SYNTHESIS OF ZINC GLYCEROLATE FROM THE REACTION OF ZINC ACETATE AND GLYCEROL BY PRODUCT BIODIESEL

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Abstract

Zinc glycerolate (Zn-GLY) raw material in pharmaceutical, cosmetic and polymer product was synthesized by zinc acetate and glycerol. The feasibility of value-added of crude glycerol by-product from biodiesel production was the aim of the study. Zn-GLY was synthesized according to a publication of Reginald M. Taylor (1989). There was the purification process to get rid of the impurity of crude glycerol, before used for preparing Zn-GLY. The molar ratio and temperature were determined during indirect heating through paraffin bath for 1 hr at temperature 200 ^{o}C, 230 ^{o}C, and 260 ^{o}C. The optimum conditions were; molar ratio 1:7 at temperature 260 ^{o}C The Zn-GLY was characterized with respect to their: physical properties and microorganism toxicity test. The IR spectroscopy, X-ray Diffraction chromatogram (XRD) and SEM results confirmed their spectroscopic properties of Zn-GLY. Zn-GLY inhibited *Micrococcus luteus* the growth at 10^{-3} M and also inhibited growth of *Staphylococcus aurous* and *Serratia Marcescens* at lowest level as 10^{-7} M. Therefore, the continuing process of Zinc glycerolate can produce from biodiesel by-product and their application also achievable.

Acknowledgments

The preparation of this important project would not have been possible without the support, hard work and endless efforts of large number of individuals and institutions.

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Chapter 1

Introduction

1.1 Motivation

Since the reservoir was first drilled in Pennsylvania, USA in 1861. Oil is widely used worldwide and demand is rapidly increasing. International Energy Agency (IEA) predicted that world oil demand will increase traffic and if oil does not change with the remaining oil reserves. Expected in another 40 years, oil will run out of the world. Current crude oil prices in world markets are likely in excess of $ 50 per barrel and not likely to drive down prices. For this reason it is essential to develop other energy sources, especially renewable energy use to replace petroleum fuel such as gasohol biodiesel. One of Renewable Energy in Thailand used instead of petroleum oil is biodiesel, a renewable energy His Majesty King Bhumibol Adulyadej. He has pioneered the production of biodiesel from palm oil and is encouraging people to produce biodiesel fuel to replace petroleum oil [1]. In addition, the Ministry of Energy has set a goal of promoting biodiesel use is 8.5 million liters per day in 2012. For this reason, it is oil biodiesel widely available in Thailand, where the majority of production will produce biodiesel from used vegetable oil to pass by Trans-esterification process will get biodiesel and glycerol. In the production of biodiesel each takes up about 10 percent glycerol by weight of biodiesel, Glycerol which is considered to occur in relatively high volumes. When compared to export value of glycerol is higher than the import value allows Thailand to produce glycerol over demand in the country [2]. Therefore, trends in glycerol prices will be lowered due to an outgrowth from glycerol biodiesel production process that is likely to increase continuously. Applying for the glycerol from the biodiesel production process to benefit in other industries is much less. Due to higher investment in the first purified before used. Do not favor the process of glycerol from biodiesel production to benefit. For this reason, it is made of glycerol occurred at a waste cause problems in the conditions, such as burn glycerol will occur acrolein, a compound that is harmful to human body and animals [3]. Therefore, this research provides interesting crude glycerol add

value by bringing a reaction with zinc acetate as a zinc glycerolate, material in the production of medicines cosmetics and zinc glycerolate is slippery when used as additives in the production of polymer is a polymer made more transparent. There are also bringing zinc glycerolate used rubber and plastic resins industry.

1.2 Objectives

1. For value-added of crude glycerol from biodiesel production. Glycerols were used as reactants for zinc glycerolate production and compare the results with the use of pure glycerol.

2. To study the reaction between glycerol and zinc glycerolate.

1.3 Scope to study

1. To study about zinc glycerolate preparatio0n in lab scale and study the chemical, molar ratio, and temperature.

2. Compare Zinc glycerolate production from crude glycerol and pure glycerol.

1.4 Expected results

1. Can add value to crude glycerol that is a waste form biodiesel production and reduce costs of biodiesel production.

2. Determine the appropriate conditions of zinc glycerolate production from crude glycerol and pure glycerol.

3. Know the physical, chemical and biological properties.

Chapter 2

THEORY AND LITERATURE REVIEW

2.1 Glycerol

Glycerol is a chemical compound also commonly called glycerin 1,2,3-propanetriol.The simplest trihydric alcohol, with the formula $CH_2OHCHOHCH_2OH$. The name glycerol is preferred for the pure chemical, but the commercial product is usually called glycerin [4]. It is widely distributed in nature in the form of its esters, called glycerides. The glycerides are the principal constituents of the class of natural products known as fats and oils.

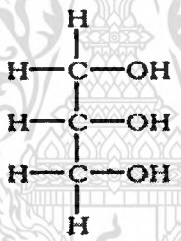

Figure 2.1 Structure of Glycerol

Pure 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.

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 [5], 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 substituents), 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.

2.1.1 Categorization

Glycerol is currently categorized by the American Dietetic Association as a carbohydrate. The FDA [6] 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.

2.1.2 Properties and structure of glycerol

2.1.2.1 Physical properties of glycerol

Pure 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.

Table 2.1 The relationship between vapor pressure and temperature of glycerol (Barbara, 1994)

Temperature(°C)	Vapor pressure(kPa)
290	101.3
266	53.3
222	13.3
204	6.67
175	2.00
152	0.67
130	0.18
100	0.03
20	<0.0001

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.

Table 2.2 The physical properties of glycerol

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

2.1.2.2 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 acidCH2CHCHOHCOOH melting at 134-135°C when pure, but usually obtained as syrupy. Some industrially important reaction products of glycerol include:

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

2.1.3 Applications of glycerol

2.1.3.1 Feedstock

Glycerol is one of the major raw materials for the manufacture of polyols for flexible foams, and to a lesser extent rigid polyurethane foam. Glycerol is used to produce nitroglycerin, or glycerol-trinitrate (GTN) [7], which is an essential ingredient of smokeless gunpowder and various explosives such as dynamite, gelignite and munitions like cordite. Reliance on soap-making to supply co-product glycerine made it difficult to increase production to meet wartime demand. Hence, synthetic glycerin processes were national defense priorities in the days leading up to World War II. GTN is commonly used to relieve *angina pectoris*, taken in the form of sub-lingual tablets, or as an aerosol

spray. Glycerol is also used to manufacture mono- and di-glycerides for use as emulsifiers, as well as polyglycerol esters going into shortenings and margarine.

2.1.3.2 Research laboratory usage

Glycerol is a common component of solvents for enzymatic reagents stored at temperatures below zero degrees Celsius due to the depression of the freezing temperature of solutions with high concentrations of glycerol. It is also dissolved in water to reduce damage by ice crystals to laboratory organisms that are stored in frozen solutions, such as bacteria, nematodes, and fruit flies. Samples are loaded into a arose gel electrophoresis mixed in loading buffers that mainly consist of glycerol; when the sample is injected into wells, the glycerol causes the solution to sink through the running buffer to the bottom of the well.

2.1.3.3 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.

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 glycerites [8].

Used as a laxative when introduced into the rectum in suppository or small-volume (2 to 10 ml) (enema) forms; irritates the anal mucosa and induces a hyperosmotic effect.

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 is possible to make glycerol soap at home [9].

Glycerol is also used in de-/anti-icing fluids, as in vitrification of blood cells for storage in liquid nitrogen [10].

In motion-picture production, glycerol is used as a non-evaporating substitute for perspiration on actors. It is also used in the formulation of some types of stage blood.

2.1.3.4 Food

Glycerin as a food is easily digested and non-toxic and its metabolism places it with the carbohydrates, though it is present in combined form in all vegetable and animal fats. For product used in food and food wrappings where direct contact with the user is involved, non-toxicity is major requirement. Since 1959, glycerine is recognized as safe by food and drug administration. In flavoring and coloring product it acts as a solvent and its viscosity lends body to the product. In candies and icings glycerin prevents crystallization of sugar. Glycerin is useful as a solvent in breaking down of cells to extract soluble proteins, since it tends to form stable association with proteins liberated, probably because of the presence of hydroxyl group in glycerol molecule, however concentration of glycerol solution employed should not exceed 85%.

2.1.3.5 Tobacco

In processing tobacco, glycerin is important part of casing solution sprayed on tobacco before leaves are shredded and packed. Along with other flavoring agents, it is applied at a rate of about 3% of the weight of the tobacco to prevent the leaves from becoming friable and thus crumbling during processing, by remaining in the tobacco, glycerine helps to retain moisture and thus prevent drying out of tobacco. It is also used in the processing of chewing tobacco to add sweetness and prevent dehydration and as a plasticizer in cigarette papers.

2.1.3.6 Wrapping and packing material

Heat casings and special types of papers, such as glassine and grease proof paper, need a plasticizers to give them pliability and toughness, as such glycerin is

completely compatible with base material used, is absorbed by them and does not crystallize and volatilize appreciably.

2.1.3.7 Lubricants

Glycerin can be used as a lubricant in places where oil can fail. It is recommended for oxygen compressor because it is more resistant to oxidation then mineral oils. It is also to lubricate pumps and bearings exposed to fluids such as gasoline and benzene, which would dissolve oil type, lubricate. In food pharmaceutical and cosmetic manufacture where there is contact with lubricant; glycerin may used to replace oils. Glycerin is often used as a lubricant because of it's high viscosity and ability to remain fluid at low temperatures make it valuable without modification. To increase its lubricating power, finely divided graphite may be dispersed in it. Its viscosity may be decreased by addition of water, alcohols and glycols and increased with polymerization or mixing with starch-pastes of such composition may be used in packing pipe joints, in gas lines or similar application. For use in high pressure gases and valves, soaps are added to glycerin to increase its viscosity and increase its lubricating ability mixture of glycerin and glucose is employed as a non-drying lubricant in the die-pressing of metals. In textile industry, glycerine is frequently used in connection with so called 'textile oils' in spinning, knitting and weaving operations.

2.1.3.8 Urethane-polymers

An important and recent use of glycerin is fundamental building block in poly-ethers for urethanpolymers. In this use it is the initiator to which propylene oxide, alone or with ethylene oxide is added to produce tri-functional polymer which on reaction with di-isocyanates, produce flexible urethane foams. Glycerine based poly-ethers have found some use too in rigid urethane foams.

2.1.3.9 Gasket and cork product

Sheets and gaskets made with ground cork and glue require plasticizers, which has some humectants action in order that they may be pliable and tough. Glycerin is used here because of its lower vapor pressure, not readily absorbed by the corks and

compatible with glue. With crown sealers and cork stoppers, which come into contact with foods, it fulfills additional requirements of non-toxicity.

2.1.3.10 Miscellaneous uses

Glycerin used in cement compounds, lubricants and pressure media. It is also used in embalming fluids, masking and shielding compounds, soldering compound and compresses, cleaning material, emulsifiers, the industrial skin protective, asphalt ceramics, photo-graphic products, leather, wood treatments and adhesives.

Table 2.3 The industry types of from glycerol

Types of Industry	%
Cosmetics and pharmaceuticals	26
Esters	17
Resins	12
Polyols	12
Food and beverages	10
Celluose	5
Nitration	4
Tobacco product	4
And others	10

2.2 Zinc acetate

Zinc acetate is the chemical compound with the formula $Zn(O_2CCH_3)_2$, which commonly occurs as a dihydrate $Zn(O_2CCH_3)_2(H_2O)_2$ [11]. Both the hydrate and the anhydrous forms are colorless solids that are commonly used in chemical synthesis and as dietary supplements. Zinc acetates are prepared by the action of acetic acid on zinc carbonate or zinc metal.

Virtually all zinc compounds, zinc acetate consists of Zn^{2+} ions. The acetate group is capable of binding to metal ions in a variety of ways through its two oxygen atoms and several connectivity are observed for the various hydrates of zinc acetate. Anhydrous zinc acetate adopts a polymeric structure consisting of zinc coordinated to four oxygen atoms in a tetrahedral environment, each tetrahedron being connected to neighbors by the acetate groups. The acetate ligands are not bidentate [12]. In contrast, most metal diacetates feature metals in octahedral coordination with bidentate acetate groups. In zinc acetate dihydrate the zinc is octahedral, wherein both acetate groups are bidentate.

2.2.1 Basic properties and structure of zinc acetate

Heating $Zn(CH_3COO)_2$ in a vacuum results in loss of acetic anhydride, leaving a residue of "basic zinc acetate," with the formula $Zn_4O(CH_3CO_2)_6$. This cluster compound has the tetrahedral structure shown below. This species closely resembles the corresponding the analogous beryllium compound, although it is slightly expanded with Zn-O distances ~1.97 vs. ~1.63 Å for $Be_4O(OAc)_6$

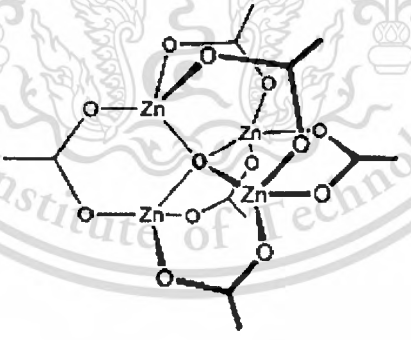

Figure 2.2 Zinc Acetate

2.2.2 Applications of zinc acetate

2.2.2.1 Dietary and medicinal applications

Zinc acetate is used as a dietary supplement and in lozenges used to treat the common cold. Zinc acetate alone is thought to be a more effective treatment than zinc gluconate [13]. Zinc acetate can also used to treat zinc deficiencies. As an oral daily supplement it is used to inhibit the body's absorption of copper as part of the treatment

for Wilson's disease. Zinc acetate is also sold as an astringent in the form of an ointment, a topical lotion; or combined with an antibiotic such as erythromycin for the topical treatment of acne. Furthermore Zinc acetate is commonly sold as a topical anti-itch ointment. In chewing gum, zinc acetate is a breath freshener and plaque inhibitor [14].

2.2.2.2 Industrial applications

Industrial applications include wood preserving; manufacturing other zinc salts, polymers, manufacture of ethylene acetate, as a dye mordant, and analytical reagent zinc acetate is a precursor via a sol-gel route to the transparent semiconductor zinc oxide.

2.3 Zinc Glycerolate

2.3.1 Preparation of zinc glycerolate

The zinc-glycerol complex particles [15] of the invention also generally maintain a hexagonal crystalline structure, thereby retaining superior lubricity and tactile properties. The invention also provides pharmaceutical and/or therapeutic compositions comprising microfine zinc-glycerol complex particles, and methods for using such compositions. The reaction between zinc acetate and glycerol in 1:1 molar ratio.

$$C_3H_8O_{3(l)} + Zn(O_2CCH_3)_{2(s)} \longrightarrow C_3H_6O_3Zn_{(s)} + 2CH_3COOH_{(l)}$$

Zinc glycerolate is white crystalline and it is a hexagonal structure (Taylor et. At, 1989)

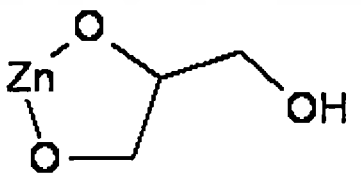

Figure 2.3 Structure of zinc glycerolate

2.3.2 Applications of Zinc glycerolate.

1. Used as feedstock in the production of pharmaceutical drugs such beeswax, burn scars vanishing, vanishing from diaper rash blister prevention finished.
2. Used in the mix. Pharmaceutical example a diabetes drug in the mix [16].
3. Used as raw material in the production of cosmetics such as sunscreen Olchaen, nourish skin cream (T. Reginald (Hawthorn, AU), B.Alan9North. Adelaide, AU).,1988)
4. Used as additives in the production of polymer such as propylene Homopolymer / copolymer polypropylene blends because Zinc glycerolate features a sleek, high when used as a feedstock in the production of polymer. Will assist in the transparency of production [17].

2.4 Xanthan gum

Xanthan gum is a gum by fermentation with Xanthomonas campestris bacteria are purified after fermentation process will be isopropyl alcohol separation by sedimentation take out dry xanthan gum [18], xanthan gum or grinding the resolution of trade names that keltol; is heteropolysaccahride. Containing glucose, mannose and glucuronic acid in a ratio 2.8:3:2 those about 4.7% acetyl and about 3 percent of pyruvic acid glucose to mannose bond with b-1, 4 mannose and a branch line to main line. 1.2 or 1.3 Commitment to the glucuronic acid bond with b-1, 2 (Figure 4.16) xanthan gum as a gelling agent, but no features of elastic themoreversible gel can be combined with Locust bean gum, and when combined with guar gum solution to a high viscosity.

Xanthan gum is soluble in cold water and hot water. The solution viscosity is high resistance to digestion by enzymes are highly stable to heat and pH viscosity of xanthan gum solution is constant even though the temperature range 0-100 $^{\circ}C$ to changes in pH or to changes in the last 1-13 xanthan gum solution, unless it also features a pseudo plastic. Characteristics appear and feel when food in the month.

Xanthan gum utilized in many food products. As additives increase the viscosity constant and make precise well as additives such as a constant if the ice cream to the mixture with xanthan gum Locust bean gum is used to hot foods caramel tomato sauce for pizza pie nucleus and

nucleus cookies is also mixed both guar gum and locust bean gum in a ratio appropriate to have a thick viscous. And specialized needs for food, such as a food product in frozen desserts, pasteurized, pasteurized process cheese spread, cottage cheese, salad dressing, sour cream and fruit syrups [19].

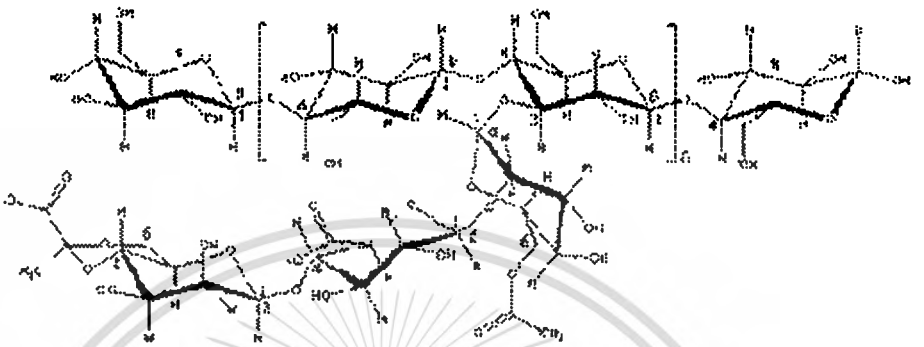

Figure 2.4 Molecular structure of Xanthan gum

2.5 Antibiotic sensitivity of bacteria to zinc glycerolate

Antibiotics play a crucial role in the manipulation, screening and killing of bacteria in a range of biotechnology processes. This kit specifically teaches the basic principles of antibiotics, bacterial resistance and susceptibility. Students learn and understand the use of antibiotic resistance in screening for infectious diseases [20]. Utilizing a bacterial strain, students learn the effects of different antibiotics and visualize bacterial sensitivity and resistance to the supplied antibiotics. This method involves the use of filter paper discs impregnated with a specified concentration of antibiotics on the surface of an agar plate containing microbial cells. This kit will enable students to analyze the inhibitory effects of different antibiotics on selected bacterial cells and then determine which antibiotic is the most suitable to treat a bacterial infection.

1. *Serratia_marcescens* is a species of Gram-negative, rod-shaped bacteria in the family Enterobacteriaceae. A human pathogen, *S. marcescens* is involved in nosocomial infections, particularly catheter-associated bacteremia, urinary tract infections and wound infections, and is responsible for 1.4% of nosocomial bacteremia cases in the United

States. It is commonly found in the respiratory and urinary tracts of hospitalized adults and in the gastrointestinal system of children [21].

Due to its ubiquitous presence in the environment, and its preference for damp conditions, *S. marcescens* is commonly found growing in bathrooms (especially on tile grout, shower corners, toilet water line, and basin), where it manifests as a pink discoloration and slimy film feeding off phosphorous containing materials or fatty substances (such as soap and shampoo residue). Once established, complete eradication of the organism is often difficult, but can be accomplished by application of a bleach-based disinfectant. Rinsing and drying surfaces after use can also prevent the establishment of the bacteria by removing its food source and making the environment less hospitable.

Figure 2.5 *Serratia marcescens* on an XLD agar plate.

2. *Micrococcus luteus* is a Gram positive, spherical, saprotrophic bacterium that belongs to the family Micrococcaceae. An obligate aerobe, *M. luteus* is found in soil, dust, water and air, and as part of the normal flora of the mammalian skin. The bacterium also colonizes the human mouth, mucosae, oropharynx and upper respiratory tract.

Although M. luteus is non-pathogenic and usually regarded as a contaminant, it should be considered as an emerging nosocomial pathogen in

immune compromised patients. *M. luteus* is resistant to reduced water potential and can tolerate drying and high salt concentrations.

M. luteus is coagulase negative, bacitracin susceptible, and forms bright yellow colonies on nutrient agar. To confirm it is not *Staphylococcus aureus*, a bacitracin susceptibility test can be performed.

M. luteus has been shown to survive in oligotrophic environments for extended periods of time. Recent work by Greenblat et al. demonstrate that *Micrococcus luteus* has survived for at least 34,000 to 170,000 years on the basis of 16S rRNA analysis, and possibly much longer [22].

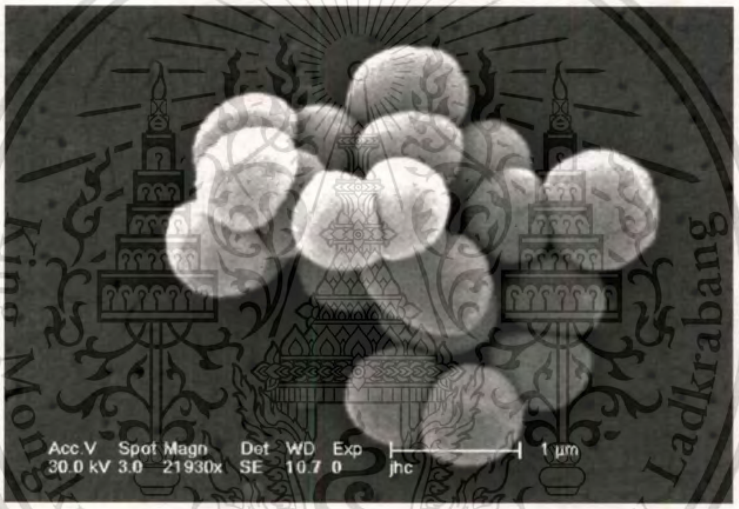

Figure 2.6 *Micrococcus luteus*

3. *Staphylococcus aureus* is the most common cause of staph infections. It is a spherical bacterium, frequently part of the skin florafound in the nose and on skin. About 20% of the population are long-term carriers of *S. aureus*. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples impetigo (may also be caused by *Streptococcus pyogenes*), boils (furuncles), cellulitisfolliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomye- litis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. Abbreviated to *S. aureus* or *Staph aureus* in

medical literature, *S. aureus* should not be confused with the similarly named and similarly dangerous (and also medically relevant) species of the genus *Streptococcus*. *S. aureus* was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses. Each year some 500,000 patients in American hospitals contract a staphylococcal infection.

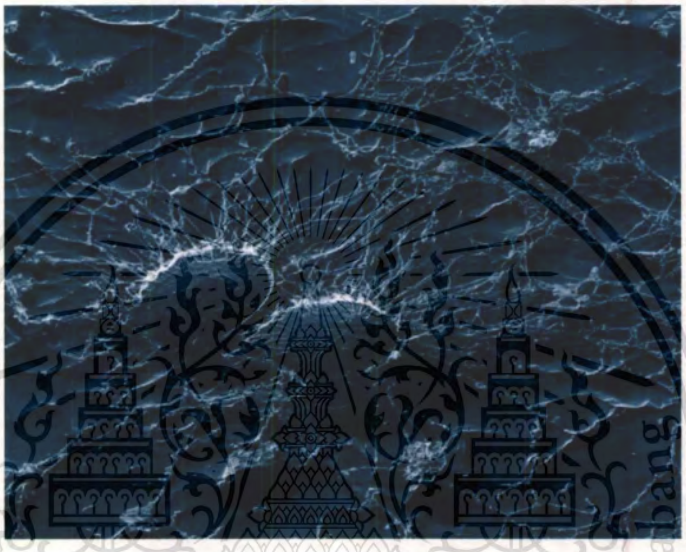

Figure 2.7 *Staphylococcus aureus*

2.6 Research related

Krongkaw Tippayasak

Zinc glycerolate (Zn-GLY) raw material in pharmaceutical, cosmetic and polymer product was synthesized by zinc oxide and glycerol. The feasibility of value-added of crude glycerol byproduct from biodiesel production was the aim of the study. Zn-GLY was synthesized according to a publication of Reginald M. Taylor (1989). The molar ratio and stirred speed were determined during indirect heating through paraffin bath for 1 hr at constant temperature 260 ^{o}C. The optimum conditions were; molar ratio 9:1, stirred speed 1500 rpm and The average yields were 97.65 $\pm$ 0.36 % (n=3).

Matkin

Matkin have studied the zinc glycerolate synthesis by zinc oxide or a zinc compound of a reaction with glycerol. Temperature at 15-105 ^{O}C. The trial add excess glycerol. When the reaction is glycerol remaining. Is eliminated zinc glycerolate by rinsing with ethanol solution C1-C2 at least 1 times. Then washed followed by isopropanol. Results from the study found that the temperature 45 degrees Celsius. Reaction with glycerol zincoxide can do better and particle size of glycerolat in the range 10-100 micron.

D.P.Fairlle

The combination between glycerol and zinc by oxidation reaction that result in a zincmonoglycerolate features: 1.Zincmonoglycerolate (ZMG) is well soluble in salt solution. But the reaction rate of ZMG will dissolve at different PH and concentration. 2. The ligand of the zinc under the skin and blood such as citrate, lactate and albumin. 3. ZMG accelerated melting faster to do this by adjusting the PH 7.3 at 25 degrees Celsius to cause the ligand zinc when ZMG melted in vitro and also to prevent irritation. The study found that ZMG consist transformation of zinc.

Claude

New method for preparation glycerolcarbonate. This includes making the reaction of urea and glycerol at temperatures between 90-220 degrees. The catalyst is related to an area Lewis acid, the only salt or metal salt or organic compound that can be react. The new type of glycerolcarbonate consists from catalytic carbomoylotion/carbonation conditions in high purity. The only metal salt or organic salt or a metal compound can be a reaction occurs.

Glycerol carbonate Caused by this new reaction. Catalytic carbomoylotion/carbonation conditions in high purity.

Chapter 3

Experimental Procedure

3.1 Apparatus and Equipment

3.1.1 Scanning electron microscope, SEM

3.1.2 Infrared spectroscopy, IR

3.1.3 X-ray Diffractometer, XRD

3.1.4 Magnetic stirrer

3.1.5 Hotplate

3.1.6 3-neck rounded bottom flask

3.1.7 pH indicator A007987 of MUA/USEEP

3.1.8 Accuracy balance Buchi B-169 vacuum system

3.1.9 Vacuum filtrator Buchi B-169 vacuum system of BUCHI

3.1.10 Oil bath

3.1.11 Glass apparatus

3.1.12 Thermometer

3.1.13 Rotary Evaporator

3.2 Chemicals

3.2.1 Commercial Glycerol ($CH_2OHCHOHCH_2OH$)

3.2.2 Crude glycerol

3.2.3 Zinc Acetate ($Zn(O_2C\,CH_3)_2$)

3.2.4 Ethanol 95%

3.2.5 Acetone (CH_3COCH_3)

3.2.6 Sulphuric Acid (H_2SO_4)

3.2.7 Sodium Hydroxide (NaOH)

3.2.8 Hexane

3.2.9 Activated carbon

3.2.10 Xanthan Gum

3.3 Glycerol's source

We received crude glycerol from The Royal Thai Naval Dockyard of biodiesel production.

3.4 Analysis the physical of crude glycerol.

- Filter the impurity out by gauze and keep it in glass bottle.
- Study the physical property of Glycerol such as color, smell, and pH.

3.5 Purify of crude glycerol. (We refer from the other project)

- Prepare crude glycerol and water in molar ratio 1:1.
- Adjust pH to 1 by sulphuric acid.
- Separate glycerol from settling.
- Wash with 25mL hexane and separate glycerol, which is lower part and repeat 3 trails.
- Adjust pH to 7 by sodium hydroxide.
- Let glycerol settling for 2 days.
- Separate salt out and evaporate by rotary evaporator to displace salt from glycerol
  (Under condition P =100 mmHg, T= 60° C).
- Filter with activated carbon for a day in ratio 7: 1 and repeat 2 trials.

3.6 The preparation zinc glycerolate with various molar ratio of glycerol.

3.6.1 The preparation of zinc acetate: commercial glycerol to prepare zinc glycerolate.

• Ratio zinc acetate: commercial glycerol (1:1 molar ratio), (1:3 molar ratio), (1:5 molar ratio), (1:7 molar ratio), (1:9 molar ratio) and (1:11 molar ratio).

3.6.1.1 Weight pure glycerol with various molar ratio (9.2g: 4.6g, 1:1 molar ratio), (9.2g: 13.8g, 1:3 molar ratio), (9.2g: 23g, 1:5 molar ratio), (9.2g: 32.2g, 1:7 molar ratio), (9.2g: 41.4g, 1:9 molar ratio), (9.2g: 50.6g, 1:11 molar ratio) and keep it in 3 necks round bottom flask bottle 100 mL.

3.6.1.2 Pour paraffin oil into oil bath.

3.6.1.3 Keep 3 necks round bottom flask in oil bath at various temperature of 200°C, and stir at medium speed for 1 h.

3.6.1.4 Cool down at room temperature.

3.6.1.5 Filter and wash the solid product with distilled water.

3.6.1.6 Wash with ethanol and acetone.

3.6.1.7 Dry the precipitate at temperature 80°C for 4 hours.

3.6.1.8 Record the weight of precipitate to find percent of product.

3.6.1.9 Analyst precipitates by IR, XRD.

3.6.1.10 Do experiment 3 trails.

3.6.2 The preparation of Zinc Acetate: Crude Glycerol to prepare Zinc Glycerolate.

• Ratio Zinc Acetate: Crude Glycerol **(1: x) and temperature x °C** at the most suitable ratio and temperature

3.6.2.1 Weight crude glycerol with the most suitable molar ratio and temperature. Then, keep it in 3 necks round bottom flask bottle 100 mL.

3.6.2.2 Pour paraffin oil into oil bath.

3.6.2.3 Keep 3 necks round bottom flask in oil bath at various temperature of 200°C, and stir at medium speed for 1 h.

3.6.2.4 Cool down at room temperature.

3.6.2.5 Filter and wash the solid product with distilled water.

3.6.2.6 Wash with ethanol and acetone.

3.6.2.7 Dry the precipitate at temperature 80°C for 4 hours.

3.6.2.8 Record the weight of precipitate to find percent of product.

3.6.2.9 Analyst precipitates by IR, XRD.

3.6.2.10 Do experiment 3 trails.

3.7 The preparation zinc glycerolate with the most suitable ratio at temperatures from 200 to 260°C.

3.7.1 Weight pure glycerol with the most molar ratio (9.2g: 32.2g, 1:7 molar ratio and keep it in 3 necks round bottom flask bottle 100 mL.

3.7.2 Pour paraffin oil into oil bath.

3.7.3 Keep 3 necks round bottom flask in oil bath at various temperatures of 200, 230 and 260 °C and stir at medium speed for 1 h.

3.7.4 Cool down at room temperature.

3.7.5 Filter and wash the solid product with distilled water.

3.7.6 Wash with ethanol and acetone.

3.7.7 Dry the precipitate at temperature 80°C for 4 hours.

3.7.8 Record the weight of precipitate to find percent of product.

3.7.9 Analyst precipitates by IR, XRD.

3.7.10 Do experiment 3 trails.

3.8 Testing property of Zinc glycerolate after prepared from ratio pure glycerol: zinc acetate (1: x) and temperature x °C that is the most suitable variable at medium speed.

3.8.1 Testing reactive of bacteria to zinc glycerol.

- Preparation concentration of sample

3.8.1.1 Prepare distilled water in PET bottle 400ml.

3.8.1.2 Weight Xanthan Gum 1.0 g and put it in PET bottle.

3.8.1.3 Shake it until mixed homogenously.

3.8.1.4 Prepare zinc glycerolate 10^{-3} mol/L, 10^{-5} mol/L and 10^{-7} mol/L.

3.8.1.5 Shake by ultrasonic machine for an hour and keep it at room temperature.

- Culture bacteria

3.8.1.1 Prepare Nutrient Agar (NA) and apparatus for feeding bacteria.

3.8.1.2 Prepare pour plate at concentration of bacteria 10^8 CFU/mL.

3.8.1.3 Spray distilled water to circle filtrated paper 0.5 cm and put it on culture medium plate.

3.8.1.4 Use new filtrated paper immerses Zinc glycerolate at varies concentration on 7 plates for a type of bacteria.

3.8.1.5 Culturing at temperature 37 °C for 12 hour.

3.8.1.6 Observe and measure size of the area that the bacteria do not grow in filtrated paper (clear zone) for each concentration.

3.8.2 Testing zinc glycerolate by SEM.

3.8.3 Testing zinc glycerolate by XRD.

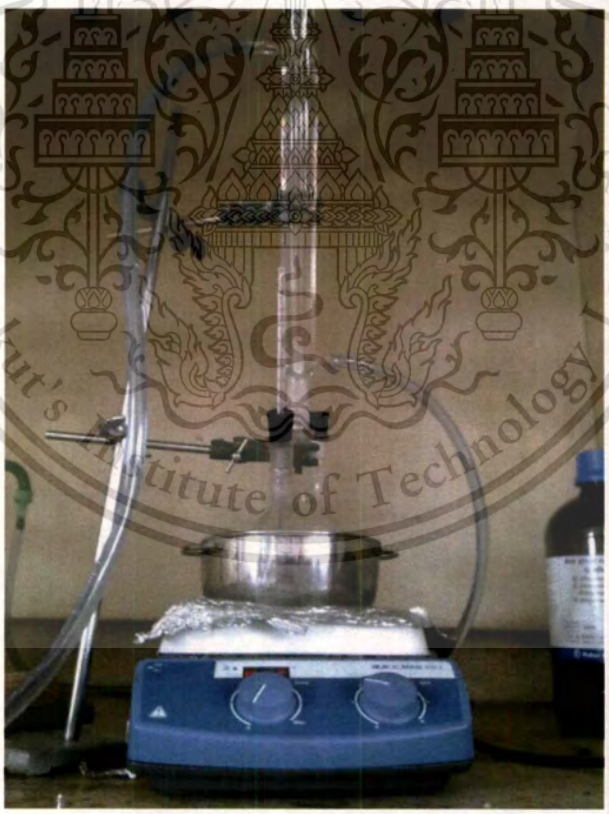

Figure 3.1 Setting the apparatus.

Figure 3.2 zinc glycerolate product.

Chapter 4

Result and Discussion

4.1 Study molar ratio of zinc acetate:glycerol.

From the experiment, we tested the factors of this experiment are the various molar ratio of zinc acetate:glycerolate by varying ratio 1.1, 1:3, 1:5, 1:7, 1:9 and 1:11. The results of percent yield are 14.30, 29.23, 38.42, 54.26, 49.99 and 53.18 %,which we had shown in figure 4.1.

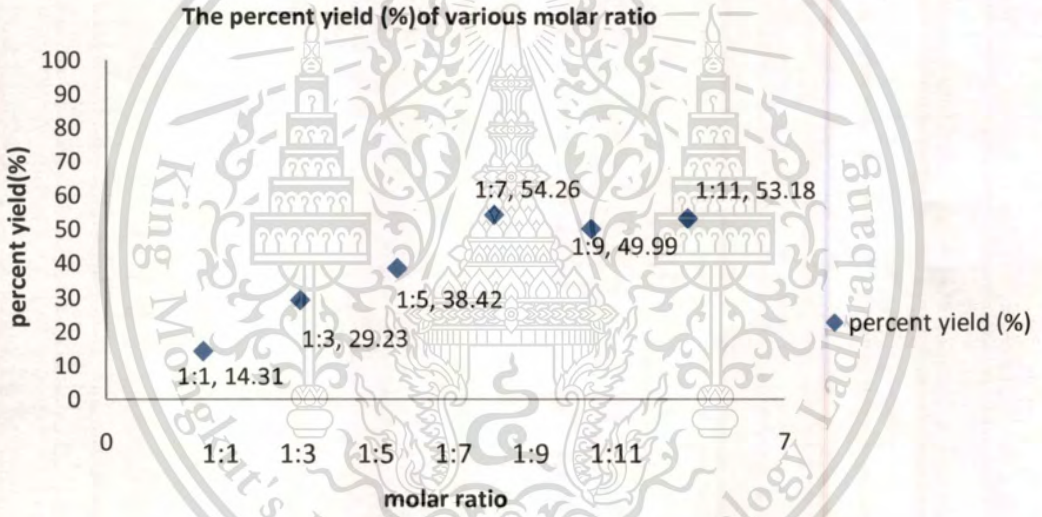

Figure 4.1 The percent yield of various molar ratios 1:1, 1:3, 1:5, 1:7, 1:9 and 1:11.

From figure, the curve of product is increasing when we increase the ratio of glycerol until 1:7 and the curve start to decline that means the most suitable molar ratio is 1:7 which is 1 portion of zinc acetate and 7 portions of glycerol that produced the highest percent yield and the reaction occurs quickly whereas we use the lowest amount of glycerol.

4.2 Study temperature to operate

From the experiment, we tested the factors of the experiment are the various temperature of zinc acetate:glycerolare which we operate the most suitable molar ratio 1:7 to vary the

temperatures are 200 230 and 260° C. The percent yield results are 54.26, 58.32 and 61.60 had shown in figure 4.2.

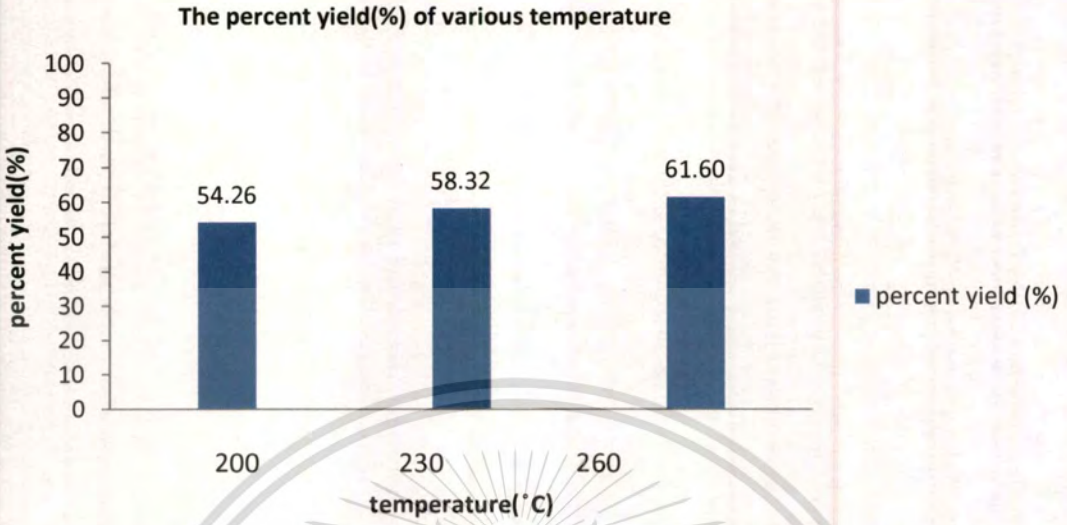

Figure 4.2 The percent yield of various temperatures 200, 230 and 260 ° C.

From graph, the curve of product is increasing relative to temperature, which show higher percent yield. We found that the most suitable temperature is 260° C, which produced the highest percent yield of zinc glycerolate product.

4.3 Study the product of zinc glycerolate from analytical grade glycerol and crude glycerol.

From the experiment, after we found the most suitable molar ratio and temperature of pure glycerol then, we tested the most suitable factors with crude glycerol which was passed the purifying process. The results of percent yield are 61.60 and 69.21, which had shown in figure4.2.

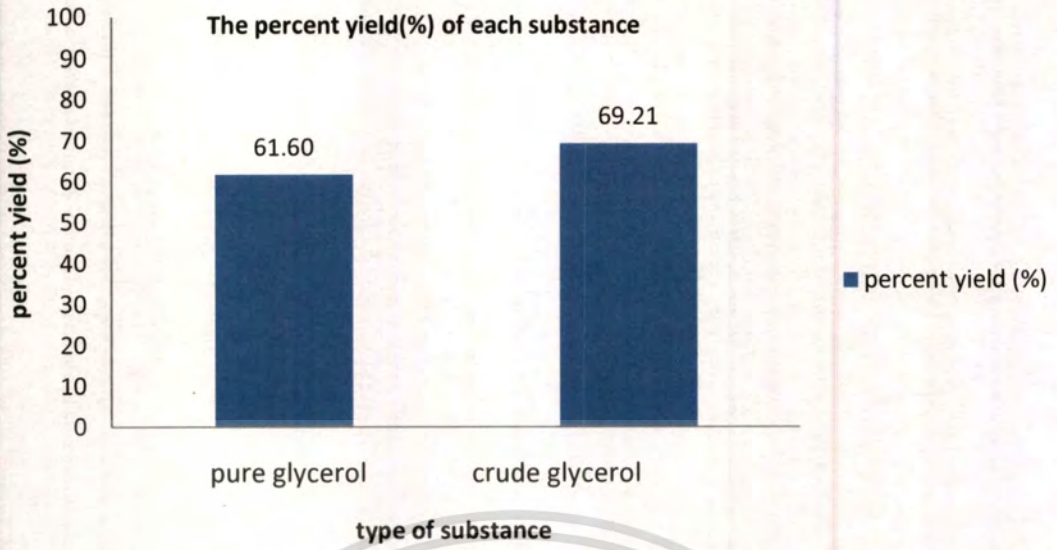

Figure 4.3 The percent yield of each substance pure and crude glycerol.

From graph, we will compare the percent yield of the product between commercial glycerol and crude glycerol which you will se the crude glycerol had higher yield but in term of quality you can compare the color which commercial glycerol had white crystals but crude glycerol had white opaque and we will analyze the comparison of these 2 products in term of testing in the next part.

4.4 Testing property of Zinc glycerolate.

4.4.1 Testing by XRD.

From the testing, we tested zinc glycerolate with XRD (X-ray diffraction) by using X-ray photo to analyze the structure of zinc glycerolate from the X-ray diffractive position affected the structure of the sample will show the peak of sample at position 2θ = 10.9 °,17.2°, 20.7°, 23.8°, 24.7°, 27.6°, 28.5°, 29.5°, 36.3°, 37.0°, 47.6 ° and 48.2° (Rodoula,Epameinondas and Frank,2006) which identify the sample structure was found the structure of zinc glycerolate that was shown in figure below.

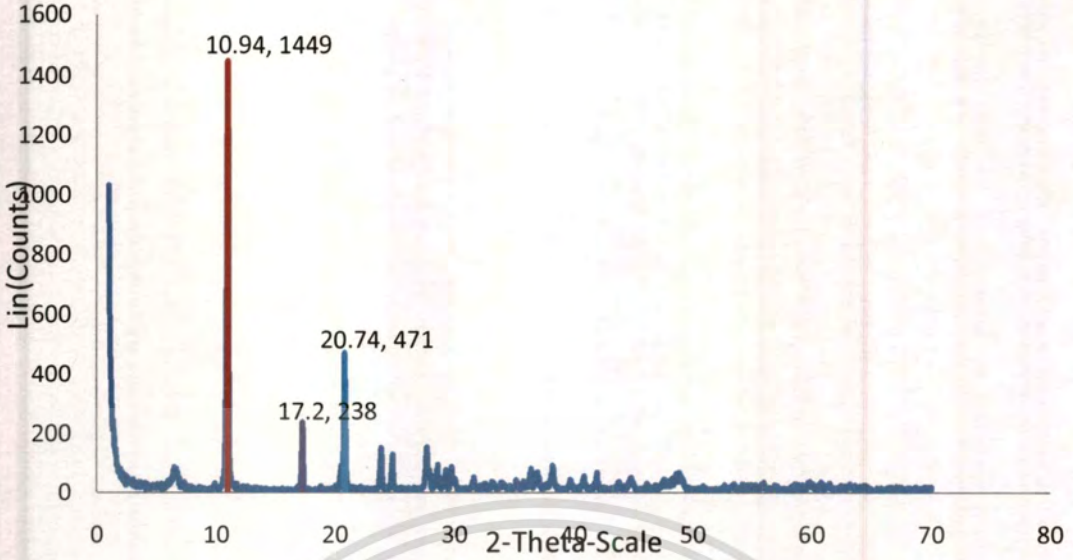

Figure 4.4 This figure show the peak which occurs to show the functional group of zinc glycerolate from pure glycerol.

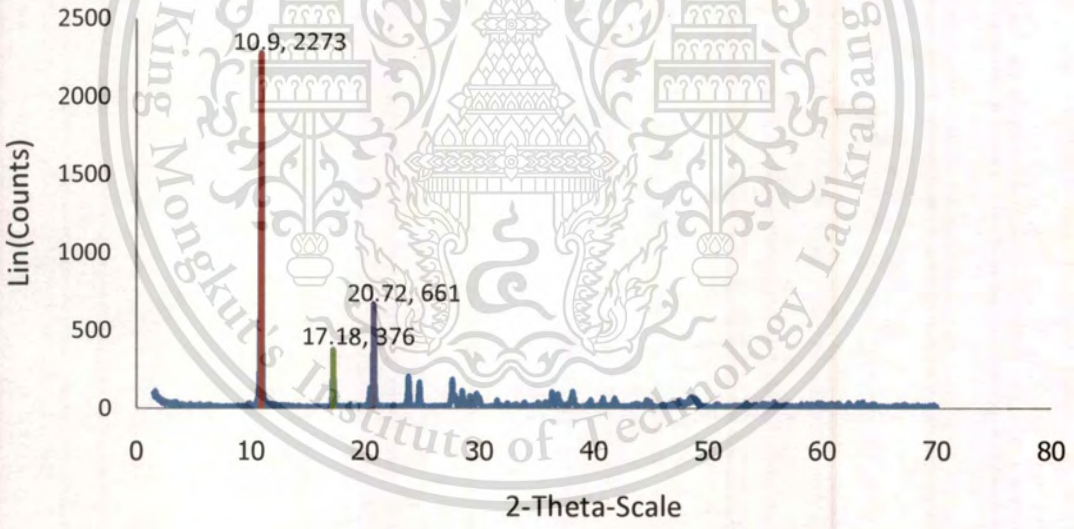

Figure 4.5 This figure show the peak which occurs to show the functional group of zinc glycerolate from crude glycerol.

From the picture, the peak of each functional group was shown by each sample. The outstanding peaks of pure glycerol , which are 10.94°, 17.2° and 20.74° and the outstanding peaks of crude glycerol , which are 10.9°, 17.18° and 20.72° .Hence we compare the outstanding of these 2 samples both pure and crude glycerol that theses peaks are similar whereas the small peaks are different in intensity.

4.4.2 Testing by IR.

From the testing, we tested sample with IR spectrum by using infrared spectroscopy machine to analyze the functional group of zinc glycerolate.The results show that sample absorb light at O-H functional group (<3200, 1640 cm^{-1}), C-H functional group (2933, 2880, 1460 cm^{-1}) and C-O functional group (1235,1050 cm^{-1}) to verify that Zinc glycerolate does not have contamination of glycerol resulted in glycerol was washed by ethanol and acetone. Glycerol was washed completely because of the solubility dissolving of glycerol which dissolve in ethanol and acetone that we had shown in the table and figure 4.4.2.

Table4.1 The peak of sample which shows the peak in range of each functional group.

Molar ratio	Functional group		
	O-H <3200,1640 cm^{-1}	C-H 2933,2880,1460 cm^{-1}	C-O 1235,1050 cm^{-1}
1:1	√	√	√
1:3	√	√	√
1:5	√	√	√
1:7	√	√	√
1:9	√	√	√
1:11	√	√	√
1:7 at 200° C	√	√	√
1:7 at 230° C	√	√	√
1:7 at 260° C	√	√	√
Pure	√	√	√
Crude	√	√	√

From the table, we can analyze the characteristic of zinc glycerolate that was shown in the picture of peak for each functional group.

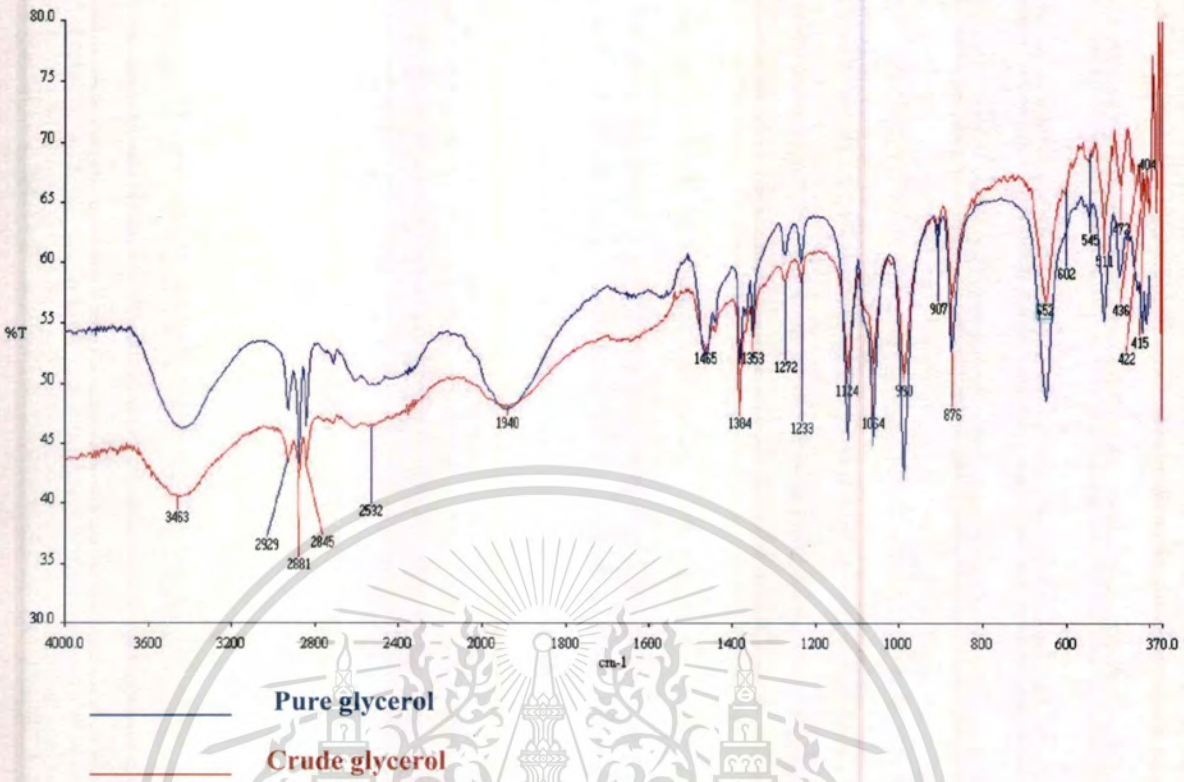

Figure 4.6 the comparison of pure and crude glycerol by IR technique.

From figure 4.6, the curves of pure and crude glycerol show the peaks of the functional group. Both pure and crude glycerol show broad peak in range of O-H functional group (<3200, 1640 cm^{-1}) and show sharp peak in range of C-H functional group (2933, 2880, 1460 cm^{-1}) and C-O functional group (1235, 1050 cm^{-1}) whereas the peaks are similar but %T are different, which pure glycerol show higher %T.

4.4.3 Testing by SEM.

From the testing, we tested sample with scanning electron microscope, SEM to analyze the size of particle both 2sample of zinc glycerolate from commercial glycerol and crude glycerol under the most suitable condition at ratio 1:7 and temperature 260° C which we had shown the picture below.

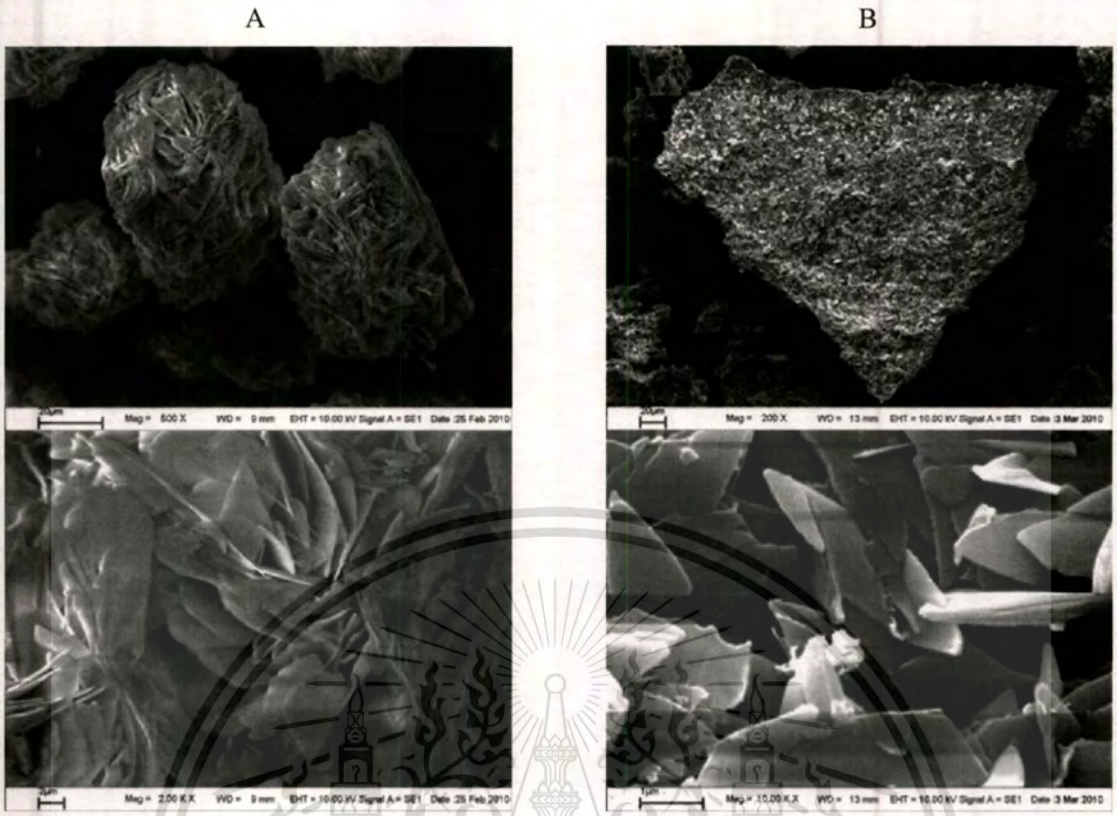

Figure 4.7 SEM image of synthesized zinc glycerolate of synthesized zinc glycerolate from a). Commercial glycerol and b). Crude glycerol which was purified

From the picture, showing the size of Zinc glycerolate that was produced from commercial glycerol has the size particle in range 20 to 2 μm micrometer at the magnification 500 to 2000 times and crystalline of crude glycerol has the size particle in range 20 to 1 μm micrometer at the magnification 200 to 10000 times Then ,the upper figures show the peak of composition both pure and crude are similar in shape and intensity ,however the crystalline of pure and crude are different ingrain size that pure glycerol has more flake and bigger grain size.

EDX technique

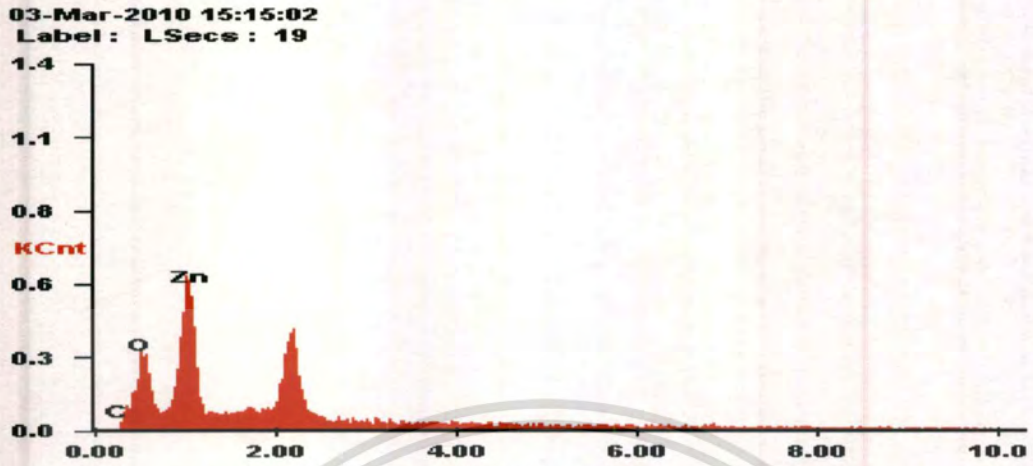

Figure 4.8 EDX picture of zinc glycerolate from pure glycerol.

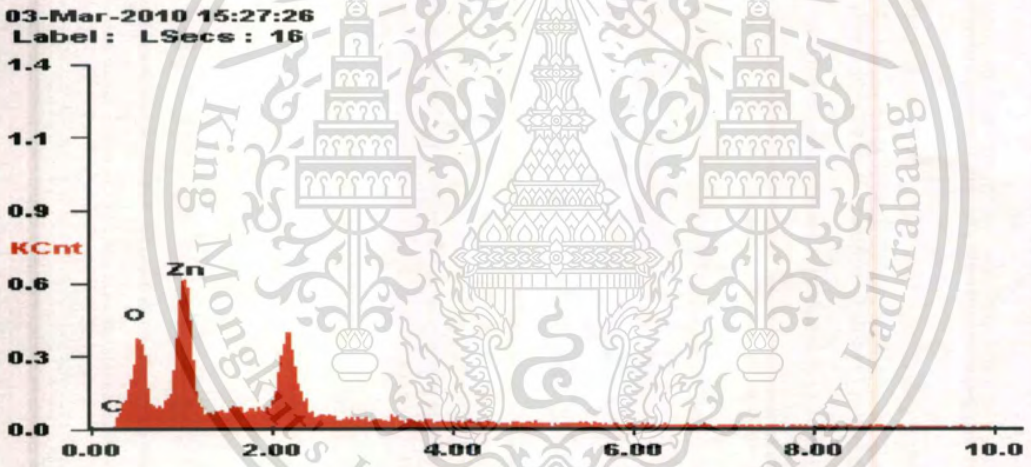

Figure 4.9 EDX picture of zinc glycerolate from crude glycerol which was purified.

4.4.4 Testing by bacteria.

The biological experiment by testing reactive of bacteria to zinc glycerol, the parameter of this is the concentration of zinc glycerolate which affect the growth of bacteria that found on the skin. Bacterias can be classified into 2 types are bacteria, which are disease of the skin and not disease of the skin. However disease of the skin includes *Staphylococcus aurens* and not disease of the skin includes *Micrococcus luteus* and *Serratia marcescens*. The difference concentration of zinc glycerolate 0, 10^{-3}, 10^{-5}, and 10^{-7} mol/L. The concentration at 0 mol/L is the control area.

Whereas, used the liquor which through disinfection and then used to control area of the experiments.

From experiment, the biological properties of zinc glycerolate ,which was prepared from pure glycerol to react with zinc acetate molar ratio 1:7 at 260°C found that zinc glycerolate can inhibit the growth of bacteria *Micrococcus luteus* at concentrations 10^{-3} mol. / L, show in the figure 4.1. However, the concentration of zinc glycerolate less than this can not inhibit the growth of the *Micrococcus luteus* tested but *Staphylococcus aurens* and *Serratia marcescens* can be inhibit the growth of bacteria minimum concentration as 10^{-7} mol / L show in the figure 4.2 respectively.

Figure 4.10 Inhibit the growth of *Micrococcus luteus* tested

(a) *Staphylococcus aurens* **(b)** *Serratia marcescens*

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

- The zinc glycerolate which was prepared from zinc acetate and glycerol in molar ratio 1:1 ,1:3 ,1:5 ,1:7 ,1:9 and 1:11. All molar ratio between zinc acetate and glycerol produced zinc glycerolate, however at 1:7 molar ratio produced the highest percentage of zinc glycerolate.

- The operation of zinc glycerolate depends on temperature.

- The zinc glycerolate was prepared from analytical glycerol had lower percent yield than crude glycerol.

- The testing of IR, XRD and SEM reported the structure, composition and surface of zinc glycerolate, which the product from analytical glycerol shows greater properties.

5.2 Recommendations

- Timing should be varied to study the effect of time.

- The operation was great when operating at open-system, then should find the method to decrease loss of percent yield.

- Testing the ability to inhibit bacterial should analyze with another type bacteria.

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Appendix-A

A.1 A by-product from biodiesel process

Pharmaceutical grade glycerine is a by-product from biodiesel process that is the best quality of glycerine. Glycerine can be used in many industries, such as food, pharmaceutical, cosmetic, dynamite and soap industries.

A.2 Demand and supply of glycerine in Thailand

Biodiesel Plant with highest capacity (100 liters/day) can produce glycerine 2,970 tons per year or estimate 10 percent by weight of biodiesel.

Table A-1 The import and export glycerine value of Thailand.

Year	Import value (1,000 Baht)	Export value (1,000 Baht)
2001	40,428	11,026
2002	7632	17,793
2003	6356	13,582
2004	4823	14,013
2005	1317	2493

Source: Thai Customs Department

From above data since 2002 the value of glycerine export is higher than the import value allows Thailand to produce glycerol over demand in the country. Therefore, trends of glycerine prices will be lowered due to outgrowth from biodiesel production process that is likely to increase continuously because glycerine is a by-product from biodiesel process.

A.3 Selling price of Pharmaceutical grade glycerine

Pharmaceutical grade glycerine sale price be equal to 10-20 baht per kilogram. Formerly, glycerine was very high economic value because of produce from specifically plant. Nowadays there are many biodiesel productions therefore glycerine price will be lower.

Table A-2 Selling price of glycerine

	Year 2007-2009	Year 2010-2012	Year 2013-…
Glycerine price (Baht/Kg)	20.00	15.00	10.00

Source: Thai Customs Department

A.4 Data of the pharmaceutical products import of Thailand

Table A-3 The value production and import of modern medicine in Thailand

Year	Modern medicine production (Million Baht)			The import of modern medicine (Million Baht)		
	For human	For animal	Total	For human	For animal	Total
2530	5,145.75	309.15	5,454.90	2,325.43	478.11	2,803.54
2531	6,708.85	181.27	6,890.12	2,570.98	592.6	3,163.58
2532	8,372.85	223.99	8,596.84	3,307.60	624.82	3,932.42
2533	8,886.02	290.5	9,176.52	3,449.08	870.18	4,319.26
2534	9,657.54	325.68	9,983.22	4,216.41	1,033.89	5,250.30
2535	10,696.54	385.05	11,081.59	4,682.61	1,114.14	5,796.75
2536	11,831.03	275.66	12,106.69	5,075.31	1,145.35	6,220.66
2537	12,969.68	284.35	13,254.03	6,086.63	1,211.63	7,298.26
2538	15,820.870	461.740	16,282.610	9,276.470	1,487.200	10,763.670
2539	18,174.431	472.571	18,647.002	10,435.337	1,628.278	12,063.615
2540	19,591.553	477.727	20,069.280	13,375.565	2,115.666	15,491.231
2541	16,726.120	352.168	17,078.288	9,739.083	2,100.566	11,839.649
2542	19,033.936	686.719	19,720.655	14,232.333	2,486.881	16,719.214
2543	20,995.923	682.521	21,678.444	16,700.346	2,517.251	19,217.597
2544	23,087.899	829.609	23,917.508	19,967.635	2,722.025	22,689.660
2545	24,144.561	542.229	24,686.790	19,867.944	2,901.874	22,769.819

2546	26,586.115	977.219	27,563.334	26,024.866	3,563.260	29,588.127
2547	31,707.647	931.882	32,639.528	30,545.543	3,101.560	33,647.103
2548	29,704.78	1,425.85	31,130.64	38,293.37	3,337.48	41,630.85
2549	30,910.918	1,831.212	32,745.129	45,004.554	3,584.495	48,589.049
2550	41,232.43	1,473.91	42,706.34	53,000.10	3,237.14	56,237.23
2551	35,322.85	1,517.68	36,840.53	64,148.13	3,169.97	67,318.09

Source: Thai Drug Control Division, Ministry of Public Health

Appendix-B

B.1 Analytical Instruments

B.1.1 pH meter [23]

A pH meter is a handheld device that tests water for its level of acidity versus base or alkalinity. If water is equally acidic and alkaline, it registers as neutral on a pH meter. The pH meter utilizes a standard pH scale for measuring these aspects of water quality. The level of concentration of positive hydrogen ions in the water determines the result.

The acidic side of the pH scale runs from 0 to 6.9, with 7.0 being neutral. Because 7.0 is neutral, 6.8 is considered only slightly acidic. Similarly, 7.2 is only slightly alkaline, with the base scale running to 10.0 or higher. Generally speaking, acidic water is soft while alkaline or base water is hard. Lacking a pH meter, water is pH-tested by adding chemical agents to a water sample. Depending on the agents used, a bright yellow result could indicate highly acidic water; blue, neutral; and deep brown, alkaline water. Hues are interpreted as positions along the scale.

Liquid pH tests can be messy and they do require some guesswork as to the reading. Conversely, a pH meter is a digital device with a connected glass electrode probe. The probe is placed into the water to be tested, and the pH of the water is digitally displayed. It takes mere seconds and is far more accurate; assuming the pH meter has been properly calibrated. Though there are many types of pH meters, common varieties will come with calibration liquids called buffer solutions. These solutions are predetermined to be highly acidic (4.01), neutral (7.01), and alkaline or base (10.01).

Calibration is accomplished by testing the liquids with the probe and adjusting the meter's readings. A pH meter should be recalibrated monthly for best results. When not in use, the tip of the probe must be stored in acidic solution. If the probe dries out, it will need replacement.

B.1.2 X-ray diffraction (XRD) [24]

X-ray diffraction is a versatile analytical technique for examining crystalline solids, which include ceramics, metals, electronic materials, geological materials, organics, and polymers. These materials may be powders single crystals, multilayer thin films, sheets, fibers, or irregular shapes, depending on the desired measurement. X-ray diffractometers fall broadly into two classes: single-crystal and powder. Single-crystal diffractometers are most often used to determine the molecular structure of new materials. Powder diffractometers are routinely used for phase identification and quantitative phase analysis but can be configured for many applications, including variable-temperature studies, texture and stress analysis, grazing incidence diffraction, and reflectometry. The operative equation in X-ray diffraction is the Bragg equation: $n\lambda = 2d\sin\theta$

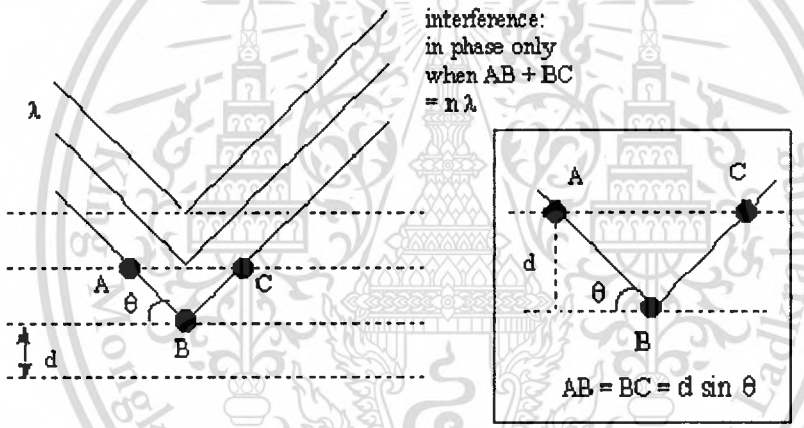

Figure B-1 Geometry of diffraction and its relationship to Bragg's law. Constructive interference occurs when AB + BC is equal to an integral number of wavelengths.

Where n is the order of a reflection (n □ {1, 2, 3 ...}), λ the wavelength, d the distance between parallel lactice planes, and θ the angle between the incident beam and a lacttice plane, known as the Bragg angle (Fig.5). When the pathlength in the crystal ($2d\sin\theta$) is a multiple of wavelength, constructive interference occurs and diffracted intensity is obtained. In general, the d-spacing is a function of the lattice parameters (a,b,c) and angles (α,β,γ) defining the unit cell, and the Miller indices (h,k,l) denoting a particular reflection. As such, it is the geometry of the crystal lattice that determines the positions of the peaks in an X-ray diffraction pattern. In general, the more symmetrical the material, the fewer peaks in its diffraction pattern. The diffracted intensities

associated with those peaks are determined by the type and arrangement of atoms within the crystal lattice.

A crystal lattice is a regular three-dimensional distribution (cubic, rhombic, etc.) of atoms in space. These are arranged so that they form a series of parallel planes separated from one another by a distance d, which varies according to the nature of the material. For any crystal, planes exist in a number of different orientations - each with its own specific d -spacing. When a monochromatic X-ray beam with wavelength λ is projected onto a crystalline material at an angle θ, diffraction occurs only when the distance traveled by the rays reflected from successive planes differs by a complete number n of wavelengths .

X-ray diffraction techniques are based on the elastic scattering of x-rays from structures that have long range order. The most comprehensive description of scattering from crystals is given by the dynamical theory of diffraction.

- Single-crystal X-ray diffraction is a technique used to solve the complete structure of crystalline materials, ranging from simple inorganic solids to complex macromolecules, such as proteins.
- Powder diffraction (XRD) is a technique used to characterize the crystallographic structure, crystallite size (grain size), and preferred orientation in polycrystalline or powdered solid samples. Powder diffraction is commonly used to identify unknown substances, by comparing diffraction data against a database maintained by the International Centre for Diffraction Data. It may also be used to characterize heterogeneous solid mixtures to determine relative abundance of crystalline compounds and, when coupled with lattice refinement techniques, such as Rietveld refinement, can provide structural information on unknown materials. Powder diffraction is also a common method for determining strains in crystalline materials.
- Thin film diffraction and grazing incidence x-ray diffraction may be used to characterize the crystallographic structure and preferred orientation of substrate-anchored thin films.

- High-resolution x-ray diffraction is used to characterize thickness, crystallographic structure, and strain in thin epitaxial films. It employs parallel-beam optics.
- X-ray pole figure analysis enables one to analyze and determine the distribution of crystalline orientations within a crystalline thin-film sample.
- X-ray rocking curve analysis is used to quantify grain size and mosaic spread in crystalline materials

B.1.3 Scanning Electron Microscope (SEM) [25]

The Scanning Electron Microscope (SEM) is a microscope that uses electrons rather than light to form an image. There are many advantages to using the SEM instead of a light microscope. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. The SEM also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. Preparation of the samples is relatively easy since most SEMs only require the sample to be conductive. The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the most heavily used instruments in research areas today.

The Electron source; the electron beam comes from a filament, made of various types of materials. The most common is the Tungsten hairpin gun. This filament is a loop of tungsten which functions as the cathode. A voltage is applied to the loop, causing it to heat up. The anode, which is positive with respect to the filament, forms powerful attractive forces for electrons. This causes electrons to accelerate toward the anode. Some accelerate right by the anode and on down the column, to the sample. Other examples of filaments are Lanthanum Hexaboride filaments and field emission guns.

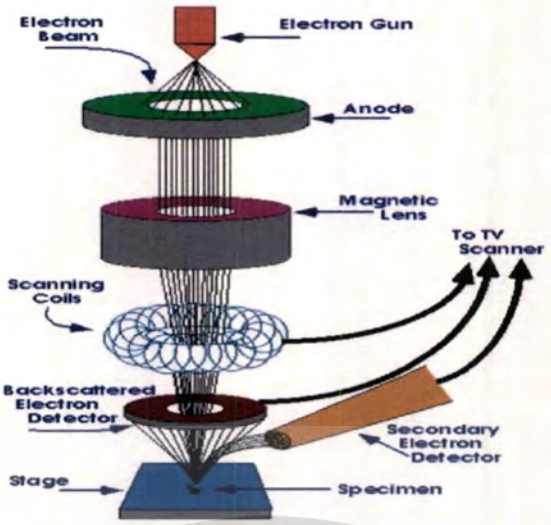

Figure B-2 The electron sources

A beam of electrons is generated in the electron gun, located at the top of the column, which is pictured to the left. This beam is attracted through the anode, condensed by a condenser lens, and focused as a very fine point on the sample by the objective lens. The scan coils are energized (by varying the voltage produced by the scan generator) and create a magnetic field which deflects the beam back and forth in a controlled pattern. The varying voltage is also applied to the coils around the neck of the Cathode-ray tube (CRT) which produces a pattern of light deflected back and forth on the surface of the CRT. The pattern of deflection of the electron beam is the same as the pattern of deflection of the spot of light on the CRT.

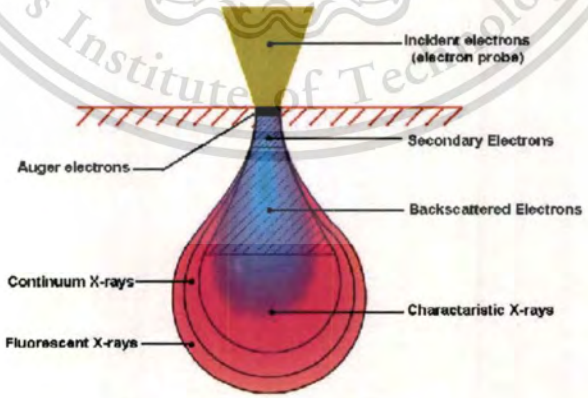

Figure B-3 The electron beam interaction diagram.

In a typical SEM, electrons are thermonically emitted from a tungsten or lanthanum hexaboride (LaB6) cathode and are accelerated towards an anode;

alternatively, electrons can be emitted via field emission (FE). Tungsten is used because it has the highest melting point and lowest vapors pressure of all metals, thereby allowing it to be heated for electron emission. The electron beam, which typically has an energy ranging from a few hundred eV to 100 keV, is focused by one or two condenser lenses into a beam with a very fine focal spot sized 0.4 nm to 5 nm. The beam passes through pairs of scanning coils or pairs of deflector plates in the electron optical column, typically in the objective lens, which deflect the beam horizontally and vertically so that it scans in a raster fashion over a rectangular area of the sample surface. When the primary electron beam interacts with the sample, the electrons lose energy by repeated scattering and absorption within a teardrop-shaped volume of the specimen known as the interaction volume, which extends from less than 100 nm to around 5 μm into the surface. The size of the interaction volume depends on the electrons' landing energy, the atomic number of the specimen and the specimen's density. The energy exchange between the electron beam and the sample results in the emission of electrons and electromagnetic radiation, which can be detected to produce an image

X-ray Diffraction sample Preparation

Metal specimens require no special preparation for SEM, except for trimming to appropriate size to fit in a specimen chamber, and make appropriate sectioning if necessary. Nonconductive solid specimens should be coated with a layer of conductive material, except when observed with Variable Vacuum or Environmental SEM. An ultrathin coating of electrically-conducting material is deposited either by high vacuum evaporation or by low vacuum sputter coating of the sample. This is done to prevent the accumulation of static electric fields at the specimen due to the electron irradiation during imaging. Such coatings include gold, gold/palladium, platinum, tungsten, graphite etc. Another reason for coating, even when there is more than enough conductivity, is to improve contrast, a situation more common with the operation of a FESEM (field emission SEM). Embedding in a resin with further polishing to a mirror-like finish could be beneficial for both biological and materials specimens, especially when imaging in backscattered electrons or X-ray microanalysis are performed. A biological specimen requires fixation to preserve its structure, which is usually performed by incubation of specimen in solution of fixative, such as gluteraldehyde or formalin. If not used in ESEM, biological specimen then should be dehydrated, usually by replacing water with organic solvents

such as ethanol or acetone, and then removing solvents. If SEM is equipped for cryo-microscopy, then cryofixation could be used. Cryofixation – freezing a specimen so fast, to liquid nitrogen or even liquid helium temperatures, that the water forms vitreous (non-crystalline) ice. This preserves the specimen in a snapshot of its solution state. An entire field called cryo-electron microscopy has branched from this technique. With the development of cryo-electron microscopy of vitreous sections (CEMOVIS), it is now possible to observe virtually any biological specimen close to its native state. Another cryo-technique for biological specimens is cryo-fracture, when frozen specimen is fractured in special apparatus, sputter coated and transferred into SEM cryo-holder while still frozen. Freeze-fracturing, freeze-etch or freeze break is a preparation method particularly useful for examining lipid membranes and their incorporated proteins in "face on" view. The preparation method reveals the lipids embedded in the protein bilayer. Freeze-fracturing where called, at the university of Harvard, for one of the most important discoveries in the world in 1967. Due to the loss of information, gold coating is often a semi-destructive process since removing a gold coating chemically requires aggressive chemicals like potassium cyanide or aqua regia. Alternative techniques, for example the low-vacuum environmental SEM, allow samples to be imaged without such plating and without the loss of natural contrast arising from the beam-specimen interaction. Gold has a high atomic number and produces high topographic contrast and resolution but the information thus produced can obscure the underlying fine detail of the specimen under examination.

Detection of secondary electron

The most common imaging mode monitors low energy (<50 eV) secondary electrons. Due to their low energy, these electrons originate within a few nanometers from the surface. The electrons are detected by an Everhart-Thornley detector which is a type of scintillator-photomultiplier device and the resulting signal is rendered into a two-dimensional intensity distribution that can be viewed and saved as a Digital image. This process relies on a raster-scanned primary beam. The brightness of the signal depends on the number of secondary electrons reaching the detector. If the beam enters the sample perpendicular to the surface, then the activated region is uniform about the axis of the beam and a certain number of electrons "escape" from within the sample. As the angle of incidence increases, the "escape" distance of one side of the beam will decrease, and more secondary electrons will be emitted. Thus steep surfaces and edges tend to be brighter than flat surfaces, which results in images with a well-

defined, three-dimensional appearance. Using this technique, resolutions less than 1 nm are possible.

Detection of backscattered electrons

Backscattered electrons consist of high-energy electrons originating in the electron beam that are reflected or back-scattered out of the specimen interaction volume. Backscattered electrons may be used to detect contrast between areas with different chemical compositions, especially when the average atomic number of the various regions is different, since the brightness of the BSE image tends to increase with the atomic number. Backscattered electrons can also be used to form electron backscatter diffraction (EBSD) image. This image can be used to determine the crystallographic structure of the specimen. There are fewer backscattered electrons emitted from a sample than secondary electrons. The number of backscattered electrons leaving the sample surface upward might be significantly lower than those that follow trajectories toward the sides. Additionally, in contrast to the case with secondary electrons, the collection efficiency of backscattered electrons cannot be significantly improved by a positive bias common on Everhart-Thornley detectors. This detector positioned on one side of the sample has low collection efficiency for backscattered electrons due to small acceptance angles. The use of a dedicated backscattered electron detector above the sample in a "doughnut" type arrangement, with the electron beam passing through the hole of the doughnut, greatly increases the solid angle of collection and allows for the detection of more backscattered electrons.

Environmental SEM

Conventional SEM requires samples to be imaged under vacuum, which means that samples that would produce a significant amount of vapor, e.g. biological samples, need to be either dried or cryogenically frozen. This means that processes involving transitions to or from liquids or gases, such as the drying of adhesives or melting of alloys, liquid transport, chemical reactions and solid-air-gas systems in general could not be observed. The first commercial development of the Environmental SEM (ESEM) in the late 1980s allowed samples to be observed in low-pressure gaseous environments (e.g. 1-50 Torr) and high relative humidity (up to 100%). This was made possible by the development of a secondary-electron detector capable of operating in the presence of water vapor and by the use of pressure-limiting apertures with differential pumping in the path of the electron beam to separate the vacuum regions around the

gun and lenses from the sample chamber. ESEM is especially useful for non-metallic and biological materials because coating with carbon or gold is unnecessary. Plastics and Elastomers can now be routinely examined, as can biological samples. Coating can be difficult to reverse, and may reduce the value of the results obtained. For example very small details on the surface of the sample may be concealed by the coating, let alone that coating is done under vacuum, which drastically alters hydrated specimens.

B.1.4 Infrared spectroscopy (IR) [26]

Infrared, or IR, spectroscopy is one type of *vibration spectroscopy*, which, as you might have guessed, is a spectroscopic technique where molecular vibrations are analyzed. To fully understand IR spectroscopy, you must first understand the principles of *simple harmonic motion*.

Imagine two spheres, or masses, connected with a spring. In case you are confused, what you are imagining should look roughly like this.

Figure B-4 Two spheres, or masses, connected with a spring

This is what is known as a *simple harmonic oscillator*. Once set into motion, the sphere will oscillate, or vibrate back and forth on the spring, at a certain frequency depending on the masses of the spheres and the stiffness of the spring. A sphere with a small mass is lighter and easier to move around than one with a large mass. Therefore, smaller masses oscillate at a higher frequency than larger masses. A very stiff spring, like a bedspring, is hard to deform and quickly returns to its original shape when the deforming force is removed. On the other hand, a weak spring is easily deformed and takes much longer to return to its shape. Therefore, a stiffer spring will oscillate at a higher frequency than a weak one. A chemical bond between two atoms can be thought of as a simple harmonic oscillator. The bond is the spring, and the two atoms, or groups of atoms, connected by the bond are the masses. Every atom has a different mass, and single, double and triple all have different stiffness, and therefore each combination of atoms and bonds has its own characteristic harmonic frequency.

When an object is vibrating at a certain frequency and encounters another vibration of *exactly* the same frequency, the oscillator will absorb that energy. Take a guitar string for example. If you were to pluck the G-string and set it "a-vibratin'," it would make that beautiful "G" sound. If you then plucked the D-string while holding it at the fifth fret, it would also make the "G" sound, but if you looked at the strings closely, you would see that not only would the D-string be vibrating, but the G-string would also be vibrating because some of energy from the vibrating D-string was transferred to the G-string, making it vibrate too. This is also true of vibrating molecules, except plucking a G-string won't affect the vibrations of chemical bonds.

At any temperature above absolute zero, all the "eensy-weensy" little simple harmonic oscillators that make up any molecule are vibrating vigorously. Infrared light just happens to be in the same frequency range as a vibrating molecule. So, if you hit a vibrating molecule with some IR light, it will absorb those frequencies in the light which exactly match the frequencies of the different harmonic oscillators that make up that molecule. When this light is absorbed, the little oscillators in the molecule will continue to vibrate at the same frequency, but since they have absorbed the energy of the light, they will have larger amplitude of vibration. This means that the "springs" will stretch further than before the light was absorbed. The remaining light which was not absorbed by any of the oscillators in the molecule is transmitted through the sample to a detector, and a computer will analyze the transmitted light and determine what frequencies were absorbed.

In the past, it was only possible to get good data by hitting the molecule with only one frequency of IR light at a time. This took a very long time because there are a lot of frequencies and to get a good spectrum, many scans must be taken. But now, thanks to the amazing Fourier Transform Algorithm, you can hit the molecule with every frequency of IR light at once, and get a perfect spectrum in only a fraction of the time. In case you are curious, here is that amazing Fourier Transform Algorithm.

$$F(\nu)=\int_{-\infty}^{\infty} f(t)\, e^{-i(2\pi)\nu t}\, dt \quad \text{and} \quad f(t)=\int_{-\infty}^{\infty} 2\pi\, F(\nu)\, e^{i(2\pi)\nu t}\, dt$$

IR spectroscopy is a very simple analytical technique. First, you need to put the material to be analyzed in some form that can be put into the infrared spectrometer. This is usually accomplished by casting a film on a sodium chloride (table salt) salt plate, or by grinding the material up with potassium bromide, KBr, and making a pellet out of it. These salts are used because they are invisible to IR light. There are other ways to make a sample, but these are the most common when dealing with polymers. Next, you place the sample into the spectrometer where Leslie is so kind to point out, close the lid, wait a few seconds for the sample chamber to purge of carbon dioxide, press the "SCAN" button on the computer, and VOILA, in less than a minute you have an IR spectrum

Appendix-C

Table C-1 The studying the various molar ratio of zinc acetate react with analytical grade glycerol.

Molar ratio	order	Wt of ZnGly (g)	Wt of glycerol (g)	Wt of filter(g)		Wt of crystalline(g)	% yield
				before	after		
1:1	1	9.2021	4.6031	0.4957	1.6137	1.1180	14.52
	2	9.2015	4.6064	0.4975	1.5939	1.0964	14.24
	3	9.2011	4.6048	0.4953	1.5863	1.0910	14.17
	Average						14.31
	SD.						0.19
1:3	1	9.2102	13.8022	0.4958	2.7635	2.2677	29.45
	2	9.2008	13.8019	0.4952	2.7421	2.2469	29.18
	3	9.2051	13.8016	0.4975	2.7351	2.2376	29.06
	Average						29.23
	SD.						0.20
1:5	1	9.2030	23.0013	0.4953	3.456	2.9607	38.45
	2	9.2017	23.0067	0.4936	3.4603	2.9667	38.53
	3	9.2061	23.0029	0.4958	3.4434	2.9476	38.28
	Average						38.42
	SD.						0.13
1:7	1	9.2016	32.2014	0.4953	4.6818	4.1865	54.37
	2	9.2047	32.2078	0.4951	4.6716	4.1765	54.24
	3	9.2014	32.2065	0.4937	4.6602	4.1665	54.11
	Average						54.26
	SD.						0.13
1:9	1	9.2033	41.4063	0.4976	4.3284	3.8308	49.75
	2	9.2014	41.4041	0.4954	4.3308	3.8354	49.81
	3	9.2045	41.4028	0.4987	4.3803	3.8816	50.41
	Average						49.99
	SD.						0.36
1:11	1	9.2036	50.6031	0.4975	4.6139	4.1164	53.46
	2	9.2048	50.6016	0.4962	4.5841	4.0879	53.09
	3	9.2091	50.6014	0.4939	4.5741	4.0802	52.99
	Average						53.18
	SD.						0.25

Table C-2 The studying the various temperature of the most suitable ratio zinc acetate:glycerol (1:7).

Temp (°C)	Order	Wt of ZnGly (g)	wt. of glycerol(g)	wt. of filter(g)		wt. of crystalline	%yield
				before	after		
200	1	9.2016	32.2014	0.4953	4.6818	4.1865	54.37
	2	9.2047	32.2078	0.4951	4.6716	4.1765	54.24
	3	9.2014	32.2065	0.4937	4.6602	4.1665	54.11
	Average						54.26
	SD.						0.13
230	1	9.2057	32.2015	0.4975	5.0097	4.5122	58.60
	2	9.2016	32.2031	0.468	4.9448	4.4768	58.14
	3	9.2046	32.2025	0.4937	4.9743	4.4806	58.19
	Average						58.31
	SD.						0.25
260	1	9.2011	32.2014	0.4978	5.2133	4.7155	61.24
	2	9.2043	32.2034	0.4986	5.2326	4.7340	61.48
	3	9.2038	32.2046	0.4968	5.2770	4.7802	62.08
	Average						61.60
	SD.						0.43

Table C-3 The studying the product of pure glycerol and crude glycerol under condition molar ratio zinc acetate:glycerol (1:7) and temperature260 °C.

Type of substance	order	Wt of ZnGly (g)	wt. of glycerol(g)	Wt of filter(g)		Wt of crystalline	% yield
				before	after		
Pure glycerol	1	9.2011	32.2014	0.4978	5.2133	4.7155	61.24
	2	9.2043	32.2034	0.4986	5.2326	4.7340	61.48
	3	9.2038	32.2046	0.4968	5.2770	4.7802	62.08
	Average						61.60
	SD.						0.43
Crude glycerol	1	9.2023	32.2031	0.4985	5.8092	5.3107	68.97
	2	9.2016	32.2024	0.4991	5.8183	5.3192	69.08
	3	9.2035	32.2027	0.4975	5.8552	5.3577	69.58
	Average						69.21
	SD.						0.33

C2.2 Chemical

C2.2.1 IR spectroscopy

- Fourier Transform Infrared Spectroscopy (FT-IR) analysis methods

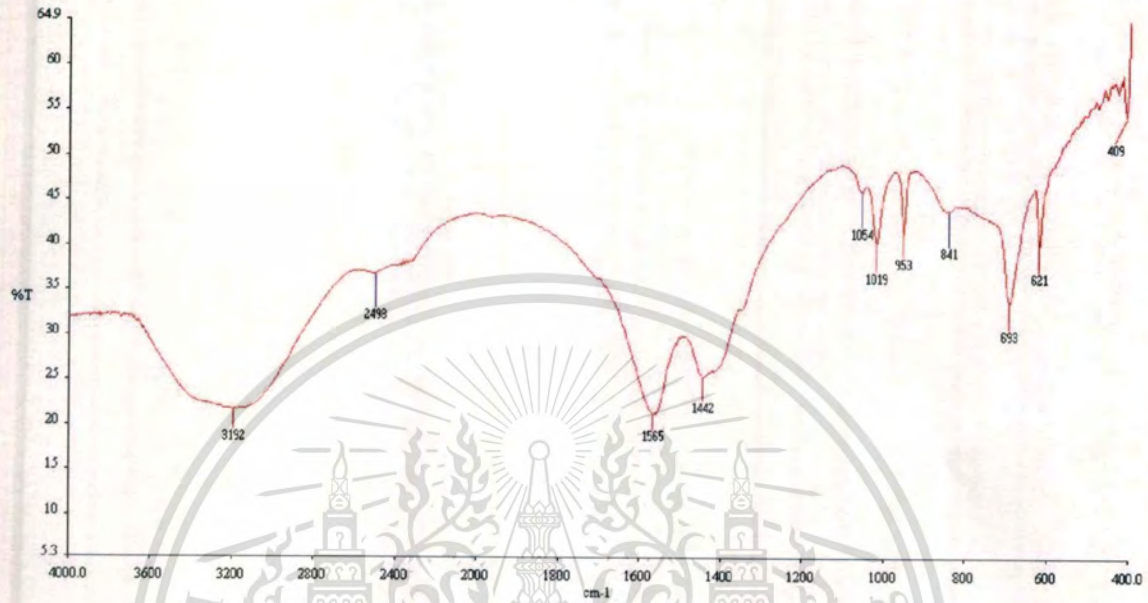

Figure C-1 The IR spectrum of zinc acetate

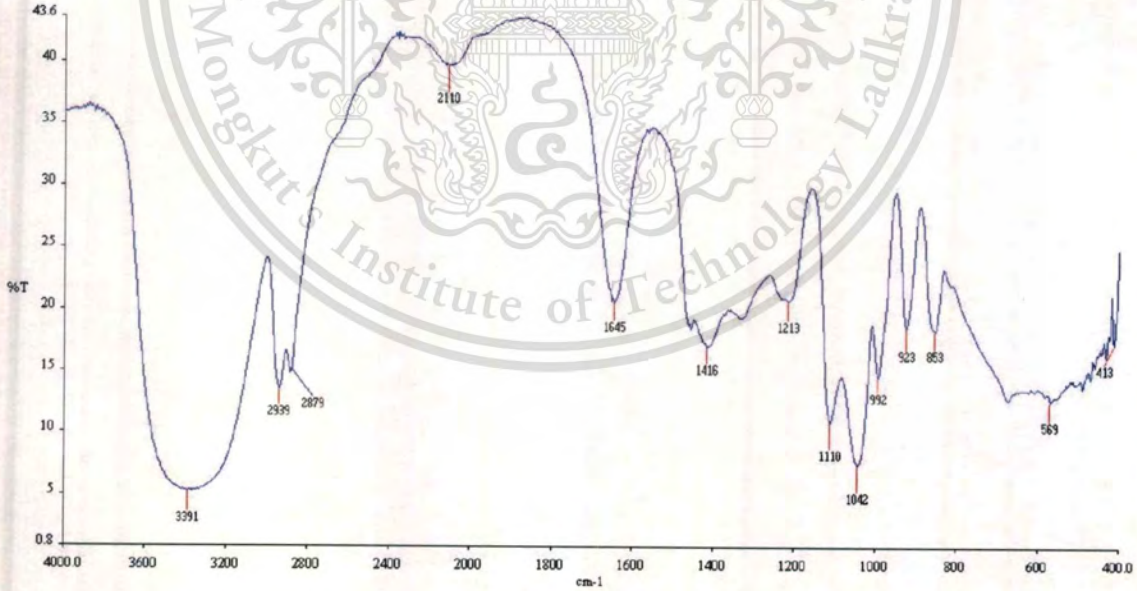

Figure C-2 The IR spectrum of pure glycerol

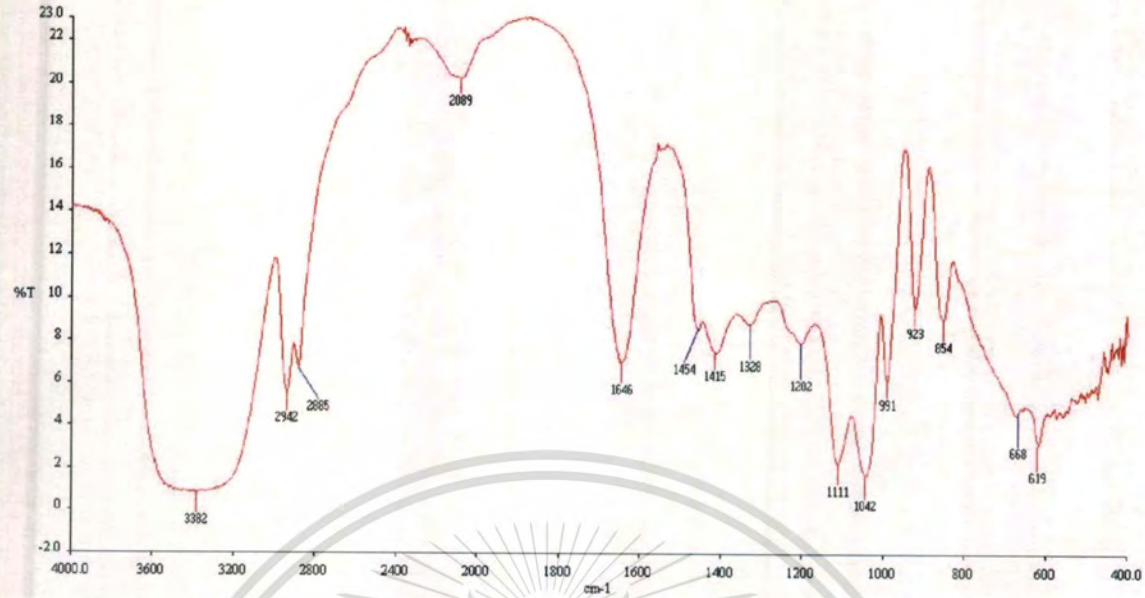

Figure C-3 The IR spectrum of crude glycerol.

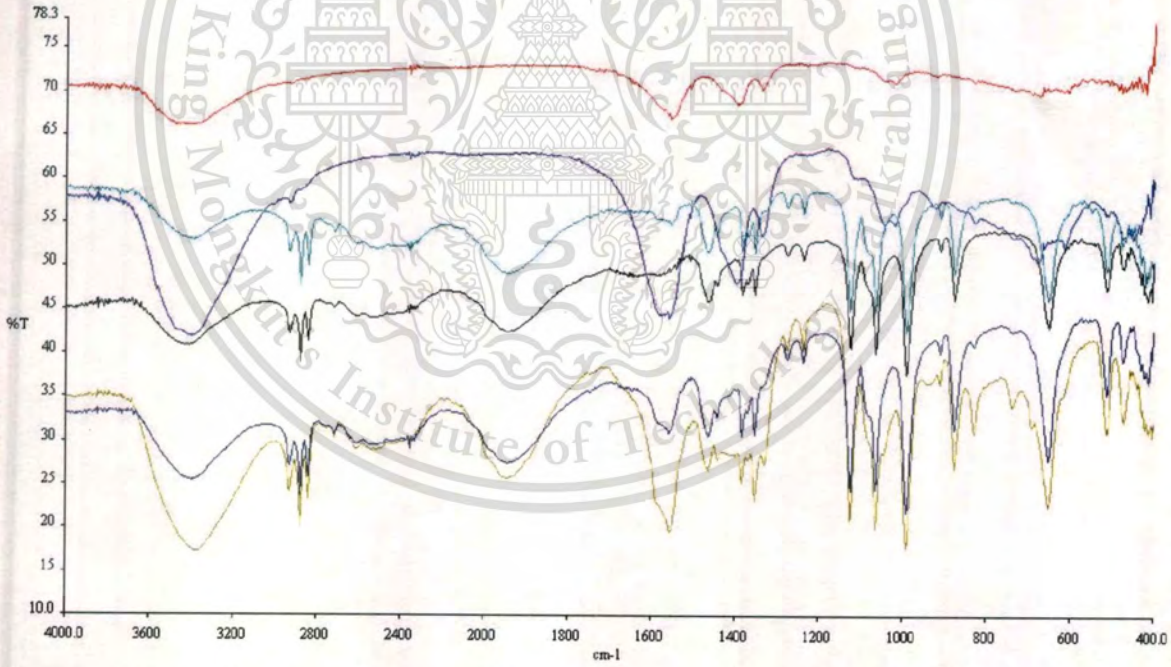

Figure C-4 The IR spectrum of zinc glycerolate at molar ratio under 200° C

Note!
- 1:1
- 1:3
- 1:5
- 1:7
- 1:9
- 1:1

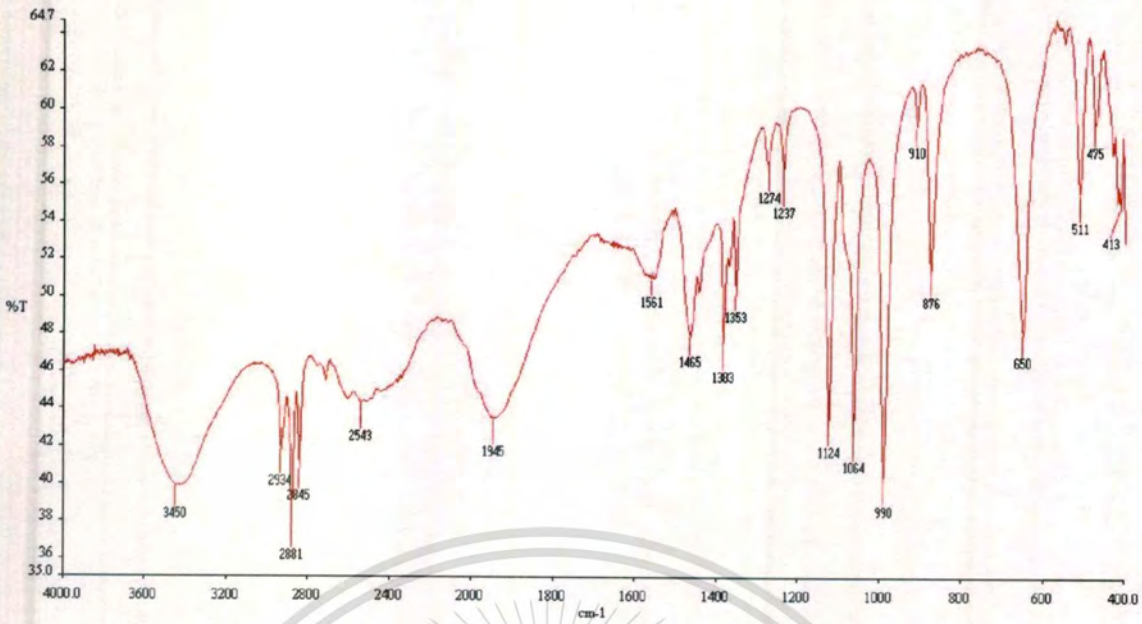

Figure C-5 The IR spectrum of zinc glycerolate at molar ratio 1:7 under 230° C

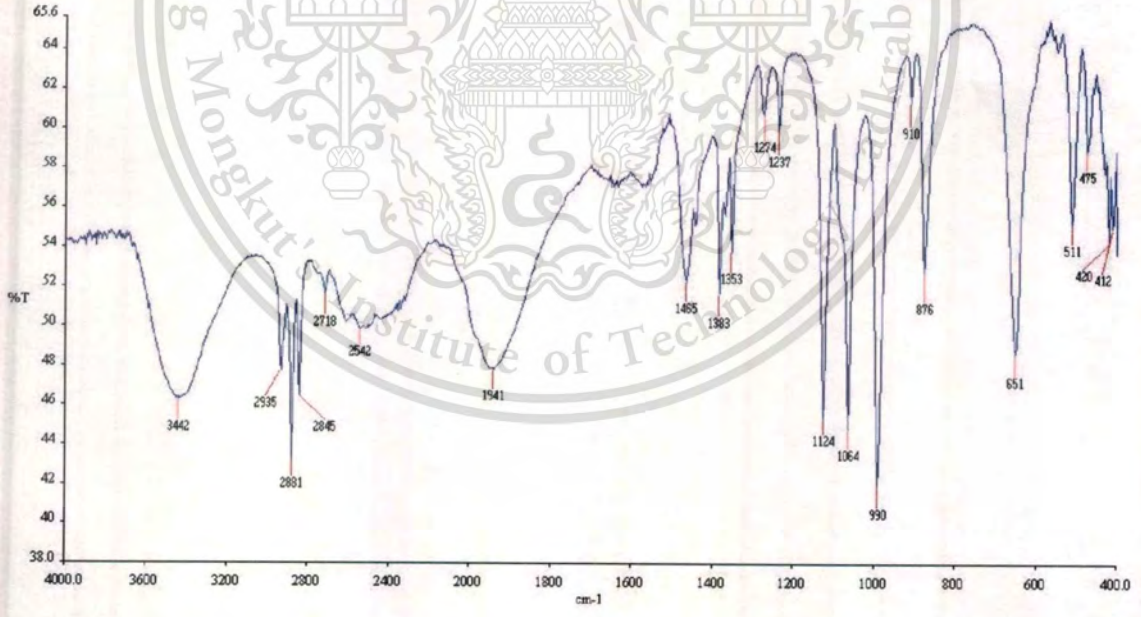

Figure C-6 The IR spectrum of zinc glycerolate at molar ratio 1:7 under 260° C

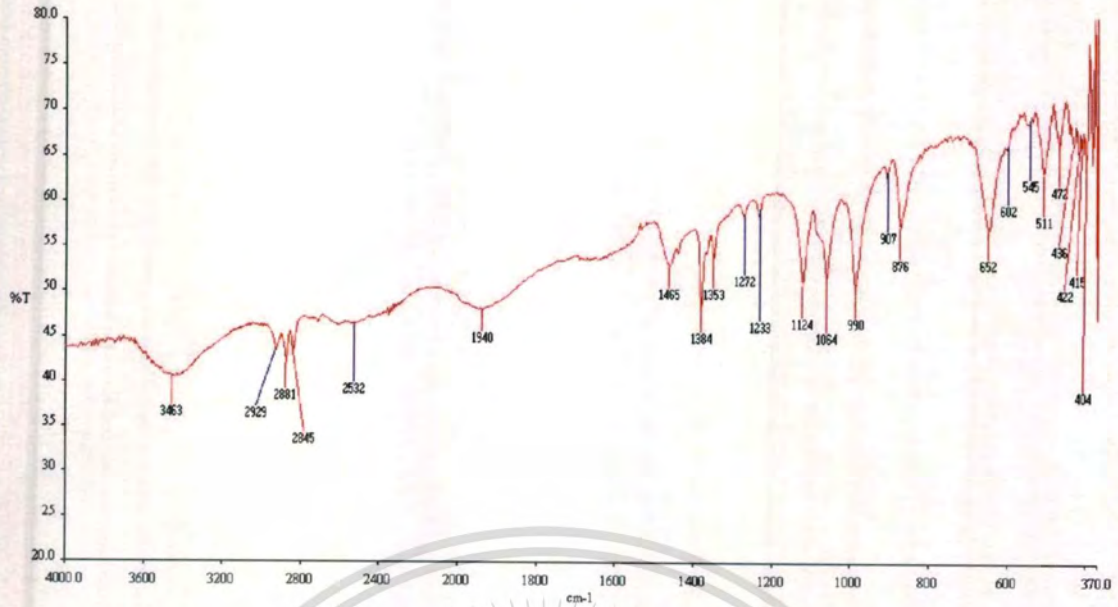

Figure C-7 The IR spectrum of zinc glycerolate from crude glycerol at molar ratio 1:7 under 260° C

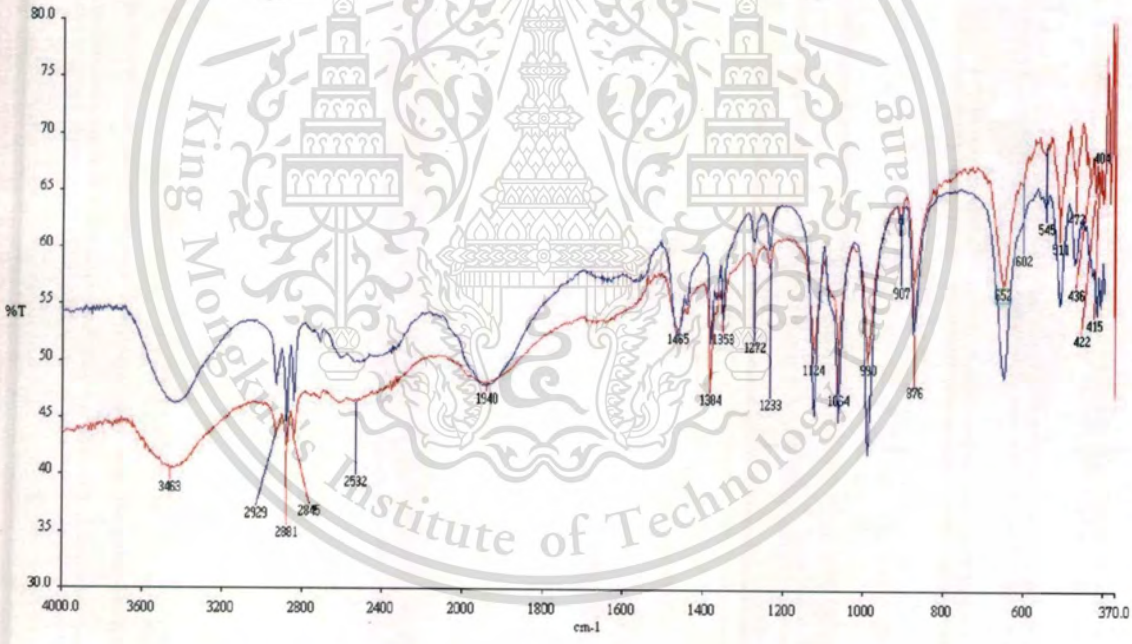

Figure C-8 The spectrum comparison of IR of zinc glycerolate from pure and crude glycerol

Note: ——— Pure glycerol

——— Crude glycerol

The IR spectrum of zinc glycerolate which preparation of pure glycerol blue line and another was red line preparation of crude glycerol .From the figure show that they were similar in every peak but different in intensity of %T of each peak.

C 2.2.2 XRD technique

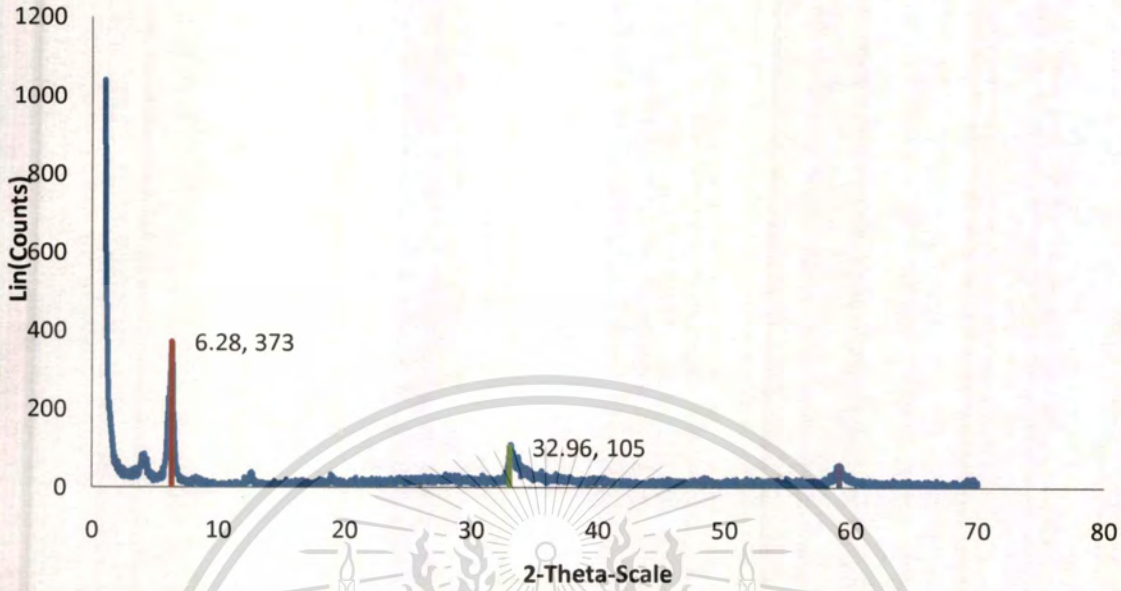

Figure C-9 The XRD peak of zinc glycerolate from molar ratio 1:1

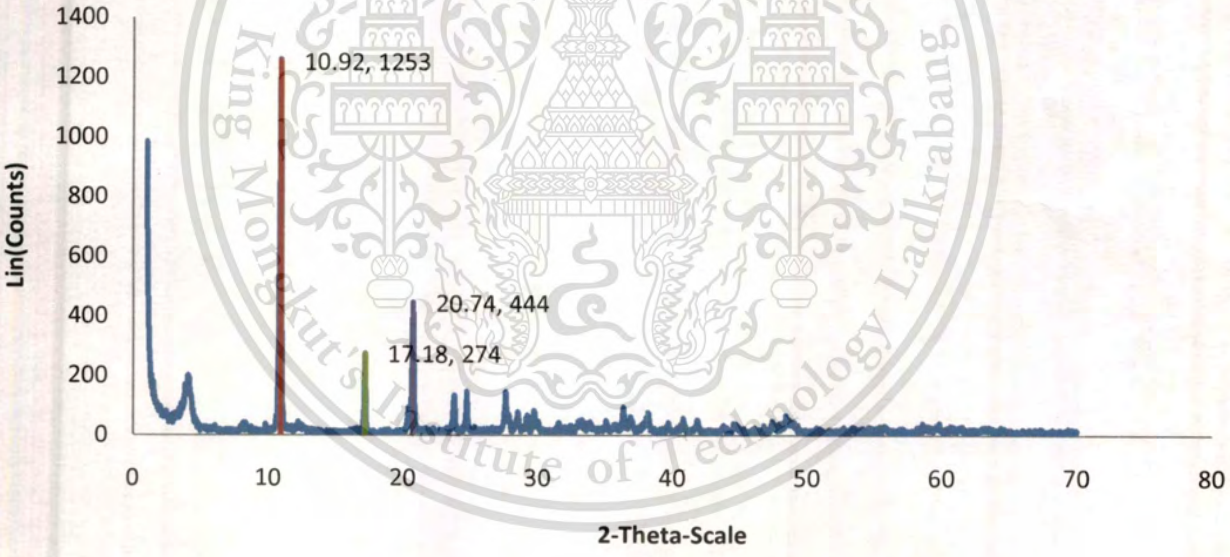

Figure C-10 The XRD peak of zinc glycerolte from molar ratio 1:3

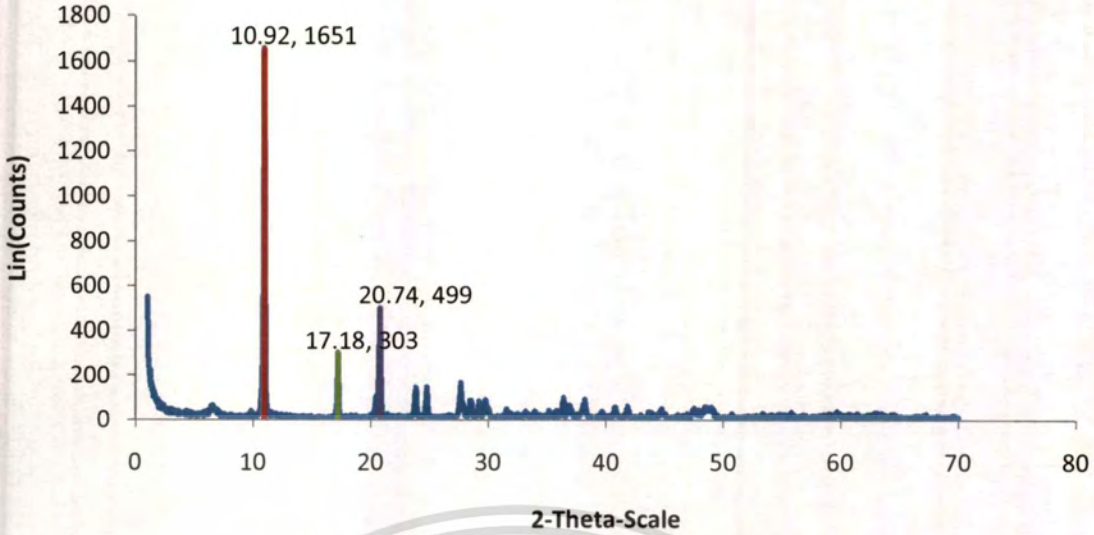

Figure C-11 The XRD peak of zinc glycerolate from molar ratio 1:5

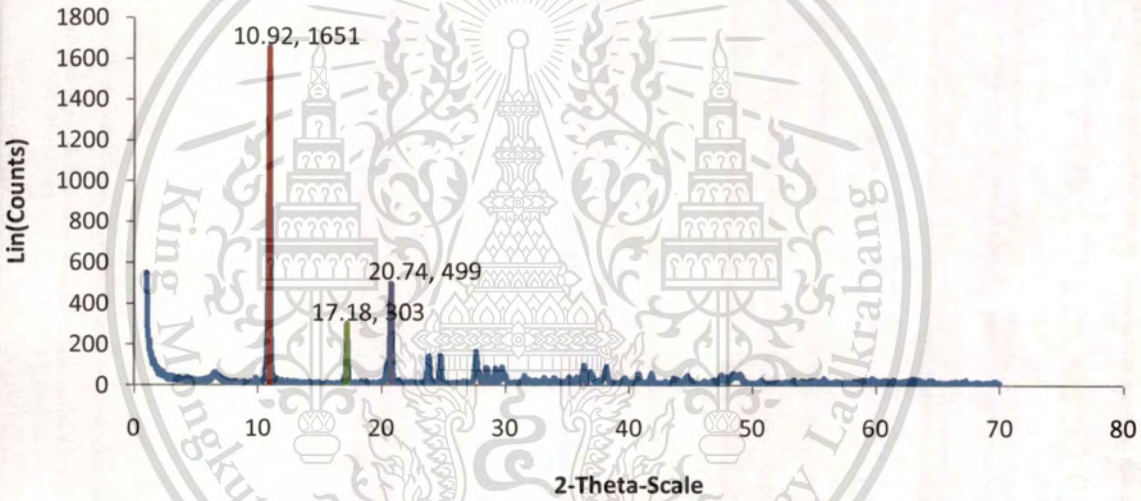

Figure C-12 The XRD peak of zinc glycerolate from molar ratio 1:7 under 200° C

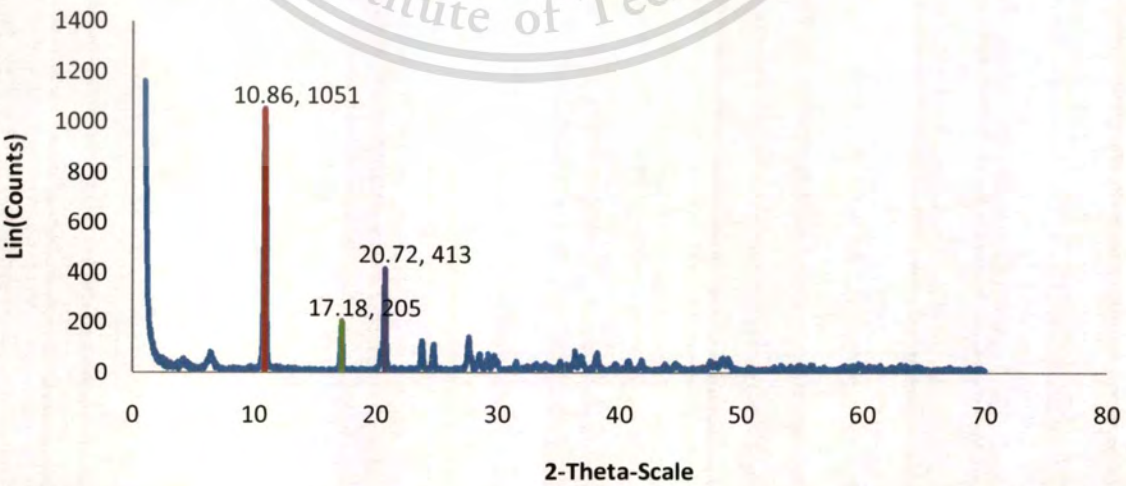

Figure C-13 The XRD peak of zinc glycerolate from molar ratio 1:7 under 230° C

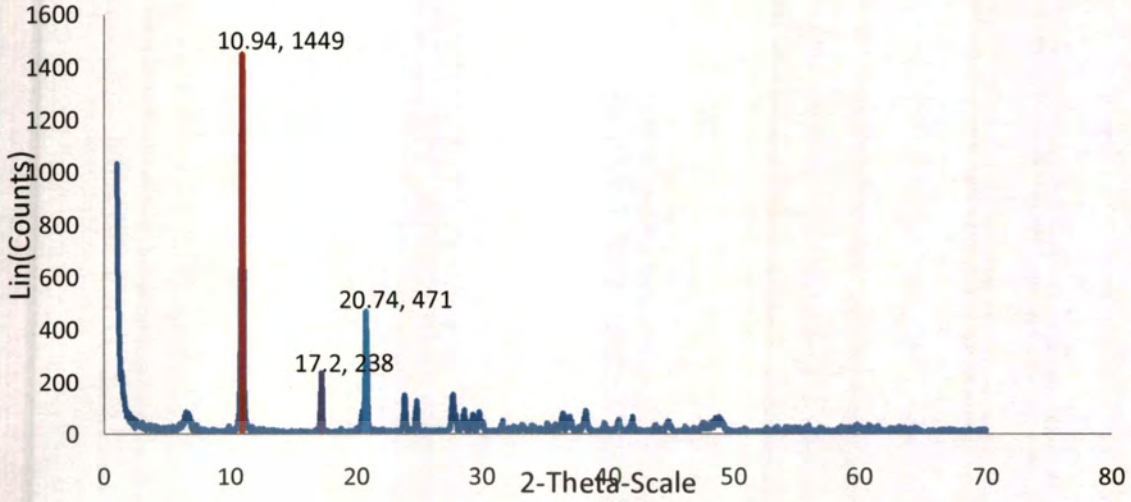

Figure C-14 The XRD peak of zinc glycerolate from molar ratio 1:7 under 260 ° C

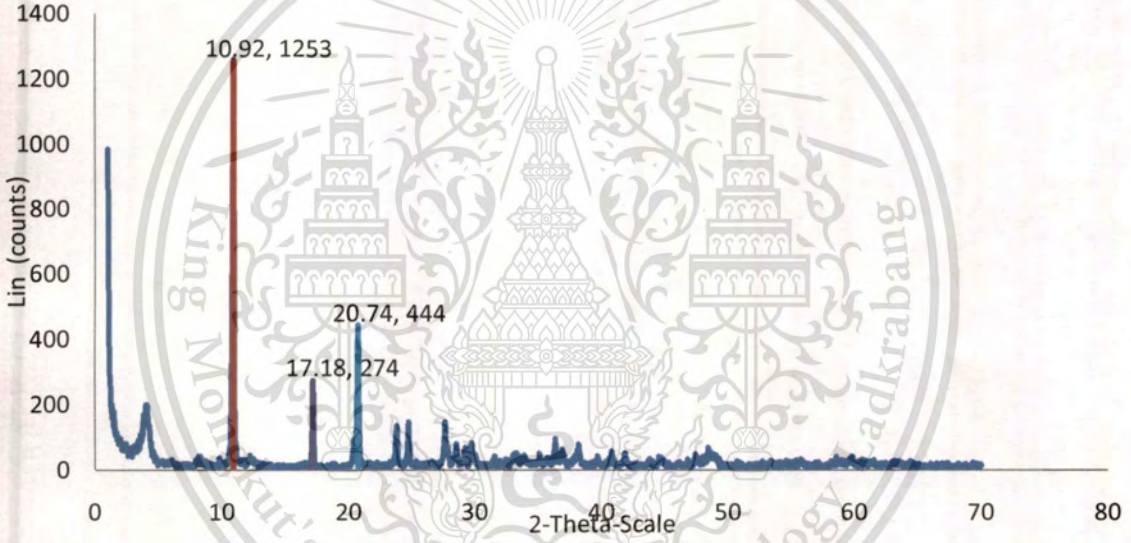

Figure C-15 The XRD peak of zinc glycerolate from molar ratio 1:9

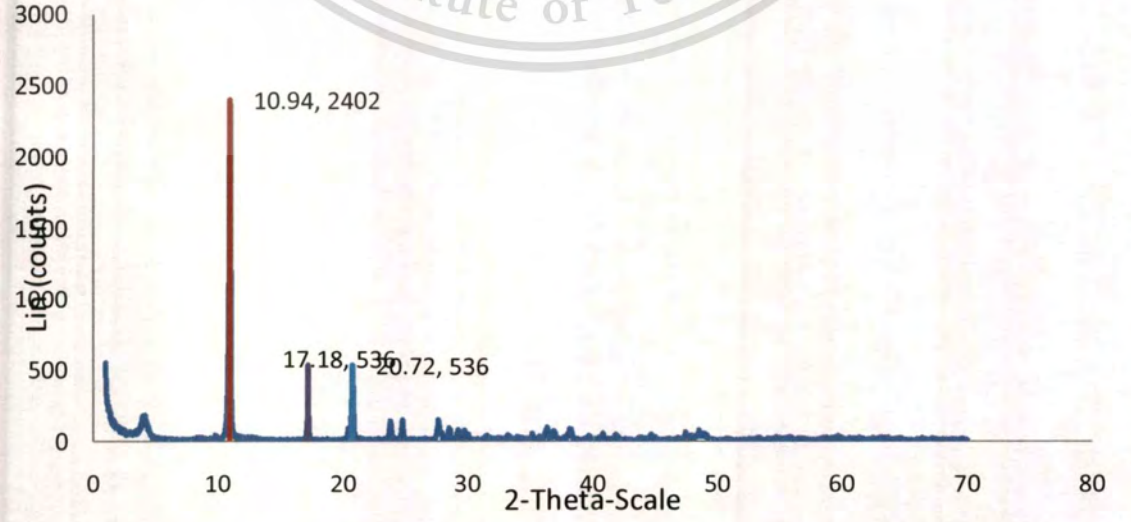

Figure C-16 The XRD peak of zinc glycerolate from molar ratio 1:11.

C2.2.3 SEM technique.

From the testing, we tested sample with scanning electron microscope,(SEM) to analyze the size of particle both 2 sample of zinc glycerolate from commercial glycerol and crude glycerol under the most suitable condition at molar ratio 1:7 and temperature 260° C which we had shown the image below.

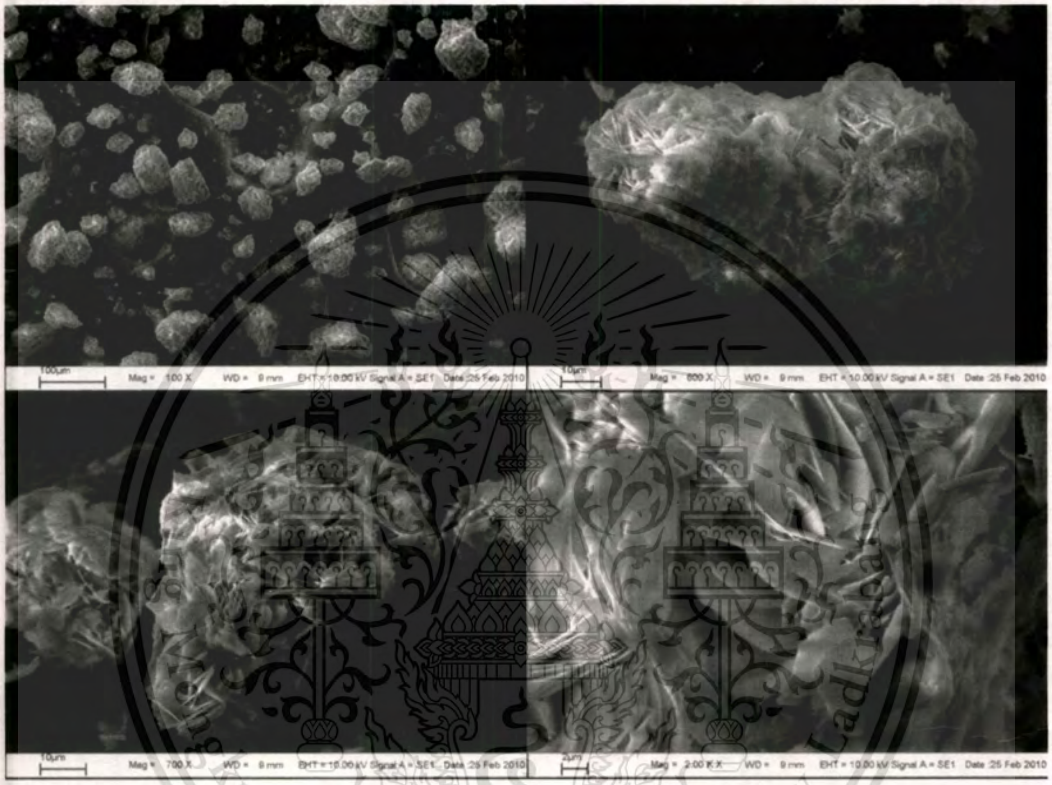

Figure C-17 SEM image of synthesized zinc glycerolate from commercial glycerol

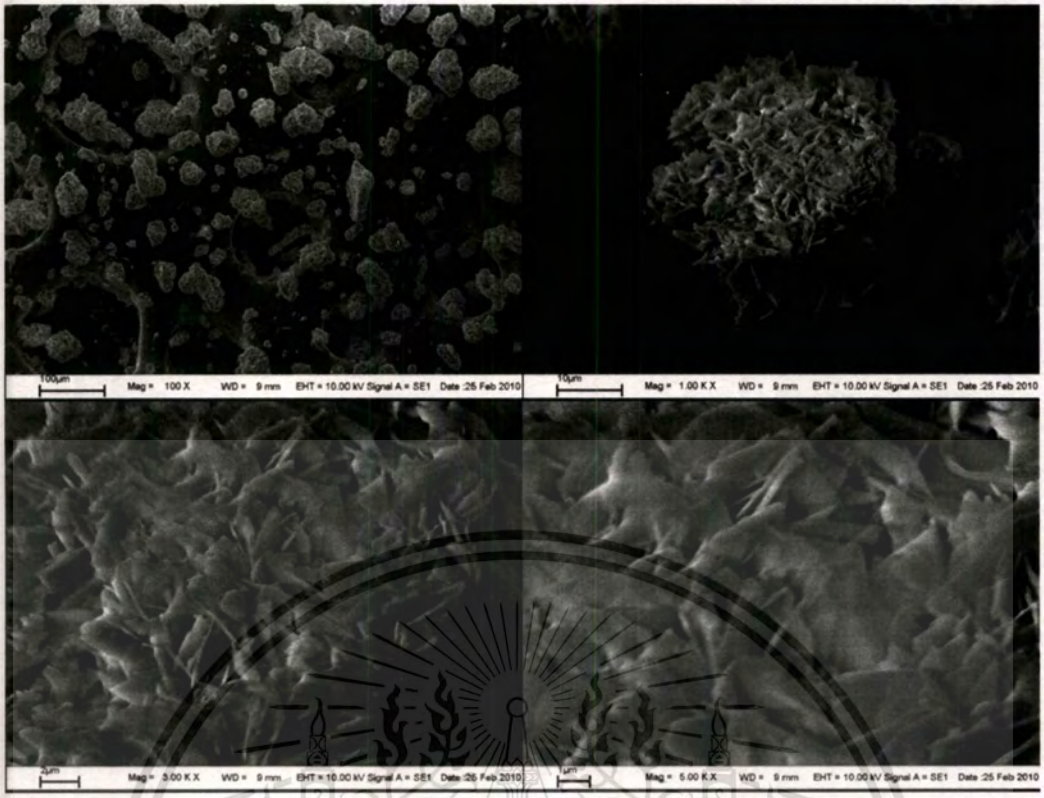

Figure C-18 SEM image of synthesized zinc glycerolate from crude glycerol.

C2.3 Biological

The biological experiment by testing reactive of bacteria to zinc glycerol, the parameter of this is the concentration of zinc glycerolate which affect the growth of bacteria that found on the skin. Bacterias can be classified into 2 types are bacteria that disease of the skin and not disease of the skin.

However, the disease of the skin includes *Staphylococcus aurens* and not disease of the skin includes *Micrococcus luteus* and *Serratia marcescens*. The difference concentration of zinc glycerolate 0, 10^{-3}, 10^{-5}, and 10^{-7} mol/L.

Table C-4 The biological experiment by testing reactive of bacteria to zinc glycerol.

Type of Bacteria	Orders	Diameter of clear zone(cm)			
		Control Area	10^{-3} M	10^{-5} M	10^{-7} M
Micrococcus luteus	1	-	0.80	-	-
	2	-	0.75	-	-
	3	-	0.90	-	-
average		-	0.82	-	-
Staphylococcus aurens	1	-	0.70	0.75	0.80
	2	-	0.85	0.90	0.75
	3	-	0.75	-	0.90
average		-	0.76	0.82	0.81
Serratia marcescens	1	-	0.8	0.7	0.9
	2	-	0.9	0.8	0.8
	3	-	0.9	0.7	0.7
average		-	0.87	0.73	0.8