# APPENDICES

# APPENDIX A

## Instrumental analysis

### 1. Weighing


**Figure 1** Balance, a product of Precisa, model XT 220A, Switzerland.

### 2. DSC


**Figure 2** Mettler DSC 821e (Mettler Toledo GmbH, Gießen, Germany).


**Figure 3** DSC 7 (Perkin Elmer, USA), product of Perkin Elmer, model DSC 7, U.S.A.

### 3. Goniometric measurement

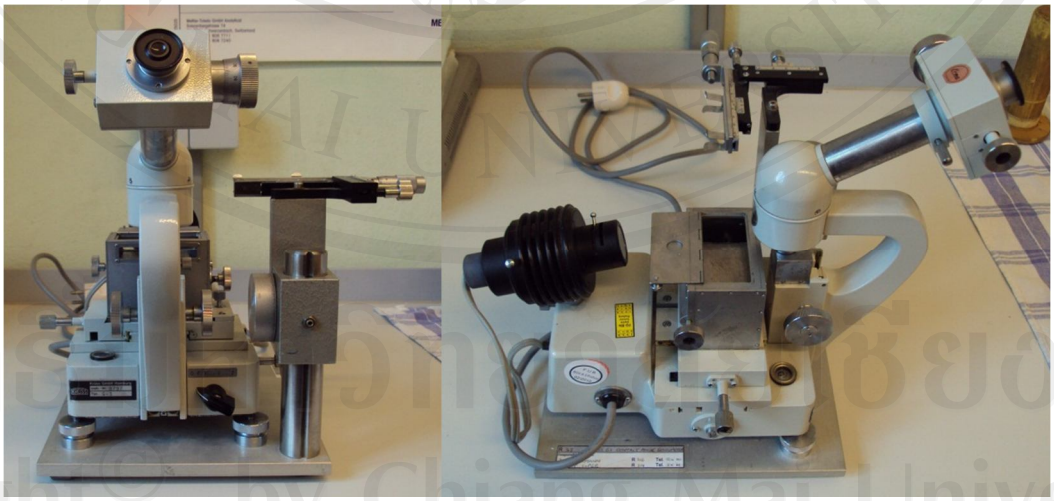

**Figure 4** Goniometer G1 (Kruss, Hamburg, Germany).

**4. High pressure homogenizer**


**Figure 5** The piston gap homogenizer; Micron LAB 40 (APV Deutschland GmbH, Unna, Germany).

**5. Particle size and zeta potential measurements**

**Figure 6** Malvern Zetasizer IV (Malvern Instruments, Herrenberg, Germany).

**6. Laser diffractrometry**

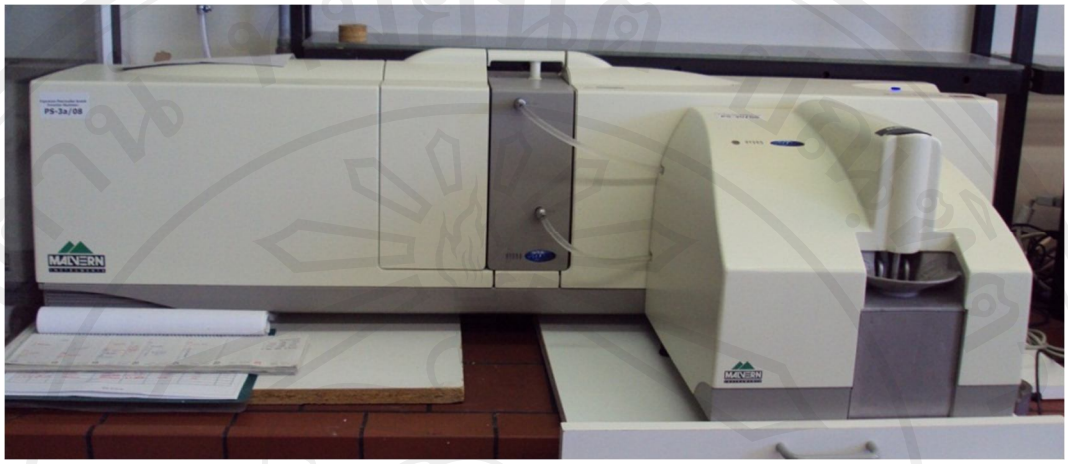

**Figure 7** Laser diffractometer LS 230 from Beckman-Coulter (Krefeld, Germany).

**7. Light microscopy and polarized light microscopy**

**Figure 8** Light microscope (Leitz, Wetzlar, Germany) equipped with a CMEX-1 digital camera (Euromex microscopes, Arnheim, The Netherlands) connected to Image Focus software version 1.3.1.4. (Euromex microscopes, Arnheim, The Netherlands).

**8. HPLC analysis**


**Figure 9** The HPLC system used consisted of an auto sampler model 360, a pump system model 420 and a UV visible detector model 430 (Kontron Instruments, Groß-Zimmern, Germany). This system was linked to a KromaSystem 2000 v. 1.83 data acquisition and process system, which also controlled the HPLC modules.

**9. UV visible spectrophotometer**

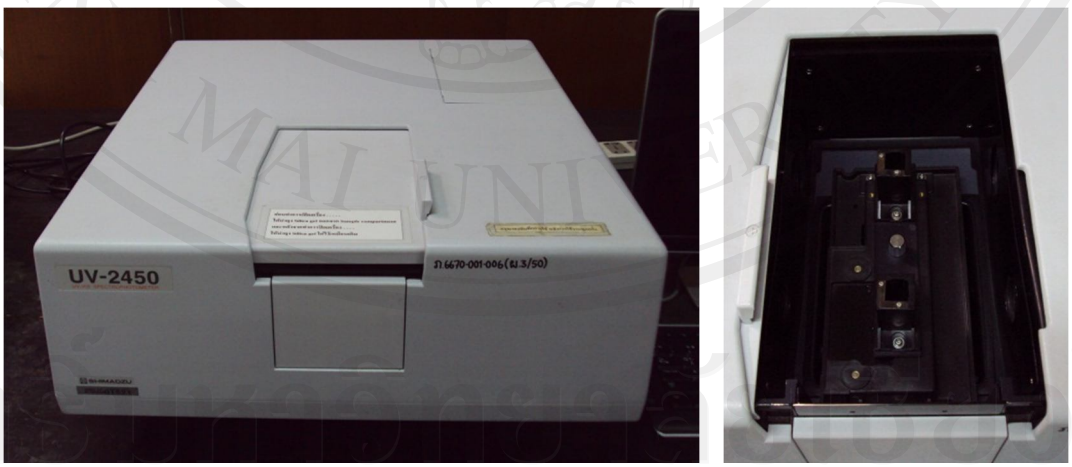


**Figure 10** UV Visible spectrophotometer, Shimadzu, model UV 2450, U.S.A.

**10. Occlusion test**

**Figure 11** Occlusion test was performed in an oven which temperature is controlled at 32°C (skin temperature) and adjust to 60±5% relative humidity (%RH).

**11. Antioxidant activity analysis**

**Figure 12** Microplate reader spectrophotometer, a product of Bio rad, Model 680, U.S.A.

# APPENDIX B

## Calculations

### 1. Percentage of crystallinity index

CI% helps to understand the percentage of lipid matrix which has re-crystallized during storage time. CI% of lipid nanoparticles (SLN or NLC) can be calculated using the following equation:

$$CI\% = \frac{\Delta H_{\text{SLN, NLC dispersion}}}{\Delta H_{\text{bulk material}} \times \text{concentration}^{*}_{\text{\%solid lipid phase}}} \times 100$$

where: CI% is the percentage of crystallinity index

$\Delta H$ is enthalpy change in J/g

*%solid lipid phase calculated from only solid lipid phase (not total lipid phase)

### 2. d-spacing value

d-spacing value was calculated using Bragg's law as following;

$$n\lambda = 2d \sin\theta$$

Where n is an integer, $\lambda$ is the wavelength of incident wave, d is the spacing between the planes in the atomic lattice, and $\theta$ is the angle between the incident ray and the scattering planes.

**3. Entrapment efficiency**

$$EE\ (\%) = \left( \frac{W \text{ initial drug} - W \text{ free drug}}{W \text{ initial drug}} \right) \times 100$$

where "*W* initial drug" is the mass of initial drug used for the assay and the "*W* free drug" is the mass of free drug detected in the supernatant after centrifugation of the aqueous dispersion.

**4. % inhibition**

$$\%\ \text{inhibition} = \frac{Abs_{control} - (Abs_{sample} - Abs_{blank})}{Abs_{control}} \times 100$$

which: Abs is an absorbance

$Abs_{control} = Abs_{positive\ control} - Abs_{negative\ control}$

**5. Occlusion factor, %F**

$$\%F = \left( \frac{A-B}{A} \right) \times 100$$

where A is the water loss without sample (reference) and B is the water loss with sample. An occlusion factor of 0 means no occlusive effect compared to the reference and 100 is the maximum occlusion factor.

## APPENDIX C

### HPLC validation method

1. **Accuracy**

The accuracy indicates the conformity of the mean of the measuring result x and the expected reference value μ. Therefore, the accuracy is equivalent to the absolute recovered amount of sample. The accuracy can be calculated using the following equation:

$$accuracy\,[\%] = \frac{\bar{x} \times 100}{\mu}$$

2. **Precision**

The precision is a measurement of the degree of reproducibility of analytical results repeating the same analytical method under the same terms and conditions. For the evaluation of the precision the relative standard deviation (RSD) can be used.

$$RSD\ [\%] = \frac{SD \times 100}{\bar{x}}$$

# CURRICULUM VITAE

**Name** Miss Pornthida Riangjanapatee

**Date of Birth** 29th October, 1985

**Education**

Graduated high school from Montfort College, Chiang Mai, in 2004

Bachelor of Science (1st Honor) from Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, in 2008

Doctor of philosophy in Pharmacy from Faculty of Pharmacy Chiang Mai University, Chiang Mai, in 2013

**Scholarship**

2004-2008 Research Professional Development Project Grant by The Institute for the Promotion of Teaching Science and Technology (IPST) (Bachelor degree)

2007 Young Scientist and Technologist Programme (YSTP) Summer Research Training Grant by NSTDA (Bachelor degree)

2007-2008 YSTP Senior Project Scholarship Grant by NSTDA (Bachelor degree)

2008-2013 The Royal Golden Jubilee (RGJ) Grant by The Thailand Research Fund (TRF) (Doctor of philosophy)

2011-2013 TRF-DAAD Project Based Personnel Exchange Programme (PPP 2011) Grant by the Deutscher Akademischer Austausch Dienst (DAAD) (Doctor of philosophy)

**Experience**

2005 Project "The prevention of color changing in peeled fruits", at Faculty of Science, Department of Chemistry, Chiang Mai University

2007 Project "Preparation and characterization of nano-titanium dioxide by sol-gel method", at NSTDA (NANOTEC), Bangkok, Thailand (Summer research training)

2007-2008 Project "Effect on catalytic activity of Pd doped on cerium dioxide nanoparticles synthesized by homogeneous precipitation", at Faculty of Science, Department of Chemistry, Chiang Mai University (B.S (Chemistry))

2008-2013 Project "Lycopene-loaded nanostructured lipid carriers for cosmeceutical applications", at Faculty of Pharmacy, Chiang Mai University (Ph. D (Pharmacy))

**List of publications**

**Patent**

1. Topical nano-cream product containing lycopene for antioxidative activities. Thai patent. (submitted).

**Journals**

1. Riangjanapatee, P., Okonogi, S. (2012). Effect of surfactant on lycopene-loaded nanostructured lipid carriers. Drug Discoveries & Therapeutics. 6(3):163-168.

2. Riangjanapatee, P., Müller, R. H., Keck, C. M., Okonogi, S. (2013). Development of lycopene-loaded nanostructured lipid carriers: Effect of rice oil and cholesterol. Pharmazie. 68.
3. Okonogi, S., Riangjanapatee, P. (2013). Potential technique for tiny crystalline detection in lycopene loaded SLN and NLC development, Drug Dev Ind Pharm (submitted).
4. Riangjanapatee, P., Okonogi, S. (2013). Physico-chemical characterization of lycopene-loaded NLC for topical administration, AAPS Pharm Sci Tech (during progression).
5. Riangjanapatee, P., Okonogi, S. (2013). Preparation and evaluation of antioxidative creams containing lycopene-loaded nanostructured lipid carrier, Int J Cosmet Sci (during progression).

**Full paper proceeding (peer review)**

1. P. Riangjanapatee, R.H. Müller, C.M. Keck, C. Ampasavate, S. Okonogi, "Potential of lycopene-loaded NLC versus nanoemulsion for cosmeceutical applications: stability, entrapment efficiency and antioxidant capacity", in The 38th Congress on Science and Technology of Thailand: Science for the Future of Mankind, Chiang Mai, Thailand, 17-19th October, 2012

**Posters**

1. P. Riangjanapatee, C. Ampasavate, S. Okonogi, "The synergistic effect of lycopene and sesame oil", in 7th Asia Pacific Conference on Clinical Nutrition, Bangkok, Thailand, 7th-9th June, 2011

2. S. Okonogi, P. Riangjanapatee, C.M. Keck, R.H. Müller, "Effect of surfactant type on physical stability of lycopene-loaded NLC", PO-233, Annual Joint Meeting, DPhG, Innsbruck, 20-23 September 2011
3. P. Riangjanapatee, R.H. Müller, C.M. Keck, C. Ampasavate, S. Okonogi, "Physicochemical characteristics and stability of skin friendly nanostructured lipid carrier (NLC)", in RGJ – Ph.D. Congress XIII, Chonburi, Thailand, 6th-8th April, 2012
4. P. Riangjanapatee, R.H. Müller, C.M. Keck, C. Ampasavate, S. Okonogi, "Nanostructural Characterization of Lycopene-loaded NLC by Digital-imaging Cryo-TEM and Its Crystallography", in The 1st Annual International Meeting of The Society of Molecular Imaging of Thailand: From Preclinical to Clinical Application, Bangkok, Thailand, 9th-11th April, 2012
5. P. Riangjanapatee, R.H. Müller, C.M. Keck, C. Ampasavate, S. Okonogi, "Protective roles of lycopene loaded NLC against skin damage from free radicals", in The 89th RGJ Seminar Series: Molecular mechanisms and technology developments in biomedical researches, Chiang Mai, Thailand, 31th August, 2012

**Oral Presentations**

1. P. Riangjanapatee, "Lipid nanoparticle by high pressure homogenization and cosmeceutical applications", in Pharmaceutical Science Seminar, Ph. D, at Faculty of Pharmacy, Chiang Mai University, semester 2/2552, on 24th February, 2010
2. P. Riangjanapatee, "Antioxidant capacities of extract from certain plants grown in Thailand", in Pharmaceutical Science Seminar, Ph. D, at Faculty of Pharmacy, Chiang Mai University, semester 1/2553, on 15th September, 2010

3. P. Riangjanapatee, “Lycopene-loaded nanostructured lipid carriers I: influence of contact angle and thermal behavior on particle size and zeta potential”, in Pharmaceutical Science Seminar, Ph. D, at Faculty of Pharmacy, Chiang Mai University, semester 2/2553, on 15th February, 2011
4. P. Riangjanapatee, “New skin friendly lycopene-loaded nanostructured lipid carrier (NLC): Physicochemical characteristics, occlusive property, and stability”, in Pharmaceutical Science Seminar, Ph. D, at Faculty of Pharmacy, Chiang Mai University, semester 2/2554, on 21th February, 2012