CHAPTER IV

CONCLUSION

This study was conducted to characterized the solid state properties of asiaticoside which is a major component of *Centella asiatica* extracts. This research include solid phase identification , solid state stability , solubility and incompatability with selected excipients.

The crystals were recrystallized by various solvents,then identified by thin layer chromatography, X-ray powder diffractometry and microscopy. The representative crystallized products and asiaticoside raw material were further identified by fourier transformed infrared spectroscopy and solution nuclear magnetic resonance. Morphology of crystals were needle-like (acicular) but different in their respective sizes.

The XRPD analysis separated the crystals into 2 polymorphs. Raw material asiaticoside , recrystallized product of methyl alcohol after heated 105°C 3 hours were the first polymorph called asiaticoside I, all other recrystallized products were found to be the second polymorph called asiaticoside II. Raw material asiaticoside and recrystallized product from methyl alcohol were chosen to be a representative of each polymorphic form for the future experiments. Although the differences in TGA thermograms were not observable, the minor difference in endothermic peaks by DSC at 40 - 70°C implied that asiaticoside II could rearranged its internal structure and recrystallized to asiaticoside I. However, the large and broad desolvation peak interfered with recrystallization peak of asiaticoside II and obscured the event which could be clearly seen otherwise.

The solubility of asiaticoside I and II were measured at 37 $\pm$ 2 °C. In early phase, asiaticoside II resulted in higher solubility than asisticoside I with the maximun at 0.5531 mg/ml in 25 mins, and then decreased until constant in 60 mins at the same saturated concentration as asiaticoside I. Therefore, the solubility data indicated that

asiaticoside II in water could transformed to asiaticoside I which was more stable than asiaticoside II.

Solid state stability studies using XRPD showed the transformation of asiaticoside II to asiaticoside I when the temperature increased or the relative humidity decreased. In addition, effect of temperature on chemical stability studies of asiaticoside I and asiaticoside II found the substance decomposed when stored at various temperatures which fitted with many kinetic equations. The reaction rate increased with increasing temperature. On the other hand, the relative humidity had only slight affect on decomposition of asiaticoside II. The moisture stability asiaticoside I implied that asiaticoside I may need an optimal %relative humidity for decomposition. Temperature effected on crystal transformation and decomposition more than the moisture. It was possible that asiaticoside II must be transformed to asiaticoside I before decomposition occurred. The incompatibility of both forms of asiaticoside and selected pharmaceutical excipients found the decrease in percent remaining of asiaticoside II when mixed talcum, magnesium stearate and silicon dioxide.

This study leads to the precaution for asiaticoside storage, formulation, and processing design because of its thermal and relative humidity sensitivity. However, the long term stability studies is still necessary because the FDA require stability testing to be done at specificed temperature which matched with country zone rather than at the higher temperature of accelerated studies. Moreover, this research points out only 2 factors, temperature and relative humidity. There are other possible factors that may affect the transformation such as UV light and packaging.

The future study should use single crystal x-ray diffraction analysis to reveal the internal structure of asiaticoside polymorphs and to provide more insights into the nature of these solid drug substance.