CHAPTER III

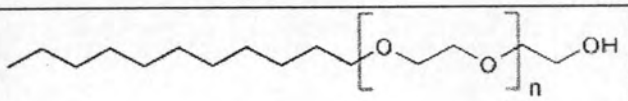
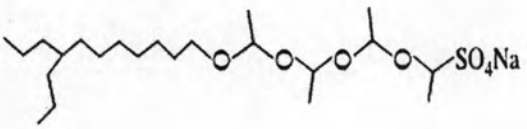
METHODOLOGY

3.1 Material

3.1.1 Surfactants

Two types of surfactant utilized in this research were nonionic surfactant (Fatty alcohol C 12-14 Ethoxylate) and anionic surfactant (Alfoterra 145-4PO). Fatty alcohol C 12-14 Ethoxylate nonionic surfactant was supported by PTT Chemical Public Co., Ltd. while Alfoterra 145-4PO was obtained from Sasol Co., Ltd. and contain 34.9% active surfactants. Chemical structure of these surfactants are shown in Table 3.1.

Table 3.1 Chemical structures of surfactant

Chemical name	Structure	Type
Fatty alcohol C 12-14 Ethoxylate		Nonionic
Propoxylate sulfate with branched C14-C15 alkyl 4 Propoxy group (Alfoterra 145-4PO)		Anionic

3.1.2 Jatropha seed

Jatropha seeds used in this study were purchased from Thai Jatropha Oil Co., Ltd. and were supplied from PTT Chemical Public Co., Ltd.

3.1.3 Jatropha oil

Jatropha oil used in this study was purchased from Thai Jatropha Oil Co., Ltd. and partly supported by PTT Chemical Public Co., Ltd.

3.1.4 Electrolytes

The electrolyte used in this research was sodium chloride, analytical grade with 99% purity, purchased from Lab Scan Co., Ltd.

3.1.5 Solvents

Solvents used in this research were hexane, methanol and acetronitrile. Hexane, analytical grade with 99% purity, was purchased from Lab Scan Co., Ltd. Methanol (C_2H_5OH), HPLC grade with 99% purity was purchased from Carlo Erba Reagent Co., Ltd. Acetronitrile, HPLC grade with 99% purity was purchased from Lab Scan Co., Ltd.

3.1.6 Dyed oil

Oil red O (solvent Red 27, CI No. 261265) was purchased from Aldrich Chemical Company, Inc. It was used for preparing the dyed oil.

3.1.7 Water

Ultra pure water was used throughout this research for preparation of mixed surfactants aqueous-based solution and other chemical solutions. Furthermore, it was used as rinse water and glassware cleaning.

3.1.8 External standard for HPLC analysis

The external standard used for phorbol esters analysis was 12-o-tetradecanoyl-phorbol-13-acetate (TPA) purchased from Lab Scan Co., Ltd.

3.2 Methodology

In order to achieve the objectives of this work, experimental design was divided into six parts as explained below.

3.2.1 Microemulsion formation and phase study

The microemulsion transition was conducted by using salinity scan for selected mixed surfactants systems. Oil and surfactant ratio at unity was carried out in flat-bottomed vial 15 mL by containing 5 mL each. Then the experimental vials were gently shaken in order to form microemulsion and were left for equilibrium at room temperature. Since the oil and surfactant have similar color, the oil was dyed with the O-red dye before using in order to easily distinguish the phase transition. The conditions of surfactant system with salinity scan are shown in Table 3.2.

Table 3.2 Surfactant systems for phase behavior study

No.	System	Salinity scan (%wt NaCl)
1	1%,3%,5% LS2 + 0.02%Alfoterra 145-4PO	0.2,0.4,0.6,0.8 and1.0
2	1%,3%,5% LS2 + 0.02%Alfoterra 145-4PO	0.2,0.4,0.6,0.8 and1.0
3	1%,3%,5% LS2 + 0.02%Alfoterra 145-4PO	0.2,0.4,0.6,0.8 and1.0
4	1%,3%,5% LS2 + 0.02%Alfoterra 145-4PO	0.2,0.4,0.6,0.8 and1.0
5	1%,3%,5% LS2 + 0.02%Alfoterra 145-4PO	0.2,0.4,0.6,0.8 and1.0

3.2.2 Raw material preparation

Jatropha seeds were carefully deshelled prior to grinding by mechanical grinder. After that, the ground Jatropha kernels were sifted using 8 mesh and 35 mesh (size between > 3.6 mm – 0.425 mm) for grain size selection. Then the ground kernels were cooked in the oven at temperature of 105 °C for 90 min for dehydration and oil yield enhancement. Lastly, the cooked kernels are kept in a desicator for further experiments. For Jatropha oil obtained from compress extraction, the oil was filtrated for small particulates removal and then the oil was kept in amber Duran bottle and stored at room temperature.

3.2.3 Jatropha oil extraction

The main method of this study was extraction by mixed surfactants aqueous-based solution; however, Soxhlet extraction which is conventional method was also conducted for the comparison of oil yield, oil quality and concentration of phorbol esters.

3.2.3.1 Soxhlet extraction using Hexane as solvent

Jatropha kernels were extracted with hexane by the Soxhlet method in order to investigate the extraction efficiency and compared with extraction by mixed

surfactants aqueous-based solution. The experimental study followed the US EPA method 3540C. After oil extraction by the Soxhlet, the solvent was evaporated and the solely oil was left in the Soxhlet. The weight of extracted oil was measured to determine the yield of extraction and referred as the total oil contained by weighting of kernels.

3.2.3.2 Mixed surfactants aqueous-based solution extraction

The extraction study was set up by weighing 1 g of Jatropha kernels in the flat-bottomed screw-capped vial 15 mL and then added 10 g of mixed surfactants aqueous-based solution from the selected systems with different salinity scan. Then the vials were brought to shake using an orbital shaker at 130 rpm for 1 min for complete mixing of kernels and mixed surfactants aqueous-based solution. After shaking, the vials were brought to separate using centrifuge at 2500x for 20 min. The microemulsion layer was then measured using cathetometer and the supernatant which was excess or free oil layer were measured and calculated for oil yield using pasture pipette and kept for further determination.

3.2.4 Oil quality investigation

As mentioned in the scope of study, the quality of extracted oil was also concerned in order to promote this method for application. The concerned parameters for oil quality were fatty acid composition and water content which were measured by GC-FID and Karl Fisher Titration coulometer, respectively. Besides these two parameters, the surfactants concentrations in aqueous phase were determined by UV-visible spectrophotometer and using titration method ASTMD1618-05 for nonionic and anionic surfactants, respectively. The surfactants concentration in aqueous phase was expected to indirectly indicate amount of surfactants partitioning into oil phase and to evaluate the surfactant loss during extraction process.

3.2.5 Phorbol esters determination

The phorbol esters determination procedures followed Hass and Mittelbach (2000) method using TPA as the external standard.

3.2.6 Analytical methods

3.2.6.1 GC-FID

GC-FID is the common technique method for analysis of fatty acids after their derivatization from nonvolatile fatty acid chemically converted corresponding to volatile methyl esters. Due to the limitation of the instruments, the oil samples were analyzed by Food Research and Testing Laboratory, Faculty of Science, Chulalongkorn University.

3.2.6.2 Spectrophotometer

Iodine-Iodide method was used for ethoxylate nonionic surfactant analysis (Baleux, 1972). Briefly, 0.25 mL of KI_3 solution (2% KI and 1% I_2) was added into 10 mL aqueous sample. After 5 minutes, the optical absorption at 500 nm was measured by UV-visible spectrophotometer.

3.2.6.3 HPLC

HPLC is the technique for analysis of phorbol esters in the extracted Jatropha oil. The sample preparation was done by weighing 10 g of Jatropha oil extracted with 10 g of methanol each four times. Then the combination of extracts was brought to centrifuge. After that, the extractant was transferred to make up volume by methanol to be 100 mL in a volumetric flask. As aforementioned, HPLC analysis condition followed Hass and Mittelbach (2000) as the analytical column used was reversed

column octadecyl as the functional group, temperature was controlled at 25°C. Isocratic acetonitrile:water ratio 80:20 (v/v) at the flow rate of 1 ml/min was set up for mobile phase. The detector used in this method was UV adsorption detector at wavelength 280 nm with sample injected volume 20 µL. TPA was used as the external standard dissolved in methanol for preparing calibration curve.

3.2.6.4 Titration methods

ASTMD1681-05

ASTMD1681-05 is the method for determination concentration of Alfoterra in aqueous phase. The method was a titration of sample with cationic surfactant, CTAB 0.005 M for quantifying Alfoterra, anionic surfactant concentration in the sample.

Karl Fischer Titration

Karl Fischer coulometry is a micro-method and is particularly suitable for samples with low water content, from 10 µg up to 10 mg. Here, the required iodine is generated electrochemically in the titration vessel by anodic oxidation from iodide contained in the coulometric reagents. The amount of consumed electric charge is used to calculate the consumption of iodine and therefore the amount of water in the sample. However, due to the limitation of the instruments, the oil samples were sent to analyze by the Petroleum and Petrochemical College, Chulalongkorn University.

3.3 Quality control

To control the quality throughout the experiment, the quality control system was also implemented. The control protocol included the source of Jatropha seeds, Jatropha oil and surfactants. The experiments have done in triplication in room

temperature, the utilized instruments were the same set and well cleaned and rinsed by DI water before used.