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Research Article

Propylene Glycol Liposomes as a Topical Delivery System for Miconazole Nitrate: Comparison with Conventional Liposomes

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Abstract. Propylene glycol (PG)-phospholipid vesicles have been advocated as flexible lipid vesicles for enhanced skin delivery of drugs. To further characterize the performance of these vesicles and to address some relevant pharmaceutical issues, miconazole nitrate(MN)-loaded PG nanoliposomes were prepared and characterized for vesicle size, entrapment efficiency, *in vitro* release, and vesicle stability. An issue of pharmaceutical importance is the time-dependent, dilution-driven diffusion of propylene glycol out of the vesicles. This was addressed by assessing propylene glycol using gas chromatography in the separated vesicles and monitoring its buildup in the medium after repeated dispersion of separated vesicles in fresh medium. Further, the antifungal activity of liposomal formulations under study was assessed using *Candida albicans*, and their *in vitro* skin permeation and retention were studied using human skin. At all instances, blank and drug-loaded conventional liposomes were included for comparison. The results provided evidence of controlled MN delivery, constant percent PG uptake in the vesicles (≈45.5%) in the PG concentration range 2.5 to 10%, improved vesicle stability, and enhanced skin deposition of MN with minimum skin permeation. These are key issues for different formulation and performance aspects of propylene glycol-phospholipid vesicles.

KEY WORDS: miconazole; nanoliposomes; propylene glycol liposomes; release; stability.

INTRODUCTION

The application of drug encapsulation in lipid vesicles has gained momentum since the 1960s in numerous delivery routes; many topical preparations utilize this technology to improve drug permeation and deposition (1). These systems range from conventional liposomes, composed primarily of a phospholipid, to niosomes prepared from non-ionic surfactants (2), and include various modified liposomes such as ethosomes and elastic liposomes (3).

The importance of lipid vesicle structure in effective skin delivery has long been recognized (4). Desired structure attributes include elasticity and size. Conventional liposomes are rigid and are often in the micron size range; both features can impede skin entry. Means of vesicle structure modification include the incorporation of additives such as edge activators (transferosomes) (5–7), bile salts (8), ethanol (ethosomes (9–13)), glycols (14), including propylene glycol (12,14,15) and polyvinyl alcohol (16). Penetration enhancers have also been used as additives in liposome preparation (17, 18).

In addition to altering liposome structure and inducing a degree of elasticity, these additives often result in nanosized vesicles which contribute to enhanced skin delivery (15, 19). The use of these additives has also resulted, in many cases, in simplifying the method of vesicle preparation by omitting the use of harmful solvents. Instead of the film hydration method or the heating method which involves a pre-hydration step, a one-step procedure is possible in which the phospholipid is dissolved in the liquid additive such as propylene glycol, before gradually adding the aqueous phase (15). The use of an optimum amount of additive is essential. Too much additive can interfere with vesicle formation. Propylene glycol (10%) provided stable lecithin vesicles (15).

One aspect of modified phospholipid liposomes which has not been sufficiently addressed is the degree of retention of the water-miscible additive by the vesicles. It can be argued that during preparation, the additive reaches an equilibrium distribution state between vesicles and surrounding medium. However, incorporation of the vesicles in the final product can cause the additive to leak out of the vesicles before application to the skin. This can compromise vesicle deformability and possible skin penetration/retention-enhancing effects.

Miconazole nitrate is a drug with a wide antifungal spectrum (20) that presents formulation challenges in topical preparations. Although a nitrate salt, it is insoluble in water and has an octanol–water partition coefficient of about 6.25 (21), emphasizing the lipophilic character of the nitrate salt. Poor skin penetration of miconazole nitrate, due probably to a low aqueous driving concentration on the skin surface, presents a problem in the treatment of cutaneous diseases by topical application (22) and suggests room for improving performance *via* topical delivery systems. Previous reported attempts for miconazole nitrate include, among others, conventional liposomes (22,23), ethosomes (23), niosomes (24), and microemulsions (25).

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The aim of the present study was to investigate the use of propylene glycol liposomes as a delivery system for topical miconazole nitrate in comparison to conventional liposomes. A point of interest was to assess the concentration of propylene glycol in the separated vesicles and monitor its diffusion away from the vesicles after repeated dispersion of the vesicles in fresh medium using the British Pharmacopoeia gas chromatography method with modification. The physicochemical properties and *in vitro* antifungal activity of both types of liposome formulations were compared. Further, the effect of encapsulating miconazole nitrate in propylene glycol liposomes on the drug *in vitro* skin permeation and retention in human skin was assessed in comparison with conventional liposomes and with blank liposomes blended with the drug suspended in water.

MATERIALS

Lipoid S 100: phosphatidylcholine (PC, 95.8%) from soybean lecithin was a kind gift from Lipoid GmbH (Ludwigshafen, Germany). Propylene glycol (PG) and chloroform were from ADWIC, El-Nasr Pharmaceutical Chemicals Co. (Abu Zaabal, Egypt). Cholesterol (99%) was supplied by Fine-Chem. Ltd. (Mumbai, India). Miconazole nitrate was kindly supplied by Al Amria Pharmaceuticals Co. (Alexandria, Egypt). Uranyl acetate-2-hydrate was from Allied Signal (Riedel-Dehaen, Germany). All other chemicals were of analytical grade and used as received.

METHODS

Preparation of Liposomes

Preparation of PG Liposomes

PG liposomes were prepared following the procedure developed in our laboratory for the preparation of cinchocaine-loaded PG liposomes (12). The concentration of PC was 4%, and that of PG was 10% of the vesicle dispersion. Briefly, the phospholipid was dissolved, at 60°C, in PG. Distilled water was added slowly in a fine stream with mixing at 700 rpm. Mixing was continued for an additional 30 min. The system was kept at 60°C throughout the preparation. For drug-loaded liposomes, miconazole nitrate was dissolved with the phospholipid in PG.

Preparation of Conventional Liposomes

Liposomes were prepared using the thin-film hydration method (12). The concentration of PC was 4%, and that of cholesterol was 1% of the vesicle dispersion. The lipid cholesterol mixture was weighed, transferred into a 100-ml quick-fit pear-shaped round-bottom flask, and dissolved in 5 ml of chloroform. Organic solvent was removed at reduced pressure, using a rotary evaporator above the lipid transition temperature. Distilled water (5 ml) was added to the deposited lipid film. The flask was rotated at a low speed at 45°C in the rotary evaporator (with no vacuum) for 30 min. The resulting dispersion was left to mature overnight at 4°C. For drug-loaded liposomes, the drug was dissolved with the lipid mixture (PC and cholesterol) in the chloroform. For both PG and conventional liposomes, no size reduction step was carried out.

Preparation of Liposomal Gel Using HPMC as Gelling Agent

For drug-loaded conventional and PG-liposomal gels, hydroxypropyl methycellulose (HPMC) powder was dispersed in the liposome dispersion, and the gel was allowed to set. The HPMC concentration in the final gel was 2%. In all systems prepared, miconazole nitrate (MN) concentration (free and entrapped) was 0.6%. The gel was prepared by dispersing the gelling agent in the vesicle dispersion (containing both encapsulated and free drug in equilibrium) rather than by incorporating the vesicle concentrate (after separating the supernatant) into the gel to avoid dilution effects which could disturb the equilibrium between encapsulated and free drug (the same applies to PG).

Transmission Electron Microscopy

Negative staining of samples (liposome dispersions) was employed, using 2% *w*/*v* aqueous uranyl acetate solution. Shots were taken at ×50,000 magnification power and at 80 kV (Jeol, JEM-100 CX electron microscope).

Vesicle Size Measurement

The submicron particle size analyzer was used (Submicron Particle Size Analyzer, PCS N5, Beckman Coulter, USA). Vesicle size was measured at an angle of 90°. The polydispersity index (PI) was determined as a measure of homogeneity (26).

Assessment of PG in Liposomes

Liposomes were separated by ultracentrifugation at 37,850×*g* at 4°C for 2 h using a 3K 30 refrigerated centrifuge (Sigma Laborzentrifugen GmbH, Germany). PG in the supernatant was determined using a GC method based on the British Pharmacopeia method for PG analysis (27) with slight modification. A PerkinElmer auto system XL gas chromatograph equipped with a Restek column (30 m length and 0.32 μm particle diameter) and a Restek Corp FID was used. The oven temperature was set initially at 200°C for 2 min before increasing to 240°C. The gas flow rate was 10 ml/min. The detector and injector temperatures were 275 and 180°C, respectively. Injector volume was 0.1 μl. The concentration of PG in vesicles was obtained by subtraction.

Retention of PG by Liposomes Following Redispersion in Distilled Water

Liposomes (separated by ultracentrifugation) were redispersed in distilled water and stored at 4°C. Stored liposome dispersions were re-centrifuged after 15 days and the supernatant analyzed for PG by GC. This cycle was repeated twice (30-day storage period) to check for potential leakage of PG into the surrounding medium over this study period.

Drug Entrapment Efficiency

Two methods of separating drug-loaded vesicles were used prior to drug assay. Ultracentrifugation (at 37,850×*g* for 2 h at 4°C), followed by lysis of liposomes with methanol, was one method. Lysis was achieved by mixing the vesicle concentrate with a sixfold volume of methanol in a stoppered tube and vortexing for 5 min, giving a clear solution. The concentration of MN was determined spectrophotometrically at λ_{max} 272 nm (22) using empty lysed liposomes as blank.

$$\textit{Entrapment efficiency}\ (EE\%) = (\textit{Drug in vesicles}/\textit{Initial drug added}) \times 100$$

The second method involved dialysis of the liposome dispersion (1 ml) for 2 h at 2–8°C using dialysis bags (Spectra/Por®, 10.000- to 14.000-Da molecular weight cutoff). Bags were suspended in distilled water (100 ml) for 2 h at 2–8°C. The free drug in the dialysate was measured spectrophotometrically at λ_{max} 272 nm.

$$\textit{Entrapment efficiency}\ (EE\%) = (\textit{Initial drug added} - \textit{Drug in dialysate}/\textit{Initial drug}) \times 100$$

Mean values of both methods were recorded.

Drug Release Studies

MN release was monitored using a dialysis method for separating released drug from liposomes (22, 28). MN liposomes (dispersion or in gel form, equivalent to 6 mg MN) were placed in dialysis bags, suspended in 100 ml of the release medium (acetate buffer, pH 5, containing 25% methanol) in capped flasks, and shaken (100 strokes per minute) at 32±0.3°C. The release medium was selected based on MN solubility data generated in acetate and phosphate buffers of different composition and pH at 32°C (data not shown). The MN solubility determined in the selected medium was 18 mg/100 ml, providing adequate sink conditions (total drug release into release medium would yield 6 mg/100 ml). Tween 80 (2%) was used in another study involving MN, as a release medium outside the dialysis bags (reported MN solubility in this medium was 0.239 mg/ml (29)). Serial sampling over 24 h with replacement was done. The drug in the dialysate was measured by second-derivative UV spectrophotometry at λ_{max} 287 nm (22) (second derivative gave the highest correlation coefficient compared to first and zero derivatives, r=0.991).

Stability Testing of Liposomes

MN-loaded PG liposomes and conventional liposomes were stored for 3 months at 4°C. Change in drug entrapment was monitored using the dialysis method at 2-week time intervals. Vesicle size was also determined at 45 and 90 days of storage. At the end of the study period, the dispersions were examined visually, for phase separation, and microscopically, for coalescence.

Freeze-Drying of Liposome Dispersion

For *in vitro* skin permeation studies, liposome dispersions (5 ml) were freeze-dried with a vacuum freeze-dryer (Cryodos-50 lyophilizer, Telstar Cryodos, Spain) using 3% mannitol as cryoprotectant. Samples were frozen at −80°C for 4 h and then placed in the lyophilizer (condenser temperature, −70°C). Lyophilization was performed at a pressure of 40 mbar and a shelf temperature of −40°C for 1 day followed by drying at 25°C for another day (30).

Antifungal Activity

Agar Diffusion Technique

MN-loaded PG liposomes were compared with conventional liposomes and with controls. Sterile agar plates were inoculated with a *Candida albicans* culture using the flooding technique. Wells were filled with the test formulae (60 µl). A constant amount of MN (360 µg) was applied in each well (formula or control). Agar plates were incubated at 37°C for 24 h. Inhibition zone diameters (in millimeters) were measured. Each experiment was done in triplicate.

Broth Dilution Technique

The MIC of MN against *C. albicans* was determined using the broth dilution technique. MN-loaded PG liposomes were compared with both conventional liposomes and controls. The formulae and controls were serially diluted twofold with sterile distilled water, and 1-ml aliquots were distributed in sterile test tubes. Each test tube received individually 1 ml of the test microorganism inocula (10^6 CFU/ml). The tubes were incubated at 37°C for 24 h and examined visually for microbial growth to determine the MIC values. Two drug-free control broth tubes (inoculated and non-inoculated) were included with the test tubes to check the adequacy of the broth to support the growth of the organism and for sterility check. The MIC value of MN against *C. albicans* was the lowest concentration of the antifungal that inhibited growth.

In Vitro Human Skin Permeation/Deposition Using Franz Diffusion Cells

In a preliminary study, excised human skin from female patients was used (permission granted from the Plastic Surgery Department of the Faculty of Medicine, Alexandria University, following approval of the Research Ethics Committee, Faculty of Pharmacy, Alexandria University). Subcutaneous fat was removed. Skin discs (3.14-cm^2 available area) were fitted onto the Franz diffusion cell. For skin pre-hydration and for generating a hydration gradient (31), an isotonic phosphate buffer with pH 7.4 and containing 0.11% formaldehyde was used as a receiver solution. Diffusion cells were incubated for 24 h.

Freeze-dried liposomes were resuspended in water; a volume equivalent to 3 mg MN (200 µl) was applied (non-occlusive) onto the skin. The receiver solution was 8 ml acetate buffer, pH 4.5. The selection of this medium was based on an equilibrium solubility study at 32°C. MN solubility determined in this medium was 0.286 mg/ml. Diffusion cells were

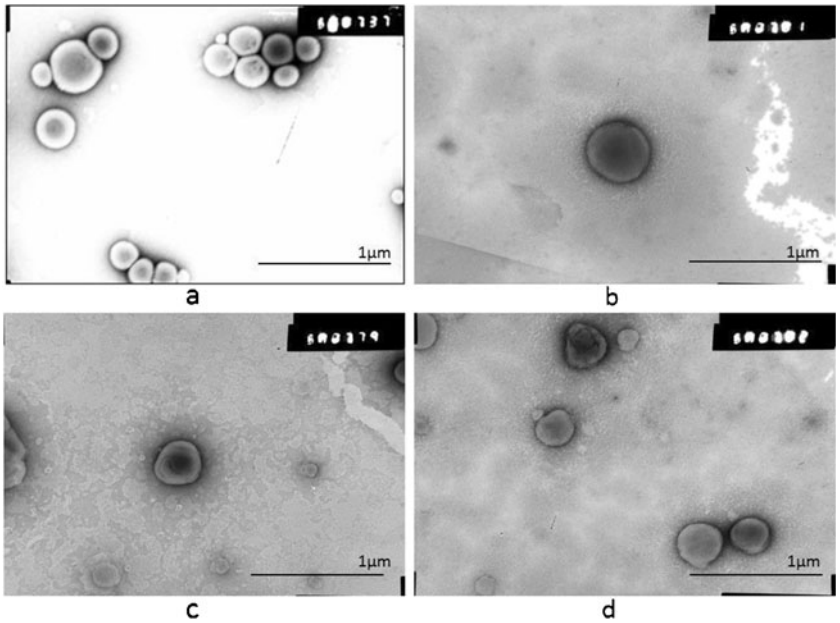

Fig. 1. TEM micrographs showing PG 10% liposomes: **a** blank, **b** MN loaded, **c** after freeze-drying, and **d** after a 3-month storage period

shaken for 24 h (100 strokes per minute) at 32°C. The receiver solution was removed and analyzed for permeated MN. The skin surface was washed with 8-ml methanol portions and the collective washings analyzed for residual drug remaining on the skin.

Drug retained in the skin was extracted with methanol (32,33). The skin was removed from the Franz diffusion cell, streaked with a knife reaching the deeper layers of the skin to facilitate extraction, and immersed in 10 ml methanol in closed vials, shaken for 24 h at 27°C (100 strokes per minute) and filtered (0.22-µm syringe filters). This procedure allowed quantitative drug extraction.

All samples collected were analyzed by HPLC. Reversed-phase HPLC (PerkinElmer Series 200 equipped with LC pump, autosampler, UV/vis detector, Series 600 interface, and Total-Chrom navigator 6.2.0.0 Computerized Chromatography Analysis Software) was used (column C18; ambient temperature; sample injection volume, 20 µl). The mobile phase was 55% methanol, 30% acetonitrile, and 15% acetate buffer, pH 5. The flow rate was 1.5 ml/min and detection wavelength was 230 nm. The retention time of MN was 3.8 min. Data presented are the average of two determinations.

RESULTS AND DISCUSSION

Nanosized PG liposomes (both blank and drug loaded, TEM micrographs, Fig. 1) were prepared by a one-step, simple method, involving dissolving PC and MN in PG before adding the distilled water (12). The same procedure, in principle, has been referred to as the Mozafari method (34) and in another reference, describing the preparation of chloramphenicol liposomes, as the polyol dilution method (35). Similarly, another study reported preparation of polyvinyl alcohol (PVA)-stabilized liposomes where PVA was again added to the lipid during liposome preparation (16). On the other hand, Manconi *et al.* (14) prepared liposomes containing glycols using the film hydration method; the glycols in this case were added to the aqueous phase.

Physicochemical and performance data generated in the present study (Tables I and II) allowed comparison of the blank with drug-loaded vesicles and comparison of conventional with PG vesicles when freshly prepared and after treatment. Individual treatments (footnotes, Tables I and II) included storing at 4°C for 90 days, freeze-drying followed by reconstitution, and formulating as gel which represents

Table I. Physicochemical Criteria of Miconazole Nitrate-loaded PG (10%) Liposomes, Compared with Conventional Liposomes

	Traditional liposomes		PG (10%) liposomes	
Comparison criteria	Fresh	Treated	Fresh	Treated
Mean size, nm				
MN-loaded liposomes	867.9	100.5[b]	351.0	293.1[b]
Blank liposomes	1,110.8	111.1[b]	539.45	457.6[b]
Polydispersity index (PI)	0.971	–	0.563	–
Entrapment efficiency (% EE)[a]	80.78	51.18[b]	95.59	70.51[b]

[a] Mean of centrifugation and dialysis methods for separating free from entrapped drug
[b] Treatment: storage at 4°C for 90 days

Table II. Performance Criteria of Miconazole Nitrate-loaded PG (10%) Liposomes, Compared with Conventional Liposomes

Comparison criteria	Conventional liposomes		PG (10%) liposomes	
	Fresh	Treated	Fresh	Treated
Release data				
Higuchi plot intercept (% released)	13.53	22.19[a] 13.84[b]	0.60	13.10[a] 13.75[b]
Higuchi plot slope (% $t^{1/2}$)	0.55	0.43[a] 0.40[b]	0.59	0.39[a] 0.24[b]
Antifungal activity				
Mean inhibition zone, mm (±SD) (agar plate)	22.00 (4.24)	19.00 (5.66)[c]	23.50 (4.95)	20.50 (4.95)[d]
MIC[e], μg/ml (broth dilution method)	2.93	5.86[c]	1.46	2.93[d]
Skin permeation				
% drug retained in skin (±SD)	5.54 (0.53)	2.44 (1.47)[f]	7.18 (2.17)	2.44 (1.47)[f]
% drug permeated through skin (±SD)	1.28 (0.51)	1.34 (0.11)[f]	1.85 (1.56)	1.34 (0.11)[f]

[a] Treatment: freeze-drying and reconstitution before testing
[b] Treatment: gelling agent added to liposome dispersion before testing
[c] System tested: MN solution in DMSO/water (2:3)
[d] System tested: PG added to MN solution in DMSO/water (2:3). Empty vesicles alone gave no antifungal activity
[e] Minimum inhibitory concentration
[f] System tested: MN suspension

the optimum dosage form for topical delivery of drug-loaded lipid vesicles.

Vesicle Size

Liposome preparation involved no sonication or extrusion to reduce vesicle size. Mean size values of blank and MN-loaded conventional liposomes were 1,110.8 and 539.5 nm, respectively. Incorporation of PG resulted in reduction in vesicle size of both blank liposomes (867.9 nm) and drug-loaded liposomes (351.0 nm) and narrower size range (lower corresponding PI values). This suggests an interaction of PG with the phospholipid bilayer allowing more bilayer flexibility and smaller vesicle size. Manconi *et al.* (14) also reported reduction in lipid vesicle size upon the introduction of glycols.

Incorporation of MN in conventional and in PG liposomes resulted in reduction in vesicle size (drug loaded compared to blank vesicles). Phospholipid vesicles carry a negative charge likely due to ionization of phosphate groups (36). Highly lipophilic drugs like MN, with a reported log P_{oct} >5, are entrapped mainly in the lipid bilayer of the liposomes (37). Reduction of repulsive forces between phosphate groups by MN could have led to closer packing of the lipid molecules. Manconi *et al.* (14) also reported a reduction in the vesicle size

Table III. Redistribution of PG Between Blank Liposomes and Fresh Dispersion Medium, at 4°C

Time (days)	PG(2.5 %) liposomes				PG(5%) liposomes				PG(10%) liposomes			
	mg PG in supernatant	mg PG in vesicles	%PG in vesicles[a]	Apparent distribution coefficient[b]	mg PG in supernatant	mg PG in vesicles	%PG in vesicles	Apparent distribution coefficient	mg PG in supernatant	mg PG in vesicles	%PG in vesicles	Apparent distribution coefficient
Day 1[c]	67.80±0.31	57.20	45.76	20.6	139.70±3.87	110.30	44.12	18.8	267±0.28	233.00	46.60	19.6
Day 15[d]	29.27±0.46	27.93	48.82	23.3	64.72±0.95	45.58	41.33	16.8	125.47±0.98	107.52	46.15	19.3
Day 30[d]	15.22±2.10	12.70	45.51	20.5	26.45±0.74	19.14	41.98	17.4	55.97±0.50	51.56	47.90	20.8

[a] In reference to total PG in dispersion
[b] An apparent distribution coefficient of PG between phospholipid vesicles and water was calculated using the equation: $\frac{\text{PG associated withvesicles (in mg)/total phospholipid added(in mg)}}{\text{PG in supernatant (in mg)/total amount of water (in mg)}}$
[c] Data are means of five batches of each formulation (n=5)
[d] Data are means of three batches of each formulation (n=3)

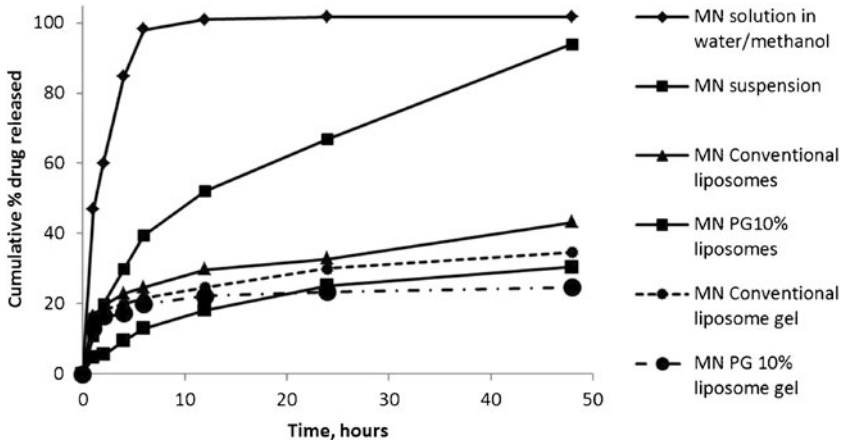

Fig. 2. Release of miconazole nitrate from solution, suspension, liposome dispersion, and liposome gel, determined at 32°C. Standard deviation values ranged from 0.85 to 7.19 at all data points (n=2–4)

of liposomes containing glycols upon loading with diclofenac (vesicle size of drug-loaded liposomes ranged from 210 to 340 nm compared to 300–350 nm for blank liposomes).

Miconazole Nitrate Entrapment Efficiency

Dialysis and ultracentrifugation methods showed good agreement in percent entrapment efficiency (% EE) results. For freshly prepared vesicles, centrifugation yielded 79.71±1.04 and 94.86±1.01%, while dialysis yielded 81.86±1.66 and 96.33±1.09% for conventional and PG liposomes, respectively. Mean values of both methods appear in Table I.

Conventional liposomes showed a mean EE of 80.78% (Table I). A similar % EE value was reported for MN-loaded multilamellar conventional liposomes using a similar drug/lipid ratio (22). Expressing drug entrapment in milligrams, 12 mg MN was entrapped per 100 mg phospholipid in the present study. Comparative value reported was 7.20–9.76 mg MN per 100 mg phospholipid (130 mg total lipid) for conventional liposomes (22).

Inclusion of PG resulted in a statistically significant increase in % EE (P<0.001) (average EE, 95.59%; Table I). Possible contributing factors include PG effect on MN solubility in the lipid phase and vesicle size reduction (12). Increased drug EE was also reported for diclofenac in glycol-containing liposomes (14), for cinchocaine in PG liposomes (12) and for calcein in PVA-stabilized liposomes (16) compared to conventional liposomes.

PG Uptake in Blank Vesicles

Propylene glycol GC assay results (Table III) indicated that increasing the initial concentration of PG, added during the preparation of vesicles, resulted in an increase in the amount of PG associated with the vesicles, while maintaining a constant percent PG uptake in the vesicles (45.49±1.26%, n=15, calculated from data in Table II, generated over PG concentration 2.5 to 10%).

PG Retention in Vesicles After Dispersion of Separated Vesicles in Fresh Medium

PG redistribution between vesicles and fresh dispersion medium was studied over 30 days. The results (Table III) indicated PG diffusion out of the vesicles when the vesicles were separated and dispersed in fresh medium. At equilibrium (after storage in fresh medium for 15-day intervals), PG remaining

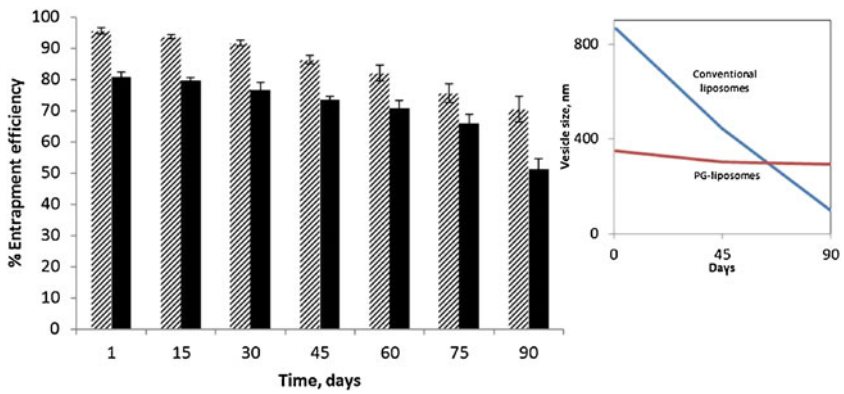

Fig. 3. Change in miconazole nitrate entrapment efficiency, as a measure of drug leakage from propylene glycol (10%) liposomes and from conventional liposomes during storage at 4°C. *Striped bars*, PG(10%) liposomes; *solid bars*, conventional liposomes. The *insert* shows change in vesicle size during storage

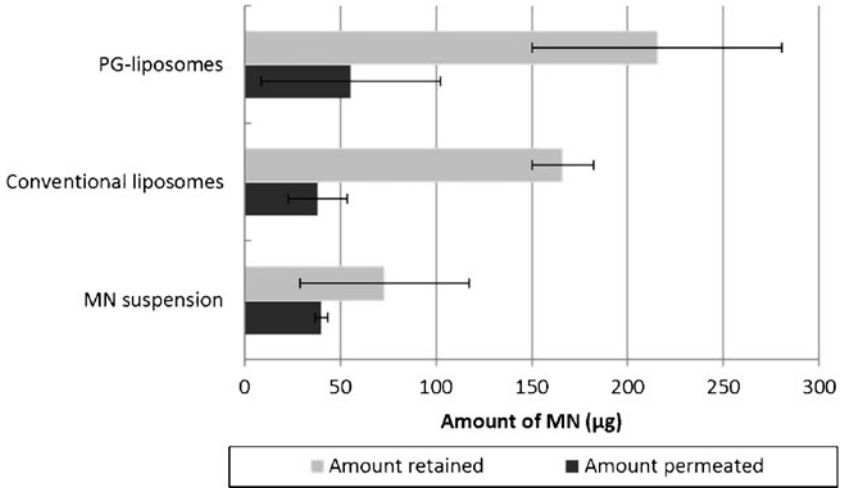

Fig. 4. MN retained in and permeated through skin after 24 h, determined at 32°C, using human skin in Franz diffusion cells under non-occlusive conditions

associated with the vesicles, relative to PG present in the system, was 45.35±3.11% (n=33, calculated from Table III).

Association of PG with the vesicles was also expressed as a distribution coefficient of PG between vesicles and medium. The values obtained, normalized for weight of both phospholipid and dispersion medium (Table III), indicated that PG is nearly 20 times more concentrated in the vesicles than in the surrounding medium.

The diffusion of PG away from the vesicles upon dilution has implication when developing a topical product containing the liposomes. During formulation, the initial equilibrium amount of PG in the vesicles needs to be maintained to make full use of PG in liposomes to modulate drug skin uptake.

Drug Release

Dialysis was used to investigate the release of MN from different systems including MN solution in 1:1 water/methanol, MN suspension in water, MN conventional and PG liposome dispersions, and liposome gels (2% HPMC) in acetate buffer, pH 5, containing 25% methanol at 32°C under sink conditions. Release was monitored in profiles are shown in Fig. 2, and Higuchi plot parameters are given in Table II. The release profile of MN solution, as a control (Fig. 2), is proof of drug dialyzability. The dialysis method was also used to assess the effect of freeze-drying of selected liposome systems on drug release. Freeze-dried powders were reconstituted to original strength before testing release.

The release profiles indicated prolonged drug release from all vesicle systems investigated (including freeze-dried vesicles) in contrast to drug release from solution and suspension (Fig. 2). For freeze-dried conventional and PG liposomes, the greater intercepts of Higuchi release plots (Table II), denoting unentrapped drug, indicated leakage of some drug out of the vesicles as a result of freeze-drying. For MN release from liposome gel, a slightly higher burst effect could be noted compared to liposome dispersion data, probably as a result of osmotic effect exerted on the gelling agent causing shrinking of the vesicles.

Stability Study

Conventional and PG (10%) liposome dispersions (both blank and MN loaded) were stored for 90 days at 4°C, as a challenge test for drug leakage out of the vesicles and to monitor changes in vesicle size during storage. Drug entrapment efficiency was determined at 2-week intervals using the dialysis method. Drug leakage was evident in both types of liposomes with higher values recorded for conventional liposomes (percent drug remaining in vesicles at 90 days was 51.18% of initial values for conventional liposomes compared to 70.51% for PG liposomes, Table I and Fig. 3). Also noted in the stability study was a variable decrease in vesicle size for all systems over 90 days, with conventional liposomes showing greater reduction in size compared to PG liposomes (Table I and Fig. 3 insert).

Antifungal Activity

Agar Diffusion Technique

Both MN-loaded conventional and PG liposomes exhibited antifungal effect against *C. albicans*, judging by

Table IV. Percent MN Retained in and Permeated Through Skin after 24 h, Determined at 32°C, Using Human Skin in Franz Diffusion Cells Under Non-occlusive Conditions

Formula tested	% MN retained in skin	% MN permeated through skin	% MN remaining on skin surface	Mass balance[b], μg
MN suspension[a]	2.44±1.47	1.34±0.11	96.23	2,935.79
Traditional liposomes[a]	5.54±0.53	1.28±0.51	93.18	3,030.01
PG (10%) liposomes[a]	7.18±2.17	1.85±1.56	90.99	2,939.28

[a] Mean of two batches for each formula tested
[b] Total MN initially added in the donor compartment was 3,000 μg

measurable inhibition zones, at 24 h (22 and 23.5 mm, respectively; Table II), compared to empty liposomes (absence of inhibition zones; Table II, footnotes c and d). MN and MN/PG mixture were used as controls and gave smaller inhibition zones than vesicle systems (19 and 20.5 mm, respectively; Table II). Maheshwari *et al.* (38) reported larger inhibition zones of clotrimazole ethosomal and ultradeformable nanoliposomes, against candidal species, compared to a commercial cream.

Broth Dilution Technique

Drug-loaded PG liposomes showed the lowest MIC value (1.46 μg/ml, Table II), followed by both drug-loaded conventional liposomes and MN/PG mixture (2.93 μg/ml each). The highest value (lowest antifungal activity) was that of MN solution (5.86 μg/ml). These results gave an indication that the presence of PG enhanced the antifungal action of MN and that the inclusion of MN in vesicles further enhanced the action. Hydrophobic interaction between liposomes and *C. albicans* cells has been suggested, and the nature of adsorption of phospholipid liposomes to *C. albicans* was examined (39).

Published studies have reported conflicting data of increased and sometimes reduced *in vitro* antifungal activity of miconazole and ketoconazole bases in liposome compared to non-liposome formulations. The antifungal activity was evaluated, in these studies, using time-kill curves. Factors influencing the observed change in antifungal activity included phospholipid and drug type and concentration, type of liposome, duration of incubation, and integrity of vesicles (40,41). The incubation time also appears to affect results of antifungal activity assessed using the cup–plate method. In a study of carbopol gel containing ketoconazole liposomes, the inhibition zone was larger for the liposome formulation at 30 h, but was smaller at 18 h, compared to the non-liposome formulations (33).

In Vitro Human Skin Permeation/Deposition Using Franz Diffusion Cells

Drug-loaded conventional and PG liposomes, both reconstituted from freeze-dried powder, were compared to controls including MN suspension. Freeze-drying and reconstitution in a limited volume of distilled water suited non-occlusive conditions aimed at driving the vesicles into the skin (31). Drugs retained in, permeated through, and remaining on the surface of skin surface were analyzed by HPLC. Mass balance was constructed from generated data to assess recovery of drug initially added (Fig. 4 and Table IV). Mass balance data obtained indicated quantitative recovery of drug from the skin.

Miconazole skin permeation and retention were improved with PG liposomes compared to both conventional liposomes and MN suspension. Compared to MN suspension, the enhancing effect was statistically significant ($P<0.1$, PG liposomes *vs* drug suspension for permeation and deposition, and $P<0.2$, conventional liposomes *vs* drug suspension for deposition only). Improved skin delivery is expected to be due, partly, to the penetration-enhancing effect of PG (12), acting synergistically with that of vesicles, in addition to increasing the deformability of vesicles. The skin retention data emphasized the drug reservoir effect in skin reported elsewhere for miconazole (22) and ketoconazole encapsulated in liposomes (33).

Miconazole permeability coefficient was calculated (1.388×10^{-4} cm/s) by normalizing MN suspension flux values (40.2 μg/3.14 cm^2/24 h) for skin area and driving concentration (determined saturation solubility, 64 μg/ml).

In conclusion, the present study has addressed some issues concerning possible performance enhancement of phospholipid vesicles applied topically, using an adjuvant such as PG. The drug loaded in the vesicles was the antifungal agent miconazole nitrate. We have shown that a constant percent uptake of PG in the vesicles (≈45.5) was maintained in the PG concentration range of 2.5 to 10%. Comparison with conventional liposomes (not containing propylene glycol) afforded means of assessing the value of the added adjuvant. Assessment of loading efficiency, release prolongation, stability on storage, antifungal activity, and skin retention all pointed to better performance of the PG phospholipid vesicles. The gel form is suggested as a suitable dosage form for the drug-loaded vesicles. The importance of maintaining the initial equilibrium state of propylene glycol in the vesicles during the preparation of the gel is emphasized. This can be achieved by adding the gelling agent to the liposome dispersion. Findings provide more insight into pharmaceutical aspects and performance of PG phospholipid vesicles.

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