APPENDICES

APPENDIX A

CHEMICAL AND PHYSICAL PROPERTIES OF THE CHEMICALS USED

1. Polyoxyethylene (4) sorbitan monostearate

Chemical name	polyoxyethylene (4) sorbitan monostearate
Synonyms	polysorbate 61, Tween61
Empirical formula	$C_{32}H_{62}O_{10}$
Molecular weight	607
Description	tan solid
HLB	9.6
Solubility	dispersible in water, soluble in ethanol
Chemical structure	chemical structure of polyoxyethylene (4) sorbitan monostearate is shown in **Figure A.1**

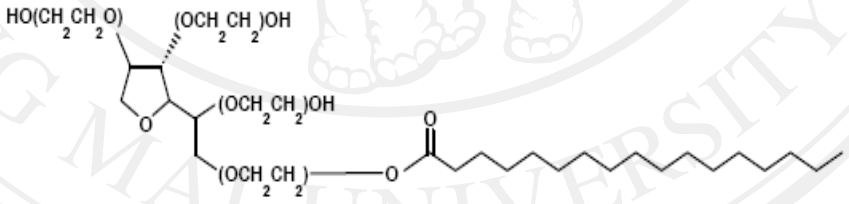

Figure A.1 Chemical structure of polyoxyethylene (4) sorbitan monostearate

2. Cholesterol

Name	cholesterol
Chemical name	cholest-5-en-3β-ol
Synonyms	cholesterin
Empirical formula	$C_{27}H_{42}O$

Molecular weight	386.67
Description	white or faintly yellow, almost odorless, needles
Melting point	147 - 150 °C
Solubility	soluble in acetone, chloroform, ether, fixed oils, practically insoluble in water
Chemical structure	chemical structure of cholesterol is shown in **Figure A.2**

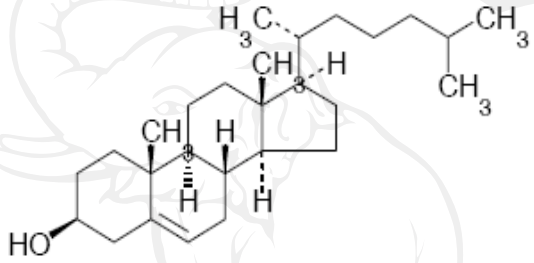

Figure A.2 Chemical structure of cholesterol

3. Cetyltrimethylammonium bromide

Name	Cetyltrimethylammonium bromide (CTAB)
Chemical name	hexadecyltrimethylammonium bromide
Synonyms	Cetrimonium bromide
Empirical formula	$((C_{16}H_{33})N(CH_3)_3Br$
Molecular weight	364.45
Description	white to off white powder
Melting point	237-243 °C
Solubility	soluble in water
Chemical structure	chemical structure of cetyltrimethylammonium bromide is shown in **Figure A.3**

Figure A.3 Chemical structure of cetyltrimethylammonium bromide

4. Benzalkonium chloride

Name	benzalkonium chloride (BZKC)
Chemical name	benzyl-dimethyl-tridecyl-azanium chloride
Synonyms	alkyldimethylbenzylammonium chloride
Empirical formula	$C_{21}H_{38}NCl$
Molecular weight	340.0
Description	Amorphous solid powder or lumps
Melting point	> 140 °C
Solubility	Easily soluble in cold water, hot water. Soluble in acetone. Very slightly soluble in diethyl ether. Very soluble in alcohol. Soluble in benzene.
Chemical structure	chemical structure of benzalkonium chloride is shown in **Figure A.4**

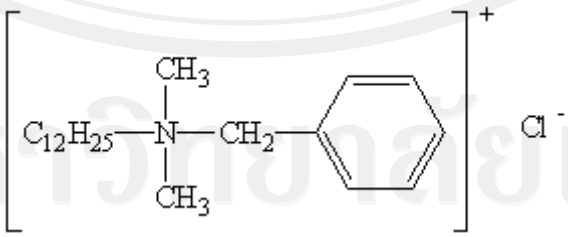

Figure A.4 Chemical structure of benzalkonium chlorid**e**

APPENDIX B

FORMULATIONS OF BUFFER AND SOLUTION USED

1. Tris-Acetate-EDTA (TAE) buffer (50X)

Tris base	242 g
Acetic Acid	57.1 ml
0.5 M EDTA (shake vigorously before use)	100 ml

Add distilled H_2O to 1 Liter and adjust pH to 8.5 using KOH

2. Loading dye

0.5 M Tris-HCl, pH 6.8	1.2 ml
Glycerol	1 ml
Bromphenol blue	2.5 mg

Add distilled H_2O to 10 ml

3. Phosphate buffer saline, PBS (10X)

NaCl	8 g
KCl	0.2 g
$Na_2HPO_4 \cdot 2H_2O$	1.44 g
KH_2PO_4	0.24 g

Add distilled H_2O to 100 ml

APPENDIX C

CERTIFICATE OF THE APPROVAL FOR THE USE OF ANIMALS

Certificate of Approval
For Use of Animals
Faculty of Medicine, Chiang Mai University

Protocol Number: **20/2553**
Title of project: **Transfolicular delivery systems of Fatty acids from natural sources entrapped in nanovessicles for anti-hair loss**
Principal investigator: **Professor Aranya Manosori**
Affiliation: **Faculty of Pharmacy**

The Faculty of Medicine, Chiang Mai University, supported by the results of Animal Ethics committee review, that the use of animals in the project conforms with international and national guidelines for ethical conduct on the care and use of animals,

Hereby approves the research proposal to be conducted under its proposed scheme. The approval is effective from **30 September 2010** and expired on **30 November 2010**

Nimit Morakote
Nimit Morakote, Ph.D.
Associate Professor
Chair
Date 30 Sept 2010

N. Nantachit
Niwes Nantachit , M.D.
Associate Professor
Dean
Date 30 Sept 2010

หนังสืออนุมัติการใช้สัตว์
คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

หมายเลขโครงการ: 20/2553

ชื่อโครงการวิจัย: ระบบนำส่งผ่านรูขุมขนของกรดไขมันจากธรรมชาติที่เก็บกักในถุงขนาดนาโนเพื่อป้องกันผมร่วง

ชื่อหัวหน้าโครงการวิจัย นางสาววรินทร รักษ์ศิริวณิช

สังกัด: ภาควิชาวิทยาศาสตร์เภสัชกรรม คณะเภสัชศาสตร์

คณะแพทยศาสตร์ โดยความเห็นชอบของคณะกรรมการจรรยาบรรณการใช้สัตว์ ได้พิจารณาโครงการวิจัย แล้ว เห็นว่าไม่ขัดต่อแนวทางสากลและประเทศในการปฏิบัติเกี่ยวกับการดูแลและใช้สัตว์

จึงอนุมัติให้ดำเนินการภายในขอบเขตของโครงการวิจัยที่เสนอมาได้ ทั้งนี้มีผลตั้งแต่วันที่ **30 กันยายน 2553** และให้หมดอายุในวันที่ **30 พฤศจิกายน 2553**

.....	.....
(รองศาสตราจารย์ ดร.นิมิตร มรกต)	(รองศาสตราจารย์ นายแพทย์นิเวศน์ นันทจิต)
ประธานคณะกรรมการ	คณบดี
จรรยาบรรณการใช้สัตว์ทดลอง	
วันที่ 30 กย 53	วันที่ 30 กย 53

APPENDIX D

CALCULATION OF NANOVESICLE COMPOSITIONS

The amount of each composition in nanovesicular formulations was calculated from the following equation:

Required amount (g) = molar ratio in the formulation × concentration of the nanovesicles (Molar) × required volume (L) × molecular weight

For example,

1. **to prepare 20 ml of neutral niosomes (20 mM) composed of Tween61: Cholesterol at 1:1 molar ratio**

(MW of Tween 61 =1311.7 and MW of cholesterol = 386.67)

The required amount of Tween61 and CHL was as follows:

Tween 61 (g) $= \frac{1}{(1+1)} \times 20 \times 10^{-3} \times 20 \times 10^{-3} \times 1{,}311.7 = 0.2623$ g

Cholesterol (g) $= \frac{1}{(1+1)} \times 20 \times 10^{-3} \times 20 \times 10^{-3} \times 386.67 = 0.0773$ g

2. **to prepare 20 ml of cationic niosomes (20 mM) composed of Tween61/ Cholesterol/CTAB at 1:1:0.5 molar ratio**

(MW of Tween 61 =1311.7, MW of cholesterol = 386.67 and MW of CTAB = 364.5)

The required amount of Tween61 and CHL was as follows:

Tween 61 (g) $= \frac{1}{(1+1+0.5)} \times 20 \times 10^{-3} \times 20 \times 10^{-3} \times 1{,}311.7 = 0.2099$ g

Cholesterol (g) $= \frac{1}{(1+1+0.5)} \times 20 \times 10^{-3} \times 20 \times 10^{-3} \times 386.67 = 0.0619$ g

CTAB (g) $= \frac{0.5}{(1+1+0.5)} \times 20 \times 10^{-3} \times 20 \times 10^{-3} \times 364.5 = 0.0292$ g

Table D Amounts of the composition in the prepared nanovesicles

Tween61: cholesterol : cationic surfactant		molar ratio		
		1:1:0.05	1:1:0.25	1:1:0.5
1. cationic niosomes with cetyl trimethyl ammonium bromide (CTAB) [mw=364.5]				
	molar*mM*ml*mw	g	g	g
Tween61	(1/2.05)*20e-3*20e-3*1311.7	0.2559	0.2332	0.2099
cholesterol	(1/2.05)*20e-3*20e-3*386.7	0.0755	0.0687	0.0619
CTAB	(0.05/2.05)*20e-3*20e-3*364.5	0.0036	0.0162	0.0292
2. cationic niosomes with cetylpyridinium chloride (CPC) [mw=358]				
Tween61	(1/2.05)*20e-3*20e-3*1311.7	0.2559	0.2332	0.2099
cholesterol	(1/2.05)*20e-3*20e-3*386.7	0.0755	0.0687	0.0619
CPC	(0.05/2.05)*20e-3*20e-3*358	0.0035	0.0159	0.0286
3. cationic niosomes with stearylamine [mw= 269.5]				
Tween61	(1/2.05)*20e-3*20e-3*1311.7	0.2559	0.2332	0.2099
cholesterol	(1/2.05)*20e-3*20e-3*386.7	0.0755	0.0687	0.0619
stearylamine	(0.05/2.05)*20e-3*20e-3*269.5	0.0026	0.0120	0.0216
4. cationic niosomes with benzalkonium chloride(BZKC) [mw=340]				
Tween61	(1/2.05)*20e-3*20e-3*1311.7	0.2559	0.2332	0.2099
cholesterol	(1/2.05)*20e-3*20e-3*386.7	0.0755	0.0687	0.0619
BZKC	(0.05/2.05)*20e-3*20e-3*340	0.0033	0.0151	0.0272
5. cationic niosomes with benzathonium chloride (BZT) [mw=448.08]				
Tween61	(1/2.05)*20e-3*20e-3*1311.7	0.2559	0.2332	0.2099
cholesterol	(1/2.05)*20e-3*20e-3*386.7	0.0755	0.0687	0.0619
BZT	(0.05/2.05)*20e-3*20e-3*448.08	0.0044	0.0199	0.0358
6. cationic niosomes with didecyl dimethyl ammonium bromide (DDAB) [mw=406.53]				
Tween61	(1/2.05)*20e-3*20e-3*1311.7	0.2559	0.2332	0.2099
cholesterol	(1/2.05)*20e-3*20e-3*386.7	0.0755	0.0687	0.0619
DDAB	(0.05/2.05)*20e-3*20e-3*406.53	0.0040	0.0181	0.0325
7. neutral niosomes (1:1)				
Tween61	(1/2)*20e-3*20e-3*1311.7	0.2623		
cholesterol	(1/2)*20e-3*20e-3*386.7	0.0773		

CURRICULUM VITAE

Name Miss Warintorn Ruksiriwanich

Date of Birth 15 June 1983

Education

1995-2000 Chiang Mai University Demonstration School , Chiang Mai, Thailand

2001-2005 Bachelor's degree in Pharmacy (B. Pharm, First Class Hons), Chiang Mai University, Thailand

2007-2012 A Ph.D candidate at Faculty of Pharmacy Chiang Mai University, Thailand under the RGJ-Ph.D Program of TRF

Work and Training Experiences

Feb, 2006 – May, 2007 Quality Operations Pharmacist at OLIC (Thailands) Limited, Bangpa-in Industrial Estate, Ayutthaya, Thailand

Oct, 2005 - Jan, 2006 Industrial Pharmacist /Training at Atlantic (laboratories) Corp., Limited, Bangkok, Thailand

Awards Obtained

2001-2005 Bronze Medal Award from the Faculty of Pharmacy, Chiang Mai University, Thailand in Best Academic Award Certificate

2005 Industrial and Research Projects of Undergraduate Students (IRPUS) from The Thailand Research Fund (TRF)

2006 1st Class Honors, Silver Medal Award from the Faculty of Pharmacy, Chiang Mai University, Thailand

2006	Very good grade from Probation Performance Appraisal from OLIC (Thailand) Limited, Ayutthaya, Thailand
2007	Very good grade from Annual Probation Performance Appraisal from OLIC (Thailand) Limited, Ayutthaya, Thailand
2007-2012	The Royal Golden Jubilee Scholarship under Ph.D. Program from the Thailand Research Fund (TRF)
2008	Outstanding Poster Presentation in RGJ-Ph.D. Congress IX. from the Thailand Research Fund (TRF).
2008	Second Prize of Poster Presentation in The Eighth National Seminar on Pharmaceutical and Biotechnology.

International Scientific Publications

1. Manosroi, A., **Ruksiriwanich, W.**, Abe, M., Sakai, H., Manosroi, W. and Manosroi, J. 2010. Biological activities of the rice bran extract and physical characteristics of its entrapment in niosomes by supercritical carbon dioxide fluid. *Journal of Supercritical Fluids***,** 54: 137-144. (Impact Factor 2.639)
2. Manosroi, A., **Ruksiriwanich, W.**, Kietthanakorn, B.-o., Manosroi, W., & Manosroi, J. 2011. Relationship between biological activities and bioactive compounds in the fermented rice sap. *Food Research International*, 44(9): 2757-2765. (Impact Factor 2.414)
3. **Ruksiriwanich W.**, Manosroi J., Abe M., Manosroi W. and Manosroi A. 2011. 5α-reductase type 1 inhibition of *Oryza Sativa* bran extract prepared by supercritical carbon dioxide fluid. *Journal of Supercritical Fluids*, 59(1): 61-71. (Impact Factor 2.639)

4. Kietthanakorn, B.-o., **Ruksiriwanich, W.,** Manosroi, W., Manosroi, J., and Manosroi, A. 2012. Biological activities of supercritical carbon dioxide fluid ($scCO_2$) extracts from medicinal flowers. *Chiang Mai Journal of Science*, 39(1): 84-96. (Impact Factor 0.34)
5. Manosroi, A., **Ruksiriwanich, W.**, Manosroi, W., Abe, M., Manosroi, J. 2012. *In vivo* hair growth promotion activity of gel containing niosomes loaded with the *Oryza sativa* bran fraction (OSF3). *Advanced Science Letters*, Accepted.
6. Manosroi A., **Ruksiriwanich W.**, Abe M., Manosroi W. and Manosroi J. 2012 Transfollicular enhancement of gel containing cationic niosomes loaded with unsaturated fatty acids in rice (*Oryza Sativa*) bran semi-purified fraction. *Eur. J. Pharm. Biopharm.*, Accepted.
7. Manosroi, A., **Ruksiriwanich, W.**, Abe, M., Sakai, H., Aburai, K., Manosroi, W., Manosroi, J. 2012. Physico-chemical characteristics of CTAB cationic niosomes loaded with the rice (*Oryza sativa*) bran semi-purified fraction (OSF3) prepared by supercritical carbon dioxide fluid ($scCO_2$). *Journal of Nanoscience and Nanotchnology*, Submitted.

Scientific Presentations

1. Manosroi A., **Ruksiriwanich W.,** Abe M., Manosroi W. and Manosroi J. "*In vivo* hair growth promotion activity of gel containing niosomes loaded with the *Oryza sativa* bran fraction (OSF3)" Seminar in Nanotechnology for Health Science, Chiang Mai Hills Hotel, Chiang Mai, Thailand, February 27-29, 2012 (Oral presentation).

2. **Ruksiriwanich W.**, Manosroi J., Abe M., Manosroi W. and Manosroi A. "5α-reductase type 1 inhibition of *Oryza Sativa* bran extract prepared by supercritical carbon dioxide fluid." The 4th International Conference on Drug Discovery and Therapy. Dubai Men's College, Dubai, UAE, February 12 - 15, 2012 (Poster presentation).

3. Aranya Manosroi**, Warintorn Ruksiriwanich**, Masahiko Abe and Jiradej Manosroi. "Biological Activities of Edible Plant Extracts Prepared by Supercritical Carbon Dioxide Fluid and Ethanolic Maceration." RGJ-Ph.D. Congress XII. Jomtien Palm Beach Resort Pattaya, Chonburi, Thailand, April 1-3, 2011 (Oral presentation).

4. Aranya Manosroi**, Warintorn Ruksiriwanich** Bang-on Kietthanakorn, Worapaka Manosroi and Jiradej Manosroi. "Gelatinolytic activity on MMP-2 inhibition in aged human skin fibroblast of Thai medicinal flower extracts entrapped in niosomes." Traditional Thai medicine, indigenous medicine and alternative medicine in the Fifth National Thai Medicinal Plants Expo, Impact Muang Thong Thani, Nonthaburi, Thailand, September 1-3,2010 (Poster presentation).

5. Aranya Manosroi**, Warintorn Ruksiriwanich,** Hideki Sakai, Masahiko Abe, Worapaka Manosroi and Jiradej Manosroi. "Comparison of physical characteristic of niosomes entrapped with rice bran extract prepared by supercritical carbon dioxide fluid and conventional methods." Traditional Thai medicine, indigenous medicine and alternative medicine in the Fifth National Thai Medicinal Plants Expo, Impact Muang Thong Thani, Nonthaburi, Thailand, September 1-3,2010 (Poster presentation).

6. Aranya Manosroi**, Warintorn Ruksiriwanich**, Masahiko Abe and Jiradej Manosroi. "Biological Activities of Rice Bran Extract and Physical Characteristics of Its Entrapment in Niosomes Prepared by Supercritical Carbon Dioxide Fluid." RGJ

Ph.D. Congress XI. Jomtien Palm Beach Resort Pattaya, Chonburi, Thailand, April 1-3, 2010 (Poster presentation).

7. Aranya Manosroi**, Warintorn Ruksiriwanich**, Masahiko Abe and Jiradej Manosroi. "Characteristics comparison of niosomes entrapped with rice bran extracts prepared by supercritical carbon dioxide fluid technique and the conventional method." The Fifth Conference of Chiang Mai University "University of Excellence Where Nature Nurtures a Beautiful Intelligence", Chiang Mai University, Chiang Mai, Thailand, November 26-27, 2009 (Poster presentation) / German-Thai Symposium on Nanoscience and Nanotechnology 2009, Chiang Mai Orchid Hotel, Chiang Mai, Thailand, September 21-22, 2009 (Oral presentation).

8. Aranya Manosroi**, Warintorn Ruksiriwanich** and Jiradej Manosroi. "Comparison of antioxidative and tyrosinase inhibition activities of Thai medicinal plant extracts prepared by supercritical carbon dioxide fluid technique and ethanolic maceration." The Fifth Conference of Chiang Mai University "University of Excellence Where Nature Nurtures a Beautiful Intelligence", Chiang Mai University, Chiang Mai, Thailand, November 26-27, 2009 (Poster presentation) / RGJ Seminar Series LXII, Faculty of Science, Chiang Mai University, September 16, 2009 (Oral presentation) / Traditional Thai medicine, indigenous medicine and alternative medicine in the Sixth National Thai Medicinal Plants Expo, Impact Muang Thong Thani, Nonthaburi, Thailand, September 2-6, 2009 (Poster presentation).

9. Aranya Manosroi**, Warintorn Ruksiriwanich** and Jiradej Manosroi. "Free radical scavenging and tyrosinase inhibition activities of fermented Thai rice for cosmeceuticals." The Fourth Conference of Chiang Mai University "Research Path Innovation for Life", Chiang Mai University, Chiang Mai, Thailand, December 19-20,

2008 (Poster presentation) / The Second International Conference on Natural Products for Health and Beauty (NATPRO), Naresuan University, Phayao, Thailand, December 17-19, 2008 (Poster presentation) / Traditional Thai medicine, indigenous medicine and alternative medicine in the Fifth National Thai Medicinal Plants Expo, Impact Muang Thong Thani, Nonthaburi, Thailand, September 3-7,2008 (Oral presentation) / The Eighth National Seminar on Pharmaceutical and Biotechnology "Practical Seminar : Entrapment of aromatic volatile oil in nanoparticle for cosmetics and preparation of aromatic volatile oil from Thai Medicinal plants for massage oil in SPA business", Lotus Pangsaunkaew Hotel, Chiang Mai, Thailand, August 13-15, 2008 (Poster presentation) / RGJ-Ph.D. Congress IX, Jomtien Palm Beach Resort Pattaya, Chonburi, Thailand, April 4-6, 2008 (Poster presentation).

10. Jiradej Manosroi, **Warintorn Ruksiriwanich**, Warangkana Loecharoenkan and Aranya Manosroi. "Development of cosmetics containing herbal extracts entrapped in nanoparticles for hair growth promotion and hair loss treatment." Thailand Innovation Awards. Central Department Store, Ladproa, Bangkok, Thailand, September, 2005 (Poster presentation) / Industrial and Research Projects of Undergraduate Students (IRPUS), MCC Hall ,The Malls Department Store, Ngarm Wongwan, Bangkok, Thailand, June, 2005 (Poster presentation).