CHAPTER V

CONCLUSIONS

From the result, GC-SLN could be prepared by solvent diffusion method and had mean particle size in nanometer size range over storage time while GB-SLN could not be prepared or showed physical instability after storage time. This might be the long chain of GB in which much difference between the melting point and room temperature could promote lipid recrystallization. The type and amounts of stabilizer and combination of stabilizers affected the physical characteristics of GC-SLN.

When using as single surfactant, tween 80 (5-15%) was the stabilizer that could produce stable white fluid dispersion even after storage for 3 months. Poloxamer 188 of 5-15 % could produce the GC-SLN but they became gel after storage time. Phospholipon 40 and Phospholipon 90 of 5-15 % could not stabilize GC-SLN over storage time. When using as combined surfactants, tween 80 with Phospholipon 40 or Phospholipon 90 showed good physical stability at the ratio of 8:2 and 12:3 whereas tween 80 with Poloxamer 188 as combined surfactants of 8:2 and 12:3 exhibited good physical appearance after storage at room temperature for 3 months. The data obtained indicated that type and concentration seem to be the crucial factor for producing stable autoclaved SLN. Poloxamer 188 could not form stable SLN after storage time. This surfactant provided no sufficient steric stabilization and gel formation occurred after storage. The reason might be the dehydration of propylene oxide portion. Upon using of Phospholipon 40 and Phospholipon 90, gel formation was found after storage time suggesting that lecithin had insufficient both steric and electro static repulsion. The appearance of GC-SLN prepared using tween 80 was white fluid dispersion.

When using as combined surfactants, at 10 % of combined surfactants, tween 80 with either Phospholipon 40, or Phospholipon 90 and tween 80 with Poloxamer 188, could stabilize GC-SLN and showed good physical stability after storage for 3 months assuming that dissolved surfactant with either Phospholipon 40, Phospholipon 90 are able to diffuse to the particle surface in a much shorter time and preventing close contact of the droplets and later particles.

For HPH method, the method consisted of two processes; preparing the pre-emulsion using high speed homogenizer and reducing the particle size by high pressure homogenizer. The condition was performed initially using homogenizeation time for 10 minutes, at 10000 psi and 5 cycles. When using as single surfactant, Poloxamer 188 could not stabilize both GC-SLN and GB-SLN. Gelation and precipitations occurred after storage at room temperature. This might be resulted from high temperature exposure during homogenization and autoclaving. Introduction of energy to the SLN systems accelerated particle growth and subsequently gelation. Phospholipon 40 or Phospholipon 90 could not stabilize of both GC-SLN and GB-SLN. As emulsifier, lecithin was easy to form vesicles to slow down the molecule movements and could not cover the naked new surface immediately.

Tween 80 alone also could not stabilize of both GC-SLN and GB-SLN. It was likely that high temperature produced high kinetic energy and might affect the layer of stabilizer. When using as combined surfactants, T-80:PL-40 (4:1) and T-80:PL-90 (4:1) in GB-SLN and GC-SLN could display good physical appearance. The combination of two emulsifiers produced mixed surfactant films at the interface thus having high surfactant coverage as well as sufficient viscosity to promote stability.

The pH of both methods was moderately acidic and osmolality of all preparations was obviously low. All preparations had low negative charge of zeta potential that was not sufficiently high enough to stabilize the dispersion solely by electrostatic repulsion force. However, stable dispersion could be obtained by the additional steric effect of single and combined surfactants.

For solvent diffusion method, AA loaded GC-SLN showed good physical stability after storage for 3 months while white precipitates occurred after storage of AS loaded GC-SLN. The particle size and zeta potential were lower than those of drug free SLN. This might be explained by improving of wetting characteristics and micellar solubilization. The pH of AA loaded GC-SLN and AS loaded GC-SLN were moderately acidic in range 3-4 and lower than drug free preparations. Incoporation of

both AA and AS did not affect the osmolality of GC-SLN due to very low amount of AA and AS that dissolve in dispersion medium.

For HPH method, AA loaded GC and GB-SLN could not be stabilized and exhibited white precipitates after storage at room temperature. Both AS loaded GC-SLN and GB-SLN could not be prepared. Hot emulsion containing AS became semisolid after standing at room temperature for both lipids. This might be that a glycoside part was rapidly hydrolyzed by hydronium ion. The sugar part might diffuse into the medium and formed gel structure.

The % release of AA from SLN was low. After 2 hours, the release was about 30 % from SLN containing T-80 with PL-40 and 20 % from SLN containing T-80 with PL-90 respectively. This result might be explained in term of smaller particle size of SLN containing T-80 with PL-40 than SLN containing T-80 with PL-90. Therefore, the smaller particle could rapidly pass through out the membrane faster than large particle. The low % release of both SLN formulations was due to the poor solubility of AA into medium.

The constant TEER value was measured for 3 times of 125±5 ohm*cm^2 for ECV-304 cell monolayer. After added AA loaded GC-SLN to upper compartment, the AA loaded GC-SLN was rapidly passed through the lower compartment within 5 minutes. This might be indicated the non integrity of ECV-304 monolayer. Measurement of cell viability, the in vitro in cell cultures revealed that AA loaded GC-SLN had toxicity. The 50 % cytotoxicity dose was 176.57μg/ml of T-80 with PL-40 and 166.55 μg/ml of T-80 with PL-90. This might due to the high concentration specific small area when AA loaded SLN attached and adhered on the cell membrane induce cell apoptosis. For cellular uptake, the results showed intensity of FITC in both formulations higher than FITC solutions without SLN formulation and no difference of FITC intensity in both formulatios. It might be that the added Phospholipids synergism with polysorbate 80 at the interface would further improve the surface properties.