CHAPTER III
EXPERIMENTAL METHOD

3.1 Materials

The *Jatropha curcas* seeds were obtained from the Nakhonratchasima Field Crops Research Center, Nakhonratchasima and the Office of Agricultural Research and Development Region 3 Khon Kaen, Thailand. The seeds were dried before oil extraction. The oil extraction was performed by the Khon Kaen Agricultural Engineering Research Institute, Khon Kaen, Thailand. The crude oil obtained was collected as shown in Fig 3.1.

Figure 3.1 The crude *Jatropha cuscas* oil

3.2 Chemicals and reagents

The chemicals and reagents in analytical reagent mainly used in this study are summarized in Table 3.1.

Table 3.1 The chemicals and reagents used in this experiment

Chemicals	Source	Grade
acetic acid glacial	J.T. Baker (USA)	AR grade
Acetone	Merck (Germany)	AR grade
Acetylacetone	Carlo Erba (Italy)	AR grade
activated charcoal	LabScan (Thailand)	AR grade
amberlite IRA-400 (Cl^- form)	Sigma-Aldrich (USA)	AR grade
amberlite 120 H^+ (H^+ form)	Sigma-Aldrich (USA)	AR grade
ammonium acetate	Carlo Erba (France)	AR grade
ethyl alcohol	Caro Erba (France)	AR grade
glycerine standard, 99.5%	Carlo Erba (Italy)	AR grade
Hexane	LabScan (Thailand)	AR grade
hydrochloric acid	Carlo Erba (France)	AR grade
methyl alcohol	Carlo Erba (France)	AR grade
phosphoric acid	J.T. Baker (USA)	AR grade
potassium hydroxide	Carlo Erba (Italy)	AR grade
sodium hydroxide	Carlo Erba (Italy)	AR grade
sodium periodate	Carlo Erba (Italy)	AR grade
sodium sulfate	Carlo Erba (Italy)	AR grade
sulfuric acid	LabScan (Thailand)	AR grade

3.3 Instrumentations

3.3.1 Gas chromatograph-mass spectrometer (GC-MS) system

Identification of composition in biodiesel from transesterification of *Jatropha curcas* oil was performed on a Trace GC chromatograph Model K07300000000010 (Italy) equipped with a Finnigan Polaris Q mass spectrometer (USA) and Combi Pal autoinjection version 1.5.2 with Xcalibur software version 1.3. An analytical column, a ZB-5 capillary column coated with 5% diphenyl and 95% dimethylpolysiloxane (30 m x 0.25 mm, 0.25 μm film thickness), Phenomenex (USA) was used for analysis.

3.3.2 Gas chromatograph-flame ionization detector (GC-FID) system

The quantitative determination of investigated glycerine was performed by a gas chromatograph, Model GC 17A, Shimazdu, (Japan), equipped with a flame ionization detector (FID) and with a DB-5 capillary column (30 m x 0.25 mm, 0.25 μm film thicknesss), J&W Scientific (USA), controlled by a CR7A Chromatopack integrator, Shimadzu, Japan.

3.3.3 UV-Vis Spectrophotometer

Absorption spectra were obtained with a spectrophotometer, Model Agilent 8453 UV-Vis Spectroscopy System (Germany). 1 cm quartz cell was used with this work.

3.3.4 FT-IR Spectrophotometer

This spectrometer, Spectrum one, Perkin Elmer, Germany, was performed using KBr pellet method.

3.3.5 Other equipments

Other equipments used for purification of residue glycerine in this work can be summarized in Table 3.2.

Table 3.2 The equipments for purification of residue glycerine

Type of equipment	Source
Analytical balance	Scaltech (Germany)
Filter paper (No.41)	Whatman International (UK)
Hot plate	Model MR 3001, Heidolph (Germany)
Low pressure rotary evaporator	Model R-114, Buchi (Switzerland)
Magnetic stirrer	SP 46920-26, Barustead Thermolyne (USA)
Micropipette (P20, P200 and P1000)	Pipetman (France)
Muffle furnace	M 110, Heraeus Instruments (USA)
Oven	D 06062 Model 600, Memmert Gmloh (UK)
pH meter	Model 1251, Denver Instrument, (USA)
Reverse osmosis water	RiOs^{TM} Type I Simplicity 185, Millipore Waters (USA)
Shaker	Model SA-31, Yamato Scientific (Japan)
Thermometer (0-100 ^{0}C)	Brannan (USA)
Vertex mixer	Model Genie 2, Scientific Industries (USA)
Water bath	Fisher Scientific (USA)

3.4 Experimental procedures

3.4.1 Production of residue glycerine from transesterification of *Jatropha curcas* oil

Weighed 100 g *Jatropha curcas* oil, was then transesterified mixed with a solution of 1 g NaOH in 22.5 g methanol to produce biodiesel (fatty acid methyl esters) and glycerine (modified from Foidl et al., 1996) (see Fig 3.2). The mixture was stirred gently at 70 ^{0}C and stirred at 200 rpm for 3 hour. The mixture was then transformed to a separatory funnel in order to separate the biodiesel and glycerine.

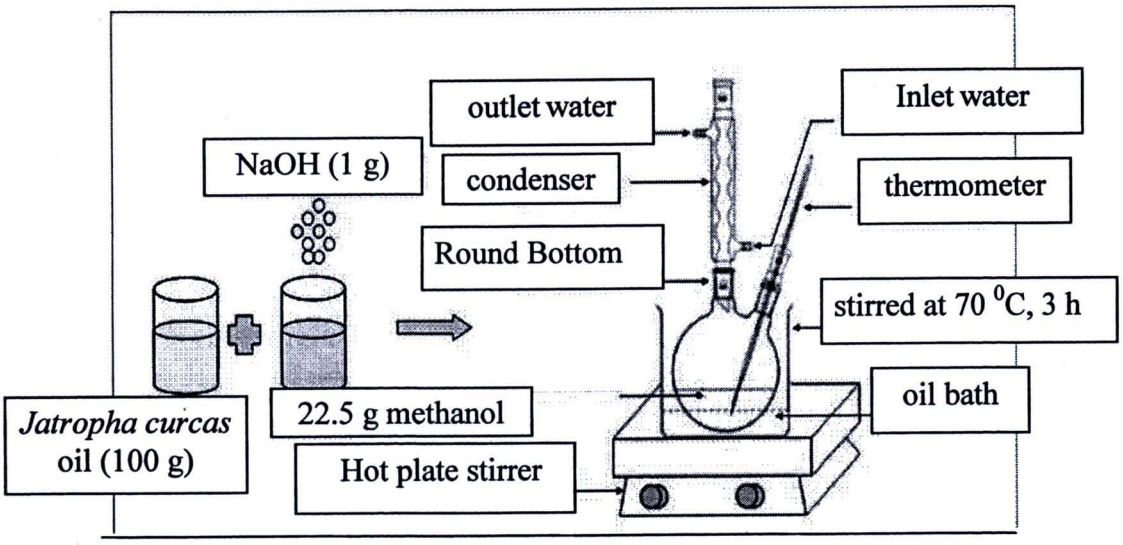

Figure 3.2 Production of glycerine residue from transesterification of *Jatropha curcas* oil (Foidl et al., 1996)

3.4.2 Study on the compositions of biodiesel obtained from transesterification of *Jatropha curcas* oil

The transesterification of oils was carried out by comparing temperature, base-catalytic, methanol percentage and some literatures as previous studies (Demirbas, 2002; Freedman et al., 1986; Freedman et al., 1984; Ma et al., 1998; Rashid & Anwar, 2008; Fukuda et al., 2001; Iso et al., 2001; Nelson et al., 1996; Nie et al., 2006; Shimada et al., 2002 Su, Wei, 2008 and Munari et al., 2003).

After biodiesel production, the glycerine layer (bottom layer) was removed and kept in a separated container. The biodiesel phase (top layer) was washed with warm water (50 ^{0}C) (Karaosmanoglu, 1996) to remove soap and glycerine trace. Until the pH of mixture were reached to 6-7. After that, water was removed by adding sodium sulfate. Finally, the mixture was further evaporated to remove solvent and redissolved with methanol.

The study on the compositions of biodiesel obtained was carried out by GC-MS technique under optimum conditions, as shown in Table 3.3. The initial column temperature was at 70 ^{0}C while the final temperature was at 325 ^{0}C. The injector temperature was at 280 ^{0}C. The temperature of transferred line was at 275 ^{0}C.

Helium gas was used as carrier gas with a flow rate of 1.0 mL/min. The injection volume was used 1.0 μL by with splitless injection mode. Mass spectra were recorded at ionization energy of 70 eV. The process for studying on the compositions of chemical *Jatropha curcas* oil by GC-MS can be summarized in Fig 3.3.

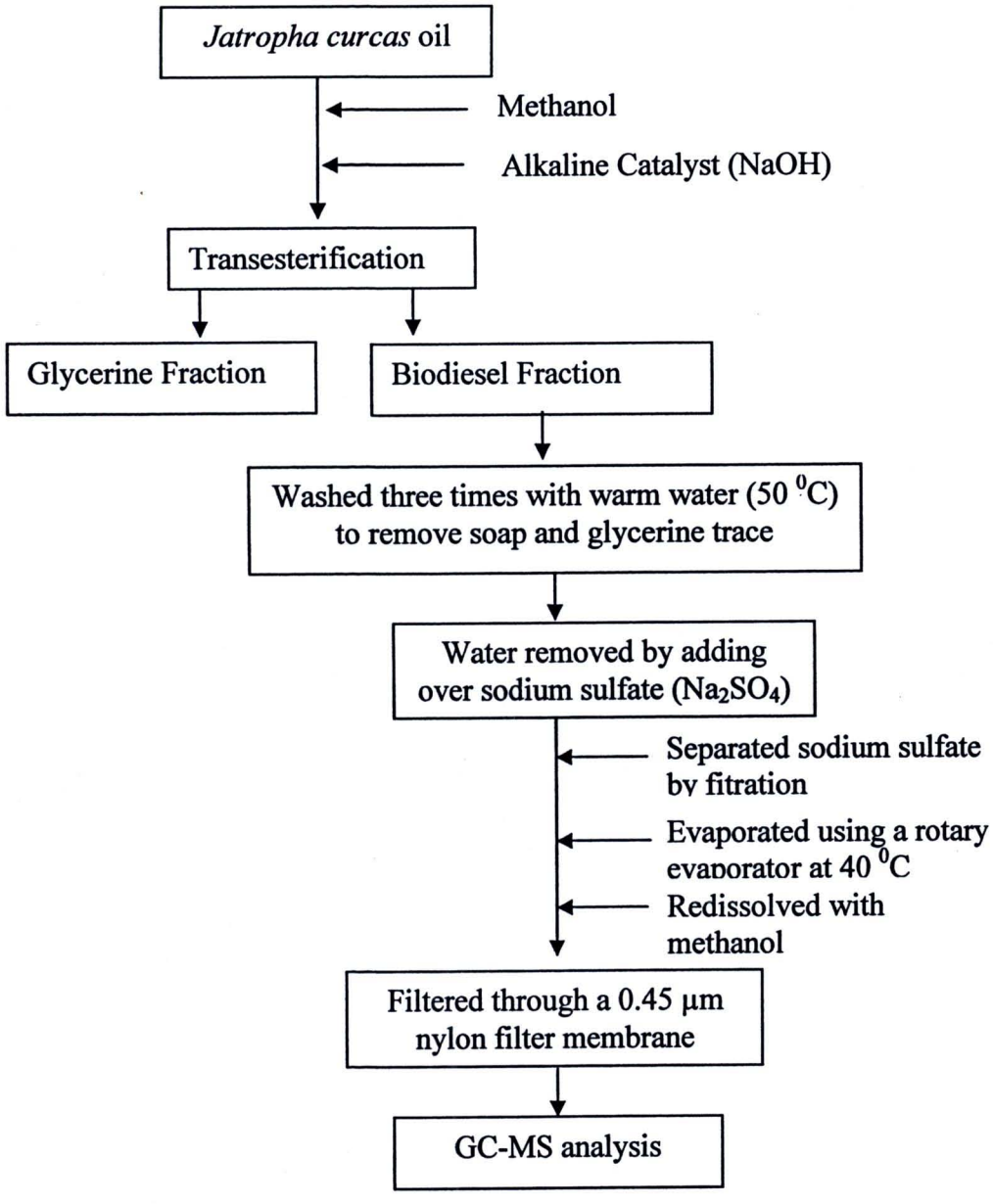

Figure 3.3 Schematic flow diagram of the process for study on the composition of biodiesel from transesterification of *Jatropha curcas* oil

Table 3.3 Chromatographic conditions for analysis of chemical components by GC-MS

System	Conditions
Column material	ZB-5 (5% phenyl – 95% dimethylpolysiloxane) Column (30 m x 0.25 mm, 0.25 μm film thickness)
Injection system	PTV splitless mode
Injector temperature	280 ^{0}C
Carrier gas	Helium
Flow rate	1 mL/min
Oven temperature	Initial temperature: 70 ^{0}C
	Final temperature: 325 ^{0}C
Injection volume	1 μL
Full scan MS	50-450 m/z
Transfer line temperature	275 ^{0}C
Ionization mode	Electron ionization (EI)

Figure 3.4 shows the temperature program profile used in this study. The GC oven temperature program for the separation of the components was used as following: 70-325^{0}C (4^{0}C/min). The identification of the components was carried out by comparing the retention times, mass spectra and literatures as used in previous studies (Su, Wei, 2008; Munari et al., 2003).

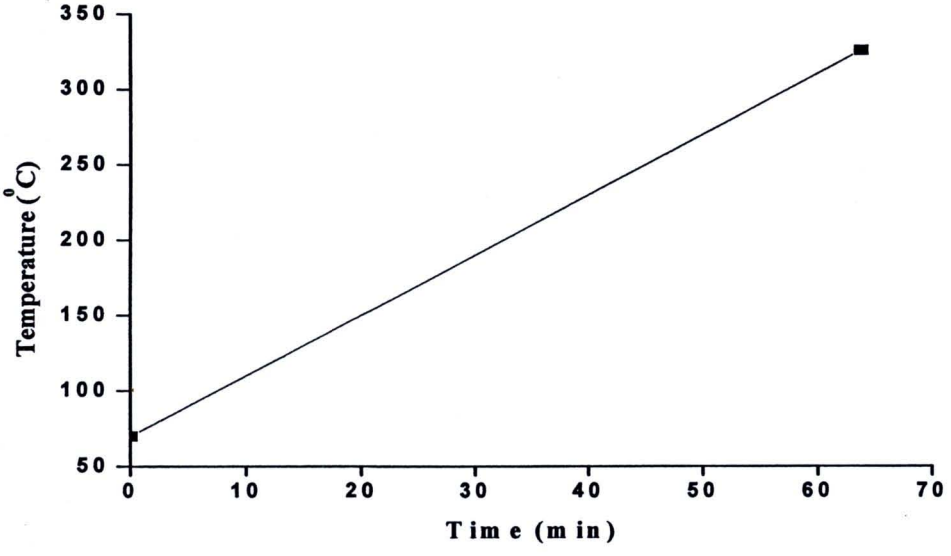

Figure 3.4 Temperature program profile for analysis of chemical components by GC-MS

3.5 Purification of residue glycerine from transesterification of *Jatropha curcas* oil

3.5.1 Purification of residue glycerine by conventional method

The conventional method was carried out followed by the report of Ooi et al. (2001). The isolation of impurity from residue glycerine was performed out by acidified, using 6% sulfuric acid to split the soap, and neutralize the residual NaOH. The residue glycerine was varied pH at 1, 2, 3, 4, 5 and 6. The charred substances obtained were filtered off. The sample was then decanted to recover the crude fatty acids, and the aqueous glycerine solution was neutralized using 50% sodium hydroxide. Subsequently, it was evaporated to concentrate the glycerine solution. The salt crystallization was removed by decantation. To purify and concentrate the solution in the next procedure, it was also extracted and filtered to remove the residual salt. Finally, the solution was evaporated to obtain the purified glycerine. The diagram of conventional method for purification of residue glycerine is shown in Figure 3.5.

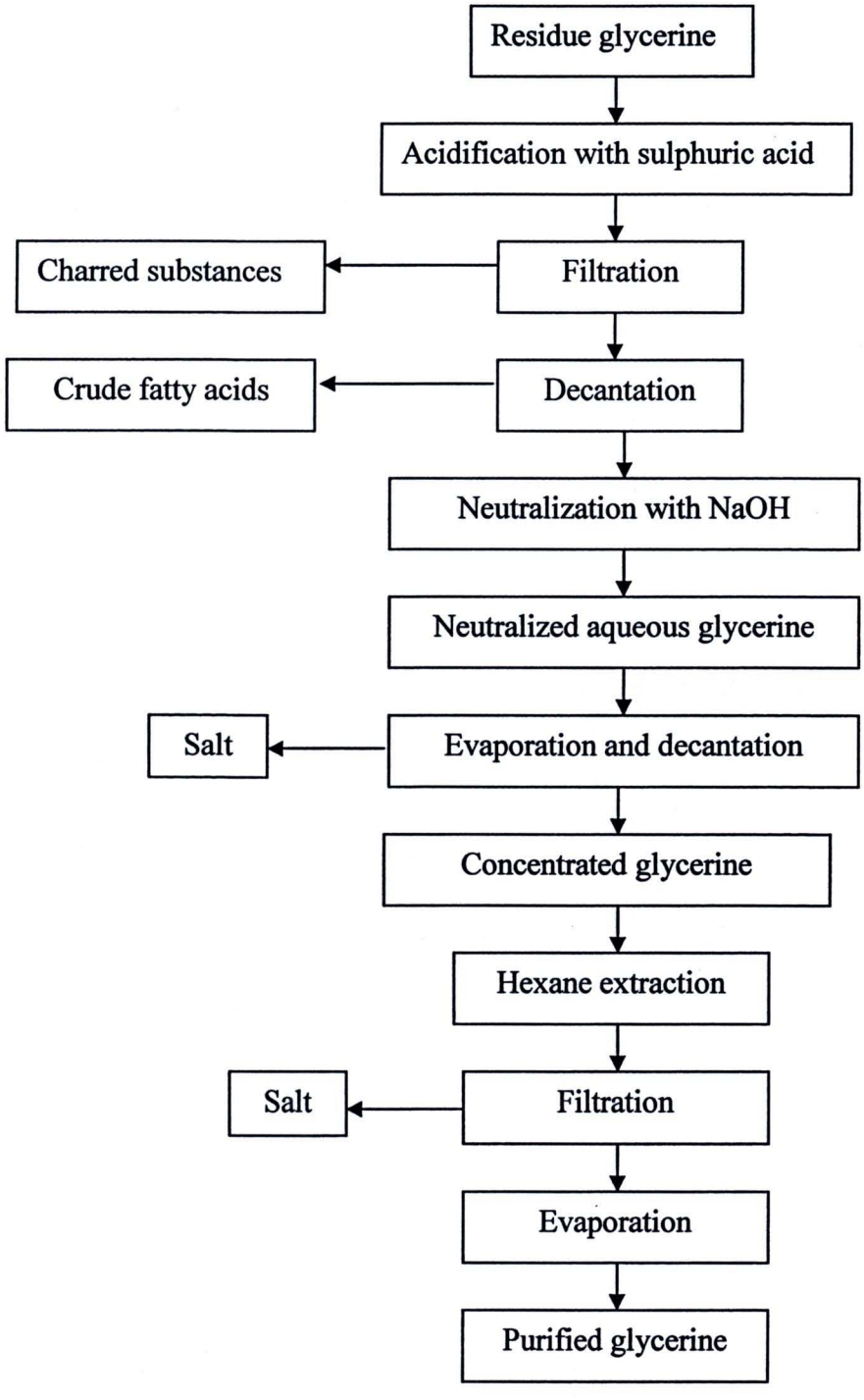

Figure 3.5 Schematic diagram of the conventional method for purification of residue glycerine (Ooi et al., 2001)

3.5.2 Purification of residue glycerine by the improved methods

The improved method was performed by using chemical and physical treatment coupled with ion exchange chromatography. This method was carried to split the soap and neutralize the residual NaOH. The charred substances obtained were filtered off. The sample was then decanted to recover the crude fatty acids, and the aqueous glycerine solution was neutralized using 50% sodium hydroxide. Subsequently, it was evaporated to concentrate the glycerine solution. The glycerine solution was further purified by ion exchange resin which was processed by batch experiments. These experiments were divided into three parts including cation exchange resin, anion exchange resin and mixed of cation-anion exchange resin. The improved method for purification of glycerine solution is shown in Figure 3.6.

3.5.2.1 Optimization conditions for purification of glycerine solution by using cation exchange resin with variation of parameters

(1) Ratio of cation exchange resin to glycerine solution (1:1, 1:2, 1:3 and 1:4 w/w) was studied, as shown in Table 3.4.

Table 3.4 Ratio of cation exchange resin (amberlite 120 H^+) to glycerine solution

Mixed ratio (w/w)	cation exchange resin (g)	glycerine solution (g)
1:1	5.0000 ± 0.0047	5.0000 ± 0.0011
1:2	5.0000 ± 0.0012	10.0000 ± 0.0029
1:3	5.0000± 0.0016	15.0000± 0.0025
1:4	5.0000 ± 0.0036	20.0000 ± 0.0018

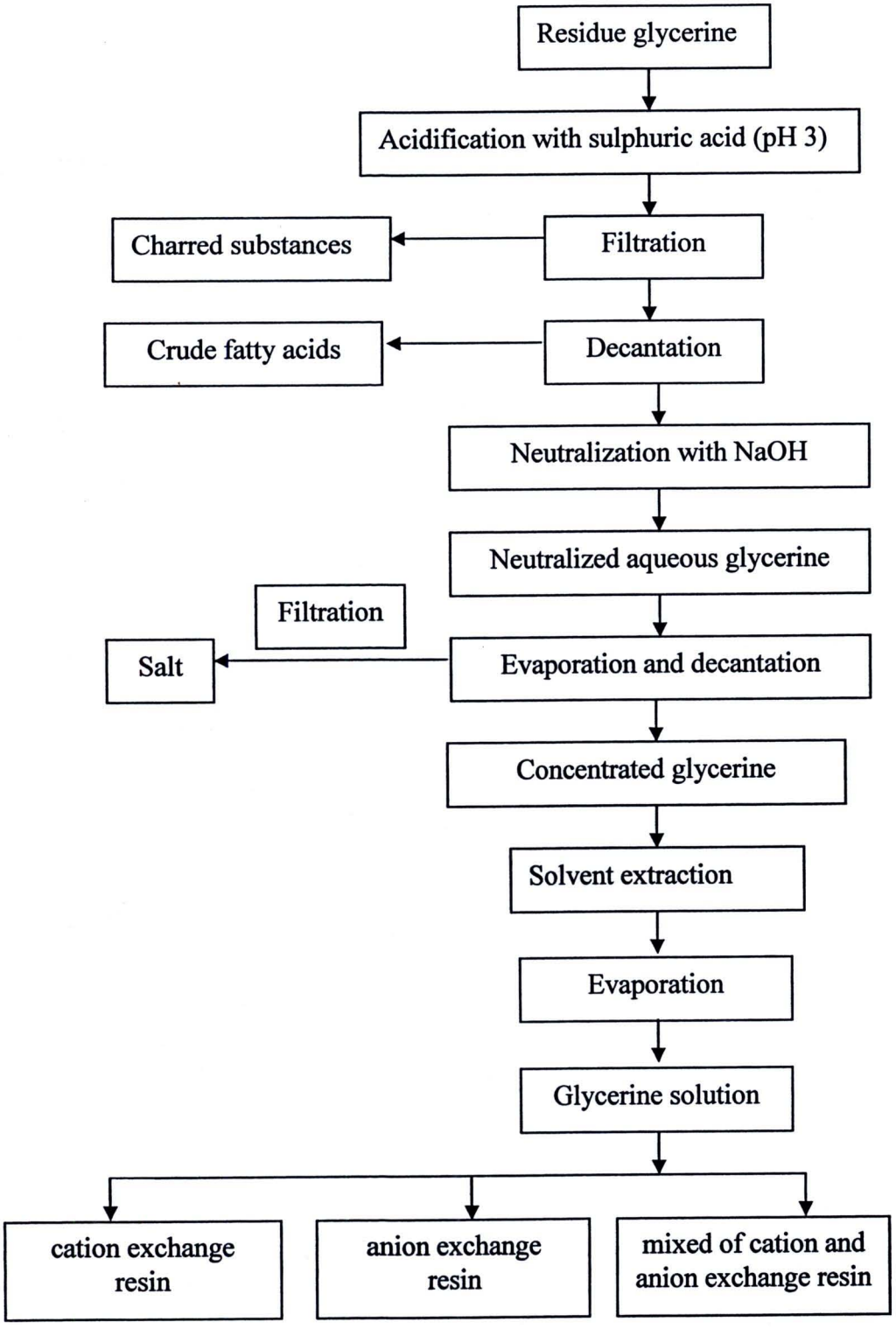

Figure 3.6 The improved method for purification of residue glycerine by ion exchange chromatography

(2) Contact time (used 30, 60, 90, 120 and 180 min).

(3) Agitation rate (used 0, 50, 100, 150 and 200 rpm).

3.5.2.2 Optimization conditions for purification of glycerine solution by using anion exchange resin with variation of parameters

(1) Ratio of anion exchange resin to glycerine solution (1:1, 1:2, 1:3 and 1:4 w/w) was studied, as shown in Table 3.5.

Table 3.5 Ratio of anion exchange resin to glycerine solution

Mixed ratio (w/w)	anion exchange resin (g)	glycerine solution (g)
1:1	5.0000 ± 0.0028	5.0000 ± 0.0027
1:2	5.0000 ± 0.0006	10.0000 ± 0.0015
1:3	5.0000 ± 0.0014	15.0000 ± 0.0029
1:4	5.0000 ± 0.0034	20.0000 ± 0.0037

(2) Contact time (used 30, 60, 90, 120 and 180 min).

(3) Agitation rate (used 0, 50, 100, 150 and 200 rpm).

3.5.2.3 Optimization conditions for purification glycerine solution by using both cation and anion exchange resin with variation of parameters

(1) Ratio of both cation and anion exchange resin to glycerine solution (1:1, 1:2, 1:3 and 1:4 w/w) was studied as shown in Table 3.6.

Table 3.6 Ratio of mixing both cation and anion exchange resin to glycerine solution

Mixed ratio	cation exchange resin (g)	anion exchange resin (g)	glycerine solution (g)
1:1	5.0000 ± 0.0031	5.0000 ± 0.0015	10.0000 ± 0.0015
1:2	5.0000 ± 0.0026	5.0000 ± 0.0007	20.0000 ± 0.0036
1:3	5.0000 ± 0.0013	5.0000 ± 0.0026	30.0000 ± 0.0041
1:4	5.0000 ± 0.0031	5.0000 ± 0.0034	40.0000 ± 0.0021

(2) Contact time (used 30, 60, 90, 120 and 180 min).

(3) Agitation rate (used 0, 50, 100, 150 and 200 rpm).

3.6 Determination of standard glycerine, residue glycerine and purified glycerine compositions

The standard glycerine, residue glycerine and glycerine purified were determined and shown as its contents of glycerine, ash, Moisture, Matter Organic Non-glycerine (MONG) and pH, as following;

3.6.1 Glycerine content

The purified glycerine was measured according to the method of (Bondioli & Della Bella, 2005). In this work, the purified glycerine obtained both conventional method and improved method was determined by using Ultraviolet-Visible (UV-VIS) Spectrophotometry.

3.6.1.1 Preparation of reagents

(1) Standard glycerine (99.5%) stock solution (3 mg/mL): Weighed app. 150 mg (accuracy ± 0.1 mg) of glycerine into a 50 mL calibrated flask. Dissolved in the working solvent and filled up to the mark.

(2) Standard glycerine working solution (0.03 mg/mL): Used a precision pipette, transfer 1 mL of glycerine reference stock solution to a 100 mL calibrated flask. Diluted and fill up to the mark using the same solvent.

(3) Acetic acid stock solution (1.6 M): Dissolved the 9.6 g of acetic acid in 100 mL of distilled water.

(4) Ammonium acetate stock solution 4.0 M: Dissolved the 30.8 g of ammonium acetate in 100 mL of distilled water.

(5) Acetylacetone solution 0.2 M: Dissolved in a test tube approx 200 µL (195 mg) of acetylacetone in 5 mL of ammonium acetate stock solution (4) and 5 mL of acetic acid stock solution (3).

(6) Sodium periodate solution 10 mM: Weighed into a test tube app 21 mg of sodium meta periodate, added 5 mL of acetic acid stock solution (3), swirl to dissolved the periodate, and after periodate is completely dissolved, add 5 mL of acetic acid stock solution (3).

(7) Working solvent: mixed equal volumes of distilled water and 95% ethanol.

3.6.1.2 Procedures

(1) Into a series of six 10 mL test tubes, transfered 0.00, 0.25, 0.50, 0.75, 1.00 and 1.25 mL of a 0.03 mg/mL glycerine solution. The working solvent in such a way as to get final volume of 2 mL in each tube as shown in Table 3.7

(2) Put 0.5 mL of a pretreated purified glycerine sample into 10 mL test tube and added 1.5 mL of working solvent into the test tube.

(3) Added 1.2 mL of sodium periodate solution 10 mM to the two former solutions (procedure 1, 2) and shaken for 30 s.

(4) Added 1.2 mL of acetylacetone solution to the former solution and put in a water bath thermostated at 70 ^{0}C for 1 min, stirring manually.

(5) After the reaction time, the sample must be immediately cooled in water.

(6) Measured the spectra for standards after measurement of spectrum for blank (Standard 1) on Quantification Standard mode.

(7) Measured the absorbance of purified glycerine on Quantification Sample mode.

Formaldehyde is generated by the reaction free glycerine in standard glycerine, residue glycerine and purified glycerine and periodate and reaction between acetyl actone and the generated formaldehyde lead to formation of the 3, 5-diacetyl-1, 4-dihydrolutidine was yellow complexes. As these complexes was generated proportionally to the amount of glycerine in sample. The determine glycerine by measuring yellow complexes had specific absorption band at 410 nm as shown in Figure 3.7.

Determination of free glycerine in residue glycerine and purified glycerine were performed using an UV-Vis Spectrophotometer equipped with a 1 cm quartz cell. Fine calibration curve had correlation value as 0.9990, as shown in Figure 3.8 - 3.9 and Table 3.7. It was obtained using an UV-Vis Spectrophotometer that had superior sensitivity and rapid capability in acquirement of data.

Table 3.7 Preparation of glycerine standard solution

Standard	Concentration (ppm)	Volume of Glycerine working solution (mL)	Volume of Working solvent (mL)
Standard 1	0.00	0.00	2.00
Standard 2	3.75	0.25	1.75
Standard 3	7.50	0.50	1.50
Standard 4	11.25	0.75	1.25
Standard 5	15.00	1.00	1.00
Standard 6	18.75	1.25	0.75

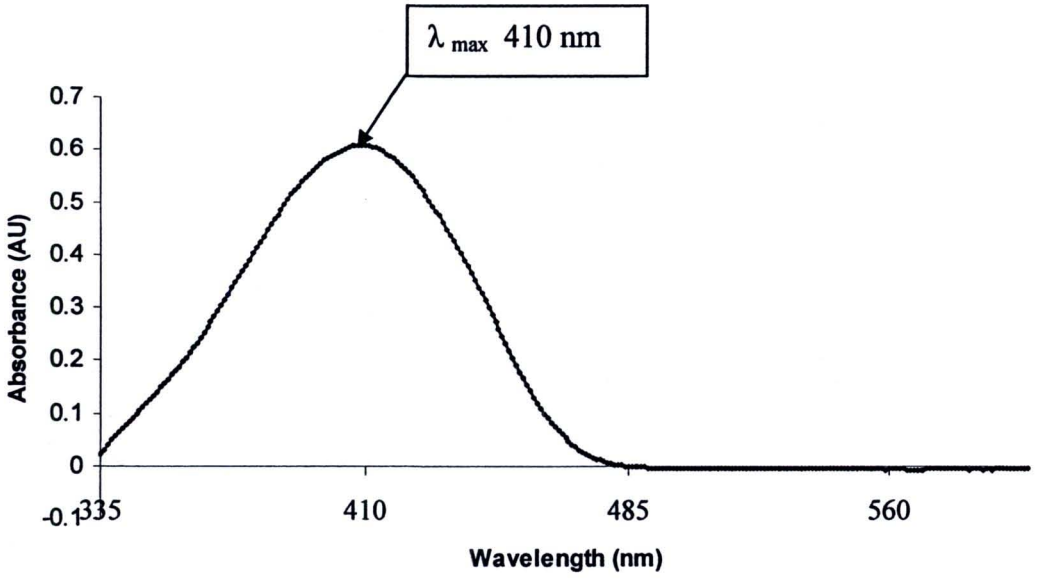

Figure 3.7 The maximum wavelength UV-Vis spectra of glycerine standard

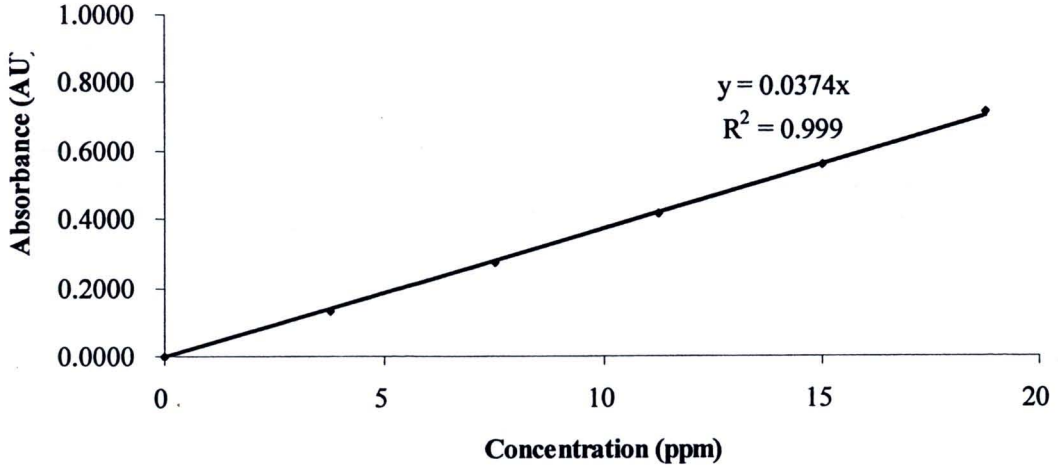

Figure 3.8 The calibration curve of standard glycerine

Table 3.8 Calibration data of standard glycerine

Standards	Concentration (ppm)	Absorbance (AU)
Standard 1	0.00	0.0000
Standard 2	3.75	0.1304
Standard 3	7.50	0.2718
Standard 4	11.25	0.4145
Standard 5	15.00	0.5565
Standard 6	18.75	0.7120

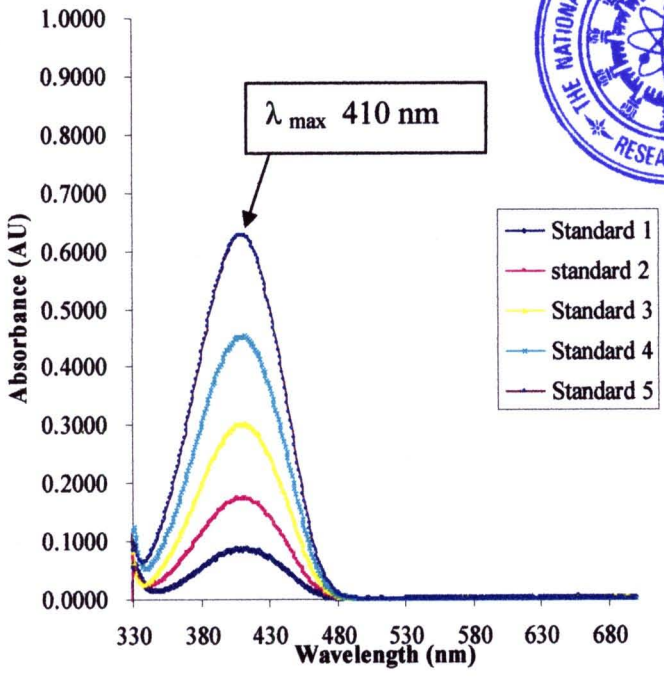

Figure 3.9 The spectra of standards glycerine

3.6.2 Determination of ash content: Standard method ISO 2098-1972

Selection of an appropriate crucible depends on the sample being analyzed and the furnace temperature used. The most widely used crucibles are made from porcelain because it is relatively inexpensive to purchase. It can be used up to high temperatures (< 1200°C) and easy to clean. The study on the ash content of purified glycerine carried out by a Muffle furnace that sample was held at 500-600 °C for 24 hours.

Table 3.9 Determination of ash content in residue glycerine, the purified glycerine obtained conventional method and improved method

No.	Sample
1	Weight of porcelain crucibles, W_1 (g)
2	Weight of porcelain crucibles + wet sample glycerine, W_2 (g)
3	Weight of porcelain crucibles + ash glycerine, W_3 (g)
4	Ash content : W (%) = $[(W_3-W_2)/(W_2-W_1)] \times 100$

3.6.3 Determination of water content: Standard method ISO 2097-1972

Cleaned a container with lid, dried and weighed it (W_1). The specimen of the sample was taken in the container and weighed with lid (W_2). The container was kept in the oven with lid removed. Dried the specimen to constant weight maintaining the temperature between 105 ^{0}C to 110 ^{0}C for 24 hours left them in the oven overnight. The final constant weighed (W_3) of the containers were recorded with dried glycerine samples.

Table 3.10 Determination of water content in glycerine residue, the purified glycerine obtained conventional method and improved method

No.	Sample
1	Weight of crucible, W_1 (g)
2	Weight of crucible + wet glycerineW_2 (g)
3	Weight of crucible + dry glycerine W_3 (g)
4	Water/Moisture content: W (%) = $[(W_3-W_2)/(W_2-W_1)] \times 100$

3.6.4 Determination of matter organic non-glycerine (MONG):Standard method ISO 2464-1973

This determination was preferred to that of the non-volatile organic residue. The matter represents, by convention, the difference obtained by subtracting from 100 sum of the contents of glycerine, ash and water. The determination of the glycerine content, determination of ash and the determination of water were carried out by following the procedure as specified.:

Calculation of Matter (Organic) Non-Glycerol (MONG) by

MONG (%) = 100 – (A+B+C)

A = Glycerine content (%)

B = Ash content (%)

C = Moisture content (%)

3.6.5 pH valve (20%)

Determined by dissolving 20.0 g of standard glycerine, residue glycerine and purified glycerine in 100 mL distilled water, and measured with a pH meter.

3.6.6 Infrared spectroscopy analysis

The infrared spectrum of standard glycerine, residue glycerine and purified glycerine were analyzed using a thin film on a potassium bromide pellet using a FT-IR spectrophotometer.