CHAPTER V
CONCLUSIONS

1. Formulation of solid lipid nanoparticles (SLN)

1.1 Preparation of drug-free SLN

1. The production parameters optimized for drug-free SLN could be transfered to drug-loaded systems, thus facilitated formulation development.

2. The WME method had limitation of types of stabilizer while several parenterally approved stabilizers could be used in HPH method.

1.2 Formulation of AmB loaded SLN

1. GB-SLN displayed bimodal size distribution while GP-SLN showed monomodal size distribution due to the longer carbon chain of the former.

2. The best formulation of SLN dispersion prepared by HPH method consisted of 3% GP and 2% P407, while the best formulation of SLN formulation prepared by WME method consisted of 10% GP, 20% CreRH and 20% Gly.

1.3 Preparation of freeze dried SLN products

1. The optimized combination of bulking agents was 7.5% mannitol and 5% sucrose which showed the good physical properties, moderate redispersion speed, and the optimum uniformity of Sf/Sc ratio.

2. However, the obtained average diameters of lyophilized products were mostly larger than 5 μm which was unsuitable and prohibited for i.v. injection possibly due to incomplete lyophilization process.

2. Formulation of AmB loaded NLC

1. The type of lipids affected the particle sizes similar to that of AmB-SLN preparations in which the formulations of GB gave larger particle than that of GP. However, they were in the nanometer range that was acceptable for i.v. therapy.

2. Reduction in zeta potential when oil was added to the system depended on the type of solid lipid which affected the recrystallization after preparation.

3. Determination of drug content

1. HPLC condition was employed for the quantitative determination of the amount of AmB from preparations with satisfactory validation results.

2. The drug content at initial of AmB-GP-NLC was lower than that of AmB-GB-NLC indicating the effect of types of lipid with different carbon chain length on the initial drug content.

4. Determination of entrapment efficiency

1. While the entrapment efficiencies of most AmB-SLN preparations were high, AmB-SLN stabilized by P407 had the lowest of entrapment efficiency but could be improved by NLC formulation for providing a higher surface area and higher accommodation probability for host molecules due to lower degree of crystallinity.

2. Lecithin in the SLN dispersions could increase the drug incorporation into the particles but less degree in entrapment was observed when the adding oil into system possibly due to the complex formation between drug and lecithin as mixed micelle or liposome which was undetected in the lipid pellet after ultracentrifugation.

5. Morphology of AmB formulations

1. The morphology of AmB-SLN with P407 was regular, spherical and uniform nanospheres with no agglomeration whereas the particles obtained by NLC had spots located on the spherical structure of particles which was attributed to the presence of liquid oil on the surface of GP particles.

2. The morphology of AmB-SLN-L showed tiny particles of lecithin within the particles and oil droplets predominantly appeared on the surface of particles in case of AmB-NLC-L which might affect the entrapment efficiency, *in vitro* release and also chemical stability.

6. Physical and chemical stability of AmB formulations

1. The AmB-SLN using either Tw80 or CreEL was physically and chemically unstable while the AmB-SLN stabilized by P407 showed good physical stability and the longest shelf-life.

2. The drug remaining in the AmB-SLN lyophilized preparations prepared by WME method was markedly higher than that from the colloidal dispersions. Contrast result from lyophilized products from HPH method that might be due to the incomplete of lyophilization process which described in term of mass and heat transfer.

3. The shelf-lives of AmB-SLN-L and AmB-NLC-L were less than 1 month. This meant the phospholipid or phospholipid with oil incorporated could not improve the drug remaining despite the drug prefer to intercalate or form complex with lecithin.

7. Physical Evaluations of various formulations

1. From NMR result, the oil molecules seemed to form clusters inside the particle and phospholipid could disturb the crystallinity of solid lipid.

2. From DSC and HSM investigations confirmed that the presence of oil or/and phospholipids affected the crystal lattice of the lipid, particularly in the combination of oil and phospholipid which had the strong effect to both melting behavior and degree of cystallinity.

3. It was demonstrated by IR spectra that there was no chemical reaction occurred between AmB and other components. The DSC, HSM, X-ray diffractograms showed that AmB in lipid matrix was in either molecularly dispersed or amorphous form except the formulation containing 5% drug loading.

4. The degree of aggregation of various AmB formulations was less than the commercial product, Fungizone® and they were ranked in the same order corresponding to type of surfactants which AmB-SLN containing M52 shown the least value.

8. Effect of drug loading in various formulations

1. The highest entrapment was observed from AmB loaded in SLN-L which used P407 and PL as stabilizers and all 2.5% AmB loaded formulations showed the highest entrapment efficiency.

2. Both 2.5% and 5% drug-loaded SLN-L and NLC-L formulations were less stable preparations. Moreover, the 5% drug loaded was too high and might be expeled from the particles into the water phase where the degradation occured and led to low chemical stability.

3. The presence of oil or/and phospholipid affected the crystal lattice of the lipid, particularly the combination of oil and phospholipid had a strong effect to both melting behavior and degree of cystallinity.

9. *In Vitro* drug release

1. Type of AmB lipid formulations and the drug loading had affect on the release profiles of AmB which described in term of aggregated from and the entrapment efficiency.

2. AmB loaded 5% in lipid formulations showed a biphasic release with the highest accumulation due to the large amount of the drug in the continuous phase could be freely passed through the membrane.

10. Biopharmaceutical characterizations of formulations

1. Various AmB lipid formulations had low hemolytic activity when compared with Fungizone® due to less aggregated form and slow drug release except the formulations stabilized by Tw20.

2. From the antifungal results in term of MIC and MFC, AmB lipid nanoparticles were equal and more effective than both AmB itself and Fungizone® indicating that low degree of aggregation of AmB from these preparations could improve antifungal activity.