CHAPTER V
DISCUSSION AND CONCLUSION

1. Evaluation of homogenization process

In this study, SLN could be prepared by hot melt homogenization method. This technique is composed of two processes: preparing the pre-emulsion by high speed homogenizer and reducing the particle size into nanometer size range by high pressure homogenizer. The homogenization processes were preliminary optimized before preparing SLN. The optimum condition using the less energy but producing the stable products was selected to prepare the colloidal dispersions for further studied. Fat emulsion containing 5% soybean oil and 2% poloxamer 407 was used as a model. It was found that the pre-emulsion could be prepared by the stirring speed of 4,080 rpm for 10 minutes. Shorter times resulted in the oil droplets on the surface of emulsion due to the insufficient kinetic energy introduced to the system. This caused the breaking of the emulsion before the particle size reduction by high pressure homogenization.

In the size reduction process, the transmittance determination was used to predict the particle size of emulsion droplets (Apte and Turco, 1992). This value was corresponding to the light intensity after absorption by the particles, by which higher transmittance was found in the dispersion containing smaller particle sizes (Ingle and Crouch, 1988). It was found that increasing the pressure and cycle of homogenization increased the transmittance. However, when using the high pressure of 12000 psi for 10 cycles the transmittance slightly decreased. This agreed with a previous study that high energy could reduce the particle size, but too high kinetic energy might be sufficient to overcome the stabilizing energy barrier of electrostatic repulsion, hence caused the droplet coalescence (Schwarz, Mehnert, Lucks et al., 1994). The optimum homogenization condition was 10000 psi for 5 cycles that produced the highest transmittance and used the least energy for the preliminary investigated emulsion. Althought this condition might not be the optimum condition for all other emulsion systems, it was used to prepare the SLN in all preparations in

order to compare the other parameters that affected their characteristics, i.e. the types and amounts of lipid, stabilizer, and drug.

From this result, the optimum condition for preparing the SLN was as followed: (i) dispersing the oily phase and aqueous phase by high speed homogenizer at 4,080 rpm, and 75°C for 10 minutes and (ii) reducing the emulsion sizes by high pressure homogenization at 10000 psi for 5 cycles.

2. Drug free SLN

2.1 Effect of stabilizers

The 5% tripalmitin SLNs were prepared using various stabilizers. These stabilizers formed condensed films around the droplets of the dispersed phase. The mechanism of stabilization was different for each stabilizer. Tween 80 is water soluble non-ionic surfactant. It could adsorb at the interface between hydrophobic oily phase and water, with the hydrocarbon moiety adhering to the hydrophobic surface and the polyethylene glycol moiety protruding into the water, where it was hydrated. After cooling down, the particle surface was thus surrounded by a thin layer of hydrated polyethylene glycol chains. This hydrophilic shell formed a steric barrier which prevented close contact between particles and, hence, coagulation. This phenomenon was steric stabilization (Schott, 1995). Poloxamer 407 also stabilized the colloidal dispersion by steric effect. It is polyoxyethylene-polyoxypropylene copolymer which could absorb at the surface of lyophobic colloid to be polymeric stabilization. The long chain of macromolecules from solution adsorbed onto the surface of droplets in the form of loops projecting into the aqueous phase rather than laying flat against the colloidal particles. Only a small portion of the chain segments of an adsorbed macromolecule was actually in contact with and adhered directly to the surface. Because of its great length, however, there were enough of such areas of contact to anchor the adsorbed macromolecule firmly onto droplets and formed a steric barrier between them that prevented the close interparticle approach necessary for coagulation (Burgess, 1990; Friberg, Goubran, and Kayali, 1990). The

commercial grade of egg lecithin used in this study contained phosphatidylcholine and the other degradation products which were apparently negatively charged. It could stabilize the colloidal dispersion by electrostatic repulsion. When adhering on the droplets, their surface layer tended to be of negative charge. Electrostatic repulsion would occur and prevent the particles from approaching closely enough to overcome the effective van der Waals force of the attractive forces, thus stabilizing the dispersion against interparticular attachments or coagulation (Schott, 1995).

Tween 80 produced the smallest nanoparticles before autoclaving, but increasing of the particle size after autoclaving occurred. Poloxamer 407 yielded the particle size of lower than 1 μm in both before and after autoclaving. Egg lecithin produced the particles in nanometer size range only when using at 4-5%. Possible explanations for these results before autoclaving were the amount of the macromolecules in dispersion medium that could diffuse to the interface between oil and water, their diffusion velocity, and the energy introduced to the systems. These stabilizers were macromolecules in aqueous phase, and could form micelles at their critical micelle concentrations (Westesen and Siekmann, 1996). Tween 80 is water soluble non-ionic surfactants, and poloxamer 407 is water soluble polymer. They could dissolve as free molecules in dispersion medium and form micelles at relatively high concentrations. Tween 80, the low molecular weight, had larger number of molecules than poloxamer 407, which had much higher molecular weight, at the same weight concentration. After addition of the oily phase and reduction by homogenization, the oily droplets could be broken to be nanoparticles, and then the surfactant macromolecules would diffuse to the surface of oily droplets and formed monolayer or multilayers in the case of high concentration of stabilizers. The diffusion velocity of the macromolecules in the low viscous aqueous phase was fast for poloxamer 407 and tween 80. Thus they could rapidly adhere to the lipid surface, and the particles were kept in the nanometer size range. Egg lecithin is an amphiphilic molecule, when using as stabilizer it could easily form aggregates after dispersing in water. Thus less amount of macromolecule units were found in dispersion medium. Moreover, it might rearrange into liquid crystals or cubic structures (Krog, 1990). Therefore the dispersion became viscous and retarded the diffusion velocity, resulted in slower adherent of macromolecules to the lipid surface

of dispersions. The aggregation or coalescence would occur to become microparticles. The particle sizes of SLN were higher than the other two stabilizers.

After autoclaving, higher temperature produced high kinetic energy and might affect the layer of stabilizer. Tween 80, which was a small molecule and showed higher hydrophilic part in the molecule, might diffuse to the aqueous medium and then rearrange to form new layers on the surface of droplets, which might form weak intermolecular bonding between tween 80 molecules that adhered to the oil droplets. This might cause aggregation and the larger particle size was obtained. Poloxamer 407 and egg lecithin were large molecules which could adhere to be the strong film on hydrophobic surfaces. High energy of autoclaving did not affect their films. Diffusion to the dispersion medium would be less or more difficult. Thus their films were not changeable and the obtained particles were in the same size range as before autoclaving.

The concentration of stabilizer markedly affected the size of SLN. For poloxamer 407, the critical concentration for marked size reduction was 2-3% which was similar to the results from a previous investigation by Seijo et al. (1990). This concentration was much higher than the critical micellar concentration of the surfactant agent. Thus, increasing the concentration of poloxamer 407 induced the formation of more micelles which led to the formation of smaller nanoparticles, provided the macromolecule concentration in dispersion medium remained constant. However, the size of SLN was very slightly decreased in higher concentrations of poloxamer 407.

Increasing the concentration of tween 80 concentration increased the viscosity of the preparations, especially those of 3% tween 80 showed the highest viscosity. This affected their particle sizes. High viscosity retarded the movement of surfactant layers around the droplet resulted by the higher energy of autoclaving, thus less destruction of the layers. Moreover, the movement of droplets to be aggregation was also difficult (Friberg et al., 1990). Therefore, increasing of particle sizes after autoclaving was then less than when using low concentration of the stabilizer.

In preparations containing egg lecithin, higher concentration of stabilizer exhibited smaller particle size. This might be due to the higher concentration had higher amount of macromolecules that could adhere more to the surface of the droplet. In addition, the particle sizes of preparations of 1-3% stabilizer decreased after autoclaving. This could explain that the higher energy from autoclaving disrupted the lecithin aggregates, accelerate the diffusion velocity and subsequently more macromolecules in the dispersion medium, thus increased the amount of macromolecules adhering on the surface of droplets which reduced their particle sizes.

This study found that the gel formation occurred in preparations containing 3% tween 80 and 3-5% egg lecithin. Westesen and Siekmann (1997) reported the possibility that the solidification of colloidal emulsified tripalmitin increased the particle surface area due to the change in overall particle shape, and in a sudden local lack of emulsifier on the particle surfaces. High concentration of stabilizers, the excess of stabilizers which formed small vesicles would diffuse to the newly created insufficiently stabilized particle surfaces. However, this diffusion velocity was slow resulting in an instability of the dispersed state and gelation via the lateral faces of the crystals. Moreover, the higher stabilizer macromolecules might form hexagonal structures, liquid crystals, or cubic structures, and the higher viscosity could be observed (Krog, 1990).

The pH and osmolality of SLN dispersions were affected by the stabilizers. It was found that preparations containing egg lecithin showed the lower pH values and higher osmolality than the other two stabilizers. The lower pH might be due to its degradation products which were acidic compounds, while poloxamer 407 and tween 80 did not affect the pH due to their neutral molecules. The higher osmolality of preparation containing egg lecithin might also be due to substances in its impurities that could soluble in water and increased the osmotic pressure. However, their osmolalities were lower than the isotonic solution. This finding was consistent with a previous study that the osmolality of a large molecular weight polymer in aqueous solution should be negligible (Viegas and Henry, 1998). The osmolality was osmotic pressure of substance that dissolved calculated by molecule

per kilogram of water. Poloxamer 407, which was a large molecular weight, showed the fewer molecules in water. It produced the least osmolality value.

The determination of the zeta potential of SLN dispersions provides useful information of the size and magnitude of the charge and its effect on the physical stability of the system with time. It was found that all preparations had the negative charge of zeta potential. It might be due to the negative dipole of oxygen atom on glyceride moiety of lipids. Increasing the concentration egg lecithin could increase the negative charge of particles due to the negative charge of its degradation products. However, these values were not sufficiently high enough to stabilize the dispersion solely by electrostatic repulsion. The zeta potentials of more than –40 millivolts are required for solely electrostatic stabilization as shown in Table f1 (Zeta meter system 3.0). While preparations containing poloxamer 407 showed no difference of zeta potential when increased the concentration due to its non-ionic property. These zeta potentials were lower than preparations containing egg lecithin. However, stable dispersions could be obtained due to the additional steric effect of poloxamer 407 (Schwarz, Mehnert, Lucks et al., 1994). Decreasing the zeta potential was only observed in tween 80 preparations. This finding was consistent with a previous study by Kayes (1977) that non-ionic surfactant, polyoxyethylene glycol monoether of hexadecanol, could decrease the zeta potential of four drugs. This behavior might be due to the adsorption of alkyl chain onto a hydrophobic portion of the surface as a hydrophobic effect and also the association of the ethylene oxide groups with some polar groups at the surface probably by hydrogen bonding. So the polar groups of particles were decreased. However, the negatively zeta potential of preparation of 1% tween 80 was higher than that of the other stabilizers. It might due to the accumulation of negative charges of aggregated particles.

These results showed that the preparation with suitable properties to be used in the parenteral administration was preparation of tripalmitin SLN containing 3% poloxamer 407. Its particle size was sufficiently small to be used intravenously. It also exhibited very low viscosity. In addition, the preparations using 2% tween 80 and 1-2% egg lecithin as stabilizers could be used in intramuscular and subcutaneous administrations due to their low viscosity.

2.2 Effect of Lipids

In this study, the SLN containing various lipids were prepared. About 3% poloxamer 407 was used as the stabilizer. It was found that preparations containing 3-7% tripalmitin could be produced in the same nanometer size range. This could be due to the sufficient amount of poloxamer 407 to stabilize their particles. Poloxamer 407 was a large molecule with highly diffusion velocity. It could diffuse and adsorb on the surface of the particles completely. Higher concentration of lipid required higher amount of stabilizer. However, 3% poloxamer 407 was more than sufficient for the suitable condensed film around the droplets. Thus, their particle sizes were lower than 1 μm and were not different in each preparation.

Both short and long chain triglycerides could be used as lipids for SLN preparations. Trimyristin and tripalmitin could produce the nanoparticles, but tristearin obtained the larger particle size. This result might be due to their molecular sizes and their lipophilicity. Trimyristin, the smallest molecular size, produced the smallest particle size. While tristearin, the biggest molecular size, produced the biggest particle size. Shorter chain fatty acid showed lower lipophilicity than the longer chain. When dispersing in aqueous medium, it was dispersed easily, and could be surrounded by the stabilizer. Therefore their particles were smaller than those of the longer chain fatty acid.

Stearic acid, however, produced the particles larger than 1 μm both before and after autoclaving. This caused by the agglomeration of their particles. Stearic acid was long chain fatty acid the same as tristearin. It had a smaller molecular size than triglyceride. When it was dispersed in aqueous solution, it could be dispersed with difficulty due to its high lipophilicity. Moreover, some acidic moiety of its molecule could be ionized to be higher nucleophilicity which repulsed with the nucleophilic moiety of oxygen atom of poloxamer 407. This might disrupt the film formation of poloxamer 407 to be thin interfacial film, and coagulation or agglomeration occurred. This result was in consistent with its low viscosity which fluctuated due to its agglomeration.

The pH and osmolality of dispersions of SLN were not affected by the types and amounts of lipid. This was because the lipid was not soluble in water. The osmolality of preparation containing stearic acid was higher than that of the other preparations due to its ionized form which could dissolve in dispersion medium.

The zeta potential of dispersions of SLN containing triglyceride was not different between various types and amounts of lipid. These preparations were stable both before and after autoclaving, and after storage for 6 months at room temperature due to their steric effect by poloxamer 407. Nevertheless, higher zeta potential value was found in SLN of stearic acid. This was due to its ionized form in aqueous medium which was negatively ion.

The viscosity of dispersions of triglyceride nanoparticles was slightly increased with increasing the fatty acid chain. This was due to the larger molecular and particle sizes, while dispersion of stearic acid nanoparticles exhibited low viscosity due to its smaller molecular size. And increasing the amount of tripalmitin also slightly increased the viscosity due to increasing the particle concentration. Their viscosities exhibited the Newtonian flow system. However, all preparations exhibited very low viscosity that could be used for parenteral administration.

Moreover, the cryo-scanning electron photomicrographs showed small agglomerate particles. Their shapes were spherical with various sizes. However, this result was in disagreement with some researchers who reported platelet-like colloidal crystals (Westesen and Siekmann, 1997) or disc like structure (Mühlen et al., 1996). This might be caused partly by the different microscopic techniques.

The infrared spectra, DSC thermograms, and X-ray diffractograms showed compatibility of tripalmitin and poloxamer 407. The crystallization of SLN obtained showed the mixture of tripalmitin and poloxamer 407. However, the endotherm of SLN showed the lower melting point of new solid lipid matrix compared with raw materials. It started to melt at 25.67°C with two peaks at 45.92 and 63.33°C. This finding agreed with previous studies that the melting point of SLN was lower than its bulk lipid substance (Westesen, Siekmann, and Koch, 1993;

Westesen and Bunjes, 1995; Westesen and Siekmann, 1996). This result was explained by Westesen et al. (1993) that the tripalmitin matrix represented an supercooled melt and might be recrystallize in the α-form (melting point was 44-45°C). In contrast, the bulk lipid recrystallized in the intermediate β′-form (melting point was 56-58°C) which was rapidly transformed into the β-form (melting point was 65-67°C). It was possible that the new solid lipid matrix had higher amount of α-form, and its melting point was reduced.

These results showed that preparations of triglyceride SLN could be used in the parenteral administration due to their small particle sizes and low viscosity. However, tripalmitin was selected to prepare the SLN for further study due to the smaller particle size and higher melting point to be solid matrix in body temperature that might sustain the drug release.

3. Drug loaded SLN

3.1 Diltiazem hydrochloride loaded SLN

3.1.1 In water dispersion medium

Diltiazem hydrochloride was loaded into SLN as a model of very water soluble drug. It was found that only poloxamer 407 could stabilize the preparations both before and after autoclaving, but not tween 80 and egg lecithin. This might be due to the high water solubility of drug and higher after autoclaving at high temperature, and it was an ionized form of positive charge which affected lower negatively zeta potential. Tween 80 could easily diffuse from the layer on the surface of droplets to the aqueous phase after autoclaving, and it might be binding with drug which dissolved in dispersion medium. Thus tween 80 could not re-adsorb to the particles and cause coalescence of melted lipid. Egg lecithin also did not stabilize the preparation because it had large amount of charge and might be incompatible with this ionized drug. Therefore egg lecithin was repulsed, could not form film on the

particles and caused coalescence of melted lipid. While poloxamer 407 could stabilize preparations because it was large molecular size and would be strong films on the oily surfaces which had low diffusivity to the dispersion medium, and nonionic nature that could not interact with ionized drug. Thus high steric effect of poloxamer 407 still stabilized the preparation.

The preparations of SLN containing diltiazem hydrochloride of 0.5-1.5% in water showed the particle size higher than 1 μm. This was higher than drug free SLN. It was possible that some drug could dissolve in dispersion medium and disturbed the poloxamer 407 diffusion velocity. Thus, the poloxamer 407 diffused to the surface of droplets slower than drug free preparation. Therefore, the higher particle size was obtained in all drug loaded preparations.

The pH value of dispersions of SLN containing diltiazem hydrochloride in water was lower than drug free preparation. This indicated that some drug was soluble in dispersion medium and affected the pH. This was in agreement with their higher osmolalities than those of drug free preparation. And increasing drug concentration could increase the osmolality due to the drug dissolved in dispersion medium.

The zeta potential of dispersions of SLN containing diltiazem hydrochloride in water was also consistent with the result above. It was found that drug caused the decreasing of the zeta potential. Increasing drug concentration could slightly decrease these values. Very low negatively zeta potential was observed. It was because some drug could dissolve in dispersion medium to be ionized form. This ionized proton could reduce the negative charge of particles. However, it was found to be the stable preparations for more than 6 months since the steric stabilization of poloxamer 407 was enough to stabilize the particles.

The viscosity of dispersions of SLN containing diltiazem hydrochloride in water was not different from drug free preparation. The low viscosities were observed in all preparations. This indicated that drug concentration did not affect the viscosity.

The infrared spectra of solid lipid matrix showed the dominant peak of tripalmitin. Only small peak of drug and poloxamer 407 appeared in this spectrum. It indicated that there was no interaction between tripalmitin and other components. Some drug and poloxamer 407 could be loaded into the lipid.

The entrapment efficiency of diltiazem hydrochloride loaded into SLN in water was low. Only 14.69-16.58% could be loaded into the solid lipid particles. This was due to its high solubility in dispersion medium, and slightly partitioned into the lipid phase. Their pH were lower than the pKa of drug (7.7) as presented in the ionized form in dispersion medium that was a hydrophilicity and had low partition to the lipid.

The comparison of the release of diltiazem hydrochloride from dispersions of SLN and saturated solution was studied. It was found that the saturated solution could sustain the release for more than 24 hours. This sustained release was due to the high drug concentration (480.30 mg/ml) and high osmotic pressure in donor phase. Increasing volume in donor compartment was observed during experiment due to the water backward from the receptor into the donor part. This effect might against the drug diffusion through dialysis membrane into receptor compartment. In addition, the rate of this release was higher than that of supernatant and preparations. Its release kinetic followed zero order model. Moreover, it was found that the release from supernatant was rapid within 10 hours. When the dispersion of SLN was tested, the drug release was also rapid due to the release of drug that was soluble in dispersion medium. Only drugs in lipid could sustain the release after the 10^{th} hours. The release profiles of both supernatants and dispersions were higher than that of saturated solution because there was no osmotic effect due to the very low concentration in preparation, i.e. 5-15 mg/ml.

These results showed the inferiority of diltiazem hydrochloride loaded SLN dispersions which had to be improved. Therefore, the phosphate buffer pH 7 was used as a dispersion medium.

3.1.2 In buffer pH7 dispersion medium

Diltiazem hydrochloride was loaded into SLN in phosphate buffer pH 7 as dispersion medium. It was found that the stable preparations could be prepared using poloxamer 407 as stabilizer. The d(v,0.5) of preparations of SLN containing 0.5-1.0% drug was lower than 1 μm and lower than drug free preparation, but higher than 1 μm in preparation of 1.5% drug. It was possible that the buffer pH 7 was close to its pKa and could change some drug molecules to be non-ionized form which was less soluble than the primary ionized form. These caused less drugs to dissolve in dispersion medium of preparations of 0.5-1.0% drug, so the less retard of diffusion velocity of poloxamer 407. Poloxamer 407 could then diffuse to the surface of droplets, and then the nanoparticles were prepared. While in the preparation of 1.5% drug, the higher drug soluble in dispersion medium occurred. This caused more disturbance of diffusion velosity, therefore poloxamer 407 was slowly adsorbed on the surface of droplets and the higher particle size was observed.

The pH value of dispersions of SLN containing diltiazem hydrochloride in buffer pH 7 was lower than 7.0. This might be due to an acidic property of drug that was soluble in dispersion medium. Increasing the drug concentration could decrease the pH value. However, these values were higher than drug free and drug loaded in water dispersion. These results agreed with the explanation above that higher drug concentration caused the higher drug soluble in dispersion medium, and also decrease the pH.

In spite of the less drug dissolved in dispersion medium, the osmolality of dispersions of SLN containing diltiazem hydrochloride in buffer pH 7 was higher than in water dispersion medium. This result could be due to the osmotic pressure of ingredients of phosphate buffer, i.e. monobasic potassium phosphate and sodium hydroxide. However, increasing drug concentration could also increase the osmolality due to the higher drug concentration in dispersion medium.

The negatively zeta potential of dispersions of SLN containing diltiazem hydrochloride in buffer pH 7 was higher than that in water. This was

consistent with the other properties that it had less drug dissolved in dispersion medium. It was lower than drug free preparation because some drug dissolved and ionized to be protonated form that reduce the negative charge of the surface of particles. Increasing the drug concentration could increase the drug soluble in dispersion medium, and so decreased the zeta potential. However, the preparations were stable due to the steric effect of poloxamer 407.

The viscosity of dispersions of SLN containing diltiazem hydrochloride in buffer pH7 was very low, and slightly lower than drug free and drug loaded preparations in water dispersion. However, the drug concentration did not affect the viscosity. This agreed with the viscosity of the other poloxamer 407 stabilized preparations. The low viscosity caused by the freely diffusion of poloxamer 407 and some drug molecule in dispersion medium.

Moreover, the cryo-scanning electron photomicrographs showed the small agglomerate particles. The agglomeration of particles, however, occurred due to the high particle concentration being frozen in liquid nitrogen. Their shapes were likely to be spherical though not clear due to their agglomeration and very small crystals fixed on their surface. These small crystals could be from the drug soluble in dispersion medium and the other buffer ingredients which recrystallized during freezing in liquid nitrogen.

The infrared spectra, DSC thermograms, and X-ray diffractograms showed the compatibility of tripalmitin, diltiazem hydrochloride, and poloxamer 407. There was no new peak in the infrared spectra. However, there was no peak of drug in both DSC thermograms and X-ray diffractograms. It indicated that the drug was not in the crystalline form in solid lipid matrix which was similar to the result from previous investigation by Cavalli, Aquilano et al. (1995). It was possible that the drug was loaded into the lipid particle as amorphous form or molecular dispersion. Moreover, the DSC thermograms showed the lower started melting point of solid lipid matrix compared with their raw materials. It was consistent with drug free preparation. However, increasing drug concentration could decrease this temperature. The result showed the initial melting point at 24.29°C and 7.29°C in

lipid matrix of preparation containing 0.5% and 1.5% drug, respectively. It indicated that higher concentration could induce the supercooling effect of lipid matrix. This might be due to drug molecule in lipid matrix could affect the solidification to higher α-form and slowly changed to the β′- and β-form, respectively. Thus the reduction of its melting point was observed.

The entrapment efficiency of diltiazem hydrochloride loaded into SLN in buffer pH7 was higher than in water. About 53.15, 45.46, and 35.88% were loaded into the particles of preparations containing 0.5, 1.0, and 1.5% drug, respectively. This could be due to the higher pH which was close to the pKa of the drug, resulting in higher non-ionized form which was less soluble in dispersion medium. This non-ionized form would partition into the lipid phase. Furthermore, increasing the drug concentration could decrease the entrapment efficiency.

The drug release from dispersions of SLN containing diltiazem hydrochloride in buffer pH7 was sustained for more than 24 hours. All preparations showed the Higuchi and power expression kinetics of release profiles.

3.2 Theophylline loaded SLN

Theophylline was loaded into SLN as a model of sparingly water soluble drug. It was found that all three stabilizers could stabilize the preparations to be the stable products both before and after autoclaving. Poloxamer 407 was an effective stabilizer for preparations of 0.25-0.75% theophylline. But the crystallization of drug occurred in preparations of 1.0 and 5.0% drug. It was due to drug could dissolve in dispersion medium and less partition to lipid phase. The higher solubility of drug in dispersion medium was obtained in the preparation process as a supersaturated solution in aqueous phase which did not precipitate when freshly prepared. Autoclaving process was of the higher temperature and could induce its solubility to be higher drug concentration in dispersion medium. Crystallization of drug from such supersaturated solution could occur after cooling for several days due to the lower solubility of drug at room temperature. Preparations of SLN containing theophylline and tween 80 2-3% precipitated after storage for 1 month at room

temperature. This was consistent to drug free preparation and could be explained by the same reason. Only preparation of SLN containing 0.25% theophylline was stable when using 1% egg lecithin as stabilizer. The preparations of SLN containing 0.50-0.75% theophylline resulted in phase separation after autoclaving, but they could be stable when increasing of concentration of egg lecithin to 2%. It was possible that the small amount of drug which was soluble in dispersion medium of preparation of 0.25% drug could slightly disturb the egg lecithin film on the surface of droplets. So the preparation was stable. Preparation of higher drug content would increase more amount of drug soluble in dispersion medium, which might sufficiently disturb the egg lecithin film stabilized particles. The amount of egg lecithin was not sufficient to stabilize oil droplets. However, higher egg lecithin level produced stable preparation due to the higher amount of stabilizer molecules in dispersion medium that could protect the film barrier.

As the same as preparations of diltiazem hydrochloride, particle size of preparations containing 0.25-0.75% theophylline and 3% poloxamer 407 was higher than that of drug free preparation. It might be due to the drug soluble in dispersion medium which slightly disrupted the diffusion velocity of poloxamer 407, so the poloxamer 407 diffused to the surface of droplets slower than in drug free preparation and then produced the higher particle size. However, the particle size decreased in higher percentage of theophylline. It was possible that some drug molecule might be loaded into lipid matrix and induced easy breaking, thus higher drug loading could further reduce the particle size. In contrast, the particle size of SLN containing drug and tween 80 or egg lecithin was lower than drug free preparation, and increasing percentage of theophylline in preparation would increase the particle size. It was possible that some drug molecule might be loaded into lipid matrix and easily broken into small particles. However, higher amount of theophylline in preparation could increase the amount drug dissolved in dispersion medium. This might reduce the diffusion velocity and then disrupt the film formation of tween 80 after autoclaving. Thus the particle size was larger than that of lower concentration of theophylline. While the film of egg lecithin might be disrupted due to interaction with the ionized form of drug in the nitrogen position 7 (Cohen, 1975), and the ionized drug in the

medium could extract lecithin from the film, thus caused the incomplete layer resulted in the aggregation or coalescence.

The result showed that theophylline did not affect the pH of drug loaded SLN. No significant difference was observed between drug free and drug loaded preparations. This could be due to the neutral pH of drug in solution. Thus, drug which was soluble in dispersion medium did not change the pH of preparations. The pH was mainly affected by types and amounts of stabilizer.

The osmolality of theophylline loaded SLN was affected by drug loading. Increasing drug concentration in preparation could increase the osmolality. This caused by the drug which was soluble in dispersion medium. Increasing the drug concentration in dispersion medium would increase the osmotic pressure as the number of molecule was increased. However, theophylline did not affect the zeta potential of preparations.

The viscosity of preparations of SLN containing theophylline was not different from drug free preparation, except for the preparation of 2% tween 80 that increasing the viscosity was observed in drug loaded preparations. The increasing of viscosities might be because the drug molecule induced the rearrangement of molecule of tween 80 to the other structures such as hexagonal, liquid crystal, or cubic structure. These caused to be the higher viscosity dispersion.

The infrared spectra showed that very low amount of theophylline could be loaded into the tripalmitin matrix. The new peak was not observed which indicated that there was no interaction between tripalmitin, drug, and other components. This result agreed with the entrapment efficiency that determined by indirect method of drug dissolved in dispersion medium. Very low entrapment efficiency was observed in preparations containing 0.25-0.75% theophylline. This could be due to the higher solubility of drug at higher temperature. And theophylline could be in the ionized form in preparation that had the pH lower than its pKa. This caused the higher hydrophilicity. Moreover, the low partition coefficient of theophylline (0.755 in octanol and buffer pH 7.4 (Cotgreave and Caldwell, 1983))

indicated that the most of drug would be in aqueous phase. However, the undetectable of interaction might be due to low drug amount in lipid matrix which could not sensitively detect by experimental instrument. Because of its low entrapment efficiency, therefore, the differential scanning calorimetry and X-ray powder diffractometry were not studied.

The release study showed the rapid drug release profile from saturated solution, poloxamer 407 stabilized preparations, and their supernatants. This indicated that most of the drug dissolved in dispersion medium, and could release rapidly through the dialysis membrane. Furthermore, drug that was entrapped in the lipid could also rapidly release to dispersion medium and then through to receptor compartment. This might be due to the low partition coefficient that the drug molecule could partition to the aqueous phase continuously. Therefore, more than 80% of drug was released into receptor compartment within 4 hours. However, the sustained release was observed in tween 80 stabilized preparations due to high viscosity of the preparations that slowed down the drug movement in solution or dispersion. Because of the high viscosity that was not suitable to be used in parenteral administration and their precipitation after storage for 1 month, thus the kinetics of drug release were not elucidated.

These results showed the inferior physicochemical properties of theophylline loaded SLN. These caused by the high water solubility at high temperature. Moreover, the low partition coefficient was the additional factor that caused the lower drug content in lipid matrix.

3.3 Piroxicam loaded SLN

Piroxicam was loaded into SLN as a model of insoluble drug in water. In this study, these preparations could not be prepared as a stable product. Piroxicam immediately and continuously precipitated to be yellowish powder after cooling. This finding was in agreement with a previous study (Klang et al., 1999). Thus, infrared spectroscopy was used to detect the incompatibility of drug with the other components. It was found that , the spectra of solid lipid matrix of poloxamer 407

stabilized preparations showed the new peak at the wavenumber of 1609 cm^{-1}. And the spectra of precipitated matter showed two broad peaks of inter- and intra-molecular hydrogen bonding at 3700-3000 cm^{-1}, and the other new peaks at 1600, 1552, and 1329 cm^{-1}. These showed the strong interaction of piroxicam and other components.

Further study was on stabilizer-free preparation. The new peak at 1600 cm^{-1} was also observed in both floating and precipitating parts of solid lipid, and showed another new peak at 1548 and 1329 cm^{-1} in precipitating part. This confirmed the incompatibility between piroxicam and tripalmitin. Moreover, the peak of N-H and O-H stretching at 3393 cm^{-1} was also shifted to 3339-3345 cm^{-1} in melted drug-lipid matrix. This indicated the changeability of the polymorphism of piroxicam (Mihalic' et al., 1986).

From this result, it could explain the precipitation of piroxicam from SLN. Piroxicam could change the tautomeric structure from keto to enol form after melted at 75°C and mixed with tripalmitin as shown in Figure 111. This showed the strong yellow colour which was observed in step of mixing into the melted tripalmitin. Furthermore, the infrared spectra showed new peak at 1600 cm^{-1} which was the C=C stretching of conjugated double bond with aromatic ring. This caused by the hydrogen atom at carbon between amide and carbonyl groups shifted to the double bond, and changed the carbonyl to be the hydroxyl group. This conjugated double bond with aromatic ring increased the chromophore of molecules and resulted in the strong yellow colour. And it could form intra-molecular hydrogen bonding of hydroxyl group with carbonyl group of amide part.

When piroxicam was mixed into the melted tripalmitin, it could bind with tripalmitin by two parts. Hydrogen bonding was bound between carbonyl groups of tripalmitin and hydrogen atom at amide nitrogen of piroxicam. This showed the broad peak at 3700-3500 cm^{-1} of inter-molecular hydrogen bonding. And the lipophilic benzothiazene part of piroxicam could interact with the lipophilic long chain alkyl part of tripalmitin.

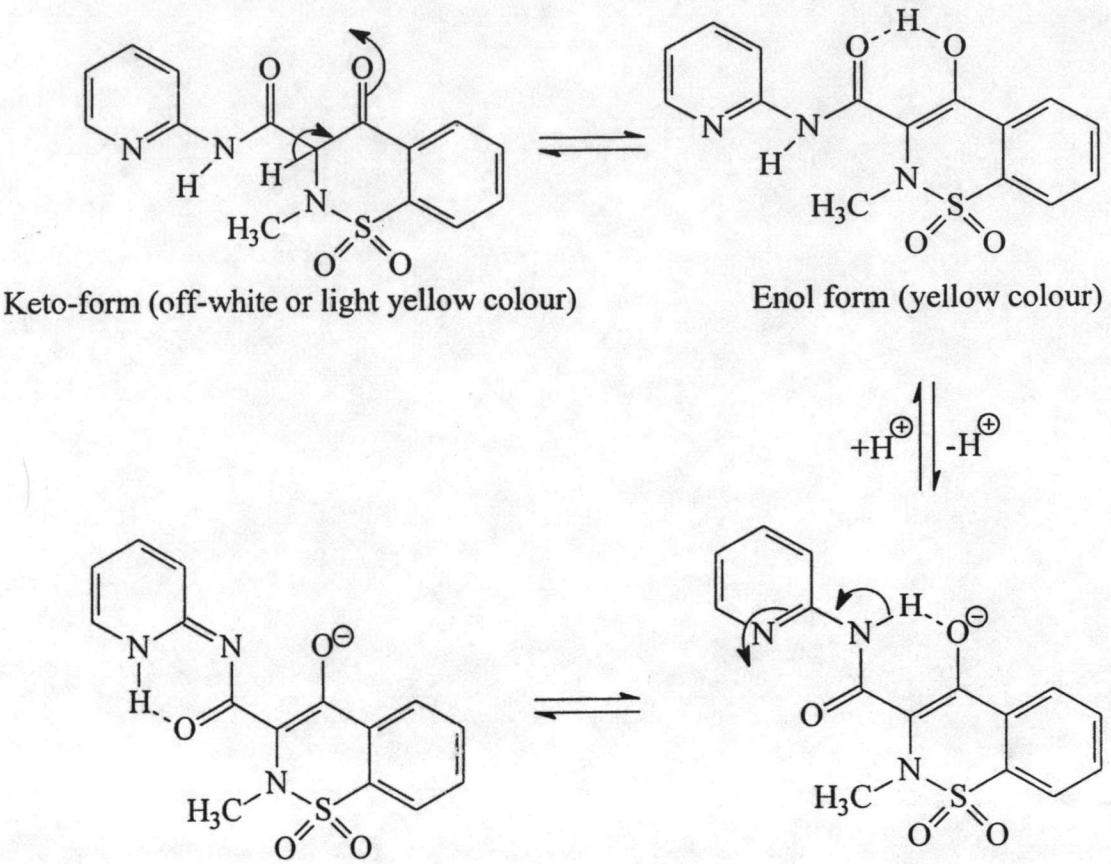

Figure 111. A schematic illustration of tautomeric equilibria and stabilization of the enolate anion for piroxicam.

After mixing the melted piroxicam-lipid into aqueous phase, piroxicam might change to be the piroxicam monohydrate which the enolic hydrogen could transfer to the pyridine nitrogen. Two intra-molecular hydrogen bonding were formed by the internal rotation of the neutral structure, i.e., between enolate oxygen and hydrogen on amide nitrogen, and between carbonyl oxygen and the hydrogen on pyridine nitrogen (Mihalic′ et al, 1986; Vrecer, Srcic, and Šmid-Korbar, 1991). Thus, the infrared spectrum presented the broad peak at 3500-3300 cm^{-1} (Colthup, Daly, and Wiberley, 1975). These caused the enolate anion of piroxicam. Water could bind with tripalmitin by hydrogen bonding at oxygen atom. Moreover, the stabilizer could increase the binding between tripalmitin and water. However, piroxicam could be expelled from tripalmitin because the enolate anion of piroxicam was repulsed by the

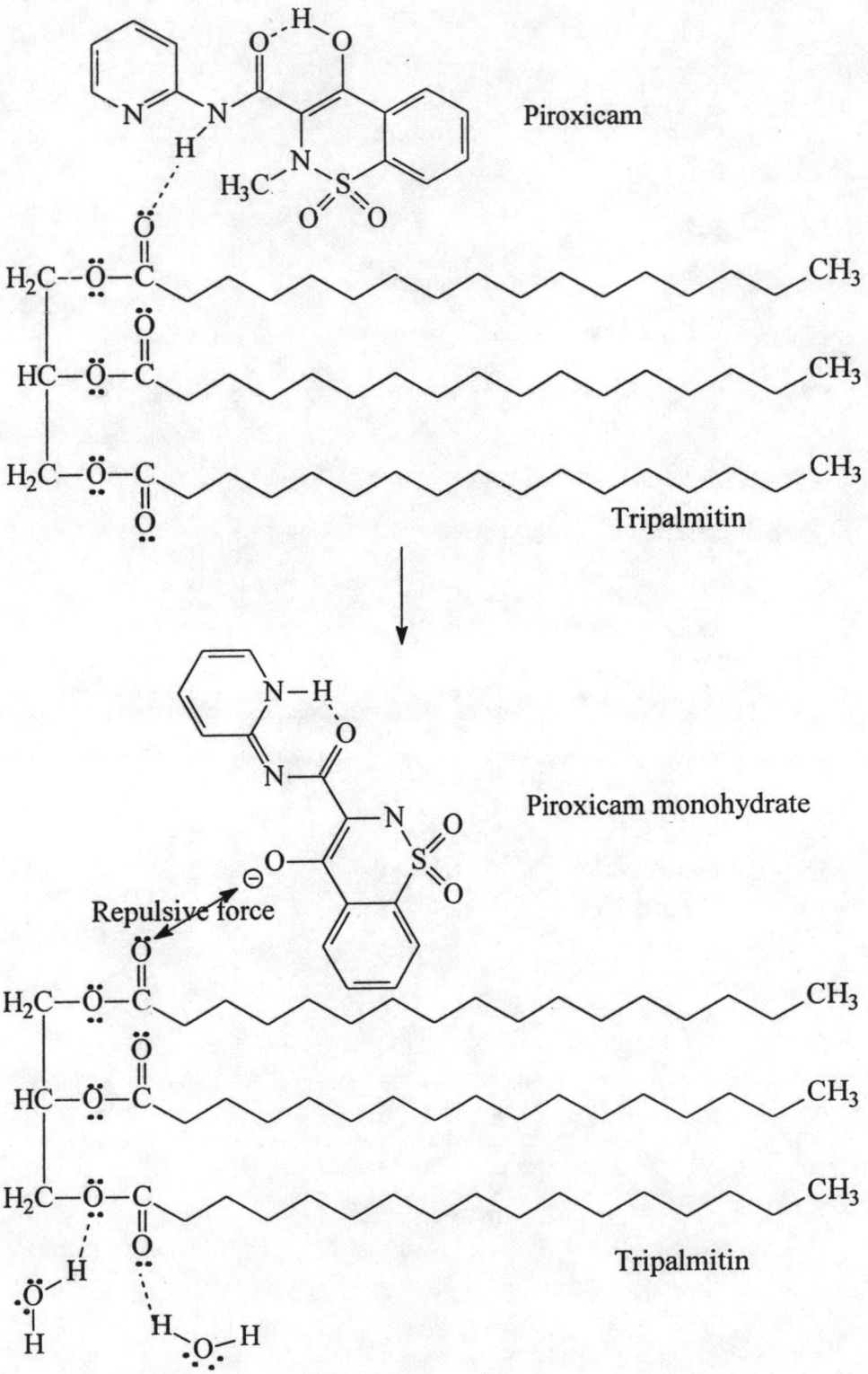

Figure 112. A schematic illustration of incompatibility of piroxicam and tripalmitin in aqueous system.

negative dipole of glyceride part of tripalmitin. Thus, the piroxicam molecule was separated from dispersions. The milky dispersion of lipid in aqueous phase and the yellow precipitated matter of piroxicam were observed. Moreover, the high melting point resulted in the crystalline form of piroxicam which could difficulty mixed in lipid matrix. This might cause the precipitation of piroxicam from dispersions after storage.

However, the piroxicam could be prepared to be the stable product in the positively charged submicron emulsion by using stearyl amine to protonate the anionic oxygen atom (Klang et al., 1999). This caused the positive charge of piroxicam-stearyl amine molecules, and could form electrostatic attractive force with lipid, and could form inter-molecular hydrogen bonding with water.

3.4 Ibuprofen loaded SLN

Ibuprofen was loaded into SLN as a model of insoluble drug in water. It was found that both preparations containing 3% poloxamer 407 and 1-2% egg lecithin could be prepared to be stable both before and after autoclaving. But tween 80 could not stabilize to be the stable products after autoclaving. The separation of oily phase occurred. Ibuprofen was amphiphilic molecule. It had both lipophilic and hydrophilic groups in the molecule. The lipophilic part would contact with lipid and showed the hydrophilic part on the surface. These could affect the stabilized films on the surface.

For preparations containing poloxamer 407, preparation of 0.5% ibuprofen was stable after autoclaving due to the low amount of drug in preparation. Poloxamer 407 could stabilize this system by the film formation of macromolecules on the surface of lipid droplets. However, the ibuprofen molecules on the surface of lipid droplets might resist the attachment of poloxamer 407 to be the film due to its amphiphilic property. However, small amount of ibuprofen could show less resistance of poloxamer 407 films formation. In addition, ibuprofen molecules might also show co-surfactant effect. Stabilized films surrounded the SLN were obtained after autoclaving. The preparation of 1.0% ibuprofen separated after autoclaving

because of the higher resistance of poloxamer 407 films formation after breaking by the autoclaving. However, the system could redispersed to be the good dispersion again because the poloxamer 407 and ibuprofen molecules in dispersion medium were fast diffusing molecules, and could rearrange to be the film by shaking. However, this system precipitated after storage for 2 months at room temperature due to the low steric effect of films. The new films were not strong and the aggregation of particles occurred. The preparation of 1.5% ibuprofen was also not stable after autoclaving. Moreover, it could not redisperse to be the good product because there were the higher ibuprofen molecules in lipid matrix and could resist the film formation of poloxamer 407. Although the repairing of these films by poloxamer 407 and ibuprofen in dispersion medium occurred after autoclaving. But high concentration of ibuprofen and low amount of poloxamer 407 in these films could not stabilize the system due to insufficient the steric effect. Nevertheless, both preparations of 0.5% and 1.0% ibuprofen were suitable to be used in parenteral administration, especially that of 0.5% could be used intravenously.

For preparations containing 2-3% tween 80, the separation of oily phase occurred in dispersions of 0.5-1.5% drug. It could be explained similar to poloxamer 407 stabilized preparations. However, tween 80 was of small molecular size. It could form films on the surface of the droplets, but exhibited lower steric effect than poloxamer 407. Therefore it could not stabilize the system.

For preparations containing 1-2% egg lecithin, the stable products could be prepared both before and after autoclaving. Similar to the other stabilizers, ibuprofen might disturb the film formation. Only some egg lecithin and ibuprofen could form films on the surface of droplets. These films could stabilize the system because there were the electrostatic repulsive effects of egg lecithin, and were also the steric effect of both egg lecithin and ibuprofen. Moreover, ibuprofen in lipid matrix could form intermolecular bonding with egg lecithin (Lichtenberger and Butler, 1999). This caused the strong lecithin films on the oily droplets. Thus, the egg lecithin stabilized preparations could be prepared.

The particle size of SLN containing either 3% poloxamer 407 or 1-2% egg lecithin was also consistent with the above explanation. For preparation containing 0.5% ibuprofen and 3% poloxamer 407, the particle size was lower than drug free preparation due to the additional steric effect of both ibuprofen and poloxamer 407 films on the particles. However, preparation of 1.0% ibuprofen gave the larger particle size. This caused by the weak film on the surface of droplet, which separated after autoclaving. Although it could redisperse to be the good dispersion, but low energy of shaking could not reduce the particle size to be in the nanometer size range. Surprisingly, the particle size of SLN containing 0.5-1.5% ibuprofen and 1-2% egg lecithin was lower than that of drug free preparation. And these were in the nanometer size range in preparations of 1.0-1.5% drug both 1% and 2% egg lecithin, and preparation of 0.5% ibuprofen in 2% egg lecithin. These might be due to the co-surfactant effect of ibuprofen. It was a small molecule, and might insert and form intermolecular bonding to egg lecithin monolayer. So stronger film would occur in these systems. Higher ibuprofen concentration could be the better co-surfactant and so reduced the droplet size.

The pH of preparations of SLN containing ibuprofen was lower than that of drug free preparations. It could be due to the acidic property of drug. Some drugs that dissolve in dispersion medium affect the pH of preparations directly. However, no difference between various drug concentrations was caused by the very low amount of ibuprofen that dissolved in dispersion medium in all preparations.

The osmolality of dispersions of SLN containing ibuprofen was not different between drug free and drug loaded preparations. These caused by the very low amount of ibuprofen that dissolved in dispersion medium in all preparations. Thus very low osmolality of preparations was observed, and it was not different in preparations of various drug concentrations.

The zeta potential of dispersions of SLN containing ibuprofen was in agreement with their physical appearances and their particle sizes. Decreasing of negatively zeta potential was observed in preparations of ibuprofen SLN using poloxamer 407 as stabilizer. This might be because the ibuprofen resisted film

formation of poloxamer 407. So there was the lower amount of poloxamer 407 on the surface of particles, which showed lower steric effect. These preparations showed the lower negatively zeta potential than that of drug free preparation. Contrarily, the zeta potential of egg lecithin stabilized preparations was not different between drug free and drug loaded preparations. This might be due to the film formation of egg lecithin with the co-surfactant of ibuprofen. Therefore, completely film formation was observed, and high zeta potential was measured.

The viscosity of dispersion of SLN containing ibuprofen was slightly affected by drug loading. The viscosity of preparations containing drug and poloxamer 407 was very low, and lower than that of drug free preparation. The viscosity of dispersion containing 0.5% drug and 1-2% egg lecithin was slightly lower than drug free preparation, and it was increased in preparations of 1.0% and 1.5% drug, respectively. It was possible that the higher amount of ibuprofen in the medium could more disturb the egg lecithin film, and obtained the higher egg lecithin in dispersion medium and form micelles or the other structures. Thus, the increasing of viscosity was observed. These egg lecithin stabilized preparations could not be used in parenteral administration because of their high viscosity. Only poloxamer 407 preparations were suitable for this route.

The cryo-scanning electron photomicrograph of preparation of SLN containing 0.5% ibuprofen and 3% poloxamer 407 showed small spherical particles. Various sizes were observed. These indicated that poloxamer 407 could stabilize the particles.

The infrared spectra, DSC thermograms, and X-ray diffractograms of ibuprofen loaded SLN showed the compatibility of tripalmitin, ibuprofen, and the other components. There was no new peak in solid lipid matrix compared with its raw materials. Similar to preparations containing diltiazem hydrochloride, DSC thermograms and X-ray diffractograms indicated that the drug was not in the crystalline form in solid lipid matrix. It was possible that the drug was loaded into the lipid matrix as amorphous form or molecular dispersion because drug could melt at preparing temperature and mix well in melted lipid. However, there was the small

peak of drug in X-ray diffractogram of 1.0% ibuprofen preparation. It might be due to the drug crystallized during drying process while preparing the specimen.

A schematic illustration the compatibility of ibuprofen and tripalmitin in aqueous medium is shown in Figure 113. The molecular of ibuprofen had both lipophilic and hydrophilic parts. When ibuprofen was mixed into the melted tripalmitin, it possibly bound with tripalmitin by both parts. Hydrogen bonding might bind carbonyl groups of tripalmitin and carboxylic group of ibuprofen. So these two compounds would be compatible. When addition to aqueous phase, water could bind with tripalmitin by hydrogen bonding at oxygen atom, and also bind with ibuprofen by hydrogen bonding at the nucleophilicity part of carboxylic group. Ibuprofen was not expelled from tripalmitin because its molecules also had hydrophilicity that could be compatible with water. Moreover, stabilizer in dispersion medium could increase the stability because it could also binding with both tripalmitin and water as well as ibuprofen. Therefore, this system could be stable. However, the intermolecular bonding of tripalmitin and ibuprofen could not be seen from infrared spectra due to less amount of drug in lipid matrix which could not be detected.

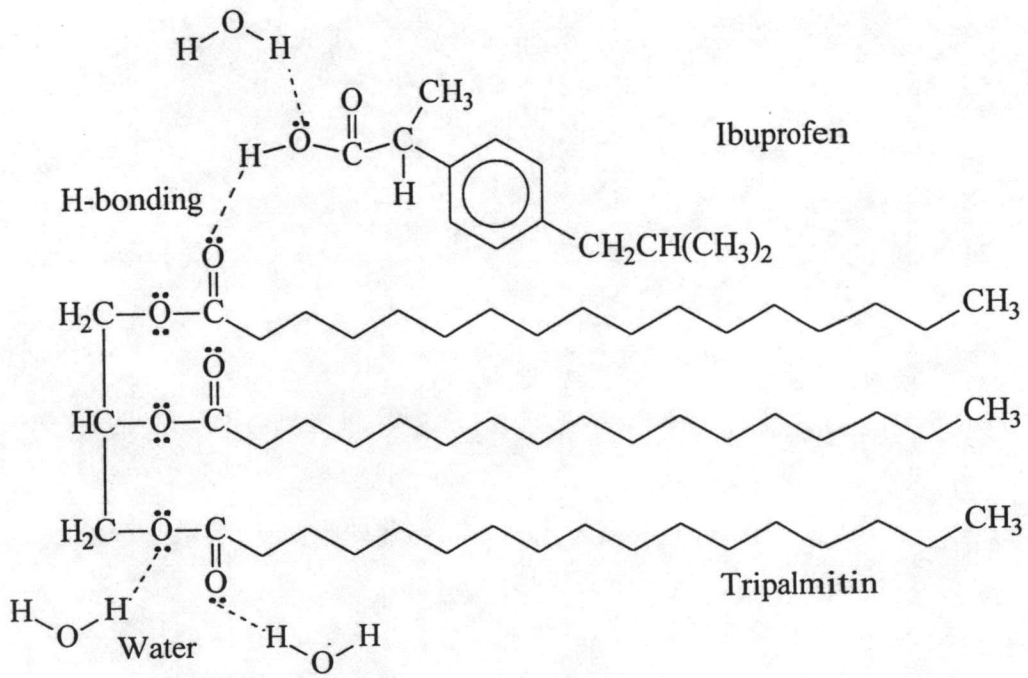

Figure 113. A schematic illustration of compatibility of ibuprofen and tripalmitin in aqueous system.

The entrapment efficiency of ibuprofen loaded into SLN was higher than 85% in all preparations. The highest value was presented in egg lecithin stabilized preparations. This caused by the low solubility of drug in dispersion medium, and drug was compatible with lipid. Thus the most of drug could load into lipid matrix and only a little drug could partition into the dispersion medium. Moreover, ibuprofen had the capacity to chemically associate with zwitterionic phospholipids both hydrophobic and electrostatic between the negatively charged carboxyl group of ibuprofen and the positively charged nitrogen of the phospholipid (Lichtenberger and Butler, 1999). These might affect the highest entrapment efficiency of ibuprofen in SLN preparations.

The saturated solution was rapidly released within 12 hours. The drug release from preparations of SLN containing ibuprofen was sustained more than 7 days. Preparations of higher drug concentration showed more sustain release of drug. Within 7 day, only 50% drug could be released from the preparation of SLN containing 1% ibuprofen and 3% poloxamer 407. While 70% drug could be released from the preparation containing 0.5% ibuprofen and 3% poloxamer 407. Different batches of these preparations showed similar physicochemical properties and release with the same pattern. The elucidations of kinetics of drug release showed that ibuprofen SLNs were Higuchi and power expression kinetics. This indicated that the release model of drug from SLN dispersion was not only affected by the drug diffusion from lipid matrix, but also effect by the other factors such as the viscosity of preparation, the particle size of particles.

These results also indicated that it was possible to prepare SLN of insoluble drugs that was compatible with the other compositions. The amphiphilic compounds were good materials that could be loaded in high entrapment efficiency and could act to be co-surfactant. These preparations could sustain the release for several days.

Conclusion

SLN could be prepared by hot melt homogenization technique to be in nanometer size range. This technique consisted of preparing the pre-emulsion by high speed homogenizer and reducing the particle size by high pressure homogenizer. The evaluation of homogenization process suggested that the stirring speed of 4,080 rpm at 10 minutes could produce good pre-emulsion, and homogenization by EmulsiFlex® C5 at 10000 psi and 5 cycles was the optimum condition for reducing the particle size of emulsion.

The various types and amounts of stabilizer and lipid affected the physicochemical characteristics of SLN. Poloxamer 407 of 1-5% was the best stabilizer that could produce the smallest particle size. The optimum concentration of 3% could produce the smallest particles and the system was stable in the same size for more than 12 months. Egg lecithin of 4-5% could also produce nanoparticles, but gel formation occurred after autoclaving and cooling to room temperature. Tween 80 was the stabilizer that also produced small particles but they became significant larger after autoclaving. Their pH, osmolality, zeta potential, and viscosity were mainly affected by the stabilizer. Egg lecithin preparations produced the lowest pH, but highest osmolality and viscosity than the other two stabilizers due to its complex mixture property. The amount of poloxamer 407 slightly affected these characteristics. Very low osmolality was observed due to the low soluble substance in preparation. The zeta potential was not sufficiently high enough to stabilize the dispersion solely by electrostatic repulsive force. However stable dispersion could be obtained due to the additional steric effect of poloxamer 407. The viscosity was very low thus suitable to be used in parenteral administration.

The chain of hydrocarbon in triglyceride molecule also affected the particle size of SLN. The nanoparticles could be prepared by all triglycerides studied. But longer chain produced the larger particle size. However, this property could slightly affect the other physicochemical properties. Their pH and zeta potential were not different. Very low osmolality and viscosity were also observed. On the contrary,

preparation of stearic acid could not be produced into the nanometer size range because aggregation occurred after cooling.

The drug solubility could mainly affect the drug entrapment efficiency in SLN. Diltiazem hydrochloride, the freely soluble drug in water, showed low entrapment efficiency. But it could be improved by changing the form to less water solubility. Increasing the pH of the system could also reduce the ionization thus decrease its solubility. The high entrapment efficiency was obtained when SLN dispersed in phosphate buffer pH 7. Theophylline, the sparingly soluble drug in water, also showed low entrapment efficiency due to higher solubility in higher temperature of processing. Moreover, its entrapment efficiency was lower than diltiazem hydrochloride due to its lower partition coefficient. Ibuprofen, the insoluble drug in water, showed the highest entrapment efficiency. Furthermore, ibuprofen as an amphiphilic molecule behaved as a co-surfactant which could produce smaller particle size due to its distribution at the interphase around the droplet surface additional to the main stabilizer. Piroxicam also an insoluble drug in water, however, could not obtained the SLN due to its incompatibility with lipid which was proven by infrared spectroscopy.

The solubility properties also affected the pH, osmolality, and zeta potential. High drug solubility in dispersion medium could change the pH of system depending on the pH of drug in solution. In addition its osmolality was nigher and zeta potential was lower than those of drug free preparation. Therefore it could affect the stability of the preparation. Their viscosities were slightly affected by their solubility properties. However, all preparations showed low viscosity that could be used in parenteral preparation.

The drug loaded into SLN was in non-crystalline state proved by DSC thermograms and X-ray diffractograms. It might be in amorphous form or molecular dispersion in solid lipid matrix. Moreover, these drugs could induce supercooling effect on lipid matrix. However, the crystalline state could not be observed which might be due to the limiting of detection of experimental instruments.

In term of drug release from these carriers system, diltiazem hydrochloride SLN in buffer pH 7 and ibuprofen SLN could sustain the release profile of drug more than 24 hours and 7 days, respectively. The release kinetics of all preparations were Higuchi model and power expression model. These indicated that there were many factors affected the release of drug from SLN carriers.

From these results, the SLN could be prepared for parenteral administration. The particles in nanometer size range were prepared using poloxamer 407 of 3% as stabilizer. Water insoluble drug could be loaded into SLN in high entrapment efficiency. Water soluble drug could also be loaded in higher levels by changing the drug molecule into insoluble form, such as changing the pH of systems. These systems could be sterilized by autoclaving and could be reproduced to be the same characteristics. It was possible to scale up to the production scale.

Suggestion for the further study

The optimum condition for preparing SLN in each system should be evaluated. The combination of lecithin and highly diffusion velocity surfactants might produce stable preparations and less quantity of these stabilizers could be used. However, percentage of phosphatidylcholine in lecithin should be assayed before used. Moreover, the effect of tonicity adjustment to the physicochemical property of SLN was one of the important factors that should be studied. And the increasing of viscosity of SLN dispersions from higher stabilizer concentration which might be due to the rearrangement of micelle structure to the other structure should be further studied.