

## CHAPTER III

### EXPERIMENTAL SECTION

#### 3.1 Chemicals

Sodium dodecyl sulfate (SDS) (99%) was used as received from Sigma (St. Louis, MO). Fatty acids with alkyl chain length, *n*, ranging from 3 to 7, were obtained from Fluka (Allentown, PA), except for pentanoic acid, which was obtained from Merck (Darmstadt, Germany). Selected properties of the fatty acids evaluated are listed in Table 3.1. Hexanes (100% as total isomer) was purchased from Mallinckrodt (Paris, KY). NaCl (99.9%) was obtained from Ajax (Auburn, New South Wales, Australia) and was thermally dried before use. Methanol (99.9%), used as a mobile phase for the HPLC, was used as received from Merck (Darmstadt, Germany).

**Table 3.1** Properties of fatty acid used in the study.

Properties Chemicals	Purity	Molecular weight	Density (g/mL)
Propanoic acid	≥99%	74.08	0.992
Butyric acid	≥99%	88.11	0.958
Pentanoic acid	>99%	102.13	0.940
Hexanoic acid	≥98%	116.16	0.927
Heptanoic acid	>98%	130.19	0.917

## 3.2 Experimental Procedures

### 3.2.1 Phase Diagram of Microemulsion

Microemulsion systems were prepared in 15 mL glass centrifuge tubes by keeping oil/brine (1:1, by vol) and SDS/fatty acid (40:60, by wt) ratios constant. SDS and NaCl were added into the centrifuge tubes followed by water to adjust the aqueous phase volume to 5 mL. The mixtures were added up to 10 mL by fatty acid and hexanes. The tubes were capped with septa and aluminum caps to prevent volatilization. The solutions were well mixed by using vortex mixer (Genie-2; Scientific Industries, Bohemia, NY) and allowed to equilibrate in water bath at 25°C for 2 weeks. Microemulsion formation was visually observed. To investigate a phase diagram, the microemulsions were systematically prepared by scanning salinity at different SDS concentrations. Systems equilibrated at 25°C for two weeks were further evaluated for solubilization enhancement and other physicochemical properties.

### 3.2.2 Effect of Temperature on Equilibrium of Microemulsion

Equilibrium of the systems was studied by increasing the temperature of the system to 85°C at some selected conditions (10.2% and 21.6% SDS for propanoic acid system, 7.8% and 18.0% SDS for butanoic acid system, 3.6% and 9.6% SDS for pentanoic acid system, 2.4% and 9.6% SDS for hexanoic acid system, and 2.4% and 6.0% SDS for heptanoic acid system). The solutions were kept at 85°C for a week. The temperature was then lowered everyday with a step of 15°C until the temperature reached 25°C. The change of the phase equilibrium was visually observed and recorded.

### 3.2.3 Solubilization Study

After being equilibrated at 25°C for two weeks, phase volumes were determined to allow calculation of the solubilization parameter, SP (milliliters of oil or water solubilized in the microemulsion per gram of SDS).

#### 3.2.4 pH Measurement of Microemulsion

After being equilibrated at 25°C, pH of Winsor type I, III and IV of the systems containing C<sub>3</sub>-C<sub>7</sub> fatty acids at the selected conditions (10.2% and 21.6% SDS for propanoic acid system, 7.8% and 18.0% SDS for butanoic acid system, 3.6% and 9.6% SDS for pentanoic acid system, 2.4% and 9.6% SDS for hexanoic acid system, and 2.4% and 6.0% SDS for heptanoic acid system) was determined by using pH paper with pH increment of one. The microemulsion phase was dropped on the pH paper and the color change was compared with the standard color in order to determine the pH of microemulsion visually. The pH measurement of microemulsion cannot be measured directly by using a pH meter and pH of deionized water used to prepare microemulsion was 5.62. Therefore, calculation of pH by using pK<sub>a</sub> and aqueous solubility of fatty acid was performed in order to compare to the experimental results.

#### 3.2.5 Measurement of Microemulsion Conductivity

Conductivity was measured at 25°C using an Alltech 550 conductivity meter (Deerfield, IL) attached to HPLC (HP 1050; Hewlett Packard, Wilmington, DE). The column was bypassed to allow the sample to flow directly to the detector. The HPLC mobile phase was methanol at a flow rate of 1.00mL/min. A conductivity calibration curve of KCl plotted between the conductivity and peak area was used to determine the microemulsion conductivity. The conductivity of KCl solutions was measured using an Orion 124 conductivity meter (Boston, MA) connected to a conductivity cell 012210.

### 3.2.6 Measurement of Microemulsion Interfacial Tension

Densities of microemulsions, excess oil and excess water phases were determined by weighing an exact volume of the solutions at 25°C. Interfacial tension (IFT) between a microemulsion phase and excess oil and/or excess water phases at 25°C was determined by utilizing a spinning drop tensiometer (SITE 04; Kruss, Hamburg, Germany). The drop size and the number of revolutions per minute were read at an equilibrium state where there was no variation of drop size relative to time. The interfacial tension was calculated by using the following formula in the instrument's manual:

$$\sigma = e(vd)^3 n^2 \Delta\rho \quad (3.1)$$

where  $d$  is the drop diameter,  $n$  is the number of revolutions,  $e$  is a unit factor given by  $e = 3.427E-7 \text{ (mN cm}^3 \text{ min}^2)/(\text{m gr mm}^3)$ , and  $v$  is enlargement factor of lenses, for this study  $v = 0.31 \text{ mm sdv}$ .