CHAPTER 5
CONCLUSION

Liposomes with the entrapped AmB were prepared by the chloroform-film method with sonication in the ratio of 0.05 mg of AmB per mg of the lipid mixture. The molar ratios of liposome compositions were hydrogenated soya phosphatidylcholine / cholesterol / charged lipid = 1:1, 7:2, 7:2:1(+) and 7:2:1(-). The obtained liposome was demonstrated as multilamellar vesicle (MLV) by TEM with particle sizes in the range of 0.115 to 0.364 μm investigated by SEM. The qualitative and quantitative analysis technique used for AmB was the high performance liquid chromatography (HPLC) with UV detection absorption at 382 nm. This technique indicated high sensitivity and high selectivity. The percentages of the entrapment of AmB was more than 85% with the highest percentage of 90% were found in the 7:2:1(+)AmB liposome formulation. This was not marked different from that from the report of Lopez-Berestein et al. (1987).

The physical notation of the deterioration of the liposome formulation can be observed showing the paler color or the increase of turbidity when compared with the freshly prepared formulation. Besides physical observation, the differential scanning calorimeter (DSC) was also studied for the prediction and comparison of thermal stability of the liposome formulations. The 7:2:1(+)AmB formulation seemed to demonstrated higher stability than other formulations because the highest ΔH was observed. The chemical stability of all samples was confirmed by a HPLC quantitative analysis of the amount of AmB remaining at 0, 5, 20, 40 and 90 days when kept at 4±1°C, 30±1°C and 45±1°C. The AmB when entrapped in liposomes appeared to be more stable than the free drug in solution or powder forms of Fungizone®. The degradation rates of AmB in all formulations were best fitted to the Higuchi model and the shelf lives predicted by the Arrhenius equation. The 7:2:1(+)AmB formulation showed the highest stability when kept at the temperature not more than 30°C and protected from light since lower degradation rate and lower shelf life than other liposome formulations were obtained.

Liposomes with the entrapped AmB facilitated the absorption of AmB through the epidermis and dermis. Although AmB in Fungizone®solution and AmB in DMSO/methanol solution gave higher absorption than the drug entrapped in liposomes but Fungizone® solution was less stable than the drug entrapped in liposomes. AmB in DMSO/methanol can also irritate the skin by the solvent effect of DMSO. The positively and negatively charged liposomes enhanced the absorption of AmB more than other liposome formulations. The positively charged liposome gave high AmB absorption in the stratum corneum whereas negatively charged liposome demonstrated high AmB absorption in the viable epidermis and dermis. The positive 7:2:1(+)AmB also showed sustained effect in some extents.

The best formulation was concluded to be the positively charged 7:2:1(+)AmB formulation which showed the highest percentage of entrapment, stability (at not more than 30°C and protected form light) and absorption with some sustained effect. For 7:2AmB, even it showed a low absorption through the skin, it demonstrated better stability at high temperature (over 30°C) than other liposome formulations. Thus, this formulation may be useful to be develop to other types of preparation beside topical preparations, such as the parenteral products.

Therefore, the results from this study have firmly demonstrated that AmB was advantageous when entrapped in liposomes. It will be challenging and interesting for the future development by incorporating these liposome systems in the preparations such as topical or parenteral products in order to promote the efficacy of an *in vivo* study and the patient with candida skin infection as well.