CHAPTER III
EXPERIMENTAL

3.1 Materials

Starting chemicals used in this research are glycerol (bidistilled 99.5% w/v) was purchesed from VWR international Inc., USA; acetone was purchased from Mallinckrodt Baker Inc., Phillpsburg, NJ, USA.; and refined palm oil which consists of triglycerides as main component was purchased from Morakot Industries Public Company Limited, Thailand. Neutralizing agents used in this research are sodium carbonate anhydrous and acetic acid were purchased from Carlo Erba Reagent, Italy. Solvents used in this research are chloroform and ethanol were purchased from Carlo Erba Reagent, Italy. Homogeneous catalysts used in this research are *p*-toluenesulfonic acid monohydrate was purchased from Carlo Erba Reagent, Italy; and sodium hydroxide was purchased from Labscan, Thailand. Heterogeneous catalyst used in this research is Dowax®M-31 (wet) ion-exchange resin was purchased from Sigma-Aldrich, Singapore. Gas used in this research are Nitrogen gas was obtained from Praxair with purity 99.99%.

3.2 Equipment

3.2.1 Reactor

3.2.1.1 Reactor for protection and deprotection step

A 250-ml three-necked flask is eqipped with a reflux apparatus for recovering the volatile substances and a thermometer was used in the experiment. The oil bath with heater was used to heat up and the temperature was digitally controlled. The experimental set-up was shown in Figure 3.1

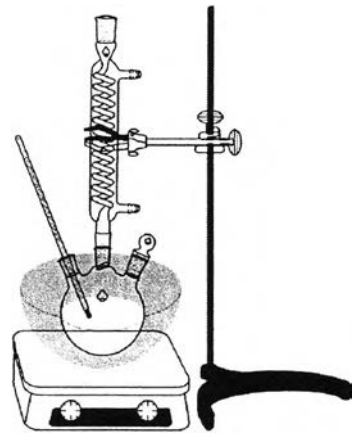

Figure 3.1 Experimental set-up used for protection and deprotection step.

3.2.1.2 Reactor for transesterification step

A 250-mL three-necked flask is equipped with a condenser, a thermometer and the a sampling port was used in the experiment. The oil bath with heater was used to heat up and the temperature was digitally controlled. The nitrogen gas is used to purged the system and provide the inert atmosphere during reaction. The experimental set-up was shown in Figure 3.2.

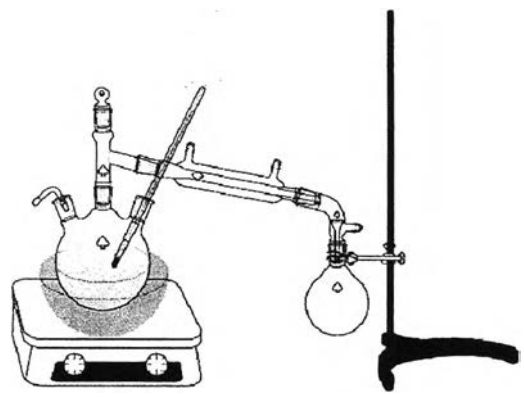

Figure 3.2 The experimental set-up used for transesterification step

3.2.2 Fourier Transform Infrared Spectroscopy (FTIR: Thermo Nicolet Nexus 670)

3.2.3 High Performance Liquid Chromatography (HPLC : Perkin Elmer LC200 with Reflective index and Ultraviolet detectors; SphereClone 5u ODS(2): 150×4.6 mm)

3.2.4 Gas Chromatography (GC: Agilent 6890N with Flame Ionization detector ; Stabilwax Capillary Column: 30 m × 530 μm × 1.0 μm)

3.2.5 Nuclear Magnetic Resonance Spectrometer (NMR 500 MHz: Varian model INOVA with CP/MAS solid probe and nano probe)

3.3 Methodology

3.3.1 Monoglycerides Synthesis

3.3.1.1 Protection of 2-adjacent hydroxyl groups on glycerol or introducting of isopropylidene group on glycerol

A mixture of glycerol (60 g), acetone (72 g), and *p*-toluenesulfonic acid (2 g) in a ratio by weight is refluxed at 80 °C for 16 hours. When the reaction is completed, the mixture of products is left untill it reach to ambient temperature. Then, Na_2CO_3 (2.25 g) is added for neutralization. The reaction mixture is purified by two-steps distillation: the first distillation had been done under atmospheric condition to remove the excess acetone and then another distillation had been done under vacuum (10 mmHg) condition in order to obtain the protected glycerol (1,2-O-isopropylidene glycerol). The final products are characterized by GC and FTIR and NMR techniques.

3.3.1.2 Transesterification of protected glycerol (1,2-O-isopropylidene glycerol) with triglycerides

Refined palm oil and protected glycerol (1,2-O-isopropylidene glycerol) with desired mass ratio; and NaOH (1 wt% based on protected glycerol) are added into the reaction flask. The mixture are stirred with 300 rpm of stirring speed and heated to 140 °C under nitrogen atmosphere for 5 hr. When the reaction completed and allow to reach the ambient condition, the acetic

acid is added for neutralization step. The complete transesterification produces two liquid phases: esters as an upper phase (oil-soluble phase), and protected glycerol as a bottom phase (aqueous phase). The mixture is left for 2 hours in order to the two phases can be separated completely. Then the two liquid phases are separated by using separatory funnel. The water is added to the ester phase for salt and others impurities removal, and the system was stirred for 5 min before separate aqueous phase out. Finally, the products are analyzed by FTIR and HPLC techniques.

The reaction parameters of transesterification are examined by varying the molar ratio of refined palm oil to protected glycerol (5:1, 7:1, 10:1, 13:1 and 17:1) and reaction temperature (120, 140, 160 and 180 °C)

3.3.1.3 Deprotection of protected monoglycerides

The protected monoglycerides (monoglyceride with isopropylidene group) from the previous step is added to EtOH 95%. The mixture is refluxed at 60 °C for 3 hours in the presence of Dowax®M-31 (wet) ion-exchange resin as catalyst. After the reaction is completed and the mixture is filtered. The final product is analyzed by FTIR and HPLC technique.

3.3.2 Product Analysis

3.3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

This technique provides a transmittance or absorbance spectrum of product showing at which IR wavelengths the product absorbs. the molecular structure of the products can be provided by their absorption characteristics which is performed by FTIR spectrophotometer, Thermo Nicolet NEXUS 670 FTIR.

3.3.2.2 Gas Chromatography (GC)

This technique is used only for protected glycerol analysis. The sample is diluted in water with known concentration and injected in to gas chromatograph, Agilent 6890N GC, with under the condition as shown below:

- Column: Stabilwax Capillary Column(30 m × 530 μm × 1.0 μm)
- Oven temperature: 80 °C to 200 °C at 8 °C/min. (hold 10 min.)
- Detector: Flame Ionization detector at 270 °C
- Carrier gas: Helium

3.3.2.3 High Performance Liquid Chromatography (HPLC)

Analysis of the products is performed by using high-performance liquid chromatography (HPLC), a Perkin Elmer Series 200 LC-pump with ultraviolet detectors. The system is controlled by a PC with a software package (Perkin Elmer Turbochrom Navigator).The 2 columns of SphereClone 5u ODS(2) column (4.6 mm×150 mm×5 μm) are seried and the mobile phases—methanol (mobile A) and isopropanol-n-hexane (mobile B) (5:4)— are used in linear gradient from 100% A to 60% A and 40% B in 20 min, then hold at this condition for 20 min with a flow rate 0.8 ml/min. The column temperature is at ambient temperature (27°C). The pump pressure is operated in the range of 300 to 1800 psi.

3.3.2.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

Analysis of the products is performed by using nuclear magnetic resonance spectrometer: NMR 500 MHz: Varian model INOVA with CP/MAS solid probe and nano probe. The sample is prepared by diluting with $CDCl_3$.