic water shaker bath at 37°C for 24 hr. Excess of drug was added in 10 ml modified buffer, pH 7.4, with 10% dioxane. After 24 hr shaking, the samples were filtered using 0.22 μm membrane filter and analyzed after appropriate dilution using HPLC method as mentioned above.

In vitro mucosa permeation of clotrimazole from liposomes/niosomes—in vitro drug permeation studies of clotrimazole in optimized liposomes/niosomes formulations and their corresponding gels were performed in the rabbit vaginal mucosa using vertical Franz diffusion cells. The receptor compartment had a volume of 7.2 ml and an effective diffusion area of 0.785 cm² (φ=0.5 cm). The receptor compartment was filled with a PBS solution containing 10% (v/v) dioxane which was constantly stirred with a magnetic bar (37°±1°C) throughout the experiments. After sacrificing the animal fresh rabbit vagina was obtained, cut open vertically, vaginal mucosa was separated immediately, stored at −20°C for experiments. Before using, samples were defrosted in saline at room temperature. The rabbit vaginal mucosal samples were mounted on the cell opening, with the mucosal epithelia facing the donor and occluded with a sheet of aluminum foil. The permeation of clotrimazole through the vaginal mucosa was studied. Optimized formulations (liposomes/niosomes and control) each equivalent to 1.5 mg of drug was applied onto the prepared vaginal...
mucosa facing the donor chamber. Samples of 200 μl were withdrawn from the receptor at specified time intervals and replaced with an equal volume of fresh receptor solution. In addition, the vaginal mucosa was cleaned on both sides and the drug accumulated in the mucosa was extracted with 100% ethanol and subjected to five sonication cycles of 30 min each in an ultrasound bath (KQ-50DB Ultrasonics Corporation, Kongshan, China). It was observed that the sonication step did not affect the stability of clotrimazole. The concentration of drug in the receiver at various times and that extracted from the mucosa was determined by HPLC. Control solution was 1% clotrimazole (w/v) PEG 400.

Results and Discussion

Particle size and size distribution of liposomes/niosomes—The micrographs in Fig. 1 confirm the formation of multilamellar structures from phospholipid and sorbitan ester nonionic surfactants by classic film method. From the microscopic observation, it is evident that large and small multilamellar vesicles were formed, the former being the major component. As for the niosomes, Yoshika et al. reported that HLB (hydrophilic-lipophilic balance) number between 4 and 8 was compatible with vesicle formation with the sorbitan monostearate surfactants. In the present study, the Span 40 was chosen to form the lipophilic drug clotrimazole-loaded niosomes, its corresponding HLB was 6.7. In addition, the mean diameter for liposome and niosome was 2.21±0.23 and 3.15±0.25 μm respectively (dynamic laser light-scattering measurement). The sizes of niosomes are bigger (t-test, P<0.05) than the liposomes, probably due to the higher HLB and the higher phase transition temperature of nonionic surfactant than the egg phospholipid.

Entrapment efficiency—In the present study, centrifugation and chromatography showed similar results for entrapment efficiencies. For simplicity, quickness and concentrating the liposomes/niosomes dispersion of centrifugation, the method was applied in entrapment efficiency determination.

Influence of process parameters—Process variables, viz. vacuum, hydration medium, hydration time, speed of rotation of flask, and agitational method of size reduction were optimized to prepare lipid vesicles of clotrimazole. The rotational speed of the flask demonstrated discernible influence on the thickness and uniformity of the lipid film. The speed of 150 rpm yielded a uniform thin, lipid film yielding vesicular preparation of desired characteristics on hydration. The hydrating temperatures used to make liposomes/niosomes were above the gel to liquid phase transition temperature of the system.

Influence of formulation component—Drug/lipid ratios of all the liposomes/niosomes formulations are shown in Table 1. The drug/lipid value increased with decreasing the total lipid below the drug:total lipid=2:10, which may be accounted to the saturation of lipid domains. In addition, when the ratio of drug and total lipid was kept constant, the ratio of

![Optical micrographs (×1000) of clotrimazole-loaded vesicles composed of egg phospholipid and sorbitan esters (Span 40) prepared by classic film method: (A) liposomes; (B) niosomes](image-url)
cholesterol and EP increased from 1:9 to 2:8, resulting in the increased entrapment efficiency, whereas the ratio when increased from 2:8 to 4:6 resulted in decrease in the entrapment efficiency suggesting importance of appropriate proportions of EP and cholesterol to maximize the entrapment.

Similar results were obtained in the niosomes preparation. The ratios of drug and total lipid and of nonionic surfactant and cholesterol are very important to optimize the higher entrapment efficiency. There was a different drug/lipid ratio between liposomes and niosomes (Table 1); liposomes had higher ratio probably due to the different nature of materials.

Formulation CPL5 and CNS4 were selected as model vesicles to investigate further studies because of their higher drug/lipid ratio.

Physical stability at storage condition and simulated physiological condition—Liposomes/niosomes were relatively stable at 4°C±1°C storage conditions (Table 2). The drug leakage percent amounts of original entrapped in liposomes/niosomes were very small and the drug amount retained in vesicles had no significant difference after 3 months as compared to the amount immediately after preparation. The presence of ionic surfactants in the formulation is generally used to stabilize niosomes by means of an increase of their zeta potential and optimized ion-dipole interaction. Since DCP is a charge inducer, it was added to all the formulations to increase the vesicles stability in the present study. In addition, 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 into higher drug leakage. Under all testing conditions, the niosomes formulation was more stable than liposomes. It may be due to the fact that the nonionic surfactant chemical nature is more stable than its egg phospholipid.

The pH value of the healthy human vagina ranges between 4.0~5.0, therefore acetic buffer (pH 4.5) was chosen to simulate normal vaginal fluid. After 24 hr the leakage was approximately 30% of original entrapped clotrimazole, which suggested that liposomes/niosomes delivery system may provide controlled and prolonged release of an adequate drug in vaginal local treatment (Fig. 2). The results further suggested that liposomes containing clotrimazole were less stable than niosomes, especially after 8 hr, which was due to the fact that molecules of Span 40 in bilayer structures were in the ordered gel state at 37°C, which is responsible for their less fluidity and permeability. Moreover, their high HLB values (Span 40 6.2) result in reduction in surface free energy which allows to form vesicles of larger particle size and hence smaller area exposed to the dissolutions medium and membrane.

In vitro mucosa permeability studies—Since the drug is poorly soluble in PBS (<0.1mg/ml), it was necessary to develop an appropriate release medium that can provide sufficient solubility for the drug to maintain below a sink condition during clotrimazole permeation studies. To promote drug release from topical preparations containing these drug substances, studies have been carried out with receptor media

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**Table 1**: Effect of lipid and Span 40 composition on encapsulation efficiency of liposomes and niosomes

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>CT: (EP or Span 40:CH) (molar ratio)</th>
<th>Entrapment levels</th>
<th>[Drug (µg)/total lipid]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPL1</td>
<td>2:16:4</td>
<td>50.09±0.24</td>
<td></td>
</tr>
<tr>
<td>CPL2</td>
<td>2:14:3.5</td>
<td>57.51±0.17</td>
<td></td>
</tr>
<tr>
<td>CPL3</td>
<td>2:12:3</td>
<td>67.10±0.52</td>
<td></td>
</tr>
<tr>
<td>CPL4</td>
<td>2:10:2.5</td>
<td>80.51±0.16</td>
<td></td>
</tr>
<tr>
<td>CPL5</td>
<td>2:8:2</td>
<td>98.63±0.24</td>
<td></td>
</tr>
<tr>
<td>CPL6</td>
<td>2:7:3</td>
<td>96.43±0.14</td>
<td></td>
</tr>
<tr>
<td>CPL7</td>
<td>2:6:4</td>
<td>92.94±0.42</td>
<td></td>
</tr>
<tr>
<td>CPL8</td>
<td>2:5:1</td>
<td>95.43±0.26</td>
<td></td>
</tr>
<tr>
<td>CSN1</td>
<td>1:8:8</td>
<td>54.62±0.44</td>
<td></td>
</tr>
<tr>
<td>CSN2</td>
<td>1:7:7</td>
<td>60.42±0.12</td>
<td></td>
</tr>
<tr>
<td>CSN3</td>
<td>1:6:6</td>
<td>71.82±0.44</td>
<td></td>
</tr>
<tr>
<td>CSN4</td>
<td>1:5:5</td>
<td>87.38±0.18</td>
<td></td>
</tr>
<tr>
<td>CSN5</td>
<td>1:4:2</td>
<td>86.34±0.12</td>
<td></td>
</tr>
<tr>
<td>CSN6</td>
<td>1:3:3</td>
<td>86.68±0.43</td>
<td></td>
</tr>
<tr>
<td>CSN7</td>
<td>1:2:4</td>
<td>87.04±0.44</td>
<td></td>
</tr>
</tbody>
</table>

*The total lipid concentration was adjusted to 20 mM; a constant amount of DCP (0.5molar ratio) was added to the formulations; CT= Clotrimazole; EP=Egg phospholipids; CH=Cholesterol; CPL=Clotrimazole Phospholipids*

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**Table 2**: Stability of liposomes/niosomes containing clotrimazole expressed as percentage of originally entrapped drug still present in liposomes/niosomes after at storage conditions at 4°C±1°C, 25°C±2°C, and 37°C±1°C

<table>
<thead>
<tr>
<th>Formulations</th>
<th>1 month</th>
<th>2 month</th>
<th>3 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C±1°C</td>
<td>CPL5</td>
<td>97.37±2.55</td>
<td>96.53±1.37</td>
</tr>
<tr>
<td></td>
<td>CSN4</td>
<td>98.22±1.54</td>
<td>97.87±3.15</td>
</tr>
<tr>
<td>25°C±2°C</td>
<td>CPL5</td>
<td>86.33±1.55</td>
<td>83.24±3.17</td>
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<tr>
<td></td>
<td>CSN4</td>
<td>90.37±1.55</td>
<td>84.83±3.11</td>
</tr>
<tr>
<td>37°C±1°C</td>
<td>CPL5</td>
<td>76.33±1.75</td>
<td>53.24±3.21</td>
</tr>
<tr>
<td></td>
<td>CSN4</td>
<td>80.37±1.52</td>
<td>64.83±3.18</td>
</tr>
</tbody>
</table>
containing surfactants and different organic/aqueous solvents. Chang et al.\textsuperscript{19} reported 0.1% M citrate phosphate buffer (pH 5.5) containing 10% Tween-80 as in vitro release dissolution medium as a thermosensitive gel of clotrimazole. In addition, Kast et al.\textsuperscript{20} also reported 0.1 M acetate buffer, pH 6 and organic resolvent dioxane (35% v/v) as clotrimazole bioadhesive vaginal gel in in vitro release medium.

According to the present results, use of surfactant resulted in foaming and formation of air bubbles during receptor mixing. The presence of air bubbles also interfered with required uniform contact between the receptor medium and the supporting mucosa membrane. Among the organic/aqueous solvent, the ethanol is commonly selected as optimum receptor medium, but it results in back diffusion to the donor chamber to influence the liposomes/niosomes drug releasing through fluidizing effect on the bilayers.\textsuperscript{14} Therefore in the present study, an amount of dioxane was selected to solubilize the drug. A medium PBS (pH 7.4) solution with 10% dioxane gave a solubility which was considered to be sufficient for the mucosal permeation study.

Figure 3a depicts the regressed lines between amount of clotrimazole permeated and the time for the optimized batches of liposomes, niosomes and drug in control. The drug-flux was obtained by plotting the cumulative amount of clotrimazole in the receptor phase per square centimeter against time. The drug-flux was obtained by plotting the cumulative amount of clotrimazole in the receptor phase per square centimeter against time. (b) Bar diagram showing the permeation flux of clotrimazole permeated from the liposomal, niosomal and control formulation. The cumulative amount of drug permeated through a unit surface area of the mucosa vs time linearity was achieved up to 24 hr.

Fig. 2—(a) Diagram depicting the regressed lines between amount of clotrimazole permeated and the time for the optimized batches of liposomes, niosomes and drug in control. The drug-flux was obtained by plotting the cumulative amount of clotrimazole in the receptor phase per square centimeter against time. The drug-flux was obtained by plotting the cumulative amount of clotrimazole in the receptor phase per square centimeter against time. (b) Bar diagram showing the permeation flux of clotrimazole permeated from the liposomal, niosomal and control formulation. The cumulative amount of drug permeated through a unit surface area of the mucosa vs time linearity was achieved up to 24 hr.
liposomes/niosomes and control clearly indicate that the liposomes/niosomes system is much more effective drug mucosa permeation carrier than control. The improved mucosa penetration of drug and the consequently enhanced drug-transport abilities through liposomes and niosomes can be explained on the basis of the presence of drug molecules in a solubilized state. The enhancing effect on transvaginal drug delivery may have two mechanisms: (i) adsorption and fusion of drug loaded vesicles onto the surface of the mucosa leading to a high thermodynamic activity gradient of the drug-mucosa interface liposomes/niosomes; or (ii) the effect of vesicles on mucosa may cause changes in drug permeation kinetics due to an impaired barrier function of the mucosa for the drug. The results of Richardson et al.\textsuperscript{21} support concept that enhanced absorption of gentamicin in presence of absorption enhancer LPC (lysophosphatidylcholine) and PCC (palmitoyl/carnitine chloride) results from an epithelial damage. Remarkably, very similar fluxes were obtained from liposomes and niosomes allowing the formulation of either egg phospholipid or non-ionic surfactant vesicles.

The in vitro permeation data showed that the liposomes/niosomes system increased the clotrimazole total penetration through the vaginal mucosa by 1.6, 1.5-fold, respectively, the accumulation of clotrimazole into the mucosa was increased by 3.1, 2.3-fold, respectively, as compared with control (Table 3). The higher drug vaginal mucosa retention in vesicles may be due to: (i) creation of reservoir effect for drug in mucosa, and (ii) deposition of other components of vesicles with drug into the mucosa, thereby increasing the drug retention capacity into the mucosa. Weiner et al.\textsuperscript{25} also suggested that the action mechanism may be that encapsulated drug may fuse, adsorb or lipid-exchange with mucosa tissue resulting in disintegrated and/or underwent rearrangement with mucosa lipids to form new entities. Therefore, the drug intercalated within the bilayer structure of the liposomes/niosomes lipids could penetrate into the mucosa as a part of mucosa with liposomes/niosomes. Further, the data obtained from Table 3 show that the clotrimazole mucosa/solution partition coefficient of liposomes is higher (t-test, \( P<0.05 \)) than the niosome formulation, indicating that the phospholipid which is more similar to the mucosa component may act as better carrier over niosomes to make the drug retain in the mucosa for modulating the drug release and tissue distribution.

### Conclusions

The present study was undertaken to prepare and investigate the liposomes/niosomes delivery system for local vaginal treatment of clotrimazole. The results show that optimized multilamellar liposomes/niosomes prepared had higher drug/lipid ratio (>85 \( \mu g/mg \) with liposome; >75 \( \mu g/mg \) with niosomes) and were stable at 4\( ^\circ \pm 1\)\( ^\circ \)C storage condition. The vesicles systems can provide sustaining release in simulated vaginal fluid at 37\( ^\circ \pm 1\)\( ^\circ \)C for 24 hr. The mucosa/solution partition coefficient results of liposomes/niosomes were significantly higher than the control, indicating that the vesicles may serve as an efficient promoter of the lipophilic drug localization into the vaginal mucosa. Thus, the liposomes and niosomes can act as a good potential drug carrier for enhancing mucosa-local drug concentration. The enhanced mucosal accumulation of clotrimazole could help significantly to optimize the targeting of the drug without a concomitant increase of the systemic side effects. In addition, it was possible to achieve an adequate pH value corresponding to physiological conditions as well as desirable viscosity\textsuperscript{9}.

### Acknowledgement

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