PURIFICATION OF CRUDE GLYCEROL & PRODUCTION OF MONOGLYCERIDES FROM PALMITIC ACID AND GLYCEROL

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ABSTRACT

The reverse transesterification of Free Fatty Acid (FFA) with purified glycerol was studied. Standard Glycerol(Oleo Chemicals Company), Crude Glycerol(The Royal Thai Naval Dockyard) and used palm oil were used as starting material. Crude Glycerol was purified by hexane extraction and de-colorization by activated carbon respectively. Purification of purified glycerol was investigated by Nuclear Magnetic resonance spectroscopy (NMR) and Refeactometer. From the resulted of NMR and refractive index were shown increasing of glycerol from 74% to 90%.

The second step was reducing of FFA in used palm oil via reverse transesterification reaction The reaction conditions of (0-12 h.) at volume ratio of plamitic acid : standard Glycerol in 1:1,1:1.5, and 1:2 were observed. A detailed experimental program was also implemented to investigate the influence of time on volume ratio of plamitic acid : purified glycerol. The titration technique was used to investigate percent conversion of palmitic acid The plamitic acid was continuously converted with glycerol to form monoplamitin The optimum condition for conversion percent of palmitic acid were 1 h. at volume ratio PA: Standard Glycerol was 1:1.5. For purified glycerol were 1 h at volume ratio of 1:3.

Keywords : Free fatty acid, Glycerol, Monopalmitin, Refractive index, Palmitic acid

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Chapter 1

Introduction

Biodiesel is a clean-burning diesel fuel produced from vegetable oils, animal fats, or grease. Its chemical structure is that of fatty acid alkyl esters (FAAE). Biodiesel as a fuel gives much lower toxic air emissions than fossil diesel. In addition, it gives cleaner burning and has less sulfur content, and thus reducing emissions. Because of its origin from renewable resources, it is more likely that it competes with petroleum products in the future. To use biodiesel as a fuel, it should be mixed with petroleum diesel fuel to create a biodiesel-blended fuel. Biodiesel refers to the pure fuel before blending. Commercially, biodiesel is produced by transesterification of triglycerides which are the main ingredients of biological origin oils in the presence of an alcohol (e.g. methanol, ethanol) and a catalyst (e.g. alkali, acid, enzyme) with glycerol as a major by-product [1,2]. After the reaction, the glycerol is separated by settling or centrifuging and the layer obtained is purified prior to using it for its traditional applications (pharmaceutical, cosmetics and food industries) or for the recently developed applications (animal feed, carbon feedstock in fermentations, polymers, surfactants, intermediates and lubricants).

Due to being renewable, having better quality of exhaust gas emissions and its biodegradability, biodiesel is becoming very popular in the European Union (EU) which has set an objective to secure for motor bio-fuels a market share of 20% of total motor fuel consumption by 2020. By 2010, the United States is expected to become the world's largest single biodiesel market, accounting for roughly 18% of world biodiesel consumption, followed by Germany. New and large single markets for biodiesel are expected to emerge in China, India and Brazil. However, one of the most serious obstacles to use biodiesel as an alternative fuel is the complicated and costly purification processes involved in its production. Removing glycerol from biodiesel is important since the glycerol content is one of the most significant precursors for the biodiesel quality. Biodiesel content of glycerol can be in the form of free glycerol or bound glycerol in the form of glycerides. In this work we refer to the total glycerol, which is the sum of

free glycerol and bound glycerol. Severe consequences may result due to the high content of free and total glycerol, such as buildup in fuel tanks, clogged fuel systems, injector fouling and valve deposits. The traditional means of removing glycerol is mainly by gravity separation or centrifugation. The methanol may in some cases be removed using flash evaporation. Neither process is 100% efficient hence a final purification stage is needed in order to meet the requirements of EN 14214 and ASTM D 6751 Standards. The untreated biodiesel contains several impurities namely; free and total glycerol, soap, metals, methanol, free fatty acids (FFA), catalyst, water, and glycerides. The engine life can be reduced by high levels of impurities [7]. Several methods have been used to separate fatty acid methyl esters (FAME) from other components. However, there are two generally accepted methods to purify biodiesel: wet and dry washing. The more traditional wet washing method is widely used to remove excess contaminants and leftover production chemicals from biodiesel. Since both glycerol and methanol are highly soluble in water, water washing is very effective in removing both contaminants and until recently was the most common method of purification. It also has the advantage of removing any residual sodium salts and soaps, the latter being a byproduct of high FFA feeds, due to their water solubility. However, the inclusion of additional water to the process offers many disadvantages, including increased cost and production time. A highly polluting liquid effluent is generated. Significant product loss can be carried out for retention in the water phase. Furthermore, emulsion formation when processing used cooking oils or other feeds with high FFA content can happen due to the soap formation [7,8]. Dry washing replaces water with an ion exchange resin or a magnesium silicate powder to neutralize impurities. Both dry washing methods are being used in industrial plants. Other methods are used to purify biodiesel such as using membrane reactors, and the addition of lime and phosphoric acid [9–11]. Nonetheless, there are numerous problems associated with the costs and complications of operating biodiesel synthesis on an industrial scale

The main objective of this work is to use reverse transesterification in the removal of total FFA from palm oil based biodiesel. The process is then optimized for the best ratio of palm

oil to FFA composition. Moreover, the palm oil to FFA ratio is also investigated in terms of its reaction time and temperature.

Motivation

1. We are interested the glut of glycerol that makes the market price of glycerol decreasing.
2. We are interested that the impurity in glycerol is too much to use for utilize to other product.
3. We are interested to make glycerol to purity for make glycerin have more valuable.
4. We are interested to mixed pure glycerol with palm oil for developing the quality of palm oil (decreased FFA in palm oil).
5. We are interested that our experiment to be use for the industrial business.

Objective

1. To study, the way to purify the glycerol. In addition, the purification steps were also studied for 2 steps as the evaporation of methanol and hexane extraction of residues lipid.
2. To study, the best condition to make the highest yield of pure glycerol.
3. To study, the characteristic of glycerol that gained from the experiment.
4. To study, the possible of the mixing of pure glycerol with FFA in palm oil to decreased the amount of FFA and convert FFA to triglyceride.
5. To study, the possibility of this experiment for apply to the industrial method.

Scope of study

1. To prepare and purify glycerol. In addition, the purification steps were also studied for 2 steps as the evaporation of methanol and hexane extraction of residues lipid.
2. To perform experiment on the best condition to make the highest yield of pure glycerol.
3. To perform experiment on characteristic of glycerol gained from the experiment.
4. To find the possibility conditions and ratio of mixing between pure glycerol and FFA in palm oil to decrease the amount of FFA in palm oil.
5. To find the possible way to archive this experiment to apply this to industry.

Expected Result

1. To purified glycerol from commercial glycerol from industrial plants.
2. To gained information about the best condition to make the highest yield and the characteristics.
3. Expected the highest yield of biodiesel that get from the mixing of pure glycerol and free fatty acid from palm oil.
4. To archive the possible way to apply this experiment to industry or business.

Chapter 2

Literature review and involve articles

2.1 Glycerol

Glycerol is an organic compound, also commonly called glycerin or glycerol. It is a colorless, odorless, viscous liquid that is widely used in pharmaceutical formulations. Glycerol has three hydrophilic hydroxyl groups that are responsible for its solubility in water and its hygroscopic nature. The glycerol substructure is a central component of many lipids. Glycerol is sweet-tasting and of low toxicity. It also is a by-product of the production of biodiesel via transesterification. The formula of glycerol was $CH_2OHCHOHCH_2OH$. [4]

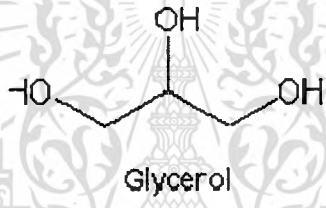


Figure 2.1 Structure of Glycerol

Glycerin is used in nearly every industry. With dibasic acids, such as phthalic acid, it reacts to make the important class of products known as alkyd resins, which are widely used as coating and in paints. It is used in innumerable pharmaceutical and cosmetic preparations; it is an ingredient of many tinctures, elixirs, cough medicines, and anesthetics; and it is a basic medium for toothpaste. In foods, it is an important moistening agent for baked goods and is added to candies and icings to prevent crystallization. It is used as a solvent and carrier for extracts and flavoring agents and as a solvent for food colors. Many specialized lubrication problems have been solved by using glycerin or glycerin mixtures. Many millions of pounds are used each year to plasticize various materials. In foods and beverages, glycerol serves as a humectant, solvent and sweetener, and may help preserve foods. It is also used as filler in commercially prepared low-fat foods (e.g., cookies), and as a thickening agent in liqueurs. Glycerol also serves as a way, along with water, to preserve certain types of leaves. Glycerol is also used as a sugar substitute. In

this regard, it has approximately 27 calories per teaspoon and is 60% as sweet as sucrose. Although it has about the same food energy as table sugar, it does not raise blood sugar levels, nor does it feed the bacteria that form plaques and cause dental cavities. As a food additive, glycerol is also known as E number E422.

In organic synthesis, glycerol is used as a readily available prochiral building block. Even if glycerol with no substitutions is symmetrical, and carbon atoms 1 and 3 are exchangeable, once one of them forms an ester or ether bond, the two are no longer exchangeable. Further bond formation and hydrolysis may lead to products substituted solely at the third carbon; due to such circumstances, to maintain both full description and conformance to the chemistry naming rules (which require carbon counting to minimize ordinal numbers of substituent), the carbons are named *sn*-1, *sn*-2, and *sn*-3, with "sn" standing for "sterospecifical numbering"

Glycerin can also serve as a substitute for petroleum based products. Glycerin derived epichlorohydrin and propylene glycol are substitutes for petroleum-based Polypropylene. [5]

2.1.1 Categorization

Glycerol is currently categorized by the American Dietetic Association as a carbohydrate. The FDA carbohydrate designation includes all caloric macronutrients excluding protein and fat. This group includes indigestible fibers, but not ash. Glycerin has a caloric density similar to table sugar, but a lower glycemic index and different metabolic pathway within the body, so some dietary advocates accept glycerin as a sweetener compatible with low carbohydrate diets. [6]

2.1.2 Physical Properties of Glycerol.

Glycerin is a colorless, odorless, viscous liquid with a sweet taste. It is completely soluble in water and alcohol but is only slightly soluble in many common solvents, such as ether, ethyl acetate, and dioxane. Glycerol is a trihydric alcohol. It melts at 17.8°C, boils with decomposition at 290°C, and is miscible with water and ethanol. It is hygroscopic; i.e., it absorbs water from the air; this property makes it valuable as a moistener in cosmetics.

Glycerol does not dissolve in water but soluble in methanol and ethanol isomer of propanol butanol. And include phenol glycol propane diolamine and heterocyclic compound which compose of nitrogen atom in the ring such as: pyridine and quinolin. Glycerol does not dissolve in almost of hydrocarbon, long chain of alcohol that came from vegetable and animal oil and for the halogen solvent such as: chloroform. Therefore glycerol was solvent that there are many useful substances of both organic and inorganic compound. It is important for many type of industry. [7]

PHYSICAL PROPERTIES	MEASURING
Molecular weight	92.09
Melting point	18.17°C
Boiling point (760mm Hg)	290°C
Density (20°C)	1.261 g/cm3
Vapor pressure	
at 50°C	0.0025 mm Hg
at 100°C	0.195 mm Hg
at 150°C	4.3 mm Hg
at 200°C	46 mm Hg
Refractive index	1.474
Surface tension at 20°C (100% glycerol)	63.4 dyne/cm
Compressibility (28.5°C)	2.1×10 MPa
Viscosity at 20°C (100% glycerol)	1499 c.p.
Specific heat at 26°C (99.94%glycerol)	0.5779 cal/gm
Heat of vaporization	
at 55°C	21060 cal/mole
at 195°C	18170 cal/mole
Heat of formation	159.6 Kcal/gm mole
Heat of combustion	1662 KJ/mole
Heat of fusion	18.3 KJ/mole
Thermal conductivity	0.29 w/°K
Flash point	177°C
Fire point	204°C

Table 2.1 The physical properties of glycerol

2.1.3 Chemical properties of glycerol

Glycerol is a reactive molecule that undergoes all the usual reactions of alcohols. The two terminal primary hydroxyl groups are more reactive than the internal secondary hydroxyl group. Under neutral or alkaline conditions, glycerol can be heated to 250°C without formation of acrolein.

Reactions with glycerol are therefore best carried out under alkaline or neutral conditions at 180°C, Alkaline glycerol begins to dehydrate forming ether-linked polyglycerols. At room temperature glycerol rapidly absorbs water. When dilute it is attacked by microorganism.
On oxidation, glycerol yields variety of product depending upon the reaction conditions. By the use of mild oxidizing agent it is possible to oxidize only one hydroxyl group to yield Glyceraldehyde.

These compounds may be considered very simple aldose and simplest ketoses respectively and mixture of two compounds obtained from glycerol as well as glyceraldehyde has been Called glycerose. Nitric acid converts glycerol to glyceric acid $CH_2CHCHOHCOOH$ melting at 134-135°C when pure, but usually obtained as syrupy. Oily liquid soluble in water and alcohol, but insoluble in ether. Some industrially important reaction products of glycerol include: Mono-,di-,and tri esters of inorganic and organic acids

Mono and diglyceride of fatty acids formed by transesterification of triglycerides (from fats). Aliphatic and aromatic esters formed by reactions with alkylatingagents respectively. Polyglycerols formed by the intermolecular alienation of water with alkaline catalyst. Cyclic 1,2- or 1,3-acetals or ketals formed by the reaction with aldehyde or ketons respectively

2.1.4 Purification of Glycerol

1) EET's glycerol purification process - EET's glycerin purification process begins with pretreatment of the glycerol to remove any solids and fouling organics and partially remove color-causing organics. The HEED® or HEEPM™ system configuration is used, with customized automated controls and control logic, providing optimal desalting of the preatreated crude glycerol. The result is a colorless liquid with low salt content. [8]

2) AMBERSEP™ BD50 Technology - Crude glycerin from storage is first heated to 194 degrees Fahrenheit on a plate heat exchanger, using energy recovered from the purified glycerin stream, plus live steam. After a safety filtration to protect the downstream processing steps from fouling by suspended materials, the hot and clean crude glycerin is carefully degassed before entering a chromatographic separator. The chromatographic separator combines the sequential simulated moving bed technology with Ambersep BD50, a high-performance chromatographic separation resin from Rohm and Haas, to purify crude glycerin with a high salt composition. The Ambersep BD50 glycerin purification system includes the option to concentrate and crystallize the salt fraction coming out of the separator. Depending on the required purity of the final glycerin, it is possible to produce a purified glycerin product with 99.5 percent purity or to add a polishing step employing an ion-exchange demineralization unit which enables the end user to produce a high quality glycerin product with 5 parts per million to 10 parts per million salt content.[9]

3) USPTO Patent Application 20090048472 - A solution of sodium borohydride and sodium hydroxide is combined with the glycerol layer to neutralize sulfuric acid and reduce colored impurities. In some embodiments of the invention, the solution of sodium borohydride and sodium hydroxide contains at least 5% sodium borohydride, alternatively at least 8%, alternatively at least 10%; the solution preferably contains less than 20% sodium borohydride. In some embodiments of the invention, the solution of sodium borohydride and sodium hydroxide contains at least 20% sodium hydroxide, alternatively at least 30%, alternatively at least 35%; preferably the solution contains less than 45% sodium hydroxide. An example of a suitable solution is sold by Rohm and Haas Company under the trade name BOROL solution, which contains about 12% sodium borohydride and about 40% sodium hydroxide. In some embodiments of the invention, the amount of solution combined with 100 g of the glycerol layer is from 0.7 g to 1.5 g, alternatively from 0.75 g to 1.3 g, alternatively from 0.8 g to 1.2 g. In some embodiments of the invention, the temperature during treatment of the glycerol layer with the solution is in a range from 15° C. to 90° C. In some embodiments of the invention, the temperature is no greater than 80° C., alternatively no greater than 70° C., alternatively no greater than 60° C., alternatively

no greater than 50° C. In some embodiments of the invention, the temperature is at least 20° C., alternatively at least 25° C., alternatively at least 30° C. In some embodiments of the invention, the contact time between the glycerol layer and the solution is at least 10 minutes. [10]

2.1.5 Usages of glycerol

1) Pharmaceutical and personal care applications

Glycerol is used in medical and pharmaceutical and personal care preparations, mainly as a means of improving smoothness, providing lubrication and as a humectant. It is found in cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products, shaving cream, hair care products, soaps and water based personal lubricants. In solid dosage forms like tablets, Glycerol is used as a tablet holding agent. It is also an ingredient in cigarettes that is used as a humectant. For human consumption, glycerol is classified by the U.S. FDA among the sugar alcohols as a caloric macronutrient.

As a 10% solution, glycerol prevents tannins from precipitating in ethanol extracts of plants (tinctures). It is also used as a substitute for ethanol as a solvent in preparing herbal extractions. It is less extractive and is approximately 30% less able to be absorbed by the body. Fluid extract manufacturers often extract herbs in hot water before adding glycerin to make glycerides.

Glycerol is a component of glycerol soap, which is made from denatured alcohol, glycerol, sodium castorate (from castor), sodium cocoate,sodium tallowate, sucrose, and water. Sometimes one adds sodium laureth sulfate, or essential oils for fragrance. This kind of soap is used by people with sensitive, easily-irritated skin because it prevents skin dryness with its moisturizing properties. It draws moisture up through skin layers and slows or prevents excessive drying and evaporation. It is possible to make glycerol soap at home.

Topical pure or nearly pure glycerol is an effective treatment for psoriasis, burns, bites, cuts, rashes, bedsores, and calluses. It can be used orally to eliminate halitosis, as it is a contact bacterial desiccant. The same property makes it very helpful with periodontal disease; it penetrates biofilm quickly and eliminates bacterial colonies. [11]

2) Alternative Chemical and fuel feedstock

A great deal of research is being conducted to try to make value-added products from crude glycerol (typically containing 20 % water and residual esterification catalyst) obtained from biodiesel production, as an alternative to disposal by incineration. [12]

Hydrogen gas production unit

Glycerine acetate (as a potential fuel additive)

Conversion to propylene glycol

Conversion to acrolein

Conversion to ethanol

Conversion to epichlorhydrin, a raw material for epoxy resins

2.1.6 Monoglyceride

A monoglyceride, more correctly known as a monoacylglycerol, is a glyceride consisting of one fatty acid chain covalently bonded to a glycerol molecule through an ester linkage.

Figure 2.2 Structure of Monoglyceride.

Monoacylglycerol can be broadly divided into two groups; 1-monoacylglycerols and 2-monoacylglycerols, depending on the position of the ester bond on the glycerol moiety. Monoacylglycerols can be formed by both industrial chemical and biological processes. They are formed biochemically via release of a fatty acid from diacylglycerol by diacylglycerol lipase. Monoacylglycerols are broken down by monoacylglycerol lipase.

Mono- and diglycerides are commonly added to commercial food products in small quantities. They act as emulsifiers, helping to mix ingredients such as oil and water that would not otherwise blend well.

The commercial source may be either animal (cow- or hog-derived) or vegetable, and they may be synthetically made as well. They are often found in bakery products, beverages, ice cream, chewing gum, shortening, whipped toppings, margarine, and confections. When used in bakery products, monoglycerides improve loaf volume, and create a smooth, soft crumb.

2.2 Biodiesel

2.2.1 Definition of Biodiesel Production

Biodiesel production is the act of producing the biofuel, biodiesel, through either transesterification or alcoholysis. The process involves reacting vegetable oils or animal fats catalytically with a short-chain aliphatic alcohols (typically methanol or ethanol).

The major steps required to synthesize biodiesel are as follows:

1) Feedstock pretreatment

If waste vegetable oil (WVO) is used, it is filtered to remove dirt, charred food, and other non-oil material often found. Water is removed because its presence causes the triglycerides to hydrolyze, giving salts of the fatty acids (soaps) instead of undergoing transesterification to give biodiesel.

2) Determination and treatment of free fatty acids

A sample of the cleaned feedstock oil is titrated with a standardized base solution in order to determine the concentration of free fatty acids (carboxylic acids) present in the waste vegetable oil sample. These acids are then either esterified into biodiesel, esterified into bound glycerides, or removed, typically through neutralization.

3) Reactions

While adding the base, a slight excess is factored in to provide the catalyst for the transesterification. The calculated quantity of base (usually NaOH) is added slowly to the alcohol and it is stirred until it dissolves. Sufficient alcohol is added to make up three full equivalents of

the triglyceride, and an excess of usually six parts alcohol to one part triglyceride is added to drive the reaction to completion.

Transesterification

A reaction scheme for transesterification is as follows

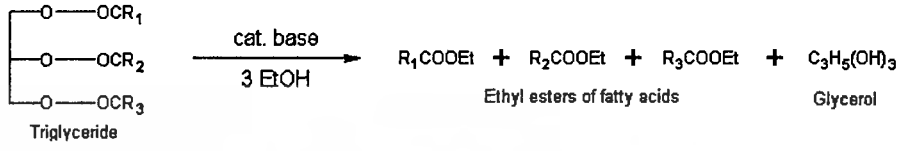


Figure 2.3 Reaction scheme of transesterification

R_1, R_2, and R_3 in this diagram represent long carbon chains that are too lengthy to include in the diagram.

Animal and plant fats and oils are typically made of triglycerides which are esters of free fatty acids with the trihydric alcohol, glycerol. In the transesterification process, the alcohol is deprotonated with a base to make it a stronger nucleophile. Commonly, ethanol or methanol are used. As can be seen, the reaction has no other inputs than the triglyceride and the alcohol.

Normally, this reaction will proceed either exceedingly slowly or not at all. Heat, as well as an acid or base are used to help the reaction proceed more quickly. It is important to note that the acid or base are not consumed by the transesterification reaction, thus they are not reactants but catalysts.

Almost all biodiesel is produced from virgin vegetable oils using the base-catalyzed technique as it is the most economical process for treating virgin vegetable oils, requiring only low temperatures and pressures and producing over 98% conversion yield (provided the starting oil is low in moisture and free fatty acids). However, biodiesel produced from other sources or by other methods may require acid catalysis which is much slower. Since it is the predominant method for commercial-scale production, only the base-catalyzed transesterification process will be described below.

An example of the transesterification reaction equation, shown in skeletal formulas:

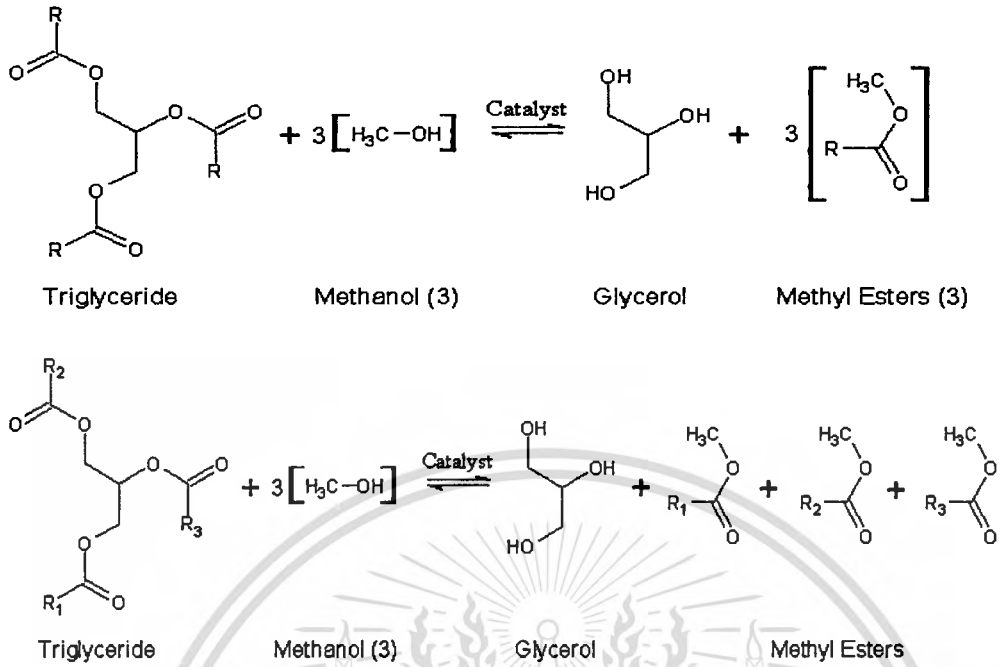


Figure 2.4 Transesterification reaction equation.

R_1, R_2, R_3 : Alkyl group.

During the esterification process, the triglyceride is reacted with alcohol in the presence of a catalyst, usually a strong alkali (NaOH, KOH, or Alkoxides). The main reason for doing a titration to produce biodiesel, is to find out how much alkaline is needed to completely neutralize any free fatty acids present, thus ensuring a complete transesterification. Empirically 6.25 g / L NaOH produces a very usable fuel. One uses about 6 g NaOH when the WVO is light in colour and about 7 g NaOH when it is dark in colour.

The alcohol reacts with the fatty acids to form the mono-alkyl ester (or biodiesel) and crude glycerol. The reaction between the biolipid (fat or oil) and the alcohol is a reversible reaction so the alcohol must be added in excess to drive the reaction towards the right and ensure complete conversion.

4) Product purification

Products of the reaction include not only biodiesel, but also byproducts, soap, glycerin, excess alcohol, and trace amounts of water. All of these byproducts must be removed, though the order of removal is process-dependent.

The density of glycerin is greater than that of biodiesel, and this property difference is exploited to separate the bulk of the glycerin byproduct. Residual methanol is typically removed through distillation and reused, though it can be washed out (with water) as a waste as well. Soaps can be removed or converted into acids. Any residual water must be removed from the fuel. [13]

2.3 Palm oil

2.3.1 Definition

Palm oil (from the African oil palm, *Elaeis guineensis*) is long recognized in West African countries, and is widely use as a cooking oil. European merchants trading with West Africa occasionally purchased palm oil for use in Europe, but as the oil was bulky and cheap, palm oil remained rare outside West Africa.

1) Refined, Bleached, Deodorized Palm Oil

Palm oil products are made using milling and refining processes: first using fractionation, with crystallization and separation processes to obtain solid (stearin), and liquid (olein) fractions. Then by melting and degumming, impurities can be removed, and then the oil is filtered and bleached. Next, physical refining removes smells and coloration, to produce refined bleached deodorized palm oil, or RBDPO, and free sheer fatty acids, which are used as an important raw material in the manufacture of soaps, washing powder and other hygiene and personal care products. RBDPO is the basic oil product which can be sold on the world's commodity markets, although many companies fractionate it further into palm olein, for cooking oil or other products. Splitting of oils and fats by hydrolysis, or under basic conditions saponification, yields fatty acids, with glycerin (glycerol) as a byproduct. The split-off fatty acids are a mixture ranging from C_4 to C_{18}, depending on the type of oil/fat.[14]

2) Nutrition

Fatty acid content of palm oil

Types of Free Fatty Acid	Percentage
Palmitic	44.3
Stearic	4.6
Myristic	1.0
Oleic	38.7
Lenoleic	10.5
Others	0.9

Table 2.2 Types of Free Fatty Acid

2.4 Free Fatty Acid

2.4.1 Definition

Fatty acids can be bound or attached to other molecules, such as in triglycerides or phospholipids. When they are not attached to other molecules, they are known as "free" fatty acids.[13]

The uncombined fatty acids or free fatty acids may come from the breakdown of a triglyceride into its components (fatty acids and glycerol). Free fatty acids are an important source of fuel for many tissues since they can yield relatively large quantities of ATP.

Examples of Fatty Acid

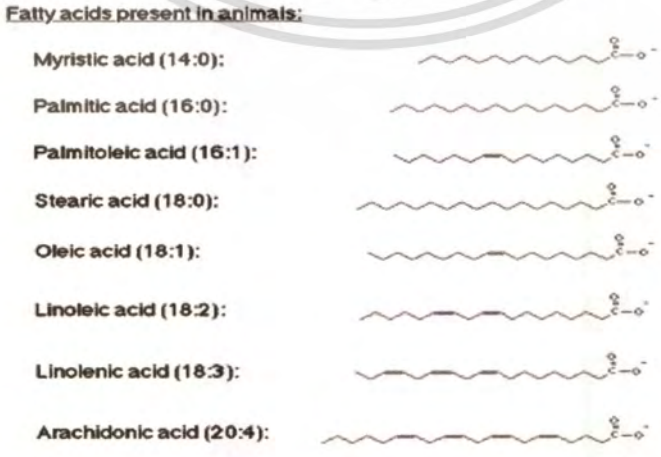
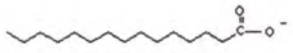


Figure 2.5 Fatty acids present in animals

Fatty acids absent in animals:

Pentadecanoic acid (15:0):



Heptadecanoic acid (17:0):

Nonadecanoic acid (19:0):

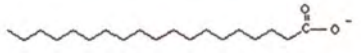


Figure 2.6 Fatty absent in animals

2.4.2 Reaction of Free fatty acid

1) Esterification – Esterification of fatty acid with alcohol inactive of acid catalyst gives fatty acid ester (soap) and water as products. [16]

Figure 2.7 Esterification of fatty acid with alcohol.

2) Reduction – Reduction of fatty acids and strong base gives Fatty Acid Alcohol [17]

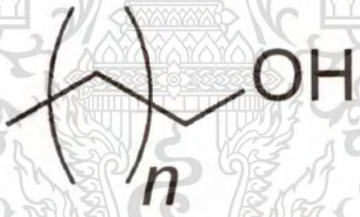


Figure 2.8 Fatty Acid Alcohol

The are three types of fatty acid alcohol; Normal-chain, Branched-chain and Phenolic alcohol.

3)Reaction between free fatty acid and glycerol – The reaction between free fatty acid and glycerol is a reverse process of transesterification to gives triglyceride and water as products.

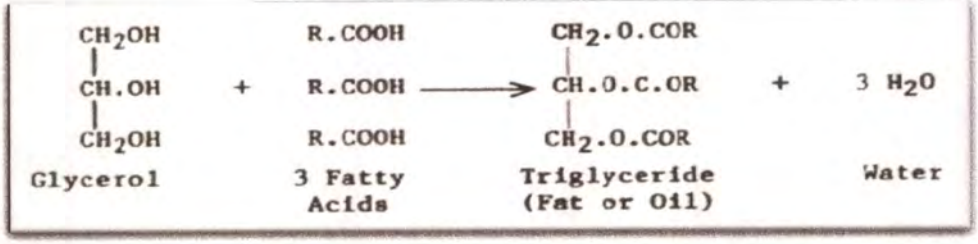


Figure 2.9 The reaction between FFA and glycerol

2.4.3 Palmitic Acid

Palmitic acid,$CH_3(CH_2)_{14}COOH$ or hexadecanoic acid in IUPAC nomenclature, is one of the most common saturated fatty acids found in animals and plants. As its name indicates, it is a major component of the oil from palm trees (palm oil and palm kernel oil). The word palmitic is from the French "palmitique", the pith of the palm tree. Palmitic acid was discovered by Edmond Frémy in 1840, in saponified palm oil. Butter, cheese, milk and meat also contain this fatty acid. Palmitate is a term for the salts or esters of palmitic acid. The palmitate anion is the observed form of palmitic acid at physiological pH.

Figure 2.10 Structure of Palmitic acid.

2.4.3.1 Physical properties of Palmitic Acid

CAS number	57-10-3
molecular formula	$C_{16}H_{32}O_2$
molecular weight	256.43
Melting point °C	62.5/63.1
Boiling point °C (mm Hg)	332.6 (512)
Density D_4^t (°C)	0.8414 (80)
Viscosity mPa·s (°C)	7.8 (70), 4.89 (90)
Refractive Index n_D^t (°C)	1.4335 (60)
Specific Heat J/g (°C)	2.73 (65/104)

Table 2.3 Physical properties of palmitic acid

2.5 Analysis

2.5.1 NMR spectroscopy

Nuclear magnetic resonance spectroscopy, most commonly known as NMR spectroscopy, is the name given to a technique which exploits the magnetic properties of certain nuclei. This phenomenon and its origins are detailed in a separate section on nuclear magnetic resonance. The most important applications for the organic chemist are proton NMR and carbon-13 NMR spectroscopy. In principle, NMR is applicable to any nucleus possessing spin.

Many types of information can be obtained from an NMR spectrum. Much like using infrared spectroscopy to identify functional groups, analysis of a 1D NMR spectrum provides information on the number and type of chemical entities in a molecule. However, NMR provides much more information than IR.

The impact of NMR spectroscopy on the natural sciences has been substantial. It can, among other things, be used to study mixtures of analytes, to understand dynamic effects such as change in temperature and reaction mechanisms, and is an invaluable tool in understanding protein and nucleic acid structure and function. It can be applied to a wide variety of samples, both in the solution and the solid state.

Nuclear Magnetic Resonance (NMR) spectra database is an electronic repository of information concerning NMR spectra. The repository can be stored as a complete self contained data set or as an online repository that can be accessed and searched remotely. The form in which the data is stored ranges from line lists that can be graphically displayed to raw free induction decay (FID) data. The data is usually annotated in a way that correlates the spectral data with the related molecular structure.[18]

2.5.2 Refractive Index

The refractive index (or index of refraction) of a medium is a measure of how much the velocity of a wave is reduced inside that medium. For example, typical soda-lime glass has a refractive index close to 1.5, which means that in glass, light travels at 1 / 1.5 = 2/3 the speed of light in a vacuum. Two common properties of glass and other transparent materials are directly

related to their refractive index. First, light rays change direction when they cross the interface from air to the material, an effect that is used in lenses. Second, light reflects partially from surfaces that have a refractive index different from that of their surroundings.

2.5.2.1 Refractometer

A refractometer is a laboratory or field device for the measurement of an index of refraction. The index of refraction is calculated from Snell's law and can be calculated from the composition of the material using the Gladstone-Dale relation.

2.5.2.2 Types of Refractometer

There are four main types of refractometers; traditional handheld refractometers , digital handheld refractometers, laboratory or Abbe refractometers, and inline process refractometers. There is also the Rayleigh Refractometer used (typically) for measuring the refractive indices of gases.[19]

Manual of traditional handheld Refractometer.[20]

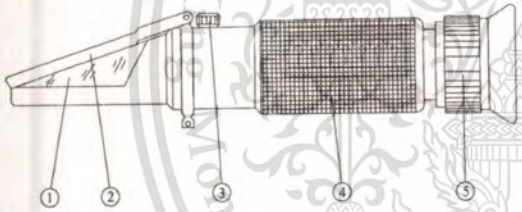


Figure 2.11 Drawing of refractometer

1) Aim the front end of the refractometer in the direction of a bright, and adjust the adjusting ring of diopter until the reticle can be seen clearly.

2) Adjustment of null: Open the cover plate and put one or two drops of distilled water on the prism. Close the cover plate and press it lightly, then adjust the correcting screw (3) to make the light/dark boundary coincide with the null line. (ATC model should be adjusted at an environmental temperature of 20°C)

3) Open the cover plate clean the surface of prism with a piece of soft cotton flannel, drop 1~2 drops of the solution to be measured. Close the cover plates, press it lightly, and then read the corresponding scale of light and dark boundary, the reading is the data of measured solution.

4) After measurement, clean the surface of prism and cover plate with moist gauze. After drying it, it should be stored carefully.

2.6 Literature Review

L.Bournay et al, were study, new continuous biodiesel production process, where the transesterification reaction is promoted by an heterogeneous catalyst. This process requires neither catalyst recovery nor aqueous treatment steps: the purification steps of products are then much more simplified and very high yields of methyl esters, close to the theoretical value, are obtained. Glycerin is directly produced with high purity levels (at least 98%) and is exempt from any salt contaminants. With all these features, this process can be considered as a green process. [21]

M.Hayyan et al, were investigated, a low cost quaternary ammonium salt–glycerine-based ionic liquid is proposed as a solvent for extracting glycerine from the transesterification biodiesel product. The separation technique was tested on palm oil-based produced biodiesel with KOH as a reaction catalyst. The study investigated the effect of DES:biodiesel ratio and the DES composition on the efficiency of the extraction process. The lab scale purification experiments proved the viability of the separation technique with a best DES:biodiesel molar ratio of 1:1 and a DES molar composition of 1:1 (salt:glycerine). The purified biodiesel fulfilled the EN 14214 and ASTM D 6751 standard specifications for biodiesel fuel in terms of glycerine content. A continuous separation process is suggested for industrial scale application.[22]

A.Demirbas, were dried ground biomass samples, being heated for 20 min in anhydrous glycerin in the presence of Na_2CO_3 or KOH, have been converted completely into water insoluble and soluble chemicals. The most important variables appear to be temperature, amount of alkali

and the nominal reaction time among the reaction parameters. The yield of total water insolubles is 68.4% from the beech wood in the presence of sodium carbonate. At these conditions, the yield of gaseous products is almost negligible. The acetone solubles from acidification of the liquefaction products was called biofuel in this study. The solubility of the biofuel in gasoline was tested as 1.96% by weight. When 10% ethanol is added to gasoline, the octane number increased by 8%. The C_3, C_4 and C_5 iso- and n-alcohols were found to be the most effective blending agents in reducing the phase separation temperature.[23]

M.Blanco et al, were use near-infrared spectroscopy to control an esterification reaction between glycerine and middle- or long-chain fatty acids performed in a laboratory-scale reactor. The process involves the initial formation of monoglycerides, which is followed by that of di- and triglycerides as well as transesterification. Establishing the end point of the process is critical with a view to ensuring that the end product will have the composition required for its intended use. PLS calibration was applied to industrial and laboratory-scale batch samples, and laboratory samples were additionally used to extend calibration ranges and avoid correlation between the concentration of the batch samples. In this way, PLS calibration models for glycerine, fatty acids, water, and mono-, di- and triglycerides, were constructed. The proposed method allows the reaction to be monitored in real time, thereby avoiding long analysis times, excessive reagent consumption and the obtainment of out-of-specification products. [24]

Maria-Isabel Galan et al, were observed the reaction kinetics for the synthesis of glycerol triacetate (triacetin) from glycerol and acetic acid has been studied in the frame of revalorization of residual glycerol in biodiesel production. The reaction has taken place in a stirred reactor at a pressure of 1070 kPa. No external catalyst has been added because the reaction performs better by using as catalyst an excess of acetic acid. Kinetic parameters were obtained at 120 and 160 C and a model of three reactions with monoacetin, diacetin and triacetin formation was proposed with a good agreement with the experimental results. Arrhenius constants were determined for the involved reactions. [25]

Sandun Fernando, Agus Haryanto, Sushil Adhikari, were found that Increasing biodiesel production has resulted in a glut of glycerin that has led to a precipitous drop in market prices. In

this study, the use of glycerin as a biorenewable substrate for hydrogen production, using a steam reforming process, has been evaluated. Production of hydrogen from glycerin is environmentally friendly because it adds value to this byproduct generated from biodiesel plants. The study focuses on nickel-based catalysts with MgO, CeO2, and TiO2 supports. Catalysts were characterized with thermogravimetric analysis and X-ray diffraction techniques. Maximum hydrogen yield was obtained at 650 C with MgO supported catalysts, which corresponds to 4 mol of H2 out of 7 mol of stoichiometric maximum. [26]

Tiancun Xiao et al, were produced hydrogen from a glycerol solution by aqueous phase reforming process was studied over supported Pt catalysts, and the affect of metal loadings and operation conditions have been examined. The reaction pathways showed that hydrogen generation is accompanied by side reactions to form alkane and liquid products. XRD study suggested that the alumina support in the catalyst was transformed into boehmite $Al_2(OOH)_2$; the elemental analysis demonstrated that carbonaceous entities was formed over the catalyst during the reaction which may cause catalyst activity drop. [27]

C.-J. Kim et al, were observed the effect of chemical doping to enhance the superconducting properties of MgB_2. In recent research activities, liquid based carbon compounds are becoming more popular for doping with an objective to achieve a homogeneous mixing. In this work, glycerin ($C_3H_8O_3$) was attempted to be used as a liquid dopant and was pre-treated with the boron powder before reacting with magnesium through an in situ process. Heat treatments were performed at 650 C or 900 C for 30 min in flowing Ar gas. The results show a slight decrease in critical temperature (Tc) and an enhancement of critical current density (Jc) at high fields for the glycerin-doped samples as compared to the un-doped samples. The FWHM results also show an improvement of the crystallinity which corresponds well with the Tc increased for the samples annealed at a higher temperature. [28]

Feral Temelli et al, were observed the effect of Esterification of free fatty acids (FFA) with glycerol in supercritical carbon dioxide (SC-CO_2) media to produce designer monoacylglycerols (MAG) for food, cosmetic, and pharmaceutical industries, was conducted to elucidate the reaction kinetics and provide the reaction mechanism. Reactions were conducted in

SC-CO_2 at 10–30MPa, 170–250 °C in a batch stirred reactor using an anhydrous glycerol to oleic acid initial molar ratio (gly/oleic) of 10:1, 1:1 and 1:2 and in supercritical nitrogen at 10MPa and 250 °C using 10:1 gly/oleic. Samples were collected as a function of time and MAG, diacylglycerol, triacylglycerol and FFA concentrations were determined using thin layer chromatography–flame ionization detection. The rate of MAG formation at 50% of equilibrium concentrations (Rate-50%) increased significantly ($p \leq 0.05$) with temperature but was not affected by pressure or supercritical media ($p > 0.05$). Rate-50% values for 10:1 and 1:1 gly/oleic were similar ($p > 0.05$) but higher ($p \leq 0.05$) than that for 1:2 gly/oleic. Equilibrium concentration of MAG significantly ($p \leq 0.05$) decreased with decreased initial glycerol concentration. The calculated rate constants provided a better understanding of the mechanism and are essential for optimal process design.[29]

Yoshihiro Sugi et al, were applied mesoporous materials for the esterification of glycerol (GL) with lauric acid (LA) in supercritical CO_2 (SCC) medium. The catalyst shows high conversion up to 93% with the formation of equal amount of lauric acid monoglyceride (Monolaurin;ML) and diglyceride (Dilaurin; DL). The catalytic activity in SCC medium was higher than those in mesitylene and without solvent under N_2 atmosphere. The coke formation was less in SCC medium compared to those in the other reaction medium. [30]

Sandun Fernando et al, were focused on the development of alternate energy resources. At present, there is a renewed interest in bio-fuels due to increasing concerns about energy security and environmental pollution. One such bio-based fuel that is being widely commercialized is biodiesel. With the increasing production of biodiesel a glut of glycerol has been created, causing market prices to plummet. This situation warrants finding alternative uses for glycerol. This study attempted to identify the possibility of blending glycerol and glycerol based co-products, such as propanediol and propanol with gasoline as oxygenates. The study revealed that there is a possibility to use glycerol and its derivatives with gasoline as an automotive fuel. Octane number and the heating value of different mixtures of gasoline, ethanol, and glycerol and its derivatives are presented. [31]

Hanny Johanes Berchmans et al, were studied a technique to produce biodiesel from crude Jatropha curcas seed oil (CJCO) having high free fatty acids (15%FFA) has been developed. The high FFA level of JCJO was reduced to less than 1% by a two-step pretreatment process. The first step was carried out with 0.60 w/w methanol-to-oil ratio in the presence of 1% w/w H_2SO_4 as an acid catalyst in 1-h reaction at 50 _C. After the reaction, the mixture was allowed to settle for 2 h and the methanol–water mixture separated at the top layer was removed. The second step was transesterified using 0.24 w/w methanol to oil and 1.4% w/w NaOH to oil as alkaline catalyst to produce biodiesel at 65 C. The final yield for methyl esters of fatty acids was achieved ca. 90% in 2 h. [32]

R. Alenezi et al, were observed the effect Non-catalytic esterification of Free Fatty Acids (FFA) with supercritical methanol was studied under reaction conditions of (250–320 C) at 10 MPa. A detailed experimental programme was implemented to investigate the influence of temperature, stirring rate and the molar ratio of methanol to FFA in the feed in a batch-type reaction vessel. The esterification products of FFA with supercritical methanol are Fatty Acids Methyl Esters (FAME; biodiesel) and water. The yield of FAME was found to increase with an increase in temperature, and with an increase in the molar ratio of methanol to FFA. At >850 rpm the yield of FAME was not affected by stirring rate. The rate constants and energy of activation have been numerically evaluated by solving an ordinary differential equation that describes the reaction kinetics. The proposed kinetic model shows a reversible second order reaction and represents all the experimental data satisfactorily, providing deeper insight into the kinetics of the reaction. [33]

Piyanuch Nakpong et al, was used coconut oil as a feedstock to produce biodiesel by a two-step process. In the first step, FFA level of the coconut oil was reduced to 0.6% by acid-catalyzed esterification. In the second step, triglycerides in product from the first step were transesterified with methanol by using an alkaline catalyst to produce methyl esters and glycerol. Effect of parameters related to these processes was studied and optimized, including methanol-to-oil ratio, catalyst concentration, reaction temperature, and reaction time. Methyl ester content of the coconut biodiesel was determined by GC to be 98.4% under the optimum condition. The

viscosity of coconut biodiesel product was very close to that of Thai petroleum diesel and other measured properties met the Thai biodiesel (B100) specification. [34]

Chapter 3

Experimental Procedure

3.1 Apparatus

1. 250 ml beaker
2. 250 ml Erlenmeyer flask
3. Condenser
4. 100 ml graduated cylinder
5. 10 ml graduated cylinder
6. Rounded-bottom flask
7. Heater for round bottom flask
8. Grabber
9. Stand
10. O-ring
11. Clamp
12. Burette
13. Dropper
14. Boiling chips
15. Hand-held refractometer (Brix Scale)
16. Digital pH meter

3.2 Chemicals

1. Ethanol
2. DI water
3. Used palm oil
4. Phenolphthalein
5. Sodium hydroxide (NaOH)
6. Potassium hydrogen phthalate (KHP)
7. Potassium hydroxide (KOH)
8. Methanol
9. Hexane
10. Iso-propanol

3.3 Glycerol's source

- We received crude glycerol from The Royal Thai Naval Dockyard of biodiesel production.
- We received Standard glycerol from Oleo Chemicals Company (Purity 99.5%).

3.4 Analysis Equipment

3.4.1 NMR Analysis : Bruker 300 Ultrashield.

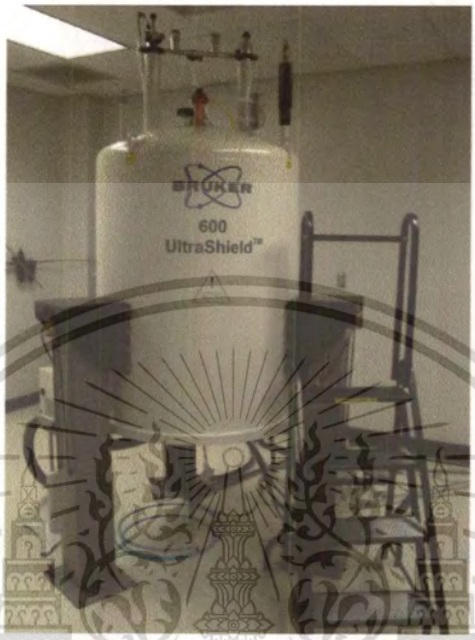


Figure 3.1 Bruker 300 Ultrashield (NMR analysis)

3.4.2 Refractometer [35]

Figure 3.2 Refractometer

3.5 Experimental Procedure

3.5.1 Preparation of crude glycerol to pure glycerol

1. Preparation a Sulfuric acid

1.1. Adding 10mL. 1M of Sulfuric acid to 100mL. in volumetric flask

1.2. Dilute with DI-water to 100mL.

2. Purification of crude glycerol

2.1 Evaporation of methanol

1) Weight crude glycerol 100g in Beaker 250 mL.

2) Heat glycerin to temperature around 100°C. and stir glycerin as well by 60-120 minutes or heat glycerol until the glycerol not have bubble inside glycerin.

3) Record the weight of the glycerol after evaporate methanol.2.2. Hexane extraction of residues lipid

2.2. Hexane extraction of residues lipid

1) Weight the glycerol that evaporate methanol already 50g. in beaker 250mL. and add DI water 50mL. and mix it together for decreased the viscosity of glycerin.

2) Adjust the alkalinity of glycerol to have pH around 6-8 by sulfuric acid in concentration of 0.1 molar.

3) Separate glycerol (in bottom-part) out form mixture and bring it to extract with Hexane 75mL. and evaporate Hexane out.

4) Extract with Hexane one more time.

5) After that bring the glycerol that evaporate Hexane already to eliminate the water out by Sodium sulfate anhydride.

6) Separate sodium sulfate anhydride out by using vacuum filter.

7) Using NMR-Spectroscopy analysis for observe a result.

3.5.2 Preparation of Phenolphthalein Indicator (Acid/Base Indicator)[36]

1. Weigh out 0.5 g of phenolphthalein.
2. Prepare a 50% ethanol solution consisting of 50ml ethanol and 50 ml water.
3. Dissolve the phenolphthalein thoroughly in the 50% ethanol solution.
4. Use from a bottle fitted with an dropper. Store the rest in a stoppered bottle.

3.5.3 Preparation of 0.1 M NaOH solution[37]

1. Weight 0.4g of dry NaOH into the beaker.
2. Dissolve with DI water in the beaker, then add them to 100 mL volumetric flask.
3. Dilute them to 100 mL with DI water.
4. Standardize NaOH solution using KHP solution titration[38]

   4.1) Add 10 ml of 0.1M KHP solution to Erlenmeyer flask

   4.2) Add 4-5 drops of phenolphthalein in to Erlenmeyer flask from (4.1)

   4.3) Titrate with unknown NaOH solution 0.5-1 ml once, shakes well. Stop the procedure when the solution appears to be faint pink after shaking for 30 seconds.

   4.4) Record the result and calculations.

$$M_1V_1 = M_2V_2$$

For examples; unknown concentration of NaOH solution titrate with 1M KHP using 10 ml KHP and 9.2 ml NaOH solution.

$$\mathbf{(1\ M)(10\ ml) = (9.2\ ml)M_2}$$

$$\mathbf{M_2 = 1.09\ M\ of\ NaOH\ solution}$$

3.5.4 Determination of Free Fatty acid in used palm oil[39]

1. Add 1 g of used palm oil to the Erlenmeyer flask and add 10 ml of iso-propanol
2. Add 2-3 drops of phenolphthalein to the flask, shake well.
3. Titrate them with NaOH by a buret, shake after each drop. Stop the procedure when the solution turns to faint pink for more than 30 seconds. Record the amount of NaOH solution used.
4. Repeat the procedure for 2 times
5. Calculations

Equation for calculate percent FFA

$$\text{FFA as \% of Palmitic acid} = \frac{mL\ NaOH \times NaOH\ Normality \times 25.6}{Weight\ of\ sample\ (g)}$$

*The calculation based on palmitic acid is substitute as 25.6 for equivalent weight.

3.5.5 Reaction of free fatty acid and glycerol

1. Add 50 ml of used palm oil to the round-bottom flask
2. Add pure glycerol according to desired ratio in the table;

Glycerol to palm oil ratio (Free fatty acid ratio)	1 : 1	1 : 1.5	1 : 2	1:3
Amount of glycerol used	1.7 ml	2.5 ml	3.4 ml	5.1 ml

Table 3.1 Volume ratio of glycerol to palm oil

3. Add 2-3 pieces of boiling chips to prevent bumping.
4. Reflux the solution for 30 mins,1 hr,2 hr,4 hr,6 hr,8 hr,10 hr and 12 hr.
5. Determine the free fatty acids in Glycerol and used palm oil samples by using the same method as the determination of free fatty acids in palm oil.
6. Determine for the optimal conditions.

Chapter 4

Results and Discussion

The experiment in this study was divided in to 2 parts; purification of glycerol and the reaction between free fatty acids and used palm oil to reduce the amount of FFA in order to increase the yield of biodiesel from transesterification process. The purification part contains 3 steps; evaporation of methanol, hexane extraction of residue lipids and de-colorization with activated carbon. The variables of this experiment are compared with two methods; (1) reflux system by various reaction time (2) reflux system by volume ratio between FFA and glycerol. The Nuclear Magnetic Resonance Spectroscopy (NMR) instrument and titration method were used to analyze the results of this study.

4.1 Standard Glycerol

This step was purified crude glycerol to have purities about 90%.

4.1.1 Analysis of standard glycerol.

Standard glycerol with 99.5% purity of this experiment was from Oleo chemicals company. The standard glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument and refractometer instrument.

Figure 4.1 Standard glycerol from Oleo Chemicals Company

Standard glycerol from Figure 4.1 was from Oleo Chemicals Company. It characteristic was colorless, high viscosity, and no odor.

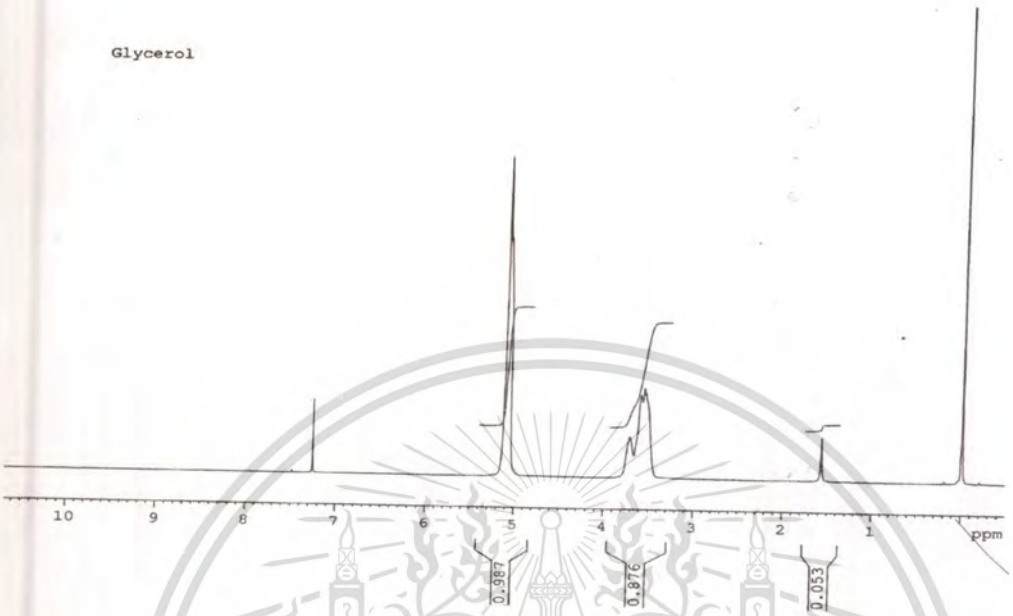


Figure 4.2 Standard glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument

From the Figure 4.2, chemical structure of Standard glycerol was present NMR peak at;

1.) 1.6 ppm for ^{1}H of OH group.

2.) 3.4-3.9 ppm for ^{1}H of CH_2O at 3.7 ppm and CHO at 3.55 ppm.

3.) 5.1 ppm for ^{1}H of H_2O or MeOH

4.) 7.25 ppm for ^{1}H of CCl_4

The refractive index resulted from refractometer in experiment was 1.4699. So, the percentage of glycerol by weight that from refractive index of glycerol-water solutions was 97%.

From the result of refractive index was shown 97% of glycerol which was lower than specification from Oleo Chemicals Company (99.5%) for 2.5%. However, the refractive index result can be able to indicate the percent purification trend of purified glycerol from purification process.

4.1.2 Analysis of crude glycerol.

Crude glycerol of this experiment was from The Royal Thai Naval Dockyard of biodiesel production. The crude glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument and Refractometer instrument.

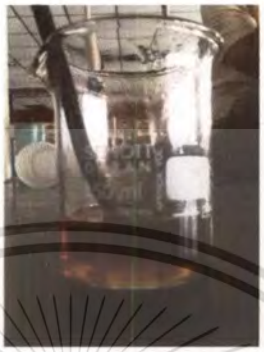


Figure 4.3 Crude glycerol from The Royal Thai Naval Dockyard of biodiesel production

The crude glycerol, brown color, high viscosity and bad smell, from the Royal Thai Naval Dockyard of biodiesel production was shown in figure 4.3.

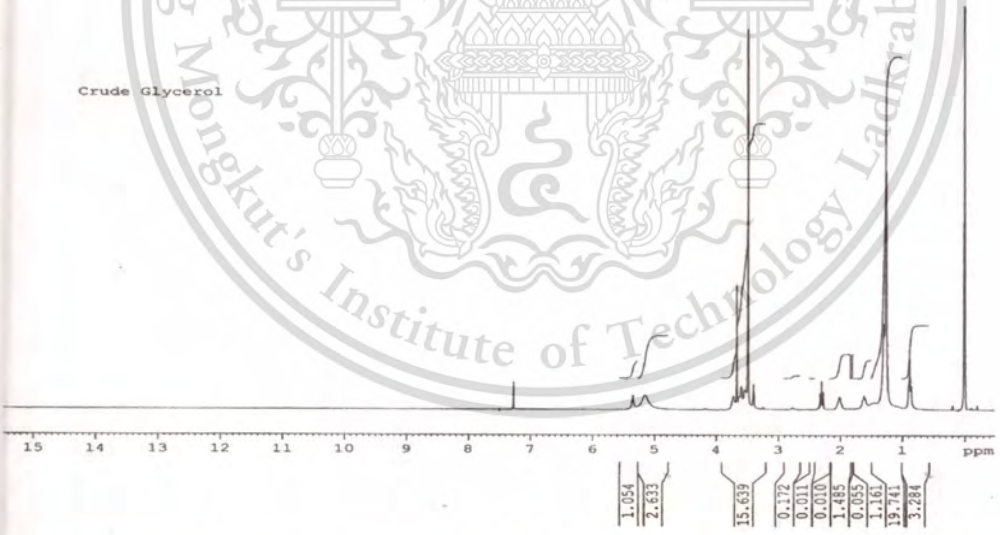


Figure 4.4 Raw glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument

The NMR spectrum of crude glycerol in figure 4.4 was shown peak of CH_2O and CHO protons at 3.7 and 3.55 ppm respectively. The impurities peak from biodiesel production was presented at 3.8, 3.5 and 1.4 ppm respectively.

The result from refractometer was shown as n_D 1.4332, that corresponding to the percent purification of glycerol at 74% (+2.5% was an error). And NMR spectroscopy was shown the lower peak area of glycerol (^1H) at 3-4 ppm than standard glycerol peak area.

From the comparison result of NMR and refractive index methods, the result was shown that the refractometer can be performed to determine the purity trend of purified glycerol. So, we will use the refractometer to analyze the purify glycerol of our experiment.

4.1.3 Analysis of purified glycerol.

The purified glycerol of our experiment was carried out in 3 steps of purification; first was evaporation of methanol, second was hexane extraction of residues lipid, and the third was de-colorization using activated carbon. The percentage of purification was analyzed by refractometer instrument.

Figure 4.5 Purified glycerol

The purified glycerol characteristic is light yellow, low viscosity and mild odor which was shown in figure 4.5. Therefore, characteristic of purified glycerol and standard glycerol were similar.

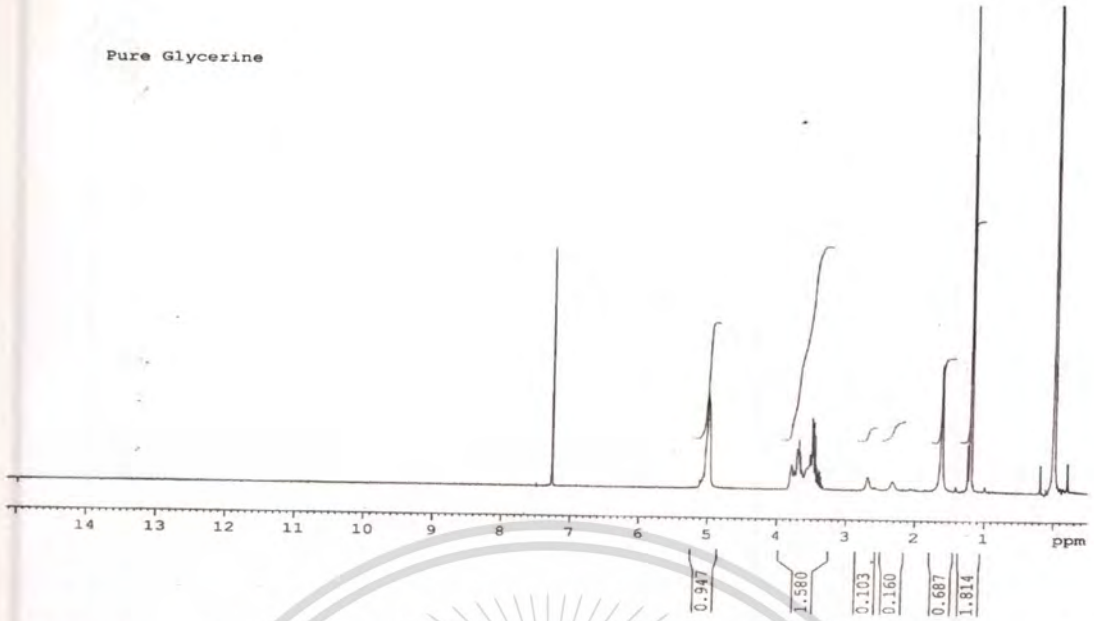


Figure 4.6 Purified glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument

From the Figure 4.6, chemical structure of purified glycerol was present in NMR peak at around 3-4 ppm, it was shown the peak area increased but still less than standard glycerol. And the impurities peak had been decreased.

The result from refractometer was shown as n_D 1.4588. So, the percentage of glycerol by weight that from refractive index of glycerol-water solutions was about 90% (+2.5% was an error).

The purification of crude glycerol were succeed to increase the percentage of glycerol from 74% to 90% purities. The percentage of glycerol in purified glycerol was not compatible for pharmaceutical, cosmetic, and food products but it can be used for the reduction of FFA in used palm oil. Then, we will use this purified glycerol in reverse transesterification of palmitic acid.

4.2 Production of monopalmitin from palmitic acid and purified glycerol.

This step was carried out to reduce the percentage of FFA content in used cooking palm oil by reflux system in various of reaction time and various of FFA and purified glycerol volume ratio.

4.2.1 Analysis of standardized NaOH.

Times	NaOH (ml)
1	9.70
2	9.85
3	9.80
4	9.80
Average	9.7875

Table 4.1 The NaOH volume in standardization of NaOH by KHP

$$M_{KHP}V_{KHP} = M_{NaOH}V_{NaOH} \quad (4.1)$$

$$0.1 \times 10 = M_{NaOH} \times 9.7875$$

$$M_{NaOH} = 0.1022$$

The standardization of NaOH solution by KHP was presented in table 4.1, the concentration of NaOH solution was 1.022 molar.

4.2.2 Analysis of FFA content in used cooking oil (Palm oil)

Times	NaOH used (ml)
1	1.40
2	1.25
3	1.20
4	1.35
5	1.20
6	1.25
7	1.20
8	1.25
9	1.25
10	1.35
AVERAGE	1.27

Table.4.2 The NaOH volume in FFA titration

The Hat-Yai fried chicken used palm oil was used as starting material. The percent FFA content in used palm oil was analyzed by titration method. The volume of NaOH solution was presented in table 4.2. The average volume of NaOH (1.27 ml) was used to calculated percent of FFA by eq. 4.2.

$$\text{FFA as \% of Palmitic acid} = \frac{mL\ NaOH\ x\ NaOH\ Normality\ x\ 25.6}{Weight\ of\ sample\ (g)} \quad (4.2)$$

$$= \frac{1.27\ x\ 0.1022\ x\ 25.6}{1}$$

$$= 3.32\%\text{FFA}$$

So, the average content of FFA in using Palm oil of 100 ml was 3.32 %FFA

Figure 4.7 The used cooking oil (Palm oil)

4.2.3 Analysis of percent FFA in reaction with standard glycerol

The table was shown the percent of FFA in waste palm oil at 1:1, 1:1.5 and 1:2 volume ratio of FFA : glycerol at various reaction time.

Ratio / Time (hr)	1:1	1:1.5	1:2
0	3.32	3.32	3.32
0.5	1.80	1.24	1.23
1	1.71	1.21	1.15
2	1.85	1.21	1.13
4	1.51	1.22	1.16
6	1.40	1.16	1.16
8	1.36	1.12	1.18
10	1.54	1.09	1.15
12	1.14	1.08	1.13

Table 4.3 Percentage of FFA with various ratio of standard glycerol to FFA ratio and reaction time

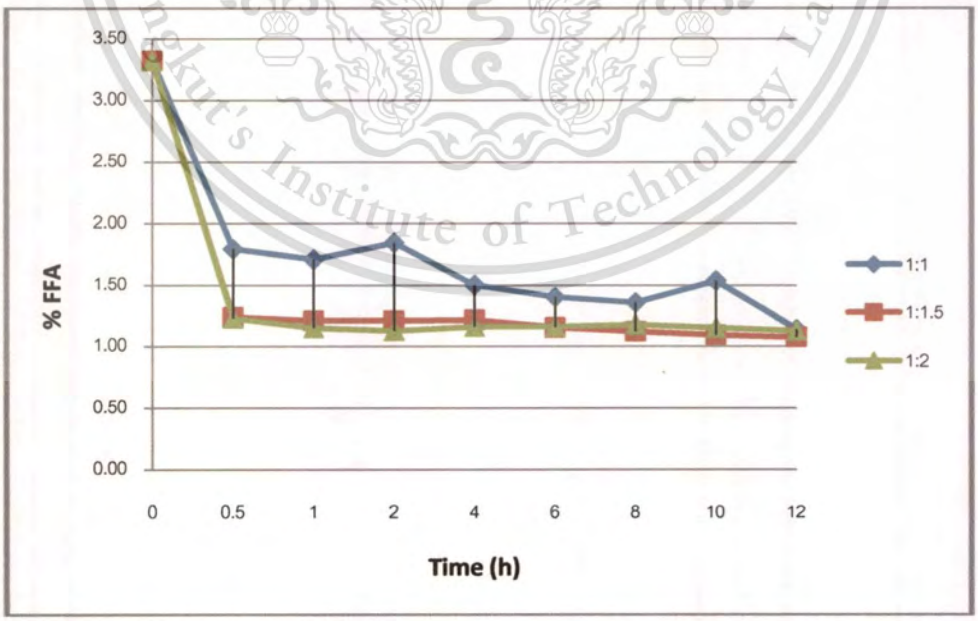


Figure 4.8 Percentage of FFA with various ratio of standard glycerol to FFA ratio and reaction time

From the figure 4.7, the percentages of FFA content are decreasing sharply while increasing reaction time from 0 to 1 hr, but for 1-12 h it slightly decreased. The reaction has been performed for 12 h, but it could observe that the equilibrium was attained after around 1 h.

For the kinetic adjustments, the reacting system is reduced to three consecutive main equilibrium reactions as follows:

Glycerol + palmitic acid ⟷ Monopalmitin + H_2O (1)

r_1 = k1 x [glycerol] x [Palmitic acid] = k-1 [Monopalmitin] x [H_2O]

Monopalmitin + palmitic acid ⟷ Dipalmitin + H_2O (2)

r_2= k2 [Monopalmitin] x [Palmitic acid]= k-2 x [Dipalmitin] x [H_2O]

Dipalmitin + palmitic acid ⟷ Tripalmitin + H_2O (3)

r_3=k3 [Dipalmitin] x [Palmitin acid] =k-3 [Tripalmitin] x[H_2O]

Feral Temelli et.al. [29] found that The conversion of glycerol to monoolein is a exist process. The oleic acid, $C_{18}H_{34}O_2$ and palmitic acid, $C_{16}H_{30}O_2$ have a similar structure. Sugi et al, [30] found that the quantity of tripalmitin and dipalmitin generated was very small in front of the monopalmitin formed. The overall reversible reaction of palmitic acid (PA), glycerol (Gly) as reactant and monopalmitin (MP), H_2O as product were described by Eq. (1) The conversion of glycerol to monopalmitin is should increase its performance to be able to treat big quantities of glycerol by optimum FFA: glycerol ratio (1:1, 1:1.5, and 1:2) and reaction time (0 to 12 h). Fig 4.9 was show glycerol conversion that was calculated according to the equation 4.3.

$$X(mol\%) = \frac{nFFA\ input - nFFA\ output}{nFFA\ input} \times 100 \qquad (4.3)$$

Where: n_{FFA} *input* and n_{FFA} *output* are the molar streams of glycerol at the input and output.

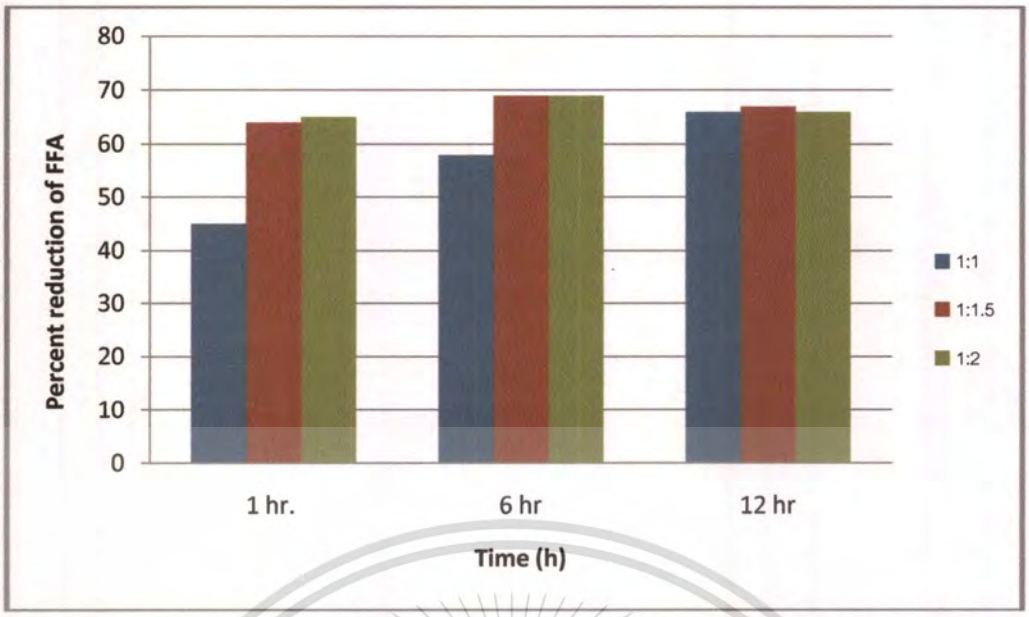


Figure 4.9 The percent reduction of FFA in used palm oil at 1,6 and 12h in various FFA to glycerol ratio

The conversion of palmitic acid to monopalmitin of free fatty acid to glycerol ratio 1:1 at 1,6 and 12h were 45%, 58% and 66% respectively.

The conversion of palmitic acid to monopalmitin of free fatty acid to glycerol ratio 1:1.5 at 1,6 and 12h were 64%, 69% and 67% respectively.

The conversion of palmitic acid to monopalmitin of free fatty acid to glycerol ratio 1:2 at 1,6 and 12h were 65%, 69% and 66% respectively.

The best performance was obtained (up to 64% of glycerol conversion) by using 1:1.5 ratios at 1 h. However, the reaction time has been investigated that the reaction time was performed for 12 h, but it could observe that the equilibrium was attained after around 2 h. Fig 4.10 show the optimum conditions were obtained at a FFA/glycerol ratio of 1:1.5, reaction time 1 h at reaction temperature above 200°C.

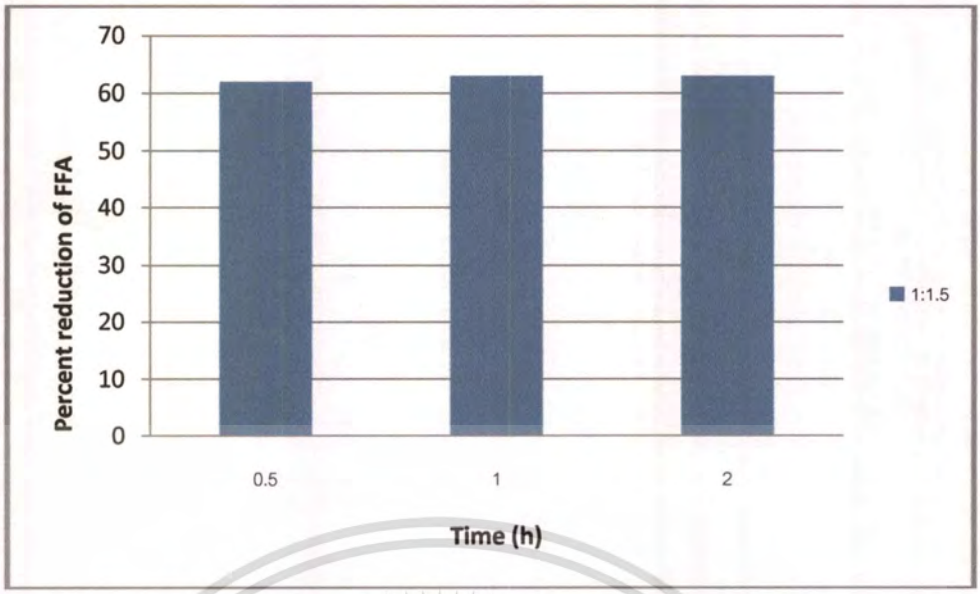


Figure 4.10 The percent reduction of FFA in used palm oil at ratio 1:1.5 at various time

4.2.4 Analysis of percent FFA in reaction with purified glycerol in optimal condition.

The used cooking oil (Palm oil) in this experiment was analyzed the %FFA content by titration method. In this experiment we use the optimal condition from the last experiment to observe the quality of our purified glycerol.

Ratio / Time (h)	1:1.5 Standard Glycerol	1:1.5 Purified Glycerol	1:2 Purified Glycerol	1:3 Purified Glycerol
0	3.32	3.32	3.32	3.32
0.5	1.24	1.52	1.35	0.93
1	1.21	1.52	1.33	0.90

Table 4.4 Percentage of FFA with various ratio of purified glycerol to FFA ratio and reaction time

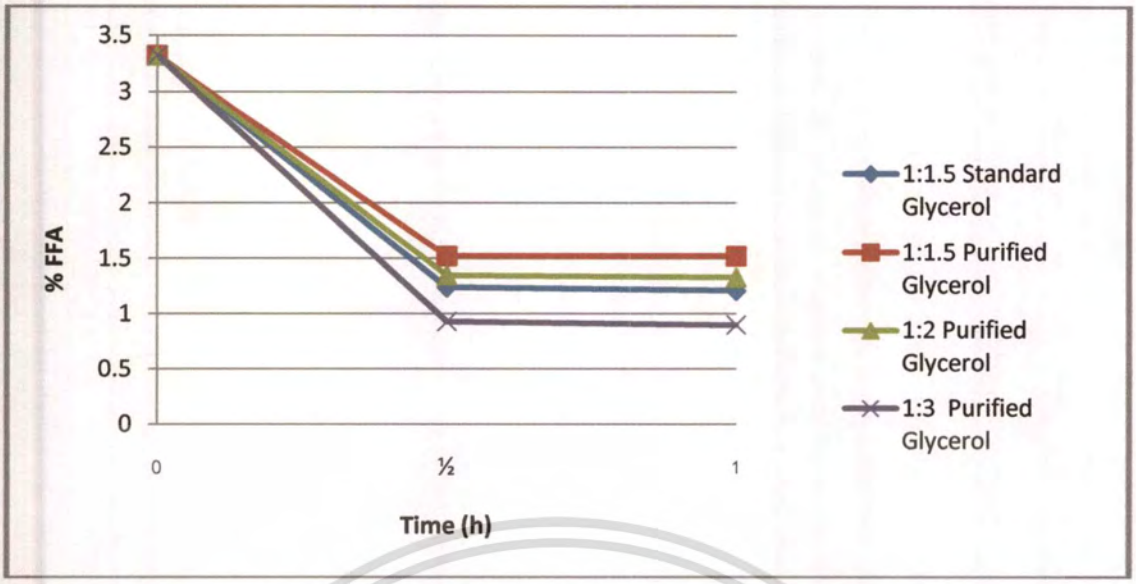


Figure 4.11 Percentage of FFA with various ratio of purified glycerol to FFA ratio and reaction time

The best performance was obtained (up to 73% of glycerol conversion) by using 1:3 ratios at 1 h was presented in figure 4.11.

Chapter 5

Conclusion and Suggestion

5.1 Conclusion

1. Characteristic of Crude Glycerol

1.1 The characteristic of crude glycerol from The Royal Thai Naval Dockyard of biodiesel production was brown color, high viscosity, and bad smell.

1.2 NMR peak of crude glycerol was presented at 3.55 and 3.7 ppm. The peak area of glycerol in crude glycerol was lower than standard glycerol. The impurities peak such as; salt, MeOH, and water were presented at 1.4, 3.5, and 3.8 ppm respectively.

1.3 The refractive index at n_D 1.4332 was corresponding to, percent purification of glycerol at 74% .

2. Characteristic of Purified Glycerol

2.1 The characteristic of purified glycerol was light yellow color, low viscosity than crude glycerol and Standard glycerol, mild odor than crude glycerol.

2.2 NMR peak of purified glycerol was presented at 3.45 and 3.7 ppm. The peak area of purified glycerol was higher than crude glycerol and impurities peak of purified glycerol was lower than crude glycerol.

2.3The refractive index at n_D1.4588 was corresponding to, percent purification of glycerol at 90%.

3.Production of monopalmitin from palmitic acid and purified glycerol.

3.1 The average content of FFA in using Palm oil was 3.32 %FFA.

3.2 The conversion of standard glycerol to monopalmitin by optimum condition at FFA : glycerol ratio 1:1.5 for 1 h was 64% glycerol conversion.

3.3 The conversion of purified glycerol to monopalmitin by optimum condition at FFA : glycerol ratio 1:3 for 1 h was 73% glycerol conversion.

5.2 Suggestion

1. Improve the purity of purified glycerol in order to increase the percent conversion of FFA.
2. Temperature should be various for study the effect of temperature.
3. Pressure should be various to study the effect of pressure.

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Appendix

Appendix-A

A.1) By-product from biodiesel production.

The major byproduct of biodiesel production is crude glycerin, also known as glycerol. Glycerin is a sweet-tasting, colorless, viscous liquid produced at a rate of 7.6 pounds (approximately one gallon) per 10 gallons of biodiesel.

A.2) Crude Glycerol

Crude glycerin can be purified for use in human products such as cosmetics, pharmaceuticals, and a variety of food items. Purification of crude glycerin is a costly and energy-intensive procedure; therefore, glycerin intended for livestock consumption is normally not purified.

A.3) Price of Glycerol

US refined glycerine spot prices were stable as most market participants focused on upcoming Q4 contract negotiations, buyers and sellers said during the week ended 9 September.

Most buyers and sellers said they expected a rollover in Q4 contract pricing, although a distributor said one of the major manufacturers might try to raise prices on its customers at the low end of the assessment.

Multiple distributors said business looked to be slow until the end of the year, while material from Argentina would prevent US producers from raising their own prices.

Many sources commented on the crash in European glycerine prices. Tallow and vegetable glycerine in the EU were averaging about 20 cents/lb as suppliers found fewer customers looking for material. That caused some US market participants to wonder if EU glycerine could find its way to the US.

One distributor said those prices did not represent the majority of the EU market, while a second distributor said EU material could eventually come to the US east coast, but not for a few months.

In Asia, refined glycerine spot prices were assessed at $530-600/tonne CFR NE Asia, unchanged from the week before.

A.4) Grade of Glycerol

Crude Glycerin - Crude glycerin contains a significant amount of methanol, water, soaps, and salts and typically has a glycerol content of anywhere between 40 to 88%. Crude glycerol is a natural by-product produced during the biodiesel production process, specifically taking place during transesterification.

Technical Grade Glycerin - Technical grade glycerin is a refined, high-purity product that is water white with most of its contaminants completely removed. Technical grade glycerin contains no methanol, soaps, salts, and other foreign matter. Biodiesel plants purchased from SRS Engineering, unlike many of our competitor's plants, produce technical grade glycerin right from the start.

USP Grade Glycerin - USP Grade Glycerin is a pharmaceutical grade glycerin suitable for food, personal care, cosmetics, pharmaceuticals, and other specialty applications. All of these products have met the US Pharmacopeia specifications (USP 30).

Properties	Crude Glycerin	Technical Grade Glycerin	99.7 -USP Grade Glycerin
Glycerol Content	40 - 88%	98.0 Min	99.70%
Ash	2.0% Max	N/A	N/A
Moisture Content	N/A	2.0% Max	0.3% Max
Chlorides	N/A	10 ppm Max	10 ppm Max
Color	N/A	40 Max (Pt - Co)	10 Max. (APHA)
Specific Gravity	N/A	1.262 (@25C)	1.2612 Min
Sulfate	N/A	N/A	20 ppm Max
Assay	N/A	N/A	99.0 - 101.0% (on dry basis)
Heavy Metals	N/A	5 ppm Max	5 ppm Max
Chlorinated Compounds	N/A	30 ppm Max	30 ppm Max
Residue on Ignition	N/A	N/A	100 ppm Max
Fatty Acid & Ester	N/A	1.00 Max	1.000 Max
Water	12.0% Max	5.0% Max	0.5% Max
pH (10% Solution)	4.0 - 9.0	4.0 - 9.1	N/A
DEG and Related Compounds	N/A	N/A	Pass
Organic Volatile Impurities	N/A	N/A	Pass
Organic Residue	2.0% Max	2.0% Max	N/A

Table A-1 Properties of Grade of Glycerol

A.5) MSDS of Glycerol

General

Synonyms: glycerin, glycerol USP, glycerine, 1,2,3-propanetriol, propanetriol, 1,2,3-trihydroxypropane, bulbold, citifluor AF 2, cristal, emergy 916, glyrol, glycerol opthalgan, glyciterol, glycyl alcohol, osmoglyn, pricerine 9091

Use: Widely used as a food additive (emulsifier, thickener, stabilizer), cosmetic agent,

lubricating agent, antifreeze etc.

Molecular formula: $C_3H_8O_3$ [structural: $CH_2OHCHOHCH_2OH$]

CAS No: 56-81-5

EC No: 200-289-5

Physical data

Appearance: viscous colourless or pale yellow liquid

Melting point: 17.8 C

Boiling point: 290 C

Vapour density: 3.17 g/l

Vapour pressure: < 1mm Hg at 20 C

Specific gravity: 1.261

Flash point: 160 C (closed cup)

Explosion limits: lower 0.9%

Autoignition temperature: 370 C

Critical temperature: 492.2 C

Critical pressure: 42.5 atm

Stability

Stable. Incompatible with perchloric acid, lead oxide, acetic anhydride, nitrobenzene, chlorine, peroxides, strong acids, strong bases. Combustible.

Toxicology

Mist is a respiratory irritant at high concentrations. Repeated contact may cause dehydration of skin. Typical TLV 10 mg/m3 (nuisance). Not hazardous according to directive 67/548/EC.

Toxicity data

IPR-RAT LD50 8700 mg kg^{-1}

ORL-RAT LD50 12600 mg kg^{-1}

SCU-RAT LD50 100 mg kg^{-1}

ORL-MUS LD50 8700 mg kg^{-1}

A.6) Refractive index of Glycerol.

Refractive Index of Glycerine-Water Solutions at 20°C (69°F)

Glycerine % by Weight	Refractive Index n_D^{20}	Difference for 1%	Glycerine % by Weight	Refractive Index n_D^{20}	Difference for 1%
100	1.47399	0.00165	50	1.39809	0.00149
99	1.47234	0.00163	49	1.39660	0.00147
98	1.47071	0.00161	48	1.39513	0.00145
97	1.46909	0.00157	47	1.39368	0.00141
96	1.46752	0.00156	46	1.39227	0.00138
95	1.46597	0.00154	45	1.39089	0.00136
94	1.46443	0.00153	44	1.38953	0.00135
93	1.46290	0.00151	43	1.38818	0.00135
92	1.46139	0.00150	42	1.38683	0.00135
91	1.45989	0.00150	41	1.38548	0.00135
90	1.45839	0.00150	40	1.38413	0.00135
89	1.45689	0.00150	39	1.38278	0.00135
88	1.45539	0.00150	38	1.38143	0.00135
87	1.45389	0.00152	37	1.38008	0.00134
86	1.45237	0.00152	36	1.37874	0.00134
85	1.45085	0.00155	35	1.37740	0.00134
84	1.44930	0.00156	34	1.37606	0.00134
83	1.44770	0.00160	33	1.37472	0.00134
82	1.44612	0.00162	32	1.37338	0.00134
81	1.44450	0.00160	31	1.37204	0.00134
80	1.44290	0.00155	30	1.37070	0.00134
79	1.44135	0.00153	29	1.36936	0.00134
78	1.43982	0.00150	28	1.36802	0.00133
77	1.43832	0.00149	27	1.36669	0.00133
76	1.43683	0.00149	26	1.36536	0.00132
75	1.43534	0.00149	25	1.36404	0.00132
74	1.43385	0.00149	24	1.36272	0.00131
73	1.43236	0.00149	23	1.36141	0.00131
72	1.43087	0.00149	22	1.36010	0.00131
71	1.42938	0.00149	21	1.35879	0.00130
70	1.42789	0.00149	20	1.35749	0.00130
69	1.42640	0.00149	19	1.35619	0.00129
68	1.42491	0.00149	18	1.35490	0.00129
67	1.42342	0.00149	17	1.35361	0.00128
66	1.42193	0.00149	16	1.35233	0.00127
65	1.42044	0.00149	15	1.35106	0.00126
64	1.41895	0.00149	14	1.34980	0.00126
63	1.41746	0.00149	13	1.34854	0.00125
62	1.41597	0.00149	12	1.34729	0.00125
61	1.41448	0.00149	11	1.34604	0.00123
60	1.41299	0.00149	10	1.34481	0.00122
59	1.41150	0.00149	9	1.34359	0.00121
58	1.41001	0.00149	8	1.34238	0.00120
57	1.40852	0.00149	7	1.34118	0.00119
56	1.40703	0.00149	6	1.33999	0.00119
55	1.40554	0.00149	5	1.33880	0.00118
54	1.40405	0.00149	4	1.33762	0.00117
53	1.40256	0.00149	3	1.33645	0.00115
52	1.40107	0.00149	2	1.33530	0.00114
51	1.39958	0.00149	1	1.33416	0.00113
			0	1.33303	–

Table A-2 Refactive index of glycerol

Appendix-B

B.1) Analytical Instruments

B.1.1) NMR Spectroscopy

Introduction

Nuclear Magnetic Resonance spectroscopy is a powerful and theoretically complex analytical tool. On this page, we will cover the basic theory behind the technique. It is important to remember that, with NMR, we are performing experiments on the **nuclei** of atoms, not the electrons. The chemical environment of specific nuclei is deduced from information obtained about the nuclei.

Nuclear spin and the splitting of energy levels in a magnetic field

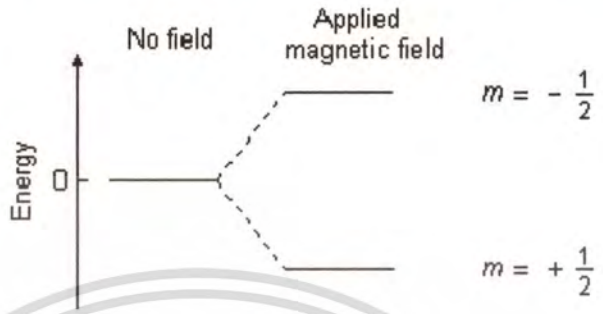
Subatomic particles (electrons, protons and neutrons) can be imagined as spinning on their axes. In many atoms (such as ^{12}C) these spins are paired against each other, such that the nucleus of the atom has no overall spin. However, in some atoms (such as ^{1}H and ^{13}C) the nucleus does possess an overall spin. The rules for determining the net spin of a nucleus are as follows;

1. If the number of neutrons **and** the number of protons are both even, then the nucleus has **NO** spin.
2. If the number of neutrons **plus** the number of protons is odd, then the nucleus has a half-integer spin (i.e. 1/2, 3/2, 5/2)
3. If the number of neutrons **and** the number of protons are both odd, then the nucleus has an integer spin (i.e. 1, 2, 3)

The overall spin, I, is important. Quantum mechanics tells us that a nucleus of spin I will have $2I + 1$ possible orientations. A nucleus with spin 1/2 will have 2 possible orientations. In the absence of an external magnetic field,

these orientations are of equal energy. If a magnetic field is applied, then the energy levels split. Each level is given a *magnetic quantum number, m.*

Energy levels for a nucleus with spin quantum number 1/2



When the nucleus is in a magnetic field, the initial populations of the energy levels are determined by thermodynamics, as described by the Boltzmann distribution. This is very important, and it means that **the lower energy level will contain slightly more nuclei than the higher level.** It is possible to excite these nuclei into the higher level with electromagnetic radiation. The frequency of radiation needed is determined by the difference in energy between the energy levels.

Calculating transition energy

The nucleus has a positive charge and is spinning. This generates a small magnetic field. The nucleus therefore possesses a magnetic moment, m, which is proportional to its spin,*I*.

$$\mu = \frac{\gamma I h}{2\pi}$$

The constant, g, is called the *magnetogyric ratio*and is a fundamental nuclear constant which has a different value for every nucleus. *h* is Plancks constant.

The energy of a particular energy level is given by;

$$E = -\frac{\gamma h}{2\pi} m B$$

Where B is the strength of the magnetic field **at the nucleus**.

The difference in energy between levels (the transition energy) can be found from

$$\Delta E = \frac{\gamma h B}{2\pi}$$

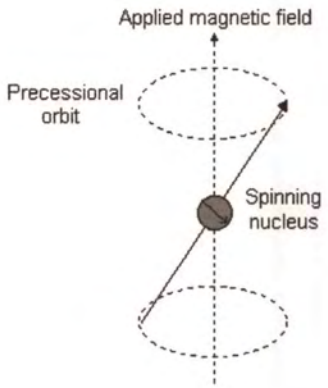
This means that if the magnetic field, B, is increased, so is DE. It also means that if a nucleus has a relatively large magnetogyric ratio, then DE is correspondingly large.

If you had trouble understanding this section, try reading the next bit (The absorption of radiation by a nucleus in a magnetic field) and then come back.

The absorption of radiation by a nucleus in a magnetic field

In this discussion, we will be taking a "classical" view of the behaviour of the nucleus - that is, the behaviour of a charged particle in a magnetic field.

Imagine a nucleus (of spin 1/2) in a magnetic field. This nucleus is in the lower energy level (i.e. its magnetic moment does not oppose the applied field). The nucleus is spinning on its axis. In the presence of a magnetic field, this axis of rotation will *precess* around the magnetic field;



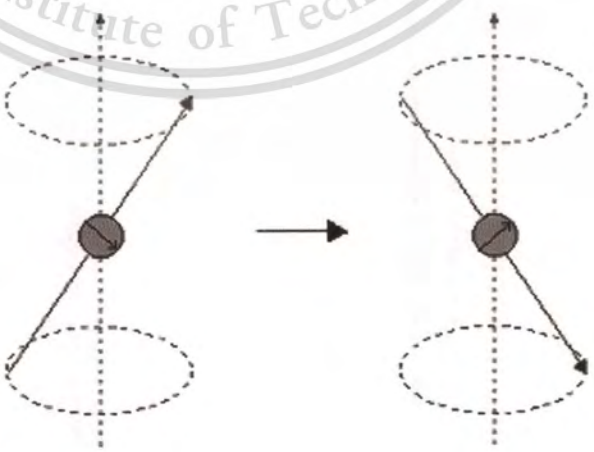
The frequency of precession is termed the *Larmor frequency*, which is identical to the transition frequency.

The potential energy of the precessing nucleus is given by;

$$E = - m B \cos q$$

where q is the angle between the direction of the applied field and the axis of nuclear rotation.

If energy is absorbed by the nucleus, then the angle of precession, q, will change. For a nucleus of spin 1/2, absorption of radiation "flips" the magnetic moment so that it **opposes** the applied field (the higher energy state).

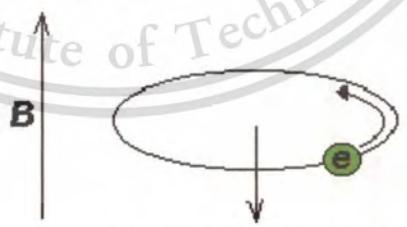


It is important to realise that only a small proportion of "target" nuclei are in the lower energy state (and can absorb radiation). There is the possibility that by exciting these nuclei, the populations of the higher and lower energy levels will become equal. If this occurs, then there will be **no** further absorption of radiation. The spin system is *saturated*. The possibility of saturation means that we must be aware of the relaxation processes which return nuclei to the lower energy state.

Chemical shift

The magnetic field at the nucleus is **not** equal to the applied magnetic field; electrons around the nucleus shield it from the applied field. The difference between the applied magnetic field and the field at the nucleus is termed the *nuclear shielding*.

Consider the s-electrons in a molecule. They have spherical symmetry and circulate in the applied field, producing a magnetic field which opposes the applied field. This means that the applied field strength must be increased for the nucleus to absorb at its transition frequency. This *upfield shift* is also termed *diamagnetic shift*.



Magnetic field produced
by circulating electron

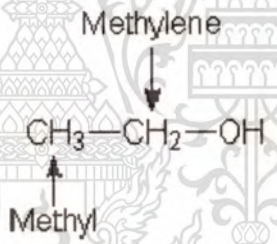
Electrons in p-orbitals have **no** spherical symmetry. They produce comparatively large magnetic fields at the nucleus, which give a *low field shift*. This "deshielding" is termed *paramagnetic shift*.

In proton (^1H) NMR, p-orbitals play no part (there aren't any!), which is why only a small range of chemical shift (10 ppm) is observed. We can easily see the effect of s-electrons on the chemical shift by looking at substituted methanes, CH_3X. As X becomes increasingly electronegative, so the electron density around the protons decreases, and they resonate at lower field strengths (increasing d_H values).

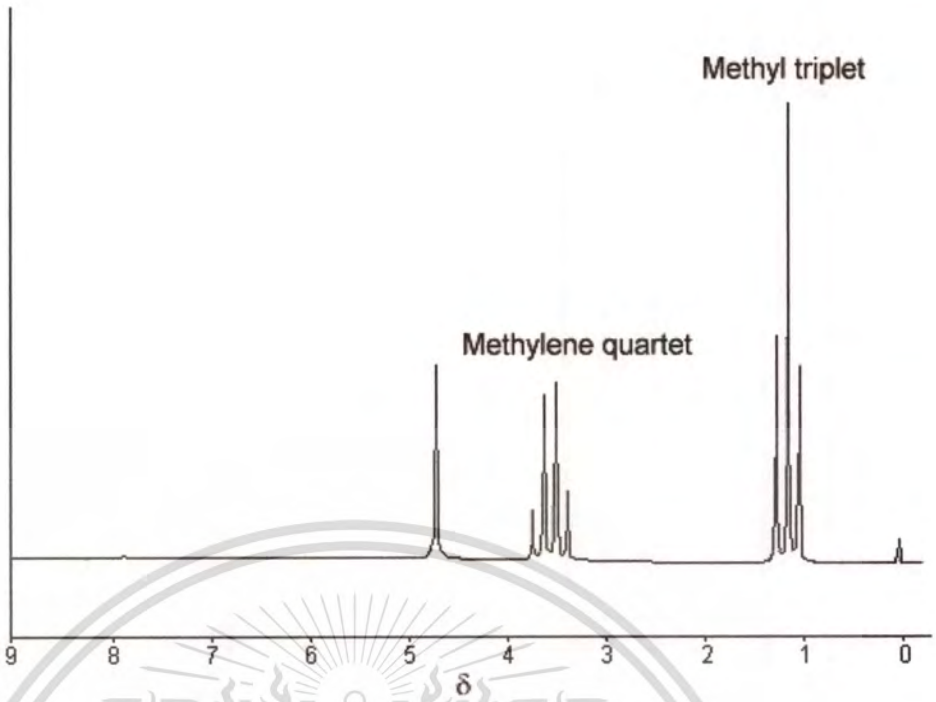
Chemical shift is defined as *nuclear shielding / applied magnetic field.* Chemical shift is a function of the nucleus and its environment. It is measured relative to a reference compound. For ^1H NMR, the reference is usually tetramethylsilane, $Si(CH_3)_4$.

Spin - spin coupling

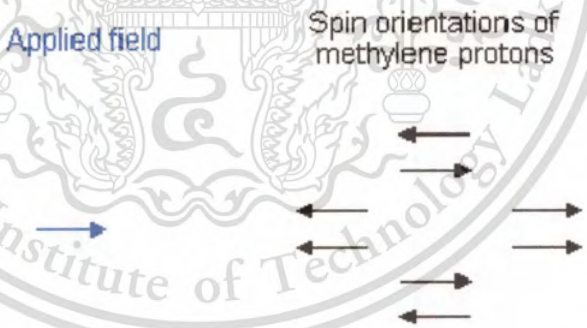
Consider the structure of ethanol;



The ^1H NMR spectrum of ethanol (below) shows the methyl peak has been split into three peaks (a *triplet*) and the methylene peak has been split into four peaks (a *quartet*). This occurs because there is a small interaction (*coupling*) between the two groups of protons. The spacings between the peaks of the methyl triplet are equal to the spacings between the peaks of the methylene quartet. This spacing is measured in Hertz and is called the *coupling constant, J.*

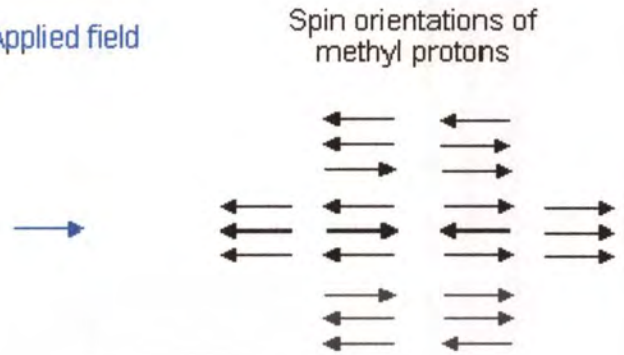


To see why the methyl peak is split into a triplet, let's look at the **methylene** protons. There are two of them, and each can have one of two possible orientations (aligned with or opposed against the applied field). This gives a total of four possible states;



In the first possible combination, spins are paired and opposed to the field. This has the effect of reducing the field experienced by the **methyl** protons; therefore a slightly higher field is needed to bring them to resonance, resulting in an upfield shift. Neither combination of spins opposed to each other has an effect on the methyl peak. The spins paired in the direction of the field produce a downfield shift. Hence, the methyl peak is split into three, with the ratio of areas 1:2:1.

Similarly, the effect of the methyl protons on the methylene protons is such that there are eight possible spin combinations for the three methyl protons;



Out of these eight groups, there are two groups of three magnetically equivalent combinations. The methylene peak is split into a quartet. The areas of the peaks in the quartet have the ration 1:3:3:1.

In a *first-order* spectrum (where the chemical shift between interacting groups is much larger than their coupling constant), interpretation of splitting patterns is quite straightforward;

- The multiplicity of a multiplet is given by the number of equivalent **protons** in **neighbouring** atoms plus one, i.e. *the n + 1 rule*
- Equivalent nuclei do not interact with each other. The three methyl protons in ethanol cause splitting of the neighbouring methylene protons; they do not cause splitting among themselves
- The coupling constant is not dependant on the applied field. Multiplets can be easily distinguished from closely spaced chemical shift peaks.

B.1.2) Refractometer

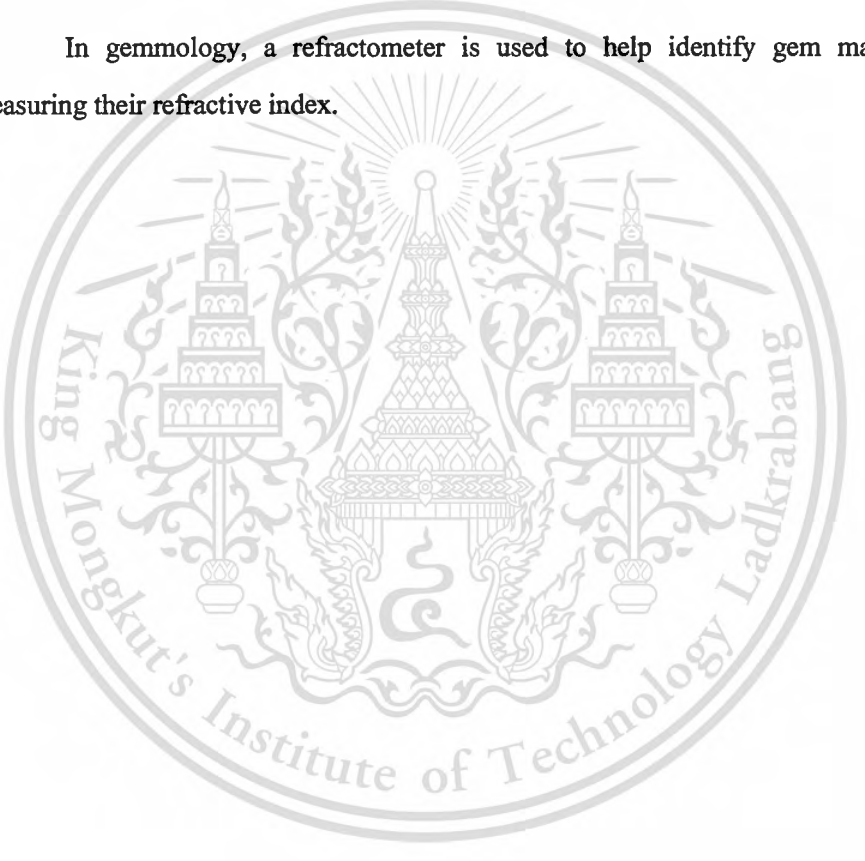
A refractometer is a laboratory or field device for the measurement of an index of refraction. The index of refraction is calculated from Snell's law and can be calculated from the composition of the material using the Gladstone-Dale relation.

Types of refractometers.

There are four main types of refractometers: traditional handheld refractometers, digital handheld refractometers, laboratory or Abbe refractometers, and inline process refractometers. There is also the Rayleigh Refractometer used (typically) for measuring the refractive indices of gases.

In veterinary medicine, a refractometer is used to measure the total plasma protein in a blood sample and urine specific gravity.

In gemmology, a refractometer is used to help identify gem materials by measuring their refractive index.



Appendix-C

C.1) Chemical

C.1.1) NMR Spectroscopy

Nuclear Magnetic Resonance Spectroscopy analysis methods

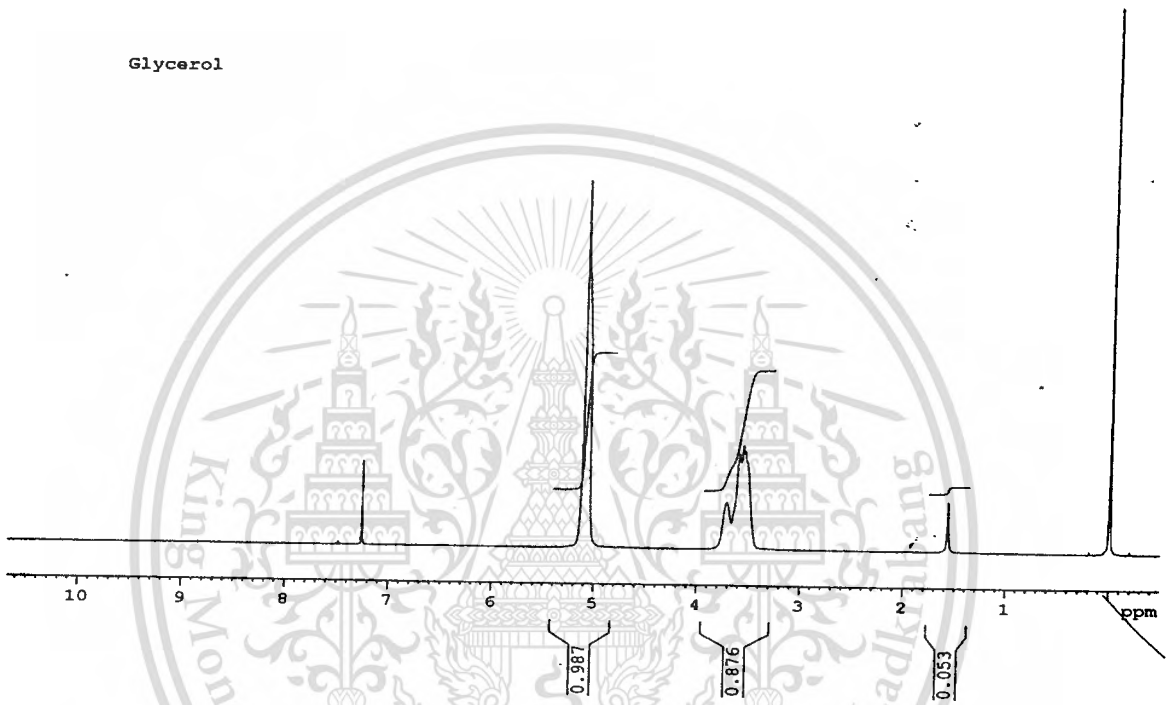


Figure C-1 Standard glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument.

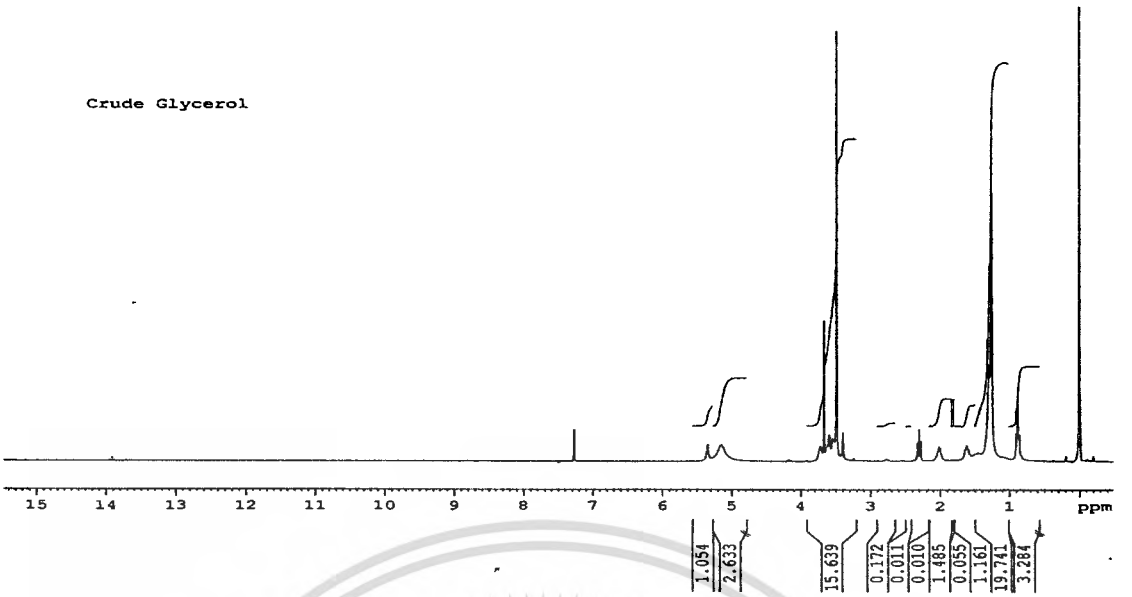


Figure C-2 Raw glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument.

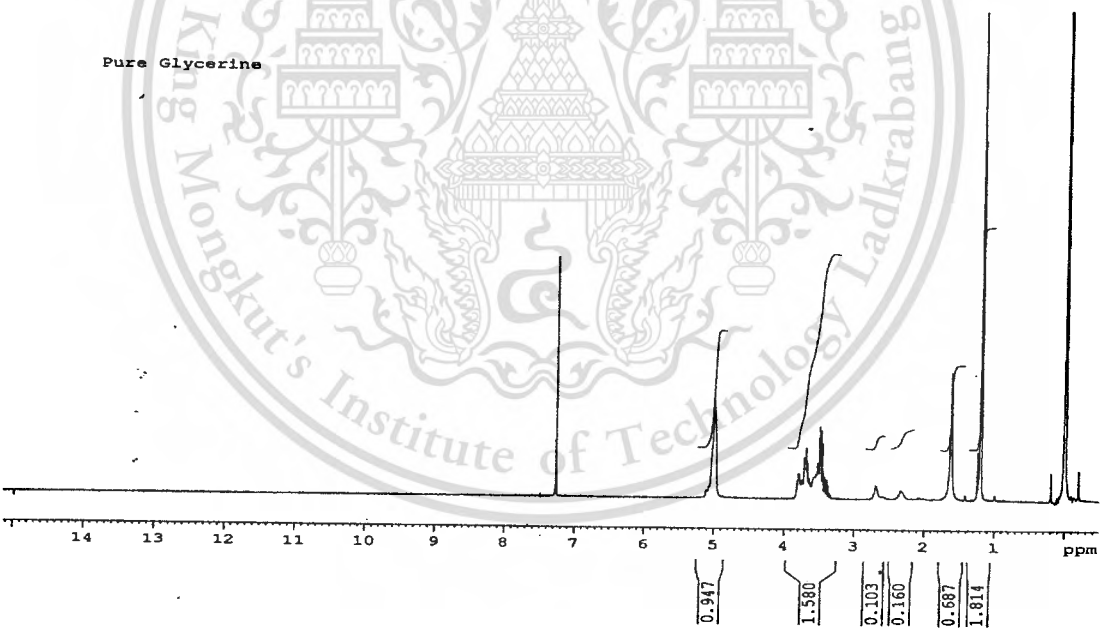


Figure C-3 Purified glycerol was analyzed by Nuclear Magnetic Resonance Spectroscopy (NMR) instrument.

C.1.2) Standardized

NaOH.

$$M_{KHP}V_{KHP} = M_{NaOH}V_{NaOH}$$

$$0.1 \times 10 = M_{NaOH} \times 9.7875$$

$$M_{NaOH} = 0.1022$$

C.1.3) Calculation of percent FFA

$$\text{FFA as \% of Palmitic acid} = \frac{mL\ NaOH\ x\ NaOH\ Normality\ x\ 25.6}{Weight\ of\ sample\ (g)}$$

$$= \frac{1.27\ x\ 0.1022\ x\ 25.6}{1}$$

$$= 3.32\%\text{FFA}$$