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Stability and transdermal absorption of topical amphotericin B liposome formulations

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Abstract

The aim of this study was to characterize the stability and transdermal absorption of amphotericin B (AmB: 0.05 mg/mg lipid) in hydrogenated soya phosphatidylcholine/cholesterol/charged lipid {dicetyl phosphate (−) or stearylamine (+)} liposomes at molar ratios of 1:1:0, 7:2:0, 7:2:1(−) and 7:2:1(+). The AmB contents in liposomes were determined by HPLC with UV detection at 382 nm. Stabilities of AmB in liposome formulations were compared with those in solution and powder forms, during storage at 4, 30 and 45 °C for 90 days. Absorption studies of AmB across the rat skin were conducted, using vertical Franz diffusion cells at 37 °C for 24 h. The slowest degradation was observed in the positive liposome (7:2:1(+)AmB), with shelf life of ∼1 year (30 °C). In comparison, the shelf lives of AmB in solution and powder were 4 and 14 days, respectively. AmB in positive liposomes seemed to demonstrate the highest flux in stratum corneum (58 ng/cm^2/h), while the highest flux in viable epidermis (23 ng/cm^2/h) was observed in negative liposomes. AmB entrapped in charged liposomes showed sustained skin absorption. The positively charged liposome might be the best formulation for AmB, due to its higher stability than other formulations.

Keywords: Stability; Absorption; Amphotericin B; Topical liposomes; Dicetyl phosphate; Stearylamine

1. Introduction

Liposomes are widely used as carriers for a variety of drugs, including their application to topical delivery (Mezei and Gulasekharam, 1982; Gabizon, 1995; Margalit, 1995; Woodle, 1995). The development of liposomes aims to improve the delivery of applied drugs through the skin. Liposomes do not only enhance the drug penetration into the skin with the slow release of drug, but also decrease the clearance of drug by minimizing its absorption into the systemic circulation.

Amphotericin B (AmB) is the drug of choice for systemic fungal infection (McEvoy, 1996). Liposomal AmB products are available for systemic, eye and lung applications (Dollery, 1991; Pleyer et al., 1995; Purcell and Corris, 1995; van-Etten et al., 1995; McEvoy, 1996; Kohno et al., 1997; Lambros et al., 1997). However, there is none for topical application. Owing to the bulky structure of AmB, it cannot be absorbed through the skin (Dollery, 1991). Since liposomes can enhance the skin absorption (Gulati et al., 1998), the liposome formulations for AmB from various lipid compositions, containing neutral, positive or negative charged lipid have been developed. We have previously found that the positive liposome composed of hydrogenated soya phosphatidylcholine/cholesterol/stearylamine at a molar ratio of

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7:2:1 demonstrated high percentage of entrapment of AmB (90%) and high rigidity of liposomal membrane (unpublished results). This liposome formulation was thus selected for further stability and skin absorption studies. The liposomal AmB is expected to be advantageous over other topical antifungal drugs for the treatment of candida infection.

The objective of this study was to investigate the physical and chemical stabilities, as well as the absorption through the rat skin of various topical AmB liposome formulations from various lipid compositions, containing neutral, positive or negative charged lipid. The stability and transdermal absorption of various AmB liposome formulations were compared with those of free AmB in solution and powder forms.

2. Materials and methods

2.1. Materials

AmB, cholesterol (CHL), dicetyl phosphate (DCP) and stearylamine (SA) were obtained from Sigma Chemical Company (St. Louis, MO). Fungizone® (FGZ) consisting of 50 mg AmB, 41 mg sodium desoxycholate and 20.2 mg sodium phosphate was a gift from Bristol-Myers Squibb (Thailand) Ltd., Bangkok. Hydrogenated soya phosphatidylcholine (Emulmetik 950®) (HSC) was kindly donated by JJ-Degussa (T) Ltd., Bangkok. Acetonitrile and methanol were of HPLC grade, ethylenediaminetetraacetic acid disodium salt dihydrate, chloroform, potassium dihydrogen orthophosphate, sodium hydroxide pellets and dimethyl sulfoxide were of analytical reagent grade, and obtained from commercial sources.

Full-thickness abdominal skin was obtained from male Wistar rats, weighing between 200 and 250 g. The hair on abdominal area was shaved off and left overnight. The rats were sacrificed and the abdominal skin was removed. The subcutaneous fat was trimmed off and the prepared skin was freshly used. The investigational protocol for all procedures adhered to the "Principles of Laboratory Animal Care".

2.2. Preparation of liposomes

Liposome dispersion samples were prepared by a chloroform film method with sonication. Four different liposome formulations composed of HSC/CHL = 1:1 and 7:2; HSC/CHL/SA = 7:2:1(+) and HSC/CHL/DCP = 7:2:1(−) at molar ratios, with the entrapped AmB (in the form of FGZ) were prepared. The AmB content was 0.05 mg/mg of total lipid, and the total lipid in 1 ml of liposome dispersion sample was 5 mg.

The lipid mixture and AmB were dissolved in 10 ml of chloroform and 30 ml of methanol, and the two solutions were subsequently mixed. The solution was vacuum-desiccated (400 mbar, 65 °C, 45 rpm) for 60 min. A formed thin film layer was further dried by vacuum-desiccator for 30 min and then flushed with nitrogen gas for 1 min. The thin film was re-suspended in 40 ml of 0.1 M phosphate buffer (pH 7.4), and swelled by swirling in a water bath (80 °C, 190 rpm) for 30 min. The liposome dispersion samples were sonicated with a microtip probe sonicator (Vibracell, Sonics & Materials, Inc., Danbury, CT) for 30 min and then flushed with nitrogen gas for 1 min. They were stored at 4 °C, protected from light, and filtered through the Whatman filter paper No. 42 (pore size: 2.5 μm) prior to use. The lamellarity of more than 15 layers was observed in the neutral 7:2 liposomes with the entrapped AmB in lipid bilayers (unpublished results).

Liposome dispersion samples (5 ml) were put into a freeze dryer (Model Lioalfa 10, Telstar, Spain), with the following prefreeze conditions: 25 °C for 1.5 h, −32 °C for 1.5 h and −36 °C for 1 h. The duration of primary (at −42 °C) and secondary (at 25 °C) drying were 10 and 3 h, respectively.

2.3. Microscopy investigations of liposomes

The particle size of freeze-dried liposome powder (100 particles) was measured by a Scanning Electron Microscope (SEM 840A JEOL, JEOL Ltd., Tokyo, Japan), at magnification ranging from 10,000 to 30,000. The powder was picked up by a toothpick and gently spread on the adhesive copper tape, attached to the brass stubs. The sample surface on stubs was covered with gold (Gold coater JFC-1100E Ion Sputtering Device, JEOL Ltd.), and the stubs were examined after coating.

2.4. Stability study

Liposomes, FGZ solution (0.554 mg/ml) in deionized (DI) water and FGZ powder were used as samples

in this study. Samples (5 ml for liposomes and FGZ solution, and 40 mg for FGZ powder) were transferred into the amber glass, prior to storage at three different temperatures, namely 4, 30 and 45 (±1) °C for 90 days. At predetermined time intervals (0, 5, 20, 40 and 90 days), samples (0.1 ml for liposomes and FGZ solution, and 5 mg for FGZ powder) were removed. For liposomes and FGZ solution, 0.1 ml of samples were diluted (1000-fold) with methanol, whereas 5 mg of FGZ powder was dissolved in 10 ml of DI water, prior to HPLC analysis (injection volume, 50 μl). At the completion of the study, samples were transferred into a clear test tube for visual observation. The experiments were performed in six replicates.

2.5. Transdermal absorption study

Liposomes, AmB solution (0.2 mg/ml) in DMSO/methanol (1:9, v/v) and FGZ solution (0.554 mg/ml) in DI water were used as samples in the skin absorption study. The vertical Franz diffusion cells (Crown Bio Scientific, Inc., Somerville, NJ) were set at 37 ± 1 °C, and the receiver chamber was filled with 12 ml of ethanol/water (1:1, v/v). The skin was fixed in the diffusion cell with the dermal side in contact with the receiver medium, and the epidermis side in contact with the donor chamber (contact area, 1.77 cm^2). The cell was clamped and the receiver medium was stirred continuously for 30 min by a magnetic bar. Samples were loaded into the donor chamber. During the study, the donor chamber and the sampling port were covered by parafilm, and the cells were protected from light. At time zero, samples loaded in the donor side were diluted (1000 times) with methanol, prior to the analysis by HPLC. Samples in the receiver chamber (1 ml) were withdrawn at time 0 and 24 h, and centrifuged at 50,000 × *g* (4 °C) for 10 min, prior to injection onto the HPLC column (injection volume, 100 μl). After 24 h, all remaining donor solution was removed. The used skin was washed twice with 2 ml of DI water. The rinsed water was collected and combined with the above remaining donor solution, and adjusted to 25 ml with methanol. Further dilution with methanol was performed when necessary, prior to the determination of AmB content by HPLC (injection volume, 50 μl). Following twice rinsing, the skin was removed from the cell and swung twice in 100 ml of DI water, and the rinsed water was discarded.

The amount of AmB in the stratum corneum (SC) was determined by stripping the skin with a 3 M Scotch Magic™ tape (1 cm × 1 cm) (Plessis et al., 1992). Nine tapes were used for each skin sample and they were pooled in a 5-ml vial, containing 5 ml of methanol. The vial was vortexed for 1 min, allowed to stand for 10 min, and vortexed again for 1 min, prior to the analysis by HPLC (injection volume, 100 μl). The amount of AmB in the viable epidermis and dermis (VED) was determined by cutting the skin into small pieces. They were pooled in a 5-ml vial, containing 5 ml of methanol, and the subsequent steps followed similar procedures as described for the determination of AmB content in SC.

2.6. HPLC assay for the determination of AmB content

Qualitative and quantitative analyses of AmB in liposome formulations and in FGZ solution and powder forms were performed by an HPLC (HP1100, Vectra XM series 4, Hewlett Packard, Waldbronn, Germany). An ODS-Hypersil C_{18} (250 mm × 4 mm i.d., 5-μm particle size) HPLC column was used. The mobile phase was a mixture of acetonitrile/2.5 mM disodium edetate in water (45:55, v/v). HPLC was performed isocratically at ambient temperature and a flow rate of 1 ml/min with UV detection at 382 nm. Under this condition, the retention time of AmB was 4.7 min. Every sample was filtered through a 0.45-μm membrane filter, prior to injection onto the HPLC column. HPLC linearity was determined from five working standard solutions (0.02−1.50 μg/ml) of AmB in DMSO/methanol (1:999, v/v). The correlation (r^2), intercept and slope of the standard curve were calculated. The peak areas of samples were calculated and the concentrations of AmB in samples were determined from the standard curve. This experiment was performed in duplicates and each replicate sample was injected for three times.

2.7. Data analysis

The proposed models (zero-order, first-order and Higuchi model) were tested, by fitting the experimental data to the appropriate equations (Table 1). The correlation was used as an indicator of goodness-of-fit of the equation to the experimental data. The observed

Table 1
Equations for the calculations of degradation rate constants and shelf lives of AmB in liposomes, solution and powder forms[a]

Types of equation	Equations
Zero-order	$C_t = C_0 + kt$
First-order	$\ln C_t = \ln C_0 + kt$
Higuchi model	$C_t = C_0 + kt^{0.5}$
Shelf life (Higuchi model)	$(t_{90})^{0.5} = (C - C_0)/\text{antiln}\, kt_{90} = ((t_{90})^{0.5})^2$

[a] Term descriptions: C_0: initial concentration, C_t: concentration at given t, k: degradation rate constant, t: time, and t_{90}: shelf life.

degradation rate constants (k_{obs}) of AmB in liposomes, solution and powder forms were estimated from the slope of the declining concentrations versus time plots, by least-squares fitting of rate equation (Table 1). The k_{obs} values of each temperature were substituted into the Arrhenius equation (Martin, 1993), to calculate the predicted degradation rate constant (k_{pred}) of AmB at a given temperature. The predicted shelf life (t_{90}), namely the time required when the AmB content remains 90%, was estimated by substituting k_{pred} into the shelf life equation for Higuchi model (Table 1).

3. Results

3.1. Liposome size

The mode of liposome size was used instead of mean, since the liposomes showed unnormal distribution. The size distribution of all liposome formulations with the entrapped AmB was in the range of 0.115–0.364 μm (Table 2). The smallest size of 0.115 μm was observed in the negative liposome {7:2:1(−)AmB} (Table 2).

Table 2
Particle sizes (μm) of various liposome formulations with the entrapped AmB[a]

Formulations	Mode	Mean	S.D.
1:1AmB	0.154	0.178	0.052
7:2AmB	0.154	0.162	0.050
7:2:1(+)AmB	0.364	0.352	0.076
7:2:1(−)AmB	0.115	0.122	0.035

[a] Experimental data represent the mode, mean and standard deviation, following measurement in 100 particles.

3.2. Determination of the AmB content by HPLC

The standard curve of AmB in DMSO/methanol (1:999, v/v) was shown to be linear ($r^2 = 0.9999$), over the concentration range 0.02−1.50 μg/ml. The following regression equation was obtained: $y = 354.34x - 8.01$, where y is the peak area of AmB (mAU × s) and x is the concentration of AmB (μg/ml). The coefficients of variation calculated during replicate assays were lower than 5.5%.

Prior to the absorption study, all related blank samples (ethanol/water (1:1, v/v), DI water, DMSO/methanol (1:9, v/v), VED without AmB, SC in stripped tape and unstripped tape in methanol) were analyzed by HPLC. The resulting HPLC chromatograms showed no peaks eluted between 4 and 5 min. Thus,

Table 3
Physical appearances of various liposome formulations with the entrapped AmB, FGZ solution and FGZ powder, either freshly prepared or stored at 4, 30 and 45 °C for 90 days

Formulations	Freshly prepared		Following storage for 90 days					
	Sediment	Supernatant	Sediment			Supernatant		
			4 °C	30 °C	45 °C	4 °C	30 °C	45 °C
1:1AmB	Yes	+1[a]	Yes	Yes	No	+2	+2	+3
7:2AmB	Yes	+1	Yes	Yes	Yes	+2	+2	+3
7:2:1(+)AmB	Yes	0[b]	Yes	Yes	Yes	+1	+1	+2
7:2:1(−)AmB	Yes	+1	Yes	Yes	No	+2	+2	+3
FGZ solution	No	0	No	No	No	0	0	+1
FGZ powder	Lyophilized powder		Powder with intense yellow color					

[a] +1 to +3 indicate an increase in the degree of turbidity of supernatant.
[b] 0 indicates clear (transparent) supernatant.

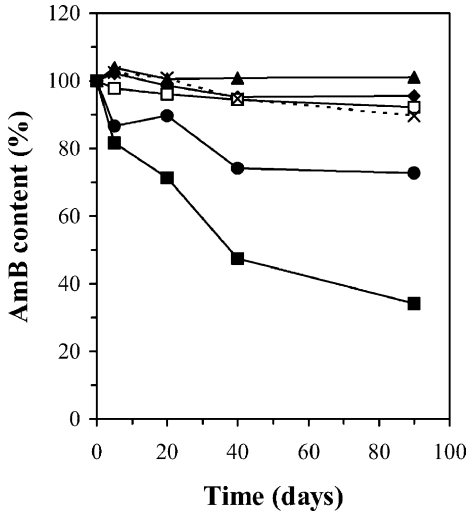

Fig. 1. Typical degradation of AmB in various liposome formulations, FGZ solution and FGZ powder during storage at 30 °C for 90 days: (◆) 1:1AmB; (□) 7:2AmB; (▲) 7:2:1(+)AmB; (×) 7:2:1(−)AmB; (■) FGZ solution; (●) FGZ powder. Symbols are the mean value of six determinations.

there was no interference on the determination of AmB content by HPLC, caused by these blank samples.

3.3. Stability of liposomes

The physical appearances of the 1:1, 7:2, 7:2:1(+) and 7:2:1(−) liposomes with the entrapped drug, FGZ solution and powder forms, either freshly prepared or stored at 4, 30 and 45 (±1) °C for 90 days are shown in Table 3. Changes in the color, formation of sediment, and increase in the turbidity of supernatant were observed in the 7:2AmB and 7:2:1(+)AmB liposome formulations, following storage at high temperature (45 °C) for 90 days, whereas FGZ solution, the 1:1AmB and 7:2:1(−)AmB liposomes showed only an increase in the turbidity of supernatant (Table 3). FGZ powder showed more intense yellowish color, after 90 days storage at 4, 30 and 45 °C than that at the initial time (Table 3).

Typical degradation kinetics of AmB in various liposome formulations, FGZ solution and FGZ powder during storage at 30 °C for 90 days are presented in Fig. 1. The observed and predicted degradation rate constants of AmB in various liposome formulations, FGZ solution and FGZ powder are shown in Table 4, while the corresponding predicted shelf lives of AmB are presented in Table 5. It was confirmed that the liposomes were still intact.

Table 5
Predicted shelf lives of AmB in liposome formulations, FGZ solution and FGZ powder[a]

Formulations	Predicted shelf lives (days)[b]		
	4 °C	30 °C	45 °C
1:1AmB	511.7	27.1	6.2
7:2AmB	210.2	38.1	16.0
7:2:1(+)AmB	5556.4	384.5	101.3
7:2:1(−)AmB	267.1	21.4	6.0
FGZ solution	11.4	4.1	2.5
FGZ powder	28.1	13.9	9.8

[a] Experimental data represent the mean value of six determinations.
[b] The predicted shelf lives (t_{90}) were estimated by substituting k_{pred} into the shelf life equation for Higuchi model (Table 1).

Table 4
Observed (k_{obs}) and predicted (k_{pred}) degradation rate constants of AmB in liposome formulations, FGZ solution and FGZ powder[a]

Formulations	C_0 (μg/ml)[b]	k_{obs} (%/day$^{0.5}$)			k_{pred} (%/day$^{0.5}$)		
		4 °C	30 °C	45 °C	4 °C	30 °C	45 °C
1:1AmB	190.9 ± 5.6	0.63	0.68	8.01	0.44	1.92	4.01
7:2AmB	212.1 ± 0.5	0.87	0.81	3.94	0.69	1.62	2.50
7:2:1(+)AmB	179.6 ± 3.7	0.34	0.03	6.25	0.13	0.51	0.99
7:2:1(−)AmB	171.1 ± 5.6	0.73	1.25	5.89	0.61	2.16	4.10
FGZ solution	220.1 ± 4.7	2.62	7.13	4.97	2.96	4.92	6.35
FGZ powder	400.0 ± 8.5[c]	1.86	2.84	3.08	1.88	2.68	3.19

[a] Experimental data represent the mean ± S.D. of six determinations.
[b] The initial content of AmB in the samples.
[c] The initial content of AmB in FGZ powder was expressed as μg/g.

Table 6
Flux of AmB in liposomes and solution forms, and FGZ solution through different strata of rat skin[a]

Formulations	C_0 (μg/ml)[b]	Flux (ng/cm^2/h)		
		SC	VED	Receiver
1:1AmB	208.3 ± 5.9	4.8 ± 0.4	2.7 ± 0.1	0
7:2AmB	214.8 ± 4.8	4.1 ± 0.8	2.2 ± 0.2	0
7:2:1(+)AmB	207.2 ± 28.4	57.6 ± 29.1	10.9 ± 3.3	0
7:2:1(−)AmB	189.0 ± 2.2	39.5 ± 6.3	22.8 ± 2.5	0
AmB solution	167.0 ± 5.8	73.3 ± 0.5	53.7 ± 24.3	4.26 ± 0.07
FGZ solution	208.4 ± 2.5	123.8 ± 9.8	127.3 ± 6.5	0

[a] Experimental data represent the mean ± S.D. of four determinations.
[b] The initial content of AmB in the donor chamber.

3.4. Transdermal absorption of liposomes

The flux of AmB in liposomes, solution (in DMSO/methanol (1:9, v/v)) and FGZ solution in DI water through different strata of rat skin is shown in Table 6. The highest flux of AmB from all formulations was observed in SC. AmB in all formulations, except AmB solution in DMSO/methanol, were found in SC and VED but not in the receiver medium, indicating not diffusing through the skin.

4. Discussion

The stability of liposomes with the entrapped AmB, FGZ solution and FGZ powder was observed physically (Table 3) and chemically (Table 4). An increase in the turbidity of supernatant (Table 3) may result from a partial transition of the sediment and the degradation of AmB at high temperature (45 °C). This was confirmed by the change of color in FGZ solution. In all kinetic studies, better correlations ($r^2 = 0.76$) were obtained when the observed data were fitted to the Higuchi model equation, comparing to those fitted to the zero- and first-order equations. Thus, Higuchi model seemed to be the most acceptable approach to be employed in this kinetic study, and was used to estimate the observed degradation rate constants and shelf lives of AmB in various forms (Table 1).

The observed degradation rate constants were compared with the respective predicted values, calculated from the Arrhenius equation (Table 4). The Arrhenius equation was not suitable for the prediction of shelf life of AmB, since the percentages of AmB remaining in liposomes were above 90%, after 90 days storage at 4 and 30 °C. In addition, the correlations of Arrhenius equation for all formulations were relatively low ($r^2 = 0.61$). As a result, the predicted shelf lives for the 1:1AmB, 7:2AmB and 7:2:1(−)AmB liposomes (at 30 °C) were less than 40 days (Table 5), whereas those observed from the experiment were approximately 90 days. The discrepancies in the predicted and observed degradation rate constants of AmB (Table 4) might be due to the facts that the Arrhenius equation should be applied for the kinetic data which exhibited the drug loss more than 50% (Rukvatin, 1995), and the Higuchi model was not the best equation to characterize the degradation kinetics of AmB in liposome formulations ($r^2 = 0.76$).

In consideration of the observed and predicted degradation rate constants (Table 4) as well as the kinetic profiles of AmB (Fig. 1), the 7:2:1(+)AmB liposome was shown to be the most stable formulation at 4 and 30 °C. However, it was less stable than the 7:2AmB and 7:2:1(−)AmB liposomes at 45 °C (Table 4). All liposome formulations with the entrapped AmB exhibited shelf lives of not less than 90 days, when stored at temperature below 30 °C and protected from light (Table 5). At high storage temperature (>30 °C), the degradation of AmB in liposome formulations was accelerated (Table 5). FGZ solution was less stable than FGZ powder, however, AmB in all liposome formulations were more stable and exhibited longer shelf lives than FGZ powder, when stored at temperature below 30 °C and protected from light (Tables 4 and 5). Thus, the entrapment of AmB in liposomes especially 7:2:1(+) formulation can protect the drug from decomposition and should be a better formulation than the free AmB in solution and powder forms. We have previously found that the

7:2:1(+)AmB liposome exhibited the highest thermal stability (ΔH) over other formulations.

AmB in FGZ solution was absorbed in SC and VED, in greater amount than other formulations (Table 6). Sodium deoxycholate present in this sample may act as a penetration enhancer, via the formation of micelles which facilitate the partition of drug into the skin. AmB in FGZ was in solution and thus, better transported as small molecules (Table 6).

AmB in all liposome formulations, especially the charged liposomes exhibited high absorption through the rat skin, but still lower than AmB in FGZ solution (Table 6). Therefore, the absorption of AmB was enhanced when it was entrapped in liposomes. The absorption of AmB entrapped in charged liposomes was higher than that in the uncharged 1:1AmB and 7:2AmB liposomes, for about 10-fold in SC and 5–10 times in VED (Table 6). The positive liposome {7:2:1(+)AmB} appeared to demonstrate higher absorption of AmB through SC than the negatively charged liposome {7:2:1(−)AmB} (Table 6). This may be due to the fact that the cell surface of the skin bears a net of negative charge (Yu and Liao, 1996). However, the 7:2:1(−)AmB liposome exhibited higher absorption of AmB through VED than the positively charged liposome (Table 6), due to its small particle size (0.115 μm) which may assist the deep penetration and distribution of the drug. The respective absorptions of AmB in various liposome formulations through SC decreased in the following order: 7:2:1(+)AmB ≥ 7:2:1(−)AmB > 1:1AmB ≥ 7:2AmB (Table 6).

The absorption of AmB entrapped in liposomes through SC can be explained by the size of liposomes. The intercellular space between corneocytes was about 0.1 μm (Roberts and Walters, 1998). Thus, the liposome particles with size smaller than 0.1 μm can easily diffuse through the skin via these pores. Nevertheless, the liposome particles with larger size than the gap can also be absorbed, since the elasticity of liposomal membrane allows the extrusion of liposomes through this gap. The 1:1AmB, 7:2AmB and 7:2:1(−)AmB liposomes with the respective sizes of 0.154, 0.154 and 0.115 μm still can be found in SC and VED (Tables 2 and 6). The smaller size of liposome {7:2:1(−)AmB} exhibited higher absorption of AmB than the larger size of 1:1AmB and 7:2AmB formulations (Table 6). However, the {7:2:1(+)AmB} liposome with larger particle size (0.364 μm) still demonstrated high absorption through SC (Table 6), which might be associated with charge effect. The positive charges on the surface of this formulation can bind to the negative charges of the skin, thereby enhancing the penetration of liposomal particles into the skin. The possible absorption mechanisms of AmB in the positively charged liposome might be the adsorption on the skin, followed by the penetration of smaller vesicles through the space between corneocytes, the disruption of multilamellar liposome vesicles resulting in the penetration of entrapped drug into the skin, and the loss of outer bilayers during the penetration of multilamellar liposomes (Mezei, 1994).

In conclusion, the AmB when entrapped in liposomes appeared to be more stable than the free AmB in solution and powder forms, when stored at low temperature (<30 °C) and protected from light. FGZ solution showed higher absorption of AmB than the drug entrapped in liposomes. However, FGZ solution was less stable than the liposome formulations. The positively and negatively charged liposomes exhibited greater absorption of AmB than the neutral liposome. The positively charged liposome demonstrated high flux in SC (58 ng/cm^2/h), while the highest flux in VED (23 ng/cm^2/h) was demonstrated by the negative liposome. The 7:2:1(+)AmB liposome also showed sustained skin absorption to some extent. Thus, the positively charged 7:2:1(+)AmB liposome might be the best formulation due to its high stability and deep penetration into the skin. Our previous study demonstrated that this liposome exhibited high percentage of entrapment of AmB (90%). It is of considerable importance to further develop this liposome formulation in the pharmaceutical dosage forms, such as topical and parenteral products.

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