CHAPTER II

EXPERIMENTAL

2.1 Materials and Chemicals

Ethyl cellulose (Ethoxy content 48%, viscosity 100.00 cps.) purchased from Aldrich (Steinheim, Germany) was used as encapsulant. Ethanol (EtOH), analytical grade reagent was purchased from Merck (Darmstadt, Germany). Citronellal was purchased from Acros Organics (Geel, Belgium). Di(hydrogenated tallow)dimethyl ammonium chloride (Arquad 2HT-75) and Ditallow dimethyl ammonium chloride (Armosoft L) were purchased from Akzo Nobel Chemicals (The Gemini, Singapore) and were used as cationic surfactants. Sodium lauryl ether sulfate (27 % active substance) (Texapon N 8000) and sodium lauryl ether sulfate (70 % active substance) (Texapon N 70) were purchased from Cognis (Bangkok, Thailand). Ammonium lauryl sulfate (EMAL AD-25) was purchased from Kao (Bangkok, Thailand) was used as anionic surfactants. Nonyl phenol ethoxylate (ECOLAT NP6) was obtained from Ecogreen Oleochemicals (Alfa Centre, Singapore) while polyoxyethylene (20) sorbitan monooleate (Tween 80) was purchased from Croda Chemicals, Ltd (East Yorkshire, England).

2.2 Instrument and Equipment

Exterior and interior morphology of all nanoparticles obtained in this research were characterized by scanning electron microscopy (SEM, JEOL JSM-6400) and Transmission electron microscopy (TEM, JEOL JEM-2100). For SEM, a drop of the particles was loaded onto a glass slide, dried overnight and coated with gold under vacuum at 15 kV for 90 min. Then the SEM image was obtained using 15 kV. TEM was acquired at an accelerating voltage of 100-120 kV in conjunction with selected area electron diffraction (SAED).

The particle size and particle size distribution of essential oil encapsulated nanoparticles were determined by dynamic light scattering technique (DLS) using Matersizer S and Zetasizer nanoseries (Mulvern Instrument, Worcestershire, UK). The results were an average values from triplicate measurements.

The turbidity of citronellal-encapsulated suspensions was examined by turbidimeter (HACH, Model HACH 2100N, Scientific Promotion Co., Ltd).

Headspace GC-MS analysis was carried out on the Agilent 6890N/5975i GC-MS equipped with a headspace oven of Agilent 7694 (Agilent Technologies, Wilmington, DE). GC was equipped with a Stabilwax 10654 (Fused silica) column (30 m x 0.32 nm ID x 1.0 μm film thickness, Restek, USA.).

2.3 Preparation of Essential oil-encapsulated nanoparticles

Two processes were experimented and compared.

2.3.1 Solvent coagulation method under stirring condition.

Ethyl cellulose (20 g) was dissolved in ethanol (800 ml) at 70°C. Twenty milligrams of citronellal oils were mixed into the cool polymer solution (1:1 of oil: polymer). Then water (1.2 liters) was slowly dropped into the solution mixture at the rate of 1 ml/min under stirring rate of 600 rpm. The final concentration of active ingredient in the obtained suspension was 10,000 ppm. Particle morphology of the obtained suspension was characterized by SEM and TEM. The particle size distribution was analyzed by DLS.

2.3.2 Solvent coagulation method under ultrasonic condition.

Encapsulation of citronellal was carried out using a similar procedure to that described in 2.3.1 excepted that during the water addition no stirring was used but the liquid was ultrasonicated (250 kHz) at 30°C. Particle morphology was characterized by SEM and TEM. The particles size distribution was analyzed by DLS.

2.4 Compatibility of various surfactants to nanoparticles

Preparation of the mixture between citronellal encapsulated nanoparticles and various surfactants.

To study compatibility of the obtained particles with surfactants, the citronellal-encapsulated spheres (concentration of citronellal = 10,000 ppm) were prepared using procedure in 2.3.2. Then, the mixtures of the obtained suspension and each surfactant (Arquad 2HT-75, Armosoft L, Texapon N 8000, Texapon N 70, EMAL AD-25, ECOLAT NP6 or Tween 80) were prepared (Table 2.1) and their physical appearances were periodically observed. Formulations were prepared at four final concentrations of surfactants (3, 5, 7 and 10 % wt). The surfactant (see exact amount in Table 2.1) was first diluted with deionized water (50 ml) with continuous stirring at 600 rpm. One hundred milliliters of 10,000 ppm citronellal-encapsulated particle suspension were mixed into the diluted surfactant solution and the final volume was adjusted to 200 ml with deionized water. The obtained mixtures (see Table 2.1) were kept in the walk-in humidity chamber (Model Nec 2640RSI, Conversant Technology Co., Ltd) at 25^{o}C, 65% RH for a period of 5 weeks. The samples were pulled from the specific condition and their physical appearances were observed daily. Samples were them immediately put back into stability chambers.

Table 2.1: Mixtures between encapsulated nanospheres and various surfactants.

Surfactant	Approximately weight of surfactant (g) and abbreviation of mixture various final concentration of surfactant			
	3 % w/v	5 % w/v	7 % w/v	10 % w/v
Arquad 2HT-75	4.0 ARQ3	6.7 ARQ5	9.3 ARQ7	13.3 ARQ10
Armosoft L	6.0 AML3	10.0 AML5	14.0 AML7	20.0 AML10
Texapon N 8000	11.1 TXN3	18.5 TXN5	25.9 TXN7	37.0 TXN10
Texapon N 70	4.3 TN73	7.1 TN75	10.0 TN77	14.3 TN710
EMAL AD-25	11.1 EMA3	18.5 EMA5	25.9 EMA7	37.0 EMA10
ECOLAT NP6	3.0 ECO3	5.0 ECO5	7.0 ECO7	10.0 ECO10
Tween 80	3.0 TWN3	5.0 TWN5	7.0 TWN7	10.0 TWN10

2.5 Fabric softener formation

2.5.1 Preparation of fabric softener using free fragrance

Six grams of citronellal oil were mixed into the surfactant (see exact amount in Table 2.2) with continuous stirring at 600 rpm (heat at 40°C to melt fat in Arquad 2HT), and the final volume was adjusted to 200 ml with deionized water. The abbreviation of the obtained fabric softeners with free fragrance were FARQ5, FARQ7, FARM5 and FARM7

2.5.2 Preparation of fabric softener using encapsulated sphere

Citronellal-loaded spheres (prepared as described in 2.3.2) with the final citronellal concentration of 20,000 ppm was used.

Four grams of free citronellal were mixed into the surfactants (see exact amount in Table 2.2) with continuous stirring at 600 rpm, then diluted with deionized water to 100 ml. A hundred milliliters of citronellal-encapsulated spheres were mixed into the obtained solution and the final volume was adjusted to 200 ml with deionized water. The abbreviation of fabric softener with citronellal-encapsulated spheres samples were EARQ5, EARQ7, EARM5 and EARM7.

Table 2.2 Amount of surfactant, encapsulated sphere (20,000 ppm stock) and free citronellal oil used for 200 ml of mixture

Mixture	Surfactant	Concentration of surfactant (%w/v)	Weight of surfactant (g)	Citronellal encapsulated nanosphere (ml)	Free Citronellal (g)
FARQ5	Arquad 2HT-75	5	13.3	-	6.0
FARQ7	Arquad 2HT-75	7	18.7	-	6.0
FAML5	Armosoft L	5	20.0	-	6.0
FAML7	Armosoft L	7	28.0	-	6.0
EARQ5	Arquad 2HT-75	5	13.3	100	4.0
EARQ7	Arquad 2HT-75	7	18.7	100	4.0
EAML5	Armosoft L	5	20.0	100	4.0
EAML7	Armosoft L	7	28.0	100	4.0

2.5.3 Stability study of fabric softening

The obtained fabric softener samples were kept at 25°C, 30°C, 40°C and under three freeze/thaw three cycles. Their appearances were visually observed.

For long term stability test, accelerating condition at raised temperature was used. The obtained fabric softeners were kept in walk-in humidity chambers (Model Nec 2640RSI, Conversant Technology Co., Ltd) at 25°C, 30°C and 40°C. At appropriate times (2, 4, 8, 12, 24, 32 and 48 weeks),

samples were pulled from the specific condition and allowed to stand at ambient temperature for 30 min and their physical appearances were examined. After examination, samples were put back into the stability chamber for further investigation.

For freeze/thaw stability test, the obtained fabric softener mixtures were kept in the freezer for 48 hrs. The mixtures were then taken out from the freezer to room temperature and allowed to stand at room temperature for 48 hrs. Then the samples were put back to the freezer again for 48 hrs and then pulled out from the frozen to room temperature for 48 hrs. The samples were freeze and thawed for three cycles and their physical appearances were examined.

2.6 The release of essential oil from essential oil-encapsulated nanospheres in fabric softener

The release profile of citronellal in fabric softener prepared using the obtained encapsulated spheres was determined with the aid of a headspace gas chromatography-mass spectrometry (Headspace GC/MS). The profile of fabric softener with free fragrance was also determined and used for comparison.

2.6.1 Preparation of headspace samples

One gram of the obtained fabric softeners (from procedure 2.5.1 and 2.5.2) was diluted with deionized water and the final volume was adjusted to 10 ml.

The headspace samples were prepared by weighing one gram of cut cotton fabric in to 20 ml flat bottom headspace vial, then added 1.0 ml of the obtained diluted fabric softener. The vials were kept open at room temperature. Then each sample was capped with head space aluminum crimp caps with PTFE/silicone septa (Agilent technologies, USA) at appropriate time (0, 1, 3, 5, 10, 15, 20 and 30 days).

2.6.2 Headspace GC-MS

The obtained capped samples were then subjected to headspace-GC-MS analysis. Samples were heated in headspace oven at 100°C for 20 min. The headspace volatile gas was filled in the loop for 1.0 min and automatically transferred to GC through transfer line which was held at 110°C. Gas sample was held in the transfer line until reaching an equilibrium (0.3 min) before being flushed into GC injection port for 2 min. The inlet temperature was 240°C. Helium (ultra high purity grade, 99.9999%, Thai industrial gas Co., Ltd, Thailand) was used as a carrier gas under a constant flow rate of 1.0 ml/min in a split mode (split ratio of 20:1). The initial temperature of oven was set as follow: 90°C held for 3 min then ramped by 10°C/min to 220°C, held for 2 min and increased to 230°C, held for 1 min. The mass spectrometer was

operated in electron ionization (EI) mode (70 eV), ion source temperature of 250°C, under total ion scan mode during the preliminary study to identify peaks and SIM mode during the quantitative analysis of samples. Quantification was obtained from peak area from the chromatogram.

Standard stock solution of citronellal was prepared in ethanol (10 g/L) and this stock was diluted with ethanol from stock solution to give solutions with the 500, 1,000, 3,500 and 5,000 g/L. One milliliter of these calibration solutions was loaded onto one milligram of cut cotton resided in a 20 ml flat bottom headspace vial and immediately capped with headspace aluminum crimp cap. Standard calibration solutions were subjected to similar headspace GC/MS analysis. Calibration curve was constructed by plotting the peak area of citronellal with concentration.