# การสังเคราะห์ไดเอทานอลเอไมด์จากน้ำมันเมล็ดในปาล์มโดยใช้เอนไซม์ไลเปส ชนิดแคนนิดา แอนแทรกทิกา

นางสาวสิริรัตน์ ขำวารี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต

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# SYNTHESIS OF DIETHANOLAMIDE FROM PALM KERNEL OIL USING CADIDA ANTRACTICA LIPASE

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for the Degree of Master of Engineering Program in Chemical Engineering
Depatment of Chemical Engineering
Faculty of Engineering
Chulalongkorn University
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| | |
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| Thesis Title | SYNTHESIS OF DIETHANOLAMIDE FROM PALM KERNEL OIL USING CADIDA ANTRACTICA LIPASE |
| By | Miss Sirirat Khomwaree |
| Field of Study | Chemical Engineering |
| Thesis Advisor | Jirdsak Tscheikuna, Ph.D. |

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สิริรัตน์ ขำวารี : การสังเคราะห์ไดเอทานอลเอไมด์จากน้ำมันเมล็ดในปาล์มโดยใช้
เอนไซม์ไลเปสชนิดแคนนิดา แอนแทรกทิกา (SYNTHESIS OF DIETHANOLAMIDE
FROM PALM KERNEL OIL USING CADIDA ANTRACTICA LIPASE) อ. ที่ปรึกษา :
อ. ดร. เจิดศักดิ์ ไชยคุนา, 76 หน้า

งานวิจัยนี้เป็นการศึกษาการสังเคราะห์สารไดเอทานอลเอไมด์ด้วยปฏิกิริยาทรานส์เอมิเดชันจากน้ำมันเมล็ดในปาล์มดิบและไดเอทานอลเอมีนโดยใช้ตัวเร่งปฏิกิริยาโซเดียมเมทอกไซด์ที่ความเข้มข้นร้อยละ1 และเอนไซม์ไลเปสชนิดแคนนิดา แอนแทรกทิกาที่ความเข้มข้นร้อยละ 4 การทดลองทำในถังปฏิกรณ์แบบกะที่อุณหภูมิ 60, 80 และ 120 องศาเซลเซียส ความดัน 5 บาร์ สัดส่วนโดยโมลของไดเอทานอลเอมีนต่อน้ำมันเมล็ดในปาล์มที่ 3:1 6:1 และ 15:1 และใช้เวลา 2- 8 ชั่วโมงสำหรับตัวเร่งปฏิกิริยาโซเดียมเมทอกไซด์ ส่วนตัวเร่งปฏิกิริยาเอนไซม์ไลเปสใช้อุณหภูมิ 40 50 และ 60 องศาเซลเซียส และเวลา 6-24 ชั่วโมง

ผลการทดลองแสดงให้เห็นว่าสภาวะที่ดีที่สุดที่ใช้ในการทำปฏิกิริยาของตัวเร่งปฏิกิริยาโซเดียมเมทอกไซด์ คือที่อุณหภูมิ 60 องศาเซลเซียสและสัดส่วนโดยโมลของไดเอทานอลเอมีนต่อน้ำมันที่ 6:1 โดยผลผลิตที่ได้มีค่าเท่ากับร้อยละ 93.24 หลังจากใช้เวลาในการทำปฏิกิริยา 4 ชั่วโมง ส่วนสภาวะที่เหมาะสมที่สุดของเอนไซม์ไลเปส คือที่อุณหภูมิ60 องศาเซลเซียสและสัดส่วนโดยโมลของไดเอทานอลเอมีนต่อน้ำมันที่ 3:1 หลังจากใช้เวลาในการทำปฏิกิริยา 24 ชั่วโมง ผลผลิตมีค่าเท่ากับร้อยละ 10.20 จากการศึกษาทางจลนศาสตร์ พบว่าปฏิกิริยาทรานส์เอมิเดชันเป็นปฏิกิริยาอันดับสองสำหรับทั้งสองตัวเร่งปฏิกิริยา โดยมีค่าคงที่อัตราเร็วปฏิกิริยา เท่ากับ 1.11 และ 1.00 ลิตรต่อโมลต่อชั่วโมงต่อกรัมของตัวเร่งปฏิกิริยา ตามลำดับ จากการทดลองนี้สรุปได้ว่าอัตราการเกิดปฏิกิริยาของตัวเร่งปฏิกิริยาที่ใช้โซเดียมเมทอกไซด์จะเกิดเร็วกว่าตัวเร่งปฏิกิริยาที่ใช้เอนไซม์ไลเปส

ภาควิชา............ วิศวกรรมเคมี..........ลายมือชื่อนิสิต......สิริรัตน์ ขำวารี..............
สาขาวิชา............วิศวกรรมเคมี..........ลายมือชื่ออาจารย์ที่ปรึกษา.....................
ปีการศึกษา.................2549...............

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KEY WORD: DIETHANOLAMIDE/ TRANSAMIDATION/ SURFACTANT

SIRIRAT KHOMWAREE: SYNTHESIS OF DIETHANOLAMIDE FROM PALM KERNEL OIL USING CADIDA ANTRACTICA LIPASE. THESIS ADVISOR: JIRDSAK TSCHEIKUNA, Ph.D., 76 pp.

Synthesis of diethanolamide using transamidation reaction from crude palm kernel oil (PKO) and diethanolamine (DEA) is investigation in this study. The catalysts used are sodium methoxide at a concentration of 1% and lipase from *cadida antractica* at a concentration of 1%. The reaction was carried out in a batch reactor at temperatures of 60, 80 and 120°C, pressure of 5 bars, molar ratios (DEA: PKO) of 3:1, 6:1 and 15:1 and reaction times from 2 to 8 hours using sodium methoxide as catalyst. Temperatures of 40, 50 and 60 °C and reaction times from 6 to 24 hours were used with lipase.

The results show that the optimum conditions when sodium methoxide was used as catalyst were a temperature of 60°C, a molar ratio of DEA to PKO of 6:1 and reaction time of 4 hours. Diethanolamide concentration of 93.24 % was obtained at this condition. When lipase was used as catalyst, the optimum conditions were a temperature of 60 °C and a molar ratio of DEA to PKO of 3:1 for 24 hours. Diethanolamide concentration in reaction product of 10.20% was obtained. The second order kinetic was found to give suitable description of this reaction rate for both catalysts, and the rate constants of PKO were estimated to be 1.11 and 1.00 l/mol hr.g-cat, respectively. It is concluded that the rate reaction using sodium methoxide as catalyst is faster than using lipase as catalyst.

Department… Chemical Engineering…Student's signature………………

Field of study…Chemical Engineering…Advisor's signature…………………

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# CHAPTER I

# INTRODUCTION

Alkanolamide, an important class of nonionic surfactant, is widely used to control viscosity, corrosion and foam properties of products used in the laundry detergents. Surfactant enters the environment in significant amounts via wastewater treatment plants or by direct release into aquatic recipients. Their possible chronic effects on sensitive species and their potential environmental impact must be minimized. The last one depends on the biodegradability and toxicity of the surfactants. Potential mineralization under aerobic and anoxic condition is important for a hazard assessment of surfactant because degradation often reduces the toxicity of these chemical (Madsen, T. et al. 1996). The alkanolamides are surfactant obtained from a fatty acid, a methyl ester, or a triglyceride such as coconut oil, as the rest of surfactants obtained from this source, are highly acceptable to the environment.

Early experiments in the preparation of alkanolamides were begun by Kritchevsky. It involves condensation reactions of fatty acids, triglycerides, esters, amides, anhydrides, and halides with an alkanolamine. The reaction was carried out at 100–300 °C at atmospheric pressure. An important improvement was made by Meade, who made use of an alkali metal alkoxide as a catalyst at 100 °C at atmospheric and slightly above atmospheric pressure. However, high temperature causes self- condensation of diethanolamine that results to from N, N-bis (2-hydroxy-ethyl) piperazine or morpholine. Thus, all of these processes are tedious and require large amounts of energy.

In view of these drawbacks, an attempt was made to develop an enzymatic reaction as an alternative low cost and low-energy-consuming industrial process. Numerous studies on enzymatic reaction of amidation used fatty acid, ester and triglyceride as the reactant.

In Thailand wherein palm kernel oil is produced abundantly, these would be a potential raw material for making alkanolamide. However, the cost of alkanolamide is main hurdle to commercialize the product. Therefore, the use of crude vegetable oil as raw material is one of the primary options to be considered.

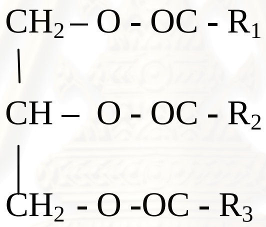
In this work, the transamidation of crude palm kernel oil on enzyme catalysts was investigated. The enzyme catalysts studied were Lipase from *Candida Antarctica* using 4 % weight of oil and studied the effect of reaction time, effect of temperature and effect of molar ratio of oil to diethanolamine and analyzed by gas chromatography. The reaction rate using these enzyme catalysts were compared to those using 1 % weight of oil of sodium methoxide as alkali catalyst in optimum condition.

# CHAPTER II

# LITERATURE REVIEWS

## 2.1 Vegetable oils

Fats and oils constitute a well-defined class of neutral and water insoluble substances that are produced in some quantity by animals and vegetable. Most vegetable oils have the triglyceride structure, which is represented in Figure 2.1. These tri-esters are derived from glycerol and carboxylic acids known as fatty acids. Therefore, vegetable oils are commonly called triglycerides, so called triacylglycerols as a more accurate name.



$$\begin{array}{l} CH_2 - O - OC - R_1 \\ | \\ CH - O - OC - R_2 \\ | \\ CH_2 - O - OC - R_3 \end{array}$$

$R_1$, $R_2$, $R_3$ : carbon chain of the fatty acids

**Figure 2.1** Chemical structure of triglyceride

The fatty acids are almost always straight chains containing between 8 and 22 carbon atom, and may be saturated, monounsaturated or polyunsaturated. Vegetable oil compositions are normally described in terms of their fatty acid content, referring to the acid ester moieties; actual fatty acids present in the oil are known as free fatty acids.

The difference chemical and physical properties of vegetable oils depend on the degree of unsaturation of the constituent fatty acids and chain length. Vegetable oils with a proportion of the saturated fatty acids are solid or semi-solid at room temperature. The melting point of the fatty acids and their glycerides decrease from polyunsaturated types, whereas the volatility and boiling points change only slightly. By chain length, the melting point, the boiling point of the fatty acids and their glycerides rises when chain length increase. There are many vegetable oils used a wide application in Thailand such as palm oil, palm kernel oil.

### 2.1.1 palm oil

The palm fruit is the source of both palm oil and palm kernel oil. Palm oil, extracted from palm fruit flesh and palm kernel or seed oil are composed of fatty acids esterified with glycerol just like any ordinary fat. Both are high in saturated fatty acids. Saturated fat, also found in coconut oil, beef fat, and milk fat are correlated with an increased incidence of atherosclerosis and coronary heart disease. The oil palm gives its name to the 16 carbon saturated fatty acid palmitic acid found in palm oil; monosaturated oleic acid is also a constituent of palm oil while palm kernel oil contains mainly lauric acid, It shows in table 2.1.

**Table2.1**. Fatty Acid Composition of Palm and Palm Kernel Oils.

| Fatty Acid | Formula | Palm Oil (%) | Palm Kernel Oil (%) |
|---|---|---|---|
| Caproic | $C_6H_{12}O_2$ | - | 0–1 |
| Caprylic | $C_8H_{16}O_2$ | - | 3–5 |
| Capric | $C_{10}H_{20}O_2$ | - | 3–5 |
| Lauric | $C_{12}H_{24}O_2$ | 0.1-1 | 44–51 |
| Myristic | $C_{14}H_{28}O_2$ | 0.9-1.5 | 15–17 |
| Palmitic | $C_{16}H_{32}O_2$ | 41.8-46.8 | 7–10 |
| Stearic | $C_{18}H_{36}O_2$ | 4.2-5.2 | 2–3 |
| Oleic | $C_{18}H_{34}O_2$ | 37.3-40.8 | 12–19 |
| Linoleic | $C_{18}H_{32}O_2$ | 9.1-11 | 1–2 |

Palm kernel oil, a co product of palm oil, comprises less than 5% of the total natural fats and oils, but they are important feedstocks of the oleochemical industry. Palm kernel oil belongs to the so-called lauric oils, which are characterized by their high lauric oil content of approximately 50%. The lauric oils are highly desirable materials in the oleochemical industry worldwide because of the importance of the lauric fraction especially in the manufacture of soaps and detergents.

## 2.2 Surfactant

Surfactants or surface-active agents make up a special class of chemicals used in practically all industries. Surfactants may be of petrochemical or natural oils origin. Surfactants are chemical compounds that possess great surface activity. They act so diversely because of the unbalanced molecular structure. A surfactant molecule may be visualized as a tadpole or a mini-racquet. The ‘‘head’’ is the hydrophilic (water-loving), strongly polar portion and the ‘‘tail’’ is the hydrophobic (oil-loving) non polar portion. The head can be an anion, a cation, or nonion. The tail is a linear or branched hydrocarbon chain. It is this characteristic configuration that makes surfactants performs such diverse function in industry.

Surfactants find broad application in practically all industries as, for example, the main ingredients of detergents and cleaners, foaming agents and emulsifiers in cosmetics and pharmaceuticals, emulsifiers for paints, scouring agents for textiles, flotation agents for the mining industry, and emulsifiers and sanitizing agents for the food industry.

### Types of Surfactants

Surfactants are classified into four categories: anionic, cationic, nonionic, and amphoteric, each with its own molecular structure and behavior.

**Anionic Surfactants.** Anionic surfactants are surface-active agents in which the hydrophobic portion is connected to an anion or negatively charged ion. In an aqueous medium, an anionic surfactant dissociates into a positively charged cation and a negatively charged anion. The latter is the carrier of the surface-active properties. Typical examples are the alcohol sulfates and the ester sulfonates.

**Cationic Surfactants.** Like the anionic surfactants, cationic surfactants also dissociate in an aqueous medium. However, the head (hydrophilic portion) is a cation, which is the carrier of the surface-active properties. The examples are the quaternary ammonium compounds.

**Nonionic Surfactants.** The nonionic surfactants do not dissociate in an aqueous medium. Their solubility is provided by the polar group, such as polyglycol ether or a polyol. The most important types of fatty nonionic surfactants are the polyglycol ethers of fatty alcohols, fatty acids, amines, and amides.

**Amphoteric Surfactants.** Amphoteric surfactants in aqueous solution contain both positive and negative charges in the same molecule. Thus, a hydrophobic fatty chain is attached to a hydrophilic group that contains both positive and negative charges. Its behavior depends on the condition of the medium or its pH value. The examples of this type are the alkyl betaines.

**Coco-Based Surfactants.** The most important coconut oil-based surfactants are fatty alcohol sulfate, fatty alcohol ether sulfate, and fatty alcohol polyglycol ether. Two relatively new coco-based surfactants are fatty acid methyl ester sulfonate and alkyl polyglycoside, which is produced from fatty alcohol and starch or sugar, both renewable materials.

## 2.3 ALKANOLAMIDES

Alkanolamides are condensation products of the reaction of a primary or a secondary alkanolamine with a fatty acid, a methyl ester, or a triglyceride such as coconut oil. The composition and functional properties of alkanolamides vary considerably, depending on the reactants employed and the reaction conditions. Such properties include super fatting, wetting, foam boosting, foam stabilizing, thickening, lubricating, emolliency, skin protection, emulsifying, and corrosion inhibition.

Alkanolamides are nonionic surface active agents that find an application in a multitude of uses. There are four major groups of fatty alkanolamides:

(1) monoethanolamides (MEA)
(2) diethanolamides (DEA)
(3) monoisopropanolamides (MIPA)
(4) ethoxylated or PEG alkanolamides

## 2.4 Amidation reaction

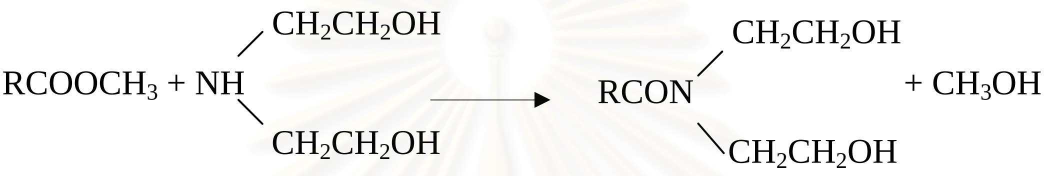
**Reaction with Fatty Acids.** When alkanolamines react with fatty acids in the ratio of 1:1 at 140–160 °C, N-alkylolamides are formed.

$$\mathrm{RCOOH} + 2\mathrm{NH}\begin{array}{l}\diagup CH_2CH_2OH\\ \diagdown CH_2CH_2OH\end{array} \longrightarrow \mathrm{RCON}\begin{array}{l}\diagup CH_2CH_2OH\\ \diagdown CH_2CH_2OH\end{array} + H_2O$$

The reaction is a two-stage reaction with the initial formation of the alkanolamine soap followed by dehydration in order to form the alkanolamide. At the same time, significant quantities of amine esters and amide esters are formed by the side reaction. If a dialkanolamine is used as the starting material, smaller amounts of amine diester and amide diester are formed as well as some morpholine and piperazine derivatives.

Reaction of dialkanolamines with fatty acids in a 2:1 ratio at 140–160 °C gives a second major type of alkanolamide. This product, in contrast to 1:1 alkanolamide, contains a considerable amount of unreacted dialkanolamine, which accounts for the aqueous solubility of the product.

**Reaction with Esters.** The reaction of an ester, such as cocomethyl ester, with an alkanolamine at 110 °C produces an N-alkylolamide.



$$RCOOCH_3 + NH(CH_2CH_2OH)_2 \longrightarrow RCON(CH_2CH_2OH)_2 + CH_3OH$$

The reaction of methyl esters of fatty acids with alkanolamines is used commercially to prepare high-purity 1:1 alkanolamide. This product, in contrast to the 1:1 alkanolamides prepared from the parent fatty acids, contains only a small quantity of the byproducts or unreacted starting material.

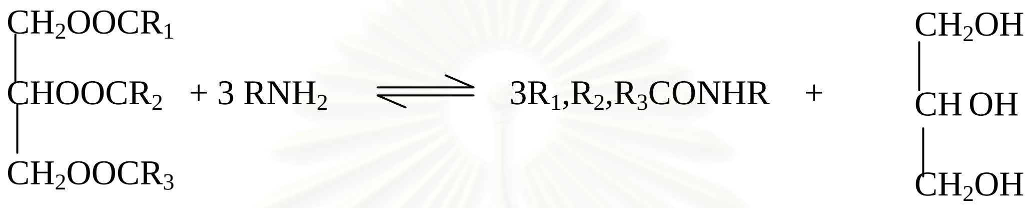
There are two competing reactions: amide formation and transesterification. The most desirable reaction is one that gives the highest amide ester byproduct. For the yield of the amide to exceed 90%, reduced pressure, lower temperature, and a slightly higher catalyst concentration were found to be necessary. Plant operations employ 0.3–0.5% catalyst, a temperature of 70–75 °C, and a vacuum of 4 kPa or less.

Another variation is the use of triglycerides, such as coconut oil, as the starting material. This involves the reaction of 1 mole of triglycerides with 6 moles of the alkanolamine. The reaction can be catalyzed by sodium methylate at 60 °C. Glycerin is a byproduct that usually remains with the final product. The typical composition of the product consists of 80–85% alkanolamide, 5–7% or less free amine, and traces of fatty acid soap and other byproducts.

The reaction is relatively mild and proceeds readily with the use of 0.2–0.3% sodium methylate catalyst at an operating temperature of 70–75 °C and at atmospheric pressure.

## 2.5. Transamidation of vegetable oil

Vegetable oil, containing naturally triglycerides, was reacted with primary amine. The products of this reaction are the mixtures of secondary amides and glycerol. This process is called transamidation of vegetable oils.



$$\begin{array}{l} \mathrm{CH_2OOCR_1} \\ | \\ \mathrm{CHOOCR_2} \\ | \\ \mathrm{CH_2OOCR_3} \end{array} + 3\,\mathrm{RNH_2} \rightleftharpoons 3\mathrm{R_1,R_2,R_3CONHR} + \begin{array}{l} \mathrm{CH_2OH} \\ | \\ \mathrm{CH\,OH} \\ | \\ \mathrm{CH_2OH} \end{array}$$

**Triglyceride** **amine** **fatty amides** **glycerol**

The mechanism of the transamidation of vegetable oils is shown in Figure 2.2. The first step (Eq.1) is the reaction of the base with the triglyceride. The nucleophilic of the amine attacks at the carbonyl group of the tridlyceride, generates a tetrahedral intermediate I. The second step is an elimination mechanism; intermediate II and anion of the diglyceride (III) are formed. The latter deprotonates the intermediate II thus gives secondary amide.

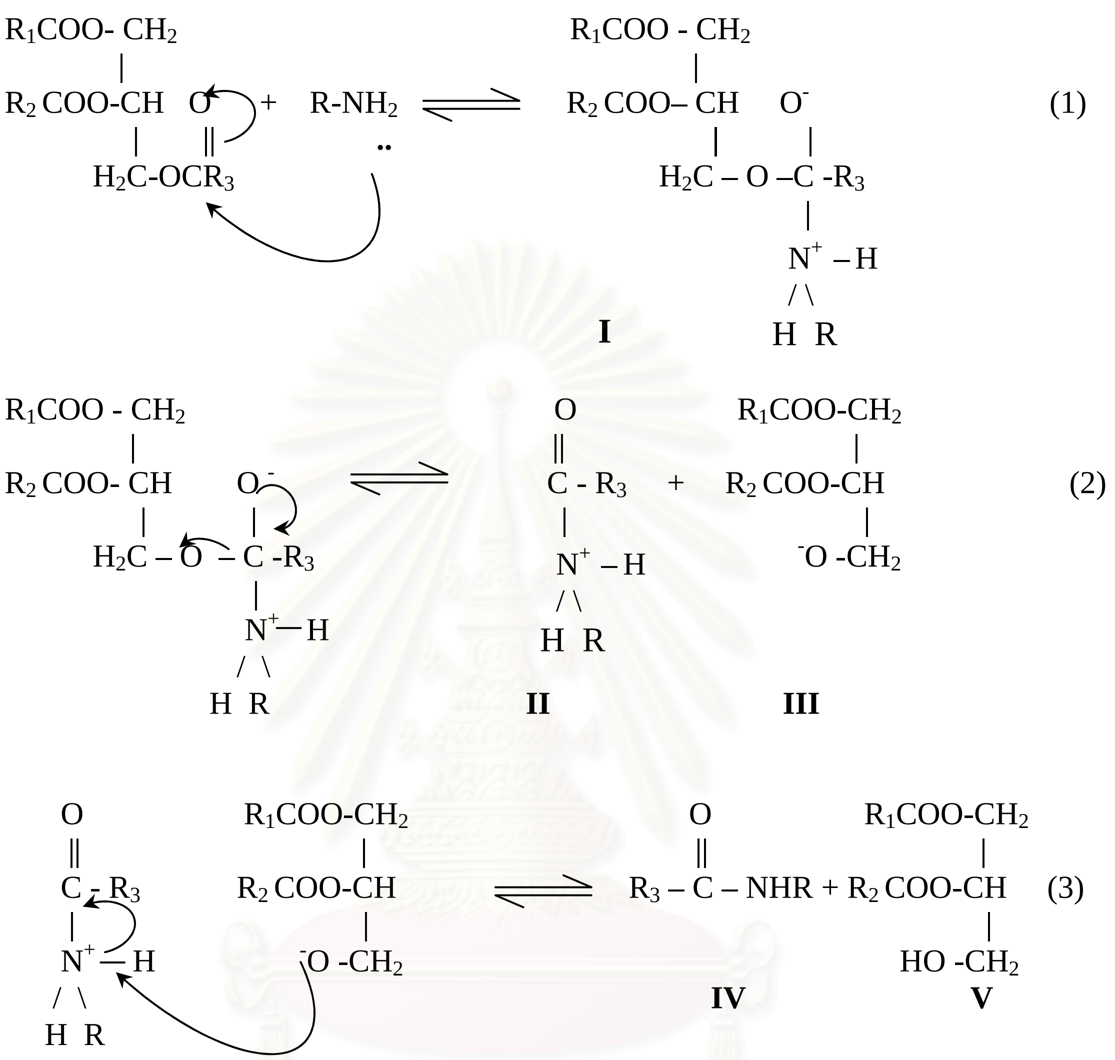


**Figure 2.2.** Mechanism of the transamidation of vegetable oils.

Early experiments in the preparation of alkanolamides were begun by Kritchevsky. It involves condensation reactions of fatty acids, triglycerides, esters, amides, anhydrides, and halides with an alkanolamine. The reaction was carried out at 100–300 °C at atmospheric pressure. An important improvement was made by Meade, who made use of an alkali metal alkoxide as a catalyst at 100 °C at atmospheric and slightly above atmospheric pressure. Further refinement was made by Tesoro, who conducted the reaction at 55–75°C and a vacuum of 4–8 kPa.

Monick (1962) patented a continuous process for making alkanolamide, which made use of a thin film reactor. Lauric diethanolamide was prepared from methyl laurate and diethanolamine in the presence of an alkaline catalyst. The conventional batch process requires cycle time of 2 -6 hr which can lead to undesirable byproducts. The results show that a reaction time of about 10 sec, product purity of 91-94% was obtained at 149 °C for an absolute pressure range of 10-760 mmHg. When reaction temperature was lowered to 110 °C, product purity became poorer as the absolute pressure was increased from 30 mm Hg to 760 mm Hg. Two studies for optimum catalyst content of either KOH or sodium methylate showed that maximum product purity of about 97% was obtained at 0.2% catalyst by weight.

Bilyk et al. (1992) developed a technique for the synthesis of monosubstituted fatty amide at low temperature and ambient pressure. This method involved the condensation of an amine with a triacyldlycerol. The primary amine (ethyl, n-butyl, n-hexyl and n-octyl were tested) acted as reagent and solvent for the fatty substrates. No additional organic solvent or catalyst was added. Tallow, vegetable oils and fish oil all served well as substrates, as did pure tripalmitin. The results showed that the rate of amidation was dependent upon temperature and the ratio of fat to amine. In a series of experiment conducted with tallow and n-butylamine at a fat:amine molar ratio of 1:16, amidation could be carried out at 20 °C, producing n-butyltallowamide in 83% yield in 24 hr. When the reaction was carried out at the boiling point of n-butylamine (78°C) and at a fat : amine ratio of 1:8, the amide yield was 93.2% in 4 hr. The reaction progressed more rapidly with higher molecular weight amine. The identity and purity of the amides was assessed by thin-layer chromatography and confirmed by elemental analyses and infrared and $C^{13}$ nuclear magnetic resonance spectroscopy.

Feairheller et al. (1994) studied amidation of fatty alkanolamides, fatty diamide and fatty aralkylamides directly from triglycerides and primary amines provides essentially quantitative yields of the various products.  The results revealed that the reactions ran to completion in 3-12 hr at temperature of 50-60 °C, approximately 100 °C lower than employed in present conventional practice.  The amines were used in excess and serve as solvent, reagent and, perhaps, as catalyst. The amides were characterized by melting point, and spectroscopic (infrared and nuclear magnetic resonance) methods.  If the mixed amide product from the various natural triglyceride mixture of fat and oils are acceptable products, this synthetic method provides these products in satisfactory quality while conserving energy and avoiding the intermediate production of free fatty acid or their esters.

Dzulkefly et al. (1997) synthesized Palm monoethanolamide (PMEA) by direct transamidation of palm oil with monoethanolamide at temperature 80 – 160 °C.  The results found that the maximum yield (85.5%) was achieved at a palm oil/monoethanolamide mole ratio of 1:3, temperature of 160 °C, reaction time of 3 hours and catalyst concentration of 0.6%.  Recrystallization using a combination of hot hexane and warm water was the best conditions to purify the PMEA, as judged by its melting point and infrared (IR) spectrum. The PMEA was not soluble in water and most hydrocarbon solvents.  However, about 60% of it dissolved in a microemulsion system containing 25% water at 50 °C. The transamidation process proceeded via the first order reaction with an activation energy of 17.4 kJ/mol.

Hakan (2004) synthesized laurel oil alkanolamide directly from laurel oil and ethanolamine at a low-temperature, was carried out in essentially quantitative yields.  The ethanolamine/laurel oil molar ratio used was 10:1. The result showed that amine served as a catalyst in the reaction and used sodium methoxide at a ratio of 0.2-2% as a second catalyst.  The reaction was complete in 1-9 hours at room temperature.  The identity of the amide was firmed by IR and $^{13}C$ NMR spectroscopy.

## 2.6 Method of Manufacture

Depending on which of the four types of alkanolamide is to be produced; the starting material could be fatty acid, methyl ester, or triglyceride (coconut oil) and monoethanolamine or diethanolamine. Normally, a batch system of manufacturing is employed.

Alkanolamide is produced from Coconut Oil. A 2:1 cocodiethanolamide (CDEA) can be produced using 6 moles of diethanolamine and 1 mole of refined and bleached coconut oil. The materials are charged with the reactor and a small amount of catalyst (0.25–0.3% sodium methylate or sodium hydroxide) is added. The temperature of the batch is increased to 70–75 °C at normal pressure. After 90 min, the reaction is completed. For a 10-t batch, total cycle time from charging the materials, heating them up, allowing the reaction to proceed to the completion, and transferring the finished product takes at least 4 h. If fatty acid is the starting material, a different operating parameter is used to drive off the water formed during the reaction.

If superamide is to be produced, coco methyl ester and diethanolamine can be used as the starting materials in a mole ratio of 1:1. These materials are charged to the reactor with 0.3–0.5% sodium methylate as the catalyst. The reaction is carried at around 100 °C and a vacuum of 4–5.3 kPa for a period of 90–120 min. The reaction temperature can be lowered to 70–75°C by employing a vacuum of less than 4 kPa. The reaction time takes longer to enable the maximum vaporization of the methanol byproduct. The methanol is rectified and recycled in order to be used in the transesterification of the coconut oil to produce the methyl ester. The final product in the liquid form is transferred into the storage tank. Post reaction can be done by keeping the product in another vessel, preferably at slightly elevated temperature for a few days. This allows further conversion of the ester byproduct to amide, thus further increasing the amide concentration.

If monoethanolamine is used as the starting material, the monoethanol amide product (CMEA) is a waxy solid. Accordingly, the finished product after the reaction is passed through a cooled flaking drum to be converted into flakes. It is subsequently packed in bags.

Although chemical amidation using alkali –catalysis process gives high conversion levels of triglycerides to their corresponding alkanolamide in short reaction times, the reaction has several drawbacks: it is energy intensive, recovery of glycerol is difficult, the alkaline catalyst has to be removed from the product. Enzymatic amidation methods can overcome the problems mentioned above. On the other hand, in general the production cost of a lipase catalyst is significantly than that of an alkaline one. Various types of fatty acid, ester and triglyceride can be employed in amidation using lipase as catalysts

Kuan et al. (2001) studied amidation of various fatty acids with diethanolamine using *Candida antarctica* lipase (Novozym 435) catalyst. Contents of fatty acids, metal ions, and water affected the yields of diethanolamides. Hexanoic acid was the best substrate among all acyl donors. The result found that yields of hexanoyl diethanolamide (HADEA), lauroyl diethanolamide (LADEA), and oleoyl diethanolamide (OADEA), obtained after 24 h of lipase-catalyzed reaction at 50 °C and 250 rpm with 90 mM fatty acid and 360 mM diethanolamine in acetonitrile, were 76.5, 49.5, and 12.1%, respectively. The addition of 1 mM metal salts increased the yields of HADEA and LADEA. Kinetic analysis showed that the yields of HADEA and LADEA in lipase-catalyzed reactions were largely associated with the rate of the forward reaction constant $k1$. Anhydrous enzyme was found to be the best for the amidation reaction. A study on the enzyme operational stability showed that *C. antarctica* lipase retained 95 and 85% of the initial activity for the syntheses of HADEA and LADEA, respectively (even after repeated use for 10 days).

Abdul Rahman et al. (2003) synthesized fatty monoethanolamides in organic solvent from palm kernel olein (PKL) and palm kernel stearin (PKS) using a lipase from Candida rugosa. The result found that the optimal yield was achieved at reaction time 72 hours for both systems with PKL and PKS as the substrates. For PKL as the substrates, the optimal reaction temperature was 40 $^{O}$C, whereas with PKS as the substrate, no optimum temperature was found where the relative yield increased with the increasing temperature. It suggested that the temperature effect on the transamidation of PKS was more significant compared to the transamidation for PKL. The best solvent for the reactions was isooctane. The increasing amount of monoethanolamine used also resulted in the increase solubility of the reactants and products, hence, increase the yield. For PKL, increasing the mole ratio of PKL : monoethanolamine to 1:15 increased the relative yield to 4.45-fold than mole ratio 1:1. Optimal ratio of enzyme/PKL (or PKS) was 0.035. In the water activity studies, the preequilibrium and the direct salt hydrate addition methods were used. Overall, PKL always showed the higher relative yield compared to that of PKS. At the optimum conditions, the yield of PKL monoethanolamide was 77.64% and the yield of PKS monoethanolamide was 39.32%.

Fernández-Pérez et al. (2003) studied kinetic for selective preparation of amide from diethanolamine with fatty acid, ester have been designed using *Candida antarctica* lipase (Novozym 435) as a biocatalyst in direct acylation and transacylation. The amidification reaction was curried out at 30°C -60 °C in organic solvent. The results found that in n-hexane, the reaction is more selective for formation of the amide by direct acylation, while in dioxane, the initial rates of direct acylation and transacylation are both higher than in n-hexane. An increase in temperature to 60 °C increases the conversion and decreases the viscosity of the n-hexane solution. At 60 °C when equimolar amounts of reactants are employed, transacylation route produces both higher conversion (71-77 mol %) and greater selectiviities to the amide (74-94%) than the direct acylation reaction (69-74 mol% conversion and 76-86% selectivity). The conversion of direct acylation reaction can be increased to 92 and 80 mol% in dioxane and n-hexane, respectively. These conversions require using a two-fold excess of diethanolamine. The resultant selectivities are 98 and 100%, respectively.

# CHAPTER III

# EXPERIMENTS

Experimental and analytical methods are described in this chapter. It is divided into four sections. The first section is materials used in the experiments. The second part describes the experimental apparatus. The third section refers to methodology of transamidation. And the last section describes analytical methods and errors of the experiments.

## 3.1 Materials

Vegetable Oils used in this research were crude palm oil from Taksinpalm Company. All chemicals used in the experiment are shown in Table 3.1.

**Table 3.1** All chemicals used in the experiment

| Number | Materials | Company |
|---|---|---|
| 1 | Diethanolamine | Fisher scientific |
| 2 | Methanol | SR lab |
| 3 | Sodium Hydroxide | Ajax finechem |
| 4 | Sodium methylate | ACROS organics |
| 5 | Tetrahydrofuran(THF) | Ajax finechem |
| 6 | Methyl palmitate, 95% | ACROS organics |
| 7 | Decanoic acid ,98% | Sigma |
| 8 | Octanoic acid,98% | Aldrich |
| 9 | Dodecanolc acid,99% | Sigma |
| 10 | Myristic acid,95% | Aldrich |
| 11 | Stearic acid,95% | Sigma- Aldrich |
| 12 | Oleic acid,90% | Aldrich |
| 13 | Glycerol | Merck |
| 14 | Lipase from *Candida antarctica* | Sigma- Aldrich |
| 15 | N- methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) | Fluka |

## 3.2 Equipments

### 3.2.1 Transamidation of vegetable oils using alkali-catalyst

The experiments were conducted in a 2 litre batch reactor. All components of the reactor are made of stainless steel to protect a system from corrosion. The maximum working pressure and temperature in reactor must exceed 1000 psig and 350 °C, respectively.

A schematic diagram of the system was shown in Figure 3.1. In this study, experiments were designed to study in transamidation of vegetable oils using alkali-catalyst.

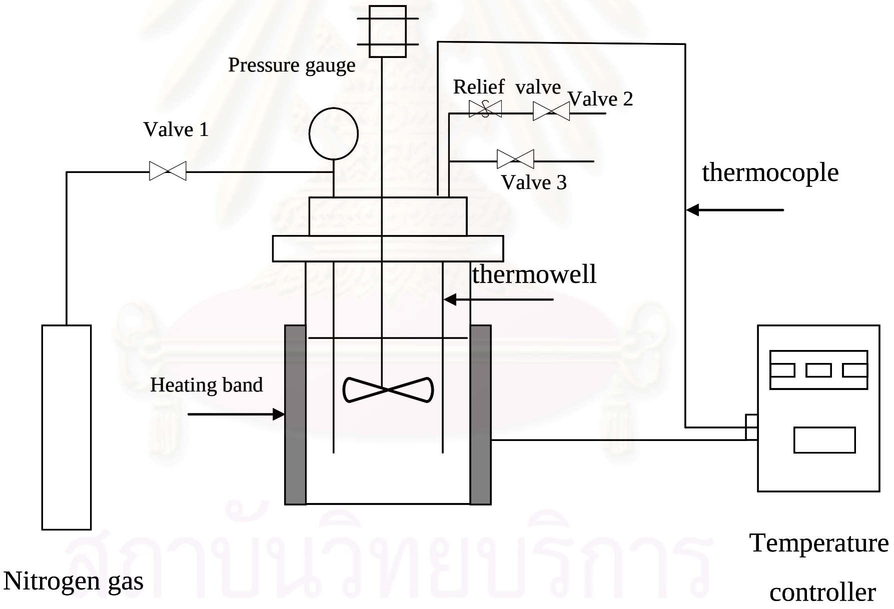


**Figure 3.1** A schematic diagram of the reactor using alkali-catalyst.

The reactants were placed in a reactor. Nitrogen gas was charged at the top trough gas inlet valve (valve 1) into the reactor. The pressure in the reactor was measured by pressure gauge, usually 5 bar, at the reactor head. A dip tube was connected to the gauge and extended to immerse the reactants. A gas relief valve was connected to the gas outlet. This valve was intended to relief the reactor pressure before it reached a dangerous level. The reactor was set in the heating band which was connected to a temperature controller. A thermocouple was used for measuring the temperature inside the reactor. This thermocouple was inserted into the thermowell on the reactor head and connected to the temperature controller. The reactant was stirred by propeller which was placed the reactant. Gas release valve (valve 2) and sample release valve (valve 3) was used to discharge the gas and sample in the reactor, respectively. Samples were taken every 2 hour over a 8 hours period. The samples were kept for GC analysis. The products were taken after each experiment.

### **3.2.2** Transamidation of palm kernel oils using enzyme-catalyst

A flask equipped with a reflux condenser and thermocouple was put in water bath. The flask was immersed in a constant temperature-water bath, set in the heater which was connected to a temperature controller and a magnetic stirrer was used to provide agitation. A schematic diagram of the system was shown in Figure 3.2. Samples were taken every 6 hour over a 24 hours period. The samples were kept for GC analysis.

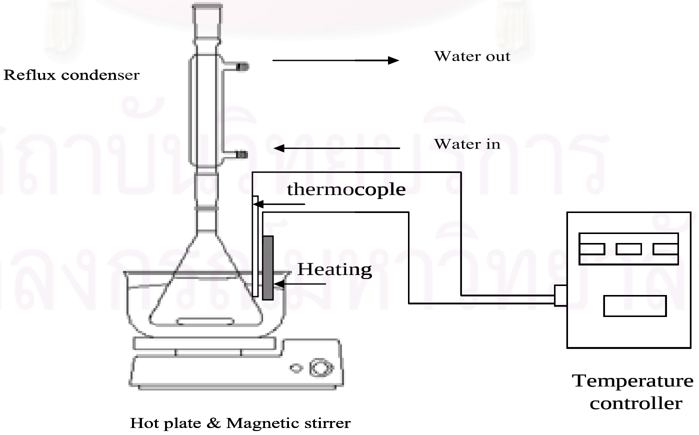


Figure 3.2 A schematic diagram of the reactor using enzyme-catalyst.

3.3 methodology

**3.3.1** Characterization of palm kernel oils

Crude palm oils were analyzed for density at 40 °C, kinematic viscosity (ASTM D-445), Acid value (AOCS Cd-3D-63) and Saponification value (AOCS Cd-3b-76 ).

**3.3.2** Transamidation of palm kernel oils using alkali-catalyst.

1. Heat oil at 100 °C to eradicate moisture.

2. Weight 500 g of crude palm kernel oil (molar ratio of diethanolamine to oil is 3:1)

3. Prepare catalyst for transamidation.

3.3.1. Weight 127.45 g of methanol (for the molar ratio of methanol to oil used is 6:1 and put in the flask.

3.3.2. Weight 6.39 g of sodium hydroxide (for the concentration of catalyst used is 1% of oil weight.

3.3.3. Mix the catalyst, sodium hydroxide and methanol, together until they were all dissolved.

4. Weight 209.37 g of diethanolamine (for the molar ratio of diethanolamine to oil is 3:1) and put in the flask (mixture of methanol and catalyst).

5. Preheat the mixture of diethanolamine and catalyst mixture and stirrer at 60 °C in 1 hour.

6. Pour the mixture of diethanolamine and catalyst mixture in crude palm kernel oil mixed thoroughly in the reactor

7. Heat sample until reach the desired temperature (60, 80 and 120 °C) for transamidation and stirrer at 500 rpm.

8. Leave the reaction continue for 8 hours mixed thoroughly and take sample every 2 hours.

9. After reach assigned reaction time. Stop heating and stirring.

10. Transfer the transamidation product to remove methanol.

11. Analyze physical and chemical properties of diethanolamide samples by GC.

12. Repeat step 1-11 for the other transamidation conditions.

**Table 3.2** Conditions used for alkali-catalyst**.**

| Sample No. | Molar ratio | React. Temp.(°C) |
|---|---|---|
| 36 | Stoichiometry ratio | 60 |
| 38 | | 80 |
| 312 | | 120 |
| 66 | 100% excess* | 60 |
| 68 | | 80 |
| 612 | | 120 |
| 156 | 400% excess* | 60 |
| 158 | | 80 |
| 1512 | | 120 |

***** 100 % excess from its stoichiometry ratio (6:1)

3.3.3. Transamidation of palm kernel oils using enzyme-catalyst.

1. Heat oil at 100 °C to eradicate moisture.

2. Weight 1 g of crude palm kernel oil (molar ratio of diethanolamine to oil is 3:1) in the volumetric flask.

3. Weight 0.42 g of diethanolamine (for the molar ratio of diethanolamine to oil is 3:1) and add in the volumetric flask .

4. Add tetrahydrofuran to bring to the total volume to 10 ml.

5. Weight 0.04 g of lipase (for the concentration of catalyst used is 4% of oil weight and put in the flask.

6. Pour themixture of diethanolamine and oil in lipase mixed thoroughly in the flask.

7. Heat sample until reach the desired temperature (40, 50 and 60 °C) for transamidation and stirrer at 150 rpm.

8. Leave the reaction continue for 24 hours mixed thoroughly and take sample every 6 hours.

9. After reach assigned reaction time. Stop heating and stirring.

10. Transfer the transamidation product to remove tetrahydrofuran.

11. Analyze chemical properties of diethanolamide samples by GC.

12. Repeat step 1-11 for the other transamidation conditions.

**Table 3.3** Conditions used for enzyme-catalyst.

| Sample No. | Molar ratio | React. Temp.(°C) |
|---|---|---|
| L34 | Stoichiometry ratio | 60 |
| L35 | | 80 |
| L36 | | 120 |
| L64 | 100% excess* | 60 |
| L65 | | 80 |
| L66 | | 120 |
| L154 | 400% excess* | 60 |
| L155 | | 80 |
| L156 | | 120 |

***** 400 % excess from its stoichiometry ratio (15:1)

### 3.3.4 Diethanolamide analysis.

The analysis of standards and the products formed were carried out on Perkin Elmer 8700 gas chromatography at Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University. A non-polar capillary column (OV-5) with an internal diameter of 0.32 mm, length of 30 m and film thickness of 0.25 .m and flame ionization detector (FID) was used. Helium was used as a carrier gas with pressure 10 psig. The injector and detector were set at 310 °C and 320 °C, respectively. The column temperature was programmed with an initial temperature 120 °C for 3 min, heating at 6 °C /min to 270 °C, then heating at 3 °C /min to a final temperature of 310 °C. Stearic acid was chosen as an internal standard. The samples undergo silylation process before being injected into gas chromatography. The silylation of sample prepared from 1-10 mg of sample into 2 ml reaction vial and dissolve sample in 300 .l of THF. Add 100 µl of silylating reagent (N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)) and 100 µl of internal standard and heat at 70 °C for 30 min and the injection volume was 1 µl. Retention time of diethanolamide is different. Therefore, the type of diethanolamide was compared retention time of each diethanolamide with diethanolamide standard in Figure 3.3 and Table 3.4.

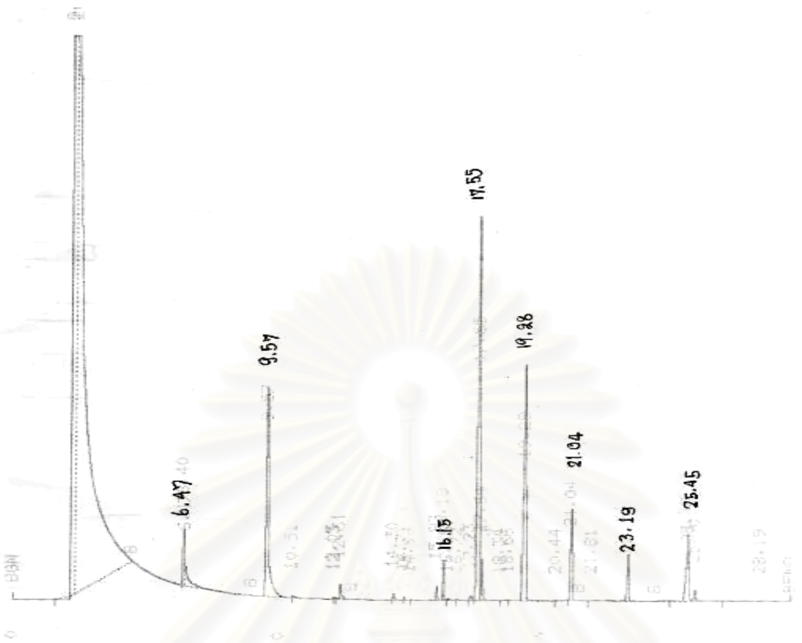


**Figure 3.3** GC chromatogram of diethanolamide

**Table 3.4** Retention time of diethanolamide in GC chromatogram

| Number of peak | Retention time (min) | Peak of sample |
|---|---|---|
| 1 | 2.54 | THF+MSTFA |
| 2 | 6.47 | glycerol |
| 3 | 9.57 | Dirthanolamine |
| 4 | 16.18 | $C_8$ diethanolamide |
| 5 | 17.55 | Stearic acid |
| 6 | 17.64 | $C_{10}$ diethanolamide |
| 7 | 19.28 | $C_{12}$ diethanolamide |
| 8 | 21.04 | $C_{14}$ diethanolamide |
| 9 | 23.19 | $C_{16}$ diethanolamide |
| 10 | 25.45 | $C_{18:1}$ diethanolamide |

### 3.4 Analytical methods and errors of the experiments.

In this section, experiments are conducted to verify repeatability, an average, and a standard deviation value of the experiment. Equation 3.1 and Equation 3.2 define an average value and percent deviation**.**

$$\text{Average value, } \overline{X} = \frac{\sum x}{n} \qquad (3.1)$$

$$\text{Percent deviation from average value} = \sqrt{\frac{\sum (X-\overline{X})^2}{n-1}} \times 100 \qquad (3.2)$$

Some experiments are repeated to study the error that might occur during each experiment of alkali-catalyst and enzyme- catalyst. The experiment is repeated for 3 times at the same condition. The results of the experiments are illustrated in Figures 3.4 to 3.5. Average the amide concentration and deviation data are shown in Tables 3.5.

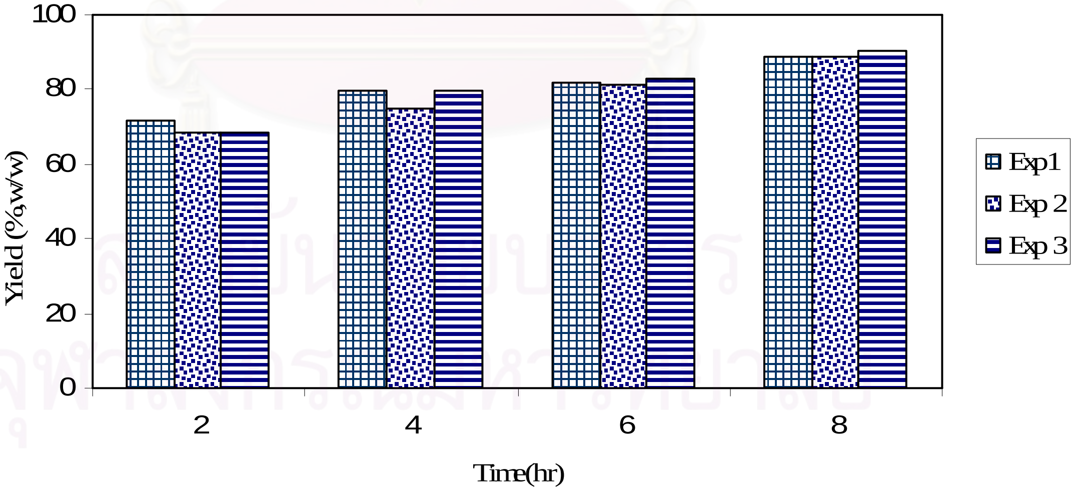


**Figure 3.4.** The yield of diethanolamide at various times on error from repeated experiment of alkali-catalyst at temperature of 120 °C.

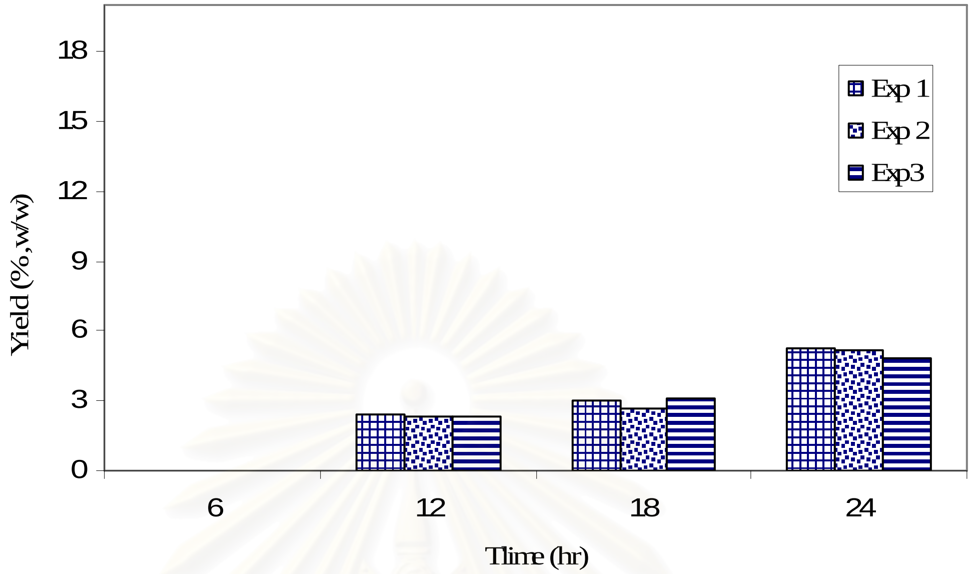


**Figure 3.5.** The yield of diethanolamide at various times on error from repeated experiment of enzyme- catalyst at Temperature of 50 °C.

**Table 3.5.** Average of the amide concentration and percentage of deviation using alkali-catalyst and enzyme- catalyst.

| Catalyst | Time(hr) | Yield (%,w/w) | | | average | % deviation |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | |
| $CH_3ONa$ | 2 | 71.64 | 68.25 | 68.36 | 69.42 | 1.92 |
| | 4 | 79.58 | 74.94 | 79.78 | 78.10 | 2.74 |
| | 6 | 82.00 | 81.08 | 83.10 | 82.06 | 1.01 |
| | 8 | 88.51 | 88.54 | 90.34 | 89.13 | 1.05 |
| Lipase | 6 | 0 | 0 | 0 | 0 | 0 |
| | 12 | 2.38 | 2.34 | 2.34 | 2.35 | 0.03 |
| | 18 | 3.01 | 2.70 | 3.09 | 2.93 | 0.21 |
| | 24 | 5.29 | 5.17 | 4.80 | 5.09 | 0.26 |

From the result above, it was certain that the amide concentration for both alkali-catalyst and enzyme- catalyst seem nearly constant. Table 3.5 shows the percentage of deviation in various times that is in range 0.03-2.74% deviate from enzyme- catalyst and alkali-catalyst. These are considered to be neglected for both alkali-catalyst and enzyme- catalyst.

# CHAPTER IV

# RESULTS AND DISCUSSION

This chapter presents the results of transmutation reaction of crude palm kernel oil with diethanolamine in presence of two catalysts: alkali-catalyst and biocatalyst (lipase). Discussions of the results are divided into 4 parts.

4.1. Characteristics of reactants and products.

4.2. The effects of different operating conditions of alkali-catalyst, such as temperature (between 60 and 120 °C) and molar ratio of diethanolamine to crude palm kernel oil (between stoichiometic ratio and 400 % excess from its stoichiometic ratio) and the reaction time is 8 hours in 1% catalyst (based on the weight of oil) on yield was determined in a batch reactor.

4.3. The effects of different operating conditions of enzyme-catalyst (*canida Antarctica lipase),* such as temperature (between 40 and 60 °C) at 24 hours in 4 % catalyst (based on the weight of oil) and molar ratio of diethanolamine to crude palm kernel oil (between stoichiometic ratio and 400 % excess from its stoichiometic ratio) on amide.

4.4. Kinetic of transamidation reaction.

4.5. The comparison between alkali-catalyst and enzyme-catalyst.

## 4.1 Characteristics of reactants and products.

### 4.1.1 Characteristics of Palm kernel oil.

Crude palm kernel oil used in this study is obtained from a palm oil mill in the southern part of Thailand. It is produced by mechanical pressing of palm kernel and is used as received without any further purification. Table 4.1 shows the properties of crude palm kernel oil used in this study. It contains relatively high free fatty acid which also results in high acid value. Free fatty acid in crude palm kernel oil increases when it is stored at ambient condition due to autocatalytic hydrolysis reaction or lipolysis reaction by fat splitting enzyme in the oil and from lipolytic microorganisms. So amount of free fatty acid must be determined every time that the experiments are conducted. Molecular weight of palm kernel oil as calculated based on saponification value and is found to be 753.25 g/mole.

**Table 4.1.** Properties of crude palm kernel oil

| Identity characteristics | CPKO |
|---|---|
| Density at 40 °C, (g/ml) | 0.9128 |
| Viscosity at 40 °C,(cP) | 25.50 |
| Acid value, (mg KOH/g) | 3.90 |
| Saponification value, (mg KOH/g) | 245 |
| % FFA (as lauric acid) | 1.38 |
| Molecular weight (g/mole) | 753.25 |

Crude palm kernel oil has its chemical structure as shown in Figure 2.1 where $R_1$, $R_2$ and $R_3$ are determined in term of fatty acid. $R_1$, $R_2$ and $R_3$ of crude palm kernel oil are determined by transesterified of crude palm kernel oil with methanol. The methyl ester products were analyzed and the amounts of methyl esters found in the sample were converted to equivalent amount of fatty acid. Table 4.2 shows the results of this study. Crude palm kernel oil used in this study consists of lauric acid group as its dominant side group with approximately half of all the fatty acid side group, oleic acid, myristic acid and palmitic acid and also found in high quantity.

**Table 4.2.** Fatty acid composition of crude palm kernel oil (wt % as methyl ester analyzed by GC)

| Fatty Acid | Formula | Palm Kernel Oil (%) |
|---|---|---|
| Caproic | $C_6H_{12}O_2$ | 0–1 |
| Caprylic | $C_8H_{16}O_2$ | 3–5 |
| Capric | $C_{10}H_{20}O_2$ | 3–5 |
| Lauric | $C_{12}H_{24}O_2$ | 44–51 |
| Myristic | $C_{14}H_{28}O_2$ | 15–17 |
| Palmitic | $C_{16}H_{32}O_2$ | 7–10 |
| Stearic | $C_{18}H_{36}O_2$ | 2–3 |
| Oleic | $C_{18}H_{34}O_2$ | 12–19 |
| Linoleic | $C_{18}H_{32}O_2$ | 1–2 |
| Average MW. of methyl ester ,g/mole | | 237.69 |

### 4.1.2 Characteristics of diethanolamine and alkanolamide.

Alkanolamide is prepared from the amidation reaction of an alkanolamine with fatty acid, fatty ester, or a triglyceride. Diethanolamine is the most popular alkanolamine used in industry. Diethanolamides prepared from different types of reactants give different physical properties. Superamide is the alkanolamide prepared directly from methyl ester at a molar ratio of diethanolamone to PKO of 1.1:1. The reaction is catalyzed by 0.5 % sodium methoxide and is operated at 110-115 °C for 4 hours. PKO is used directly in transamidation reaction of 1 mole of PKO with 6 mole of diethanolamine. The reaction is catalyzed by 0.5% sodium methoxide and is operated at 120 °C for 8 hours.

When sodium methoxide (NaOH+$CH_3OH$) is used as a catalyst, by products, methanol and glycerol are formed. The reaction is conducted at 120 °C and 5 bar for 8 hours. Diethanolamide products were analysed by gas chromatography (GC) while fatty acid diethanolamide, such as lauric diethanolamide, oleic diethanolamide were prepared in our laboratory and used as reference standards. In this preliminary, three different kinds of diethanolamide were prepared from palm kernel oil as show in Table 4.3.

Two commercial diethanolamide samples were obtained from local supplier and were used for comparison. Superamides compose of a small amount of the ester, diethanolamine and diethanolamide consisting of lauric, myristic, oleic diethanolamide. Since it is prepared from methyl ester, no glycerol is detected in superamide. PKO diethanolamide has the same components as superamide with the presence of glycerol. It increased viscosity and made a dark colour, as seen in Figure 4.1. PKO diethanolamide using sodium methoxide were prepared from sodium hydroxide and methanol as catalysts is the higher yield than PKO diethanolamide using sodium methoxide and reduced the process when compared with superamide. In this experiment, sodium methoxide were prepared from sodium hydroxide and methanol as catalyst.

**Figure 4.1** Picture of PKO (left), superamide (middle), PKO diethanolamide (right).

**Table 4.3** The properties and composition of diethanolamide.

| component | composition | | | | | |
|---|---|---|---|---|---|---|
| | PKO DEA (standard1) | PKO DEA (standard2) | DEA | Super amide | PKO DEA | PKO DEA(OH) |
| Diethanolamide | 92.03 | 87.57 | - | 96.99 | 86.27 | 90.73 |
| $C_8$ amide | 2.54 | 2.65 | - | 2.02 | 2.28 | 2.48 |
| $C_{10}$ amide | 3.73 | 4.34 | - | 4.62 | 2.68 | 5.60 |
| $C_{12}$ amide | 59.22 | 57.14 | - | 62.56 | 54.02 | 58.51 |
| $C_{14}$ amide | 10.34 | 9.18 | - | 11.12 | 9.64 | 9.00 |
| $C_{16}$ amide | 5.26 | 5.10 | - | 5.47 | 5.75 | 4.64 |
| $C_{18:1}$ amide | 10.94 | 9.16 | - | 11.21 | 11.90 | 10.50 |
| Diethanolamine | 4.37 | 1.10 | 99 | 0.45 | 7.54 | 8.10 |
| glycerol | 4.73 | 8.35 | - | 0 | 1.29 | 2.13 |
| pH | 9 | 9 | 9 | 10 | 10 | 10 |
| Viscosity at 40 $^{O}$C, (cP) | 295 | 366 | 187 | 308 | 374 | 366 |
| Density at 40 $^{O}$C, (g/ml) | 0.978 | 0.989 | 1.09 | 0.972 | 1.016 | 0.99 |

DEA-diethanolamine.

PKO DEA - palm kernel oil diethanolamide using sodium methoxide as catalyst. The reaction is conducted at 120 °C in 8 hour.

PKO DEA (OH) - palm kernel oil diethanolamide using NaOH and $CH_3OH$ as catalysts at 120 °C in 8 hour.

## 4.2 Transamidation of palm kernel oils using alkali-catalyst

Methanol–NaOH mixture and diethanolamine were added to a flask. Based on stoichiometic, the chemical reaction of palm kernel oil and diethanolamine to produce diethanolamide are 1 mole to 3 moles and amount of catalyst must plus amount of neutralized catalyst. The mixture was stirred with a magnetic stirrer and was heated to 60 $^{O}$C using a hot plate for 1-hour. PKO and diethanolamine mixture were put in a pressure reactor. The reaction was conducted at a pressure of 5 bar and at temperature of 60, 80 and 120 °C at constant stirring rate. The samples were taken every 2 hours over an 8 hours period. The samples were kept for analysis. The analyses are shown in Appendix B. The diethanolamide studied by GC appear in the retention time at 30 min. The yield was calculated using the internal standardization method and is shown in equation 4.1

$$\text{Yield (\%, w/w)} = \frac{\text{Mass of diethanolamide}}{\text{Theoretical mass of diethanolamide from mass balance}} \times 100 \qquad (4.1)$$

### 4.2.1 Effect of reaction time.

The objective of this section is to find a suitable reaction time for the transamidation reaction. The experiments were conducted at the molar ratio of diethanolamine to oil is 3:1(stoichiometic ratio) and a pressure of 5 bar. The temperatures were varied from 60 °C to 120°C (60, 80, and 120 °C). Sodium methoxide at concentration of 1 % weight (based on oil) was used as a catalyst and reaction time are varied from 2 to 8 hours. The result is shown in Table 4.4, at reaction time of 2, 4, 6 and 8 h.

**Table 4.4** The yield of diethanolamide at a various temperature and stoichiometic ratio using alkali-catalyst.

| Time(hr) | Yield,% | | |
|---|---|---|---|
| | 60 °C | 80 °C | 120 °C |
| 2 | 70.49 | 79.83 | 73.19 |
| 4 | 82.03 | 82.15 | 78.49 |
| 6 | 92.62 | 86.55 | 84.88 |
| 8 | 90.88 | 89.30 | 89.57 |

The suitable length of time was evaluated by conducting experiments with variation of time, from 2 hours to 8 hours. The results, as shown in Table 4.4, show that yield depends on the length of time. Figure 4.2 shows that the yield increases rapidly in the initial period (0-6 hours) and approaches a constant value at the final period (6-8 hours). The suitable time from this study is 6 hours, while Gregorio, C.G. (1996) studied the transamidation reaction of refined coconut oil using sodium methoxide as catalyst. They concluded that diethanolamide contains 80% of the desired amide at a temperature of 75 °C in 1.5 hours, which is the short reaction time because of the lower percentage of free fatty acid than crude palm kernel oil.

From the previous section, the results show that the highest yield is 92.62 % at reaction time of 6 hours and declined. The results found are in accordance with Dzulkefly, K et al. (1997). They reported that increasing reaction time might have increased the side products, lowered the conversion of monoethanolamide from palm oil. An increase in temperature from 60 to 120 °C did not substantially affect the yield in stoichiometic ratio at 8 hours.

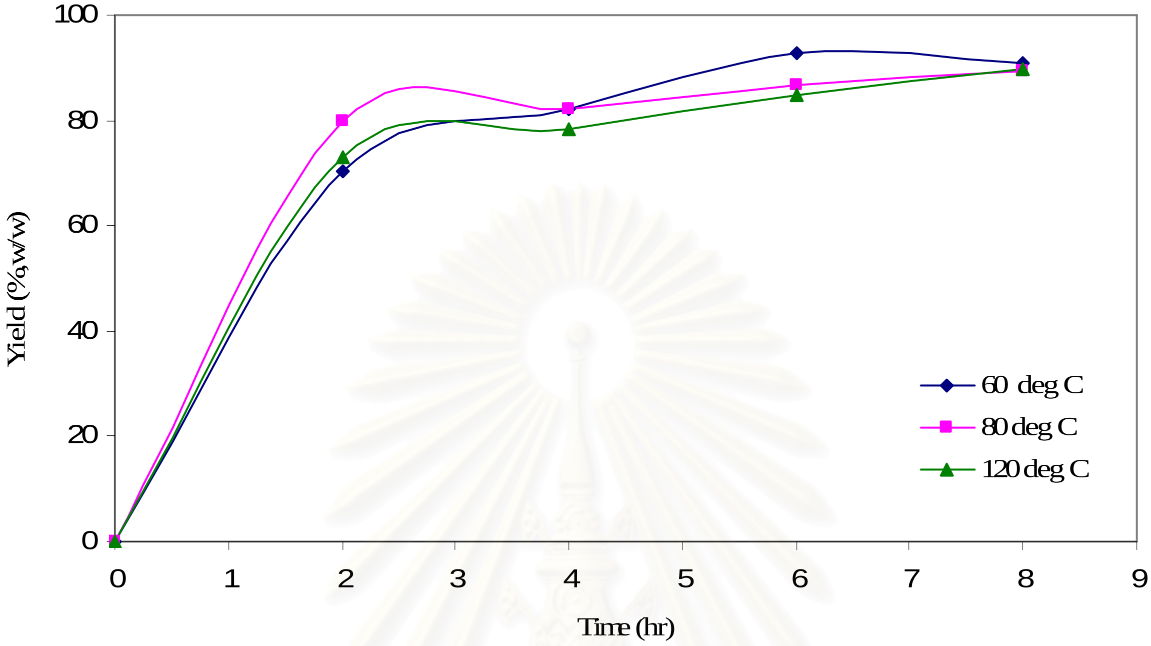


**Figure 4.2** Effect of reaction time on the formation of diethanolamide and stoichiometic ratio using alkali-catalyst.

### 4.2.2 Effect of Molar ratio of diethanolamine to oil.

This section is designed to study the effect of molar ratio of diethanolamine to oil. The study is performed at difference conditions in order to compare the yield. The transamidation stoichiometrically, 3:1 molar ratio of diethanolamine to oil is needed (Arthur, 1977). The experiments were conducted at a temperature of 60 °C and 5 bar for 8 hours. The molar ratio of diethanolamine to oil is varied from stoichiometic ratio to 400 % excess from its stoichiometic ratio. Sodium methoxide was used as catalyst at amount of 1 wt% based on oil.

**Table 4.5** The yield of diethanolamide at a various molar ratio using alkali-catalyst and at temperature of 60 °C

| Time (hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | stoichiometic ratio | 100% excess* | 400% excess* |
| 2 | 70.49 | 86.67 | 71.10 |
| 4 | 82.03 | 93.58 | 71.15 |
| 6 | 92.62 | 90.42 | 76.04 |
| 8 | 90.88 | 79.05 | 64.23 |

***** 400 % excess from its stoichiometic ratio (15:1)

The result of the effect of diethanolamine to oil for crude palm kernel oil is shown in Table 4.5 and is plotted in Figure 4.4. The yield of diethanolamide increases continually from stoichiometic ratio to 100 % excess from its stoichiometic ratio. It indicates that diethanolamine excess was required to shift the equilibrium of the resversible transamidation reaction to the right leading to the improvement of the yield of product. However, the yield of diethanolamide decreased when molar ratio was 400 % excess from its stoichiometic ratio. According to Arzamendi, G. et. al. (2006), molar ratio of diethanolamide to oil increased as the same amount of catalysts, 1 wt% based on oil, which was kept constant. Therefore, in these reactants, the catalyst concentration decreased with the diethanolamine to oil ratio.

The result from this study was in accordance with Gregorio, C.G. (1996). They reported that molar ratio of diethanolamine to oil was 100% excess from its stoichiometic ratio.

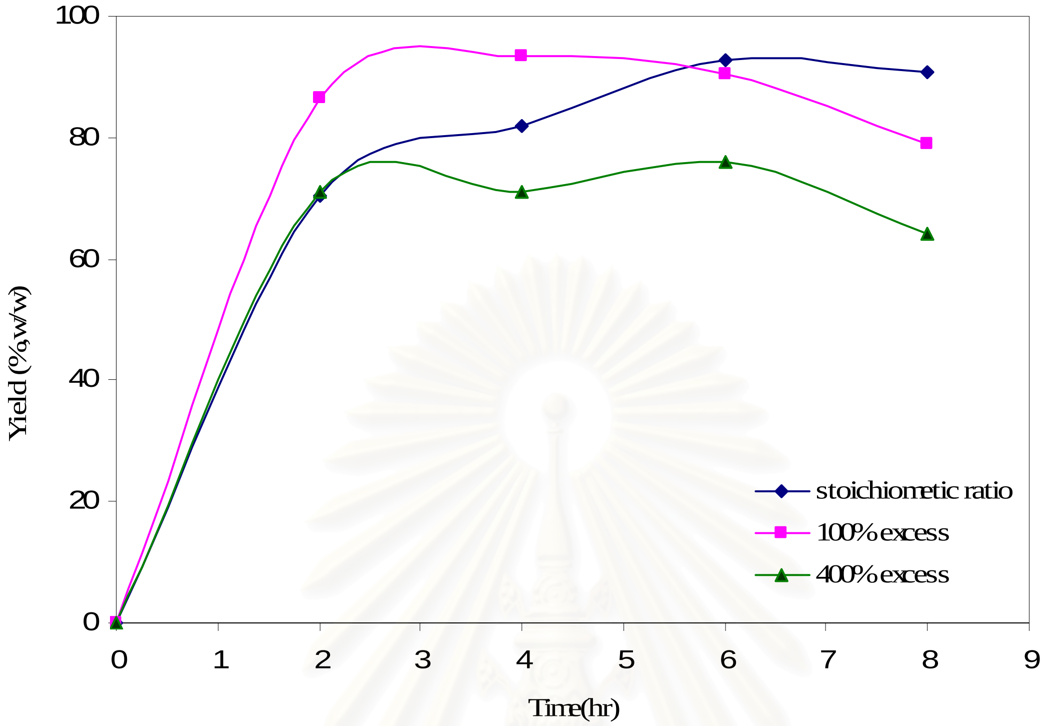


**Figure 4.3** Effect of molar ratio (DEA:PKO) on the formation of diethanolamide at a temperature of 60°C using alkali-catalyst.

### 4.2.3 Effect of Temperature

This section is designed to study the effect of operating temperatures for transamidation reaction. The experiments were conducted at temperature of 60 to 120°C for 8 hours. Sodium methoxide are used in this study as a catalyst. The results are shown in Table 4.6.

The results in Figure 4.4 indicated that the yield of diethanolamide decreased when temperature increased because temperature of reaction excesses the boiling point of methanol (64°C), methanol is vaporized. In other words, it affected the rate of reaction, so the yield of product decreased. The trend in the results for molar ratio from stoichiometic ratio is similar to those at 100% excess from its stoichiometic ratio and 400% excess from its stoichiometic ratio.

**Table 4.6** The yield of diethanolamide at a various temperature and 1various molar ratio using alkali-catalyst in 6 hours.

| Molar ratio | Yield (%,w/w) | | |
|---|---|---|---|
| | 60 °C. | 80 °C. | 120 °C. |
| Stoichiometic ratio | 92.62 | 86.55 | 84.88 |
| 100% excess* | 90.42 | 81.96 | 82.00 |
| 400% excess* | 76.04 | 71.25 | 74.45 |

***** 100 % excess from its stoichiometic ratio (6:1)

This effect of temperature is corresponded to Jungerman, E. (1966). He reported that methyl ester was used as raw material while lower temperature could be used, preventing the formation of by product which tended to inhibit the foam-boosting properties of the product. It indicated that there are some competing between amidation and transesterification reaction in the experiment, so by the product of diethanolamide are glycerol and methanol.

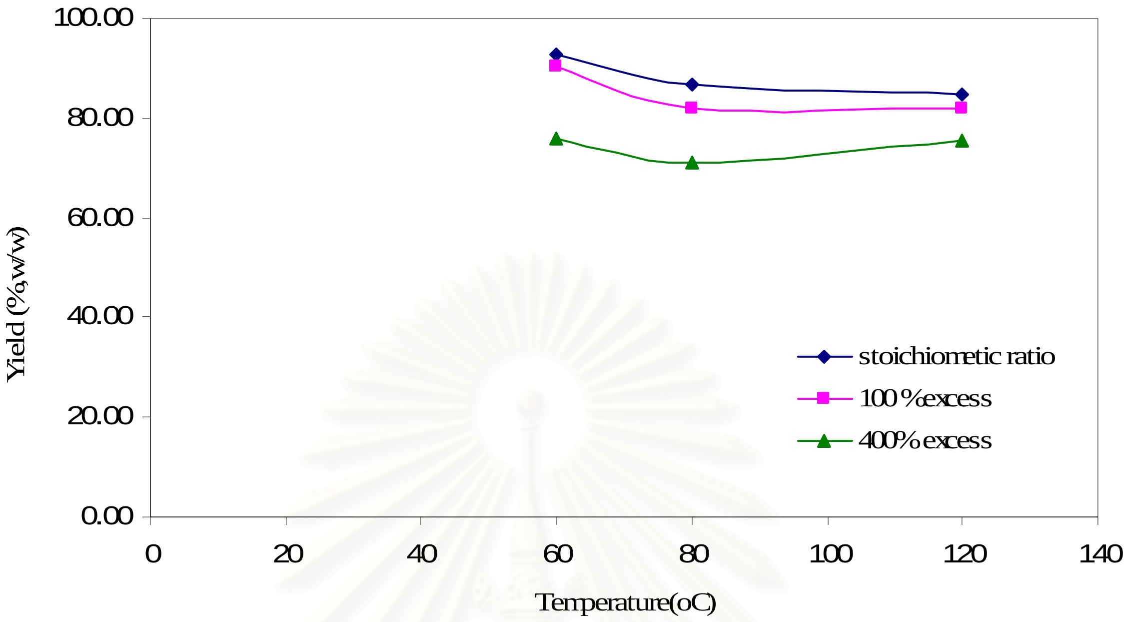


**Figure 4.4**. Effect of temperature on the formation of diethanolamide and various molar ratio using alkali-catalyst.

### 4.2.4 Physical Properties of diethanolamide

The product from various transamidation conditions were analyzed for physical properties, such as density, viscosity. Physical properties of the produced diethanolamide are compared with a standard diethanolamide.

Physical properties of diethanolamide are in the range of the limit of diethanolamide standard. If viscosity is low, there will be a problem with an application of diethanolamide. As shown in Table 4.5, in this study viscosity of product found in the range of 250- 350 cP, which are close to the standard. While the density of the products found in the range of 0.97-1.03 kg/m$^3$ which are rather close to the standard. It indicates that the yield depends on the density and viscosity. When the molar ratio increases which means the density also increases, as a result the yield decreased because of transamidation at higher molar ratio. This finding can help identifying that the reaction is not complete.

**Table 4.7** Physical Properties of diethanolamide at various conditions

| Experiament | | viscosity at 40 $^{O}$C (cP) | Density at 40 $^{O}$C (kg/m$^3$) | Yield (%,w/w) |
|---|---|---|---|---|
| Molar ratio | React. Temp.($^{O}$C) | | | |
| Stoichiometic ratio | 60 | 278.5 | 0.972 | 92.62 |
| | 80 | 320 | 0.975 | 86.55 |
| | 120 | 320 | 0.979 | 84.88 |
| 100% excess* | 60 | 270 | 0.992 | 90.42 |
| | 80 | 350 | 1.000 | 81.96 |
| | 120 | 320 | 1.003 | 82.00 |
| 400 % excess* | 60 | 250.5 | 1.033 | 76.04 |
| | 80 | 317.5 | 1.033 | 71.25 |
| | 120 | 287.5 | 1.033 | 78.45 |
| Standard1 | | 295 | 0.978 | 92.03 |
| Standard2 | | 366 | 0.989 | 87.57 |

***** 100 % excess from its stoichiometic ratio (6:1)

## 4.3 Transamidation of palm kernel oils using enzyme-catalyst

The reaction mixture consists of PKO (1 g), diethanolamine (0.4 g) and lipase (0.04 g) and an addition of organic solvent which brings to the total volume to 10 ml. Klibanov, A.M. (1997) reported that the use of biocatalyst in organic solvents offered many advantages over using pure water, such as the increasing of solubility of poorly water-soluble organic substrates, avoiding unwanted side reactions and degradation of common organic reagents, as well as the ability to shift the thermodynamic equilibrium of many processes to the synthetic way, thus favoring product recovery. In this study, THF was used as organic solvent to accordance with Kuan, J et al. (2001). He suggested that the best organic solvents for the synthesis of diethanolamides by *C. antarctica* lipase are hydrophilic organic solvents (namely, acetonitrile and tetrahydrofuran). Therefore, the hypothesis that hydrophobic organic solvents with high log *P* are the most acceptable for biocatalysis cannot be universally applicable. The reaction mixtures were incubated at 40, 50 and 60 °C for 24 hours and were stirred at 200 rpm in a water bath. The samples were taken every 6 hours over a 24 hours period. The samples were kept for analysis. The analyses are shown in Appendix B-3.

### 4.3.1 Effect of reaction time.

The effect of reaction time on enzymatic tranamidation reaction study gives an insight into the performance of an enzyme as the reaction progresses. The transamidation reactions of PKO were carried out at a fix stoichiometic ratio. The temperature was varied from 40 to 60 °C. Table 4.6 shows the result of the yield and is potted figure 4.5.

**Table 4.8** The yield of diethanolamide at a various temperature and stoichiometic ratio using enzyme-catalyst

| Time(hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | 40 °C. | 50 °C. | 60 °C. |
| 6 | 3.57 | 4.40 | 5.10 |
| 12 | 3.60 | 5.97 | 7.20 |
| 18 | 4.68 | 7.70 | 9.00 |
| 24 | 4.87 | 8.50 | 10.20 |

The profile of diethanolamide produced at various time, from 6 hours to 24 hours is presented in Figure 4.5. The yield was increased with the increasing reaction time. Novozym 435 gave highest yield within a reaction period of 18 hours.

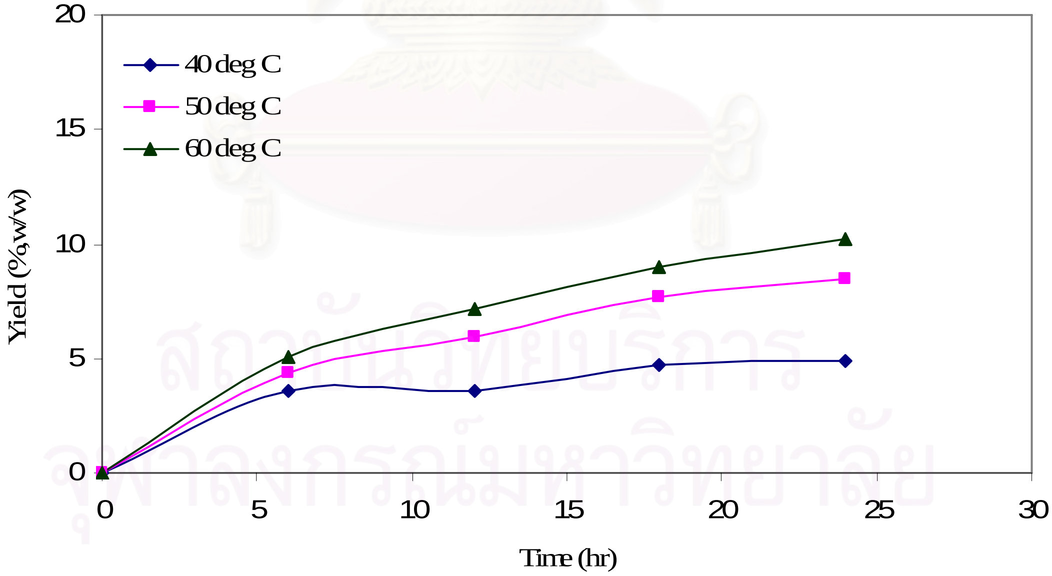


**Figure 4.5** Effect of reaction time on the formation of diethanolamide and stoichiometic ratio using enzyme-catalyst

After 18 hours at temperature of 40 °C, the yield was relatively constant. It indicates that transamidation of PKO achieved the equilibrium. At temperature of 60 and 50 °C, the yield was slightly increased in 24 hours, so the suitable reaction time from this study is 24 hours. Similar finding was reported by Kuan, J.et al. (2001). The amidation reaction used immobilized *Candida antarctica* lipase in 24 hours of reaction time.

### 4.3.2 Effect of molar ratio of diethanolamine to oil

The stoichiometic of this transamidation reaction requires 3 mol diethanolamine per 1 mol PKO to yield 3 mol diethanolamide and 1 mole glycerol. In this experiment, transamidation reaction of PKO using lipase catalyst was carried out at a fix temperature of 60 °C. The molar ratio was varied from stoichiometic ratio to 400% excess from its stoichiometic ratio. The effect of molar ratio of diethanolamine to oil on the transamidation reaction is shown in Table 4.9. The yields depend on the effect of molar ratio.

**Table 4.9** The yield of diethanolamide at a various molar ratio and a temperature of 60 °C using enzyme-catalyst

| Time (hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | stoichiometic ratio | 100% excess* | 400% excess* |
| 6 | 5.10 | 0.00 | 0.00 |
| 12 | 7.20 | 1.87 | 1.55 |
| 18 | 9.00 | 3.63 | 2.68 |
| 24 | 10.20 | 5.64 | 4.42 |

***** 100 % excess from its stoichiometic ratio (6:1)

The result is illustrated in Figure 4.6, which shows the reaction rate increasing when the molar ratio was decreased. The yield increased from 1.55 % to 10.20% as the molar ration decreased at 24 hours of reaction. The result of the optimal molar ratio was 3:1 (stoichiometic ratio) because of molar ratio of diethanolamide to oil increased as the same amount of catalysts, which was kept constant. Therefore, in these reactants the catalyst concentration decreased with the diethanolamine to oil ratio. THF is increased solubility of PKO and diethanolamine. In general, THF is soluble in oil; however, it has low solubility in diethanolamine..

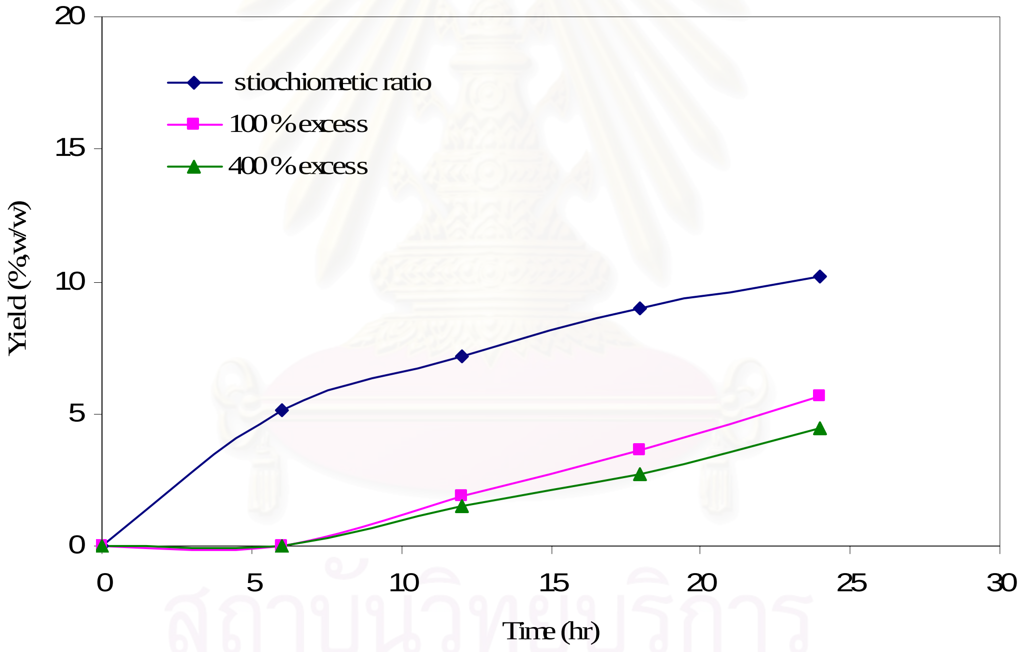


**Figure 4.6**. Effect of molar ratio (DEA:PKO) on the formation of diethanolamide at a temperature of 60° C using enzyme-catalyst.

However, at a lot of THF, it mixes with PKO and helps reducing the viscosity of the oil. The decrease in viscosity could be attributed to the higher mass transfer between diethanolamine and oil, thus increases the yield. When excess diethanolamine hinders the interaction frequency between reactants and lipases and increases the viscosity, thus decreases the yield. On the contrary, Abdul rahman M.B et al. (2003) reported that the optimal molar ratio which was used to produce monoethanolamide was 15:1( 400 %excess from its stoichiometic ratio ). Increasing the amount of the monoethanolamine used results in the increasing solubility of the reactants and products, so the yield of monoethanolamide increases.

### 4.3.3 Effects of temperature

The reaction temperature is an important parameter in enzyme catalyst. In this experiment, transamidation reactions of PKO were carried out at 24 hours. Molar ratio was varied from stoichiometic ratio to 400% excess from its stoichiometic ratio and temperature was varied from 40 to 60 °C. The effect of reaction temperature on transamidation reaction is shown in Table 4.10. Transamidation reaction showed an increment of yield as the temperature was increased from 40 °C to 60 °C.

**Table 4.10** The yield of diethanolamide at a various temperature and various molar ratio using enzyme-catalyst in 24 hours

| Molar ratio | Yield (%,w/w) | | |
|---|---|---|---|
| | 40 °C. | 50 °C. | 60 °C. |
| Stoichiometic ratio | 4.87 | 8.5 | 10.2 |
| 100% excess* | 4.05 | 5.29 | 5.74 |
| 400% excess* | 1.72 | 4.22 | 4.42 |

***** 100 % excess from its stoichiometic ratio (6:1)

As shown in Figure 4.7, the yield was increased with increasing temperature from 40 °C to 60°C. The result is similar to that of Kuan, J. et al. (2001). They reported that Novozym 435 was optimally used at 40 °C to 60 °C. Higher temperature can give a faster transformation but too high will lead to enzyme denaturing. The result found that the lower yield may have been deactivated of lipase. Mat Radzi, S et al. (2005) found that the conversion decreased which probably caused by the vibration and movement of the enzyme molecule would affect the hydrogen bonds and other bonds in the lipase structure. Consequently the catalytic power of lipase will be reduced, because denaturation process has occurred.

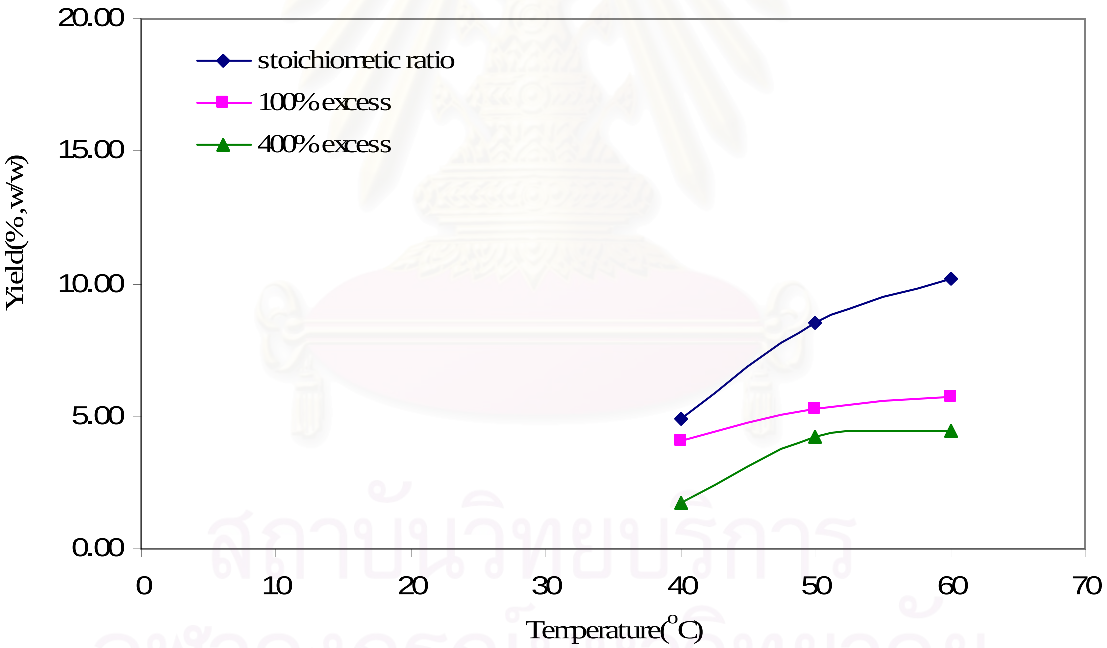


**Figure 4.7** Effect of the temperature of diethanolamide at a various temperature and various molar ratio using enzyme-catalyst

### 4.4. Kinetic of transamidation reaction.

A study of the kinetics of transamidation will provide parameters that can be used to predict the extent of the reaction at any time under particular conditions. In this study, the model of kinetic reaction will be based on overall reaction and transamidation reaction is assumed to proceed in the n$^{th}$ order reaction as a function of the concentration of triglyceride, diethanolamine, and reaction time both alkali-catalyst and enzyme-catalyst, as shown below.

$$-r_A = \frac{dC_A}{dt} = kC^n_{TG}C^m_{DEA} \qquad (1)$$

Since DEA is in excess for the reaction, the rate equation could be simplified as:

$$-r_A = \frac{dC_A}{dt} = kC^n_{TG} \qquad (2)$$

There have been a few attempts to develop kinetic models for transamidation of triglyceride in diethanolamide, such as Jundermann, E et.al (1966). They found that the reaction rate of diethanolamide was 2$^{nd}$ order reaction.

The second order rate equation has the following form,

$$-r_A = \frac{dC_A}{dt} = kC^2_{TG} \qquad (3)$$

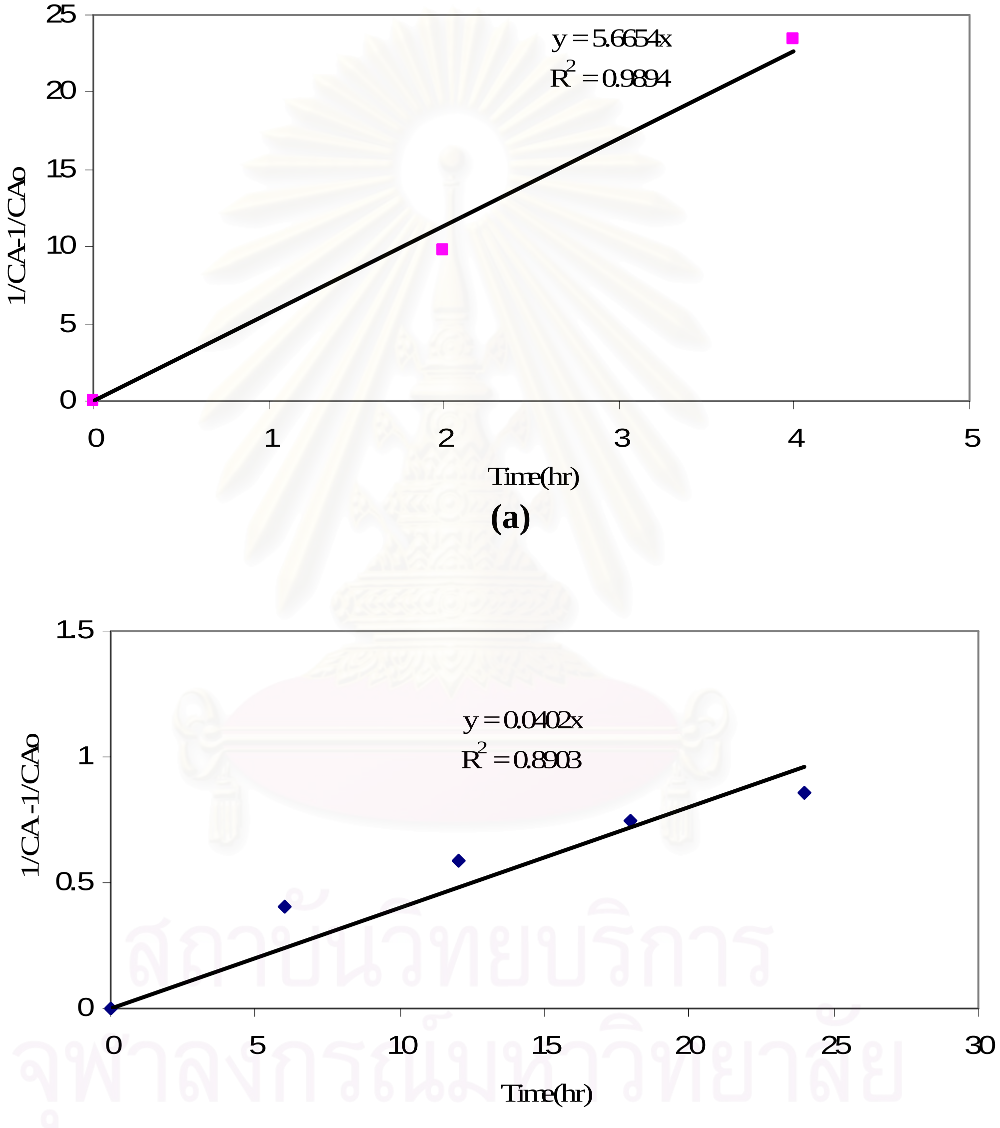
where k is the rate constant. Integration this equation obtains

$$-\int_{C_{AO}}^{C_A} \frac{dC_A}{C^2_A} = k\int_0^{tr} dt \qquad (4)$$

or

$$\frac{1}{C_A} - \frac{1}{C_{AO}} = kt \qquad (5)$$

A plot of ($1/C_{A}$ - $1/C_{AO}$) vs. t gives a straight line through the origin for rate of equation. If the experimental data seems to be fitted by a curve than by straight line, the second order reaction does not satisfactorily fit the data; another rate law should be attempted.



**(a)**

**(b)**

**Figure 4.8** Second order kinetic plot (optimum condition)

(a) alkali-catalyst (b) enzyme-catalyst

The second order kinetic was found to be the best in describing the experimental results both alkali-catalyst and enzyme-catalyst as shown from a straight line plot of ($1/C_{A}$ - $1/C_{AO}$) versus time in Figure 4.8. A plot for the first order does not give good agreements and this plot can be seen in Appendix C. For the second order kinetic, the rate constant could be evaluated from the slope of line in Figure 4.8, the results (k) and rate constant per gram catalyst ($k^{'}$) are tabulated in Table 4.11.

**Table 4.11.** The rate constant and rate constant per gram catalyst at alkali and enzyme catalyst.

| Variable | alkali-catalyst | enzyme-catalyst |
|---|---|---|
| k (l/ g. hr ) | 5.6654 | 0.0402 |
| $k^{'}$(l/mol hr.g-cat) | 1.108 | 0.101 |
| Catalyst (g) | 5.111 | 0.04 |
| Rate ((l/mol hr.g-cat) | 1.108 $C^{2}_{TG}$ | 0.101$C^{2}_{TG}$ |

## 4.5 The comparison between alkali-catalyst and enzyme-catalyst.

This section demonstrates the comparison between the results of PKO and diethanolamine by sodium methoxide and lipase as catalysts operating under the optimum condition. It was found that the optimum conditions for both systems are quite different (see Table 4.12).

**Table 4.12** Comparison of different technologies to produce diethanolamide.

| Variable | Alkali catalyst | Enzyme catalyst |
|---|---|---|
| Reaction time (hr) | 4 | 24 |
| Reaction temperature (°C) | 60 | 60 |
| molar ratio DEA:oil | 6:1 | 3:1 |
| %Yield | 93.24 | 10.20 |
| Recovery of catalyst | Difficult | Easy |
| Production cost of catalyst | Cheap | Relatively expensive |
| Reaction rate (l/mol hr.g-cat) | 1.108 | 0.101 |

To consider the rate of transamidation reaction (at optimum condition), alkali-catalyst is 1.11 l/(mol hr.g-cat) while enzyme-catalyst is 1.00 l/(mol hr.g-cat). In both cases, diethanolamide is the main product. So we can conclude that alkali-catalyst gives higher rate than enzyme-catalyst.

The remarkable differences in optimum conditions between the two catalysts are the yield of product; alkali-catalyst is nine times higher than enzyme-catalyst. The difference indicates that lipase, as a catalyst, shows its low catalytic activity in transamidation of crude palm kernel oil in comparison to sodium methoxide because of alkali-catalyst dissolve easily in liquild-phase reaction, which they form uniform mixtures with the reactants so mass transfer limitations is minimum with high reaction rates.  Although chemical transamidation using alkali catalysis process gives high conversion levels of triglycerides to their corresponding alkanolamide in short reaction times, the reaction has drawbacks: the alkali catalyst has to be removed from the product.  If lipase is used instead of sodium methoxide, the reaction time and amount of catalyst are increased.

# CHAPTER V

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Conclusion

The following conclusion are drawn from this study

5.1.1. Diethanolamide can be produced by transamidation of crude PKO using lipase –catalyst. The product of reaction contains only diethanolamide and glycerol since no catalyst was produced.

5.1.2. The optimum condition for transamidation reaction using alkail–catalyst was 100 % excess from its stoichiometry ratio (6:1) at a temperature of 60°C and 4 hours in which 93.24 % yield was produced.

5.1.3. The optimum condition for transamidation reaction using enzyme –catalyst was stoichiometry ratio (3:1) at a temperature of 60 °C and 24 hours in which 10.20 % yield was produced.

5.1.4. Increasing operating temperature did not substantially affect the yield for transamidation using alkali-catalyst but increased yield for transamidation using enzyme-catalyst.

5.1.5. Increasing operating molar ratio tended to increase yield for transamidation using alkali-catalyst but decreased yield for transamidation using enzyme-catalyst.

5.1.6. The rate constants of second- order reaction to form diethanolamide at alkali-catalyst and enzyme-catalyst is 1.11, 1.00 l/lmol hr.g-cat, respectively. It is concluded that the rate reaction using sodium methoxide as catalyst is faster than using lipase as catalyst

## 5.2 Recommendations

Recommendations for future studies and researches are as follows;

5.2.1 A similar study should be conducted in continuous flow reactor, such as fix flow reactor in order to study capacity.

5.2.2 In order to improve process of lipase, the percentage amide of diethanolamide should be increased, such as a reaction time of enzyme-catalyst.

5.2.3. The same experiment set should be conducted by using another type of lipase and another type of organic solvent.

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# APPENDICES

# Appendix A

## A-1 Raw data in the characterization of palm kernel oil.

**Table A-1.1.** Density and viscosity measurement.

| PKO | Density | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| W prcnometer,g | 17.4708 | 17.4710 | 17.4709 |
| W pycno+ oil,g | 26.6005 | 26.5980 | 26.5990 |
| W oil,g | 9.1297 | 9.1270 | 9.1281 |
| V Pycno,ml | 10.0000 | 10.0000 | 10.0000 |
| Density,g/ml | 0.9130 | 0.9127 | 0.9128 |
| Ave.Density,g/ml | | 0.9128 | |
| Viscosity | 25.5000 | 25.0000 | 26.0000 |
| Ave.Viscosity,Cp | | 25.5 | |

**Table A-1.2.** Acid value using  AOCS Cd-3D-63

| | 1 | 2 |
|---|---|---|
| W oil,g | 5.0546 | 5.0234 |
| V diethyl ether+ethanol | 50.0 | 50 |
| Conc $_{KOH}$, N | 0.1 | 0.1 |
| $V_{KOH}$used ,ml | 3.5 | 3.5 |
| Acid Value | 3.8845 | 3.9087 |
| Average A V | 3.8966 | |
| FFA(%Lauric acid) | 1.3824 | 1.391 |
| Average FFA | 1.3867 | |
| Amt needed to neutralize oil<br>g Naoh/100 g oil | 2.7783 | |

Formula:

A.V.  =   $56.1 \times N \times V_{KOH} / W_{oil}$

FFA (% Lauric acid) =  A.V./ 2.81

Amount needed to neutralize oil =A.V. x 40 /5 6.1

**Table A-1.3.** Saponification value using AOCS Cd-3b-76

| | Time | |
|---|---|---|
| | 1 | 2 |
| W oil,g | 1.9122 | 1.85 |
| $V_{0.5\ N\ NaOH}$ ,ml | 25.0000 | 25 |
| Conc HCl,N | 0.5 | 0.5 |
| $V_{HCl}$used ,ml | 5.1 | 5 |
| S.V. | 219.248 | 228.0632 |
| Average A V | 223.6557 | |

Formula:

S.V. = $56.1 \times N \times (V_0 - V_1) / W_{oil}$

**Calculation of molecular weight of palm kernel oil**

Mean Molecular weight of triglyceride

$$M.MW. = (3)(MW.) + 38$$

$$MW. = \frac{16108 - (12.67)(S.V._{neutral}}{S.V._{neutral}}$$

$$S.V.\ neutral = \frac{56108 \times S.V.}{56108 + 12.67(A.V.)}$$

Where

M,MW = Mean molecular weight of triglyceride.

MW = molecular weight of the combined fatty acid in neutral pure triglyceride.

S.V. neutral = If the triglyceride contain free fatty acid than the S.V can be corrected for the free fatty acids(A.V)

## A-2 Standard calibration curve of diethanolamide

**Table A-2.1** Standard calibration curve data of Caprylic diethanolamide.

| Concentration of $C_8$ amide, (g/ml) | $A_I$ | $A_{C8}$ | $A_{C8}/A_I$ |
|---|---|---|---|
| 0.002382 | 26.6603 | 8.034 | 0.301347 |
| 0.00397 | 26.3885 | 12.0666 | 0.457267 |
| 0.005558 | 25.4934 | 16.9935 | 0.666584 |
| 0.00794 | 25.3835 | 24.473 | 0.96413 |

**Table A-2.2** Standard calibration curve data of Capric diethanolamide.

| Concentration of $C_{10}$ amide, (g/ml) | $A_I$ | $A_{C10}$ | $A_{C10}/A_I$ |
|---|---|---|---|
| 0.0039588 | 22.0543 | 7.6904 | 0.348703 |
| 0.006598 | 25.2569 | 15.0681 | 0.596593 |
| 0.0085774 | 27.7735 | 20.2266 | 0.72827 |
| 0.013196 | 32.0516 | 35.8142 | 1.117392 |

**Table A-2.3** Standard calibration curve data of Lauric diethanolamide.

| Concentration of $C_{12}$ amide, (g/ml) | $A_I$ | $A_{C12}$ | $A_{C12}/A_I$ |
|---|---|---|---|
| 0.004176 | 18.3924 | 3.5152 | 0.191122 |
| 0.00696 | 18.5724 | 5.0074 | 0.269615 |
| 0.009744 | 18.3033 | 6.9391 | 0.379117 |
| 0.01392 | 21.0377 | 12.4089 | 0.589841 |

**Table A-2.4** Standard calibration curve data of Myristic diethanolamide.

| Concentration of $C_{14}$ amide, (g/ml) | $A_I$ | $A_{C14}$ | $A_{C14}/A_I$ |
|---|---|---|---|
| 0.0031884 | 18.1561 | 5.4266 | 0.298886 |
| 0.005314 | 21.0096 | 10.1988 | 0.485435 |
| 0.0074396 | 21.8468 | 16.0554 | 0.734909 |
| 0.010628 | 21.3504 | 20.4213 | 0.956483 |

**Table A-2.5** Standard calibration curve data of Palmitic diethanolamide.

| Concentration of $C_{16}$ amide, (g/ml) | $A_I$ | $A_{C16}$ | $A_{C16}/A_I$ |
|---|---|---|---|
| 0.002916 | 22.6158 | 4.504 | 0.199153 |
| 0.00486 | 22.0669 | 7.6677 | 0.347475 |
| 0.006804 | 22.7549 | 10.8542 | 0.477005 |
| 0.00972 | 22.2864 | 15.2287 | 0.683318 |

**Table A-2.6** Standard calibration curve data of Oleic diethanolamide.

| Concentration of $C_{18:1}$ amide, (g/ml) | $A_I$ | $A_{C18:1}$ | $A_{C18:1}/A_I$ |
|---|---|---|---|
| 0.0020539 | 25.3952 | 4.1741 | 0.164366 |
| 0.004668 | 26.8946 | 11.3798 | 0.423126 |
| 0.0065352 | 29.4556 | 16.4715 | 0.559198 |
| 0.0088692 | 29.2525 | 19.5214 | 0.667341 |

**Table A-2.7** Standard calibration curve data of Diethanolamine

| Concentration of DEA, (g/ml) | $A_I$ | $A_{DEA}$ | $A_D/A_I$ |
|---|---|---|---|
| 0.001202 | 28.1119 | 1.6646 | 0.059213 |
| 0.003006 | 28.1111 | 6.7711 | 0.240869 |
| 0.006011 | 28.1111 | 16.0151 | 0.569707 |

**Table A-2.8** Standard calibration curve data of Glycerol.

| Concentration of glycerol, (g/ml) | $A_I$ | $A_{glycerol}$ | $A_g/A_I$ |
|---|---|---|---|
| 0.001086 | 28.1119 | 4.738268 | 0.16855 |
| 0.002716 | 28.1111 | 9.8678 | 0.351029 |
| 0.005432 | 28.1111 | 28.9876 | 1.03118 |

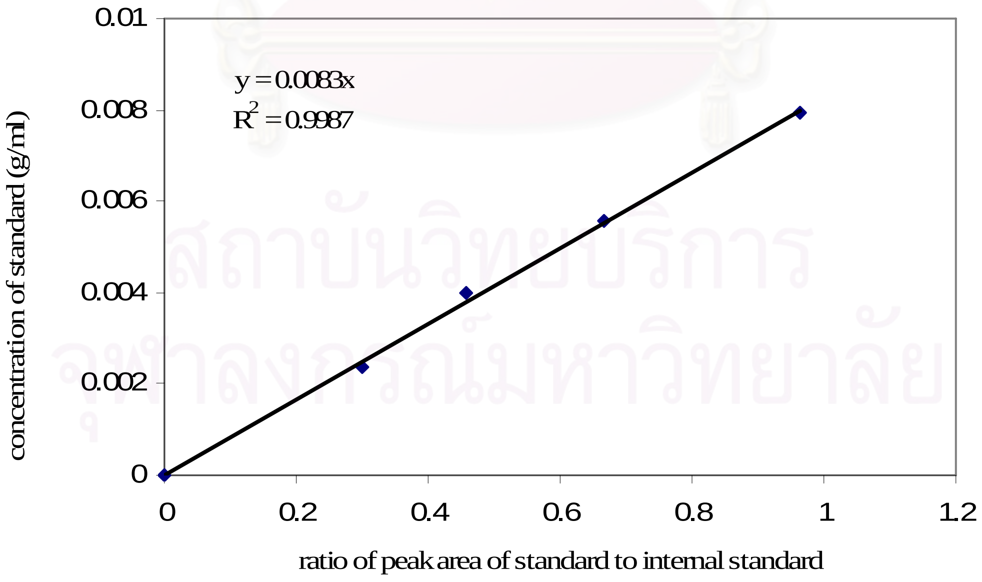

**Figure A-2.1** Standard calibration curve data of Caprylic diethanolamide.

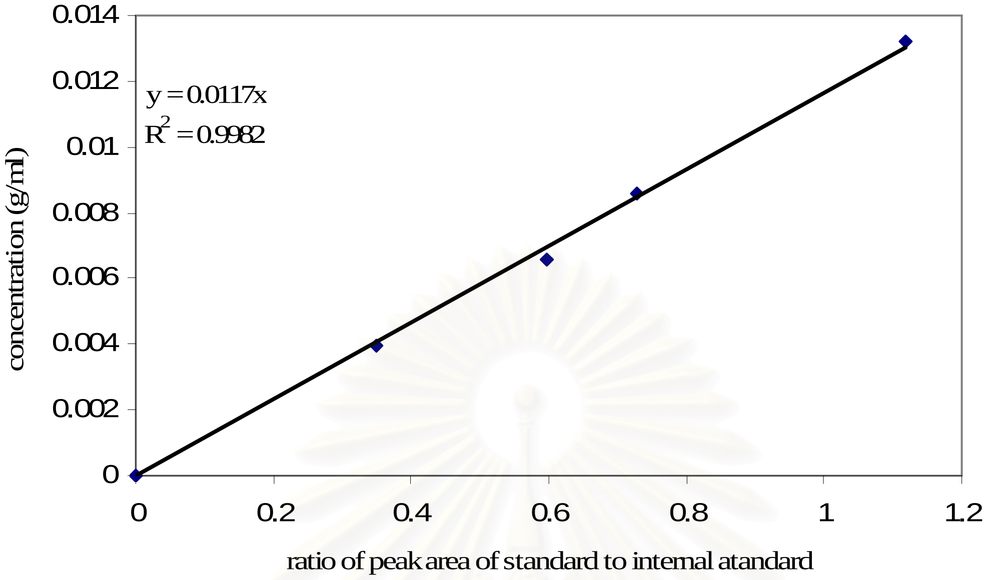

**Figure A-2.2** Standard calibration curve data of Capric diethanolamide.

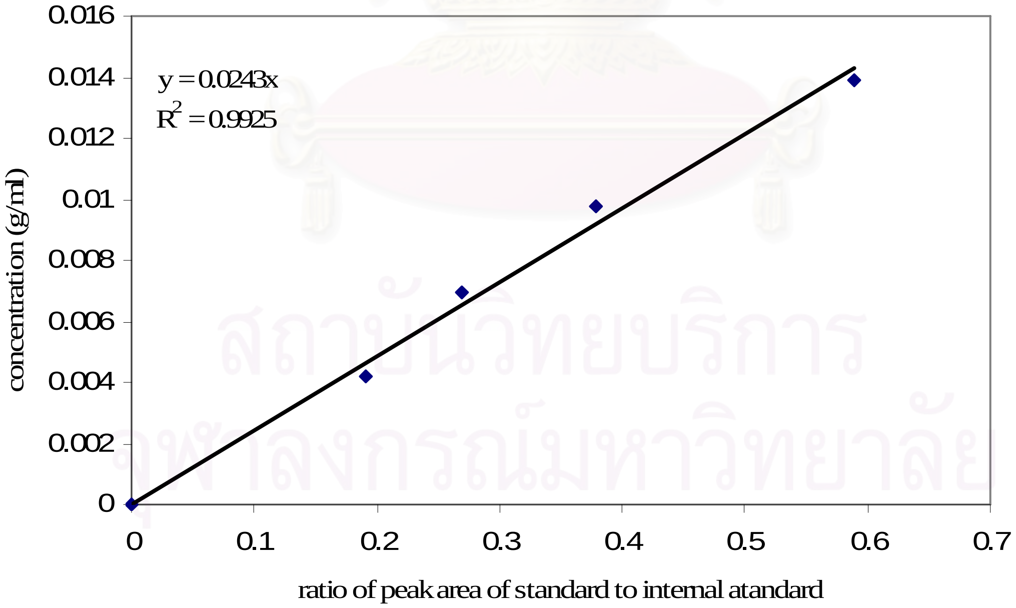

**Figure A-2.3** Standard calibration curve data of Lauric diethanolamide

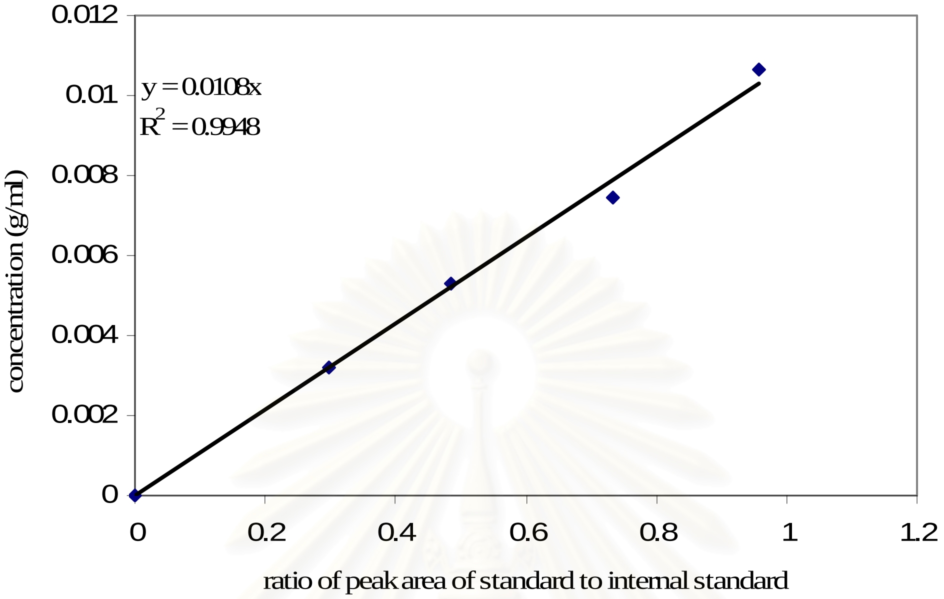

**Figure A-2.4** Standard calibration curve data of Myristic diethanolamide.

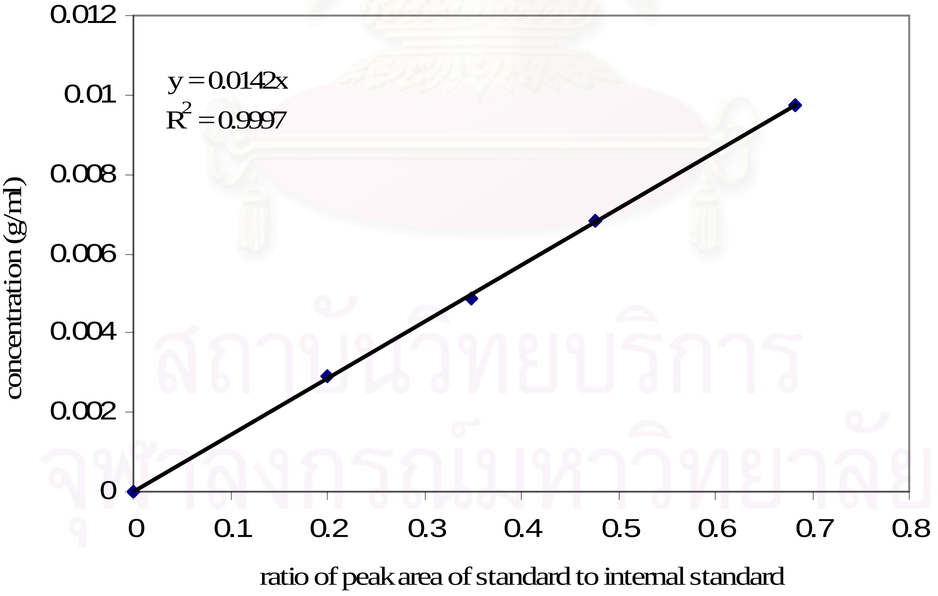

**Figure A-2.5** Standard calibration curve data of Palmitic diethanolamide

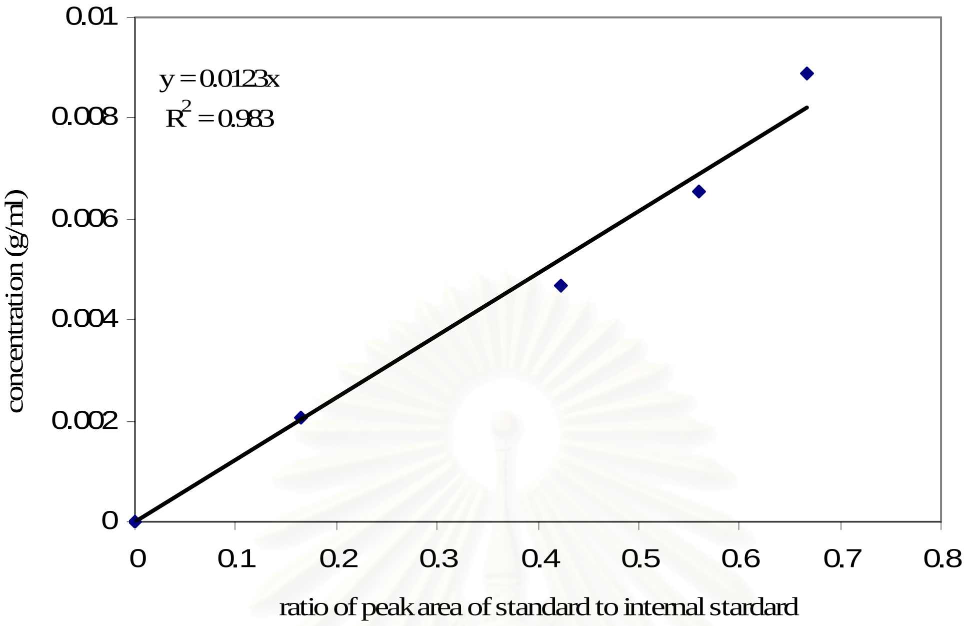

**Figure A-2.6** Standard calibration curve data of Oleic diethanolamide

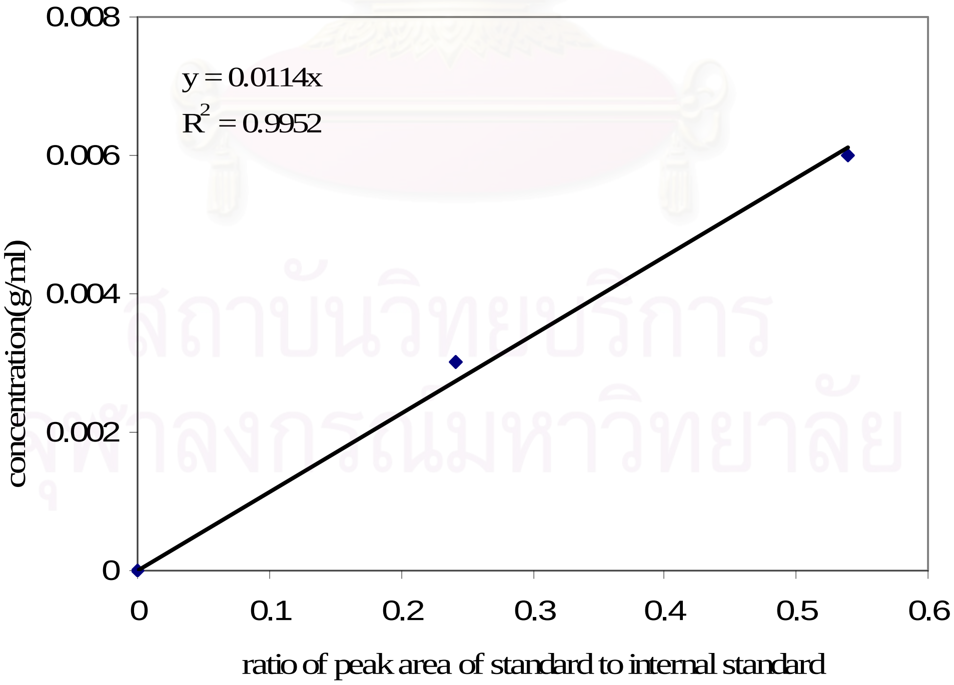

**Figure A-2.7** Standard calibration curve data of Diethanolamine

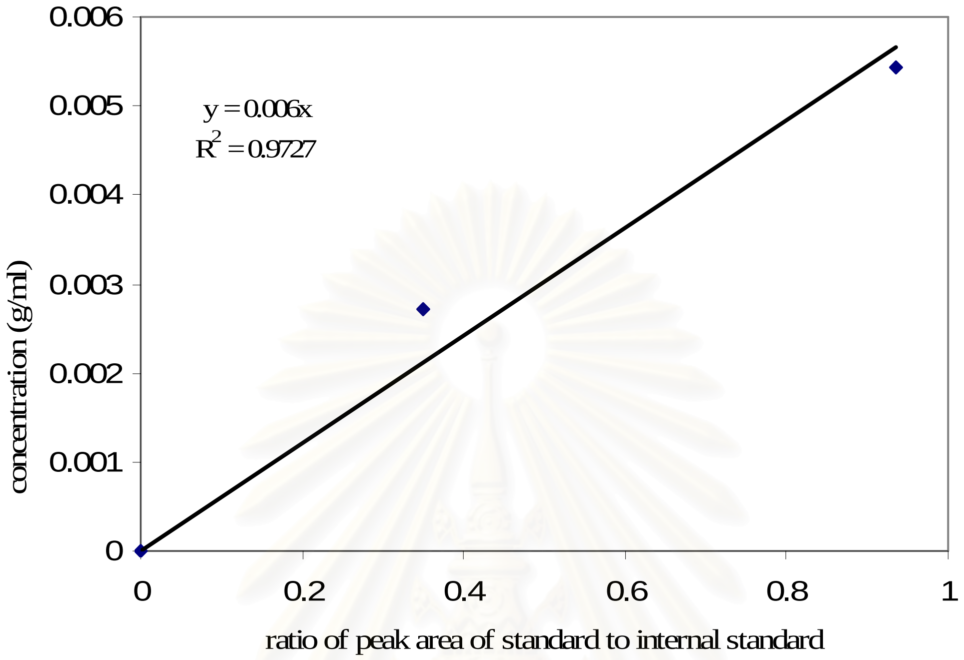

**Figure A-2.8** Standard calibration curve data of glycerol

# Appendix B

## B Diethanolamide products and calculation percent of amide

### 1. Diethanolamide

Analysis of diethanolamide in product by gas chromatography (GC). The retention time of diethanolamide is different. Therefore, for find the type of diethanolamide by compare retention time of each diethanolamide with diethanolamide standard.

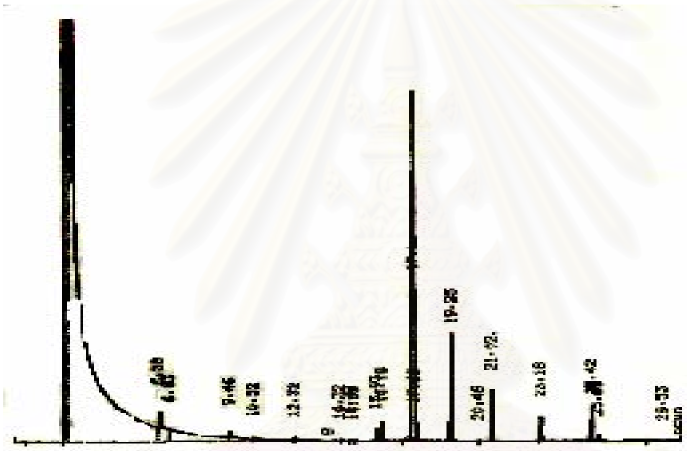

**Figure B1**. GC chromatogram of diethanolamide standard 1

**Table B1** The percentage of diethanolamide standard 1

| component | PKO DEA (standard1) |
|---|---|
| $C_8$ amide | 2.54 |
| $C_{10}$ amide | 3.73 |
| $C_{12}$ amide | 59.22 |
| $C_{14}$ amide | 10.34 |
| $C_{16}$ amide | 5.26 |
| $C_{18:1}$ amide | 10.94 |
| Total | 92.03 |
| Mw of diethanolamide, g/mole | 291 |

## 2. Calculation of yield of diethanolamide

$$\text{Yield (\%,w/w)} = \frac{W_D}{W_T} \times 100$$

$$W_D = \frac{C \times V_{TD} \times W_P}{W_s}$$

Where

$W_D$ = weight of diethanolamide obtained (g)

C = concentration of each diethanolamide from calibration curve (g/ml)

$V_{TD}$ = total volume dilute (ml)

$W_S$ = weight of diethanolamide(g)

$W_T$ = weight of diethanolamide from mass balance(g)

$W_P$ = total weight of diethanolamide (g)

## 3. Calculation of concentration of triglyceride.

The concentration of triglyceride at ant time t can be obtained from the reaction stoichiometry:

$$C_A = \frac{Wu - Wr}{V}$$

$$Wr = \frac{MW_x \times W_D}{3 \times MW_y}$$

where

$C_A$ = concentration of triglyceride.(mole/l)

Wu = weight of triglyceride used (g)

Wr = weight of triglyceride react (g)

V = Total volume of reactant (l).

MWx = Molecular weight of triglyceride(g/mole).

MWy = Molecular weight of diethanolamide(g/mole).

**<u>Example1.</u>** Find yield of diethanolamide and concentration of triglyceride at 100 % excess from its stoichiometic ratio, temperature of 60 °C, 4 hours using alkali catalyst. The volume of product 690 ml and 0.0069 g of product add to 0.4 ml of THF and 0.1 0f MSTFA.

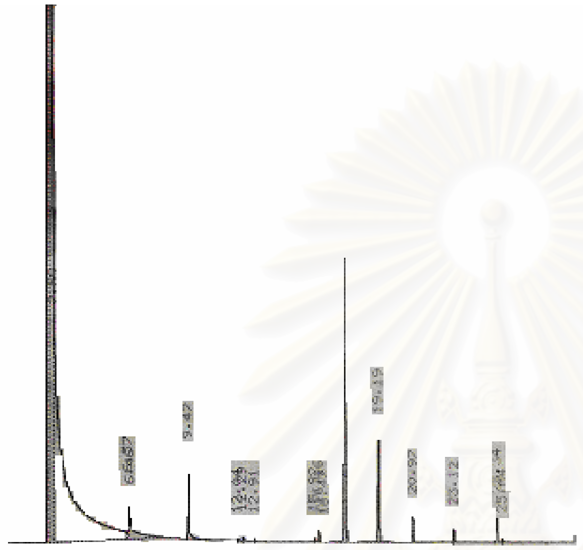

**Figure B2.** GC chromatogram of diethanolamide at 100 % excess from its stoichiometic ratio and a temperature of 60 °C using alkai-catalyst.

**<u>Weight of each diethanolamide.</u>**

Weight of $C_8$ amide = $\frac{0.000174 \times 0.5 \times 690}{0.0069}$ = 8.72

Weight of $C_{10}$ amide = $\frac{0.000433 \times 0.5 \times 690}{0.0069}$ = 21.63

Weight of $C_{12}$ amide = $\frac{0.00590 \times 0.5 \times 690}{0.0069}$ = 295.19

Weight of $C_{14}$ amide = $\frac{0.00073 \times 0.5 \times 690}{0.0069}$ = 36.51

Weight of $C_{16}$ amide = $\frac{0.000479 \times 0.5 \times 690}{0.0069}$ = 13.94

Weight of $C_{18}$ amide = $\frac{0.000916 \times 0.5 \times 690}{0.0069}$ = 45.79

$W_T$ = 463.74 g

$$\text{Yield \%} = \frac{(8.72 + 21.63 + 295.19 + 36.51 + 13.94 + 45.79)}{463.74} \text{ X } 100$$

Yield % = 93.24 %

**The concentration of triglyceride**

$$\text{Wr} = \frac{753 \times 431.79}{3 \times 291} = 372.44 \text{ g}$$

V = 0.9018 L

$$C_A = \frac{400 - 372.44}{0.9018 \text{ l x } 753} = 0.04 \text{ mol/l}$$

**Table B2** The concentration of triglyceride using alkali catalyst.

| Time (hr) | Concentration $C_A$, mol/l | $\frac{1}{C_A} - \frac{1}{C_{AO}}$ | $-\ln\left(\frac{C_A}{C_{AO}}\right)$ |
|---|---|---|---|
| 0 | 0.589 | 0 | 0 |
| 2 | 0.087 | 9.80 | 1.91 |
| 4 | 0.04 | 23.43 | 2.69 |

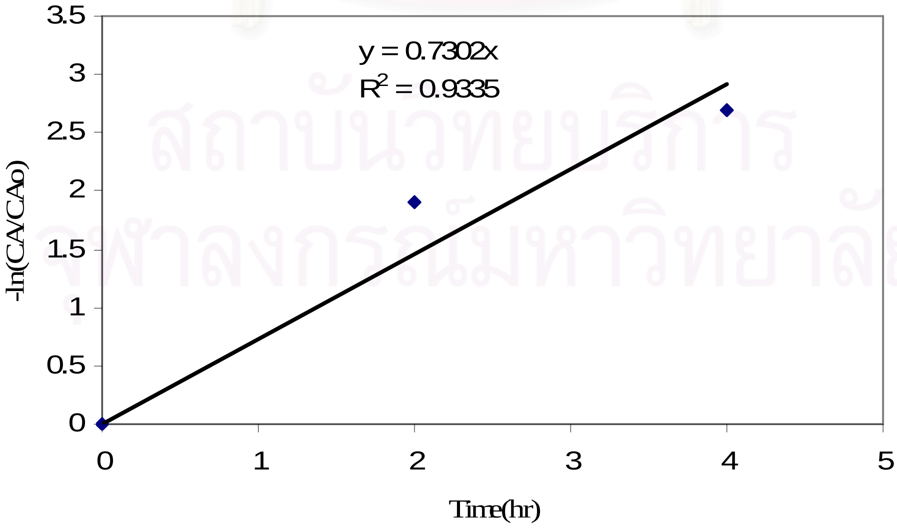

**Figure B3** first order kinetic plot (optimum condition) using alkali catalyst.

**Example2.** Find yield of diethanolamide and concentration of triglyceride at 100 % excess from its stoichiometic ratio, temperature of 60 °C, 4 hours using enzyme catalyst.  The volume of product 0.62 ml and 0.0084 g of product add to 0.4 ml of THF and 0.1 0f MSTFA.

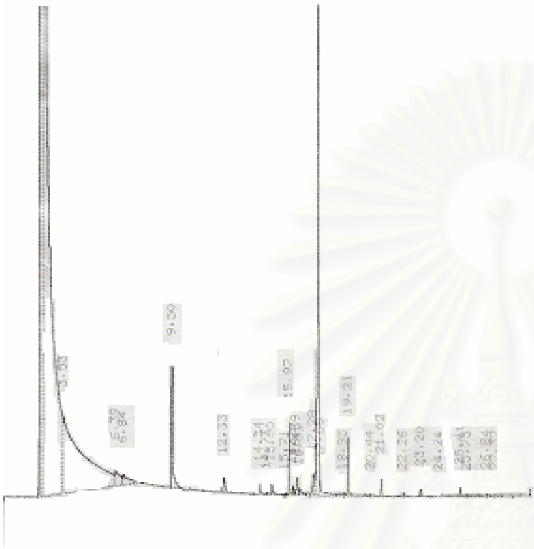

**Figure B4**. GC chromatogram of diethanolamide at stoichiometic ratio and a temperature of 60 °C using lipase-catalyst.

**Weight of each diethanolamide.**

Weight of $C_8$ amide = $\frac{0.08\times10^{-3}\times0.5\times0.62}{0.0084}$ = $2.84\times10^{-3}$

Weight of $C_{10}$ amide = $\frac{0.63\times10^{-3}\times0.5\times0.62}{0.0084}$ = $23.21\times10^{-3}$

Weight of $C_{12}$ amide = $\frac{1.96\times10^{-3}\times0.5\times0.62}{0.0084}$ = $72.26\times10^{-3}$

Weight of $C_{14}$ amide = $\frac{0.28\times10^{-3}\times0.5\times0.62}{0.0084}$ = $10.17\times10^{-3}$

Weight of $C_{16}$ amide = $\frac{0.134\times10^{-3}\times0.5\times0.62}{0.0084}$ = $4.95\times10^{-3}$

Weight of $C_{18}$ amide = $\frac{0.131\times10^{-3}\times0.5\times0.62}{0.0084}$ = $4.83\times10^{-3}$

$W_T$ =  1.159 g

$$\text{Yield \%} = \frac{(2.84 + 23.21 + 72.26 + 10.17 + 4.95 + 4.83) \times 10^{-3}}{1.159} \quad \text{X } 100$$

Yield % = 10.20 %

**<u>The concentration of triglyceride</u>**

$$\text{Wr} = \frac{753 \times 118.3 \times 10^{-3}}{3 \times 291} = 0.102 \text{ g}$$

V = 10 L

$$C_A = \frac{1 - 0.102}{0.01 \text{ x } 753} = 0.1193 \text{ mol/l}$$

**Table B3** The concentration of triglyceride using enzyme catalyst.

| Time (hr) | Concentration $C_A$, mol/l | $\frac{1}{C_A} - \frac{1}{C_{AO}}$ | $-\ln\left(\frac{C_A}{C_{AO}}\right)$ |
|---|---|---|---|
| 0 | 0.1328 | 0.0000 | 0.0000 |
| 6 | 0.1260 | 0.4045 | 0.0523 |
| 12 | 0.1232 | 0.5845 | 0.0748 |
| 18 | 0.1208 | 0.7452 | 0.0944 |
| 24 | 0.1193 | 0.8555 | 0.1076 |

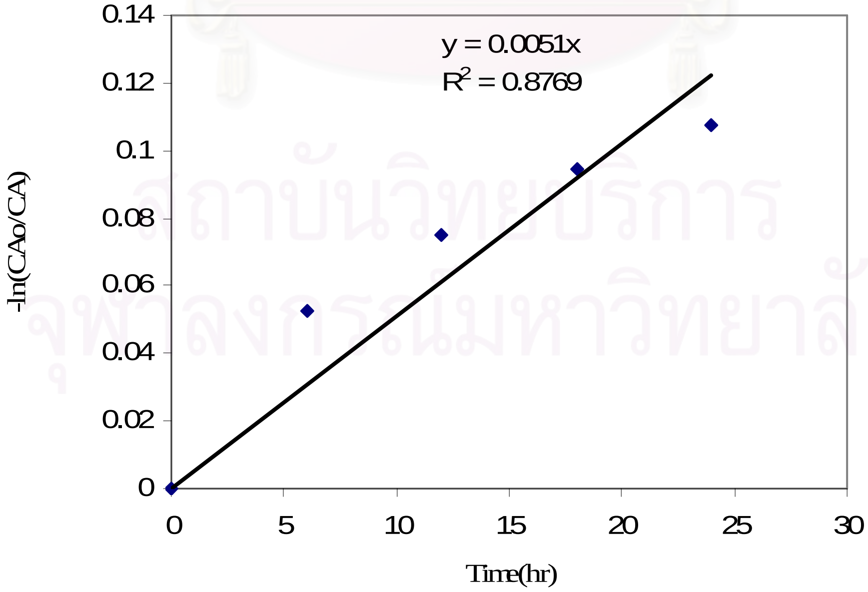

**Figure B5** first order kinetic plot (optimum condition) using enzyme catalyst.

# Appendix C

## C Raw Data for diethanolamide using alkali-catalysts and enzyme-catalysts.

**Table C1.** PKO dirthanolamide using alkali-catalyst in 6 hours.

| sample No. | wt. oil,g | wt.DEA,g | wt.MeOH, g | wt.NaOH, g | Yield % |
|---|---|---|---|---|---|
| 36 | 500 | 209.37 | 127.44 | 6.389 | 92.62 |
| 38 | 500 | 209.4 | 127.5 | 6.39 | 86.55 |
| 312 | 500 | 210 | 127.45 | 6.393 | 84.88 |
| 61 | 400 | 335 | 101.95 | 5.11 | 90.42 |
| 68 | 400 | 334.99 | 102 | 5.12 | 81.96 |
| 612 | 400 | 335.5 | 102 | 5.11 | 82.00 |
| 156 | 250 | 523.42 | 63.72 | 3.194 | 76.04 |
| 158 | 250 | 523.5 | 63.72 | 3.2 | 71.25 |
| 1512 | 250 | 523.4 | 63.7 | 3.19 | 78.45 |

**Table C2.** PKO dirthanolamide using enzyme-catalyst in 24 hours.

| sample No. | wt. oil,g | wt.DEA,g | wtTHF, ml | wt.NaOH, g | Yield % |
|---|---|---|---|---|---|
| L34 | 1 | 0.418 | 10 | 0.04 | 4.87 |
| L35 | 1 | 0.42 | 10 | 0.04 | 8.50 |
| L36 | 1 | 0.42 | 10 | 0.04 | 10.20 |
| L64 | 1 | 0.832 | 10 | 0.04 | 4.05 |
| L65 | 1 | 0.840 | 10 | 0.04 | 5.29 |
| L66 | 1 | 0.834 | 10 | 0.04 | 5.64 |
| L154 | 1 | 2.09 | 10 | 0.04 | 1.72 |
| L155 | 1 | 2.10 | 10 | 0.04 | 5.38 |
| L156 | 1 | 2.10 | 10 | 0.04 | 4.42 |

**Table C3** The yield of diethanolamide at temperature of 120 °C using alkai-catalyst.

| Time(hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | stoichiometic ratio | 100% excess from stoi ratio | 400% excess from stoi ratio |
| 2 | 73.19 | 71.64 | 72.22 |
| 4 | 78.49 | 79.58 | 73.84 |
| 6 | 84.88 | 82.00 | 74.45 |
| 8 | 89.57 | 88.51 | 78.45 |

**Table C4** The yield of diethanolamide at temperature of 80 °C using alkai-catalyst.

| Time(hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | stoichiometic ratio | 100% excess from stoi ratio | 400% excess from stoi ratio |
| 2 | 79.83 | 70.03 | 65.63 |
| 4 | 82.15 | 80.50 | 67.76 |
| 6 | 86.55 | 81.96 | 71.25 |
| 8 | 89.30 | 88.04 | 72.18 |

**Table C5** The yield of diethanolamide at temperature of 60 °C using alkai-catalyst

| Time(hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | stoichiometic ratio | 100% excess from stoi ratio | 400% excess from stoi ratio |
| 2 | 70.49 | 85.23 | 71.10 |
| 4 | 82.03 | 93.24 | 71.15 |
| 6 | 92.62 | 90.42 | 76.04 |
| 8 | 90.88 | 79.05 | 64.23 |

**Table C6** The yield of diethanolamide at stoichiometry ratio using enzyme-catalyst

| Time(hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | 40 °C | 50 °C | 60°C |
| 6 | 3.57 | 4.40 | 5.10 |
| 12 | 3.60 | 5.97 | 7.20 |
| 18 | 4.68 | 7.70 | 9.00 |
| 24 | 4.87 | 8.50 | 10.20 |

**Table C7** The yield of diethanolamide at 100% excess from its stoichiometic ratio using enzyme-catalyst

| Time(hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | 40 °C | 50 °C | 60°C |
| 6 | 0.00 | 0.00 | 0.00 |
| 12 | 3.19 | 2.38 | 1.87 |
| 18 | 3.96 | 3.01 | 3.63 |
| 24 | 4.05 | 5.29 | 5.64 |

**Table C8** The yield of diethanolamide at 400% excess from its stoichiometic ratio using enzyme-catalyst

| Time(hr) | Yield (%,w/w) | | |
|---|---|---|---|
| | 40 °C | 50 °C | 60°C |
| 6 | 0.00 | 0.00 | 0.00 |
| 12 | 0.69 | 1.74 | 1.55 |
| 18 | 0.96 | 3.68 | 2.68 |
| 24 | 1.72 | 5.38 | 4.42 |

# VITA

Miss Sirirat Khomwaree was born on January 1, 1982 in Saraburi, Thailand. She received the Bachelor degree of chemical engineering from Institute of Engineering, Kasetsart University in May 2004. The last education is studying in Master Degree in Chemical Engineering, Chulalongkorn University that began in 2004.