Chinese Academy of Medical Science & Peking Union Medical College[1], Centre of Drug Controlled Release Research, National Research Institute for Family Planning[2], Beijing, P.R. China

Preparation and characterization of EP-liposomes and Span 40-niosomes

M. Ning[1,2], Y. Guo[2], H. Pan[2], S. Zong[2], Z. Gu[2]

Received February 16, 2005, accepted April 26, 2005

Zhongwei Gu, Centre of Drug Controlled Release Research, National Institute for Family Planning, No. 12, Da Hui Si Haidia District. Beijing 100081, P.R. China
zwgu@public.bta.net.cn

Pharmazie 61: 208–212 (2006)

Clotrimazole, an imidazole derivative antifungal agent, has been widely used for the treatment of mycotic infections of the genitourinary tract. In order to develop alternative formulations for the vaginal administration of clotrimazole which would provide sustained and controlled release of the appropriate drug for local vaginal therapy, liposomes/niosomes were evaluated as delivery vehicles. To optimize the preparation of the liposomes/niosomes with regard to size and entrapment efficiency, multilamellar liposomes/niosomes containing the drug were prepared by a lipid hydration method. The liposomes/niosomes thus prepared were evaluated for their stability as drug-loaded liposomes/niosomes in simulated vaginal fluid at 37 ± 1 °C. The two vesicle systems were also evaluated with regard to rat and rabbit vaginal irritation.

1. Introduction

Liposomes are microscopic vesicles composed of one or more lipid layers arranged in a concentric fashion enclosing an equal number of aqueous compartments (Bangham 1965; Kim 1997; Valenta 2000; Schmid 1994; Farshi 1996; Pavelié 1999, 2001). Analogous to liposomes, niosomes are formed from the self-assembly of nonionic amphiphiles in aqueous media resulting in closed bilayer structures. They require energy to form the vesicles, and offer several advantages over liposomes such as higher chemical stability, intrinsic skin penetration enhancing properties and lower costs (Yoshika 1994). Therefore, niosomes have also been widely studied as drug carriers for controlled and targeted delivery (Uchegbu 1995; Varshosaz 2003; Manconi 2001; Perini 1996; Shahiwala 2002; Vora 1998; Fang 2001; Agarwal 2001). However, few studies have attempted to compare their characteristics.

The purpose of the present work was to develop mulitlamellar vesicles (MLV) using a conventional lipid film evaporation method and to determine their different characteristics. The model drug employed here was the lipophilic drug clotrimazole, which is an imidazole derivative, and is widely and effectively used for the treatment of vulvovaginal candidiasis. The drug has poor water solubility and high lipophilicity which make it an excellent candidate for liposome/niosome encapsulation. Formulations composed of egg phospholipids and nonionic surfactant compositions were characterized in respect of particle morphology, viscosity, encapsulation efficiency and in vitro release behavior. Furthermore, the two vesicle systems were evaluated in terms of rat and rabbit vaginal irritation.

2. Investigations, results and discussion

2.1. 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 a classic film method. From the microscopic observation, it is evident that both large and small multilamellar vesicles were formed. The mean diameter was $4.1 \pm 1.9\ \mu m$ for liposomes (CPL6) and $3.4 \pm 2.2\ \mu m$ (CSN6) for niosomes determined by dynamic laser light-scattering measurement. There was no significant difference between the sizes of the liposomes (CPL6) and niosomes (CSN6) ($p > 0.05$). As regards the niosomes, a previous publication reports that an HLB

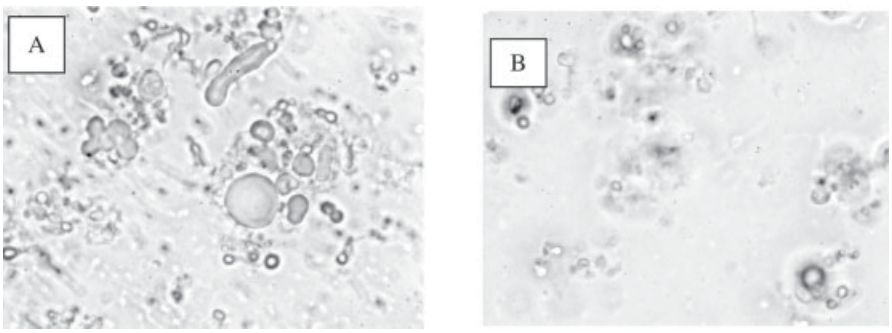

Fig. 1:
Optical micrographs (× 1000) of clotrimazole-loaded vesicles composed of phospholipid and sorbitan esters (Span™) prepared by a classic film method: (A) CPL6 liposomes; (B) CSN6 niosomes; CPL = clotrimazole, phospholipid and liposomes. CSN = clotrimazole, surfactant and niosomes

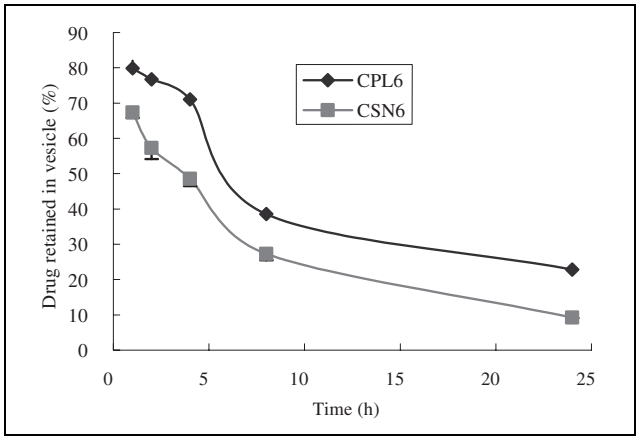

Fig. 2: Stability of liposomes/niosomes containing clotrimazole expressed as percentage of originally entrapped drug still present in liposomes/niosomes after exposure to simulated vaginal fluid (pH 4.2) at 37 ± 1 °C (n = 3 ± S.D.). CPL6 = clotrimazole : egg phospholipid : cholesterol (2 : 7 : 3 molar ratio); CSN6 = clotrimazole : Span 40 : cholesterol (1 : 8 : 2, molar ratio)

number between 4 and 8 was found to be compatible with vesicle formation with sorbitan monostearate surfactants (Yoshika 1994). In this study, we chose Span 40 whose corresponding HLB is 6.7 to form the lipophilic clotrimazole-loaded niosomes. In addition, there was no significant difference between the sizes of optimized vesicles for the liposomes CPL6 and niosomes CSN6 ($p > 0.05$). The size distribution of the vesicles tends to be fairly wide, although this can be modified by altering the hydration time and degree of shaking.

2.2. Rheological properties

Table 1 compares the relative viscosity of suspensions of lipsomes/niosomes formed by a lipid hydration method. At 20 °C, 25 °C, 37 °C and 45 °C, the viscosity of niosomes is higher than that of liposomes. The viscosity of both decreases with increasing temperature due to reduced vesicle-solvent interactions.

Table 1: Relative viscosity of liposomes/niosomes

Formulation codes	20 °C	25 °C	37 °C	45 °C
CPL6	1.171	1.167	1.149	1.112
CSN6	1.251	1.232	1.228	1.224

SPL6 = clotrimazole : egg phospholipid : cholesterol (2 : 7 : 3, molar ratio)
CSN6 = clotrimazole : Span 40 : cholesterol (1 : 8 : 2, molar ratio)

Table 2: Effect of lipid composition on encapsulation efficiency of liposomes (n = 3 ± S.D.)

Formulation codes	CT : EP : CH (molar ratio)	Drug/lipid ratio (μg/mg)	Entrapment efficiency (%)
CPL1	2 : 16 : 4	41.56 ± 0.70	89.26 ± 1.51
CPL2	2 : 14 : 3.5	53.13 ± 0.25	92.38 ± 0.44
CPL3	2 : 12 : 3	58.73 ± 0.32	87.53 ± 0.48
CPL4	2 : 10 : 2.5	73.94 ± 0.15	91.84 ± 0.19
CPL5	2 : 8 : 2	89.33 ± 0.50	88.76 ± 0.50
CPL6	2 : 7 : 3	92.79 ± 1.55	87.18 ± 1.46
CPL7	2 : 6 : 4	90.20 ± 1.69	79.87 ± 1.50
CPL8	2 : 9 : 1	89.44 ± 0.86	93.72 ± 0.91

[a] The total lipid concentration was adjusted to 20 mM; a constant amount of DCP (0.5 molar ratio) was added to the formulations. S.D. = standard deviation; CT = clotrimazole; EP = egg phospholipids; CH = cholesterol; DCP = dicetylphosphate; CPL = clotrimazole, phospholipid and liposomes

2.3. Determination of content and entrapment efficiency

The regression equation for clotrimazole content (μg/ml) in methanol ranging from 10 to 500 μg/ml was: $C = 18013A - 28.537$, ($R^2 = 0.9995$), where C (μg/ml) and A represented the concentration and peak area of clotrimazole, respectively. The mean recovery was 98.5 ± 1.7% (n = 3). The precision assay showed that relative standard deviations within 1 day and among every other day were all below 3%. This method was validated in terms of specificity, linearity and reproducibility. The limit of quantification was 1 μg/ml. The exact amounts of clotrimazole were determined using a calibration curve.

In the present study, a dialysis method was used for determination of entrapment efficiency. Recovery of drug was determined for all samples and was between 94.5% and 96.2% of the amount used in preparation.

The contents of clotrimazole-liposomes/niosomes determined by HPLC are shown in Table 2 and Table 3. For liposomes increasing the cholesterol level (molar ratio of lipid: cholesterol from 1 : 9 to 6 : 4) decreased the entrapment of drug from 93.7 ± 0.9% to 79.8 ± 1.5%. For niosomes, similarly to the results of liposomes, with increasing cholesterol, the entrapment efficiency increased from 60.1 ± 3.8% to 88.1 ± 0.7% as cholesterol: lipid ratio increased from 5 : 5 to 2 : 8. On the basis of the results for entrapment efficiency and drug/lipid ratio, formulations CPL6 (clotrimazole : phospholipid : cholesterol = 2 : 7 : 3) and CSN6 (clotrimazole : Span 40 : cholesterol = 1 : 8 : 2) were chosen for further experimentation.

Process variables, hydration medium, hydration time, and speed of rotation of the flask were optimized to prepare lipid vesicles of clotrimazole. The rotational speed of the flask demonstrated a discernible influence on the thickness and uniformity of the lipid film. A speed of 100 rpm yielded a uniform, thin lipid film yielding a vesicular preparation with the desired characteristics on hydration. The hydrating temperatures used to make liposomes/niosomes were above the gel to liquid phase transition temperature of the system. During the preparation of niosomes, although the surfactant film was thin, it had finite thickness and hydration initially occurred at the surface of the glass. As a result, full hydration of the surfactant film was difficult, but the preparation of liposomes with a lower molar ratio of cholesterol did not produce such phenomena. The hydration of phospholipid films was relatively easy.

The presence of ionic surfactants in the formulation is generally used to stabilize niosomes by means of an increase in zeta potential and optimized ion-dipole interaction. DCP is a charge inducer, and dicetylphosphate was added to all the formulations to increase the vesicles' stability in our present study.

Table 3: Effect of lipid composition on encapsulation efficiency of niosomes (n = 3 ± S.D.)

Formulation codes	CT : Span 40 : CH (molar ratio)	Drug/lipid ratio (μg/mg)	Entrapment efficiency (%)
CSN1	1 : 10 : 10	24.38 ± 0.70	55.80 ± 1.60
CSN2	1 : 12 : 8	26.20 ± 1.68	60.19 ± 3.85
CSN3	1 : 14 : 6	32.04 ± 0.22	73.92 ± 0.51
CSN4	1 : 16 : 4	38.07 ± 0.31	88.18 ± 0.72
CSN5	1 : 18 : 2	58.65 ± 1.05	68.20 ± 1.22
CSN6	1 : 8 : 2	75.74 ± 1.65	87.71 ± 1.91
CSN7	2 : 8 : 2	102.25 ± 1.41	59.20 ± 0.82

[a] The total lipid concentration was adjusted to 20 mM; a constant amount of DCP (0.5 molar ratio) was added to the formulations. S.D. = standard deviation; CT = clotrimazole; CH = cholesterol; DCP = dicetylphosphate; CSN = clotrimazole, surfactant and niosomes

Table 4: Stability of liposomes/niosomes containing clotrimazole expressed as percentage of originally entrapped drug still present in liposomes/niosomes after storage at 4 ± 1 °C

	Formulations	1 month	2 months	3 months
4 ± 1 °C	CPL6	97.37 ± 2.55	86.53 ± 1.37	69.36 ± 2.32
	CSN6	98.22 ± 1.54	77.87 ± 3.15	67.27 ± 2.19
25 ± 2 °C	CPL6	86.33 ± 1.55	73.24 ± 3.17	55.28 ± 2.79
	CSN6	90.37 ± 1.55	71.83 ± 4.11	50.24 ± 2.20
37 °C	CPL6	76.33 ± 1.75	53.24 ± 3.29	25.28 ± 2.15
	CSN6	80.37 ± 1.52	64.83 ± 3.18	45.24 ± 1.10

SPL6 = clotrimazole : egg phospholipid: cholesterol (2 : 7 : 3, molar ratio)
CSN6 = clotrimazole : Span 40 : cholesterol (1 : 8 : 2, molar ratio)
RT (room temperature about 25 ± 2 °C), 37 ± 1 °C (n = 3 ± S.D.)

2.4. Physical stability under storage conditions

Table 4 shows that liposomes/niosomes were relatively stable when stored at 4 ± 1 °C. The drug leakage as a percentage of the amount of originally entrapped in the liposomes/niosomes was small after 1 month and was not significant ($p < 0.05$) but there was a significant difference after 3 months compared with the situation immediately after preparation ($p > 0.05$). The results of drug retention studies show higher drug leakage at higher temperatures. This may be due to the higher fluidity of the lipid bilayers at higher temperatures resulting in higher drug leakage. At the test temperatures below 25 °C, the niosome formulation was less stable than liposomes. This result might be due to the fact that the multiniosomes formed with the nonionic surfactant were less stable than multiliposomes. When the test temperature was above 25 °C, contrary results were found, which might result from the fact that the Span 40 niosomes have a higher transition temperature than the liposomes.

2.5. In vitro release studies under simulated physiological conditions

It is known that the pH value of the healthy human vagina ranges between 4.0 ~ 5.0. In our study, simulated vaginal fluid was chosen as the *in vitro* release medium. Fig. 3 shows the release profiles of liposomes/niosomes. Clotrimazole showed release from the vesicles of about 70% within 8 h. After 24 h, the percentages of the original entrapped drug retained in liposomes and niosomes were 22.86% and 9.25% respectively, with a significant difference between the two vesicles ($p < 0.05$). The results shown in Figure 2 indicated that niosomes containing clotrimazole were less stable than liposomes, especially after 8 hours.

2.6. Tolerability of clotrimazole vesicles in a rat and rabbit tissue model

Liposome gels did not alter the morphology of vaginal tissues. Fig. 4 shows the histopathology of the vaginal mucosa after intravaginal application of CT-containing liposomal and niosomal gels. As compared to the control with no treatment, the liposomal vesicle gel-treated group showed no visible sign of inflammation or necrosis, while the niosomal vesicle gel-treated group showed only mild edema.

Histological evaluation of 3 different regions of the vaginal tissues of rabbits (Fig. 4) receiving daily intravaginal application of vesicles for 7 consecutive days showed a lack of significant vaginal irritation. None of the rabbits treated with control revealed epithelial ulceration, edema, leukocyte influx, and vascular congestion characteristic of inflammation. As compared to the controls with no treatment, the liposomal vesicle gel-treated group showed no visible sign of inflammation or necrosis, while the niosomal vesicle gel-treated group showed mild vascular congestion in the lower vaginal region.

3. Experimental

3.1. Materials

Clotrimazole and egg phospholipids (EP) (> 98%) were generously donated by Drs Fu and Cheng (Xi'an Libang Liposomes Pharmaceutical Company). Sorbitan monoesters (Span 40, HLB = 6.7), dicetylphosphate (DCP) and cholesterol (CH) were brought from Sigma. Cellulose nitrate membrane filters (0.22 μm, Whatman, Maidstone, UK). Phosphate buffered saline (PBS) buffer (pH 7.4) was made up with 8 g NaCl, 0.2 g KCl, 0.025 g $Na_2HPO_42H_2O$ and 0.050 g $NaH_2PO_42H_2O$ per 1L. All other reagents used in the study were of analytical grade.

3.2. Methods

3.2.1. Preparation of liposomes/niosomes

To study the effect of the composition of the vesicles containing clotrimazole, a series of formulations containing different compositions with EP, sorbitan ester (Span™) and cholesterol were designed (Tables 1, 2, CPL = Clotrimazole Phospholipids Liposomes; CSN = Clotrimazole Span 40 Niosomes). Conventional multilamellar vesicles (MLV) were prepared by a thin lipid evaporation method. The formulations containing phospholipid or nonionic surfactants, cholesterol and DCP were resolved in ethanol, and 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 for 12 h. The dried lipid film was hydrated when required with PBS buffer, 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 blank liposomes/niosomes. Conventional, drug-containing liposomes/niosomes were prepared by adding the drug (clotrimazole was dissolved in ethanol previously) to the surfactant mixture prior to evaporating the organic solvent.

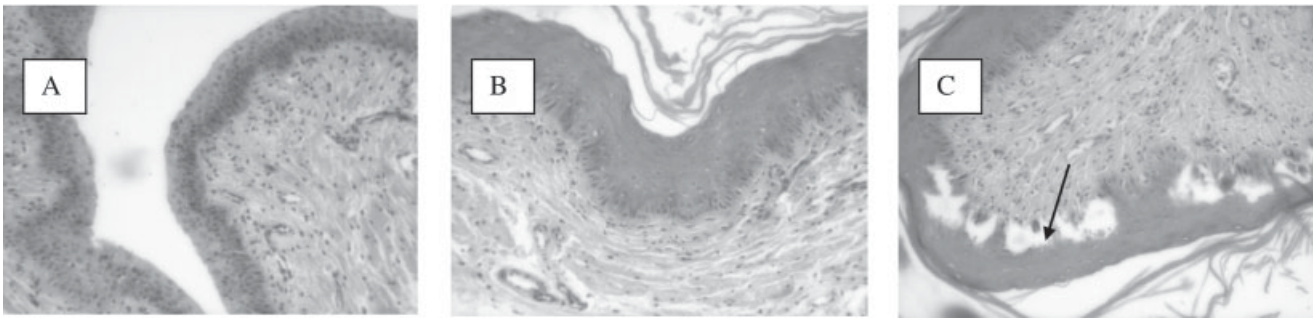

Fig. 3: Morphology of rat vaginal tissues after application of liposome/niosome gels. Liposome/niosome gels were administered into the vagina of the rats at a CT dose of 25 mg/kg. The vaginal tissues of the blank gel treated rats (A) and liposome gel-treated rats (B) and niosome gel-treated rats (C) were isolated, fixed in 10% neutral carbonate-buffered formaldehyde, embedded in paraffin wax, and cut into slices. After hemotoxylin-eosin staining, the slices were observed under a light microscope (100). The arrow region indicated mild edema

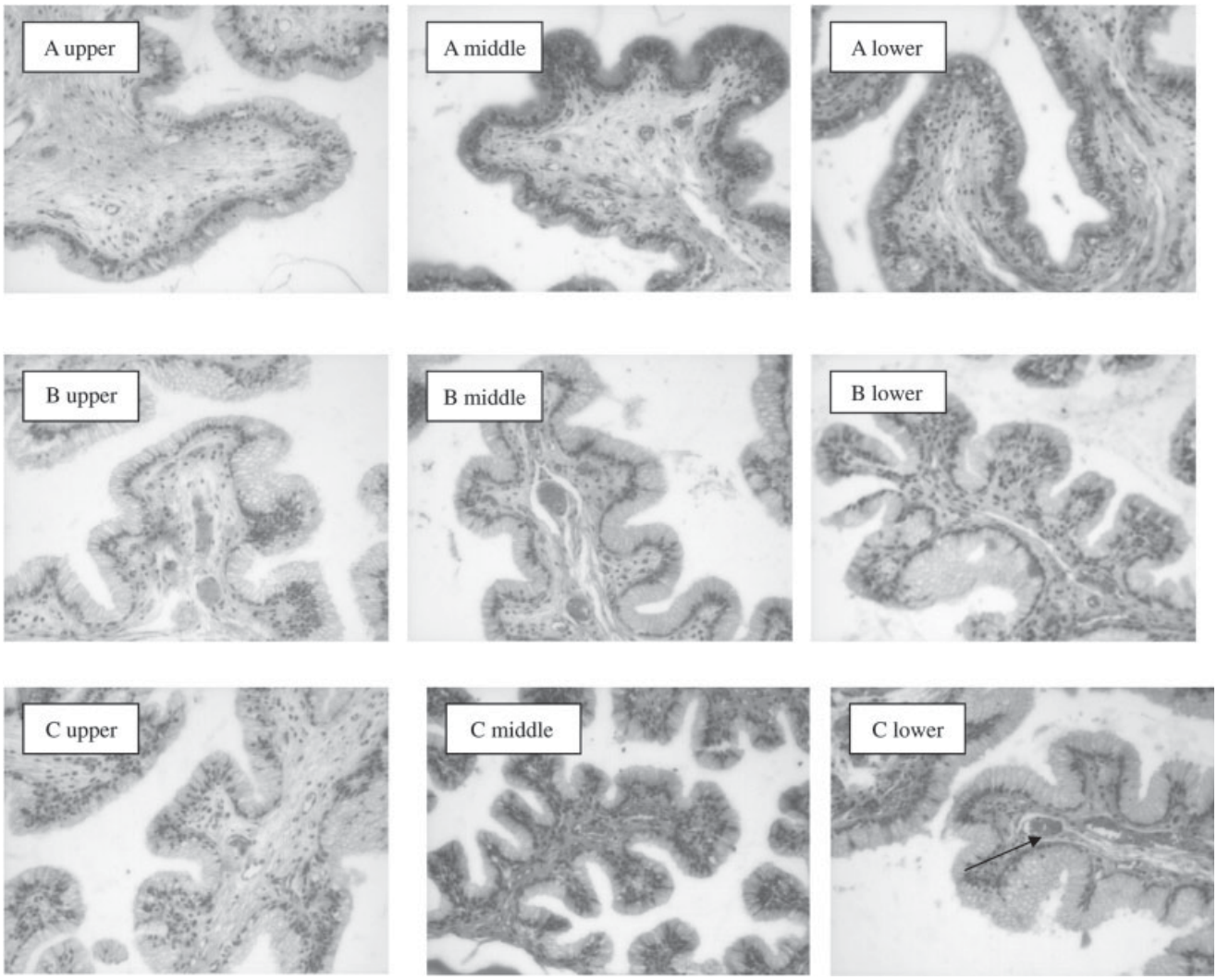

Fig. 4: Morphology of rabbit vaginal tissues after application of liposome/niosome gels. Liposome/niosome gels were administered into the vagina of the rabbits at a CT dose of 25 mg/kg. The vaginal tissues of the blank gel treated rabbits (A) and liposome gel-treated rabbits (B) and niosome gel-treated rabbits (C) were isolated, and parts of upper, middle, and lower regions of each vagina were fixed in 10% neutral carbonate-buffered formaldehyde, embedded in paraffin wax, and cut into slices. After hemotoxylin-eosin staining, the slices were observed under a light microscope (100). The arrow region indicated mild vascular congestion

3.2.2. Purification of the resultant liposomes/niosomes

The unencapsulated materials as well as residual organic solvent were removed by dialysis against PBS (pH 7.4) solution overnight using cellulose membrane tubing with molecular-weight cut off at 8000 ~ 12000, which had been stored in PBS (pH 7.4) before use. The system was maintained at 25 °C. The dialysis solution was continuously stirred with a magnetic bar stirrer.

3.2.3. Morphology and size of liposome/niosome particles

Multilamellar vesicles after dilution with 5% mannitol were viewed under an optical microscope with photograph auto-selecting system (Axiokop 40, ZEISS, UK) to observe the shape and lamellar nature of the vesicles. Some of photomicrographs are shown in Fig. 1 and were prepared using the photograph auto-selecting system.
The size distribution of the resultant dispersion was characterized by laser diffraction using a Beckman LS Particle Size Analyzer (Beckman Coulter, USA).

3.2.4. Rheological properties

The rheological properties of the niosomes were studied using an Ubbelohde suspended-level viscometer at various temperatures. Samples were diluted with water to the required concentrations and left to equilibrate for 1 h. Relative viscosity (η_{rel}) was calculated by comparing the efflux time with that of distilled water.

3.2.5. Entrapment efficiency of drug in the liposomes/niosomes

One milliliter of clotrimazole liposomal/niosomal suspensions in each case before and after dialysis, was diluted and adjusted to volume with methanol in a 10-mL volumetric flask, and the amount of drug was determined by HPLC. The system was maintained at 25 °C, 150 rpm by means of a shaker. The percentage entrapment efficiency of the drug was calculated by:

$$\%\ \text{Entrapment efficiency} = [\text{content of clotrimazole in post-dialyzed vesicles}/(\text{content of Clotrimazole in pre-dialyzed vesicles})] \times 100 \quad (1)$$

The HPLC system consisted of a Gold Nouveau software workstation, a Beckman 126 NM solvent delivery system, a Beckman 508 autosampler with a 100-μL loop, and a Beckman 168 NM PDA detector. The column used was a Beckman C18 dp 5 μm, 4.6 mm × 25 cm (Beckman, USA). The mobile phase consisted of methanol and H_2O (pH 3.0) (95 : 5, v/v). The flow rate was 0.8 ml/min. The chromatogram was monitored at a wavelength of 220 nm.
This method was validated in terms of specificity, linearity and reproducibility. The limit of quantification was 1 μg/ml. The exact amounts of clotrimazole were determined using a calibration curve.

3.2.6. Stability of vesicles under various conditions

Samples CPL6 and CSN6 were stored in glass vials after purging with nitrogen and kept in a refrigerator (4 ± 1 °C), at room temperature (RT, 25 ± 2 °C), and at 37 ± 1 °C for 3 months. Samples of the liposomes/niosomes were withdrawn at specified time intervals, and the residual amount of drug in the vesicles was determined as described above.

3.2.7. In vitro release studies under simulated physiological conditions

In order to investigate the stability of the formulations under simulated physiological conditions, amount of the drug retained in the liposome/niosome vesicles was tested in simulated vaginal fluid (SVF) (pH 4.2) at 37 ± 1 °C for 24 h. The percentage of drug retained in the vesicles are shown in Fig. 3.

The simulated vaginal fluid (Owen 1999) was prepared as follows (weight (g) per 1 L): NaCl, 3.51 g; KOH, 1.40; $Ca(OH)_2$, 0.222; bovine serum albumin, 0.018; lactic acid, 2.00; acetic acid, 1.00; Glycerol, 0.16; urea, 0.4; and glucose, 5.0. After these compounds were combined, the mixture was adjusted to pH 4.2 using HCl.

3.2.8. Tolerability of clotrimazole vesicles in the rat and rabbit tissue model

For the vaginal irritation study, female Sprague-Dawley rats weighing 200 ± 10 g were used. Liposomal/niosomal gels (liposomes or niosomes in 2% Carbopol gel) were administered into the vaginas of the rats at a CT dose of 25 mg/kg once a day for 7 days, and the vaginal tissues of the blank gel treated rats (A) liposomal gel-treated rats (B) and niosomal gel-treated rats (C) were isolated, fixed in 10% neutral carbonated-buffered formaldehyde, embedded in paraffin wax, and cut into slices. After hemotoxylin-eosin staining, the slices were observed under a light microscope.
In addition, female rabbits were treated intravaginally with the vesicle formulations (2% Carbopol gel), 25 mg/kg once per day for 7 consecutive days. Animals were killed on day 8 and the reproductive tract was examined visually and microscopically. The vaginal tissues were rapidly removed and parts of the upper (cervico-vagina), middle, and lower (urovagina) regions of each vagina were fixed in 10% neutral-buffered formalin. Tissues were embedded in paraffin wax, sectioned at 4 to 6 mm and stained with hematoxylin and eosin and examined under 100 magnification using a Zeiss light microscope interfaced with an image analysis system in conjunction with a camera for observation and analysis. Each of the three regions of the vagina was examined for epithelial ulceration, edema, leukocyte infiltration, and vascular congestion. The results are shown in Figs. 3 and 4.

3.2.9. Data analysis

Data were analyzed statistically by the one way ANOVA analysis using Microsoft Excel 2000 software and by Student's t-test (level for significance $p < 0.05$).

References

Agarwal R, Katare OP, Vyas SP (2001) Preparation and *in vitro* evaluation of liposome/niosomal delivery systems for antipsoriatic drug dithranol. Int J Pharm 228: 43–52.

Bangham AD, Standish MM, Watkins JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 13: 238–252.

Fang JY, Hong CT, Chiu WT, Wang YY (2001) Effect of liposomes and niosomes on skin permeation of enoxacin. Int J Pharm 219: 61–72.

Farshi FS, Ozen AY, Ercan MT (1996) *In vitro* studies in the treatment of oral ulcers with liposomal dexamethasone phosphate. J Microencapsul 13: 537–544.

Kim MK, Chung SJ, Lee MH, Cho AR, Shim CK (1997) Targeted and sustained delivery of hydrocortisone to normal and stratum corneum-removed skin without enhanced skin absorption using a liposome gel. J Control Release 46: 243–251.

Manconi M, Sinico C, Valenti D, Loy G, Fadda AM (2001) Niosomes as carriers for tretinoin. I. Preparation and properties. Int J Pharm 234: 237–248.

Owen DH, Katz DF (1999) A vaginal fluid stimulant. Contraception 59: 91–95.

Pavelié Ž, Škalko N, Jalšenjak I (1999) Liposomes containing drugs for treatment of vaginal infections. Eur J Pharm Sci 8: 345–351.

Pavelié Ž, Škalko N, Jalšenjak I (2001) Liposomal gels for vaginal drug delivery. Int J Pharm 219: 139–149.

Perini G, Saettone MF, Carafa M, Santucci E, Alhaique F (1996) Niosomes as carriers for ophthalmic drugs: *in vitro*/*vivo* evaluation. Bull Chem Pharm 135: 145–146.

Schmid MH, Korting HC (1994) Liposomes: a drug carrier system for topical treatment in dermatology. Crit Rev Ther Drug Carrier Syst. 11: 97–118.

Shahiwala A, Misra A (2002) Studies in topical application of niosomally entrapped nimesulide. J Pharm Pharm Sci 5 (3): 220–225.

Uchegbu IF, Double JA, Turton JA, Florence AT (1995) Distribution, metabolism and tumoricidal activity of doxorubicin administered in sorbitan monostearate (Span-60) niosomes in the mouse. Pharm Res 12: 1019–1024.

Valenta C, Wanka M, Heidlas J (2000) Evaluation of novel soya-lecithin formulations for dermal use containing ketoprofen as a model drug. J Control Release 63: 165–173.

Varshosaz J, Pardakhty A, Hajhashemi VI, Najafabadi AR (2003) Development and physical characterization of sorbitan monoester niosomes for insulin oral delivery. Drug Deliv 10: 251–262.

Vora B, Khopade AJ, Jain NK (1998) Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Control Release 54: 149–165.

Yoshika T, Sternberg B, Florence AT (1994) Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, Span 40, Span 60 and Span 80) and a sorbitan trimester (Span 85). Int J Pharm 105: 1–6.