CHAPTER 4
DISCUSSION

4.1 Physical properties of the prepared liposome formulations

4.1.1 pH measurement

The narrow variation of the pH range of all eight liposome formulations were observed since the eight liposome formulations were dispersed in phosphate buffer (pH 7.4 ± 0.1) with the pH's between 7.48 to 7.54 (Table 3.2). Thus, it appeared to be advantageous from using buffer as continuous medium for the liposome formulations.

4.1.2 Charges

Charge characterization of the eight liposome formulations revealed that 1:1, 7:2, 7:2:1 (-), 1:1AmB, 7:2AmB and 7:2:1(-)AmB liposome formulations were negatively charged. The 7:2:1(+) and 7:2:1(+)AmB formulations were positively charged with some particles showed negatively charged. The negatively charged presented in the liposome formulations even without the composition of the negatively charged lipid may be due to the free fatty acid from the partial decomposition of lipids at high temperature (80°C) during the process of liposome preparation (Talsma *et al*, 1989). However, in comparing zeta potential values (Table 3.3 and Figure 3.3), the 7:2:1(-) and 7:2:1(-)AmB formulations had higher surface negatively charged density than other liposome formulations since these liposome formulations contained negatively charged lipid (dicetyl phosphate).

The entrapped AmB liposome formulations had a tendency of higher zeta potential than the unentrapped AmB liposome formulations. Thus, low coaggulation should be presented. However, the entrapped AmB liposome formulations showed the more effect of coaggulation or sedimentation than the unentrapped AmB liposome formulations because the sodium ion in Fungizone® that may accelerate coaggulation by decreasing the electrical double layers of the particles (Müller, 1991). This zeta potential appeared to be due to the dilution effect of the samples that may mask the effects of the sodium ion. It was essential to

dilute the sample before the measurement since the concentrated buffer may give high conductivity that can deviate the particle movement.

4.1.3 Sizes

The size determination of eight liposome formulations by SEM showed an unnormal size distribution. Thus, the mode was used in stead of mean for size determination by SEM. The mode of sizes of eight liposome formulations (Table 3.12) were in the vicinity of 0.115 μm to 0.154 μm, except for the 1:1 (0.231μm), the 7:2:1(-) (0.280 μm) and the 7:2:1(+)AmB (0.364 μm). The 7:2:1(-) AmB formulation showed the smallest size (0.115 μm).

The size distribution of the eight liposome formulations was in the range of 0.115 to 0.364 μm (Table 3.12) and the distribution curves of liposomes without the entrapped AmB demonstrated more distribution than the liposomes with the entrapped AmB (Figure 3.12 and 3.13). The entrapment of AmB in liposome gave the smaller and the more uniform size than the liposomes without the entrapped AmB. This indicated the better liposome formulation with the presence of AmB may be the transition temperature of AmB in the form of Fungizone® (93 to 164°C) was in the between of HSC (75°C) and CHL (148°C) (Figure 3.16 to 3.18) thus the lipid bilayers can be formed easier in the process of hydration from the lipids with AmB than the lipids alone.

4.1.4 Lamellarity

The lamellarity determination of the 7:2AmB liposome formulations by TEM showing more than 15 layers was obviously seen. However, lamellartity of other liposome formulations were not presented since suitable sample preparation technique could not obtain before testing by TEM. Also, this technique had low potential to get the liposome particle on the grid. Nevertheless, the existing of all liposome particles was already proved by SEM.

4.1.5 Physical appearances

The physical appearance of four liposome formulations without the entrapped AmB (1:1, 7:2, 7:2:1(+), 7:2:1(-)) gave the translucent white dispersion while the other four liposome formulations with the entrapped AmB (1:1AmB, 7:2AmB, 7:2:1(+)AmB, 7:2:1(-)AmB) showed the translucent yellow dispersion (table 3.1). The translucent appearance of the formulation indicated the light scattering property of the liposomal colloid.

One day after the preparation kept in the refrigerator at 4±1°C, the sediment at the bottom of the containers was observed especially for the 1:1, 7:2, 1:1AmB, 7:2AmB, 7:2:1(+) AmB and 7:2:1(-)AmB liposome formulations but not seen in 7:2:1(+) and 7:2:1(-) formulations.

The size and sedimentation do not appeared to be related, especially in the case of blank liposomes. The 7:2 and 7:2:1(+) formulations had an equal size of 0.143 μm whereas the 7:2:1(-) had the larger size of 0.280 μm. However, the sediment appeared in the 7:2 but not in the 7:2:1(+) and 7:2:1(-) formulations. Thus, charges and the surface charge density had effects on the sedimentation than the size. Moreover, the charged or highly surface charged density particles tended to be more dispersibility than the uncharged ones. In the case of liposomes with the entrapped AmB which showed sedimentation were already described in 4.1.2.

4.1.6 The DSC study

From the DSC curves, eight formulations of the lyophilized liposomal powder gave two peaks of each formulation between 30°C to 250°C (Figures 3.19 to 3.22). The transition temperature (T_c) and the enthalpy of transition (ΔH) were evaluated and showed in Tables 3.17 and 3.18. But, for the ΔH of the second peak of 1:1 and 1:1AmB formulations, they can not be obtained for accurate values because decomposition occurred.

The DSC curves of cholesterol, dicetyl phosphate and stearylamine showed single endothermic peaks at 148, 69 and 46 °C respectively. For the hydrogenated soya phosphatidyl choline (Emulmetik 950®), its DSC curves showed two endotermic peaks at 61 and 76 °C. The first small peak was the pretransition and the second peak was the main transition (Cevc, 1993).

The AmB powder from Fluka BioChemika Co. demonstrated gradually decomposition above 135°C (Figure 3.15) whereas AmB in the form of Fungizone® did not show decomposition and gave two endothermic peaks (Figure 3.16). This may be due to the effects from micelle formation of sodium deoxycholate present in Fungizone® thereby protecting the drug from decomposition. The first peak may indicated the melting of sodium deoxycholate and the second peak may indicated the interaction of the AmB and the micelles.

The two endothermic peaks occurred in all liposome formulations with and without the entrapped AmB. The first peak demonstrated the transition of the gel phase to the liquid phase of the lipid mixtures while the second peak expressed the physical interaction between the lipid compositions. For the comparison of T_c's between all liposome formulations and the free drug (Fungizone®) in the first peak, the neutral liposomes (1:1AmB, 7:2AmB) and the positively charged 7:2:1(+)AmB showed higer T_c of about 44 and 15 °C than the free drug but with lower T_c of about 10°C than the free drugs in the negatively charged 7:2:1(-) AmB formulation. This may indicate the increase stability of the drug in neutral and positive liposomes than in the negative liposomes. The ΔH 's values of all liposome formulations with and without the entrapped AmB showed lower enthalpy changes than the free drug, except for the positively charged system which gave higher ΔH of about 260 and 75 J/g than the free drug in 7:2:1(+) and 7:2:1(+)AmB formulations respectively.

For the comparison of ΔH and T_c's of the liposome formulations with and without the entrapped AmB, the T_c of the first and the second peak of liposome formulations were in the range of 131 to 152°C and 200 to 215°C respectively with the exception for the first peak of 7:2:1(+)AmB and 7:2:1(-)AmB which were at 108.7°C and 84.9°C respectively. The ΔH of the first and the second peak of liposomes with and without the entrapped AmB were in the range of 119 to 250 J/g and 24 to 90 J/g respectively for the 7:2AmB which was higher than 7:2 and no difference in the case of 1:1 and 1:1AmB. For the first peak, the ΔH of blank 7:2:1(+) was higher than other liposome formulations whereas the entrapped 7:2:1(+)AmB gave lower ΔH than the blank 7:2:1(+), but still higher than other liposome formulations, for the second peak, the 7:2:1(-)AmB showed higher ΔH than other formulations while the blank 7:2:1(-) formulations gave similar ΔH to other liposome formulations.

The neutral 1:1 formulation gave no difference in T_c and ΔH of the with and without the entrapped AmB. This indicated no interaction between the entrapped drug and the lipid membrane. However, with the increase HSC in the formulation in the cases of 7:2 and 7:2AmB, although they gave similar T_c to the neutral 1:1 systems, their ΔH's were higher since some interaction may occur especially with the entrapped AmB (7:2(+)AmB). The -OH groups in AmB may play an important role in this interaction.

Thus, the T_c and ΔH values of the charged liposome formulations were different from the uncharged liposome formulations. The high ΔH in the first peak of 7:2:1(+) formulations demonstrated strong binding between the positively charged lipid (stearylamine) and the negatively charged free fatty acid of the HSC (please see the topic of charges) in the lipid bilayers. However, the 7:2:1(+)AmB formulations gave lower ΔH and T_c. This was due to the effect of the zwitterion of AmB which may enhance the aggregation of AmB with the positively charges in the lipid bilayer thereby causing the heterogeneity (lowering of the ΔH) of the lipid bilayer. This also caused the enhancement of the phase separation (lowering of the T_c) (New, 1990).

For the decrease T_c in the first peak and increase ΔH in the second peak of the 7:2:1 (-)AmB formulations, this may be affected by the zwitterion property of AmB as well as the positive charge from sodium salt in Fungizone® and the negatively charged lipid (dicetyl phosphate) in liposomes enhanced the aggregation. Therefore, the phase separation and the lower T_c were resulted. The high ΔH of the second peak in this liposome system may be due to the interaction between the positive sodium ion and the negative charge of the liposomes.

Thus, when the temperature was increased, the charged liposome with the entrapped AmB showed phase separation more than the uncharged liposomes due to the charged interaction of the lipid bilayer with the drug. The T_c and ΔH values can not only be used to predict the interaction of the entrapped drug with the lipid membrane but also to the stability of the liposome system as well. The stability of the liposome systems estimated in the orders of high to low was 1:1~ 7:2 ~ 1:1AmB ~ 7:2AmB, 7:2:1(+)AmB, 7:2:1(-)AmB when arranged from T_c of the first peak, and was 7:2:1(+), 7:2:1(+)AmB, 7:2AmB, 7:2:1(-)AmB, 7:2:1(-) ~ 7:2, 1:1 ~ 1:1AmB when arranged from ΔH. These sequence arranged from the consideration of transition temperature seemed to be more reasonable than the enthalpy of transition since higher T_c showed more influence on thermal stability than the ΔH values.

4.2 Quanlitative and quantitative analysis of amphotericin B, Fungizone® and the drug in liposome formulations by HPLC

From the determination of UV absorption of AmB by the scanning wavelengths from 200 to 500 nm, the UV absorption of AmB in the forms of amphotericin B reference standard, AmB in the drug mixture (Fungizone®) and AmB in lipid mixture's (as liposomes) gave the

same absorption peaks at four wavelengths of 406, 382, 363,and 345 nm (Asher *et al.*, 1977). However, the lipid mixtures in the form of liposomes without the entrapped AmB did not show absorption at the wavelengths more than 300 nm (Figures 3.23 and 3.24). Thus, the analysis of AmB by UV absorption in the range of 300 to 500 nm of AmB in liposome formulations did not interfere by the lipid mixtures or liposomes.

Nevertheless, HPLC was used to assay AmB instead of a spectrophotometer because of the high resolution with high sensitivity especially in the case of very low concentration of AmB. The reverse phase column of ODS-Hypersil C_{18}, 5 μm, 250 x 4 mm, the mobile phase (acetonitrile and 2.5 mM disodium edetate, 45:55 v/v), the 1 ml/min of pumping rate and the UV detection at 382 nm were the selected conditions of the HPLC which gave the high selectivity for all forms of AmB's peak at retention time of 4.6 mins (Wang *et al*, 1992).

The standard curve was constructed for the quantitative analysis of AmB contents in Fungizone®, the percentage of entrapment in liposomes, the remaining drug at different temperatures and time intervals of the stability test and the transdermal absorption study. The standard curve gave linearity at the concentrations between 0.02 to 1.50 μg/ml with the coefficient of variation not more than 5% (Table 3.20). This showed good correlation coefficient of the data (r^2 = 0.9999) (Figure 3.26). The equation of the standard curve was as the following :

$$y = 354.34x - 8.01$$

where : x = concentration of AmB (μg/ml)

y = peak area of AmB (mAU*s)

The contents of AmB in Fungizone® was investigated and showed the amount of 42± 3.7% which was close to the labelled amount of 45%. The little difference of AmB in this case may be due to the degradation of the drug during the transportation and storage, if the drug is exposed to high temperature of over 4°C. It has been recommended the storage of Fungizone® in the refrigerator.

4.3 The percentages of the entrapment of amphotericin B in liposomes

The percentages of the entrapment of AmB in liposomes was determined and calculated from the total amount of AmB in the formulation, the entrapped AmB in the pellets and the unentrapped AmB in the supernatant of liposomes assayed by HPLC. The chromatograms of the liposomes with the entrapped AmB of the 1:1AmB, 7:2AmB, 7:2:1(+) AmB and 7:2:1(-)AmB liposome formulations both the total and the pellet showed the exact retention time at 4.6 mins. But, the supernatant of all formulations gave the shift retention time to 4.0 mins (Figure 3.27). This may be due to the effect of insufficient dilution with methanol of the liposome formulations. The buffer in the formulation may increase the polarity of the nonpolar condition of the column (C_{18}) resulting the shorter retention time of the drug since the drug was a non polar compound. However, this retention time was still used in the experiment because if the samples were too diluted, the AmB amount was too small and an accurate result could not be obtained. There was also no interference of the blank liposome on AmB analysis by HPLC (Figure 3.28).

The percentages of the entrapment of AmB in the 1:1AmB, 7:2AmB, 7:2:1(+)AmB and 7:2:1(-)AmB liposome formulations showed similar values of more than 85%. The highest percentages of entrapment of AmB in liposome was observed in the 7:2:1(+)AmB formulation because of the larger size of the 7:2:1(+)AmB formulation while gave the higher volume of entrapment. The 7:2:1(-)AmB gave the similar percentages of the entrapment to the 7:2AmB. Thus, the charges did not show significance effects on the percentages of the entrapment in liposome formulations. The percentages of the entrapment calculated from the entrapped AmB gave the coefficient of variation of 1:1AmB, 7:2AmB, 7:2:1(+)AmB and 7:2:1(-)AmB in the range of 2 to 7% (Table 3.38), whereas those calculated from the free AmB in the supernatant showed high variation of 7:2AmB, 7:2:1(+)AmB and 7:2:1(-)AmB of about 20 to 56% and of 1:1AmB of about 107%. Thus, the percentages of the entrapment obtained from the free AmB in the supernatant were not used since they appeared not to be reliable.

The loading of AmB in liposomes (Table 3.39) gave the values in the ranges of 32 to 36 μg of AmB per mg of lipid. This value will be important for the calculation of doses of AmB in liposome formulations.

4.4 The stability study of various liposome formulations, Fungizone® solution and Fungizone® powder

The stability study of liposomes with the entrapped AmB, Fungizone® solution and Fungizone® powder were observed physically and chemically. In Table 3.40, the change in color and sedimentation as well as the increase of turbidity of the supernatant were observed at accelerated temperature (45°C) in 90 days of the 1:1, 1:1AmB, 7:2AmB and 7:2:1(-)AmB while the 7:2:1(+) and Fungizone®solution gave only the increase in turbidity (Figure 3.31). The 7:2:1(+)AmB formulation indicated the pale color of the sediment and the 7:2, 7:2:1(-) did not have any changes. Fungizone®powder gave more intense yellowish color (Figure 3.31).

The disappearance of the sediment may be due to the accelerated high temperature that dispersed or made a partial transition of the sediment thereby affecting the increase in turbidity of the supernatant. The other reason of the increase of turbidity of supernatant, especially in the case of liposomes with the entrapped AmB, was the decomposition of Fungizone®. This was confirmed by the change of color of the Fungizone®solution.

Temperatures at 4°C and 45°C were controlled in a refrigerator and an incubator respectively, but at 30°C all samples were kept in a room. Therefore, the 1:1, 7:2:1(-)AmB, Fungizone®solution and Fungizone®powder at 30°C showed more variation of changes than at others controlled temperatures. Also, the water bath which was placed near the test samples of the 1:1, 7:2:1(-)AmB liposomes, Fungizone® solution and Fungizone® powder may also affect the stability of the drug and caused the variation (Figure 3.29 to 3.30). This observation was also used for further discussion of chemical stability of these formulations.

The chemical stability of all test samples was observed and the curve profile in Figure 3.32 and the information in Table 3.47 were obtained. The two terms of degradation rate and shelf life were determined. The degradation rate was calculated by fitting the data to the zero, the first order and the Higuchi model (Table 3.48). The correlation coefficient from the Higuchi model showed higher relation than the zero and the first order. Thus, the degradation rate from the Higuchi model was used to predict the shelf life interpreted by the Arrhenius equation. The degradation rate was compared between the value from the experiment and the value from the Arrhenius equation as presented in Table 3.55. The predicted shelf life values at different temperature was shown in Table 3.56.

The Arrhenius equation appeared not to be suitable for the prediction of the shelf life of AmB in this experiment since the percentages of the remaining drug in liposomes with the entrapped AmB were more than 90% at 4°C and 30°C after 90 days and also the correlation coefficient of the Arrhenius equation of each formulation was quite low.

As a result, the predicted calculated shelf life for the 1:1AmB, 7:2AmB and 7:2:1(-) AmB was less than 40 days at 30°C whereas that obtained from the experiment was about 90 days. Also, for the 7:2:1(+)AmB, it had the predicted shelf life of 101 days at 45°C whereas from the experiment was less than 5 days. This error of the predicted shelf life was mainly due to the fact that the application of the Arrhenius equation should be used in the data that had the drug loss more than 50% (Rukvatin, 1995) and the Higuchi model was not the best fit for this degradation kinetic of liposome formulation.

In consideration of the degradation rates (Table 3.55) and profile curve (Figure 3.32 and Table 3.47), the 7:2:1(+)AmB was the most stable formulation at 4°C and 30°C. However, it showed less stability than the 7:2AmB and 7:2:1(-)AmB at 45°C. The 7:2AmB and 7:2:1(-) AmB were less stable than the 7:2:1(+)AmB and 1:1 AmB at 4°C and 30°C. But, they were more stability was than 7:2:1(+)AmB and 1:1 AmB at 45°C. The 1:1AmB was less stable than the 7:2:1(+)AmB at 4°C and 30°C but it was at 45°C less stable than 7:2AmB, 7:2:1(+)AmB and 7:2:1(-)AmB. At 4 and 30°C, the order of high to low stability was 7:2:1(+)AmB, 1:1 AmB, 7:2AmB and 7:2:1(-)AmB. At 45°C, the order of high to low stability was 7:2AmB, 7:2:1(-)AmB, 7:2:1(+)AmB and 1:1 AmB.

This can be concluded that the liposome formulation with the entrapped AmB had the shelf life of not less than 90 days when kept at temperature not more than 30°C and protected from light. At the temperature more than 30°C, the AmB liposome formulations tended to be accelerated for degradation. The Fungizone®solution was less stable than Fungizone® powder, however AmB in liposome formulations were more stable than Fungizone®solution and Fungizone®powder when kept at temperature not more than 30°C and protected from light. Thus, the entrapment of AmB in liposomes especially in the 7:2:1(+) formulation can protect the drug from decomposition and should be a better formulation than the free drug in solution or in powder since better physical and chemical stability were obtained.

4.5 The transdermal absorption of amphotericin B liposome formulations through the full-thickness rat skin, by the vertical Franz diffusion cells

All related control samples were first validated by HPLC analysis. These samples included the 50:50 v/v of ethanol/water solution in the receiver chamber, viable epidermis and dermis, stratum corneum stripped in tapes, tapes in methanol, deionized water, phosphate buffer (pH 7.4) and 1:9 v/v of DMSO/methanol solution. The HPLC chromatogram of these samples were demonstrated in Figures 3.40 to 3.46. There were no peaks at retention time between 4 to 5 mins where the peak of AmB appeared. The 1:1, 7:2, 7:2:1(+) and 7:2:1(-) liposomes showed no peak at the retention time of 4 to 5 mins as already presented in Figures 3.28 in the entrapment topic. For the control systems when loaded in the donor chamber, deionized water, DMSO/methanol solution, phosphate buffer (pH 7.4), and the 1:1, 7:2, 7:2:1 (+) and 7:2:1(-) blank liposomes, samples from the stratum corneum, viable epidermis and dermis and the receiver chamber gave no peak at the retention time at 4 to 5 mins. Thus, no interference on the assay by HPLC of AmB in using these control samples.

From the transdermal absorption study, the amount (μg) and flux (ng/cm^2 per hr) of AmB of different formulations in different strata of the rat skin were shown in Tables 3.58 to 3.59 and Figure 3.47. For the mass balance of AmB, it found that the amount at initial was more than the sum of the amount in different strata and that in the receiver medium of about 20% in the case of Fungizone® solution, AmB in DMSO/methanol solution, 1:1 AmB, 7:2AmB, 7:2:1(+)AmB and 7:2:1(-)AmB. But, AmB in phosphate buffer (pH 7.4), it was 54%. The loss of AmB may be resulted from the loss during the process of rinsing since 200 ml of the rinsed water was discarded. Also, the instability of AmB occurred when AmB exposed to 37°C for 24 hrs. The sample of AmB in phosphate buffer (pH 7.4) gave the highest loss because of the low solubility of AmB which was dispersed in particles in the buffer causing high loss when swung in water.

From the preliminary study, the amount of AmB in different strata of the rat skin and the receiver medium at time intervals before 24 hrs was very low and the AmB peak can not be detected even when assayed by HPLC. Thus, no sampling before 24 hrs. was performed in order to let the accumulation of AmB in the skin and the receiver chamber so that and quantitative analysis of the amount of AmB can be accurately determined.

The results from the transdermal absorption study were compared as in Table 3.59 and Figure 3.47. The highest AmB contents from all formulations was found in stratum

corneum. Every formulation, except for the AmB in DMSO/methanol solution, AmB can not penetrated through the skin since AmB in the receiver chamber was not found. The Fungizone® solution sample was absorbed in the stratum corneum, viable epidermis and dermis more than other formulations because of sodium deoxycholate presented in the sample which may act as an enhancer. Sodium deoxycholate can form micelles which can facilitate the drug to be partition to the rat skin. Also, AmB in Fungizone® was in solution and can have better transportation as small molecules while AmB dispersed as particles in phosphate buffer (pH 7.4) was slightly absorbed in stratum corneum and can not penetrated to viable epidermis and dermis. AmB in all liposome formulations especially the charged liposomes showed higher absorption through the skin than the free AmB in phosphate buffer (pH 7.4) but less than AmB in Fungizone® solution. Therefore, this indicated the absorption enhancement of AmB through the rat skin when AmB was entrapped in liposomes. In comparing different liposome formulations, the AmB in charged liposome formulation was absorbed higher than the uncharged 1:1AmB and 7:2AmB for about 10 folds in stratum corneum and 5 to 10 folds in viable epidermis and dermis. The positively charged liposome (7:2:1(+)AmB) appeared to give high absorption of AmB in stratum corneum than the negatively charged (7:2:1(-)AmB) liposome. This may be due to the fact that at physiological pH, the cell surface bears a net negative charge (Yu and Liao, 1996). The positively charged liposomes can have better adsorption on the rat skin. However, the negatively charged liposome (7:2:1(-)AmB) showed higher absorption of AmB in viable epidermis and dermis than the positively charged liposome (7:2:1(+)AmB because of the smaller particle size of the negatively charged than the positively charged liposome that can assist the deeper penetration and distribution of the drug. The order of high to low absorption of the liposome formulations were 7:2:1(+)AmB ~ 7:2:1(-)AmB) and 1:1AmB ~ 7:2AmB.

The result of the absorption of AmB entrapped in these liposome formulations through the SC can be explained by the size of liposomes. The gap between corneocytes has been found to be about 0.1 μm (Robert and Walter, 1998). Thus, the liposome particles with size smaller than 0.1 μm can easily pass through the skin by this pores. Nevertheless, the liposome particles with larger size than the gap can also be absorbed through the skin since the elasticity of liposomal membrane can extrude through this gap. In case of the 1:1AmB, 7:2AmB and 7:2:1(-)AmB formulations, their sizes were 0.154, 0.154 and 0.115 μm

respectively which were larger than 0.1μm and still can be found in the skin. Thus, the smaller size of (7:2:1(-)AmB) showed higher absorption than the 1:1AmB and 7:2AmB formulations. The absorption mechanism of the 1:1AmB, 7:2AmB formulation may be the extrusion through the gap. However, although the size of 7:2:1(+)AmB formulation was 0.364 μm, the absorption in SC was still the highest. This may be the effect from charges. The positive charges of this formulation can bind to the negative charges of the skin thereby enhancing the absorption of liposomal particles through the skin. In fact, there are 3 possible events of liposomal absorption proposed by Mezei in 1994 which are :

1. The penetration of the smaller vesicles through the pore between corneocytes
2. The disruption of the multilamellar liposome vesicle and the entrapped contents are penetrated into the skin.
3. The loss of the outer bilayers during penetration of the multilamellar liposome

Thus, the possible absorption mechanism of our positively charged AmB liposomes should be first the adsorption on the skin and followed by the above proposed events.